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Interpretation of the Clinical Course and Surgical Treatment of Grade III and IV Prolapsing Haemorrhoidal Disease Using Morphological and Bioinformatic Data Analysis

Summary of the Doctoral Thesis for obtaining the scientific degree "Doctor of Science (*PhD*)"

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The Doctoral Thesis was developed at the Surgery Clinic of Pauls Stradiņš Clinical University Hospital, the Interdepartmental Electron Microscopy Laboratory of the Institute of Anatomy and Anthropology at Rīga Stradiņš University, and the Institute of Microbiology and Virology of Rīga Stradiņš University, Latvia.

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Abbreviations used in the Thesis

Abbreviation	**Explanation**
AI	Artificial intelligence
ANO1	Anoactimin-1
ASA	American Society of Anesthesiologists
CD105	Endoglin
CD 34	Transmembrane glycoprotein
DNN	Deep neural network
FFPE	Formalin- Fixed Paraffin- Embedded
FI	Faecal inkontinence
GIT	Gastrointestinal tract
H&E	Haemotoxylin and eosin
HD	Haemorrhoidal disease
IAS	Internal anal sphincter
ICC	Interstitial cells of Cajal
IHC	Immunohistochemistry
IL	Pro-inflammatory cytokine
KIT (CD 117/c-KIT)	Type III receptor tyrosine kinase
LH	*LigaSure* haemorrhoidectomy
MMP	Matrix metalloproteinase
PPH	Circular steipler haemorrhopidopexy (Longo modification)
PSCUH	Pauls Stradiņš Clinical University Hospital
RSU	Rīga Stradiņš University
TGF β	Transforming growth factor β
TMEM	Transmembrane preotein
TNF-α	Tumor necrosis factor α
VAS	Visual Analogue Scale
WSI	Whole slide images

Introduction

Haemorrhoidal disease (HD) is a pathological enlargement of the uniquely formed anal canal wall submucosal cushions, characterised byprolapse and/or bleeding. Nowadays, colorectal surgeons use several classifications of HD based on Goligher's classification, which does not reflect possible etiological factors, prolapse symptoms, enteropathogenesis or age and gender differences (Rubbini et al., 2019). Clinically important is the third grade, wherein haemorrhoidal tissue prolapses below the dentate line, causing clinical symptoms and serious functional complaints, and where prolapse repositioning is not spontaneous (Mott et al., 2018; Gardner et al., 2020). In grade IV HD, repositioning of prolapsed haemorrhoidal tissue is not possible even manually, and the condition involves chronic inflammation with maceration, mucosal atrophy, and ulceration (Garg, 2017; Lohsiriwat, 2018; Margetis, 2019).

HD is very common, especially in developed countries with high socioeconomic status, and is increasingly found in younger people. The true incidence is unknown, but various sources report 4–40 % in the population (Lohsiriwat et al., 2015). Some studies report that the prevalence of HD is as high as 88 %, depending on the HD classification variant used (Hong et al., 2022; Kumar Jayaswal et al., 2022). Approximately 50 % of people worldwide over the age of 50 have complaints related to HD (Sahin et al., 2020; Sadeghi et al., 2021).

Despite extensive research and accumulated knowledge on the etiology and pathogenesis of HD over the past decade, the mechanisms responsible for the development of this prolapsing disease are not fully understood. It was previously believed that haemorrhoidal disease arises from the descent of improperly loaded blood vessels into the anal cushions (Li et al., 2015; Lohsiriwat, 2018; Fontem et al., 2020; Erol et al., 2025). However, while anal cushion prolapse explains the prolapse of haemorrhoidal nodes, it cannot explain recurrent bleeding in patients with non-prolapsed nodes. This bleeding could be secondary to age-related destructive changes in connective tissue (Sardinas et al., 2016); however, this theory does not explain the presence of prolapsing nodes in young patients (< 45 years) without connective tissue or autoimmune diseases, previous surgeries, pregnancies, etc. It is possible that this phenomenon is due to the depletion of Treitz's muscle capacity in these patients (Guttadauro et al., 2018).

Previous studies have demonstrated that metalloproteinases (MMP) and their inhibitors play a role in the remodelling of prolapsed tissue (Serra et al., 2015). A correlation has been found involving the effect of proteolytic enzymes, which cause inflammatory reactions, microangiopathy, blood vessel dilation, and progressive connective tissue weakness (Bergan et al., 2006), but no markers capable of determining predisposition have been found. The composition of collagen has also been studied, with changes being associated with prolapsed haemorrhoidal nodes (Nasseri et al., 2015). Reduced type I collagen and increased type III collagen are found in HD tissue compared to healthy tissue and neonatal tissue (Sardinas et al., 2016). Studies have also demonstrated reduced collagen protein synthesis in HD grades III and IV (Nasseri et al., 2015). However, these studies found no association with patient age or gender. Contradictory are the studies that find a link between increased collagen type III in tissues and advanced age (Sardinas et al., 2016). It is also unclear whether changes in collagen protein synthesis are caused by exogenous or endogenous factors (Sandler et al., 2019).

There are studies that describe changes in the blood vessel wall as hyperplasia, pathological lumen dilatation in varicose veins, and hyperperfusion, but structural changes in HD have not been fully investigated (Margetis, 2019). In studies by other authors, venous outflow disorders are associated with sinusoidal overload (Kuivaniemi et al., 2019). Venous insufficiency in the anal canal, associated with reflux or hypertension in the small pelvis, is found in about one-third of women with HD grades III/IV (Holdstock et al., 2015). One of the factors causing venous insufficiency is the overproduction of CD105 in the vascular bed (Lohsiriwat, 2018). Several vasodilatory factors have been identified in HD, including changes in vascular endothelial receptors and the presence of TGF-β (Li et al., 2015; Lohsiriwat et al., 2017). Endothelin receptor B is found three times more frequently than endothelin receptor A in cases of HD (Lohsiriwat et al., 2017). The severity of HD has been shown to correlate with blood vessel diameter (Aigener et al., 2006). Studies have shown that a significant increase in the terminal branches of the superior rectal artery increases blood supply to haemorrhoidal nodes compared to healthy individuals, which is associated with the degree of HD (Tian et al., 2018). It is possible that HD develops secondarily, as the use of superior rectal artery embolisation in HD grade III does not cause ischemic changes in the colon wall (Tradi et al., 2019).

In addition to vascular and connective tissue structures, interstitial cells of Cajal (ICC) play a significant role in regulating motility and sphincter function in the gastrointestinal tract (GIT). These are specialised cells located in the muscular layer of the intestinal tract and are closely associated with enteric neurons, facilitating coordinated smooth muscle activity. ICC are responsible for generating bioelectric rhythms that drive peristalsis and relaxation reflexes in the rectum, including the internal anal sphincter (IAS). Their density and structure vary across different tissue layers, and even minor changes in cell number or architecture can affect motility, contributing to defecation disorders or creating conditions conducive to the progression of HD. Furthermore, ICC are sensitive to inflammatory mediators such as TNF-α, IL-1β, and IL-6, suggesting a potential interaction between local inflammation and motility disorders (Kaji et al., 2023; Mah et al., 2020). Consequently, ICC provide important insights into motoric and sphincter regulation, complementing our understanding of how changes in tissue structure influence symptom development. Modern microscopy and automated image analysis methods allow for a more detailed assessment of the organisation and functionality of the cellular network, offering opportunities for both the identification of new biomarkers and more precise planning of individualised therapy, particularly for patients with progressive grade III–IV HD (Zhang et al., 2016; Ward et al., 2000). Such an interdisciplinary approach combines morphological and functional aspects and potentially facilitates the application of artificial intelligence (AI) in the analysis and treatment of HD.

Likewise, studies have been conducted to evaluate possible correlations between episodes of upper GIT bleeding and patients' AB0 blood groups (Bayan et al., 2009; Merle et al., 2018; Zheng et al., 2021). Although some analyses suggest a possible association between the AB0 blood group and the frequency of haemorrhoidal bleeding, consistently statistically significant results have been observed primarily in patients with blood group 0, whereas this association has been less frequently observed in individuals with blood groups B and AB (Dahlén et al., 2021; Matkovic et al., 2021). The current understanding of the association between the development of haemorrhoids and different AB0 blood groups remains incomplete.

HD is a multifactorial disease whose pathophysiological causes are not fully understood. This disease negatively affects the quality of life. Surgical treatment guidelines based on Goligher's classification do not ensure a complete cure in the long term. Surgical therapy presents several complications, and relapses of the disease are also common. The International Society of Coloproctology is working intensively to improve the classification of HD and develop new guidelines that, in turn, will increase the possibility of treating patients not only symptomatically, but also etiologically, taking into account comorbidities, age, and gender (Rubbini and Ascanelli, 2019). Furthermore, with the expansion of knowledge and a deeper understanding of the development and progression of the disease, it has become clear that markers are needed to improve preoperative assessment and more accurately determine the patient's prognosis (Beksac et al., 2018). This, in turn, would allow the selection of patients who would be indicated for more invasive treatment in order to avoid complications and disease recurrence. Surgical data science is a new field that aims to "improve the quality and value of healthcare interventions by acquiring, organising, analysing, and modelling data" (Maier-Hein et al., 2017), particularly using AI-based methods. AI advances are predicted to impact surgery, but due to its specific characteristics and complexity, progress in this field has been slow. AI has been successful in object recognition, speech recognition, and natural language processing (Esteva et al., 2019). There is strong support for radiologists in the diagnosis of colorectal, ovarian, breast, and pancreatic cancer images (Yao et al., 2024), but surgical data analysis is not often performed due to the large amount of annotated data required for training.

In view of the above, interdisciplinary clinical and morphological research of HD is relevant, using a combination of several research methods and applying AI frameworks.

Aim of the Paper

The aim of the study is to comprehensively analyse the clinical course of grade III and IV prolapsing HD, the surgical treatment methods used and their outcomes, by integrating morphology, bioinformatics and AI methods into the data analysis.

Tasks of the Paper

The following tasks have been set to achieve the aim of the Doctoral Thesis:

1. To collect and analyse the medical history and clinical data of patients with grade III and IV prolapsing HD, describing the clinical characteristics of the disease; and to evaluate the effectiveness of various surgical treatment methods by analysing their relationship with clinical symptoms and the postoperative period.
2. To evaluate possible correlations between the clinical manifestation of HD, patient demographics and clinical indicators, morphological findings, and AB0 blood group, using bioinformatics data analysis methods.
3. To understand the morphological abnormalities characteristic of the development of anal canal mucosal prolapse, and to conduct a detailed study of resected haemorrhoidal tissue using histopathology, histochemistry, and immunohistochemistry methods to evaluate connective tissue structure, blood vessel changes, and signs of inflammation.
4. Using immunohistochemistry methods, to determine the electrically active ICC that coordinate smooth muscle contractions and peristalsis, as well as ANO1 (Ca^{2+}-activated Cl^{-} channel protein) expression and their role in HD pathogenesis. At the same time, using deep neural network (DNN) models trained for immunohistochemical (IHC) image analysis, to determine the presence and quantity of ICC in resected anal canal tissues in patients with HD, interpreting the role of these cells in regulating intestinal motility and expanding our understanding of the pathophysiological processes of the disease.

Hypotheses of the Paper

1. Regardless of gender, patients with grade III and IV prolapsing HD have similar morphological characteristics of the rectal wall, but there are differences between different age groups, and the incidence of the disease increases proportionally with the patient age.
2. Patients with blood group 0 HD exhibit different morphological features compared to patients with other blood groups.
3. The density of ICC in anorectal tissues is associated with the severity of HD.

Novelty of the Paper

This study is innovative because it addresses a significant knowledge gap in Latvia regarding the prevalence, clinical characteristics, and treatment of symptomatic HD. Unlike previous studies, it combines detailed clinical, histological, and morphometric analysis, providing a comprehensive overview of HD. In addition, the study applies advanced quantitative tissue assessment tools and an integrated AI approach (DNN models) in ICC analysis.

To date, no complex analysis of anorectal tissues stratified by lesion location and appropriate surgical technique has been performed in the existing literature. This Doctoral Thesis is the first in Latvia to compile and analyse the clinical, morphological, and bioinformatic data of patients with grade III and IV prolapsing HD, including a nine-month postoperative follow-up.

The study evaluates for the first time the possible links between the clinical manifestations of HD, the surgical methods applied, and the AB0 blood groups of patients, while also analysing the structure of prolapsed and resected anorectal tissue. By combining clinical, morphological, and bioinformatic data, the work provides a new perspective on the pathogenesis and treatment of HD, highlighting the interrelationships between clinical symptoms, surgical approaches, and structural changes in anorectal tissue.

Practical value of the Paper

This study is the first to develop and validate a DNN-based artificial intelligence model for evaluating IHC reaction results in tissues obtained from patients with grade III/IV HD after resection surgery employing the recently approved *LigaSure* device. The development of such a model, the training of surgeons, and the approval of the interpretation of IHC reaction results remain a challenge. This is the first study outside the field of oncology, which further highlights the added value of this study.

Ethical aspect of the Paper

The research was conducted in accordance with the 1964 Helsinki Declaration. Each participant in the study received and signed an informed consent form. All parts of the study were conducted in accordance with ethical principles, ensuring strict patient anonymity and confidentiality. The study was approved by the Institutional Ethics Committee of the Pauls Stradiņš Clinical University Hospital and Rīga Stradiņš University (decision No. 22-2/264/2021).

1 Materials and methods

1.1 Research design

This doctoral thesis comprises a study designed to analyse the clinical manifestations and complaints associated with grade III and IV HD, alongside the surgical treatment methods applied and the outcomes achieved with the *LigaSure* device. The dynamics of clinical symptoms in the postoperative period, patient care and management, as well as patient satisfaction nine months after surgery, were also evaluated. Furthermore, the study investigated morphological changes in anal canal tissues, the distribution of ICC and TMEM-16 expression, interpreting these data through bioinformatics frameworks and AI-based analysis. The research incorporates both retrospective and prospective components. Ethical approval for the study was obtained from the Institutional Ethics Committee of the Pauls Stradiņš Clinical University Hospital and Rīga Stradiņš University (Decision No. 22-2/264/2021).

1.1.1 Characteristics of the patients included in the retrospective part of the study

The study population was selected from a database of 316 patients who underwent surgical treatment at the Pauls Stradiņš Clinical University Hospital in Riga, Latvia, between September 2020 and June 2021. This comprehensive database included HD patients from a regional medical centre, thereby improving the representativeness of the cohort. Sixty adults, equally divided between male and female patients, presenting with symptomatic grade III and IV HD, met the eligibility criteria for inclusion in the study and constituted the target group. Inclusion criteria were defined as follows: age over 18 years, and symptomatic grade III or IV HD that was refractory to conservative therapy with persistent clinical manifestations over the past year. Exclusion criteria comprised concurrent inflammatory diseases of the anorectal region (fistula, abscess, or inflammatory bowel disease), a history of anorectal neoplasm surgery, the need for additional or repeat surgery, and active use of immunosuppressants (due to increased susceptibility to infections). Patient medical records included a carefully documented history and physical examination, including a clinical examination – digital rectal examination and anorectoscopy. HD classified as symptomatic according to Goligher's criteria. All patients underwent either haemorrhoidectomy or haemorrhoidopexy. Postoperative follow-up included a clinical assessment tailored to the specifics of the surgical procedure performed, followed by an anorectal examination four weeks later. This retrospective study used the medical records and anorectal tissue specimens obtained from these patients. A board-certified surgeon compiled the data, thereby ensuring accuracy and consistency. Information on gender, age, blood group, symptoms, type of surgery, complications, and histopathological examination results was obtained from the medical records and evaluated for each patient. To minimise observer bias and ensure objectivity and consistency in data collection, standardised data collection protocols and measurement tools were strictly employed. The number of recorded complaints was categorised as follows: 1 – one complaint; 2 – two complaints; 3 – three complaints; and 4 – four different complaints recorded. Any sensitive information was excluded, and due to the retrospective nature of the study, the need for informed patient consent was waived; however, data were only included from patients whose medical records contained a signature confirming consent for the transfer of postoperative material for use in medical research at Rīga Stradiņš University, alongside a signed healthcare services contract at the Pauls Stradiņš Clinical University Hospital permitting the use of personal data in clinical training and academic research.

