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**ASPECTS ON LOCAL
RECURRENCE OF RECTAL
CANCER**

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**Karolinska
Institutet**

Stockholm 2009

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Published by Karolinska Institutet. Printed by [name of printer]

Layout Ringvor Hägglöf

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ISBN 978-91-7409-432-9

*To acquire knowledge, one must study;
but to acquire wisdom, one must observe.*

Marilyn vos Savant

ABSTRACT

Rectal cancer is a common malignant disease in Sweden which is diagnosed in about 1,800 patients annually. Local failure, defined as residual or recurrent disease within the pelvis, is of major concern in rectal cancer treatment. Preoperative radiotherapy and refinements in surgery have increased local control and survival during the past two decades. Since extensive surgery and radiation have documented side effects, a more individualized therapy aiming at local control and cure with a minimized risk of morbidity is sought.

The aims of this thesis were to define localization, analyze possible causes, and explore potential prognostic factors for local failure in rectal cancer, ultimately aiming at improved local control in rectal cancer treatment with minimized morbidity.

The basis for the study was a consecutive population-based cohort of abdominally operated rectal cancer patients identified from the registry of the Regional Oncologic Center in Stockholm and registers in Norrköping and Uppsala. The patients were operated upon in centers that had adopted modern principles in rectal cancer surgery, including total mesorectal excision (TME) and were treated with preoperative radiotherapy as described in regional care programs. In the studies, revision and analysis of registered data, analyses of images of the recurrent tumor and histological analysis of the invasive front of the primary tumors were performed.

It is concluded that lateral lymph node metastases are not a major cause of local failure.

Partial mesorectal excision may be associated with an increased risk of local recurrence of tumors in the upper rectum. Locally recurrent tumors are situated in the lower $\frac{3}{4}$ of the pelvis, suggesting that lowering of the upper limit of the radiation could be introduced. The anal sphincter complex with surrounding tissue can be excluded from the target volume in patients with primary tumors more than 5 cm from the anal verge.

It is also concluded that tumor-specific factors such as distal tumor location, advanced T and N stage, and treatment-specific factors such as omission of radiotherapy, residual disease, and treatment at a center with a lower caseload are independent risk factors for local failure.

Analyses of the invasive front of the primary tumor revealed that tumor budding determined by antibody clone MNF-116 might serve as an additional predictive marker for local recurrence.

Despite advances in rectal cancer management over the past decades, locally recurrent tumor growths develop in a number of patients. This thesis provides some insights with respect to localization, clinicopathological risk factors, and biological tumor markers. Since locally recurrent rectal cancer is a condition that is often extremely difficult to treat and is so devastating for the patient, further research in this field is warranted.

LIST OF PUBLICATIONS

This thesis is based on following papers, which will be referred to by their Roman numerals as indicated below:

- I **Radiological findings do not support lateral residual tumour as a major cause of local recurrence of rectal cancer.**
E. Syk, M. R. Torkzad, L. Blomqvist O. Ljungqvist and B. Glimelius
British Journal of Surgery 2006; 93: 113-119

- II **Local recurrence in rectal cancer: Anatomic localization and effect on radiation target**
E. Syk, M. R. Torkzad, L. Blomqvist , Per J Nilsson and B. Glimelius
Int. J Radiation Oncology Biol. Phys. 2008; 77:658-664

- III **Factors influencing local failure in rectal cancer: analysis of 2315 patients from a population-based cohort**
E. Syk, B. Glimelius and PJ. Nilsson
Submitted for publication

- IV **Tumour budding correlates with local recurrence of rectal cancer.**
E. Syk, C Lenander ,PJ. Nilsson, C Rubio, B Glimelius
In manuscript

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LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
APR	Abdominoperineal resection
AR	Anterior Resection
CEA	Carcinoembryonic antigen
CI	Confidence interval
CRM	Circumferential resection margin
CT	Computed tomography
CTV	Clinical target volume
EMVI	Extramural vascular invasion
FAP	Familial adenomatous polyposis
Gy	Gray
HAR	High anterior resection
H-E	Hematoxylin and Eosin
HR	Hazard ratio
LAR	Low anterior resection
LPLD	Lateral pelvic lymph node dissection
LR	Local recurrence
MDT	Multidisciplinary team
MRI	Magnetic resonance imaging
PME	Partial mesorectal excision
R	Residual tumor status
RT	Radiotherapy
RW	Rectal washout
SRCT	Swedish Rectal Cancer Trial
TEM	Transanal endoscopic microsurgery
TME	Total mesorectal excision
TNM	Tumor, Nodes, Metastasis
TRUS	Transrectal ultrasonography
TRG	Tumor regression grade
UICC	Union International Contre le Cancer

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INTRODUCTION

Colorectal cancer rank third in frequency of incidence and mortality among all cancers in the world. The incidence is high in North America, western Europe, Australia/New Zealand, and southern South America, and low in Africa and Asia.[1, 2] This large geographic difference probably corresponds to the effect of different environmental exposures where dietary differences have been suggested as the most important factor[2]. Red and processed meat has been associated with an increased risk for rectal cancer and a protective role of fish has been observed [3]. A high fibre intake has also been suggested to be a risk-reducing factor. A reduced fecal transit time with reduced exposure to carcinogens may be the explanatory mechanism for this observation. A certain percentage of colorectal cancers (3-5%) are inherited in an autosomal manner. The most well-known hereditary syndromes are familial adenomatous polyposis (FAP), and hereditary nonpolyposis colorectal cancer (HNPCC) [4]. The majority of colorectal cancer cases (around 80%) are sporadic and the rest (15%) are inherited in an unknown manner. First-degree family relatives have a twofold greater risk of developing bowel cancer compared to the general population [5]. The life-time risk for colorectal cancer in Sweden is 5%. [6] Nearly 5500 patients are diagnosed annually with an adenocarcinoma of the large bowel[7]. About one third of them are situated in the rectum[8], defined as the part of the bowel below the sacral promontory or within 15 cm of the anus. Cancer of the rectum is rare in younger age groups (<50 years). The median age at the diagnosis of rectal cancer is above 70 in Sweden. The incidence of rectal cancer is higher among men than among women[7]. The treatment for rectal cancer has improved during the past decades in terms of reduced local recurrence rates and improved survival. There is however, variability between different countries and different surgical centers. The survival from colorectal cancer has been estimated for most European countries and a substantial variability between them was observed. The lowest relative survival was observed in Poland and Czech republic (38-43%) whereas the highest was observed in Switzerland, Norway and Sweden (58-60%) [5]. In Sweden, cancer of the rectum has presently a better prognosis than cancer of the colon[9]. More than 90 percent of all rectal cancer patients undergo tumor resection[8]. Local or loco-regional tumor recurrence in rectal cancer refers to a recurrence of the tumor within the pelvis after a presumed curative resection. In Sweden a local recurrence rate of 9.5% has recently been reported[8]. Previously, much higher rates above 30% were seen.

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BACKGROUND

LOCAL RECURRENCE

Local recurrence rates of 15-35% were reported in the 70s and 80s when conventional surgery alone was the treatment for rectal cancer[10, 11] During the latest decades, new treatment techniques have been introduced with the aim to reduce local failure rates and improve survival. Adjuvant radiotherapy has been administered either preoperative (mostly Europe)[10, 12-14] or postoperatively (mostly North America)[15, 16]. In the Swedish Rectal Cancer Trial short course preoperative radiation, where radiation is given five succeeding days the week before surgery, resulted in better local control and improved overall survival[13]. In an earlier trial the Uppsala Trial, this short-course radiotherapy was superior to prolonged high dose postoperative radiotherapy[17]In the beginning of the 80s Mr Heald introduced a new surgical technique were a total excision of the mesorectum (TME) was performed with a refined dissection technique[18]. The first results of TME was published by Heald in 1986[19] and showed that local recurrence rate of 3% could be achieved with surgery alone in specialty center. Since the introduction of TME surgery, local recurrence rates of 10-15% have been reported from population-based series and when combined with preoperative radiotherapy recurrence rates of 5–8 percent have been reported [8, 20, 21]. In randomized studies in which TME has been combined with short course preoperative radiotherapy, significant reduction of local recurrence in the radiation arms have been shown[10, 22].

Time to local recurrence is variable. Some 55-80% of the recurrences are diagnosed within the first two years following the primary operation and recurrence rarely develop after five years[23]. It has been reported that recurrences after an

anterior resection occur slightly earlier than after an abdominoperineal resection (APR) [23]. The majority of local recurrences present as isolated tumors within the pelvis and are unaccompanied by disseminated disease[23]. In Stockholm 41% of the recurrent tumors showed signs of disseminated disease at the time of recurrence[24]. Recurrent tumors are mostly diagnosed based on the occurrence of symptoms[25]. Pain is the predominant symptom, which probably reflects overgrowth on adjacent nerves and other tissues. Altered bowel habits and rectal bleeding are symptoms of an anastomotic recurrence or recurrence within the bowel. In the Dutch TME trial it was found that the clinical nature and prognosis for a local recurrence was different after TME with preoperative radiation compared to a local recurrence after TME alone[26]. In the radiated group, the survival was shorter and a larger proportion of patients had generalized disease at the time of the local recurrence.

Patients with a local recurrence have unpleasant symptoms and a short life expectancy without treatment [27]. Radiotherapy with or without chemotherapy allows symptomatic improvement with less pain and bleeding in most patients but the five year survival is usually less than 5% with this treatment alone[28]. Radical resection of the recurrent tumor is the only treatment that offers a significant improvement in the prognosis[29-31]. In a population-based study in Stockholm, 40% of the patients with local recurrent were selected for surgery. A potentially curative resection (R0) was achieved in 44%[24]. The proportion of patients who underwent a curative resection was in the range of 37-62% at different centers[29, 32-34] Variance in patient selection and aggressiveness in therapy may explain differences both in

proportion of patients undergoing surgery and in proportion of patients having R0 surgery.

It is important to identify features that can predict the ability to achieve an R0 resection since the surgical trauma is severe and palliative surgery does not improve survival compare to no surgery[35]. Predictors for resectability of a tumor are young age at diagnosis, early stage at the primary operation, and initial treatment by a sphincter-saving procedure[35]. CT and MRI imaging findings that have been reported to predict a non-resectability of the a recurrent tumor are pelvic sidewall involvement and hydroureteronephrosis[36] although this may differ between centers. Wiig reported a better disease free survival for patients with a normal CEA level at the time of recurrence[25]. *En bloc* resection of the tumor and adjacent organs is preferred. In a recent study by Sagar[37] *et al*, a composite abdominosacral resection for locally recurrent rectal cancer was performed in 40 patients and R0 resections were achieved in 50% of the patients with a mean disease-free interval of 55 months.

Since local recurrence is associated with significant morbidity and the treatment of a recurrence is palliative for the majority of patients, research has been focused on the treatment of the primary tumor with the motto "the best way to treat a local recurrence is to avoid one".

TREATMENT OF RECTAL CANCER

Multidisciplinary team

Treatment of rectal cancer involves specialists from multiple disciplines.

Colorectal surgeons, oncologists, radiologists and pathologists are all involved in deciding on the treatment for the individual patient. Multidisciplinary team (MDT) conferences are routinely held before treatment with the purpose to improve decision-making regarding neoadjuvant RT and/or chemotherapy and the type of surgery to be performed[38]. Postoperatively, the team conference, based on the histopathology report, decides if the patient should be considered for adjuvant treatment. The risk of local recurrence is

considered for each rectal cancer patient and is an essential issue in the decision on treatment

DIAGNOSTIC IMAGING OF THE PRIMARY TUMOR

Rectal cancer diagnosis is dependent on histopathology on biopsies, generally taken using endoscopy. It is important to accurately verify local tumor growth and spread preoperatively. Digital rectal examination and endoscopy are generally employed to verify the location, fixation, level and extent of the tumor, however an adequate staging of extra-luminal tumor growth and local lymphatic spread is impossible to assess by these methods, and thus, additional imaging methods are used.

