Aspects on Minimally Invasive Surgery for Rectal Tumours

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Gothenburg 2014

Cover illustration: Kustmotiv, Christina Swartling

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ISBN 978-91-628-8992-0
http://hdl.handle.net/2077/35441

Printed in Gothenburg, Sweden 2014
Ineko

Teach me knowledge and good judgement, for I believe in your commands.
Psalm 119:66

To Christina, Mathias, Jonathan, Cornelia and Sakarias

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ABSTRACT

Background Transanal endoscopic microsurgery (TEM) and laparoscopic rectal resection are minimally invasive methods of surgery for rectal tumours. One aim of this thesis was to analyse the inflammatory response after minimally invasive surgery compared with open resection. Other aims were to investigate patient selection using magnetic resonance imaging (MRI) and endorectal ultrasound (ERUS) and to investigate the outcome of TEM for rectal cancer.

Methods Inflammatory mediators were measured using enzyme-linked immunosorbent assays (ELISA) in patients undergoing TEM, laparoscopic or open resection. Assessments of tumours using MRI and ERUS were compared with histopathology. Registry data from TEM procedures and salvage resection for rectal cancer were analysed. Low-risk tumours were defined as tumour stage T1, submucosal invasion Sm1-2, <3 cm, without adverse features, and these were separately analysed for outcome.

Results The increases of interleukin-6 and C-reactive protein were less pronounced after TEM and laparoscopic resection than after open resection. The staging accuracy using MRI was increased from 0.65 to 0.83 by combining lymph node assessment using MRI with bowel wall assessment using ERUS. There were no local recurrences after TEM for low-risk tumours.

Conclusions The inflammatory response after TEM and laparoscopic resection was limited compared with open resection. The staging accuracy was increased by a combined use of MRI and ERUS. The population-based oncological outcome of TEM for low-risk tumours was excellent.

ISBN:978-91-628-8949-4
http://hdl.handle.net/2077/35441

SAMMANFATTNING PÅ SVENSKA

Bakgrund Transanal endoskopisk mikrokirurgi (TEM) och laparoskopisk resektion av rektum är minimalinvasiva metoder för operation av tumörer i ändtarmen. Ett syfte med avhandlingen var att studer det inflammatoriska svaret vid minimalinvasiv kirurgi och öppen resektion. Andra syften var att studera den preoperativa selektionen av patienter med hjälp av magnetkamera (MR) och endorektalt ultraljud (ERUS) och att studera utfallet av TEM och kompletterande resektion för rektalcancer.

Metoder Mätning gjordes med enzyme-linked immunosorbent assay (ELISA) av inflammtoriska markörer i blod från patienter som opererades med laparoskopisk och öppen resektion samt TEM. Bedömning av tumörer med MR och ERUS jämfördes med patologisk anatomisk undersökning. Resultat av TEM och kompletterande resektion efter TEM för rektalcancer analyserades med hjälp av registerdata. Lågrisktumörer definierades som tumörstadium T1 med submukosainvasion Sm1-2, en diameter <3 cm och utan illavarslande tecken. Utfall för lågrisktumörer analyserades separat.

Resultat De inflammatoriska markörerna interleukin-6 och C-reaktivt protein ökade i lägre grad vid laparoskopisk resektion och TEM än vid öppen resektion. Stadiumindelningens tillförlitlighet för selektionen av patienter genom användning av MR ökades från 0.65 till 0.83 vid kombinerad användning av MR för bedömning av lymfkörtlar med ERUS för bedömning av väggpenetration. TEM för lågrisktumörer medförde inga lokalrecidiv inom tre år medan TEM för samtliga stadium I tumörer som var mindre respektive större än tre cm resulterade i lokalrecidiv hos 1 av 23 respektive 4 av 11 patienter. Kompletterande resektion efter TEM för stadium I respektive stadium I-III medförde perforation av resektionspreparatet vid 4 av 16 respektive 9 av 28 operationer.

Konklusion Det inflammatoriska svaret vid TEM och laparoskopisk resektion var mer begränsat än vid öppen operation. Tillförlitligheten vid stadieindelning av tumörer genom användning av MR enbart kunde ökas från 0,65 till 0,83 genom en kombinerad analys av undersökning av lymfkörtlar med MR och vägginvasion med ERUS. Det populationsbaserade resultatet av TEM för lågrisktumörer var utmärkt. Kompletterande resektion efter TEM medförde en förhöjd risk för perforation av den tarm som opererades bort, vilket är en känd riskfaktor för återfall.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Kvarnström, A, Swartling, T, Kurlberg, G, Bengtsson, J-P, Bengtsson, A. **Pro-inflammatory Cytokine Release in Rectal Surgery: Comparison between Laparoscopic and Open Surgical Techniques.** *Archivum Immunologiae et Therapiae Experimentalis 2013; Aug 8.*

II. Kvarnström, A, Sokolov, A, Swartling, T, Kurlberg, G, Mollnes, T.E., Bengtsson, A. **Alternative pathway activation of complement in laparoscopic and open rectal surgery.** *Scandinavian Journal of Immunology 2012 Jul; 76(1): 49-53.*

III. Swartling, T Kvarnström, A, Bengtsson, A, Kurlberg, G. **Inflammatory response to transanal endoscopic microsurgery for tumours of the rectum.** *Manuscript*.

IV. Swartling, T, Kälebo, P, Derwinger, K, Gustavsson, B, Kurlberg, G. **Stage and size using magnetic resonance imaging and endosonography in neoadjuvantly-treated rectal cancer.** *World Journal of Gastroenterology2013 Jun 7; 19(21): 3263-71.*

V. Swartling, T, Kodeda, K, Derwinger, K, Kurlberg, G. **A population-based study of transanal endoscopic microsurgery and salvage total mesorectal excision as treatments for early rectal cancer.** *Manuscript*.

CONTENT

ABBREVIATIONS

2D/3D	Two-dimensional/three-dimensional
ADF	Alive disease-free
APR	Abdominoperineal resection
AR	Anterior resection
ASA	American Society of Anesthesiologists physical status classification system
AU	Arbitrary unit
AWD	Alive with disease
Bb	Bb complement activation product
CRP	C-reactive protein
C3bc	C3bc complement activation product
C4d	C4d complement activation product
DCR	Death cancer related
DNCR	Death non cancer related
DR	Distant recurrence
EDTA	Ethylene Diamene Tetraacetic Acid
ELISA	Enzyme-linked immunosorbent assay
ERUS	Endorectal ultrasound/endosonography
G	Tumour grade
G-CSF	Granulocyte colony-stimulating factor

HP	Hartmann's procedure
ICAM	Intracellular adhesion molecule
IL	Interleukin
IQR	Interquartile range
LMM	Linear Mixed Models
LR	Local recurrence
MCP	Monocyte chemotactic protein
MODS	Multiple organ dysfunction syndrome
MRI	Magnetic resonance imaging
p	probability value
PAI	Plasminogen activator inhibitor
pN	Pathological node
pT	Pathological tumour
R0	Radical surgery macro- and microscopically
R1	Surgery not radical either macro- or microscopically
rTME	Total mesorectal excision for recurrence
SCRCR	The Swedish Colorectal Cancer Registry
SCTR	The Scandinavian TEM Registry
SIRS	Systemic inflammatory response syndrome
Sm	Submucosa
SPSS	Statistical Package for the Social Sciences

sTME	Salvage total mesorectal excision
T	Tesla
TNF	Tumour necrosis factor
WBC	White blood cell
TNM	Tumour node metastasis
TEM	Transanal endoscopic microsurgery
Th	T-helper cell
TCC	Complement terminal C5b-9 complex
TME	Total mesorectal excision
VCAM	Vascular cell adhesion molecule

DEFINITIONS IN SHORT

alpha-value	the significance level, usually taken at 0.05; the probability of type I error
accuracy	proportion of true results (both true positives and true negatives); (true positives + true negatives) divided by all results.
beta-value	the probability of type II error
dysplasia	premalignant changes in the epithelium of adenomas. Adenomas with high-grade dysplasia were previously defined as cancer in situ.
high-risk patient	patient at risk for big surgery
low-risk tumour	low risk for recurrence after TEM; In this thesis T1, Sm 1-2, < 3 cm, without adverse features such as high tumour grade.
M0	No distant metastasis
M1	Distant metastasis
MX	No information on distant metastasis
N0	No lymph node metastasis
N1	1-3 lymph node metastases
N2	>3 lymph node metastases
null hypothesis	hypothesis that there is no true difference between two groups; hypothesis that there is no relationship between two measured phenomena.
NX	No information on lymph nodes available

power	(1-beta); the probability of not committing a type II error; the probability that the test will reject the null hypothesis if the alternative hypothesis is true; used to calculate the minimum sample size needed to detect a difference between groups
p-value	the probability of getting the observed or more extreme results, given that the null hypothesis is true
R0	No residual tumour; Radical surgery macro- and microscopically
R1	Microscopic residual tumour
R2	Macroscopic residual tumour
sensitivity	true positive rate; a diagnostic intervention with high sensitivity will detect the vast majority of patients with the disease it is meant to detect and few patients with the disease will be missed.
significant	statistically significant = a p-value below 0.05; clinically significant = a clinically detectable phenomenon
Sm 1	invasion of upper one-third of submucosa
Sm 2	invasion of middle the one-third of submucosa
Sm 3	invasion of lower one-third of submucosa
specificity	true negative rate; a diagnostic intervention with high specificity will identify only those patients who truly have the disease it is meant to detect and it will not falsely identify as positive, those patients who do not have the disease