1.1.2 Characteristics of patients included in the prospective part of the study

This study cohort comprised forty-two patients who, between January 2021 and December 2022, underwent excisional haemorrhoidectomy using the *LigaSure* device at the Pauls Stradiņš Clinical University Hospital in Riga, Latvia. Inclusion criteria for the cohort were patients over 18 years of age with symptomatic grade III or IV HD, clear indications for excisional haemorrhoidectomy, an American Society of Anesthesiologists (ASA) Physical Status Class I or II, and a signed informed consent form. Exclusion criteria included concomitant anal pathologies (anal sphincter damage, rectal prolapse, fistulas, or inflammatory diseases), a history of anal incontinence surgery, and existing severe incontinence. Patients with an ASA class III score or a planned hospitalisation duration exceeding three calendar days due to other comorbidities were also excluded. The following clinical parameters were recorded, compiled, and analysed: age, sex, clinical symptoms, complaints, intraoperative data, immediate postoperative complaints, complications, and observations during the patient's visit. Postoperative digital and anoscopic examinations were performed during six-week postoperative follow-up visits until complete wound healing, with a final follow-up visit at 9 months after surgery. The tissue specimens obtained during surgery were sent for histological examination.

1.2 Description of the surgical techniques used

1.2.1 Open Milligan-Morgan haemorrhoidectomy

Patient preparation began 12 hours before surgery, with a light lunch permitted and dinner restricted. The patient was instructed to increase fluid intake the day before, as well as to perform cleansing enemas the night before and on the morning of the surgery. The surgery was performed under general or regional anaesthesia, with the patient in the lithotomy position.

Premedication, analgesia, and prophylactic antibacterial therapy with a 2-gram dose of cefazolin were administered prior to surgery. After antiseptic preparation of the surgical field, a V-shaped incision was made around the haemorrhoidal node, followed by precise subcutaneous dissection with scissors to separate the node from the surrounding tissues. The pedicle was ligated with Vicryl 2.0, and the distal end was resected. The procedure was repeated for all nodes, preserving skin bridges to prevent anal stenosis. A tampon (SPONGOSTAN™ Anal Sponge, model MS0007) was placed in the rectum for up to 24 hours postoperatively. Postoperative care included warm sitz baths, granulation-promoting and analgesic topical applications, flavonoids, analgesia, and a light diet, tailored to standard surgical protocols. Patients were discharged from the hospital 12–24 hours after surgery. Wound checks were performed 1–2 and 4 weeks after surgery, then at 8–10 weeks, with a final follow-up visit at 9 months.

1.2.2 Excision-type haemorrhoidectomy using the *LigaSure* device

Preoperative patient preparation began 12 hours before surgery, allowing a light lunch and restricting dinner. An increased fluid intake regimen and a cleansing enema were prescribed the night before, with the cleansing enema repeated on the morning of the surgery. The surgery was performed under general or regional anaesthesia, with the patient in the lithotomy position. Premedication, analgesia, and prophylactic antibacterial therapy with a 2-gram dose of cefazolin were administered prior to surgery. Following antiseptic preparation, the procedure was performed at the 5, 7, and 11 o'clock positions. After dilation of the anal canal and insertion of the anoscope, the haemorrhoidal nodules were clamped with Allis forceps. A V-shaped incision approximately 5 mm long was made at the junction between the skin and the anal canal, after which the node was retracted, and the *LigaSure* device was positioned 2–3 mm above the IAS. The node was resected without the use of sutures. For haemostasis control, electrocoagulation was required in 1.3 % of cases, and a haemostatic suture in 0.2 % of cases. A tampon (SPONGOSTAN™ Anal Sponge, model MS0007) was placed in the rectum for up to 24 hours postoperatively, applied with a lidocaine-containing gel (Instillagel®). Postoperative care included warm sitz baths, flavonoids, analgesia, and a light diet. Patients were discharged from the hospital 12–24 hours after surgery. Wound follow-up was performed at 1–2, 4, and 8–10 weeks after surgery, with a final visit at 9 months.

1.2.3 Haemorrhoidopexy with a circular stapler

Preoperative preparation of the patient began 12 hours before surgery and included a light lunch, increased fluid intake the day before, and cleansing enemas the night before and on the morning of the procedure. The surgery was performed under general or regional anaesthesia, with the patient in the lithotomy position. Premedication, analgesia, and prophylactic antibacterial therapy with a 2-gram dose of cefazolin were administered prior to surgery. After antiseptic preparation, the anal canal was dilated and a circular retractor was inserted and secured to the perianal skin. A circular suture was placed approximately 2.5 cm above the dentate line, at the level of the mucosa and submucosa only, avoiding involvement of deeper structures. The PPH device was then inserted, and a circular mucosal resection was performed with simultaneous stapling and haemostasis. The resulting mucosal fragment was ring-shaped, of uniform thickness, and free of muscle tissue. In cases of incomplete haemostasis, separate Vicryl 3.0 sutures were placed along the circular suture line in 4.3 % of cases. In rare (two) cases, a small piece of gauze was placed over the suture line to control bleeding and was removed within 24 hours after surgery. For postoperative care, patients were prescribed warm sitz baths, flavonoids, and analgesia, depending on the operating surgeon's preferences. Discharge from the hospital occurred 12–24 hours after surgery. Follow-up visits were conducted 2, 4, and 8–10 weeks after surgery, with a final evaluation at 9 months.

1.3 Histopathological, histochemical, and immunohistochemical methods

1.3.1 Collection and preparation of surgical specimens for morphological studies

Tissue samples for the study of the morphological characteristics of the rectum were obtained from surgical specimens following haemorrhoidectomy or haemorrhoidopexy procedures. In the prospective part of the study, patients were informed of the study details prior to surgery and signed informed consent forms. Tissue sections for morphological examination from patients included in the retrospective part were obtained from the archives of the Institute of Pathology at the Pauls Stradiņš Clinical University Hospital. Tissue samples measuring 4–5 mm were obtained from the resected tissues for research purposes. The formalin-fixed, paraffin-embedded (FFPE) tissue material was sectioned into 5 µm-thick sections using a semi-automatic rotary microtome (Leica RM2245, Leica Biosystems Richmond Inc., USA). The sections were transferred to microscope slides (code-6130603; Histobond®+, Paul Marienfeld GmbH & Co. KG, Germany), placed in a thermostat for drying, then deparaffinised in xylene, and gradually hydrated using alcohols of varying concentrations. The sections were then stained with H&E, dehydrated in solutions of increasing alcohol concentration, cleared in xylene, and mounted with a mounting medium (PERTEX, Histolab, Gothenburg, Sweden) and cover slips (H875.2; Carl Roth GmbH + Co, Germany) (Lillie et al., 1976).

1.3.2 Histopathological and histochemical examination of haemorrhoidal tissue

A total of 102 surgically obtained FFPE anorectal tissue samples were prepared according to the method described in Section 1.3.1. Routine histopathological staining with H&E was used to confirm the diagnosis of HD, determine the location of pathological changes relative to the dentate line, and verify the type of epithelium. Van Gieson and Picro Sirius Red histochemical methods were used to detect collagen in connective tissue, with the latter also analysed under polarised light to enhance visualisation of collagen fibres. The density of collagen fibres was assessed semi-quantitatively on a scale from 0 to 4. Additionally, the condition of the anal canal mucosal crypts, inflammatory cell infiltration, damage to blood vessel walls, and the presence of haemorrhage were assessed by analysing five representative microscopic fields in each region. Tissue sections were analysed using light microscopy, and the images obtained were digitised for further evaluation.

1.3.3 Immunohistochemical examination of haemorrhoidal nodule tissue

Immunohistochemical analysis was performed using 4–5 μm-thick FFPE tissue sections mounted on SuperFrost Plus slides, following standardised antigen retrieval and incubation protocols. In the retrospective part of the study, the expression of type V collagen and CD34 in vascular walls was assessed, while in the prospective part, the ICC markers CD117 (c-Kit) and ANO1 (TMEM16A) were identified. Immunoreactions were visualised using the HiDef Detection HRP polymer detection system and a diaminobenzidine substrate kit, with haematoxylin used for nuclear counterstaining. Primary antibodies were not used in the negative controls. The results were independently evaluated by two experts, unaware of the clinical data, using light microscopy and image digitisation. ANO1 expression was assessed semi-quantitatively on a scale from 0 to 3, according to the proportion of positive cells.

1.4 Statistical data analysis

Statistical analysis of the data was performed using IBM SPSS 26, GraphPad Prism 9, and JMP 16 software. Continuous variables were described using the mean and standard deviation or the median and interquartile range, while categorical variables were described using frequency and percentage distribution. Data normality was tested using the Shapiro–Wilk test. The Student's t-test or Mann–Whitney U test was used for group comparisons, while the Pearson or Spearman correlation coefficient was used for correlation analysis. Propensity score matching was performed to improve the comparability of patient groups. Histopathology, histochemistry, and IHC data were analysed using the Wilcoxon signed-rank test, while the distribution of categorical variables was assessed using the chi-square test. Hierarchical cluster analysis (using Ward's method) and scatter plots were used to examine data structures and interrelationships. The analysis was performed on both prospective and retrospective data, ensuring methodological consistency. Statistical significance was set at $p < 0.05$.

1.5 Artificial intelligence models and algorithms

In the study, with the advisory support of E. Edelmers, acting researcher at the RSU Center for Medical Education Technology, several models of various sizes were trained, from which one was selected to ensure optimal accuracy relative to computational requirements. A total of 40 whole-slide images (WSI) were obtained, each with a resolution of 2048x2048, depicting IHC-detected ICC. These images were initially acquired at maximum magnification. To meet the requirements of the YOLOv11 architecture, each image was divided into smaller 1024 ×1024 sections, resulting in a total of 160 images. All images were carefully annotated, resulting in 1,871 labelled masks for training purposes. Data augmentation methods were applied to improve model accuracy and mitigate potential performance variations due to differences in immune response intensity. The model training dataset included 376 images, as well as an additional 32 unaugmented images used for validation.

2 Results

2.1 Clinical characteristics of patients with prolapsing HD

The findings from the retrospective study of anorectal tissue samples from 60 participants, in relation to patient age, sex, HD grade, symptoms, comorbidities, and blood group is summarised in Figures 2.1, 2.2, and 2.3.

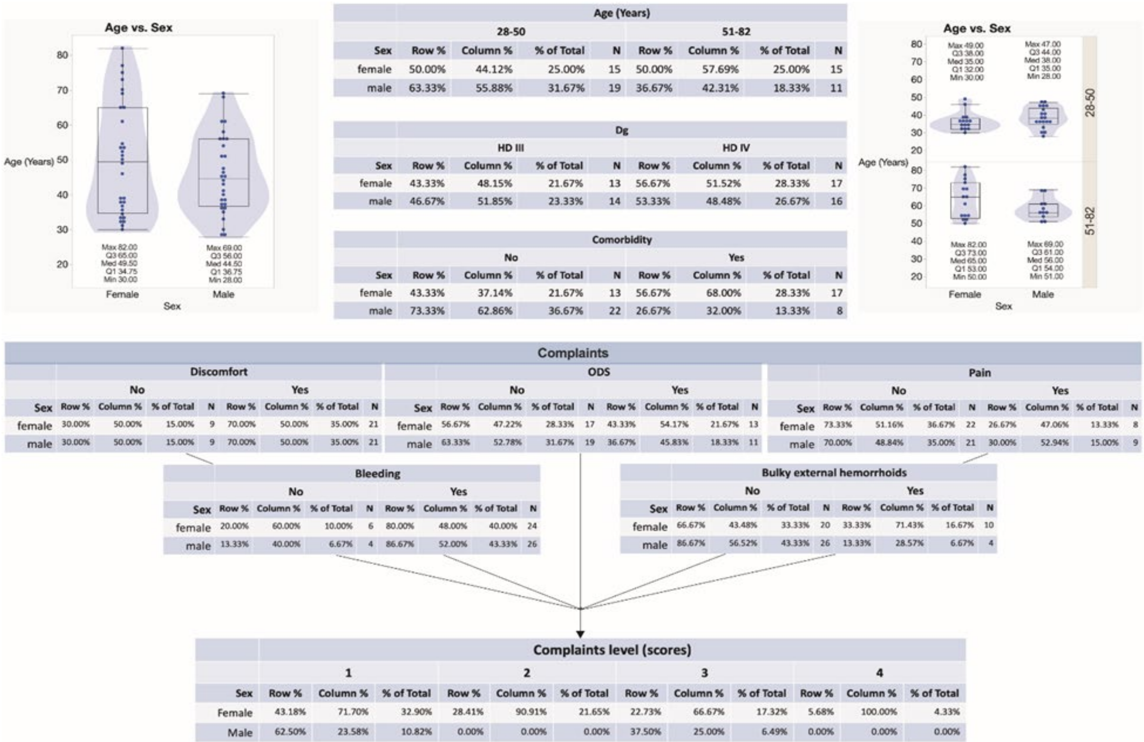
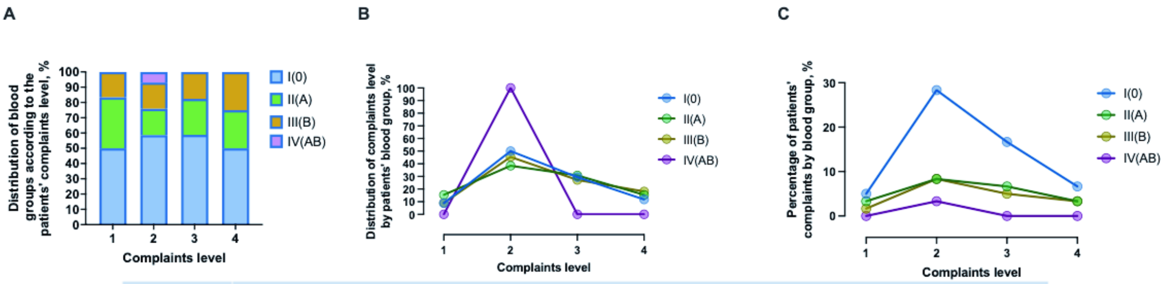


Figure 2.1 **A summary of patients' characteristics related to age, sex, HD staging, complaints, and comorbidities**

Of all patients included in the study, 13 (21.67 %) women and 14 (23.33 %) men were diagnosed with grade III HD, while 17 (28.33 %) women and 16 (26.67 %) men were diagnosed with grade IV HD (Figure 2.1). In this study, the majority of patients were found to have blood group 0. Among patients with grade III HD, 55.56 % had blood group 0, 22.22 % had blood group A, 14.81 % had blood group B, and 7.41 % had blood group AB. In contrast, among patients with grade IV HD, 57.58 % had blood group 0, 21.21 % had blood group A, and 21.21 % had blood group B; no patients in this group had blood group AB. Two male patients with grade III HD had blood group AB. Our study did not reveal any significant differences between men and women regarding blood group distribution. An equal number of women was observed in the cohort aged 28 to 50 years and in the cohort aged 51 to 82 years. In contrast, 19 (31.67 %) men were in the cohort aged up to 50 years, while 11 (18.33 %) men were in the cohort over 51 years of age, where the mean age was lower than that of the female cohort (Figure 2.1). It should be noted that women presented with significantly more complaints than men ($p = 0.0094$). At the same time, no differences were found between the complaints of men and women across different age groups (Figure 2.2 D). The widest range of complaints was observed in the 35–45 age group.