Transrectal ultrasonography

Transrectal ultrasonography (TRUS) enables high-resolution images of the intestine within a limited field of view. The method is highly "operator-dependent" and has its limitation when the tumor strictures the lumen or is situated in the proximal rectum[39]. TRUS is accurate for assessing tumor growth within the bowel wall[40]. The technique is more precise in distinguishing between benign tumors from invasive cancer and is also accurate for distinguishing invasion of muscularis propria, and differentiating T1-T2 tumors from more advanced ones [40]. The technique is not accurate in the detection of metastatic lymph nodes[41]. However, TRUS-guided fine-needle aspiration has been reported to be a reliable method[42].

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is well tolerated by the majority of patients and is nowadays the most frequently used imaging method in the Stockholm region as well as in most Western countries. A second reviewer can use the images acquired by this method. Reliable staging of the tumor for the multidisciplinary team facilitates planning of the neoadjuvant therapy and surgery.

Tumor stage

MRI in particular when combined with contrast agents or external coils is accurate for detecting the more advanced tumors and invasion of adjacent organs[43, 44]. The layers of the bowel wall can usually be clearly identified on T2 weighted images and perirectal fat appears as a high signal surrounding the low signal of the muscularis propria[44]. The staging of T3 lesions is based of the presence of tumor signal discontinuity extending into the perirectal fat. The distance beyond the muscularis propria is measured and the tumor is classified into T3 subgroups[44] (Table 1).

Using MRI after a patient has received radio(chemo)therapy it is difficult since it is not always possible to distinguish tumor from fibrosis and inflammatory reactions. Most surgeons therefore use the pretreatment imaging to determine resection planes even if the patient has been radiated.

Circumferential resection margin

MRI can be used to assess the distance from the tumor to the mesorectal fascia and thereby predict the circumferential resection margin (CRM) status in patients undergoing TME[45]. The mesorectal fascia is seen as a fine, low-signal layer enveloping the perirectal fat and rectum. A meta analysis comparing MRI against histology after TME, including data from nine studies, found that MRI predicts tumor involvement of the CRM with a sensitivity of 94% and a specificity of 85%[46]. A potential positive margin is defined as a tumor lying within 1 mm of the mesorectal fascia[45], even if others have suggested that a margin of 2 mm would be more accurate in discriminating between those with a low and high recurrence rate[47].

The nodal stage

Nodal staging has traditionally relied on size, however criteria based on the outline of the node

Table 1. T staging on MRI according to Taylor *et al* 2008

Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor invades submucosa: low signal in submucosal layer, replacement of submucosal layer by abnormal signal not extending into circular muscle layer
T2	Tumor invades but does not penetrate muscularis propria: intermediate signal intensity (higher signal than muscle, lower signal than submucosa) in muscularis propria; outer muscle coat replaced by tumor of intermediate signal intensity that does not extend beyond outer rectal muscle into rectal fat
T3	Tumor invades subserosa through muscularis propria: broad-based bulge or nodular projection (not fine spiculation) of intermediate signal intensity projecting beyond outer muscle coat
T3a	Tumor extends < 1 mm beyond muscularis propria
T3b	Tumor extends 1–5 mm beyond muscularis propria
T3c	Tumor extends 5–15 mm beyond muscularis propria
T3d	Tumor extends > 15 mm beyond muscularis propria
T4	Tumor invades other organs: extension of abnormal signal into adjacent organ, extension of tumor signal through peritoneal reflection

and features of signal intensity have been shown to be more reliable[45]. Nodes with a uniform appearance and a homogenous signal intensity are not considered to be suspicious of metastatic growth whereas irregular borders and mixed signal intensity are features that arouse suspicion of a metastatic node. Despite the identification of lymph nodes as small as 2-3 mm on modern planar imaging, reliable detection of nodal metastases is currently very difficult[41]. The diameter of benign and malignant nodes are often similar. Greater accuracy is achieved using other signs such as an irregular border or a mixed signal intensity of the lymph node[45].

MRI using lymph node-specific contrast agents has been reported to yield promising results. Ultra-small iron oxide particles have shown promising results in terms of identifying small foci of tumors within mesorectal nodes[48]. However, the inability to detect microscopic metastases in lymph nodes suggests that a negative MR examination should not be used to select patients for local excision surgery[45]

Extramural Vascular invasion

MRI is the only imaging modality that reliably can preoperatively demonstrate extramural vascular invasion (EMVI) in rectal cancer[49]. EMVI is characterized as a discrete serpiginous or tubular projection of intermediate signal intensity into perirectal fat following the course of a visible perirectal blood vessel (usually a vein)[45].

The presence of MRI-detected EMVI is associated with a poor clinical outcome, with a fourfold higher risk of distant metastasis and a reduction in relapse-free survival at 3 years [49].

SURGERY

The main treatment of rectal cancer is surgery and since the beginning of the 20th century, different surgical methods have been developed. In 1908 Miles described a combined abdomino-perineal excision[50]. Surgery with sphincter preservation started in the 1940s[5]. A substantial change in the number of sphincters preserved became obvious in the mid-1970s when stapling techniques became available[51].

Abdominoperineal resection

Rectal tumors situated < about 6 cm from the anal verge may require an abdominoperineal resection (APR) to ensure an adequate distal margin. The procedures follow the TME principles and the entire rectum, anal canal and anus are removed. A permanent stoma is created. A significant reduction in the rate of APR has been achieved since the introduction of the TME technique[21, 52]. In Sweden 25-30% of patients with a rectal cancer are treated with APR [8].

A number of studies have reported worse outcomes in terms of local recurrence[53, 54] and survival [53-55] after APR compared to AR. Recently, it has been noted that the APR-specimen frequently has a waist with the resection lines close to the tumor and the bowel wall[56]. Therefore, it has been proposed that the technique for APR should include a more extended perineal approach with a wider excision of the pelvic floor and the patient in the prone-jack knife position[57]. Using this technique one may have a better chance of avoiding the waist and to prevent the specimen from having a positive CRM[56, 58]. One of the major problems associated with APR is perineal wound complications. Pelvic radiation therapy increases the risk of wound complications and infection [59, 60]. For this reason, a number of reconstruction procedures have been proposed such as gluteus maximus[57], rectum abdominis[61] or gracilis[62] flaps, respectively.

Anterior Resection

Surgery without a permanent stoma became increasingly possible for low-level rectal cancers when the introduction of stapling devices made a low anastomosis possible. In Sweden 50% of the patients are treated with an anterior resection (AR) [8]. In this procedure the entire or the upper part of the rectum is removed and anastomosis between the left colon and the remaining rectal stump or anal canal is made. The part of colon connected to the distal remnant is usually termed "neo rectum". A major concern after AR is the risk of an anastomotic leakage. In a large population-based study, 12% of the patients had symptomatic anastomotic leakage after AR. Low anastomosis, occurrence of adverse intraoperative events

and male gender were independent risk factors for symptomatic leakage[63]. In a randomized multicenter study, a defunctioning loop stoma significantly decreased the risk of symptomatic anastomotic leakage[64]. Anastomotic leakage is associated with increased morbidity and mortality[65]. However, conflicting results have been reported concerning the risk of local recurrence. No effect of anastomotic leakage on local recurrence was found in a large cohort study of TME operated patients in Norway[66] but other studies have found anastomotic leakage to be an independent predictor of local recurrence[67, 68].

Total mesorectal excision

Heald first introduced total mesorectal excision (TME) in the early 1980s[18]. The technique has been widely adopted and is now considered to be the gold standard in rectal cancer surgery[5]. TME relies on sharp dissection of the avascular plane between the mesorectum and the surrounding parietal tissues down to the distal extremities of the pelvis. The surgically removed specimen includes an intact mesorectal fascia which encloses the rectum with perirectal fat, blood vessels, lymph nodes and nerves[69]. The dissection should be performed under direct vision with preservation of the hypogastric and sacral nerves, leaving sexual and micturitional functions intact[18].

Since the majority of rectal cancers with transmural spread (with or without regional nodal metastases) are restricted to the mesorectum[70], removal of the mesorectum intact maximizes the likelihood of surgical cure. TME optimizes the surgical clearance around the tumor and removes all mesorectal regional lymph nodes *en bloc*, including those distal to the tumor that may be involved by metastatic disease. In a series of 135 rectal cancer patients, Heald reported a local recurrence rate of less than 5% without preoperative radiotherapy using TME [19]. These excellent results can be influenced by patient selection but can also reflect very high surgical excellence.

Partial mesorectal excision

Partial mesorectal excision (PME) has been suggested for tumors situated in the upper rectum (10 to 15 cm above the anal verge)[71]. Since

a 4 cm distal spread within the mesorectum has been described[72, 73], a distal margin of 5 cm within the bowel and mesorectum has been suggested[71]. Operations with PME are associated with shorter operation time, shorter hospital stay and less blood loss compared to TME [74]. The risk of anastomotic leakage is lower [74] probably because the risk depends on the level of the anastomosis[63]. A higher level of rectal division with a longer rectal remnant also increases the chance of a better functional result with less frequency, urgency and incontinence of stool[75].

Lateral lymph node dissection

Studies on the lymphatic drainage of the rectum support a different lymphatic spread of tumors situated in the lower rectum than in the upper rectum[76, 77]. The lower rectum, (defined in Japan as being situated below the peritoneal reflection), has lateral lymphatic channels as a supplement to the upward drainage within the mesorectum. The lateral pelvic lymph nodes are situated in the pelvic wall outside mesorectum and therefore are not removed in standard TME surgery. In Japan, removal of the lateral pelvic lymph nodes is considered essential for patients with low rectal cancer[76, 78]. A recent meta-analysis of 10 large Japanese studies in which lateral lymph node dissection was performed for low rectal tumors, reported a median incidence of 17.8% lateral pelvic node involvement was found[76]. Clinicopathological factors that were reported to predict lateral involvement are: involved mesorectal nodes, female sex, advanced T stage, low tumor differentiation and lymphovascular invasion[76]

Lateral pelvic lymph node dissection (LPLD) is associated with significant morbidity such as increased operative blood loss[79, 80], postoperative bladder dysfunction[79, 80] and impotence[80]. However Moriya et al report acceptable urinary function and blood loss with refined nerve-sparing techniques in a series of 133 patients operated with LPLD.[81]. The latest Japanese guidelines suggest LPLD for T3/4 cancers with a lower edge below the peritoneal reflection[82].

Local excision

Transanal endoscopic microsurgery (TEM) is a new technique used for early T1-T2 tumors[83]. An anoscope with three ports for instrumentation seals the anal canal. The rectum is insufflated with carbon dioxide and the tumor is removed locally but no lymph nodes can be assessed. TEM and other local surgical procedures are part of routine treatment for very early rectal cancers where the risk of nodal metastases is very low (preferably in T1 tumors with only superficial growth in the submucosa)[84-86] This technique can also be considered acceptable in elderly patients with comorbidity and an increased risk of local recurrence in order to avoid major abdominal surgery even if the risk of local failure is comparably high[83].