Stage I	T1-2 N0 M0
Stage II	T3-4 N0 M0
Stage III	T1-4 N1-2 M0
Stage IV	T1-4 any N M1
T0	Benign tumour including cancer in situ
T1	Cancer penetrating into submucosa
T3	Cancer penetrating into perirectal fat
T4	Cancer penetrating beyond serosa
tumour grade, high	G1 = low grade = well or highly differentiated
(TNM 7th edition 2010)	G2 = intermediate grade = moderately differentiated
	(TNM 6th edition 2002)
tumour grade, low	G3 = high grade = poorly or low differentiated
(TNM 7th edition 2010)	G4 = high grade = undifferentiated
	(TNM 6th edition 2002)
type I error	falsely accepting an effect of chance as a true difference; alpha error
type II error	falsely rejecting a true difference as an effect of chance; beta error

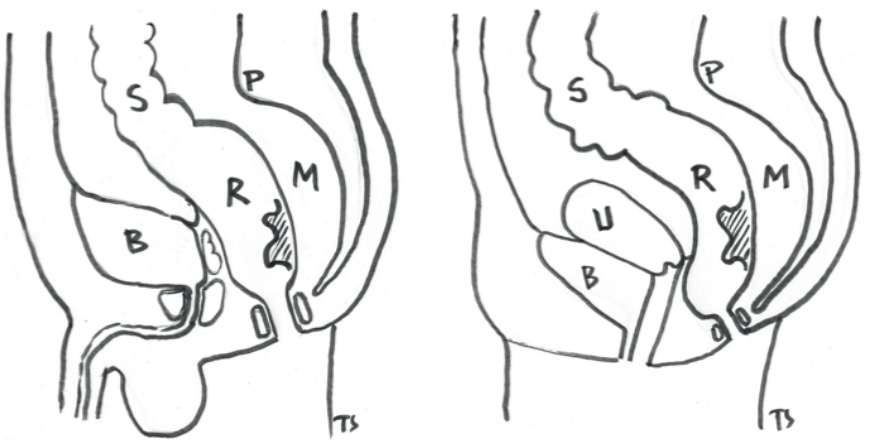
1 INTRODUCTION

1.1 General background

Rectal cancer is detected in approximately 2000 patients per year in Sweden. Together with colon cancer (approximately 4000 per year), it is the third most common cancer in Sweden after cancers of the breast and prostate. Rectal cancer results in death in about 40 % of patients and local recurrence in about 10% of the patients within five years. The anatomical position of rectal tumours makes surgical removal intricate, and local recurrence detrimental[1-3]. Modern management of rectal cancer has reduced the incidence of local recurrences from 30-40% a few decades ago[4-6] to 10% or below[7], through the selective use of radiotherapy[7, 8] in combination with the surgical technique known as total mesorectal excision (TME), which is traditionally performed as open surgery[9-12]. However, open total mesorectal excision implies a significant surgical trauma and considerable side-effects. To reduce the side-effects of the surgical trauma of open mesorectal excision, minimally invasive techniques have been developed.

1.2 Anatomy

Figure 1. *Sagittal view of male and female pelvises.* ***S*** *sigmoid colon.* ***P*** *promontory.* ***R*** *rectum with a posterior tumour.* ***M*** *mesorectum.* ***U*** *uterus.* ***B*** *bladder. The seminal vesicles and the prostate are located anteriorly to the rectum in the male and the vagina and uterus in the female.*

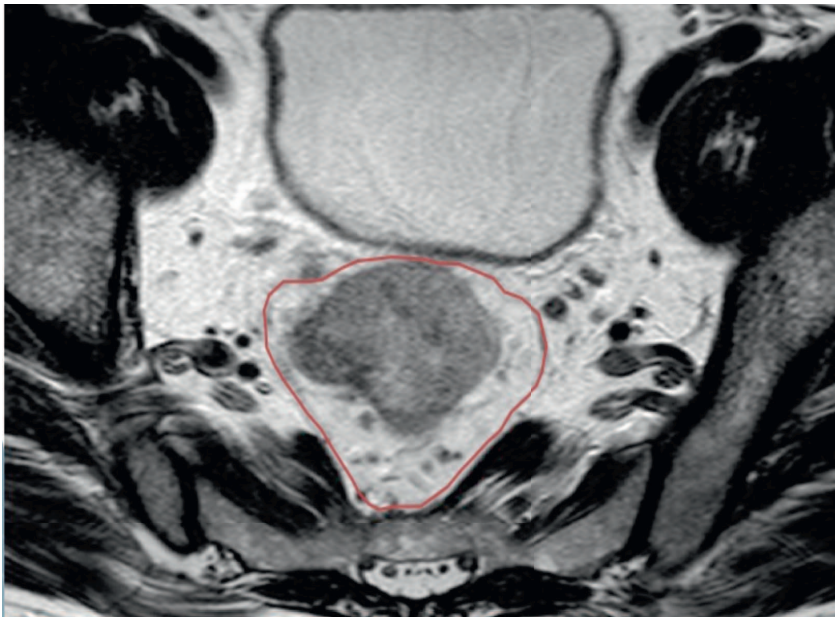


The rectum is the last part of the bowel and includes the anal canal above the dentate line. The dentate line is situated approximately two cm inside the anal verge and represents the border between the squamous epithelium of the skin and the glandular epithelium of the bowel. The anal canal is approximately 3-4 cm of bowel surrounded by the internal and external sphincter muscles and is more prominent and longer in men than in women.

In the Swedish Colorectal Cancer Registry an adenocarcinoma is registered as located in the rectum if it is found between 0 and 15 cm from the anal verge during examination in the left lateral position with a straight proctoscope being withdrawn backwards (on the way out). The verge is identified where the proctoscope enters the bowel lumen.

The definition of the rectum coincides more or less with the anatomical part of the bowel that is located beneath the sacral promontory. A large portion of the rectum is confined to the posteroinferior part of the abdomen surrounded by other organs in the pelvis and is attached to the immobile mesorectum which contains the arterial blood supply and venous and lymphatic return of the rectum. The mesorectum is enveloped by a sheath, the mesorectal fascia, which creates a compartment within the body.

Figure 2. *A tumour filling the lumen of the rectum, and the surrounding mesorectal fascia outlined in red. Anteriorly lies the bladder, posteriorly the sacrum and the piriformis muscles and to each side the ureters and branches of the internal iliac vessels. MRI image in the transverse plane.*



The rectum is a reservoir for faeces and its function is to expel the bowel contents at will. The reservoir function is lost if the rectum is surgically removed, but some of the function may be substituted by bowel connected to the rectal remnant.

The rectum is located between other vital structures that should not be injured during surgery. The vagina and the uterus are located anterior to the rectum in women and the prostate and seminal vesicles are located anterior to the rectum in men. In both sexes the ureters are located just lateral to the outermost part of the mesorectal fascia and care must be taken not to injure them. The hypogastric nerve plexus runs closely postero-laterally to the plane of the proximal mesorectal dissection and the pelvic nerve plexus runs just laterally to the distal dissection plane and anteriorly to the dissection plane between the rectum and the seminal vesicles and prostate. These nerves contribute to sexual, bladder and rectal functions and care should be taken not to injure them.

1.3 Rectal tumours

The tumours included in the studies in this thesis are adenoma and rectal cancer (adenocarcinoma). Both arise from the glandular epithelium above the dentate line but may extend below the dentate line through growth. Rectal cancer must be distinguished from anal cancer (squamous cell cancer) which arises from the squamous epithelium below the dentate line but may extend into part of the rectum through growth. Anal cancer is treated differently and is not included in this thesis. However, other tumours in the rectum include carcinoids, melanomas, sarcomas and lymphomas as well as overgrowth from cancers in surrounding organs.

According to the adenoma-carcinoma sequence theory, adenomas transform into invasive cancers over time[13-16]. For this reason, adenomas are removed and followed even if they do not cause symptoms. Furthermore, because preoperative assessment of rectal tumours frequently understages early cancers, tumours assessed as adenomas should be removed to attain a histopathological analysis of the complete lesion. Even if biopsies are benign, other parts of the adenoma may have transformed into a cancer. The risk of transformation into cancer increases with increasing tumour size.

Tumours in the rectum typically spread first to the mesorectum through the lymphatic vessels and lymph nodes. A rectal tumour without dissemination can be cured by complete removal of the visible and palpable tumour. A rectal tumour with early dissemination can theoretically be cured by

removing the mesorectum en-bloc together with the rectum, as the dissemination is contained within the compartment of the mesorectum.

1.4 Tumour stage

It is important to stage rectal tumours preoperatively to optimise treatment. The dominating classification of stage is the pathologic tumour node metastasis (TNM) classification system. Preoperative staging may be abbreviated cTNM for clinical assessment, uTNM for assessment with endorectal ultrasound (ERUS) or mTNM for assessment with magnetic resonance imaging (MRI). Preoperative staging is more or less accurate, whereas the gold standard is the pathological staging of the excised surgical specimen (pTNM).

Adenomas are classified as serrated, tubular, tubulo-villous and villous. Villous adenomas carry a higher risk of development into an invasive cancer[17]. High-grade dysplasia indicates a higher risk of imminent invasion beyond the mucosa, and it is sometimes reported as a cancer in situ. In Sweden it is currently most often reported as adenoma with high-grade dysplasia.

Cancers are classified according to the tumour node metastasis (TNM) classification, summarised as stages I-IV, (see Definitions in short for details). The risk of lymph node metastases is connected to stage and it is a predictor of outcome, especially in local surgery.