Complaints level	Blood group											
	I (0)			II (A)			III (B)			IV (AB)		
	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)
1	50.00%	8.82%	5.00%	33.33%	15.38%	3.33%	16.67%	9.09%	1.67%	0.00%	0.00%	0.00%
2	58.62%	50.00%	28.33%	17.24%	38.46%	8.33%	17.24%	45.45%	8.33%	6.90%	100.00%	3.33%
3	58.82%	29.41%	16.67%	23.53%	30.77%	6.67%	17.65%	27.27%	5.00%	0.00%	0.00%	0.00%
4	50.00%	11.76%	6.67%	25.00%	15.38%	3.33%	25.00%	18.18%	3.33%	0.00%	0.00%	0.00%

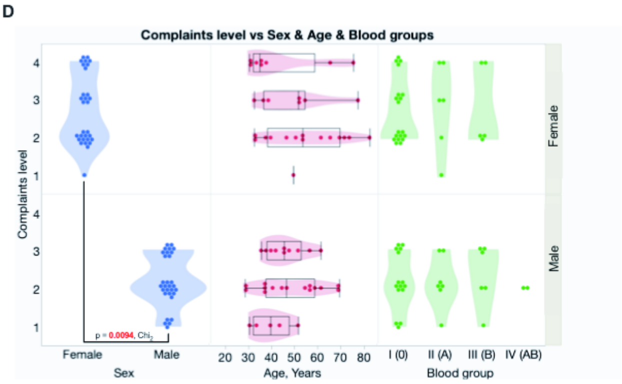


Figure 2.2 **A spectrum and distribution of complaints reported in HD patients with different blood groups**

The prospective part of the study included 42 patients with verified grade III and IV HD who underwent isolated radial excision haemorrhoidectomy using the *LigaSure* (LH) device, with anorectal tissue samples collected for analysis. In this patient cohort, the mean age was 53 years for women and 46 years for men, with a total age range of 24–72 years. Three women (15 %) and seven men (31.81 %) were younger than 40 years. Among the patients, 50 % of women and 45.45 % of men were classified having grade III HD, while 50 % of women and 54.55 % of men were classified as having grade IV HD. For grade III HD patients, the main preoperative complaint for both genders was bleeding (75 %), followed by tissue prolapse (45 %), with no significant differences observed between men and women. Men reported faecal smearing/incontinence (20 % vs. 10 %) and perianal tissue masses (20 % vs. 10 %) more frequently than women. Patients with grade IV HD most commonly complained of bleeding (72.7 %) and perianal tissue masses (40.9 %), while pain was reported more frequently by women (38.4 %) than by men (4.5 %). Men in this group also more frequently reported faecal smearing/incontinence (18.2 %) and persistent discomfort (18.2 %), while women most commonly reported itching and burning in the anorectal area (18.2 %). The average duration of

HD symptoms was 4.6 years for women and 7.8 years for men. Although a numerically higher number of lesions was excised in women regardless of the HD severity, this was not statistically associated with patient complaints.

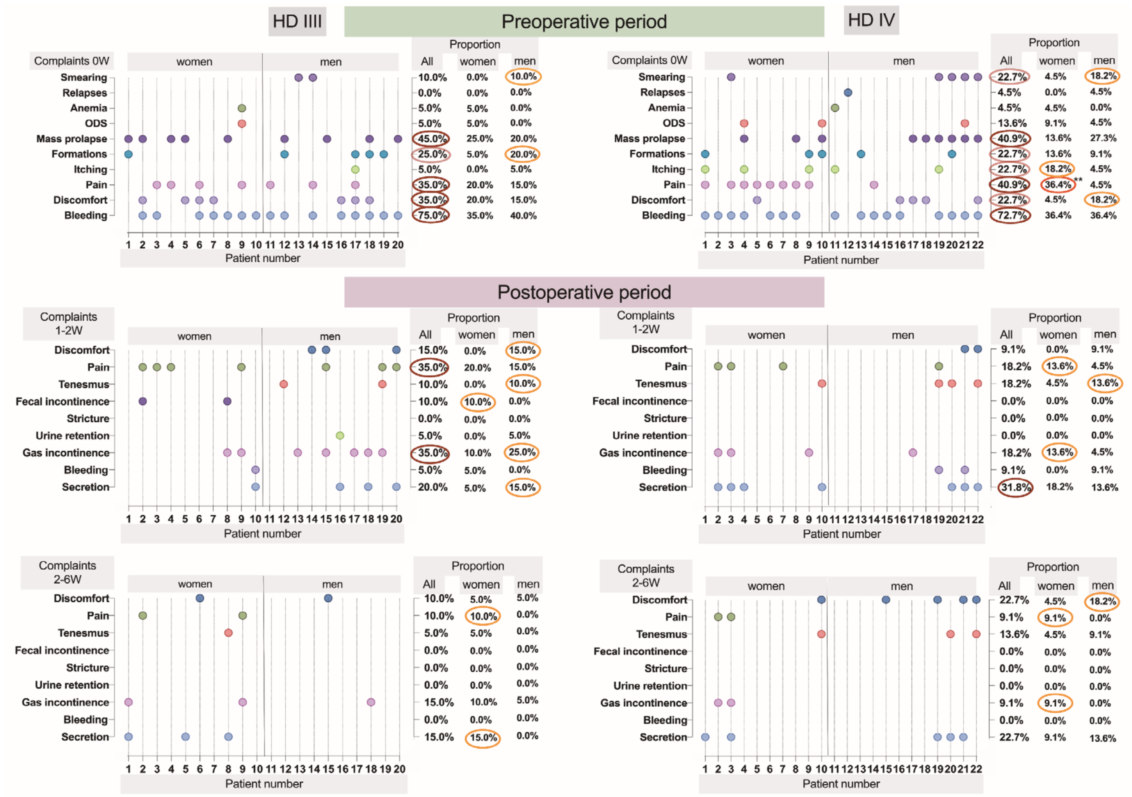


Figure 2.3 **Comparison of complaints regarding gender and HD grades in preoperative and postoperative periods**

In the retrospective study, haemorrhoidal bleeding, anal and perianal discomfort, and ODS were the most commonly reported complaints. Nine men and eight women reported pain, while four men and ten women reported large external haemorrhoidal nodules (Figure 2.1). All individuals with blood group A reported only one complaint. In this study, individuals with blood group 0 most frequently reported multiple complaints. Men typically experienced two or three complaints, while women presented with three or more complaints. Significantly more complaints were found among women with HD than among men ($p = 0.0094$). At the same time, no differences were found between men and women in different age groups (Figure 2.1 D).

In the *LigaSure* group, the main complaint for both genders was bleeding, followed by tissue prolapse and pain, whether related to or unrelated to defecation. For men, faecal smearing was identified as a significant complaint in both grades, which was not recorded in the retrospective part of the study. Only one man underwent repeat surgery due to HD recurrence. In grade III HD, a numerically higher number of complaints was observed in women, whereas in grade IV HD, a significantly higher number of complaints was observed in men (p = 0.0010). Although the duration of surgery did not differ significantly between patients with the two grades of prolapsing HD, in cases of grade IV HD, it was longer for men than for women, which may be attributed to anatomical differences between the genders and pronounced tissue prolapse, as verification of the sphincter complex is more time-consuming in male patients.

2.2 Analysis of postoperative outcomes in the *LigaSure* group

It was found that at the first postoperative visit 1–2 weeks after LH, discomfort and maceration were more pronounced in men (15 %), and 25 % of men reported bleeding without defecation following haemorrhoidectomy for grade III HD, compared to no cases following treatment for grade IV HD. FI occurred in 10 % of men following LH for grade III HD, but not in those who underwent surgical treatment for grade IV HD. Treatment of both grades of HD caused tenesmus in men, more pronounced after surgical treatment of grade IV HD (13.6 %). In contrast, women had fewer postoperative complaints; FI occurred in 10 % of cases following grade III HD treatment with LH. Following grade IV LH, 13.6 % of women experienced bleeding without defecation. Maximum pain (VAS score 8) was reported by 6 (30 %) women aged 43 to 66 years (mean age 56.8), during the first 24 hours; of these, one woman had been diagnosed with reduced anal sphincter tone prior to surgery, and none of the women had received a presacral block. In the male group, only 2 (9.09 %) patients reported peak pain between the ages of 30 and 52 years, both of whom presented with abnormal anal sphincter tone during the preoperative period. Three men (aged 27, 29, and 44 years) continued to experience persistent symptoms for more than 6 weeks postoperatively, including discomfort, swelling, and recurrent preoperative symptoms such as bleeding, tissue prolapse, FI, discomfort/itching, perianal lesions, and obstructive defecation (Figure 2.3). All patients were prescribed analgesic therapy postoperatively (100 %); however, at the first postoperative visit, 35 % of all operated patients complained of pain (Figure 2.4). Men more frequently reporting gas incontinence, accounting for 25 % of cases following grade III HD therapy. Management of the postoperative period, based on data from outpatient charts, was dynamically adjusted; however, it varied significantly depending on individual surgeon preference (Figure 2.5). Postoperative pain in the first 24 hours was assessed using the visual analog scale (VAS). The mean pain intensity score following treatment of grade III HD with the LH method was 1–7 VAS points, whereas following grade IV HD, it was 1–8 VAS points during the first 24 hours. However, it should be noted that of the 42 patients, only 16 (38.09 %) underwent a presacral block, although it is known that a presacral nerve block with bupivacaine reduces postoperative pain and increases patient satisfaction.

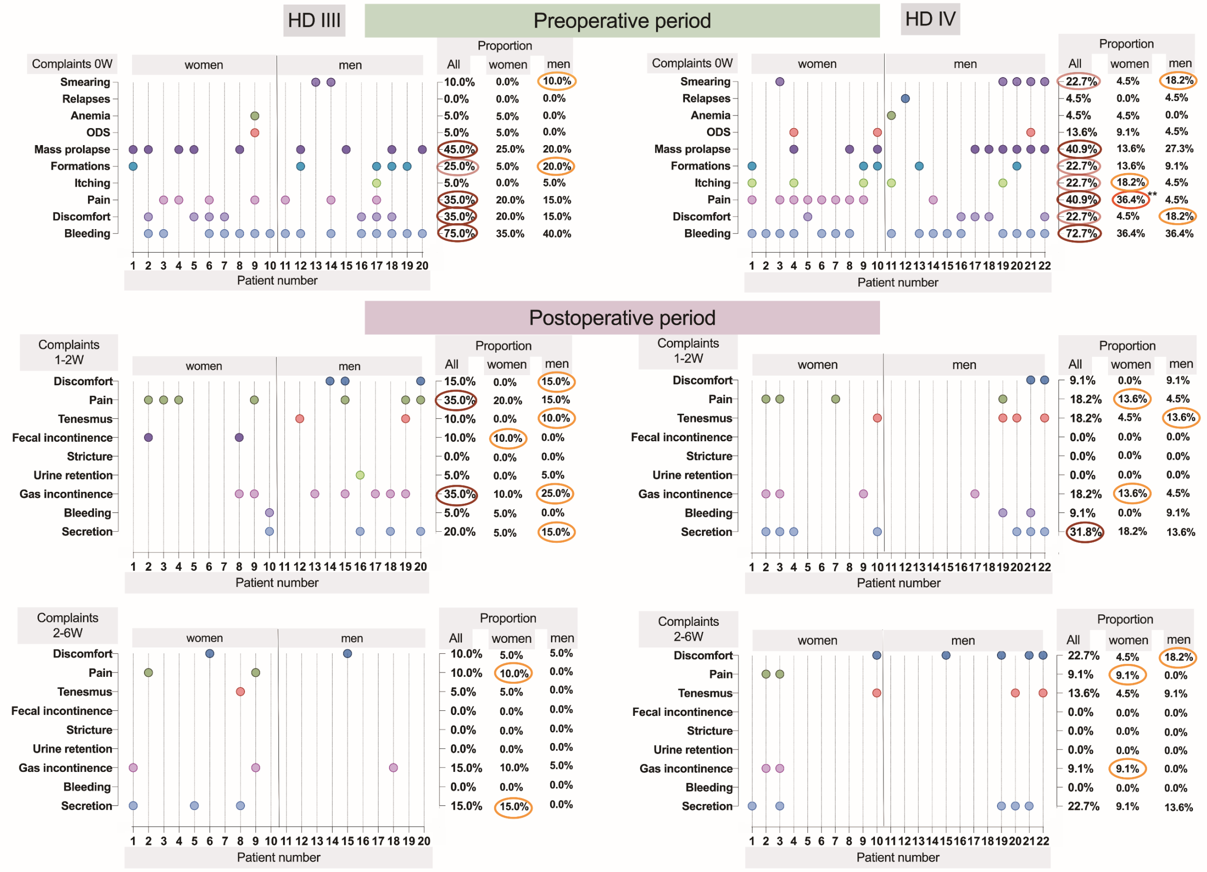


Figure 2.4 **Distribution of preoperative symptoms by gender and HD stage, as well as symptoms observed during the postoperative period according to HD stage and gender**

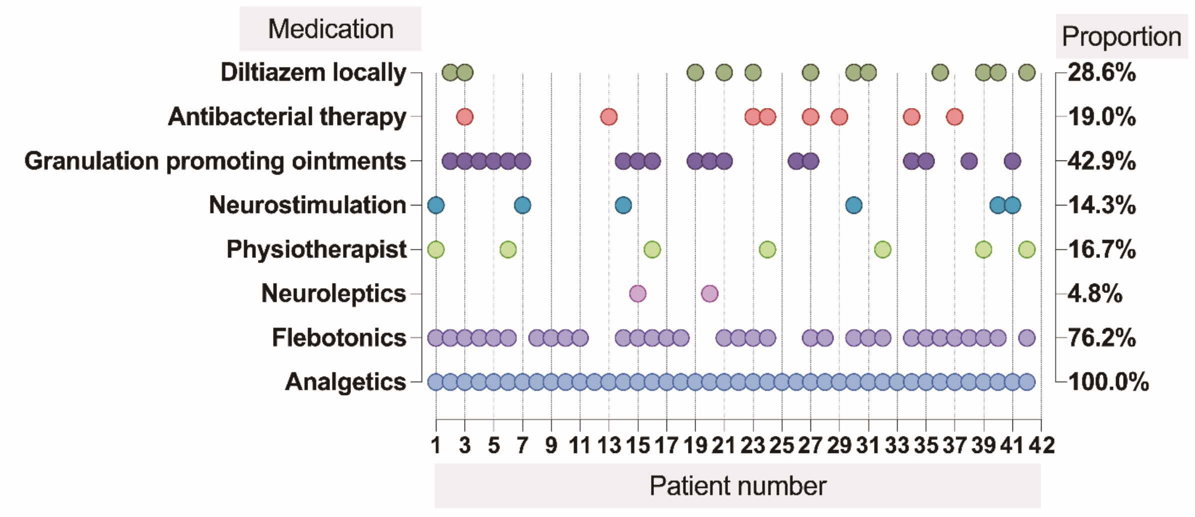
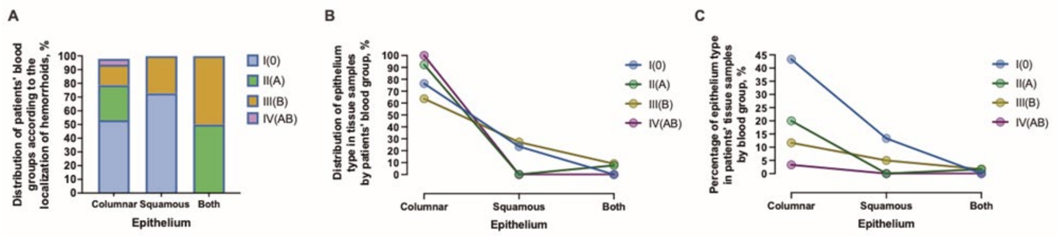


