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Abstract

In Sweden, 2000 patients are diagnosed with rectal cancer annually. In 1995, the Swedish Rectal Cancer Registry (SRCR) was launched to supervise and assure the quality of the management of rectal cancer. Advances in the management of rectal cancer have reduced the local recurrence (LR) rate and improved survival. To improve the outcome further, identification of prognostic and predictive factors is important for optimal, personalised neoadjuvant/adjuvant treatment and follow-up strategies.

This thesis identifies potential risk factors of tumour recurrence and reduced survival – i.e., surgery-related and tumour biology-related prognostic factors – in a cohort of patients registered in the SRCR between 1995 and 1997 with 5-year follow-up. SRCR data were used and for subgroups additional data from the original medical records were retrieved. In addition, SRCR data were validated.

In Paper I, preoperative radiotherapy (RT) significantly reduced the LR rate irrespective of the tumour height. Moreover, preoperative RT and rectal washout reduced the LR rate after incidental perforation. Preoperative RT prolonged time to LR. LR was an isolated tumour manifestation in 39% of the patients with LR. Paper II showed that anastomotic leakage had no impact on the oncological outcome. In Paper III, incidental perforation was a significant risk factor of increased LR and overall recurrence rates as well as reduced overall and cancer-specific 5-year survival. In Paper I–III, the validity of SRCR data was acceptable. In Paper IV, high immunohistochemical expression of the tumour marker ezrin in primary tumours from patients with LR correlated to earlier occurrence of LR. A linkage of high ezrin expression and aggressive biological behaviour is suggested.

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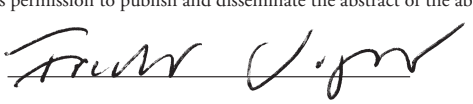
Signature  Date August 24, 2010

I must fight this sickness, find a cure.
Robert Smith

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Contents

List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I: Jörgren F, Johansson R, Damber L, Lindmark G. Risk factors of rectal cancer local recurrence: population-based survey and validation of the Swedish Rectal Cancer Registry. *Colorectal Dis.* 2009 Apr 27. [Epub ahead of print];doi:10.1111/j.1463-1318.2009.01930.x

II: Jörgren F, Johansson R, Damber L, Lindmark G. Anastomotic leakage after surgery for rectal cancer: a risk factor of local recurrence, distant metastasis and reduced cancer-specific survival? *Colorectal Dis.* 2009 Nov 14. [Epub ahead of print];doi:10.1111/j.1463-1318.2009.02136.x

III: Jörgren F, Johansson R, Damber L, Lindmark G. Oncological outcome after incidental perforation in radical rectal cancer surgery. *Int J Colorectal Dis.* 2010;25:731-40.

IV: Jörgren F, Nilbert M, Rambech E, Bendahl P-O, Lindmark G. Ezrin expression in rectal cancer predicts time to development of local recurrence. *Manuscript*.

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Abbreviations

ACF	Aberrant crypt foci
AJCC	American Joint Committee on Cancer
AL	Anastomotic leakage
APR	Abdominoperineal resection
AR	Anterior resection
CRC	Colorectal cancer
CRM	Circumferential resection margin
CT	Computed tomography
DM	Distant metastasis
DRM	Distal resection margin
EUS	Endorectal ultrasound
FAP	Familial adenomatous polyposis
Gy	Gray
HA	Hartmann's procedure
HNPCC	Hereditary non polyposis colorectal cancer
IHC	Immunohistochemistry
ITC	Isolated tumour cells
LAR	Low anterior resection
LR	Local recurrence
MDT	Multidisciplinary team
MRI	Magnetic resonance imaging
OAR	Overall recurrence
PCR	Polymerase chain reaction
PET	Positron emission tomography
PME	Partial mesorectal excision
PRM	Proximal resection margin
SRCR	Swedish Rectal Cancer Registry
RT	Radiotherapy
TMA	Tissue microarray
TME	Total mesorectal excision
TNM	Tumour, Node, Metastasis
UICC	Union Internationale Contre le Cancer

Abstract

In Sweden, 2000 patients are diagnosed with rectal cancer annually. In 1995, the Swedish Rectal Cancer Registry (SRCR) was launched to supervise and assure the quality of the management of rectal cancer. Advances in the management of rectal cancer have reduced the local recurrence (LR) rate and improved survival. To improve the outcome further, identification of prognostic and predictive factors is important for optimal, personalised neoadjuvant/adjuvant treatment and follow-up strategies.

This thesis identifies potential risk factors of tumour recurrence and reduced survival – i.e., surgery-related and tumour biology-related prognostic factors – in a cohort of patients registered in the SRCR between 1995 and 1997 with 5-year follow-up. SRCR data were used and for subgroups additional data from the original medical records were retrieved. In addition, SRCR data were validated.