Wash out

Exfoliated malignant cells have been found in the liquid of resection margins, in rectal stumps, and on circular stapling devices[87-89]. Furthermore the viability and metastatic potential of these exfoliated malignant cells have been demonstrated[88, 89]. Chemical washout of the rectal stump prior to division may eliminate exfoliated malignant cells[90]. Irrigation of the rectum (rectal washout) has been recommended to prevent implantation of viable intraluminal tumor cells in the anastomosis after anterior resections[91]. The advantage of this procedure has not been proven in a randomized trial and in a recent meta-analysis of five published studies, Constantinides *et al.* could not find a statistically significant superiority with this procedure [92]

RADIOTHERAPY

In the Swedish Rectal Cancer Trial[13] it was shown that short-course preoperative radiotherapy (5x5Gy) reduces the risk local recurrence. Since then, the Dutch TME trial[10], where TME surgery was combined with short-course preoperative radiotherapy has shown that the beneficial effect of radiotherapy is seen also when good surgery is used. A later report of the SRCT showed that preoperative radiotherapy reduced the risk of local failure following TME also after long-term follow-up[93]. In a systematic

review article covering 8507 patients from 22 randomized trials comparing outcomes of surgery for rectal cancer combined with preoperative or postoperative radiotherapy with those of surgery alone, it was stated that preoperative radiotherapy reduces the risk of local recurrence and death from rectal cancer[94]. Several randomized trials have shown that postoperative radiation therapy has less effect and is more toxic than preoperative radiation[95-97]. In a locally advanced rectal cancer with overgrowth into adjacent nonresectable structures, preoperative radiotherapy together with chemotherapy may allow a R0 resection and results in better control than radiotherapy alone[98].

Radiation target

Radiotherapy is presently delivered with linear accelerators using 8-16 megavolt photons, nowadays almost always using a multiple field techniques were a conformal dose distribution is used to irradiate the target volume containing tumor cells while sparing surrounding organs at risk[99]. The definition and delineation of the target volume should ideally be based on knowledge of the extent of infiltrative growth close to the tumor and local lymphatic tumor spread and of the location of local recurrent tumors[100]. Lymphatic tumor spread occurs mainly in mesorectal nodes upward into the inferior mesenteric nodes and lateral into the internal iliac nodes[100]. Metastatic disease in the inguinal and external iliac nodes is rare unless the tumor infiltrates organs anterior to rectum in the smaller pelvis or grows below the anal dentate line. Most studies on the location of local recurrences were conducted before the introduction of TME and revealed that the mesorectal, posterior and inferior subsites were the most common locations of recurrent disease[100]. The standardized target volume prescribed in the regional care program in Stockholm during the time periods the clinical materials were collected (see below) is shown in figure 1. The superior border of the beams was located in the middle of the L5 vertebra. The inferior border was 1 cm below the anal verge when an APR was scheduled and the inferior

border is 3-4 cm above the anal verge for all others. The lateral borders were located 1 cm outside the pelvic brim. For the lateral beams the posterior border was located behind the sacrum and the anterior border about 2.5 cm in front of the promontory unless the tumor is located in the upper portion of the rectum and grew anteriorly, in which case this distance instead was 3.5 cm. The most commonly used radiation regimen in Sweden is the short-course, or hypofractionated, 5x5 Gy schedule. Hypofractionation (giving the radiation in fewer fractions with doses above 2 Gy) has the advantage of shortened treatment time and a reduced burden on the radiation departments. Theoretically the disadvantage of this regimen is an increased risk of late toxicity compared to long-course therapy when the fraction sizes are lower[101]. However, besides the size of the radiation fraction, also the total radiation dose is important. Using 5 Gy fractions, the total dose is 25 Gy compared to a dose of 46-50.4 Gy using prolonged schedules[101]. Complications after the hypofractionated regimen of 5x5 Gy have been extensively evaluated in

randomized trials[12, 13, 59]. In the TME trial, early complications that become apparent during the radiation therapy or up to 3 months later were nausea, diarrhea, dermatitis, enteritis and proctitis[59]. Most early complications were of low grade, transient and required no intervention. However, a few patients suffered from acute neurological toxicity causing pain in the legs and gluteal region. The rate of surgical complications was found to be slightly higher when the patients had radiation therapy. Irradiated patients had more blood loss during the operation and showed more perineal complications following APR[59]. Studies of long-term complications have shown an increased rate of small bowel obstruction, anal sphincter damage with incontinence, sexual dysfunction, urinary dysfunction, frequent stools, osteoradionecrosis and peripheral nerve injuries[102-107]. An increased risk of a second cancer within or adjacent to the irradiated volume was reported by Birgisson *et al*[108]. The risk of these complications is dependent upon the radiation burden meaning both the radiation dose and the volume irradiated.

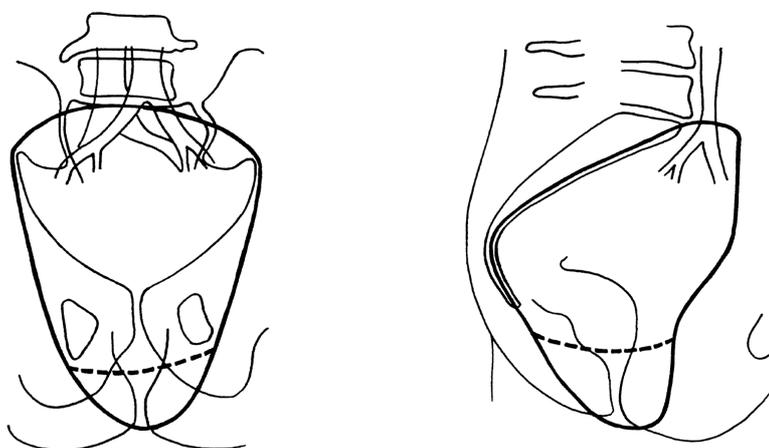


Figure 1. The target volume according to the care program in Stockholm: frontal and sagittal image of the pelvis. The dotted line indicates blocking of the anal sphincter.

Tumor regression / prediction of therapy response
 Histological changes following preoperative radiotherapy affect both the tumor and the normal tissue. Increased necrosis, stromal fibrosis, absence of vascular invasion and a decreased lymphocytic response are seen in the tumor [109]. An overall reduced number of lymph nodes has been reported after long course radiotherapy[110], and also in the TME trial in which short-course radiotherapy was used[10].

Different pathologic staging systems have been suggested for tumor regression grading (Table 2). Bozetti *et al* [111] used the tumor regression grade (TRG) proposed by Mandard *et al* [112] for esophageal carcinoma, in a study on rectal cancer patients treated with preoperative radiotherapy. Dworak[113] suggested another grading of regression grade corresponding to fibrosis and remaining tumor cells.

A proportion of patients who receive radiotherapy or chemoradiotherapy have a complete clinical and pathological response to the therapy. Glynne-Jones reviewed 38 studies in which radiologic response was reported[114]. In 10 of these studies not a single complete response was reported and, in the remaining 28 complete clinical responses ranged from 3% to 53%. Habr-Gama[115] reported 28% complete responders following chemoradiotherapy for patients with distal rectal cancer. The complete clinical responders were

then followed with a "wait-and-see" policy and examined carefully with digital and endoluminal examinations every 1-2 months and CT scans every 6 months the first year and then yearly 2nd and 3rd year. In these series 5% local failures were seen and the 5-year disease-free survival was 93%.

Since the separation between transmural fibrosis, actinic ulcers and residual tumor is difficult, clinical identification of complete responders by digital-rectal, endoscopic and imaging studies is hazardous. In a study by Guillem *et al* [116] only 3 of 14 patients with complete response were correctly identified by digital examination. There is no clinical way to determine which patients' tumor will respond to radiotherapy at the moment; however, molecular markers have been assessed in efforts to predict radiation response. Immunohistochemistry studies have demonstrated an association with radiotherapy response using p 53 gene mutation [117-120], endogenous p21[120-122] and tumors with a high spontaneous apoptosis[123-125]. In two recent studies on rectal cancer using pretreatment gene expression profiling; Watanabe *et al*[126] were able to predict the radiotherapy response with an accuracy of 82.4% and Ghadami *et al*[127] predicted tumor response with 78% sensitivity and 86% specificity.

Table 2.
 Tumor regression grade according to Bozetti *et al*

TRG 1	(complete regression) absence of residual tumoral cell
TRG 2	presence of rare residual cancer cells and prominent fibrosis
TRG 3	increased number of cancer cells, but predominated fibrosis
TRG 4	numerous cancer cells and little fibrosis
TRG 5	absence of regression

TRG 1, 2 and 3 correspond to a regression exceeding 50% of the tumour

Tumor regression grade according to Dworak *et al*

Grade 0	no regression
Grade 1	dominant tumor mass with obvious fibrosis and/or vasculopathy
Grade 2	dominantly fibrotic changes with few tumor cells or groups (easy to find)
Grade 3	very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucous substance
Grade 4	no tumor cells, only fibrotic mass (total regression or response)

Chemotherapy in addition to preoperative radiotherapy

Neoadjuvant therapy in rectal cancer can include chemotherapy in addition to the radiotherapy. The chemotherapeutic agents can serve as radiosensitizers and to exert a cell kill effect on the primary tumor and to kill micrometastatic disease in order to avoid distant metastases. The value of chemotherapy with radiotherapy has been explored in recent randomized clinical trials in locally advanced rectal cancer[98, 128, 129]. In all three studies the addition of chemotherapy to the same conventionally fractionated RT improved local tumor control. There was no clear influence on the risk of systemic disease or overall survival. The combined modality treatment increased the risk of acute toxicity where the predominant form of toxicity was diarrhea but nausea, stomatitis, and hematological toxicity were also reported.

CLINICOPATHOLOGICAL PARAMETERS

Macroscopic appearance of the specimen

A macroscopic assessment of the specimen was performed in the Dutch TME study and in the recently reported MRC CR07 trial[130, 131]. In the MRC study The quality of the plane of surgery was classified into three grades[131]:

1. Complete (mesorectal plane): Intact mesorectum, with no defect deeper than 5mm, No coning, a smooth circumferential margin on slicing.
2. Nearly complete (intramesorectal plane): Moderate bulk to the mesorectum, but irregularity of the mesorectal surface. Moderate coning of the specimen is allowed. At no site is the muscularis propria visible, with the exception of the insertion of the levator muscles.
3. Incomplete (muscularis propria plane): Little bulk to mesorectum with defects down onto muscularis propria and/or very irregular circumferential resection margin.

In both studies it was found that the risk for local or distant recurrence was statistically significant increased if the mesorectum of the specimen was incomplete[130, 131].

Distal margin of the rectal wall

When performing an anterior resection for tumors in the lower part of the rectum the distal resection margin of the specimen is of importance. Reports from the 1990s noted that intramural submucosal spread, found in 40% of the patients, extended for more than 1 cm in only 4-6% of cases[132, 133] and concluded that a 1-cm distal margin could be considered to reflect good surgery. In the American National Guidelines for Rectal Cancer Treatment in 2000[134] a 2-cm margin was recommended but it was also concluded that a distal mural spread beyond 1 cm rarely occurs and is associated with tumors of advanced stage or histologically aggressive disease. An advanced stage at diagnosis, a poorly differentiated tumor, lymphovascular/perineural invasion, and tumor budding have been found to be associated with distal intramural spread beyond 1 cm[135].

Distal margin of the mesorectum

The distal margin of the mesorectum is of interest when purposely transecting the mesorectum when performing a PME. Blockage of the upward lymphatic flow by a locally advanced tumor may cause a downward spread in the mesorectum. Patients with a distal spread to the lymphatics of the mesorectum have a worse outcome than patients who do not[72, 136]. Hida et al noted that the extent of distal spread was related to tumor location[73]. The longest distance of spread (4 cm) was in the upper rectum where as this was shorter in the lower rectum and in rectosigmoid tumors. Zhao et al reported an 18% rate of distal mesorectal spread with a maximum distance of 3.6 cm[137] in a study of 45 patients.

Circumferential resection margin

The circumferential resection margin (CRM) also known as the radial, lateral or mesorectal resection margin is the distance between tumor cells and the nonperitonealized surface of the rectal specimen created by mesorectal dissection at surgery. Tumor growth in connection with the non peritonealized surface, often expressed as involvement of the CRM, has been shown to be the single most critical factor in predicting LR [138, 139]. The TNM definition of a positive margin is 0 mm.