Figure 2.5 **Medications and therapeutic interventions used in postoperative care**

2.3 Analysis of histopathologically stratified tissue samples

This study retrospectively analysed sixty tissue samples obtained surgically from patients with grade III and IV HD. Histopathologically, the tissues were divided into two groups: tissues in which the mucosal lining consisted of either simple columnar epithelium or stratified squamous epithelium, and tissues in which both types of epithelium were present, connected in the transition zone, reflecting the tissues of the internal and external haemorrhoidal nodes, respectively (Figure 2.6). Crypts of various shapes were located in the loose connective tissue of the mucosal lamina propria (Figures 2.6 E and F). In contrast, stratified squamous epithelium was visualised over denser bundles of connective tissue located beneath the basement membrane (Figure 2.6 G and H). Patients with blood group 0 constituted the largest cohort from whom surgical specimens were obtained above the dentate line, followed by individuals with blood groups A, B, and AB, at 55.32 %, 25.53 %, 14.89 %, and 4.26 %, respectively. In turn, the presence of simple columnar epithelium was confirmed in 72.73 %, 0.00 %, 27.27 %, and 0.00 % of individuals with blood groups 0, A, B, and AB, respectively (Figure 2.6 A). Finally, surgical specimens with a transition zone and both types of epithelium accounted for only 50 % of individuals with blood groups A and B (Figures 2.6 A, B, and C). In female patients, surgical specimens were more frequently located below the dentate line than in male patients; tissue specimens were found behind the transition zone in only two males following conventional excisional haemorrhoidectomies (Figure 2.6 D). The prospective study utilised 42 surgically obtained tissue samples from patients with grade III and IV HD. Histopathological examination most frequently revealed both types of haemorrhoidal nodes (internal and external) – 47.6 %, while isolated external nodes were found in only 4.8 % of cases (Figure 2.7). Similarly, in anorectal tissue resected using the *LigaSure* method, marked oedema was present in 40.5 % of cases, followed by marked varicose veins in the submucosa in 38.1 % and haemorrhages in 35.7 %, which were verified as non-iatrogenic. Finally, in the examined tissues following haemorrhoidectomies, hyperplastic polyps were found in 7.1 % of cases and exclusively in male patients; one case of villous adenoma was identified in each sex, along with three malignant anorectal lesions (7.1 %).



	Blood group											
	I (0)			II (A)			III (B)			IV (AB)		
Epithelium	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)
Columnar	55.32%	76.47%	43.33%	25.53%	92.31%	20.00%	14.89%	63.64%	11.67%	4.26%	100.00%	3.33%
Squamous	72.73%	23.53%	13.33%	0.00%	0.00%	0.00%	27.27%	27.27%	5.00%	0.00%	0.00%	0.00%
Both	0.00%	0.00%	0.00%	50.00%	7.69%	1.67%	50.00%	9.09%	1.67%	0.00%	0.00%	0.00%

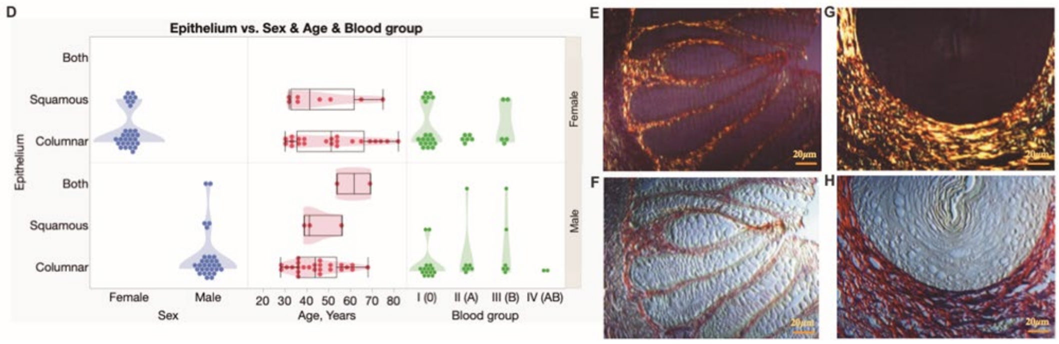


Figure 2.6 **Distribution of histopathological samples above and below the dentine line in patients with HD (men and women) by blood group type (A–D)***

*This figure demonstrates a pattern of the histopathological stratification of tissue samples localized above and below the dentate line in HD males and females presented with different blood groups (**A-D**). Elongated and paralleled crypts that are embedded in the loose connective tissue can be viewed in a polarized light (**E**) and a differential interference mode (**F**). A stratified squamous epithelium that rests on the basement membrane is followed by tightly packed collagenous bundles supporting the basement membrane and is assessed using a polarized light (**G**) and a differential interference mode (**H**). When observed under polarized light *the lamina propria mucosae* reveals type I and type III collagen fibres stained in yellow-red and green, respectively. Picro Sirius red staining (E-H). Scale bars: 20 µm.

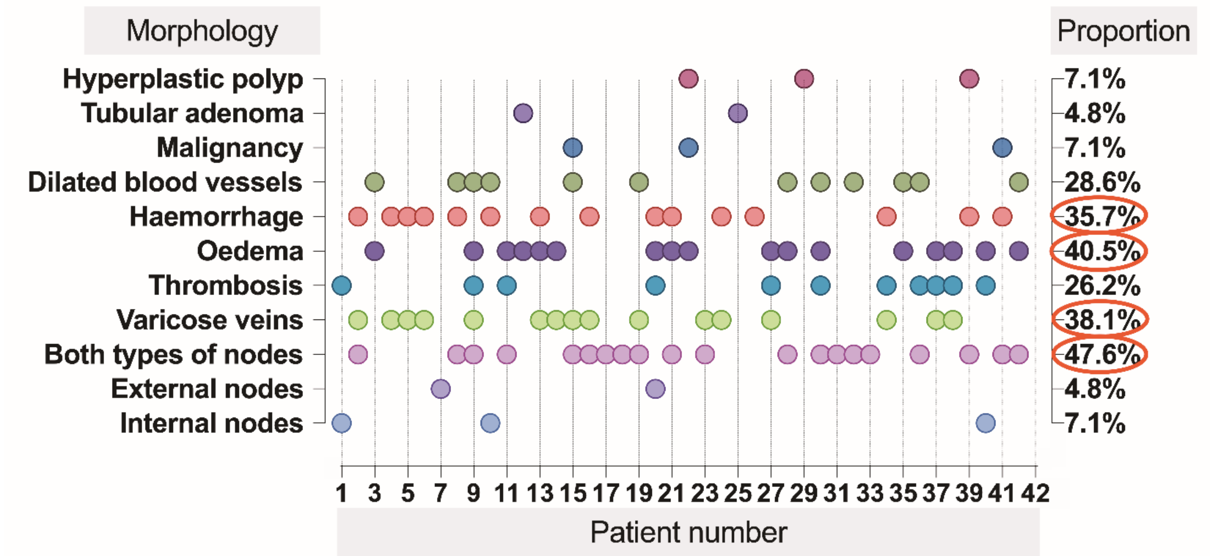


Figure 2.7 **Results of routine histopathological examination for the *LigaSure* group**

2.4 Structural changes in anorectal tissue in patients with prolapsing HD

Chronic structural changes in the rectal mucosa, including inflammatory cell infiltration, crypt deformation, and crypt atrophy, were observed in tissue samples obtained from patients with HD (Figures 2.8 A and B). In this study, anorectal tissue obtained from men was significantly more frequently inflamed (96.7 % vs. 3.3 %) than samples obtained from women with HD (Figure 2.8 C).

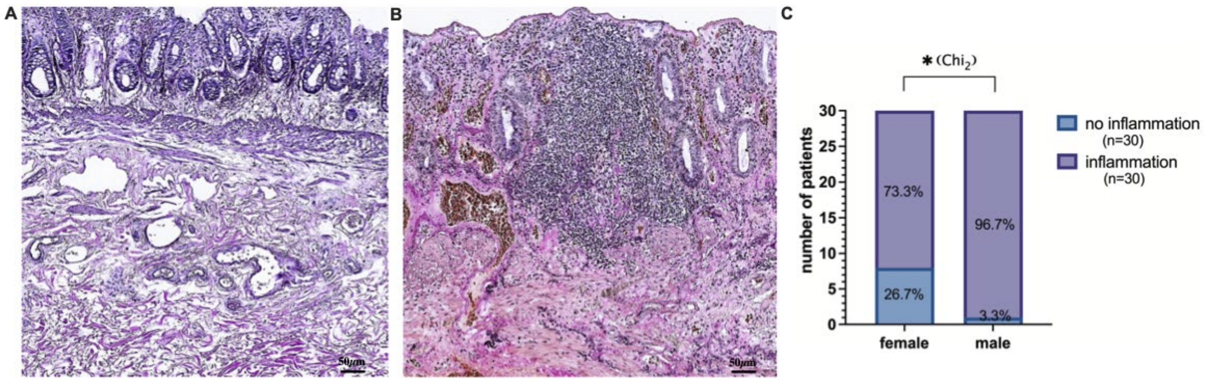
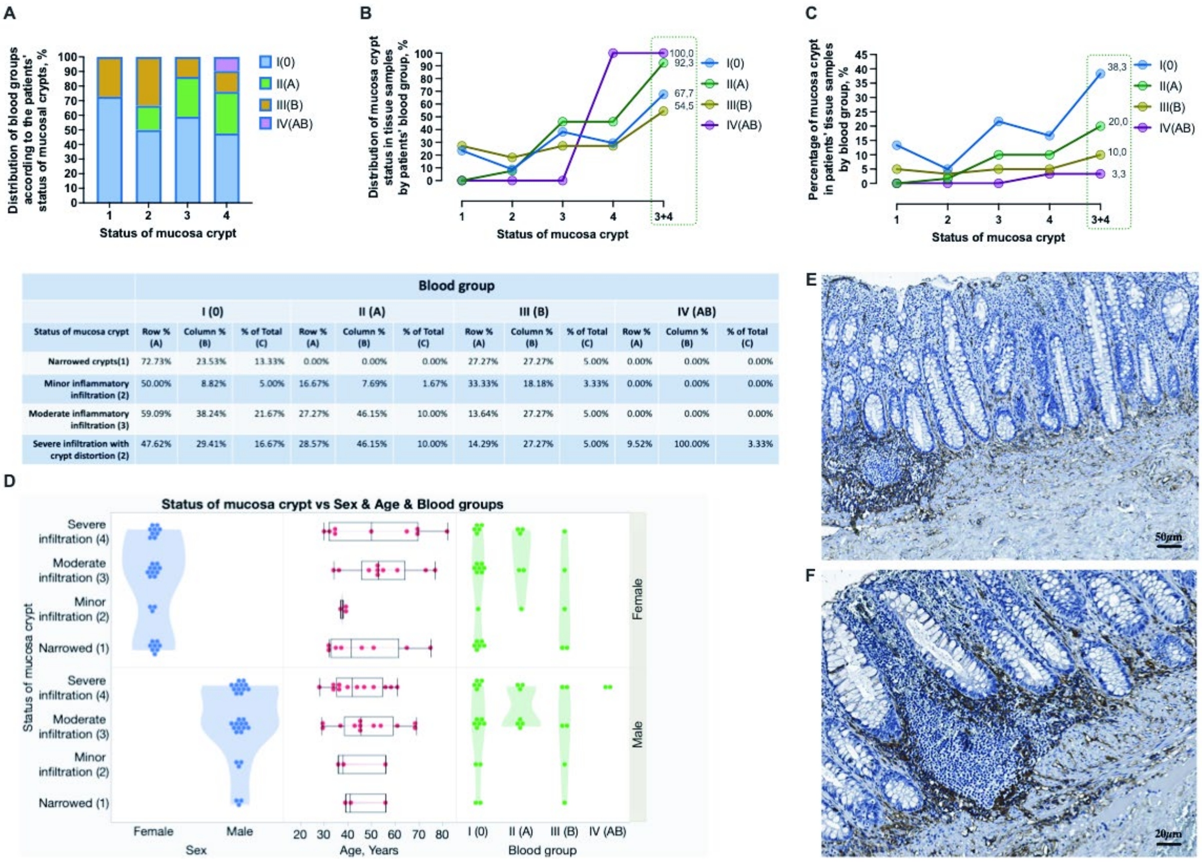


Figure 2.8 **Histopathological changes in the mucosa and submucosa of the rectum observed in HD, depending on the presence of inflammation***

*A representative image that depicts the histopathological features of mucosa and submucosa in HD in the absence (**A**) and presence (**B**) of inflammation. (**C**) The extent of inflammation in the anorectal samples obtained from males is significantly higher than that in females. Asterisks represent the significance level (**$p < 0.01$). (**A**) The rectal mucosa reveals paralleled crypts; however, the glands do not rest on the *muscular mucosae*; the submucosa houses thin- and thick-walled vessels. **(B**) The culprit bleeding vessels are visualized in the rectal mucosa. Inflammatory infiltrate brings to the reduction and distortion of crypts and produces insertions toward the haphazardly patterned *muscular mucosae*. Van Gieson's staining (**A, B**). Scale bars: 50 µm.

In addition, to better investigate chronic mucosal lesions in HD, IHC staining was performed to determine the involvement of blood vessels and immune cells in tissue changes. CD34 IHC was used to analyse the vascularity of the mucosal lining, the presence of immune cells, and their distribution. Endothelial cells of small mucosal blood vessels reacted positively with the anti-CD34 antibody, making them easily visualisable and analysable. It was found that the surface molecule CD34 marks immunocompetent cells that infiltrate the mucosa (Figure 2.9 E and F). Mucosal crypts were evaluated, and the degree of crypt damage was determined (Figure 2.9 A–D). Markedly damaged and inflamed mucosa was found in 47.62 %, 28.57 %, 14.29 %, and 9.52 % of individuals with blood groups 0, A, B, and AB, respectively (Figure 2.9 D).