In Paper I, preoperative radiotherapy (RT) significantly reduced the LR rate irrespective of the tumour height. Moreover, preoperative RT and rectal washout reduced the LR rate after incidental perforation. Preoperative RT prolonged time to LR. LR was an isolated tumour manifestation in 39% of the patients with LR. Paper II showed that anastomotic leakage had no impact on the oncological outcome. In Paper III, incidental perforation was a significant risk factor of increased LR and overall recurrence rates as well as reduced overall and cancer-specific 5-year survival. In Paper I–III, the validity of SRCR data was acceptable. In Paper IV, high immunohistochemical expression of the tumour marker ezrin in primary tumours from patients with LR correlated to earlier occurrence of LR. A linkage of high ezrin expression and aggressive biological behaviour is suggested.

Introduction

General background

The incidence of rectal cancer varies worldwide. High incidences are found in Europe, North America, Australia and New Zealand and low incidences are found in Asia, Africa and South America[42]. In Sweden, 2047 patients in 2008 were diagnosed with rectal cancer, defined as adenocarcinoma located, completely or to some part, within 15 cm from the anal verge[229]. Rectal cancer is the 7th most common cancer in men and women in the country[229]. Taken the genders together, it is the 8th most common malignancy. Rectal cancer is more common among males. In 2008, the age standardized incidence in Sweden was 29 per 100 000 in males and 19 per 100 000 in females[229]. Figure 1 shows the incidence according to the Swedish Rectal Cancer Registry (SRCR)[230]. These figures are somewhat lower than the incidence presented by the National Board of Health and Welfare[229], because in the SRCR tumours located in the rectosigmoidal junction above 15 cm from the anal verge and tumours diagnosed at autopsy are excluded. Since the 1980s, the total number of diagnosed patients with rectal cancer has increased in Sweden; however, when standardized for increasing population and age among the population, only a slight increase in incidence is seen over the last decades[175, 229]. The median age at diagnosis is 72 years in the SRCR[230]. The risk of developing rectal cancer before the age of 75 is 1.6% in males and 1% in females[229].

Risk factors for developing rectal cancer include heredity, increasing age, male gender, previous colonic polyps or colorectal cancer (CRC), obesity, diabetes mellitus, and inflammatory bowel disease (ulcerative colitis and Chron's disease). Dietary risk factors include high intake of processed red meat and a high-fat diet; inadequate fibre intake may also increase risk although this has not been established. In addition, lifestyle factors such as smoking, high intake of alcohol, as well as low physical activity are also associated with increased risk of rectal cancer. The dietary risk factors, smoking habits, and alcohol consumption may explain the differences in incidences between genders and the geographical variations of rectal cancer. Several of the risk factors are associated with a Western lifestyle. Increasing incidences of CRC have been demonstrated in less-developed countries adopting a Western lifestyle. Migration studies have also reported a higher incidence among immigrants to high-incidence Western countries in comparison to the population remaining in the low-incidence, less-developed countries: both these findings indicate the importance of lifestyle factors. Calcium and vitamin D intake may have a preventive effect as well as use of non-steroidal anti-inflammatory drugs[42, 44, 52, 95, 96, 174, 175].

Adenoma is the precursor to rectal carcinoma. About 10% of adenomas progress to invasive carcinoma through a sequence of well-defined histological steps called the adenoma-carcinoma sequence. This process takes 10–15 years[245]. In approximately 80% of the patients, the cancer is sporadic; in the rest it is hereditary. Approximately 5% of the patients with CRC belong to a defined hereditary syndrome, and the remaining 15% that are inherited represent familial CRC without any up to now identified genetic cause. The most common defined hereditary syndromes are non-polyposis colorectal cancer syndrome (HNPCC), accounting for 2–5% of all CRC, and familial adenomatous polyposis syndrome (FAP), accounting for <1%[52, 113, 174, 175].

The primary treatment modality in rectal cancer is surgery. In the past two decades, advances in the management of rectal cancer have reduced the local recurrence (LR) rate and increased the overall survival rate. Today, the LR rate is below 10% and the 5-year cancer-specific survival is above 60% in Sweden[182]. Standardised preoperative staging, in-