But a distance of 2 mm has also been regarded as a cut-off due to a significantly increased risk of a local recurrence[47]. In general the larger the distance of the tumor from the CRM is the better the prognosis[139]. According to a recent meta-analysis, CRM involvement is also a powerful predictor of distant metastasis and survival[139]. Different types of CRM involvement have been described: direct tumor spread, discontinuous tumor spread, lymph node metastases, venous invasion, lymphatic invasion and perineural tumor spread. There is a correlation of CRM positivity with TNM stage. The more advanced the stage is the greater the chance of CRM involvement[139]. Increased depth of tumor invasion, presence of tumor deposits and lymph node involvement all contribute to this correlation. Histological factors such as poor differentiation and vascular invasion are also associated with a positive CRM[138, 140]. Variations between surgeons and the improvement over time support the view that there is also a surgical factor that influences positive CRM[141]. More positive margins have

been reported in tumors located in the lower rectum[142]. Higher CRM positivity is also seen in patients who have undergone APR compared to AR [142]. In a subgroup analysis of the Dutch TME study it was concluded that radiotherapy does not compensate, in terms of local recurrence risk for positive CRM[143].

T stage

The pathologically determined T and N stages are considered to be more accurate than the clinically derived T and N. The AJCC/UICC stage according to TNM classification[144] with complements on T stage[145] is shown in Table 3. In T3 tumors, an extramural extension of more than 5 mm appears to have an adverse prognosis[146]. Penetration of the parietal peritoneum is classified as a T4b and is of importance for the tumors in the upper rectum. A free perforation of a tumor into the peritoneal cavity is also classified as a T4b. In a study of 209 patients, the outcome for these patients was significantly worse, with a higher risk of local recurrence[147].

Table 3. AJCC/UICC and TNM system for classification of rectal cancer

AJCC/UICC staging system	TNM system
Stage I	T1-2 N0 M0 T1= Involvement of the submucosa T2= Invasion into but not through the muscularis propria N0= No nodal involvement M0= No distant metastases
Stage II	T3-4 N0 M0 T3= Penetration through the muscularis propria into the serosa or perirectal fat T3a=Minimal invasion:<1mm beyond the border of the muscularis propria T3b=Slight invasion:1-5 mm beyond the border of the muscularis propria T3c=Moderate invasion:5-15mm beyond the border of the muscularis propria T3d=Extensive invasion:>15 mm beyond the border of the muscularis propria
Stage III	Any T N1-2 M0 N1=Metastases in 1-3 perirectal lymph nodes N2=Metastases in ≥ 4 perirectal lymph nodes
Stage IV	Any T any N M1

N stage

Metastatic disease of the regional lymphnode is a adverse prognostic factor for local recurrence[146, 148-150]. It has been shown that at least 12-15 regional lymph nodes must be examined to accurately determine node-negative colorectal cancer[151, 152]. The number of lymph nodes involved is of importance. Patients with N1 (1-3 involved nodes) have a better oncologic outcome compared to N2 (>3 involved nodes). An index of metastases derived from the number of metastatic nodes divided by the number of nodes examined has been suggested to be a better prognostic factor of more importance than N stage itself[153].

It has been observed that neoadjuvant chemoradiation therapy reduces not only the rate of metastatic lymph nodes but also the overall number of retrieved nodes[154]. It has also been observed that an absence of lymph nodes retrieved from the resected specimen for patients treated with chemoradiation is associated with lower T status, decreased risk of perineural invasion and good disease-free survival[155].

Micrometastases

Lymphatic micrometastases are metastatic deposits smaller than 2mm found in lymphnodes[156]. In a recent study of 67 rectal cancers, micrometastasis was found using cytokeratin staining with immunohistochemistry technique in 30 % of N-negative nodes examined with hematoxylin and eosin (H-E) staining[157]. The clinical relevance of these metastases is however not known.

Budding

Tumor budding is a feature that has been observed in different cancers and also in colorectal adenocarcinomas[158]. The term denotes that at the invasive front of the tumor, tumor cells singly or in small aggregates become detached from the neoplastic glands. Biologically, tumor budding is closely related to the epithelial-mesenchymal transition. The budding cells are de-differentiated in the meaning that they have lost their ability to form glands as epithelial cells, demonstrated in an electron microscope as a lack of desmosomes and basement membranes. [158].Recent studies have found that these cells can form podia which

theoretically enable the cells to migrate by amoeboid movement[159].

Several studies have addressed tumor budding as a prognostic factor in colorectal cancer[160-163]. Patients with tumors with a high grade of budding cells have a higher risk for metachronous metastases[162, 164] and a poorer survival [163, 164]. In early invasive colorectal carcinomas a high grade of budding is correlated with a higher risk of synchronous regional lymph node metastases [165, 166]. Absence of budding has also been correlated with the presence of intratumoral and peritumoral lymphocytes, suggesting an immune reaction responsible for the destruction of buds and therefore improved prognosis[167].

Laminins constitute a family of trimeric extracellular matrix proteins localized in the basal membrane (BM). The isoform, laminin-5 is composed of three chains units $\alpha 3$, $\beta 3$ and $\gamma 2$. The $\gamma 2$ chain is expressed in the cytoplasm of epithelial human cancer cells located at the advancing edge of the tumor.[168] Laminin-5 $\gamma 2$ chain expression has been found to be upregulated in more advanced colorectal carcinomas and facilitates the detection of budding cells.[169]

Tumor grade

The grading of rectal adenocarcinoma is based on both architectural and cytological features (e.g., hyperkromatism and pleomorphism) determined by the pathologist[146]. The degree of gland formation is regarded as the most important feature in grading. The estimation is largely subjective. There are a number of grading schemas in the literature[146]. Most systems stratify tumors into 3 or 4 grades:

Grade 1-Well differentiated

Grade 2-Moderately differentiated

Grade 3-Poorly differentiated

Grade 4-Undifferentiated

In most studies documenting the prognostic power of tumor grade, the data analyses are based on two grades : low grade (grades 1 and 2) and high grade (grades 3 and 4). High grade has been shown by multivariate analyses to be a stage-independent risk factor for relapse [170, 171] in two studies including 1400 rectal cancer patients.

Erik Syk

AIMS OF THE THESIS

The overall aims of this thesis were to define localization, analyze possible causes and explore potential prognostic factors for local failure in rectal cancer, ultimately aiming at improved local control and minimized morbidity in rectal cancer therapy.

SPECIFIC AIMS

- To determine the sites of local recurrence following TME with clear resection margins in an effort to elucidate possible reasons for recurrence.
- To determine the sites of local failure after rectal cancer surgery and to assess the possible impact on the radiation target volume.
- To assess the prognostic impact of clinicopathological variables with regard to local failure
- To explore the potential for tumor budding as a predictor of local failure

Erik Syk

METHODOLOGY

Patients and treatment (Paper I-IV)

The patients included in all four papers were all operated on in centers that had adopted TME surgery and most of the surgeons who treated colorectal patients during the study period were familiar with the TME technique. The TME concept was introduced at workshops in the Stockholm area initiated in 1994 [21]. During the study period, rectal cancer surgery was performed at nine hospitals in Stockholm. The primary study population was the cohort of abdominally operated rectal cancer patients from Stockholm during 1995-2004 (Paper III). In Paper I the subgroup of patients who underwent R0 surgery was included and in Paper II this group was extended to include patients with R1 and R2 resections as well. In Paper IV, patients from Norrköping (1995-2000) and Uppsala (1985-95) were also added (Table 4). The TME concept was introduced at these centers prior to the time periods studied.

The surgical procedures performed were: (i) low anterior resection (LAR), i.e. a TME with a coloanal anastomosis or an extended Hartmann's

procedure, (ii) high anterior resection (HAR), i.e. a partial mesorectal excision (PME) with a colorectal anastomosis or a Hartmann's procedure, or (iii) an abdominoperineal resection (APR), i.e. a TME with removal of the anal canal.

During the study period the regional care program prescribed preoperative radiotherapy with 25 Gy in 5 Gy fractions on five successive days followed by immediate surgery as the reference treatment for patients with a resectable tumor considered to be at some risk of local failure. Patients with early cancers could undergo surgery alone (local procedure for some, abdominal for most) whereas patients with a non-resectable cancer (all T4) had long-course RT (25-28x1.8-2 Gy) alone or with chemotherapy. Some of the irradiated patients were included in a randomized study comparing 5x5 Gy with immediate surgery, 5x5 Gy with delayed surgery or 25x2 Gy with delayed surgery [101]. Most of the locally advanced non-resectable tumors were included in another randomized study comparing radiation with chemoradiation [98].

Table 4. Patients abdominally operated for rectal cancer included in the studies.

Paper	Cohort	Data collected	Number of patients in the study	Follow-up
I	Stockholm-Gotland	1995-1999	880 patients (R0) 42 local recurrences	1995 to Jan 2002
II	Stockholm-Gotland	1995-2004	2315 patients (R0-2) 155 local failures	1995 to Jan 2005
III	Stockholm-Gotland	1995-2004	2282 patients (R0-2) 154 local failures	1995 to Jan 2005
IV	Stockholm-Gotland Uppsala Norrköping	1995-1999 1985-1995 1990-2000	1180 patients (R0) 58 local recurrences	1995 to Jan 2002 1985 to May 1998 1990 to Jun 2002

In Stockholm, the radiotherapy was delivered in either of two institutions employing a four-field box technique.

Since 1995 all patients with rectal cancer in the Stockholm Gotland region have been registered in a local data registry at the Regional Oncologic Center. The responsible surgeon reports clinicopathological data such as age, sex, tumor site and stage, adjuvant treatment, type of surgery, resection status (R-stage) and postoperative morbidity and mortality. Based on the assessment of the specimen, the pathologist reports pathological findings as tumor stage, resection margin and local spread. The database also includes follow-up data. In this database, a rectal cancer was defined as a rectal adenocarcinoma with a distal margin <16 cm from anal verge and a local recurrence was defined as any recurrence of rectal cancer within the pelvis. Data on Norrköping and Uppsala patients included in Paper IV were collected from local databases at each surgical center.

In Papers I and IV, in which local recurrence after R0 surgery was studied, the patients included had a radical abdominal operation in the form of an optimal surgical resection, i.e. an abdominal or abdominoperineal operation with a total or partial (distal margin of at least 5 cm) mesorectal excision. The operation was defined as radical if the surgeon reported complete excision of the tumor in a patient with no distant metastases, and the pathologist reported tumor-free margins for the specimen. In Papers II and III, in which local failures were studied, patients were also included if the surgical procedure resulted in an R1 or R2 resection.

Analyses of clinicopathological factors influencing local failure (Paper III)

In the studied cohort comprising 2315 rectal cancer patients registered in the Stockholm Oncologic Center database a total of 154 patients with local failure after abdominal resection were identified. Variables studied included sex, age at primary resection, study period (early: 1995-1999, late: 2000-2004), tumor location (low: 0-5 cm, middle: 6-10 cm and high: 11-15 cm from the anal verge) as measured by the surgeon with

a rigid sigmoideoscope, tumor size (more or less than 40 mm) as measured transversely by the pathologist, T-stage (T1-2 or T3-4), N-stage (N0, N1, N2 or Nx), tumor differentiation (moderate/high or poor), preoperative irradiation or not, type of surgery, irrigation of rectal stump prior to division or not, inadvertent rectal perforation or not and resection status (R0 or R+). Data on case load and the crude local failure rate at each of the nine surgical departments in Stockholm were obtained from the registry. The hospitals were divided into two groups in two different ways based on either case load or failure rate: (i) the three hospitals with the largest case load were compared with the remaining six hospitals, and (ii) those with a crude local failure rate exceeding 7% were compared with those with a local failure rate below 7%.