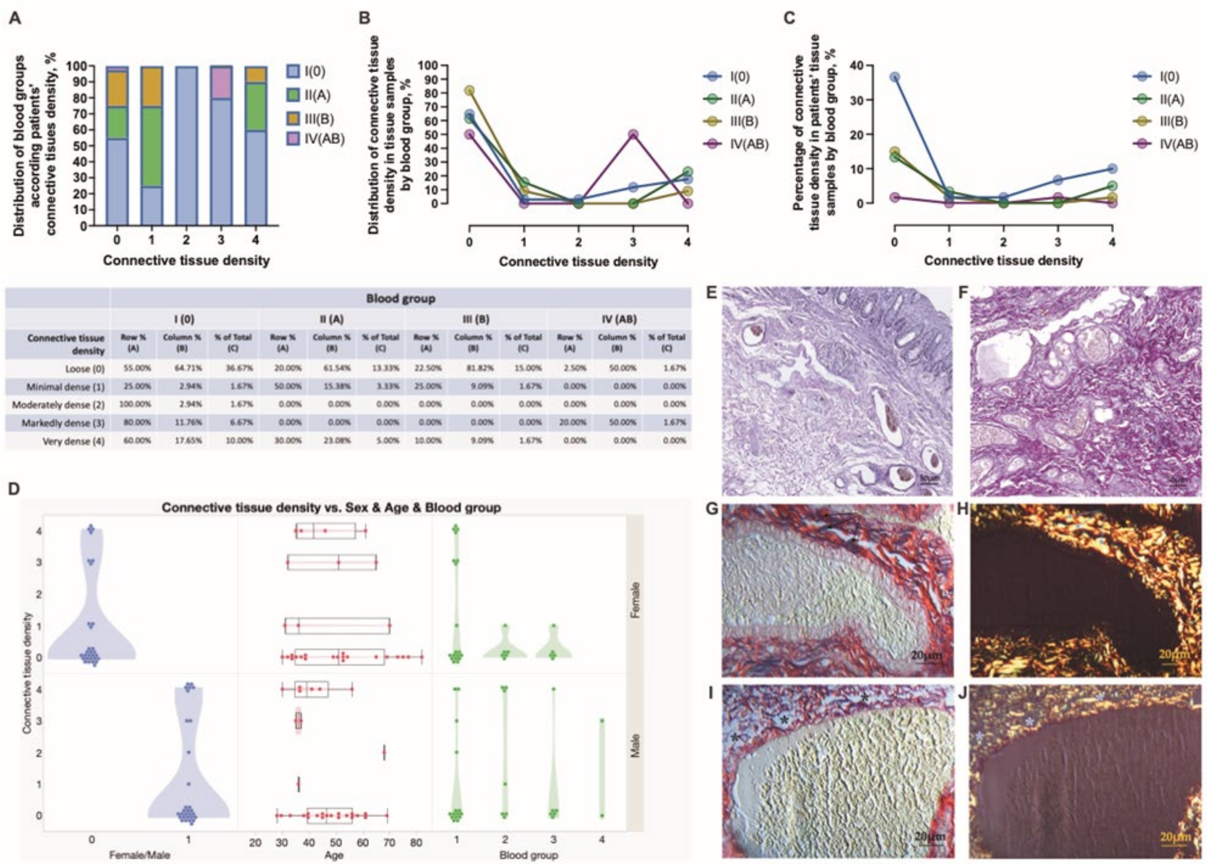


	Blood group											
	I (0)			II (A)			III (B)			IV (AB)		
Status of mucosa crypt	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)
Narrowed crypts(1)	72.73%	23.53%	13.33%	0.00%	0.00%	0.00%	27.27%	27.27%	5.00%	0.00%	0.00%	0.00%
Minor inflammatory infiltration (2)	50.00%	8.82%	5.00%	16.67%	7.69%	1.67%	33.33%	18.18%	3.33%	0.00%	0.00%	0.00%
Moderate inflammatory infiltration (3)	59.09%	38.24%	21.67%	27.27%	46.15%	10.00%	13.64%	27.27%	5.00%	0.00%	0.00%	0.00%
Severe infiltration with crypt distortion (2)	47.62%	29.41%	16.67%	28.57%	46.15%	10.00%	14.29%	27.27%	5.00%	9.52%	100.00%	3.33%

Figure 2.9 **Histopathological assessment of chronic mucosal lesions in men and women with different blood groups***

*The histopathological assessment of chronic mucosal lesions in HD males and females presented with different blood groups. The schematic presentation of the structural appearance of crypts in HD patients of 0, A, B, and AB blood groups (**A, B, C**). The status of mucosal glands is graded and estimated in HD males and females of different age groups **(D)**. A representative image that depicts the histopathological features of inflamed mucosa HD (**E, F**). CD34 immunohistochemistry (**A, B**). Scale bars: 50 µm (**A**), 20 µm (**B**).

Both women and men in all blood groups exhibited marked damage to the rectal mucosa. No significant differences were found between women and men, or among different age groups of patients with various grades of HD, when assessing chronic mucosal damage. Evaluation of anorectal tissue was performed down to the submucosal level, and the integrity and density of collagen fibres, including perivascular fibres, were examined using specific histochemical stains – Van Gieson's and Picro Sirius Red (Figure 2.10 E–J), which were summarised in the results (Figures 2.10 A–C). Analysis of tissue samples from HD patients confirmed collagen changes in connective tissue. The presence of a loose collagen arrangement in tissue samples was confirmed and further evaluated in proportion to the representative number of HD subjects studied. This was observed in a large proportion of HD individuals with blood group 0 – 42.86 %, followed by HD individuals with blood groups A and B – 28.57 % and 28.57 %, respectively. When evaluating collagen density in HD male and female samples and across different age groups, no significant differences were found (Figure 2.10 D).



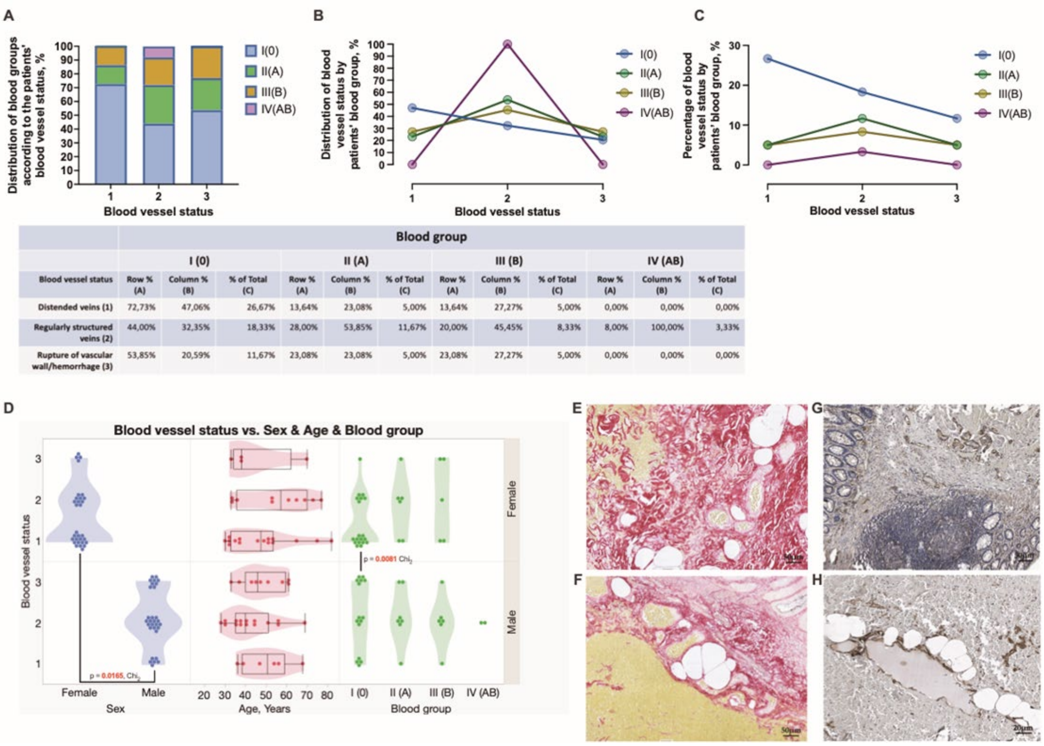
	Blood group											
	I (0)			II (A)			III (B)			IV (AB)		
Connective tissue density	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)
Loose (0)	55.00%	64.71%	36.67%	20.00%	61.54%	13.33%	22.50%	81.82%	15.00%	2.50%	50.00%	1.67%
Minimal dense (1)	25.00%	2.94%	1.67%	50.00%	15.38%	3.33%	25.00%	9.09%	1.67%	0.00%	0.00%	0.00%
Moderately dense (2)	100.00%	2.94%	1.67%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Markedly dense (3)	80.00%	11.76%	6.67%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	20.00%	50.00%	1.67%
Very dense (4)	60.00%	17.65%	10.00%	30.00%	23.08%	5.00%	10.00%	9.09%	1.67%	0.00%	0.00%	0.00%

Figure 2.10 **Assessment of collagen fibres in connective tissue in both sexes with HD and different blood groups***

*The histochemical assessment of connective tissue collagen fibres in HD males and females presented with different blood groups (**A-D**). Loosely (**E**) or more densely (**F**) structured submucosa houses thick- and thin-walled congested blood vessels, Van Gieson's staining (**E, F**). Scale bars: 50 µm. Perivascular collagen is assessed in thick- (**G** and **H**) and thin-walled (**I** and **J**) vessels using a differential interference (**G** and **I**) and a polarized light mode (**H** and **J**). When observed under polarized light type I and type III collagen fibres are stained in yellow-red and green, respectively. Picro Sirius red staining (H and J). Scale bars: 20 µm.

IHC staining was used to assess the expression and distribution of type V collagen and to investigate its role in vascular wall integrity. The results were evaluated using Picro Sirius Red staining and type V collagen immunohistochemical reactions in all anorectal tissue samples (Figures 2.11 E–H).

The presence of dilated and ruptured subcutaneous veins was confirmed in 72.73/ 13.64/ 13.64 and 0 %, as well as 51.35/ 27.03/ 18.92 and 2.70 % of HD individuals with blood groups 0, A, B, and AB, respectively (Figure 2.11 A–C). When assessing vascular integrity in male and female HD samples, significant differences were found, but not across age groups (Figure 2.11 D). This study demonstrated that in cases of HD, vascular bed ruptures occur significantly more frequently in males than in females ($p = 0.0165$). Furthermore, in men with blood group 0, prolapsing HD was more frequently associated with bleeding compared to women with blood group 0 ($p = 0.0081$).



	Blood group											
	I (0)			II (A)			III (B)			IV (AB)		
Blood vessel status	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)	Row % (A)	Column % (B)	% of Total (C)
Distended veins (1)	72,73%	47,06%	26,67%	13,64%	23,08%	5,00%	13,64%	27,27%	5,00%	0,00%	0,00%	0,00%
Regularly structured veins (2)	44,00%	32,35%	18,33%	28,00%	53,85%	11,67%	20,00%	45,45%	8,33%	8,00%	100,00%	3,33%
Rupture of vascular wall/hemorrhage (3)	53,85%	20,59%	11,67%	23,08%	23,08%	5,00%	23,08%	27,27%	5,00%	0,00%	0,00%	0,00%

Figure 2.11 **Characteristics of collagen architecture and changes in blood vessel walls in HD patients***

* This Figure illustrates the architectural peculiarities of collagen and its' contribution to the maintenance of vascular integrity. Small arterioles are embedded in a collagenous matrix that reveals a haphazard orientation of fibres partly inserted into the haemorrhage area (**E**). Congested and markedly enlarged submucosal vessels (**F**). Inflamed mucosa with crypt distortion and thickened muscular mucosa; small mucosal and submucosal vessels are labelled with the anti-collagen type V antibody (**G**). Small dilated submucosal vessels labelled with the anti-collagen type V antibody are surrounded by a large haemorrhage (**H**). Picro Sirius red staining (**E** and **F**). Collagen type V immunohistochemistry (**G** and **H**). Scale bars: 50 µm (**E, F, G**), 20 µm (**H**).

In turn, CD117 and ANO1 IHC staining was used to determine the presence and distribution of ICC in the tissue, as well as to assess the membrane expression of ANO1 – a calcium-activated chloride channel – membrane expression, which was visualised in both crypt and smooth muscle cell membranes. IHC staining demonstrated that ICC are branched cells scattered among smooth muscle cells. The density of ICC associated with the muscular component of the anal canal wall varied significantly in the study group of patients with HD (Figure 2.12 a–c). As the muscular component of the anal canal wall decreased and large, dilated blood vessels appeared, ICC were found in reduced numbers, often in a perivascular location (Figure 2.12). ANO1 immunoreactivity was primarily observed in the membranes of crypt wall epithelium and smooth muscle cells (Figure 2.13). The level of ANO1 expression in tissue samples from the study cohort varied significantly – in some cases, no membrane expression was detected, while in other samples it was assessed semi-quantitatively with a score of "3," corresponding to expression in more than 50 % of structures.

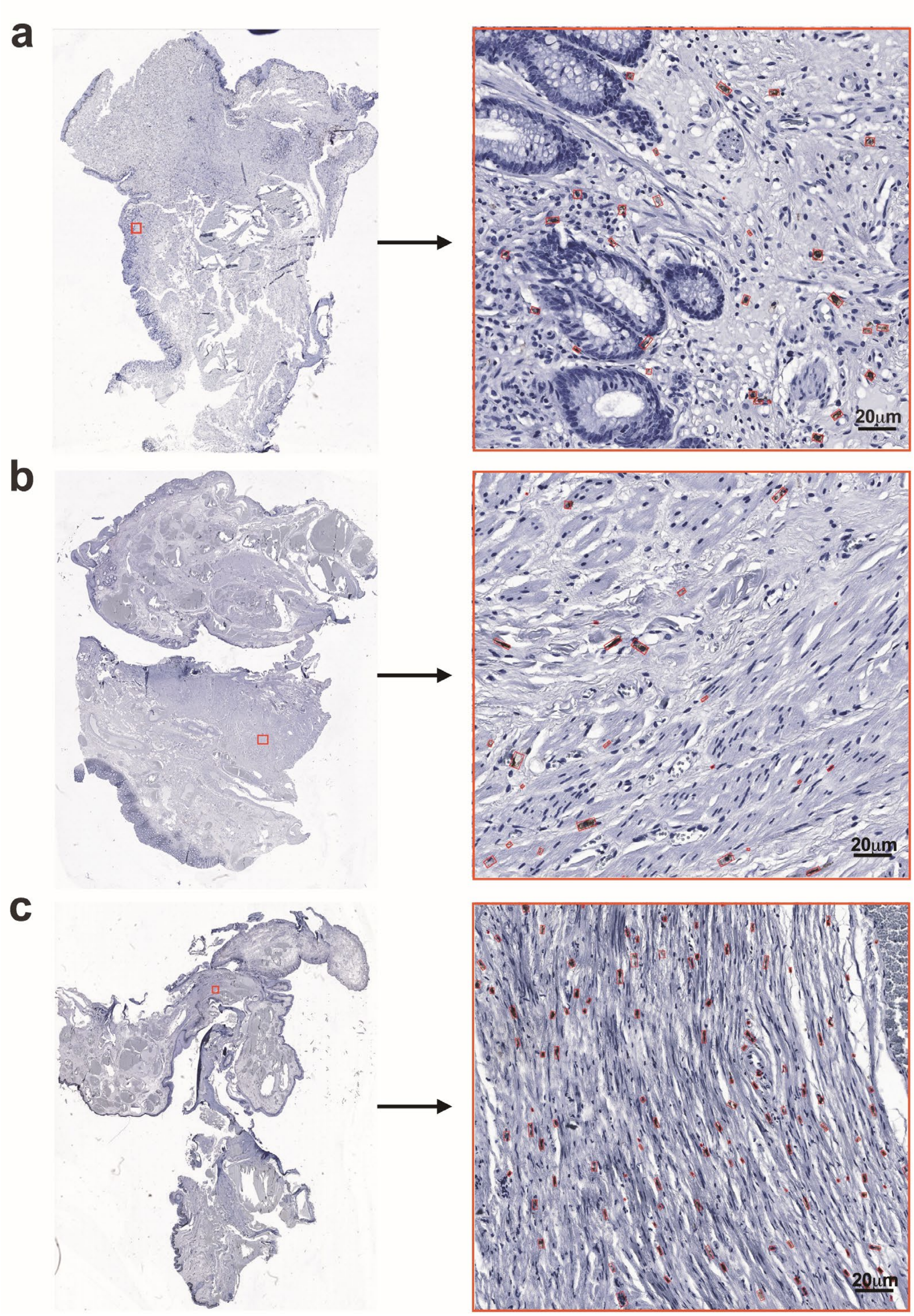


Figure 2.12 **Scanned whole-mount samples of anorectal tissue with region selection and IHS labelling using the DNN algorithm***