In one estimation T1, T2, T3 and T4 carry 0-12%, 12–28%, 36– 66% and 53–79% risks of lymph node metastases, respectively[18, 19]. Furthermore, T1 tumours are classified according to the degree of submucosal invasion (Sm), (see Definitions in short for details). Nascimbeni et al found that Sm 1, Sm 2 and Sm 3 carries a 3%, 8% and 23% risk of lymph node metastases, respectively[20].

Cancers are classified according to tumour grade. This classification has changed several times, which may cause some confusion. Until 2010, histopathological analyses were reported in four grades, whereas from 2010, a dichotomised classification was used: high or low grade, according to the 7th edition of the TNM staging system. During the first half of the study period, the histopathological findings were classified as well (highly), moderately, poorly (low) and undifferentiated. The reports gradually changed to grades G1-4, where high grade corresponds to poorly (low) differentiated and low grade corresponds to well (highly) differentiated. The World Health

Organisation classification of tumour grade in a biopsy or a specimen is related to the percentage of tumour with gland-like structures; grade G1 >95%, G2 50-95%, G3 5-50% and G4 <5% gland-like structures[21], (see Definitions in short for further details).

It is essential to point out that tumour grading of a preoperative biopsy provides a hint of the final tumour grading but is not conclusive. In one study grading of biopsies was consistent with the grading of the surgical specimen in 75% of cases [22].

Low-grade (G1) T1-tumours carry 0-3% risk of lymph node involvement, whereas high-grade (G3) T1-tumours carry 12% risk of lymph node involvement[18], and Saraste et al found that the presence of high tumour grade increases the risk for lymph node metastases with an odds ratio of 6.5[23].

Location is important. Early rectal cancers in the lower third of the rectum carry a sixfold higher risk than tumours in the upper two-thirds of the rectum[17].

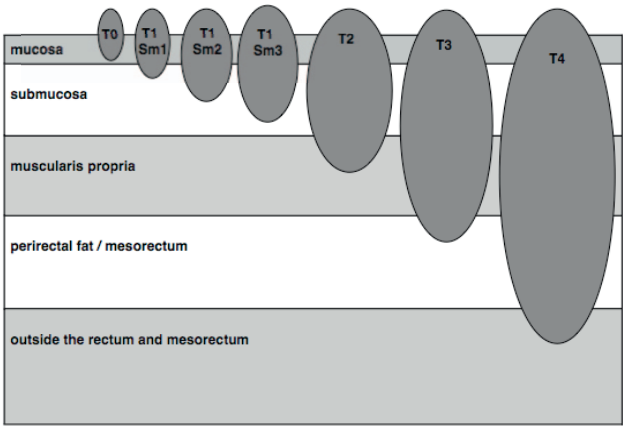


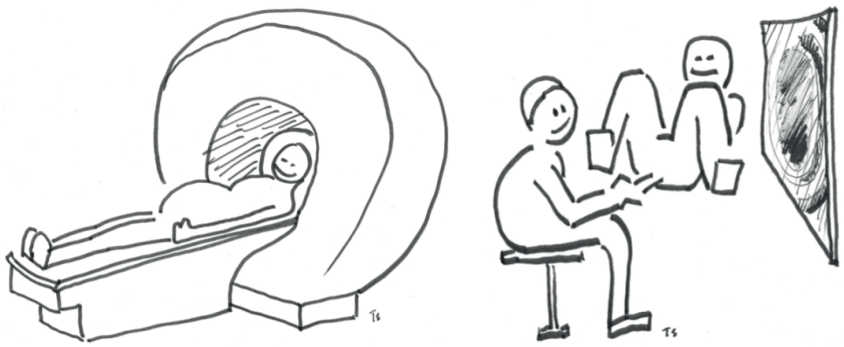
Figure 3. *The T- and Sm-stages of rectal tumours. The layer of the bowel wall are shaded as they appear using ERUS.*

1.5 Preoperative assessment

Rectal tumours are assessed and diagnosed first of all by palpation and proctoscopy with a straight or flexible instrument. The texture of the tumour is assessed as hard, soft or firm. It may be fixed, mobile or tethered (which is in between fixed and fully mobile). The presence of a central depression or ulceration should be noted as adverse features. The surface pattern of the tumour is assessed. In short, a regular structured pattern formed by the microvasculature indicates a benign diagnosis. An irregular pattern indicates high-grade dysplasia and a more distorted pattern indicates a malignant

diagnosis. The pattern may be better visualised with narrow banding imaging, a green-coloured light, which emphasises the architecture of the surface pit pattern of the tumour[24].

Figure 4. *During MRI the patient is required to lie still for approximately 40 minutes after the examining table has moved the patient into the MRI scanner. ERUS takes 10-15 minutes and is performed using a probe that is inserted into the anus through a normal-sized proctoscope.*



Biopsy of the tumour may provide a malignant diagnosis based on histopathology, in which case the entire tumour is treated as a malignancy. However, a benign biopsy does not guarantee that the entire lesion is benign, and the lesion has to be evaluated together with the other available information.

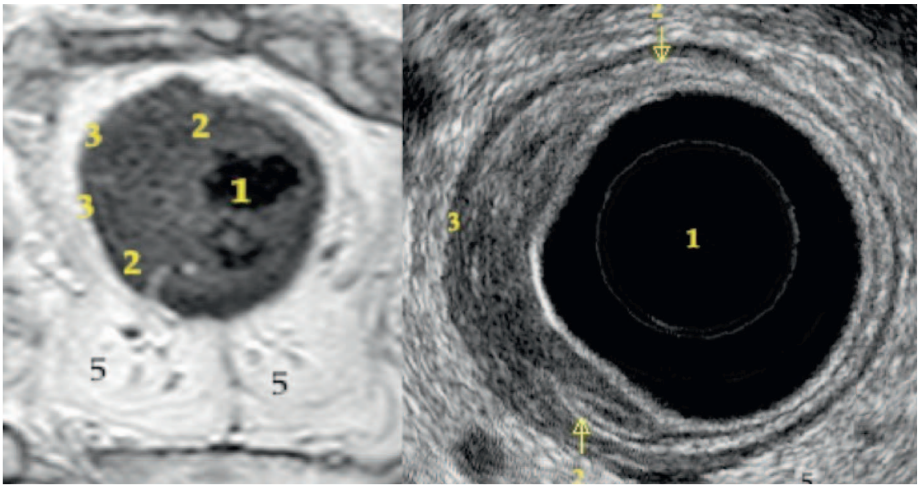
Imaging of rectal tumours may be obtained with MRI or ERUS. In Sweden the patient is sent to a radiologist for MRI, whereas ERUS is commonly performed by a colorectal surgeon who is specialised in this method. ERUS is often used for the assessment of early tumours, whereas MRI has a more obvious place in advanced tumours. The indications for the methods overlap each other.

Distant dissemination is ruled out by computer tomography of the abdomen and thoracic cavity, or by ultrasound of the liver and plain chest x-ray. Colonoscopy or computer colonography is performed to rule out synchronous tumours in the colon.

The accuracy of the assessment is represented by the proportion of true results (both true positives and true negatives). In other words, the accuracy of the assessment is the number of true positives plus the number of true

negatives divided by all results. The sensitivity of the assessment is the true positive rate. The specificity of the assessment is the true negative rate.

Figure 5. *A T2N0 rectal cancer imaged in the transverse plane. MRI to the left and ERUS to the right.* ***1*** *Lumen, with probe in the ERUS image.* ***2*** *Submucosa, the middle white line in ERUS, disappearing in the tumour, typically for T2.* ***3*** *Border between muscularis and perirectal tissue, respected in this tumour, unlike in T3-tumours.* ***5*** *Mesorectum with a benign enlarged lymph node.*



1.6 Surgical and endoscopic treatment for rectal tumours

Neoadjuvant treatment is administered before surgery in the form of radiotherapy, chemotherapy or a combination of these. In short-term radiotherapy, the patient is administered 5 Gray of radiation once a day for five days. Short-term radiotherapy is currently indicated for patients with stage III and stage II with risk factors and for patients who will undergo an abdominoperineal excision. Long-term radiotherapy is normally administered together with chemotherapy for five weeks, currently indicated for tumours involving the surgical margin.

Removal of rectal tumours can be performed as a local excision or as a resection of a whole bowel segment. Local excision removes only the tumour and a limited portion of the surrounding tissue. Resection removes any length of the rectum. Total mesorectal excision (TME) is a resection that removes

the attached mesorectum together with the rectum without opening the surrounding mesorectal fascia.

Local excision can be performed as transanal excision (TAE), transanal endoscopic microsurgery (TEM), endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD). One advantage of local excision is the limited surgical trauma. The surgical trauma is even more limited in EMR and ESD, which may be performed without general anaesthesia. A drawback of EMR and ESD is that it is more difficult to obtain a surgical specimen in one piece with a sufficient amount of normal tissue surrounding the tumour. TAE may be performed under local anaesthesia but is usually performed under general anaesthesia to achieve a safe excision. TAE may be performed for tumours that can be pulled down for excision under direct vision from below. Given the availability of TEM, TAE is only used for those tumours that are easily detected a few cm inside the anus. TEM enables excision under microscopic visualisation inside the rectum. The method requires general anaesthesia and muscle relaxation. TAE and TEM enable good local specimens in one piece with adequate margins of normal tissue.