Interpretation of CT/MRI scans (Papers I-II)

In Papers I and II the radiological images from the time of recurrence were retrieved from the hospitals involved. All available CT and MRI scans of the abdominal and pelvic regions suggestive of local tumor relapse were reviewed. Two radiologists reviewed the images independently in the first 37 patients included in Paper I. One radiologist evaluated the additional patients in Paper 2 using a simplified protocol. The radiologists were aware of the diagnosis of a local recurrence (Papers I and II) or local failure (Paper II), but did not know which, if any, of the cross-sectional imaging modalities had shown the failure. The radiologists were blinded to clinical data, laboratory tests and the results of other imaging modalities. The radiological definition of a recurrence or failure was any infiltrative, expansive or asymmetrically located pelvic mass with some degree of contrast enhancement that could not be explained by normal or postoperative changes. The first imaging study in which a failure had been identified was considered to be the reference examination. If the mass could be inferred retrospectively on a previous image, the earliest such examination was considered to be the reference examination. In the reference examination, the axial image that showed the largest tumor burden was selected as the

reference image. This image was considered to be representative of the site of relapse or residual tumor if the surgery was not radical.

When determining pelvic length, the upper border of the puborectal ring was considered to be the previous or present anorectal junction; the upper border of the pelvis was defined as the level of the L5-S1 interspace. In Paper II, the pelvic length was divided in every patient using a scale of 100 in order to obtain a measurement rendering inter-individual comparison possible (Figure 2). The anatomical site of the local failure on the reference image was then calculated in relation to the pelvic length. Thus, for each tumor, the site of the failure was classified in percentage of the distance between the puborectal muscle and the L5-S1 interspace. In failures located in the upper part of the pelvis, the relation to the anterior border of the S1-S2 interspace was documented. Furthermore, the relation of the local failure in the reference image to the pelvic floor, defined as the levator

ani or puborectal muscles, was documented for recurrences situated down low in the pelvis when the primary cancer had been at least 5 cm from the anal verge, i.e. middle and high primary cancers. Additionally, all failures were classified anatomically as either anastomotic (recurrence at the anastomosis), presacral midline (recurrence in the midline immediately ventral to the sacrum), presacral asymmetric (recurrence immediately ventral to the sacrum asymmetrically located on either the left or the right side), on the pelvic floor, at the pelvic wall medial to the pelvic fascia or at the pelvic wall lateral to the pelvic fascia. For tumors extending to both sides of the pelvic fascia, medially as well as laterally, the larger portion was documented. If a tumor extended to several of these sites, they were all recorded. When fatty tissue completely surrounded the remaining rectum at any level, this was regarded as a sign of residual mesorectal fatty tissue, i.e. remaining mesorectum (Figure 3).

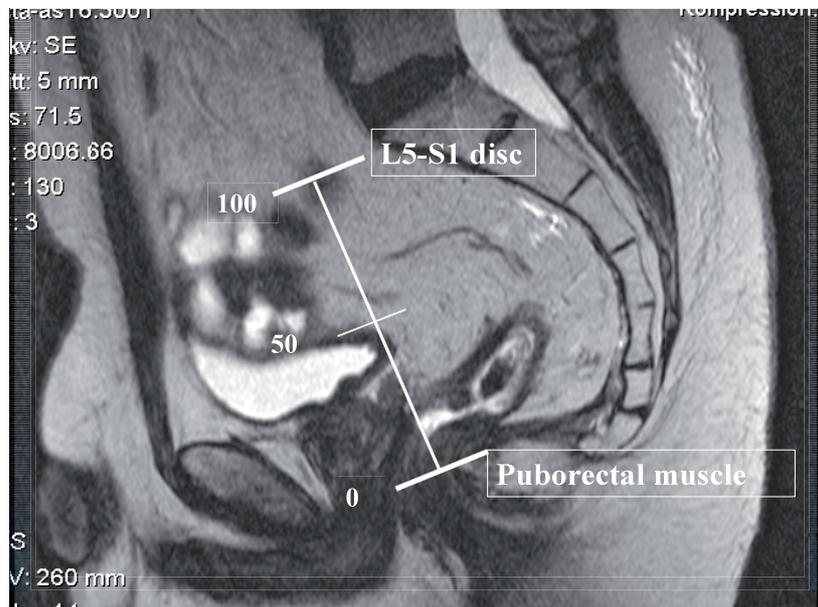


Figure 2. The anatomical landmarks for the determination of the pelvic length



Figure 3. Remaining mesorectal fat encircling the neorectum

In Paper II, the medical records and radiology reports were studied also for those patients whose images failed to show tumor recurrence or whose images were unavailable or in whom no imaging was done. Based on the available data, the location of the recurrent tumor in these patients was classified as lower pelvis (recurrence in the vagina, anus, subcutis or below 8 cm in the neorectum) or upper pelvis (above 8 cm in the neorectum or in the upper pelvis).

Analyses of the invasive front of the tumors. (Paper IV)

A nested case-control study was designed for 58 patients with a local recurrence identified from a total of 1180 patients having undergone R0 resection in the Stockholm, Norrköping and Uppsala region. Two controls were selected from the study population for each local recurrence case. The controls were matched for gender and preoperative radiation, otherwise they were randomly selected. For each control, an observation period free from locally recurrent disease at least as long as that of the matched local

recurrence case was required. From the cohort of 174 patients (58 cases and 116 controls) it was possible to retrieve 129 (48 cases and 81 controls) formalin-fixed paraffin wax-embedded blocks containing the surgical specimen with the tumor cells from the primary operation.

The original hematoxylin-eosin (H-E) glass slides were re-examined by a senior pathologist to confirm the original diagnosis and the representativity of the sections used. Sections including the invasive front were selected for staining. From the representative paraffin-embedded section, 4 μ m tumor sections were cut and immunohistochemical staining was performed employing a standard technique. The following antibodies were used: Cytokeratin, Clone MNF 116, Monoclonal Mouse Anti-Human, (DakoCytomation, Denmark) 1:75 and Laminin-5 gamma-2 chain (Ln-5 γ 2) clone 4G1, Monoclonal Mouse Anti-Human, (DakoCytomation, Denmark) 1:50, and the amplification system EnVision+System-HRP (DAB, DakoCytomation, Denmark). Tumor sections were deparaffinized in xylene, and then

stepwise hydrated in decreasing concentrations of ethanol. To quench the endogenous peroxidase activity the sections were incubated in hydro peroxidase. Goat serum was used to reduce of non-specific background staining. The primary antibodies were incubated over night followed by incubation with the amplification system. The peroxidase reaction was visualized using diaminobenzidine tetrahydrochloride (DAB) and finally the slides were counterstained with Mayer's hematoxyline.

The immunohistochemically stained slides were assessed jointly by two investigators using a double objective microscope. All evaluations were performed in a coded manner without prior knowledge of clinical and pathological data on the patient. Presence of tumor budding according to the criteria stated by Ueno *et al.* in H-E staining studies (an isolated cancer cell or a cluster composed of fewer than five cancer cells appearing to bud from a large cancer gland at the invasive front[163] was determined using both markers (Ln-5 γ 2 and MNF 116). All 129 slides were scanned at low power magnification (10x) to identify areas with the highest density of budding and to measure the invasive front in millimeters. The number of budding foci was counted simultaneously by the two investigators in a selected field in which budding frequency was considered maximal using 40x magnification. The mean of the two counted values was used and if the sum differed by more than 10%, the counting was repeated until consensus was reached.

To determine the extension of budding, the areas containing MNF-116 stained budding cells were measured and calculated as a percentage of the total invasive front. The degree of Ln-5 γ 2 staining was studied in the part of the invasive front where the staining frequency was considered maximal and was evaluated in terms of the percentage of immunopositive cancer cells along the invasive front using 40x magnification. The staining grade of the tumor was classified semi-quantitatively as high or low with a cut off at more or less than 20% stained cells. To verify reproducibility, a senior pathologist assessed the frequency of budding on both the MNF-116 and Ln-5 γ 2 slides.

Statistical methods

All statistical analyses were performed in STATISTICA (StatSoft Inc, Tulsa, OK,US).

All tests were two-sided and $p < 0.05$ was regarded as statistically significant.

In Paper I, the statistical analysis of patient characteristics was carried out using the chi-square test. In Paper III, univariate comparisons of survival between groups were made using log-rank test. A Cox proportional hazards regression model was used for the exploratory analyses of clinicopathological factors predicting local failure. Variables tested in the multivariate model were gender, age, study period, tumor location, tumor size, T-stage, N-stage differentiation,, radiotherapy, type of surgery, TME, intraoperative perforation of the rectum, residual status and case load. Exploratory analyses of discriminators for hospitals with high and low failure rates were done using a logistic regression analysis, including characteristics of treatment, i.e. radiotherapy or not, type of surgery, TME, intraoperative rectal perforation, and tumor characteristics, i.e. T-stage, N-stage, and differentiation. Univariate analyses were performed using the chi-square test.

In Paper IV, exploratory analyses of discriminators for the odds of local recurrence were performed using a logistic regression analysis, including Ln-5 γ 2 expression, Ln-5 γ 2 budding frequency, MNF-116 budding extension and MNF-116 budding frequency. The goodness of fit, i.e. model prediction against observed values, was estimated for all analyses, and presented as a measure of agreement. The cut off value of MNF-116 and Ln-5 γ 2 budding frequency were obtained using the logistic regression analysis. The Mann – Whitney U-test was used to perform analyses of the difference in MNF-116 stained budding by radiation. A logistic regression model was used to perform univariate- and multivariate analyses on TNM-stage and MNF stained budding in relation to local recurrence. The agreement, sensitivity and specificity were calculated from the same model.

Erik Syk

RESULTS AND DISCUSSION

LOCAL FAILURE IN RELATION TO TREATMENT

Case load

In Paper III, the prognostic impact of the surgery performed was studied in data from 154 local failures stemming from a cohort of 2315 patients in the Stockholm region. In the comparison between different hospitals the issue of case load was studied. The total number of rectal cancer operations performed during the 10 year period in each hospital varied between 617 and 52 (Table 5). When comparing the three centers with the higher case load to the six centers with a lower case load, the crude local failure rates were 5% and 9%, respectively. This difference was statistically significant and, moreover, when data were entered in a multivariate Cox analysis, case load was an independent risk factor for local failure (Table 6). The surgeon's experience [172], degree of specialization [173] and case load [174, 175] have all been suggested as risk factors for an adverse outcome in the treatment for rectal cancer. However, since the difference in case load between the different centers in

Paper III was hardly discernable, analysis with respect to produced outcomes was performed. Comparison of the four centers with a failure rate below 7% ("low failure") to the five centres with a local failure rate exceeding 7% ("high failure") revealed no differences with respect to gender, age, study period, T-stage, N-stage, size or tumor location between the two groups. However, in centers with a low failure rate, a significantly larger proportion of patients underwent LAR with rectal washout and had a TME (Table 7). The "low failure" centers also irradiated a larger proportion of patients, although this difference was not statistically significant in the multivariate analysis. The results in Paper III indicate that there may be a case for further centralizing rectal cancer treatment to departments with excellent results. On the other hand, these results may also be interpreted to mean that in some centers with very low local failure rates, the use of preoperative radiotherapy could be decreased.