*(a) Representative image showing the thinning of the muscularis mucosae, random orientation of myocytes, and brown staining of some ICC. (b) A small number of ICC in the muscularis externa. (c) Densely distributed ICC in the muscularis externa. Scale: 20 μm

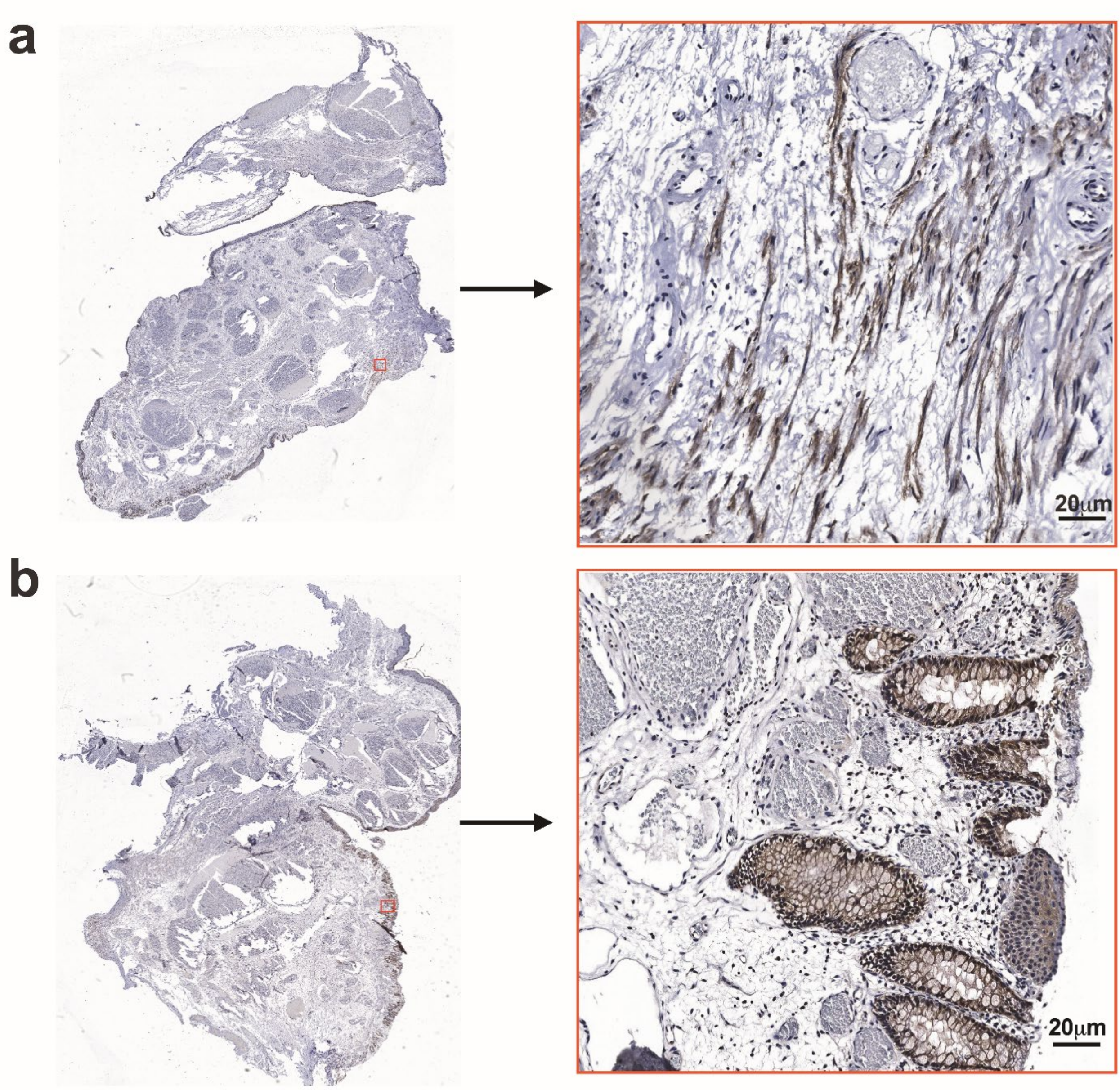
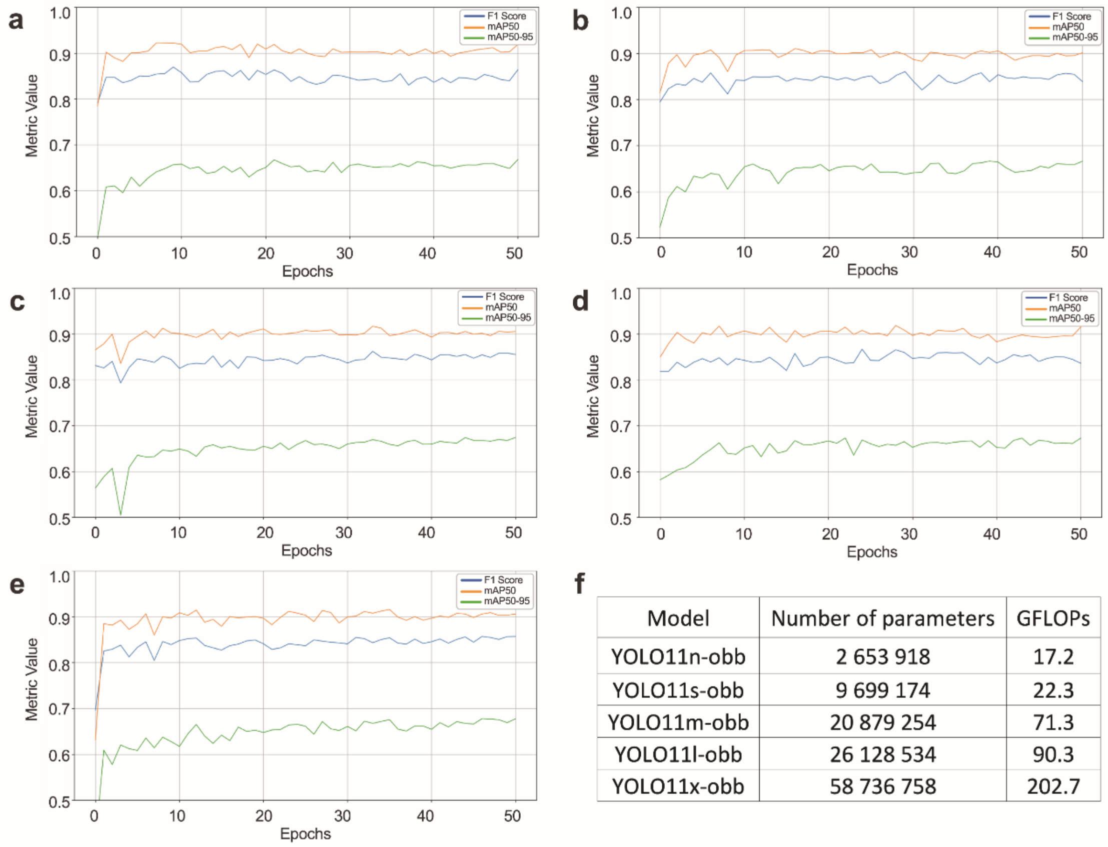


Figure 2.13 **Expression of ANO1 in the membranes of myocytes (a) and crypt wall epithelial cells (b)**

Several models of varying sizes were trained to identify the ICC, as shown in Figure 2.14. Training was performed for up to 50 epochs, evaluating model performance using three primary metrics: mAP50, mAP50-95, and F1 score. All AI models demonstrated similar performance, achieving an mAP50 of 92 %, which is considered sufficient for cell counting tasks. When selecting the model, priority was given to efficiency, balancing a low number of parameters to improve performance on low-power devices and achieve faster inference speeds. Thus, the YOLOv11n-obb AI model was selected, which is optimised for both accuracy and resource efficiency. The program's surface area quantification module provided a method for evaluating histological sections and identifying specific tissue regions. Using either the resolution metadata embedded in TIFF files or a user-defined pixel size, the algorithm converts pixels to area in square millimetres. Using morphological operations, the program generates a tissue mask that facilitates the calculation of the tissue-occupied area relative to the entire slide. Following model training, the "MorpHista" software was developed to efficiently process large H&E slides and identify ICC. It integrated automated detection and post-processing, addressing the challenge of reassembling segmented regions after drawing conclusions, in order to combine all detected features into a single image file while compiling relevant statistics, including the number of ICC. This tool has been made publicly available, ensuring reproducible ICC analysis and fostering broader research innovation in digital pathology (Figure 2.14).



Model	Number of parameters	GFLOPs
YOLO11n-obb	2 653 918	17.2
YOLO11s-obb	9 699 174	22.3
YOLO11m-obb	20 879 254	71.3
YOLO11l-obb	26 128 534	90.3
YOLO11x-obb	58 736 758	202.7

Figure 2.14 **Performance of trained models***

*(a) YOLO11n-obb, (b) YOLO11s-obb, (c) YOLO11m-obb, (d) YOLO11l-obb, (e) YOLO11x-obb, and (f) parameters of the trained models.

Despite the efforts to implement a segmentation-based approach for the quantitative determination of ANO1 expression, the model has not achieved a sufficient level of accuracy (≥ 90 %). This shortcoming highlights the complexity of pixel-level classification tasks, particularly given the variability in staining intensity and tissue morphology in our dataset. A robust linear regression analysis simultaneously confirmed an inverse relationship between the number of ICC and ANO1 expression in anorectal tissues from patients with prolapsing HD. Tissue samples with reduced ANO1 expression in myocyte membranes had a higher number of ICC, whereas samples with increased ANO1 expression had a lower number of ICC (Figure 2.15).

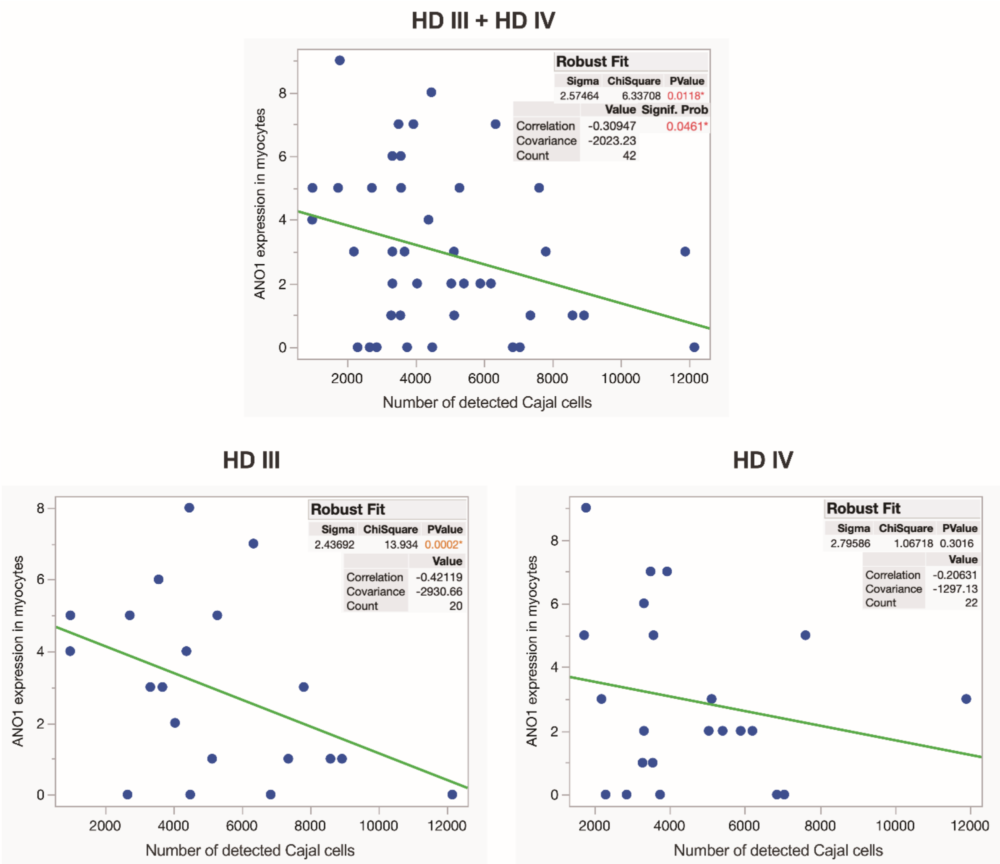


Figure 2.15 **Scatter plots with robust linear regression for comparing the number of ICC and ANO1 expression intensity in smooth myocytes***

*In the pooled sample (HD grade III+IV, n = 42), a moderate but statistically significant negative correlation was observed (r ≈ −0.30; p = 0.046). For grade III HD (n = 20), the inverse relationship is stronger and highly significant (r ≈ −0.42; p = 0.0002), whereas in the case of grade IV HD (n = 22), it becomes weak and statistically non-significant (r ≈ −0.21; p = 0.30).

To conduct an in-depth analysis of the role of ICC in HD development, a correlation analysis was performed to assess the relationship between the number of ICC identified and the area of the corresponding tissue sample. The calculated ICC density in surgical specimens of various shapes and sizes is shown in Figure 2.15.1.

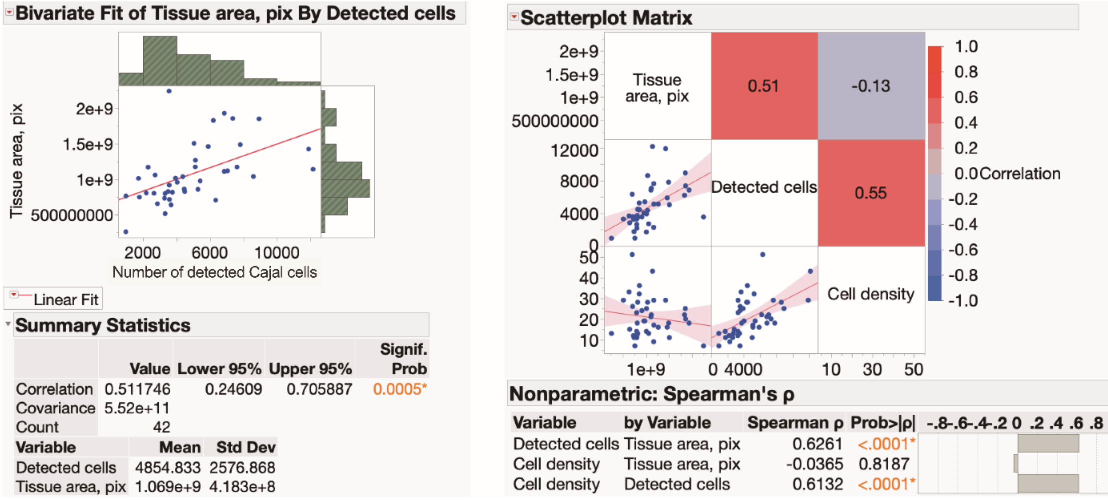


Figure 2.15.1 **Density of ICC in tissue samples of various shapes and sizes**

An unsupervised clustering method was also used to analyse the study data, incorporating clinical data, the results of IHC analyses for ICC and ANO1, and data on the quantitative assessment of ICC obtained using DNN. Both five (Figure 2.16) and six (Figure 2.17) principal components were included in the cluster analysis. Of all patients, 43 % of women and 57 % of men were diagnosed with grade III HD, while 52 % of women and 48 % of men were diagnosed with grade IV. The distribution of ICC was observed almost evenly across two large clusters of HD patients characterised by grade III and grade IV disease, accounting for 58 % and 57 %, respectively, of the anorectal tissue samples obtained from patients with grade III and grade IV HD (Figure 2.16). A cluster analysis incorporating six factors revealed that only one-third (36 %) of patients with grade III HD exhibited high ICC density in surgical specimens. In contrast, 60 % of patients with grade IV HD were found to have a high density of these cells. In both HD grades – III and IV – patients reported complaints of discomfort, pain, and bleeding both before and after surgery. In the preoperative period, pain was reported by 35 % of patients with grade III HD and 41 % of patients with grade IV HD; however, after surgical treatment, its frequency decreased significantly in both groups. A statistically significant reduction in bleeding symptoms was observed during the postoperative period ($p < 0.0001$). However, two patients who continued to bleed in the postoperative period and were diagnosed with grade IV HD, exhibited high ICC density in the anorectal tissues ($p = 0.0041$), which may indicate a possible association between ICC density and the severity of HD.