Figure 6. *TEM procedure performed through a large proctoscope inserted into the anus. The seated surgeon makes use of the stereoscope, but a video screen is also available for the surgeon to change positions of the head and neck.*

A standard TEM procedure is a full-thickness excision including a secure margin of healthy bowel wall around the lesion as described by Buess[25]. The standard procedure may be modified to include only a superficial part of the bowel wall for benign lesions or tumours with high anterior locations, and it may be extended to include the mesorectum adjacent to a rectal cancer. Diathermy which was used in the early years of the study, has been replaced by the harmonic scalpel in western Sweden. The defect may be sutured or left open to heal by secondary intention. Perforations of the peritoneal cavity are always sutured. The patients are postoperatively mobilised and allowed to eat on the day of surgery and may be discharged within 12-24 hours.

Depending on the location of the tumour and the patient's anatomy, resection can be performed as an anterior resection (AR), abdominoperineal resection (APR) or Hartmann's procedure (HP). AR is a sphincter-saving procedure,

whereas APR and HP are not. In APR, the entire rectum and anus are removed. In HP, the rectum is divided and left in place without reconnection to the proximal bowel. A colostomy is part of the procedure in both APR and HP. APR is performed when there is not a sufficient margin to divide the rectum safely below the tumour. HP is performed when APR is not necessary but the risk of a low anastomosis should be avoided. Resections are performed as total mesorectal excision for rectal tumours. This means that the whole compartment surrounded by the mesorectal fascia is removed without being opened, from the division of the inferior mesenteric artery (the feeding artery) to the distal end of the mesorectal compartment. The theory is that most cancer cells are confined within the mesorectal fascia until later stages in the disease, and the complete removal of the entire compartment increases the possibility of a cure. For tumours in the upper part of the rectum (with a distal border above approximately 12-13 cm), it may be sufficient to perform a partial mesorectal excision, which includes at least 5 cm of undamaged mesorectal tissue beneath the distal border of the tumour.

All resections can be performed as open or as laparoscopic procedures. The open procedures are normally performed via a midline incision through the anterior abdominal wall. In most procedures the incision is at least 20 cm in length, from the pubic bone and 5-10 cm beyond the umbilicus. If the left flexure of the colon needs to be mobilised (which is the case in the majority of the procedures according to many surgeons), the incision often needs to be extended further cranially making it approximately 25-30 cm long. From the division of the feeding artery, the mesosigmoid and the mesorectum are mobilised in the embryological plane between them and the surrounding structures. After mobilisation of the tissue, the rectum is divided above the anal sphincter in both an anterior resection and in Hartmann's procedure. In an abdominoperineal excision, the mobilisation of the tissue continues to the outside until the entire rectum and anus is mobilised. After division of the sigmoid colon at a convenient level, the tissue is removed without having opened the compartment of the mesorectum and the adjoining mesosigmoid.

The laparoscopic resections are performed according to the same principles as the open resections. In anterior resection and in Hartmann's procedure the challenging aspect is the dissection and division of the distal part of the mesorectum and rectum. The laparoscopic abdominal dissection in abdominoperineal resection is less challenging because the distal rectal dissection and division are not required. Furthermore, laparoscopic abdominoperineal resection does not require an abdominal incision for removal of the specimen because the procedure is completed by dissection and removal of the specimen from below in the same way as in the open procedure.

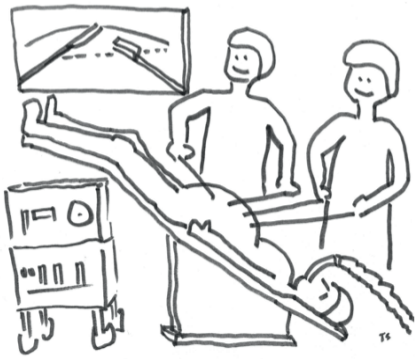


Figure 7. *A laparoscopic resection performed by two surgeons standing on the patient's right side. The inclination of the table helps exposure. Surgery is done under direct vision via one of several video screens positioned around the table.*

Adjuvant treatment with chemotherapy can be recommended for healthy patients below a biological age of approximately 80 years if lymph node metastases are detected in the surgical specimen postoperatively and for some other high-risk tumours. Chemotherapy is administered over a 6-month period as soon as the patient has recovered from surgery. A common regimen is intravenous treatment every other week.

1.7 Low-risk tumours

Definitions of tumours that are considered oncologically low-risk for treatment with TEM alone vary somewhat in the literature[17, 18, 26-29]. In this thesis the definition of a low-risk tumour is a T1, Sm1-2 tumour with a diameter less than three cm and without adverse features such as a high tumour grade. (Other adverse features including tumour budding and lympho-vascular infiltration were rarely seldom available in this study). [17]

1.8 Inflammatory response to surgery

Local inflammation was described by Celsus around the birth of Christ as rubor (redness), tumor (swelling), calor (heat), and dolor (pain). Functio laesa (loss of function) was later added to this description which is still valid today. Inflammation is a response triggered by damage to the body by infection or

trauma. One purpose of the inflammatory response is to restore homeostasis and commence healing [30]. During this process, the system is checked and balanced by an entire network of mediators working in harmony through action and counteraction. Blood flow and permeability through blood vessel walls are increased. Fluids, proteins and white blood cells migrate from the blood vessels into the damaged tissue[31]. Inflammatory responses may be caused by a tumour which is regarded as foreign by the body. Likewise radio- and chemotherapy cause an inflammatory response because of the damage done to cells in the body. The trauma caused by surgery induces a substantial inflammatory response in patients.

While a balanced inflammatory response will contribute to optimal wound healing and recovery[32 33], an excessive response is associated with the development of systemic inflammatory response syndrome. If the local inflammatory response is inappropriate, its components increase in the systemic circulation. Taking it one step further, the entire body is affected by the systemic inflammatory response, in a systemic inflammatory response syndrome (SIRS) which in severe cases may worsen to multi-organ dysfunction syndrome (MODS), which may be fatal[34]. The definition of SIRS is at least two of the following findings:
(1) Temperature greater than 38 C or less than 36 ˚C.
(2) Heart rate greater than 90 beats/min
(3) Respiratory rate greater than 20/min
(4) White blood cell count greater than 12.0 or less than 4.0 x 10^9 per litre [35].

The following inflammatory mediators were analysed in this thesis: C-reactive protein (CRP); white blood cell count (WBC); interleukin 1-α (IL-1-α); IL-1 receptor antagonist (IL-1RA); IL-6; IL-8; IL-10; IL-11; tumour necrosis factor-α (TNF-α); granulocyte colony-stimulating factor (G-CSF); E-selectin; P-selectin; intercellular adhesion molecule-1 (ICAM-1); vascular cell adhesion molecule-1 (VCAM-1); monocyte chemotactic protein-1 (MCP-1); active plasminogen activator inhibitor-1 (aPAI-1); total plasminogen activator inhibitor-1 (tPAI-1); fibrinogen; complement factors C4d, C3bc, and Bb; and the terminal complement complex of C5b-9 (TCC).

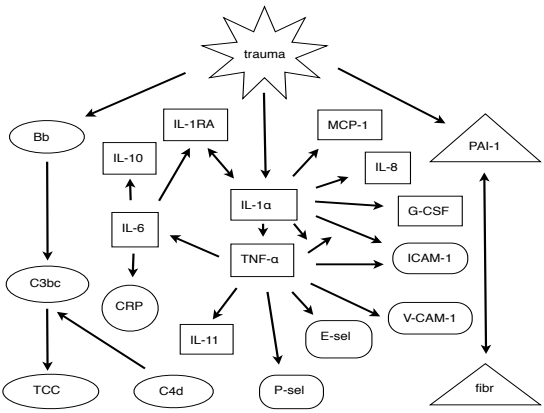


Figure 8. *The inflammatory mediators that were analysed in this thesis.*

Cytokines are small proteins released from leukocytes in response to trauma or infection. The activation of the first cytokine triggers a cascade that directs the immune function to the damaged or infected part of the body. The cytokine cascade is initiated by the activation of IL-1 after trauma. IL-1 activates TNF-α and TNF-α stimulates the production of IL-6, which triggers the liver to produce the acute phase reactant CRP. The production of IL-8 is triggered by IL-1 and the production of IL-10 is triggered by IL-6. IL-1, IL-6 and IL-8 are pro-inflammatory cytokines. Their basic function is to increase the inflammatory response. IL-10 is an anti-inflammatory cytokine, and its basic function is to inhibit the inflammatory response. In addition to their basic functions, many cytokines have dual functions depending on the surrounding conditions.

C-reactive protein (CRP) is a plasma protein produced by the liver when triggered by IL-6. It plays a role in the clearance of bacteria and damaged cells[36]. CRP is often used in everyday healthcare to assess the level of tissue damage caused by trauma or infection.

The adhesion molecules (e.g. E-selectin; P-selectin; ICAM-1and VCAM-1) play a role in collecting leukocytes from the blood, attaching them to the walls of vascular capillaries and facilitating their passage through the capillary walls. E-selectin and p-selectin stimulate the selection of leukocytes from the circulation to the endothelium and platelets. ICAM-1 and VCAM-1 continue the process by stimulating adherence, rolling and transmigration through the vessel wall to the damaged tissue.

Fibrinogen and plasminogen activator inhibitor type 1 (PAI-1) reflect the activities of the coagulation-fibrinolysis systems. These cascades are activated by each other to accomplish a balance of coagulation and

fibrinolysis. Furthermore, they are activated by an inflammatory response, e.g., via complement. Fibrinogen is the precursor to fibrin, the end-product of the coagulation cascade and the building material for the blood clots. It circulates in the blood where it is activated by the coagulation cascade in response to trauma[37]. Plasminogen activator inhibitor type 1 (PAI-1) is a glycoprotein produced mainly by the endothelium of blood vessels. It plays a crucial role in the regulation of fibrinolysis and has also been implicated in other biological processes including inflammation and wound healing. PAI-1 is the main physiological inhibitor of urokinase-type and tissue-type plasminogen activators. Elevated plasma levels of PAI-l are correlated with a higher risk of deep venous thrombosis[38]. PAI-1 is relatively unstable. Under physiological conditions, human PAI-1 has a half-life of 2 h and spontaneously converts to an inactive, "latent" form[39]. For this reason, both active and total PAI-1 are analysed.