Table 5. Local failures after abdominal surgery for rectal cancer in the nine hospitals in the Stockholm and Gotland region 1995-2004

Hospital	No of patients	Local failures	Crude failure rate (%)
1	617	25	4.0
2	352	24	6.8
3	297	18	6.1
4	292	22	7.5
5	277	25	9.0
6	239	24	10.0
7	92	10	11.0
8	64	1	1.6
9	52	5	9.6

Table 6. Hazard ratios and 95% confidence intervals for local failure after abdominal rectal cancer surgery from Cox proportional hazards regression analyses

Variable		Univariate		Multiple Cox regression		
		n (events)	p (log rank)	HR	95% Confidence interval	p
Gender	male:female	89:65	0.72	1.28	0.92-1.77	0.14
Age	≤ 71: >71	72:82	0.04	1.06	0.92-1.77	0.14
Study period	95-99:00-04	88:66	0.42	1.10	0.79-1.52	0.56
Tumour location	0-5cm	64		1	reference	
	6-10 cm	37		0.47	0.28-0.80	0.005
	11-15 cm	53		0.65	0.38-1.14	0.13
Tumour size	≥ 40 : < 40 mm	78:52	0.00032	1.21	0.84-1.74	0.30
T-stage	T1-2: T3-4	20:131	<0.00001	0.42	0.25-0.70	0.001
N-stage	N0	56		1	reference	
	N1	47		1.62	1.07-2.43	0.02
	N2	37		2.13	1.35-3.36	0.001
	Nx	14		1.94	1.06-3.53	0.03
Differentiation	Poor: Moderate or High	41:109	0.001	1.41	0.96-2.07	0.08
Radiotherapy	no:yes	89:65	<0.00001	2.78	1.92-4.00	<0.001
Type of surgery	LAR +rw	48		1	reference	
	APR	52		1.03	0.61-1.74	0.90
	LAR-rw	24		1.03	0.59-1.79	0.91
	HAR+rw	14		1.48	0.45-4.80	0.51
	HAR-rw	16		2.38	0.73-7.70	0.15
TME	No:Yes	32:122	0.03	1.47	0.52-4.12	0.46
Rectal perforation	Yes:No	16:123	0.03	1.10	0.64-1.80	0.73
Residual status	R+:R0	52:100	<0.00001	3.03	2.06-4.40	<0.001
Case load	6 low-vol.: 3 high-vol.	87:67	0.0008	1.61	1.15-2.21	0.006

APR: Abdominoperineal Resection. LAR: Low Anterior Resection.

HAR: High Anterior Resection. rw: Rectal Washout.

Table 7. Clinical characteristics and discriminative factors of 2282 patients classified into two hospital categories; Low and High failure centers

		Low failure	%	High failure	%	p (chi2)	Odds Ratio	95% Confidence interval	p
Gender	N	1330		952					
	M	770	58%	556	59%	0.78			
	F	560	42%	396	41%				
Age	≤ 71	720	54%	463	49%	0.01			
	>71	610	46%	489	51%				
Tumor location	≤ 5 cm	404	30%	306	32%	0.65			
	6-10 cm	492	37%	345	36%				
	≥ 11 cm	434	33%	301	32%				
Tumor size	< 40 mm	557	43%	389	41%	0.15			
	≥ 40 mm	650	57%	399	42%				
	Missing data	123	1%	164	17%				
T-stage	T1-2	389	29%	268	28%	0.30	1.05	0.83-1.31	
	T3-4	887	67%	671	71%		1		
	Missing data	54	4%	13	1%				
N-stage	N0	692	52%	485	51%	0.68	1		
	N1	301	23%	236	25%		1.07	0.84-1.07	0.58
	N2	235	18%	166	17%		1.18	0.89-1.56	0.24
	NX	102	8%	65	7%		0.93	0.62-1.40	0.74
Differentiation	Poor	243	18%	198	21%	0.17	0.77	0.60-0.98	0.04
	Moderate/								
	High	1036	78%	732	77%		1		
	Missing data	51	4%	22	2%				
Radiotherapy	No	493	37%	426	45%	0.0003	1		
	Yes	837	63%	526	55%		1.12	0.92-1.37	0.24
Type of surgery	APR	333	25%	269	28%	<0.0001	0.60	0.47-0.76	<0.001
	LAR+rw	734	55%	338	36%		1		
	LAR-rw	108	8%	169	18%		0.25	0.19-0.35	<0.001
	HAR+rw	107	8%	91	10%		1.63	0.65-4.06	0.30
	HAR-rw	48	4%	85	9%		0.82	0.32-2.14	0.70
TME	Yes	1163	87%	749	79%	<0.00001	2.85	1.21-6.68	0.016
	No	167	13%	200	21%		1		
	Missing data	0	0%	3	0%				
Rectal perforation	Yes	86	7%	81	9%	0.07	0.90	0.62-1.3	0.58
	No	1151	86%	811	85%		1		
	Missing data	93	7%	60	6%				
Residual status	R0	1131	89%	776	81%	0.02	1		
	R+	199	10%	176	19%		1.05	0.78-1.41	0.73

Rectal stump wash-out.

Irrigation of the rectum (rectal washout) has been recommended to prevent implantation of viable intraluminal tumor cells into the anastomosis after an anterior resection [91]. The advantage of this procedure has not been proven in a randomized trial, and in a recent meta-analysis of five published studies, Constantinides *et al.* could not find any statistically significant superiority with this procedure [92]. Also in Paper III, rectal washout was non-significant in the multivariate analysis (Table 6). Theoretically, rectal washout could be a surrogate marker for an overall superior management which is reflected in increased use of preoperative radiotherapy, lower rate of APR, more TMEs, fewer rectal perforations and, ultimately, a lower failure rate.

Remaining mesorectum

Using TME surgery alone, single centers have reported a recurrence rate of less than 5% [69, 176]. Heald stated that the mesorectum is the clue to pelvic recurrence and suggested that “complete removal of the mesorectum encompasses the most dangerous and prevalent field of spread and its excision is as logical as that of any mesentery in close proximity to a cancer” [18]. The quality of the excised mesorectum can be determined by the pathologist. Quirke *et al.* [131] recently classified the specimen into three grades (good, moderate and poor plane of surgery) and found that the plane of surgery achieved i.e. the quality of the TME was an independent risk factor for local recurrence. Preoperative radiation reduced the rate of local recurrence in all three planes of surgery and LRs were almost abolished in short-course preoperatively irradiated patients who had a resection in the mesorectal plane.

In Paper II, remaining mesorectal fat was studied in MRI images of the locally recurrent tumors. Evidence of residual mesorectal fat was found in 50 of the 99 patients studied. Of the 54 patients who underwent surgery for a primary tumor in the upper two thirds of the rectum, 40 (74%) had regions of localized presacral fatty tissue with a surrounding fascia visible on CT or MRI, suggesting residual distal mesorectum. When performing a TME, removal of the upper

mesorectum is less technically demanding than complete removal of the distal mesorectum, especially in a patient with a bulky tumor or a narrow pelvis. Thus, the risk of inadvertently leaving parts of the mesorectum is greater in the lower pelvis. This may explain the high proportion of remaining mesorectum among the patients with a local recurrence. It may also explain why no recurrences were detected in the upper 25% of the pelvis.

Cancer spread to mesorectal lymph nodes within a remaining mesorectum, or diffuse spread, e.g. within vessels might be the origin of a local recurrence. N-stage determined from the nodes in the mesorectum and the distal mesocolon in the surgical specimen was an independent risk factor for local recurrence in Paper III, which has also been the case in many other studies [177, 178]. Spread within the mesorectum distally to the primary tumor has been detected in up to 20% of patients with rectal cancer [72, 137], and discontinuous mesorectal tumor deposits 4 cm distally of the tumor mass have been reported [18, 137]. It has been reported that patients with spread to the lymphatics of the distal part of the mesorectum have a worse outcome than patients who do not [72, 136]. Hida *et al.* noted that the extent of spread within the mesorectum distal to the primary was related to tumor location. The longest distance of mesorectal spread (4 cm) was in the upper rectum and a shorter distance was found in the lower rectum and rectosigmoid tumors [73].

In the Dutch TME trial, the effect of the radiation therapy was mainly seen in patients with lymph node metastases, uninvolved CRM and tumors of the mid rectum [93]. After combining the results of the Dutch TME trial with the observation of remaining mesorectum in patients suffering a local recurrence, one may speculate that the main effect of radiation therapy is to eradicate remaining mesorectal lymph node metastases/small metastatic deposits outside lymph nodes.

Partial mesorectal excision with a 5 cm distal margin in the mesorectum, intentionally leaving the distal mesorectum, has been reported to be an

adequate operation for tumors in the upper rectum [74, 179]. In Stockholm (Paper III), 258(35%) of the 735 patients with primary tumor in the upper rectum underwent PME surgery. The local failure rate following PME was 9%, which corresponds to the recurrence rate observed following APR (9%). However, PME was not a statistically significant risk factor for local failure in the multivariate analysis including tumor characteristics and radiotherapy (Table 6). Based on the present evidence, a recommendation to perform a PME on the more advanced tumors in the upper rectum can be questioned. A prospective randomized trial in Germany is currently recruiting patients to compare PME vs TME for tumors of the upper third of the rectum[180]. The oncological outcome in this trial may give the answer to this question. In a study of colon cancer in Stockholm, Sjövall *et al.* found a local recurrence rate of 12% for tumors in the sigmoid colon [181]. The results in Paper III support the view that there is a need for improvement in the surgical technique for PME when operating on malignant tumors of the distal sigmoid and the upper rectum. The PME technique has been discussed in a recent Japanese study [182]. The authors proposed that rectal mobilization should be followed by blunt dissection of the rectal wall, and then, as the next step, division of the rectum. Having achieved a good visual field, transection of the mesorectum could be completed in an improved manner.

Involved margins

An obvious reason for a local failure is a non-radical (R+) operation where the surgeon reports residual disease or the pathologist reports involved surgical margins. In Paper III, a non-radical operation was reported in 16% of all resections. R+ operated patients had an increased risk of local failure (HR 3.03, $p < 0.001$) and contributed to 33% of all recurrences in the study. A previous study has found that radiotherapy fails to compensate for an involved surgical margin [143]. In Paper IV it was found that patients with irradiated tumors had an increased count of tumor buds at the invasive front, i.e. the outermost boundary of the tumor. It is likely that the invasive front will be left *in situ* in the patient in a non-radical operation. It has

been reported that CRM-positive resections carry an increased risk of local failure[10, 139] and that radiation therapy fails to fully compensate this risk[143]. Radio-resistance of the budding cells may explain why radiation fails to compensate for an involved CRM.

Lateral pelvic lymph nodes

The lateral pelvic lymph nodes are situated in the pelvic wall outside of the mesorectum and therefore are not removed in standard TME surgery. Metastatic disease in these nodes could theoretically be a cause of locally recurrent disease. In Japan, lateral pelvic lymph node dissection is routinely performed for T3-4 tumors below the peritoneal reflection, in order to remove these nodes[76]. A median incidence of 17.8% lateral pelvic nodal involvement was found in a review of Japanese studies [76]. According to the American Guidelines for Colon and Rectal Surgery from 2000[134], dissection should be attempted to remove clinically suspected lateral lymph nodes when technically feasible. Dissection of the lateral lymph nodes was not performed on a regular basis in Stockholm; however nodes suspected of harboring cancer cells at surgery have occasionally been removed. Also, the indication for preoperative radiochemotherapy was liberal when a suspected involved node was found on preoperative imaging.

In Paper II, radiological evidence of lateral lymph node recurrence was found in 6 out of 83 patients with a local failure. The majority (4/6) of these recurrences had the primary tumor below 6 cm from the anal verge and three of these were not treated with preoperative radiation. The results indicate that lateral lymph node metastasis was not a major cause of local recurrence. In a recent study, Kusters *et al.*[183] compared the recurrence pattern for Western and Japanese patients with low rectal tumors. Extended surgery with lateral lymph node dissection was compared with TME surgery with or without radiotherapy. The recurrence rate in the lateral pelvis was 2.2%, 0.8 % and 2.7% in the Japanese, RT+TME and TME group, respectively. The authors concluded that both extended surgery and RT+TME result in good local control as compared with TME alone

and, furthermore, that preoperative radiation can sterilize lateral extramesorectal deposits. In a study from Korea including 366 patients with advanced tumors (T3-4) below 8 cm from the anal verge, recurrence in the lateral lymphnodes was seen in 7 per cent of all patients and was found to be the major cause of locoregional recurrence [184]. The patients in the study population all underwent TME and had preoperative radiotherapy and the follow-up was intense with CT scanning of the pelvis every 6 months.