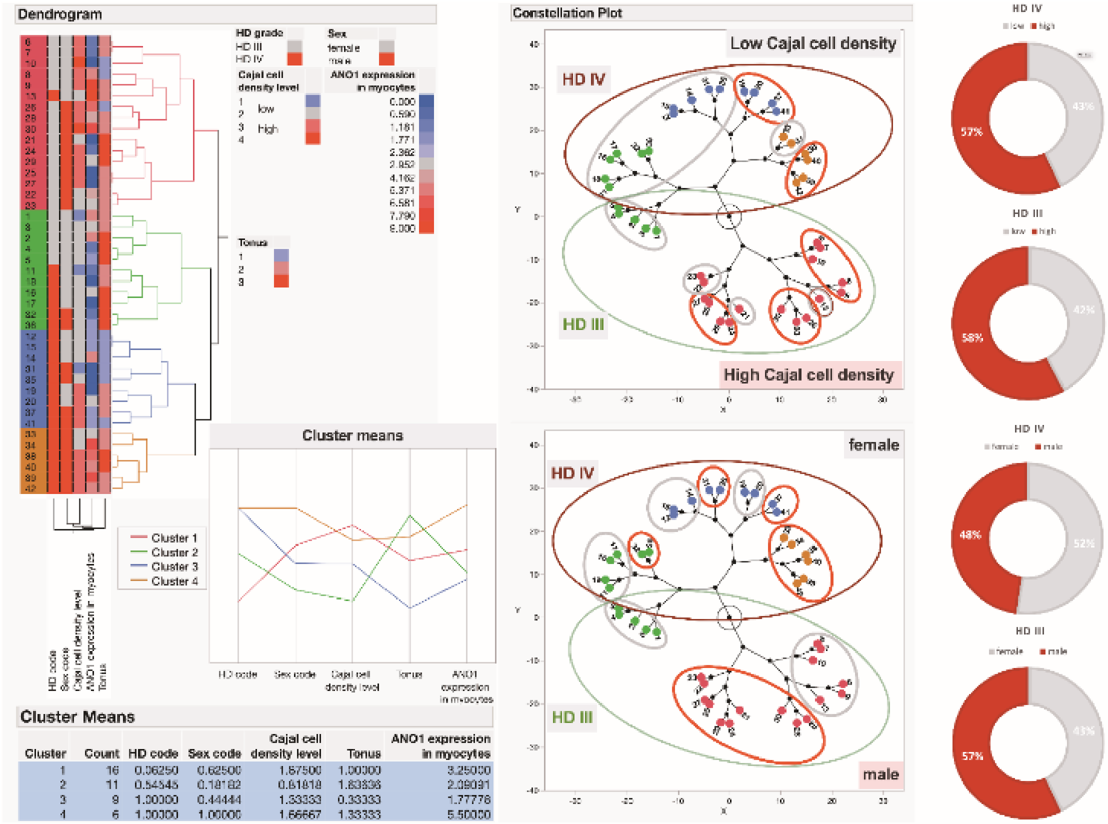


Figure 2.16. **Hierarchical clustering of HD patients and a visual representation of ICC density, muscle tone, and disease severity in male and female participants of the study cohort**

Using hierarchical cluster analysis, groups of patients with similar characteristics were identified, in which the variables within a specific cluster are more similar to one another than they are between the five clusters identified in this study. The hierarchical clustering dendrogram shown in Figure 2.18 illustrates the formation of distinct patient groups within the HD cohort. Scatter plots further visualise the distribution of HD patients across clusters based on different levels of the analysed variables. In turn, the pie charts provide a visual comparison between patients with grade III and IV HD classified by low or high ICC density, as well as between patients with and without pain syndrome manifestations.

The hierarchical clustering method was also used to assess the similarities and differences among the clinical and morphological factors under study. Data on HD patients with blood group 0 were analysed separately from data on HD patients with blood groups A, B, and AB (Figure 2.18).

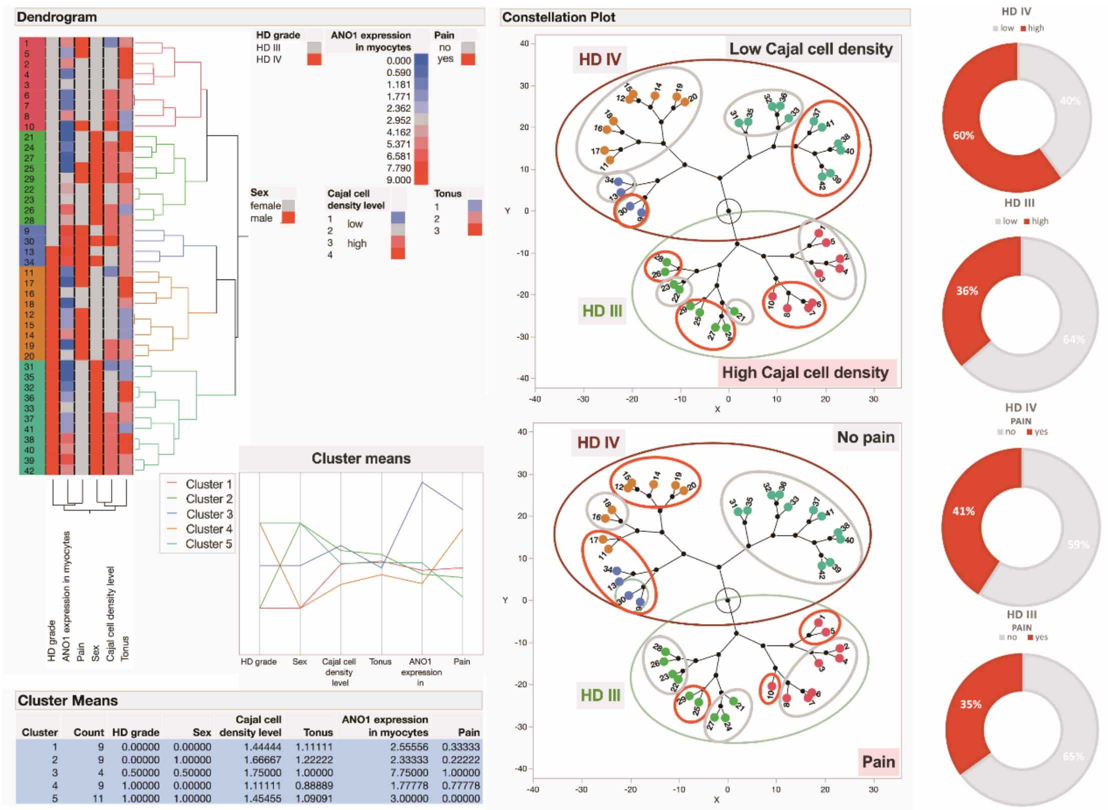


Figure 2.17 **Hierarchical clustering of HD patients and a visual representation of ICC density, pain expression, muscle tone, and disease severity for men and women in the study cohort***

*The dendrogram depicts a two-dimensional tree structure showing the nested cluster sequence in the studied group of HD patients with respect to ICC density, pain expression, muscle tone, ANO1 expression levels in myocytes, patient gender, and disease severity. The variables are color-coded on a scale from blue to red, reflecting a gradient from the lowest to the highest observed values. The dendrogram shows that distinct clusters of HD patients were identified primarily based on disease stage, patient gender, as well as ICC density and pain expression. Patients in the red, yellow-green, and one-sided blue clusters had HD grade III, while the rest of the study group had grade IV. In particular, HD patients were grouped in the blue-green cluster, whereas the majority of patients in the red cluster did not report pain. In contrast, HD patients in the blue cluster, as well as about half of the patients in the orange cluster, reported pain. All men with HD identified in the blue-green cluster, as well as nearly all women in the red cluster, were pain-free.

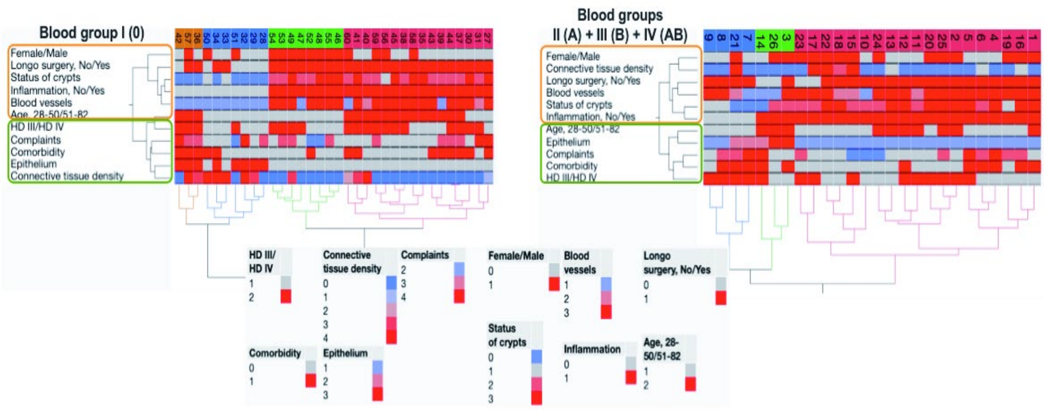


Figure 2.18 **The information depicted in the branches and leaves of the dendrogram includes histopathological assessment data on various anorectal tissue parameters for 60 patients with stage III and IV HD who underwent surgical treatment, as well as data on patient age, gender, symptoms, and comorbidities**

Patients in the HD of 0 blood group were divided into four distinct clusters. In addition, a small, orange cluster was identified exclusively in the 0 blood group. Notably, certain similarities were observed regarding patient complaints and comorbidities, but not regarding the type of surgery, among patients in the blue cluster, which represented patients with HD of blood groups A, B, and AB. The green cluster, consisting solely of men with 0 blood group who underwent haemorrhoidopexy (PPH) surgery, exhibited inflamed mucosa, severely deformed crypts, and ruptured blood vessels associated with bleeding. In the red cluster, which consisted of HD patients with blood groups A, B, and AB who also underwent PPH surgery, it was found that the levels of several analysed factors – particularly the presence of inflammation, crypt deformation, and blood vessel damage – were similar to those observed in HD patients with blood group 0.

2.5 Distribution and interrelationships of categorical variables in groups of HD patients: analysis of scatter plots

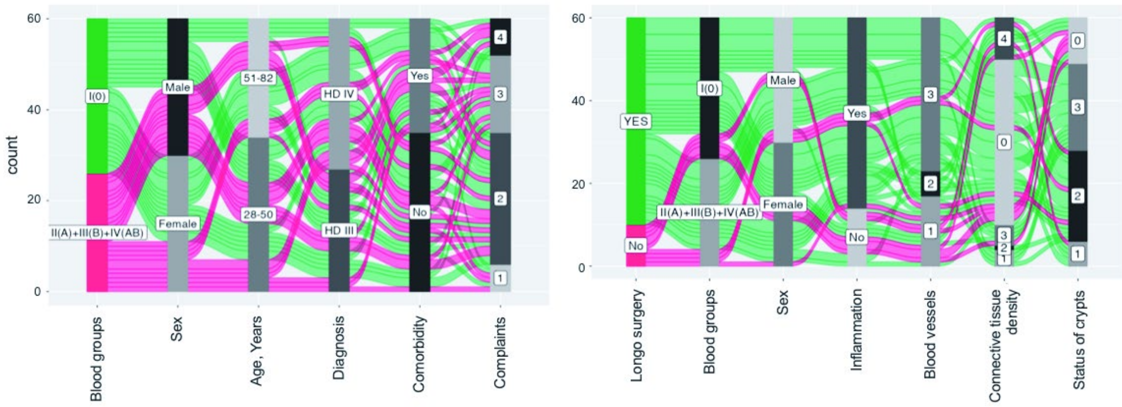


Figure 2.19 **Alluvial diagrams***

*Alluvial diagrams illustrate the flow or directional relationships between categorical variables, which are represented as nodes. In these diagrams, each individual observation is represented in a row, while the characteristics of the variables are shown in columns. Each connecting line and its flow path is determined by the proportion of the respective category in the total data set. In the left panel, the diagram shows the distribution of HD patients by blood group (0 vs. A, B, and AB), gender (male/female), age group (under or over 50 years old), HD grade (III or IV), comorbidities, and the number of reported symptoms. The right panel, in turn, depicts the proportion of HD patients (men and women) who underwent PPH surgery or other types of surgical treatment, with data grouped by blood group (0 vs. A, B, and AB) and histological features – inflammation, vascular and mucosal gland damage, as well as the degree of connective tissue density in the examined specimens.

In addition, scatter plots were created to illustrate the distribution of associations among the categorical variables. This visualisation approach was employed to more clearly demonstrate the individual variation within the studied variables – particularly between HD patients with blood group 0 and those with blood groups A, B, and AB, as well as between patients who underwent PPH surgery and those treated with other surgical methods. The diagrams also illustrated the interrelationships between variables such as gender, age, HD grade, comorbidities, symptoms, PPH surgery, and the histological characteristics of the anorectal tissue (Figure 2.19).

2.6 Multifactorial analysis of factors contributing to HD

Multivariate analysis was used to better examine the relationships among several variables simultaneously. The correlation matrix, depicted and shown in Figure 2.20, indicates that in this study, women reported complaints slightly more frequently than men ($r = -0.30$).

Older patients with HD were found to have a higher likelihood of developing inflammation than younger patients ($r = 0.24$). The number of patient complaints was positively correlated with the presence of comorbidities ($r = 0.35$). The use of the PPH method was associated with better outcomes in patients whose tissue samples showed simple columnar epithelium, compared to those whose samples showed stratified squamous or transitional zone epithelium ($r = -0.52$). This method was used more frequently in patients with cryptic changes ($r = 0.46$), bleeding ($r = 0.45$), and inflammation ($r = 0.39$). Inflammatory infiltrate in the cryptic region was detected more frequently than in cases of stratified squamous epithelium ($r = -0.63$), whereas bleeding was more frequently observed in the mucosa covered by simple columnar epithelium ($r = -0.62$). A decrease in connective tissue density correlated with bleeding ($r = -0.34$) and more pronounced inflammatory infiltration ($r = -0.34$). Markedly deformed crypts were closely associated with ruptured blood vessels, bleeding, and more pronounced inflammatory cell infiltration ($r = 0.74$). Finally, a moderate correlation was found between the presence of ruptured blood vessels and bleeding and tissue inflammation ($r = 0.59$).

Consequently, a comparative analysis was conducted on the proportions of the main categorical variables, which in this study were examined in HD patients with blood group 0 and those with other blood groups (Figure 2.21). Individuals with blood group 0 HD underwent a proportionally higher number of surgeries below the dentata line compared to patients with other blood groups (24 % vs. 11 %). Furthermore, individuals with blood group 0 were more frequently found to have unchanged mucosal glands compared to patients with other blood groups (24 % vs. 11 %). It should be noted that the distribution of the proportions of minimally and densely arranged collagen fibres in the connective tissue differed significantly between HD patients with blood group 0 and patients with other blood groups. Furthermore, dilated submucosal veins were twice as common (38 % vs. 16 %) in samples from HD individuals with blood group 0 compared to those with other blood groups. At the same time, anorectal tissue obtained from individuals with blood group 0 was less inflamed (29 % vs. 15 %) compared to samples from other blood groups.