Complement is part of the innate immune system. The complement system consists of more than 30 proteins circulating in the blood, ready to be activated by bacteria or trauma. Activation of complement leads to opsonisation and lysis of foreign or damaged cells and this process works together with the rest of the inflammatory cascade for removing unwanted cells[40].

There are three known main pathways of complement activation; the classical pathway, the lectin pathway and the alternative pathway. C4 represents the classical pathway, and Bb represents the alternative pathway. Both pathways join together in C3. The end-product is TCC. Trauma and surgery activate complement via the alternative pathway[41].

The clinical significance of the inflammatory response

The inflammatory response measured by inflammatory mediators in the blood is one way to measure the impact of surgical trauma on the body. Other parameters related to the inflammatory response are body temperature, heart rate and respiratory rate. The inflammatory response could be related to short-term clinical outcomes such as blood loss, duration of surgery, complications, deaths and length of stay.

In the literature on inflammatory response after trauma or surgery, some of the most commonly used markers of systemic inflammatory response have been IL-6, IL-10, IL-8, IL-5, IL-4, IL-2, IL-1, TNF-α, human leukocyte antigen (HLA-DR), interferon-gamma, granulocyte/monocyte colony stimulating factor and CRP. Earlier studies of trauma and surgery have shown consistently elevated levels of IL-6, IL-10 and CRP, consistently lower expression of HLA-DR, conflicting data on IL-8 and consistently no

change for the rest[42].

Earlier studies have shown lower systemic levels of CRP and IL-6 for laparoscopic procedures compared with open cholecystectomy, gynaecological procedures, and colorectal cancer surgery[43-48]. In several studies, laparoscopy has been associated with faster recovery and a shorter hospital stay compared with the corresponding open procedure[49].

For rectal cancer surgery, short-term benefits of the laparoscopic technique such as shorter time to feeding, first bowel movement and hospital discharge, as well as less morphine use and better functional score at 3 months have been demonstrated[49, 50]. However, comparisons of inflammatory responses after laparoscopic versus open rectal cancer surgery have produced conflicting results. Some studies showed a less pronounced inflammatory response after laparoscopic compared with open procedures while at least one study failed to show any difference between laparoscopic and open procedures in this respect[51-54].

TEM has resulted in shorter duration of surgery, less blood loss and shorter hospital stay compared with resection surgery for rectal tumours[55-58]. One experimental study on the levels of inflammatory mediators after TEM in comparison with a natural orifice procedure through the umbilicus has been published[59], but no clinical studies on inflammatory mediators after TEM were found.

1.9 Ethics

Clinical research mandates approval from patients when it may influence their treatment or follow-up. If a study is conducted using patient records or registry data, the approval of the local ethics committee is enough. The studies in this thesis were performed according to the principles stated in the Declaration of Helsinki and approved by the Regional Ethical Review Board of Gothenburg, Sahlgrenska University Hospital, Sweden. Written informed consent was obtained from all patients in the studies reported in papers I-IV.

1.10 Concluding background

In the management of rectal cancer the competing issues of oncological and functional outcomes have to be addressed. With the oncological outcome in focus, efforts are directed at preventing local and distant spread and recurrence, while the issue of functional outcome focuses on limiting the side-effects of the surgical trauma and the radio- and chemotherapy.

Open total mesorectal excision is oncologically safe but typically results in 400 ml blood loss, 9 days hospital length of stay, 37% morbidity and 2% 30-day mortality[50]. The abdominal incision in open surgery may lead to wound dehiscence, incisional hernias and bowel obstruction due to adhesions[60]. Furthermore, bowel, sexual and urinary disorders are side-effects of rectal resection[61-64]. Temporary or permanent stomas are frequently needed.

Laparoscopic total mesorectal excision seems to result in lower incidences of small-bowel obstruction[65, 66] and incisional hernia[67] as well as improved short-term outcome with equivalent long-term oncological outcome compared with the open technique[49, 50, 68].

In analogy with the development of organ-sparing surgery for breast cancer, a similar concept is being developed for rectal cancer. Local excision has traditionally been reserved for benign tumours and early small cancers in the lower one-third of the rectum, but with TEM the indication for local excision has expanded to the full length of the rectum and new limits of indications in terms of tumour stage are delineated with and without additional treatment.

One possible benefit of minimally invasive surgery for rectal tumours may be a less pronounced inflammatory response reflecting a limited surgical trauma. A limited inflammatory response could coincide with or result in a faster short-term recovery. Studies with this purpose on laparoscopy for rectal tumours have been conflicting and there are no clinical studies on TEM as far as we are aware. The selection of the optimal treatment requires accurate preoperative assessment of the tumour. There is a need to clarify the place for MRI or ERUS or a combination of the methods in the preoperative assessment of rectal tumours. Finally, the outcome of transanal endoscopic microsurgery for rectal cancer has been analysed in individual hospitals, but population-based analyses are scarce and needed to validate studies from individual centres in an entire population.

2 AIM

The aims of this thesis were to analyse the following:

- the inflammatory response of transanal endoscopic microsurgery and laparoscopic rectal resection compared with open rectal resection for rectal tumours.

- preoperative assessment of stage and size of neoadjuvantly treated rectal cancer by magnetic resonance imaging (MRI) and endorectal ultrasound (ERUS) compared with postoperative histopathology of the surgical specimen.

- the Western Sweden Health Region population-based outcome of transanal endoscopic microsurgery and salvage rectal resection for rectal cancer.

3 PATIENTS AND METHODS

3.1 Papers I-III

Patients

In papers I-II, patients scheduled for laparoscopic and open rectal cancer surgery were included prospectively. The patients were already included in a large randomised study of the outcomes of laparoscopic and open rectal cancer surgery (COLOR II)[50], which was simultaneously being conducted. From the COLOR II trial, 12 patients from each arm of randomisation were included in this study. The randomisation of the COLOR II study was 2 to 1, with 2 laparoscopic procedures for each open procedure, whereas the proportion in this study was 1 to 1. Therefore, this study was not randomised in the strict sense as for the COLOR II trial. Paper III describes a prospective single-centre study. Patients scheduled for TEM because of an adenoma or early rectal cancer were prospectively included in the study, and compared with the patients in papers I and II.

Methods

Surgery was performed as described in the introduction. For analysis of inflammatory mediators, blood samples were centrifuged, frozen and kept in a freezer outside the operation theatre complex. The entire process was performed within half an hour to limit possible break-down of inflammatory mediators.

C-reactive protein (CRP) and white blood cell count (WBC) were analysed in the hospital laboratory. The following were analysed with a multiplex sandwich enzyme-linked immunosorbent assay (ELISA) in a plate with multiple wells: interleukin 1-α (IL-1-α); IL-1 receptor antagonist (IL-1RA); IL-6; IL-8; IL-10; IL-11; tumour necrosis factor-α (TNF-α); granulocyte colony-stimulating factor (G-CSF); E-selectin; P-selectin; intercellular adhesion molecule-1 (ICAM-1); vascular cell adhesion molecule-1 (VCAM-1); monocyte chemotactic protein-1 (MCP-1); active plasminogen activator inhibitor-1 (aPAI-1); total plasminogen activator inhibitor-1 (tPAI-1) and fibrinogen were obtained with a multiplex sandwich enzyme-linked immunosorbent assay (ELISA) in a plate with multiple wells. The plates were washed six times before adding the detection antibodies. After incubating with detection antibodies for 30 min, the plates were washed three times and incubated for 30 min with streptavidin-horseradish peroxidase. The plates were again washed before adding luminescent substrate. The plates were immediately imaged and the results were calculated using a software system.

Complement factors Bb and C4d were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits. Complement factors C3bc, and the terminal complement complex of C5b-9 (TCC) were analysed using a laboratory ELISA method invented by Mollnes and colleagues at Rikshospitalet in Oslo. The blood samples were kept in EDTA tubes, and refrigerated within half an hour after centrifugation, which was performed minutes after drawing the blood from the patient. The tubes were sent to the laboratory for analyses using ELISA. Blood plasma from each patient was poured into a small well in a plastic tray. The surface of the wells in the tray was coated with an antibody. Each kit carried one antibody for one factor. The factor contained in each blood sample will attached to the antibody coating at a concentration that was proportional to the concentration in the plasma. The plate was then washed. A solution of antibodies attached to detectable dye was poured into the wells in the tray. The concentration of dye was measured using a machine that transmits light through the solution and registers the concentration of dye in the solution (photospectrometry).

Methodological and statistical considerations

The rational for choosing the mediators were as follows. To compare with earlier studies, the following most commonly investigated mediators were included: CRP, IL-1, IL-6, IL-8, IL-10 and TNF-α. These represent central pathways of the cytokine cascade. IL-11, MCP-1 and G-CSF are also part of the cytokine cascade but have not been as frequently explored. They were included for the purpose of exploring lesser known mediators in addition to the well-known ones and because they represent other parts of the cascade. The adhesion molecules (E-selectin; P-selectin; ICAM-1 and VCAM-1) were selected to reflect changes in the process of recruiting leukocytes from the blood to the damaged tissue. Fibrinogen and PAI were selected to reflect the activities of the coagulation-fibrinolysis systems. The rational for selecting Bb and C4d was that they are relatively stable and represent the alternative and classical pathways, respectively. C3 represents the common pathway but is unstable. However, the C3bc fraction is relatively stable and was selected for analysis. TCC represents the end-product; it is stable, and its measurements are more reliable than the other components.