There are different explanations for the low incidence of lateral recurrences reported in Papers I and II. Preoperative radiation with a target that covers the lateral pelvic lymphnodes was delivered to all lower tumors. Lateral lymph node metastases may constitute a more generalized disease with a high risk of distant metastasis. Asymptomatic lymph node metastasis in the pelvic nodes may then not be observed in a patient with liver or lung metastasis. The true incidence of lateral node metastasis might therefore be higher than that found in the Papers I and II.

Radiation therapy

The care program for rectal cancer in Stockholm during the time period for these studies recommended preoperative radiation for all patients with a resectable rectal cancer except for those with an early cancer at very low risk of having a local failure. The therapeutic decision is made preoperatively, nowadays often in a multidisciplinary team (MDT) meeting including the surgeon, oncologist and radiologist. The effect of radiotherapy in TME operated patients has been proven in randomized trials [10, 97].

The impact of radiotherapy on local failure was studied in Paper III. In this study, 919 (40%) out of 2282 patients were selected not to receive preoperative radiotherapy and omission of radiotherapy was found to be an independent risk factor for local failure (Table 6). An interesting observation in Paper III is that omission of radiation remained as a risk factor despite the fact that the care program recommended radiation for the patients with the higher risk. Although the MDT meeting serves as an aid in decision-making, it is difficult to assess on which grounds

the recommendation to give or omit radiotherapy in an individual patient is based. Interestingly, T-stage and N-stage (assessed on the surgical specimen) did not appear to influence the selection for the radiation therapy in Paper III. However, down-staging among irradiated patients may influence this finding. Theoretically down-staging would reduce the number of advanced tumors among patients that received radiation. Significant downsizing of the tumors and less nodal metastasis was reported after short course therapy in the SRCT [185]. However there was no significant difference in TNM stage in the Dutch TME trial [10]. Patients in whom radiation was not delivered were significantly older and had a higher proportion of tumors located in the upper two thirds of the rectum. The finding of an unexpectedly high rate of local failure for tumors located >10 cm above the anal verge (7%) may be explained by the omission of preoperative radiotherapy in combination with a PME, rather than a TME, for some of these patients. According to an earlier report from the Swedish Rectal Cancer Registry [186], fewer women, independently of age and tumor status, received radiation therapy than men. This difference in the preoperative treatment approach did not result in any significant differences with respect to local failure. This finding may be interpreted such as that the relative difficulty of doing excellent surgery in men can be compensated for by increased usage of preoperative radiation therapy.

Clinical target volume (CTV)

The CTV should include tissues at risk of harboring residual cancer cells after TME surgery. To minimize normal tissue toxicity and adverse effects the CTV should be kept as small as possible and the radiation technique should be optimised not to irradiate tissues outside CTV unnecessarily.

In Paper II, pelvic images of 99 of the 155 patients with a local failure were studied to evaluate the location of the locally recurrent tumor. In addition, the medical records and/or the radiology report describing the site of recurrence was found for 42 of the 72 remaining patients in whom the failure was not radiologically visualized. The

site of the recurrence was in the lower half of the pelvis in more than two-thirds of the patients and in the lower 75% of the pelvis for all patients with a radiologically identified recurrence (Figure 4). Moreover, the center of the four most cranially located failures was situated below the S1-S2 interspace. Data based on the medical records of the patients for whom imaging studies were not available or did not reveal the site also showed that most (33 out of 42) recurrences were in the lower half of the pelvis. Eight were found in the perineum, 4 in the vagina, 18 in the anastomosis, and 3 in the bowel less than 8 cm from the anal verge. These findings strongly suggest that the upper limit of the CTV may not need to be located at the promontory, as usually has been the case at many centers around the world. In a recent review by Roels *et al.* [100], the definition and delineation of CTV in rectal cancer was discussed. The CTV was defined from articles reporting on the incidence and the predominant location of the recurrent tumor and the distribution of lymphatic spread. Seven of the reviewed articles reported on the location of local recurrences. In five of these studies, TME was not performed and in the other two studies, it is unclear whether TME was performed or not. Since local recurrence rates have decreased considerably after the introduction of TME [176, 187, 188] there is limited value in analyzing

recurrence patterns in studies including non-TME surgery. However, in two of the more recently published studies reviewed, it was found that most recurrences (>300 cases analyzed) appeared at the level at or below the anastomosis [189] or that 64% occurred at the site of or posterior to the anastomosis [190]. The results in Paper III are in agreement with these reports.

Also in Paper III, the majority of the recurrences (26/44), in patients with the primary tumor in the upper two thirds of the rectum were seen in the anastomosis. Less common sites were the presacral midline (13), presacral asymmetric (8), or pelvic sidewall (12) (Table 8). The level of recurrence in these patients was only rarely (5 of 44) in the lowest 20% of the pelvis (Figure 4) and only 2 patients had relapses in the pelvic floor. Among patients with primary tumors in the lower third of the rectum (n=39) relapses were seen in a more diverse pattern with recurrences more equally distributed to presacral midline (14), the presacral asymmetric (12), pelvic wall (11), pelvic floor (19), and anastomoses (7). However, the majority (22/39) had relapses in the lower third of the pelvis.

Although radiation therapy in the population studied in Paper III did not include the external and internal sphincters in patients scheduled for an AR, only five (11%) patients with a primary tumor above 5 cm from the anal verge relapsed

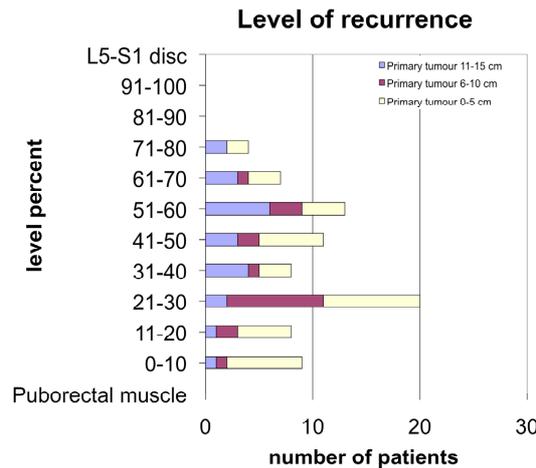


Figure 4. Level of recurrence for patients with radiologic evidence of locally recurrent tumor according to level of primary rectal cancer

in the lowest 20% of the pelvis (Figure 4). This continues to support a CTV that should exclude the external and internal sphincters, as suggested practised already in the Uppsala trial starting patient inclusion in 1980[17] and recommended in most trial protocols. The finding also raises the question of the necessity to include the pelvic floor (puborectal and levator muscle) in patients with a primary tumor above 5 cm from the anal verge for whom a sphincter-saving procedure is planned. Exclusion of the puborectal muscle and the levators, while still including the entire distal mesorectum in patients with tumors located 5-9 cm from the anal verge and not involving the pelvic floor does not markedly raise the lower radiation border but requires more conformed techniques than those used in the past. In this way, it is hoped that late adverse effects of impaired anal function and fecal incontinence can be minimized.

In the study of the invasive front of the tumors in

Paper IV, an increased count of budding cells was found at the invasive front of irradiated tumors, both among the patients with recurrent tumors and the controls. Although the study was not designed to explore radio-sensitivity, this interesting finding raises the question as to whether budding cells are less sensitive to radiation than the more differentiated cells in the more central parts of the tumor. Rubio found that the proliferation marker Ki 67 detected only a small fraction of the budding cells labelled by MNF 116 and speculated on that the majority of the budding cells are in cell cycle arrest (G0) which is a feature associated with radio-resistance [191]. A finding of radio-resistant cells at the invasive front suggests that the surgical margin, for a down sized tumor after radiation therapy, should include the invasive front assessed from the pre-treatment imaging.

Table 8. Radiological findings for 83 patients with radiological evidence of recurrence (When recurrence extended to several sites, all were recorded)

Primary tumor level	No. of pat.	TME	Visible meso-rectal fat	Localisation of the recurrent tumor						
				Anastomotic	Presacral midline	Presacral assymet.	Pelvic wall Med lat		Pelvic floor	
0-5 cm	all	39	38	8	7	14	12	7	4	19
	RT-	13	13	3	2	3	7	1	1	7
	RT+	26	25	5	5	11	5	6	3	12
6-10 cm	all	20	18	13	10	9	5	5	2	1
	RT-	11	10	6	4	4	4	2	2	0
	RT+	9	8	7	6	5	1	3	0	1
11-15 cm	all	24	16	21	16	4	3	5	0	1
	RT-	15	8	13	11	2	2	2	0	1
	RT+	9	8	8	5	2	1	3	0	0

Abbreviations: TME= total mesorectal excision; RT- = no preoperative radiotherapy; RT+= preoperative radiotherapy

PREDICTION OF LOCAL RECURRENCE

The prediction of a local recurrence is of the utmost importance in preoperative treatment planning. A proper selection of patients at risk for a local failure enables an optimized treatment for the individual patient and minimizes the side effects of extensive surgery and preoperative radiation. In Paper III, clinicopathological risk factors for local failure, which theoretically all could have been evaluated preoperatively, were studied in 154 local failure patients stemming from a population-based cohort of 2315 patients, and in Paper IV, the possible predictive value of tumor budding for local recurrence was explored.

Location

The location of the tumor, determined preoperatively by the use of a rigid endoscope, was an important risk factor for local failure in Paper III. The lower tumors were significantly more likely to fail locally than tumors situated in mid rectum (9 vs. 4%, $p < 0.005$), but not significantly more than the upper tumors (9 vs. 7%, $p = 0.13$) (Table 6). In the Dutch TME trial, the local recurrence rate was significantly higher for lower tumors compared to tumors of both the mid- and upper rectum. However, a statistically significant effect of radiotherapy on the local recurrence rate was only seen for tumors in the mid-rectum [93]. An increased risk of an involved surgical margin with a limited effect of radiation may explain the higher proportion of local recurrences in patients with tumors situated in the lower third of the rectum. PME surgery and omission of radiotherapy may explain the higher rate of recurrence of the upper tumors seen in Paper III.

T-stage and N-stage

The results in Paper III showed a significantly lower risk of local failure among the less advanced tumors (T1-2) (Table 6). This finding is in concordance with earlier studies [148, 192, 193]. A meta analysis of MRI studies shows that preoperative MRI can predict T1-T2 tumors with a sensitivity of 82% and a specificity of 76% [194]. MRI may thus be defined as a robust tool with respect to T-staging preoperatively and should be

used as an aid in predicting the risk of local failure in rectal cancer management.

Nodal stage was also of importance for local recurrence in Paper III. In the multivariate analysis N2 tumors had a two-fold risk of local failure compared to N0 (Table 6). However, the N-stage in Paper III was assessed in the surgical specimen and there are many influencing factors to consider. It is known that neoadjuvant therapy reduces the number of lymph nodes that can be found [110]. On the other hand, N-stage in the specimen was not affected by radiation in the randomized trials [10, 97]. The number of investigated lymph nodes may also depend on the pathologist and the surgical procedure performed [195]. Ideally the evaluation of lymph nodes should be based on pretreatment findings. Despite the identification of lymph nodes as small as 2-3 mm on modern planar imaging, reliable detection of nodal metastases is difficult at present with MRI [194]. The use of morphological, rather than size criteria improves nodal staging [41]. In the Dutch TME trial a main effect of radiation on the recurrence rate was seen in N+ patients and there was no statistically significant effect of radiotherapy in T1-3 N0 patients [93]. In the MRC CRO7 trial, an effect of radiation was seen in all TNM stages [22]. One may conclude that because nodal status is difficult to assess preoperatively, and also postoperatively in irradiated patients, no firm conclusions with respect to the predictive value of N-stage can be drawn.