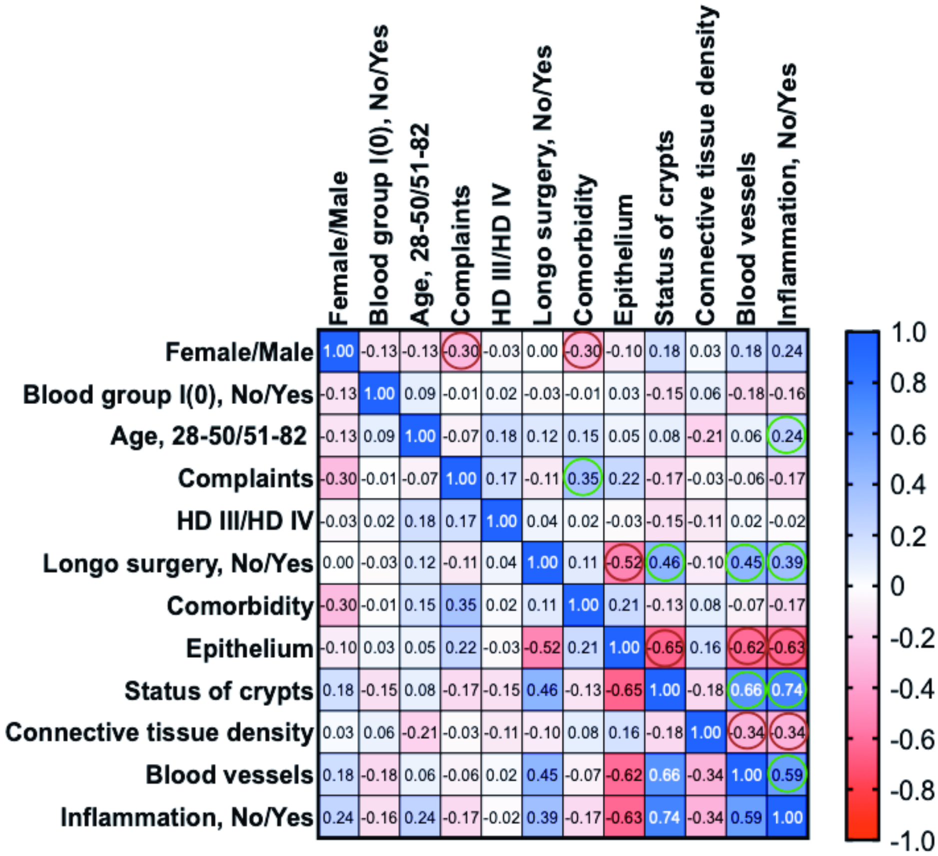


Figure 2.20 **Correlogram of the studied variables***

*In this graph, correlation coefficients are colour-coded according to their values. Positive correlations are shown in blue, while negative correlations are shown in red. The colour intensity is proportional to the correlation coefficients. Coloured circles indicate associations with a significance level of $p < 0.05$.

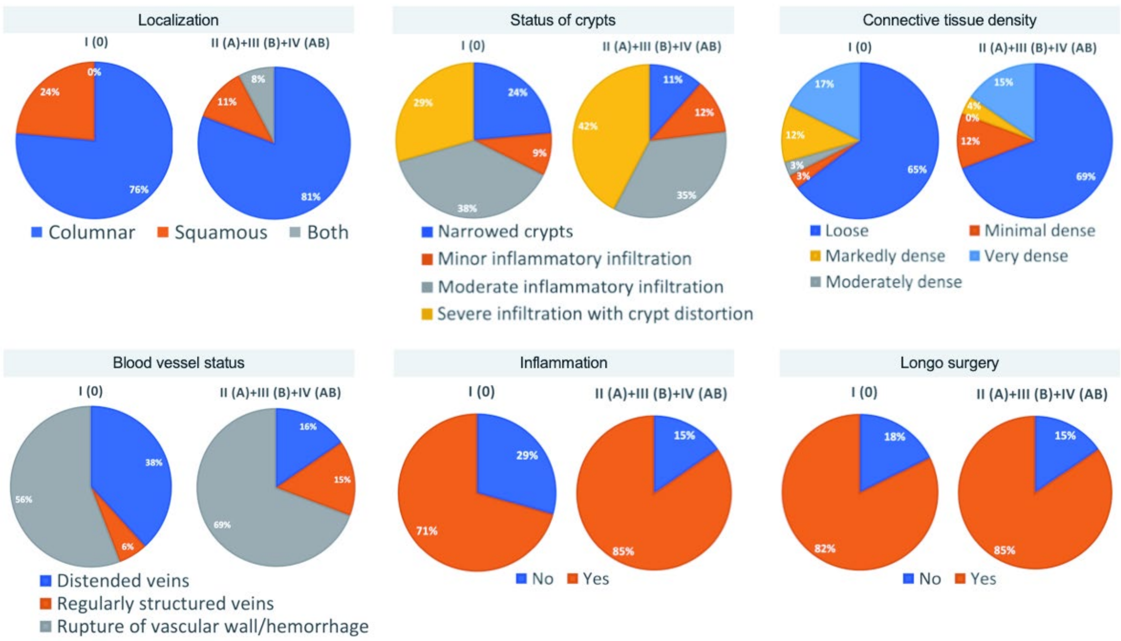


Figure 2.21 **Comparison of the proportion of categorical variables among patients with blood group 0 and other blood groups**

2.7 ICC content and density in haemorrhoidal tissue samples

ICC are cells located between and within the smooth muscle layers of the GIT, from the oesophagus to the IAS, and are characterised by CD117 (c-Kit) immunopositivity and morphological heterogeneity (Torihashi et al., 1999; Al-Sajee et al., 2012). The relationship between ICC and anal canal function in cases of prolapsing HD has not been sufficiently studied. This study employs DNN models trained on IHC images to assess the presence and abundance of ICC in anal canal tissues affected by HD. A machine learning (ML) model based on YOLOv11 was trained on 376 images to automate the detection of ICC using the CD117 marker. The DNN model accurately identified ICC in healthy sections of anal canal wall tissue, revealing that one-third of grade III HD patients and 60 % of grade IV HD patients had high ICC density. Furthermore, 35 % of grade III and 41 % of grade IV HD patients reported postoperative pain, which was likely related to ICC density. IAS hypertonicity and hypercontractility are associated with pain, and IAS spasms may also be a postoperative complication. In HD, pain is associated with IAS spasm and ICC activity; however, this correlation does not imply a direct causal relationship. Studies by other authors indicate that in patients with defecation difficulties, ICC are more densely distributed in the anterior wall of the rectum than in the posterior wall, and their density in the IAS is higher than in the control group (Hagger et al., 1998; Brunenieks et al., 2017). In cases of GIT inflammation, the number of ICC decreases, and their functional and ultrastructural changes contribute to the development of GIT dysmotility (Kaji et al., 2023). Recently, a link between ICC and gastrointestinal stromal tumors (GIST) has been demonstrated, as ICC with similar genetic alterations can act as progenitors or form a supportive microenvironment for tumor growth (Wu et al., 2019). ICC are electrically active cells that generate and transmit slow waves, thereby coordinating smooth muscle contractions and peristalsis (Yin et al., 2008; Chevalier et al., 2020). Under the influence of local tissue factors that activate paracrine mechanisms, this stimulatory function can exceed physiological norms, leading to pathological contractions (Hwang et al., 2009; Forrest et al., 2009; Lees-Green et al., 2014; Sanders, 2019). ANO1, a Ca^{2+}-activated Cl^{-} channel, is a key molecule in the regulation of slow wave mechanisms (Hwang et al., 2009; Lees-Green et al., 2014; Sanders, 2019). Furthermore, ICC are distributed throughout smooth muscle layers to regulate rhythmic contractions (Sweet et al., 2023), a function complemented by their mechanosensitivity and sodium channel activity, which provide inhibitory and excitatory motor neuron input (Strege et al., 2003; Won et al., 2005). ICC dysfunction may contribute to impaired motor function in conditions such as slow-transit constipation, chronic idiopathic pseudo-obstruction, Hirschsprung's disease, diverticular disease, and FI (Huisinga et al., 2021; Choi et al., 2023). ICC decreases with aging, contributing to reduced relaxation and poorer digestive motility (Truong et al., 2023). However, the specific role of ICC in various diseases is not yet fully understood, highlighting the need for more detailed characterisation.

Conclusions

1. Hemorrhoidal disease (HD) is a consequence of rectal prolapse, not its cause. Comorbidities increase the variety and severity of clinical symptoms. In women, regardless of age, more pronounced tissue prolapse and a wider variety of clinical manifestations of HD are more commonly observed.
2. The effectiveness of surgical treatment depends on appropriate patient selection and correct tissue excision. In cases of circular mucosal prolapse, haemorrhoidopexy with a resection line ~3 cm above the dentate line is optimal, whereas in cases of isolated node prolapse, excisional haemorrhoidectomy is more effective. For grade III HD, haemorrhoidopexy is recommended (especially for men), while for grade IV – excisional haemorrhoidectomy, including with the *LigaSure* device.
3. Following haemorrhoidopexy, the early postoperative period is milder if the suture is placed at the optimal height, however, in the long term, the highest patient satisfaction is achieved with radial haemorrhoidectomy using *LigaSure*. The incidence of complications does not depend on patient age; however, recovery after excision surgery may be easier for older patients, possibly due to a decrease in the density of ICC. The duration of rehabilitation correlates with the duration of symptoms prior to surgery, but the risk of inflammatory complications increases with age due to tissue ischaemia and loss of elasticity.
4. Specific clinical and morphological features have been found in patients with blood group 0: reduced collagen density, an altered ratio of type I to type III collagen, and more frequently observed dilated submucosal veins. Women with blood group 0 are characterised by lower levels of inflammation, whereas men experience more frequent bleeding. In patients with blood groups A and B, symptoms are more frequently associated with the presence of combined haemorrhoidal nodes.
5. In cases of prolapsed HS, morphological changes are similar in both sexes, but their severity varies by age and gender. Pronounced mucosal damage and inflammation intensity that increases with age are observed. Vascular ruptures are more common in men. A decrease in connective tissue density and changes in the type I/III collagen ratio contribute to tissue protrusion and disease progression, although the total collagen density does not differ between the genders.
6. The ICC concentration and density influence rectal motility, indirectly modulating the inflammatory process. A reduced number of ICNs correlates with dilated blood vessels and large nodules. In cases of grades III and IV of hemorrhoidal disease (HD), an inverse relationship has been observed between the number of ICC and ANO1 expression, which decreases as the disease progresses. Higher ICC density is associated with more intense

postoperative pain. Morphologicy-based DNT models, integrated with clinical data, improve the prediction of surgical outcome in patients with prolapsing HD.

Proposals, practical recommendations

1. It is recommended to revise the HS classification to include potential etiological factors, symptoms, patient age, gender, and comorbidities.
2. It is necessary to establish standardised surgical protocols with specific guidelines for technical execution and postoperative management for each specific method. The indications for the use of a specific surgical technique must be clearly defined, justified, and consistent.
3. When planning invasive treatment for prolapsed HS, priority should be given to selecting a pathogenetically justified method that ensures minimal postoperative pain, a low complication rate, and the lowest risk of recurrence.
4. During surgical intervention, it is recommended to perform the minimum necessary tissue dissection and resection, using devices with a controlled thermal component with maximum precision.
5. Considering the clinical symptoms in male patients, the morphological findings of anorectal tissue, and the characteristics of the postoperative period, it is recommended to avoid the use of thermal devices in cases of prolapsed HS in the 40–60 age group. In cases with appropriate indications, the PPH surgical method should be prioritized for these patients; however, in cases of Grade IV HS, the use of this method should be strictly limited.
6. Starting on the 14th postoperative day, when tissue oedema subsides and tissue healing intensifies, following excisional hemorrhoidectomies, including LH, it is standard practice to prescribe granulation-promoting ointments for local application, local calcium channel blockers, and medications to alleviate or prevent peripheral neuropathic pain.
7. Continue to perform routine histopathological examinations of resected tissue to verify for possible malignant cellular changes.
8. Prophylactic rectal examination should be recommended starting at age 35.
9. Recommend that the Latvian Association of Surgeons update the certification requirements for the subspecialty of proctology, including mandatory training courses on the use of specific surgical devices provided by the manufacturer or its authorised representative.

List of publications, reports and patents on the topic of the Thesis

Publications:

1. Fišere, I., Groma, V., Goldiņš, N. R., Gardovskis, A. and Gardovskis, J. 2021. Worldwide Disease – Haemorrhoids. How Much Do We Know? Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences., vol.75, no.1, pp.1-10. https://doi.org/10.2478/prolas-2021-0001
2. Fišere, I., Groma, V., Svirskis, Š., Strautmane, E., Gardovskis, A. 2023. Evaluation of Clinical Manifestations of Hemorrhoidal Disease, Carried Out Surgeries and Prolapsed Anorectal Tissues: Associations with ABO Blood Groups of Patients. J Clin Med. 2023 Aug 4;12(15):5119. doi: 10.3390/jcm12155119. PMID: 37568521; PMCID: PMC10420034.
3. Fišere, I., Edelmers, E., Svirskis, Š., Groma, V. 2025. Utilisation of Deep Neural Networks for Estimation of Cajal Cells in the Anal Canal Wall of Patients with Advanced Haemorrhoidal Disease Treated by *LigaSure* Surgery. *Cells*. 2025; 14(7):550. https://doi.org/10.3390/cells14070550

Reports and theses at international congresses and conferences:

1. RSU Research Week 2021 (March 22-26, 2021, Riga, Latvia) and within the framework of the RSU annual scientific conference with a poster report: "Clinical characteristics of patients with prolapsing haemorrhoidal disease".
2. RSU Research Week 2021 (March 22-26, 2021, Riga, Latvia) and within the framework of the RSU annual scientific conference with a poster report: Hemorrhoidal disease III and IV: histopathological assessment in women of different age groups.
4. Colorectal cancer and women's pelvic floor diseases: an interdisciplinary approach to achieving better treatment results. Women's pelvic floor health from the perspective of a proctologist. Latvian Association of Surgeons. Evisit.lv 28.02.2022.
5. Consultations for the preparation and presentation of student scientific papers and reports at the conference: Strautmane E. Morphological Peculiarities of the Rectum in Patients with Grade III and Grade IV Types of Hemorrhoids, XXVI Student international conference of Morphology Sciences, Riga, May 13, 2022.
6. RSU Research Week 2023 (March 29-31, 2023, Riga, Latvia) and within the framework of the RSU annual scientific conference with a poster presentation on the topic: "CHARACTERIZATION OF PROLAPSING HAEMORRHOIDAL DISEASE IN DIFFERENT AGE GROUPS OF FEMALE".
7. Circular sutures in the treatment of hemorrhoids. Is the era over? Latvian Association of Surgeons. Riga, Latvia 27.05.2023.
8. PSKUS Surgery Clinic Clinical Conference with an oral report on complications after hemorrhoidectomy PSKUS 24.09.2023.
9. Oral presentation: Deep Learning-Based Software for Automated Cell Detection and Counting in Whole-Slide Histological Image Analysis. 11th Baltic Morphology Meeting13–15 November 2024 Institute of Anatomy and Anthropology (Anatomicum), Rīga Stradiņš University 9 Kronvalda bulvāris, Riga, LV-1010).

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