Comparison between the surgical groups for the time course of each mediator could be analysed using overall mean, the area under the curve or repeated-measures ANOVA. In this study a linear mixed model was chosen. The linear mixed model is a regression model that limits the possible influence of mass significance due to multiple results. This model allows condensation of the results from all the time points into one comparison between surgical types.

3.2 Paper IV

Patients

This study was linked to a study of neoadjuvant chemotherapy in rectal cancer[69]. The study included 37 patients who underwent MRI and ERUS before and after chemotherapy. Patients with stages I-III disease were included, except early T1-tumours that were amenable to local excision and advanced tumours in which the surgical margin required chemoradiotherapy. The early T1-tumours would be assessed with ERUS and the advanced tumours with MRI. The indications for MRI and ERUS overlapped in the study group, and the appropriateness of each method was not as obvious as in the early and the advanced tumours.

Methods

All surgically removed specimens were routinely sent for histopathological examination, which also provided a comparison between MRI, ERUS and the fixed specimen in terms of both stage and size. The supero-inferior length was compared between MRI, ERUS and the fixed specimen, which was the gold standard. The maximal transaxial area in a dimension perpendicular to the long axis of the rectum at the site of the cancer was compared between MRI and ERUS. The MRI machine was a Philips Intera 1.5 Tesla and was used together with a cardiac 5-channel synergy surface coil. The ERUS machine was a B-K Medical Falcon 2101 EXL with a 3D-Probe.

Stage and size

Stage and size were assessed using MRI and ERUS before and after preoperative chemotherapy.

Histopathology

The histopathological assessments of the resected surgical specimens were performed after fixation. The specimens were immersed in 10% formaldehyde for 48-72 h. The specimens were then sliced and again immersed in formaldehyde for another 12 h before dehydration overnight. Then, the specimens were embedded in paraffin and sectioned. Routine staining was performed. The slides were analysed by specialist pathologists according to the routine protocol utilised in the pathology department. The diameter in three dimensions and the TN staging were recorded.

Methodological considerations

The shape of the tumour could change with compression by the ultrasound probe, whereas no internal probe was used for MRI. This could influence the calculations of tumour size. No attempt was made to compensate for this possible difference between the methods.

3.3 Paper V

Patients

Patients were identified via the Scandinavian TEM Registry (SCTR) and the Swedish Colorectal Cancer Registry (SCRCR). SCRCR, launched in 1995, is a well-validated prospective database with a coverage exceeding 98% including data on all patients in Sweden who have been diagnosed with rectal cancer[23, 70-72]. SCTR is a web-based registry of TEM procedures connected to the Swedish national cancer database INCA. The TEM registry was started in 2009 and was aimed at prospectively including all TEM procedures from 2009 onwards and retrospectively including all previous TEM procedures. The SCTR was validated by checking surgical files, hospital data bases and the SCRCR.

Patients who had undergone TEM for rectal cancer in the Western Sweden Health Care Region (Region Västra Götaland and Northern Region Halland) were identified through the registries. The region comprises 1.75 million inhabitants, and during the study period TEM procedures were performed at two of the region's hospitals: the Sahlgrenska University Hospital/Östra and the Northern Älvsborg County Hospital.

The data files from the SCRCR allowed T-classification only from 2007 and TNM-stages I-IV before 2007. Stage I was the most detailed classification available for the entire period.

After TEM for rectal cancer, patients were followed with palpation and straight proctoscopy 3 and 12 months postoperatively and then yearly up to five years postoperatively. In the study, patients were assumed to be free of recurrence when no signs of reappearance of the tumour were found in medical records of the particular hospital or in the registry reports. In this respect, the study conformed to the methodology of the SCRCR. In addition, most patients were followed with clinical examination and/or radiology. Causes of death were confirmed by claimed reports from the Swedish National Cause of Death Registry.

Methods

Tumours of the rectum were staged by histopathology of the specimen according to tumour node metastasis (TNM) stages I-IV. Stage I included pathological tumour (pT) category 1-2, which were pathological node negative (N0). Due to the lack of lymph nodes in the TEM specimens, pN could not be assessed (pNX). Preoperative investigations represented ERUS-

staged tumours as uT0-4N0-2 and MRI-staged tumours as mT0-4N0-2. Postoperative staging by histopathology was the gold standard (pTNM).

Adverse histopathological features were defined as high tumour grade (previously poor or low differentiation), tumour budding and lymphovascular invasion.

Low-risk tumours were defined as tumour stage T1 with submucosal invasion Sm1-2, <3 cm, without adverse features, and these were separately analysed for outcome.

In the SCTR, patients are assigned to one of four preoperative indications for TEM; benign, curative for cancer, excisional biopsy or compromise. In the benign indication, the tumour was preoperatively assessed as a benign lesion. Curative for cancer was used when the lesion was known to be a cancer preoperatively and the surgeon expected TEM to be the definite curative treatment. The term excisional biopsy was used for lesions in which the preoperative assessment could not provide enough information to decide whether TEM or resection was required. The TEM procedure for excisional biopsy was performed in the same manner as for the other indications. In this way, TEM for an excisional biopsy could be accepted as a definite curative procedure for low-risk cancers. The compromise indication implied that TEM was an oncological compromise that was accepted because of comorbidity, old age or patient refusal to undergo primary TME surgery. The SCTR mandates a postoperative decision be reported after obtaining the result of the histopathological analysis of the TEM specimen: curative, further treatment or accept-compromise.

Salvage total mesorectal excision (sTME) was recommended for low-risk patients with unfavourable histopathology or involved margins (high-risk tumours). Re-TEM was an option for high-risk patients. Early sTME was defined as surgery within three months, and late was defined as sTME more than three months after primary TEM. TME for local recurrence (rTME) was performed after the detection of locally recurrent disease. TME was performed as abdominoperineal resection (APR), anterior resection (AR) or Hartmann's procedure (HP).

Methodological considerations

The study was a retrospective analysis of prospectively collected data from databases. The data had to be validated and checked against patient records and surgery files. Therefore, the study was essentially retrospective in nature. A prospective study would be preferable, because of more structured and

controllable information than in a retrospective study. However, the same study period would not be ready for analysis for another 12 years.

The fact that all patients in the entire health care region are included diminishes selection bias, which is possible when only patients from one centre are included. Selection bias means that the results can only be applied to those patients who are selected to be treated in the centre of interest. This study, however, involved a real patient population, which means that the results are valid for all patients in the region, and not just for those selected from one centre. However, there is an expected selection bias because the patients have been scheduled for TEM as opposed to resection surgery. Adjusting for this possible bias would require randomisation, which has not been performed in this study. Therefore, the conclusions have to be considered with this in mind. It is not clear whether there are any differences in the conditioins under which the treatments were administered to patients.

With this limitation in mind, it was of interest to relate the outcome of TEM and salvage resection to primary total mesorectal excision for rectal cancer in the same region or the entire country during the same period. Patients from the entire country with adjusted tumour stage and distance from the anal verge were chosen to represent the outcome of primary total mesorectal excision.

4 RESULTS

4.1 Papers I-III

Results
The median changes from baseline levels of IL-6 and CRP were higher in open resection than in laparoscopy and TEM. IL-6 change from baseline peaked at 102.58 (29.59-169.47) pg/ml in TEM, 152.32 (76.72-249.68) in laparoscopic resection and 274.56 (156.79-388.92) pg/ml in open resection. CRP change from baseline peaked at 39.00 (6.75-60.00) mg/l in TEM, 52.00 (44.75-64.00) mg/ml in laparoscopic resection and 91.50 (56.75-122.25) mg/ml in open resection. The differences were significant when analysed using linear mixed models. Further data are provided in the manuscripts. In papers I-II the results of the levels of the inflammatory markers were presented in absolute values, and in paper III they were presented as changes from the baseline levels. The demographics were comparable between the groups. Tumour stage, radiotherapy and chemotherapy were different between TEM and resection groups. The median blood loss was 10 ml in TEM, 200 ml in laparoscopic surgery, and 1150 ml in open surgery.

Comments on the results
Blood loss may be expected to be higher in open surgery than in less invasive procedures; however, the blood loss in the open group was unusually high in our study. This unexpected finding may be attributable to the difference in IR. In 153 possible inclusions who underwent open or laparoscopic surgery at the same department during the same time period (35 laparoscopic, 103 open, and 15 converted), the median blood loss was 200 ml via laparoscopy and 750 ml via open surgery. Blood loss in open/laparoscopic surgery was 400/200 ml in the COLOR II trial[50] and 328/127 ml in a study performed by Veenhof et al[54].

The elevations of IL-6 and CRP were lower for laparoscopy than for open surgery, but this could be explained by a lower proportion of APR and expected blood loss compared to the open group. However, IL-6 remained significantly lower for laparoscopy also after correction for expected blood loss and decreasing the proportion of APR. This shows that the difference is explained by the type of surgery. Furthermore, different types of surgery resulted in different volumes of blood loss which is consistent with other studies. The difference in blood loss could partly explain the difference in the inflammatory response.

Among other weaknesses of this study were its limited number of patients and the large number of assessed factors. With the numerous results obtained, the question of the influence of mass significance arises, and it could be argued that the any differences are due to chance.