CRM is one of the most important factors influencing the risk of local failure and mortality [139, 196], and it is also possible to predict CRM with MRI [197]. Data on CRM was not available for patients in Paper III and this risk factor was therefore not analyzed.

Grade of differentiation

In the cohort of patients in Paper III, tumors with a low grade of differentiation were more likely to fail locally, although not statistically significantly in the multivariate analysis (Table 6). Grade of differentiation does not influence the response of rectal cancer to preoperative radiochemotherapy [198]. However, in a study by Ueno *et al.*, grade of differentiation along with tumor budding and vascular invasion constituted risk factors

for distal intramural spread [135]. Wang *et al.* found microscopic spread within the mesorectum in patients with tumors with medium and low grades of differentiation, but never in highly differentiated tumors [135]. However, one should bear in mind that tumor grade was assessed in the postoperative specimen in Paper III. Also, grade of differentiation within a single tumor may be heterogeneous and thus, a parameter difficult to assess in preoperative biopsy specimen.

Tumor budding

The possible predictive value of a local recurrence for tumor budding was studied in Paper IV. When the MNF-116 antibody was used, the predictive value of budding frequency was statistically significant in the univariate analysis ($p=0.02$). When included in a multivariate analysis with TNM-stage it remained as an independent significant predictor of local failure ($p=0.02$) (Table 9). The obtained specificity of 89% and sensitivity of 31% in the multivariable model supports the view that the frequency of budding detected by MNF-116 may serve as an additive marker to TNM-stage with a better capacity to select patients at a low risk of local recurrence than to select patients with an increased risk. The present finding is in accord with earlier studies showing that tumor budding correlates with the

risk of local cancer metastasis in the pelvis [199] and intramural spread [135], parameters that may influence the risk of residual tumor deposits in the pelvic region after primary surgery. Previous studies have also shown that tumor budding is a prognostic factor for generalized disease and survival [160-162, 200]. Due to the design used in Paper IV, this was not explored. An earlier study by Ueno found it possible to adequately assess preoperative histopathology findings from the invasive region in the submucosal layer by means of transanal biopsy specimens meticulously taken from the edge of the anal side of the tumor [201]. Budding, together with vascular invasion and tumor differentiation assessed in these biopsy specimens, correlated with extranodal tumor deposits and involved nodes including lateral pelvic lymph node metastasis. To verify the validity of MNF-116 determined tumor budding as a predictor of local recurrence, further studies are needed where budding frequency is determined from preoperative biopsies and analyzed together with other preoperative predictors, e.g. those detected using MRI. The preoperative biopsy specimens must then be taken with greater care than previously was the case, when the chief purpose was to confirm a malignant diagnosis [202].

Table 9. Uni- and multivariate logistic regression analyses including TNM stage, MNF-116 and Ln-5 $\gamma 2$ on local recurrence.

	Univariate			Multivariate				
	OR	p	95% CI	Agreement*	OR	p	95%CI	Agreement**
TNM stage				67%				68%
I	1				1			
II	3.4	0.04	1.03-11.4		2.4	0.16		
III	8.6	0.0007	2.5-29.0		7.0	0.003		
MNF tumour buds								
≤35	1				1			
>35	2.6	0.02	1.2-5.9		2.7	0.02	1.14-6.29	
Ln-5 $\gamma 2$ tumour buds								
≤10	1							
>10	2.0	0.09	0.9-4.5		n.s.			

*TNM stage alone, sensitivity 52% and specificity 77%

** TNM stage and MNF-116 stained budding, sensitivity 31% and specificity 89%.

SUMMARY

Rectal cancer is a common malignant disease in Sweden and is diagnosed in about 1800 patients annually. Despite progress with respect to surgical technique and radiation therapy, some 5-15% of patients still suffer a local recurrence after rectal cancer treatment. Planning of therapeutic approach, preferably in a MDT setting, is highly important in order to balance risk of suboptimal therapy to risk of treatment related morbidity. MRI is an essential tool in preoperative staging. Population based data in this thesis indicate that treatment variables such as omission of radiation therapy for certain subgroups and surgical performance remain as important risk factors for local recurrence and could therefore be subjects for further improvement. Further centralization of rectal cancer management may lead to improved results. After the introduction of the TME technique, new delineation for the clinical target volume should be considered. A lowering of the upper limit of the CTV could be introduced, since local recurrences appear to be situated in the lower $\frac{3}{4}$ of the pelvis. The anal sphincter complex with surrounding tissue can be excluded from the CTV in patients with primary tumors > 5 cm from the anal verge not growing

into or adjacent to the levator muscles. Inclusion of the most distal part of the mesorectum means, however in practise that it is not possible to exclude the cranial parts of the sphincters.. The results also indicate that lateral lymph node dissection should not be recommended as standard treatment for lower rectal cancers since lateral lymph node metastases are an infrequent cause of local failure and the lateral node dissection appears to be connected with increased morbidity. Improvements in the treatment for tumors in the upper rectum appear plausible and the indications for a PME and radiotherapy in these patients need further revision. Tumor location, T-stage and CRM-positivity are predictive factors for local recurrence that are possible to assess preoperatively. N-stage is an important predictive factor but difficult to assess with present imaging techniques. Analyses of tumor budding at the invasive front of the primary tumor may serve as an additive predictive marker but further studies where tumor budding is determined from preoperative biopsy specimen are needed.

Erik Syk

CONCLUSIONS

- Lateral pelvic lymph node metastases do not appear to be a major cause of local recurrence after TME in Swedish patients with rectal cancer. Partial mesorectal excision may be associated with an increased risk of local recurrence for tumors in the upper rectum.
- Local failures appear not to occur in the upper 25% of the pelvis suggesting that a lowering of the upper limit of the radiation target volume may be introduced. This means that for most patients less dose is given to small bowel loops and that the increased risk of late bowel complications could be lower than before. The anal sphincter complex with surrounding may also be excluded for patients with primary tumors >5cm from the anal verge. The latter has been practised at most sites, including Stockholm during the past decade, but was not always the case at some centers prior to that. More fine tuning of the radiation technique is needed. In addition it is important to explore the size of the margin distal to the tumor needed to reach a very low risk of anastomotic/presacral recurrences.
- Tumor specific factors such as distal tumor location, advanced T and N stage are variables of predictive value for local recurrence. Treatment variables such as omission of radiation therapy and surgical performance are important additional risk factors to consider when optimizing the treatment for patients with rectal cancer. The decrease in the proportion of patients irradiated, particularly for tumors in the upper third of the rectum, in Stockholm and otherwise in Sweden during the past 3-5 years is probably a step in right direction. However, the finding that the risk of local failure is not very low for high, non-irradiated tumors tells that the preoperative staging must be further improved. This is important so that the ambition to limit the number of irradiated patients does not come at a price of increased local recurrence rates. Late toxicity may be very serious, like a secondary cancer, but a local failure is often a catastrophe for the patient.
- Tumor budding detected using MNF-116 may be of value when estimating the local recurrence risk in rectal cancer patients. Further studies in which tumor budding and other tumor characteristics are determined from preoperative biopsies are needed for confirmation and clarification of possible clinical implications.

Erik Syk

FUTURE PERSPECTIVES

A technical development in surgery, radiotherapy, imaging and pathology enables refined treatment for rectal cancer. For surgery, a variety of different surgical procedures can be used and an individualized treatment of the patient can be achieved, e.g. local excisions with endoscopic surgery for “early” cancers and the elderly patients. For these patients solid predictive factors for loco-regional spread is of great importance. The treatment for the tumors in the upper rectum needs to be improved. The results from ongoing trials may guide us on indication for radiotherapy and on the surgery of choice. The locally more advanced tumors need a more extensive therapy including neoadjuvant chemoradiotherapy and extensive surgery in specialized centers. The timing of the surgery after the radiation therapy and knowledge in how to handle complete responders to the neoadjuvant treatment are here research issues of clinical interest. Predictive factors for radiation response are needed.

For the radiotherapy, the outlining of the target volume will be of more importance when new techniques, such as intensity-modulated radiotherapy, enables a more precise determination of the target and a more precise delivery of treatment. A more individualized target could be introduced if a reliable preoperative staging of the pelvic lymph nodes was found.

Imaging techniques have developed rapidly. The new generation of high resolution MRI enables us to visualize the pathology of the tumor prior to adjuvant treatment and surgery. The clinical outcome determined by MRI findings has been focused on lately and will probably be of more importance with this new technique. Development of new contrast agents will hopefully improve the discrimination of malignant and non- malignant lymph nodes. PET/CT will become increasingly available and may aid in preoperative staging. In pathology the development in biogenetics will give us tools to analyse gene profiles on which patient will respond to what adjuvant treatment. The preoperative analyses of the tumor will be of more importance and proper biopsy techniques are needed.

In the future, the more individualized treatment based on high-quality preoperative staging including clinical assessment imaging and biological markers will hopefully lead to further reduction in local failure rate and a decrease in treatment related morbidity.

Erik Syk

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all of those who played an important role in the completion of this work. I would especially like to thank:

Professor Bengt Glimelius, my main tutor, for excellent teaching in how to perform clinical research and how to write scientific papers, providing me with knowledge either by personal communication or by showing up as an author of a paper on PubMed in almost every issue in this field of research;

Dr. Sven Goldman, head of the Ersta Hospital Department of Surgery, for introducing me to research (and Bengt Glimelius) and generously teaching me colorectal surgery at Ersta Hospital;

Dr. Per Nilsson, my co-tutor and friend, for all the rewarding discussions, constructive criticism, and necessary support;

Dr. Claes Lenander, my co-tutor, colleague, and friend, for your encouraging optimism and never-ending enthusiasm during hours of cell-counting or cancer surgery;

Professor Olle Ljungqvist, co-author, for all your valuable, professional help as a co-tutor in my first study;

Dr. Lennart Blomqvist and Dr. Michael Torkzad, co-authors, for fruitful collaboration and professional work on my first two papers;

Dr. Carlos Rubio, coauthor, for enthusiastically sharing thoughts about the front of the cancer while being in the front of cancer research;

Dr. Åke Öst, for all your help with the evaluation of the tumor blocks while teaching me the history of Stockholm;

Marja Hallström, for excellent assistance with the staining of specimens.

Jan Kowalski, for excellent statistical advice.

Toom Singnomklo, for help with providing me the data on the patients and for all your work pertaining to the quality of the database, upon which this thesis is based;

Mikael Machado, Jonas Nygren, Stefan Carlens, Ulf Gustafsson, Marit Kressner, Solveig Elmér and Josefin Segerman, for being fantastic colorectal team colleagues at Ersta. It is great to work with you!

Erik Syk

All staff on the surgical wards, outpatient clinics and in the operating rooms at Ersta Hospital for all your support, and avdelning 5: "Tack för kaffet"!

Isaac Austin, for correcting my English;

Ringvor Hägglöf, for excellent help with the final layout of this thesis;

Last, but not least, Hélena, Cecilia, and Adam, my beloved family, for keeping up with a distracted absent-minded family father. You are my best friends; I will be back!

This work has been supported by the Swedish Cancer Society and the Cancer Society in Stockholm. Financial support was also provided through the regional agreement on medical training and clinical research (ALF) between the Stockholm County Council and Karolinska Institutet.

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