TEM caused a less pronounced inflammatory response based on IL-6 and CRP between TEM vs and based on TCC between TEM and laparoscopy with correction for mass significance using Bonferroni's method.

In addition , without Bonferroni's correction there were differences with similar patterns between TEM and open resection based on the levels of IL-8, G-CSF, aPAI-1, tPAI-1 and C3bc and between TEM and laparoscopic surgery based on the levels of IL-8 and C4d. The results are affected by reverse relationships between TEM and open surgery for E-selectin, P-selectin and ICAM-1, and between TEM and laparoscopic surgery for E-selectin. It appears that the adhesion molecules behave differently.

Seekamp et al found that a moderate surgical trauma activates cytokines but not adhesion molecules[73], which could be a possible explanation for the opposite results for the adhesion molecules in this study.

Because of the different indications for TEM and resection surgery in the present study, it was not possible to include a resection control group with matching tumour stages within the proposed study period. The indication for standard resection was stages T1–3, whereas stages T0-1 were indications for TEM. The indications overlapped only in patients with small T1 tumours.

Weaknesses with comparisons of the inflammatory response and short-term outcomes between resection surgery and TEM are the different indications and nature of the procedures. The ideal study would be a comparison between resection surgery and TEM for patients with tumour eligible for both procedures, which are low-risk early stage small cancers. Such a study would be better if it could be randomised.

However, open or laparoscopic resection was performed in the same standard fashion for tumours with stages T0-3 confined within the mesorectal surgical margins. In general, laparoscopic or open resection for an adenoma of the rectum would not be less traumatic than such surgery for a cancer confined within the mesorectum. The standard dissection procedure would be the same. Transanal endoscopic microsurgery was performed in the same standard fashion for tumours with stages T0-1 tumours. However, if the surgeon was aware that the lesion was a cancer, an excision aimed at larger

margins could be expected. Thus, comparisons between groups of patients with different tumour stages could influence the results, but overall, the procedures were standardised regardless of tumour stages within T0-3.

The results showed statistically significant differences between the surgery types for some mediators but we cannot assume that the lack of differences for other mediators was due to a lack of power although it is one possible reason. Other reasons include the accuracy/variability of the measurements.

It is reasonable to interpret the results as not just the product of random variation. IL-6, IL-8 and IL-10 and maybe even TNF-alpha all showed a similar pattern with open surgery values increasing more than laparoscopic values at 3 and 6 hours before both decreased. If there was no difference between the techniques we might not expect this consistent pattern.

In conclusion, the significant differences after adjustment for mass significance highlight a less pronounced inflammatory response after TEM compared with open and laparoscopic surgery, respectively. For the typical markers of inflammatory response, IL-6 and CRP, there was a significant difference between TEM and open resection and between laparoscopic resection and open resection, but not between TEM and laparoscopic resection. Furthermore, significant differences before adjustment for mass significance highlight a less pronounced inflammatory response based on the more well-known inflammatory markers with consistent patterns of reaction, while the opposite results for the adhesion molecules and the small sample motivate some caution in the interpretation of the results.

4.2 Paper IV

Feasibility
The staging feasibility was 37 of 37 for MRI and 29 of 36 for ERUS. The feasibility of the size assessment was 36 of 37 for MRI and 25 of 34 for ERUS. The inability to lie still for approximately 40 minutes was the reason for MRI noncompliance, and stenosis or pain on examination was the reason for ERUS noncompliance. In a number of patients, air pockets within the bowel lumen and remnants of bowel contents compromised the quality of the ERUS but not of the MRI.

Stage
The staging accuracy of the perirectal tissue penetration after chemotherapy using MRI and ERUS was 0.52 and 0.78, respectively. The staging accuracy of lymph node metastases after chemotherapy using MRI and ERUS was

0.74 for both methods. The accuracy of the TNM staging after chemotherapy, with stage II as the cut-off, using MRI, ERUS and the combined MRI and ERUS examinations was 0.65, 0.78 and 0.83, respectively. The post-chemotherapy staging by MRI alone was improved by a combination of MRI assessment of the lymph nodes and ERUS assessment of the perirectal tissue penetration (P=0.046, Wilcoxon Signed Rank Test).

Size
MRI had several advantages for use in the measurement of tumour size. The probe used for ERUS provided a view that measures a maximum of six cm in supero-inferior length. Tumours larger than this could not be reliably measured with ERUS because of the necessity for multiple recordings. Thus, MRI was more consistent with histopathology in the tumours with a supero-inferior length greater than five cm. For tumours smaller than five cm, measurements of the supero-inferior length using ERUS compared with MRI were within 1.96 standard deviations of the difference between the methods (18 mm). Consistency with the histopathology of the resected specimen after fixation was within 1.96 standard deviations of the difference between imaging and pathology for MRI (15 mm), and consistency with the histopathology of the resected specimen after fixation was within 1.96 standard deviations of the difference between imaging and pathology for ERUS (22 mm) for tumours that did not exceed five cm.

Comments on the results
In neoadjuvantly treated rectal cancer, assessment using magnetic resonance imaging (MRI) was more feasible than endorectal ultrasound (ERUS) in large and stenotic tumours. However, staging accuracy using MRI alone was improved from 0.64 to 0.83 using a combination of lymph node assessment with MRI and assessment of perirectal tissue penetration with ERUS, with stage II as the cut-off. A combined examination may especially be considered in tumours at the cut-off between stage I and II for more accurate use of preoperative radiotherapy and for selection of local excision with TEM. To measure tumour size, MRI was better suited in large and stenotic tumours, while ERUS was a sufficient alternative in small tumours (<5 cm) that were passable by proctoscopy. The study showed the results of assessments of neoadjuvantly treated stage II-III tumours and the sample was limited. Other studies are needed to confirm the results in larger samples of patients with all stages of tumours with and without neoadjuvant chemo- or radiotherapy.

4.3 Paper V

Validation of the TEM registry

The registry was valid for one of the two involved hospitals in the Western Sweden Health Region. However, no patients were registered from the other hospital. Data were complemented by visiting the hospital and searching the surgical files in combination with the Swedish Colorectal Cancer Registry (SCRCR). The registry data were validated by examination of operation logs, local registries and chart reviews.

Results
With at least three years' follow-up, TEM only for low-risk tumours resulted in no local recurrences in 16 patients, and TEM only for 25 T1-tumours smaller than 3 cm resulted in one local recurrence. In the Western Region, salvage resection for stage I and for stages I-III resulted in perforations of the resection specimens in 4 of 16 and 9 of 28 patients, respectively. In Sweden, primary resection for tumours positioned 2-12 cm from the anal verge with stage I and and stages I-III resulted in 87 specimen perforations out of 2490 (3.5%) and 599 of 11018 (5.4%) of the patients, respectively. Sphincter-saving procedures were performed in 3 of 16 salvage resections for stage I-tumour; in 3 of 13 tumours measuring at least 6 cm from the anal verge. Preoperative assessment was benign in one-third of the patients with rectal cancer based on the TEM specimens. Further data are provided in the manuscript.

Comments on the results
Good results have been published for TEM performed individual centres for low-risk tumours. This population-based study confirms that the results are valid for an entire population. There was a high perforation rate in salvage resection procedures. Although the sample is small and direct comparison to the national results could be biased, perforation rates of one-quarter to one-third of the specimens seem to be in another category related to the 3-5% perforations in primary resections. The explanation could be the fibrotic scar around the wound after the TEM procedure making exact dissection more difficult in addition to a fragile bowel wall in the wound area. Salvage resection after TEM could therefore be considered advanced rectal cancer surgery.

5 DISCUSSION

One possible benefit of minimally invasive surgery for rectal tumours may be a less pronounced inflammatory response than in open resection. In this study the inflammatory responses of transanal endoscopic microsurgery and laparoscopic rectal resection were less pronounced compared with open resection for rectal tumours. However, the study could be criticised for underpower making the results hypothesis generating rather than hypothesis testing. A larger study needs to be performed to confirm the results.

One important issue is whether the differences in inflammatory response based on inflammatory mediators affect the clinical outcomes. The response exhibited a large interindividual variation, which cannot be detected before surgery. Additionally, it is difficult to determine the critical level of inflammatory response for an individual patient. The ideal situation would be a molecular marker the preoperatively provides information to enable a tailored limitation of the total surgical trauma related to the indication for surgery. Secondary measurements such as length of hospital stay are difficult as they are susceptible to issues such as culture; in addition, the expectations of the patients and the surgeons may also contribute to some of the clinical effects, such as mobilisation and hospital stay. Johansson et al showed that patients who were blinded regarding whether they underwent open or laparoscopic cholecystectomy exhibited similar short-term outcomes in this respect[74].

No patients in these studies suffered from any serious inflammatory response syndrome such as multi-organ dysfunction syndrome (MODS). Other studies have shown differences in the inflammatory response based on the levels of inflammatory mediators in the blood within the first 1-24 hours after surgery or trauma with corresponding prognostic significance concerning death, time in ICU etc. In this light, a less pronounced inflammatory response as a result of minimally invasive surgery may be one factor that influences the final outcome in patients with complications that initiate a second or third inflammatory response. Much research is aimed at reducing the inflammatory response after substantial surgeries such as heart surgery or transplant surgery. In these cases, a pronounced inflammatory response is considered detrimental with clinical implications.

It could be assumed that an initial high peak of IL-6 or CRP after surgery for rectal tumours does not affect the final outcome in most cases. However,

patients with high levels could be assumed to be in a worse position than patients with low peaks of inflammatory mediators, if they should protract a second or third inflammatory response through complications that mediate dangerously high levels of inflammatory mediators along with signs of clinical deterioration. Furthermore, adding the burden of adjuvant chemotherapy required by some patients, a low inflammatory response is assumed to be advantageous for enabling an early start of and tolerance to the long period of adjuvant chemotherapy after surgery. Therefore, every measure should be taken to decrease the inflammatory response in association with surgery.

Other studies confirm a lesser pronounced inflammatory response, an attenuated immune depression and improved short-term outcomes[49, 50, 68] for laparoscopic resection compared with open resection for rectal tumours[49, 50, 52-54]. Furthermore, some studies have showed improved short-term outcomes after TEM compared with open[55, 56] or laparoscopic[75] resection for rectal tumours.

With this in mind, patients with rectal tumours should be considered for TEM if the selection criteria are met and for laparoscopic resection for other tumours except T4 tumours that have invaded into other organs where open resection remains unchallenged at large, although good results have been published also for T4-tumours undergoing laparoscopic resection in highly specialist centres[76, 77].

In neoadjuvantly treated rectal cancer, assessment using magnetic resonance imaging (MRI) was more feasible than endorectal ultrasound (ERUS) in large and stenotic tumours. However, staging accuracy using MRI alone was improved from 0.64 to 0.83 using a combination of lymph node assessment with MRI and assessment of perirectal tissue penetration with ERUS, with stage II as the cut-off. A combined examination may especially be considered in tumours at the cut-off between stage I and II for more accurate use of preoperative radiotherapy and for selection of local excision with TEM. The study showed the results of assessments of neoadjuvantly treated stage II-III tumours and the sample was limited. Other studies are needed to confirm the results in larger samples of patients with all stages of tumours with and without neoadjuvant chemo- or radiotherapy.

The population-based outcome of transanal endoscopic microsurgery for rectal cancer was excellent for low-risk tumours. Furthermore, TEM was useful as a compromise treatment in high-risk patients in whom the oncological risk was balanced against the risk of undergoing substantial

surgery or non-cancer-related death. Preoperative assessment was benign in one-third of the patients with rectal cancer based on the TEM specimens. This problem has been addressed by other authors. For example, Morino recently reported understaging in one-fifth of the patients undergoing preoperative endorectal ultrasound[78], and in a populatin-based study of TEM for rectal cancer Bach found that 44% of the T1-tumours were preoperatively thought to be benign[26]. Clearly, the preoperative assessment needs to be improved in order to limit the ratio of two-stage procedures.

Further surgery with salvage resection was performed in one-third of the patients who underwent TEM for rectal cancer with a non-compromise indication. Salvage resection yielded a high ratio of microscopically radical specimens, but the quality of surgery was negatively affected by perforations of the surgical specimens in one-fourth of the patients. Specimen perforation is one of the main risk factors for local recurrence and should be avoided. Even if no increase in local recurrence was observed in our study, measures should be taken to limit specimen perforation in salvage resection after TEM. The surgeon should consider this procedure a case of advanced rectal cancer. Care should be taken to dissect outside the fibrotic TEM scar. Undue traction on the specimen should also be avoided. Finally, the preoperative investigation needs to be more accurate to limit TEM followed by salvage resection surgery in high-risk tumours.

6 CONCLUSION

- All types of surgery caused an inflammatory response.

- The inflammatory response caused by laparoscopic resection in this study was less pronounced than that caused by open resection for rectal tumours.

- The inflammatory response caused by transanal endoscopic microsurgery in this study was less pronounced than that caused by open and laparoscopic resection for rectal tumours.

- For selection of the type of surgery and preoperative radiotherapy, preoperative assessment using magnetic resonance imaging was more feasible than endorectal ultrasound in the assessment of neoadjuvantly treated T2-3 rectal cancer.

- The staging accuracy of magnetic resonance imaging alone for neoadjuvantly treated T2-3 rectal cancer was improved by a combination of nodal assessment using magnetic resonance imaging with tumour assessment using endorectal ultrasound.

- Measurement of the size of tumours with a diameter larger than five cm was more accurate using magnetic resonance imaging than endorectal ultrasound, although the methods were comparable for small tumours.

- The population-based oncological outcome of TEM for low-risk rectal cancer was excellent and it was acceptable for all T1-tumours smaller than 3 cm.

- In patients with rectal cancer in the specimen after transanal endoscopic microsurgery one-third of the patients with a non-compromise indication underwent further surgery with salvage resection.

- Salvage resection after TEM implied a challenging dissection with a high risk of specimen perforation, which is a risk factors for local recurrence.

- The local recurrence rate after salvage resection was low.

- The rate of sphincter-saving procedures in salvage resection was low.

7 FUTURE PERSPECTIVES

Scientific perspective

Papers I-III showed a less pronounced inflammatory response in minimally invasive surgery compared with open resection, but the sample was small. The result needs to be confirmed in larger studies. Furthermore, larger studies would be needed to clarify if differences in inflammatory response correspond to any differences in short-term and/or long-term outcome.

Paper IV showed an improvement in the accuracy of preoperative assessment of rectal tumours by combining results of assessment of lymph node involvement using MRI with results of bowel wall penetration using ERUS. This result should be confirmed in other larger studies. Furthermore, other possible ways to increase accuracy in preoperative assessment should be investigated.

Paper V showed excellent outcome after TEM for low-risk tumours but a high rate of perforations of the specimen in salvage resection. These results should be confirmed in larger population-based studies involving the whole of Sweden or Scandinavia.

Clinical perspective

One central issue in preoperative staging is the possible presence of tumour-affected lymph nodes. This issue is important in standard resection surgery because the risk of node involvement affects the use of short-course preoperative radiotherapy before surgery. Preoperative staging of lymph node involvement is certainly central when considering local resections and could be of great value when assessing patients who could benefit from a neoadjuvant chemotherapy regimen. As previously reported, MRI tends to be superior for the assessment of the lymph nodes, whereas ERUS tends to be superior for the assessment of the penetration of the bowel wall and perirectal tissue, especially in the early stages; combining the strengths of each modality in the same patient could be a way to increase accuracy of preoperative assessment. Furthermore, there have been some promising developments in MRI using superparamagnetic iron oxide particles to improve the assessment of lymph node stage. Other possible ways of increasing the diagnostic accuracy could be Narrow Band Imaging (NBI), sono-elastography and genetic markers. ERUS-guided needle biopsy of uncertain lymph nodes and tumour full-thickness biopsies may add

information to the assessment. A method for sentinel node biopsy may be established to remove the first lymph nodes that recieve cells from the tumour.

One drawback with TEM is the lack of histopathology of lymph nodes. However, with additional chemo- and/or radiotherapy, the indications for TEM could expand. In combination with radiochemotherapy, TEM could be used as a big biopsy of the marked tumour site to assess if there is any remaining viable tumour after treatment, and to remove any such remaining tumour. Furthermore, TEM could be used to remove visible tumour, as definite treatment in combination with radiotherapy or radiochemotherapy. A Dutch study on this theme has just closed[79], and a British study is just starting[80].

Transanal endoscopic microsurgery and laparoscopic resection in a combined procedure enables total mesorectal excision from below and from above simultaneously or entirely from below. This procedure is being explored [81]. Thus, the most critical aspect of the procedure from above may be performed more securely under direct microscopic visualisation from below. It could have the potential to improve the results of treatment for rectal cancer with anterior resection in men with narrow pelvises, and scarless surgery may be possible with oncological safety through resection from below alone.

Other possible developments could be laparoscopic resection for T4-tumours with invasion into other organs or limited peritoneal carcinosis. The techniques have been demonstrated in small series in highly specialised centres[76, 77]. Thus, the advantages of the laparoscopic technique could be expanded to benefit those patients who potentially have most to gain from a minimally invasive approach.

ACKNOWLEDGEMENT

Deo, parentibus et magistris non potest satis gratiae rependi, that is, To God, to parents, and to teachers we can never render sufficient gratitude and compensation. The large Catechism of Martin Luther, page 27

I wish to express my gratitude to all who have contributed to or supported the completion of this thesis. In particular I thank:

Göran Kurlberg, main supervisor who never gives up and always looks for new challenges and possibilities. Thank you for your endurance and high perspective.

Kristoffer Derwinger, assisting supervisor, for being organised, quick and supportive. Thank you.

Andreas Kvarnström, co-researcher, for sharing in the sufferings and the joys of this work.

Anders Bengtsson, co-supervisor, for always looking at the positive side of things.

Peter Kälebo, co-researcher, for sharing an interest in rectal tumour imaging.

Karl Kodeda, co-researcher for your inspiring commitment.

Hans Brevinge, clinical mentor, for your combined clinical and scientific curiosity.

Bengt Gustavsson, for joint venture in paper IV and scientific interest.

Elisabeth Lindholm, for helping me look for patient records in the region.

Svante Nordgren, former supervisor, for scientific motivation.

Peter Naredi, professor, for kind and positive support.

Anders Hyltander and Ulf Angerås, present and former heads of department, for being supportive of my research.

Lars Börjesson and Jonas Bengtsson, collegues, for encouragement.

My colleagues at the department of surgery for support and understanding in

times of despair and of inspiration.

Christina for your beautiful smile and love.

Mathias, Jonathan, Cornelia and Sakarias for being my super children.

Pelle och Gudrun for being my lovely parents, Gunnar, Lisa, Janne for being my dear brothers and sister, and Doris for being my dear mother-in-law.

This thesis was supported by the Anna-Lisa and Bror Björnsson Foundation, Göteborgs Läkaresällskap, the Lions Cancerfond Väst, and the Swedish Cancer Foundation.

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