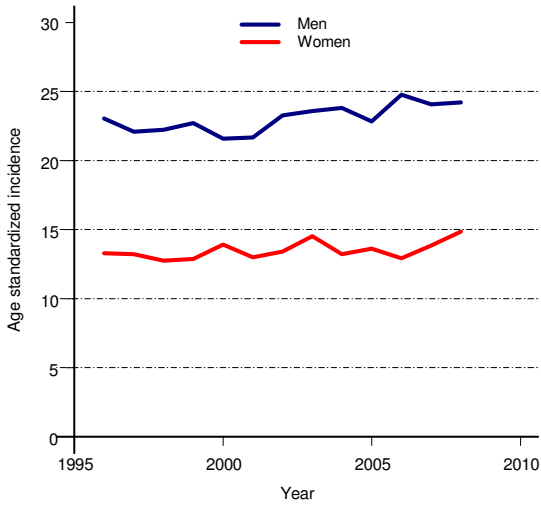


Figure 1. The age standardized incidence in the Swedish Rectal Cancer Registry for 1996-2008.

creased use of pre- or postoperative radiotherapy (RT) and chemotherapy, refined surgical technique [total mesorectal excision (TME) technique], centralisation of surgery to specialist colorectal units, and introduction of the multidisciplinary team (MDT) conference approach may contribute to these reductions[75, 98, 146, 182, 187, 217].

Despite the advances in the management of rectal cancer, tumour recurrence still affects many patients. Although promising results have been reported from dedicated centres, the majority of patients with tumour recurrence will not survive[43, 99, 110, 184, 205, 219, 220, 244]. Risk factors for tumour recurrence may be related to the patient (advanced age and male gender), the treatment (suboptimal use of pre- and postoperative treatment and surgical technique with circumferential or distal margin involvement), or the tumour (low tumour, poor tumour differentiation, vascular invasion, lymphatic vessel invasion, advanced TNM stage, and intrinsic biologic aggressive behaviour)[2, 49, 272]. The morbidity of tumour recurrences is also vast.

To improve the results further, identification of surgical and tumour biological risk factors of tumour recurrence is of utmost importance.

The Swedish Rectal Cancer Registry

In 1995, the Swedish Board of Health and Welfare founded the SRCR to supervise and assure the management of patients with rectal cancer. The SRCR is a national population-based registry that prospectively collects data for all patients with rectal cancer[182]. Sweden is divided into six healthcare regions each with a regional oncology centre. The departments of surgery provide data on each patient with newly registered rectal cancer to the regional oncology centres where the data are checked for accuracy and completeness of registration. The revised data are forwarded to the Umeå Regional Oncology Centre for compilation and analysis. Primary data – information about the patient (age and gender) the tumour (TNM-stage), the preoperative assessment, surgical treatment, local radicality and early postoperative complications – are registered

after the performed surgery or at diagnosis in patients with no surgical treatment. Follow-up data – including information on late complications, recurrences, and death – are registered annually for five years from surgery or from diagnosis in patients with no surgical treatment. Validation by an independent observer and by several research projects has shown that the validity is acceptable with around 5% discrepancy, but the postoperative morbidity is underestimated.

The registry has over 97% degree of covering, and 5-year follow-up data are available for more than 98% of the patients. The SRCR is continuously linked to the Swedish Cancer Registry and to the Causes of Death Registry. Reports from the SRCR on primary and follow-up data are compiled annually[230]. The SRCR is continuously changing. In 2007, online registration was introduced, and several new variables were added to the primary registration form. Data concerning preoperative staging, surgical procedure, and pathology were more detailed. In 2009, a special form concerning oncological treatment was added. From the start in 1995 until 2010, data on approximately 24 000 patients with rectal cancer have been collected in the registry. Data of several variables in the SRCR and influence on the oncological outcome are given in Table 1.

Local recurrence

Definition

Definition of LR varies in the literature[150]. In the SRCR, LR is registered if there is the presence of tumour growth at the anastomotic site, perirectally, in the lesser pelvis, perineum, or at another site as documented by clinical, radiological, or pathological examination or examination at surgery or autopsy. LR in the lesser pelvis includes tumour growth in the vagina, bladder, or lateral pelvic lymph nodes. LR at another site includes tumour growth in the rectal stump after Hartmann's procedure (HA) or at the top of the stoma after abdominoperineal resection (APR) or HA, which is synonymous with the proximal resection margin.

In the 1970s and 1980s, LR rates between 30–70% were reported[181], which had a major impact on survival. Today, with modern management, LR rates below 10% are presented from centres of excellence[98], multicentre trials[187, 216] as well as population-based registries[118, 182, 255]. When comparing LR rates in different studies, one must consider that the definition of LR varies, the strategies for neoadjuvant/adjuvant treatment are different, and the duration of follow-up might not be similar. Some studies report the crude rate and some studies report the rate after curative surgery[53, 150].

Time to development

It has been reported that 90% of the LR occurs within two to three years after primary surgery[105, 163]. Neoadjuvant treatment may lengthen the time to LR possibly increasing the proportion of LR diagnosed later than two to three years after primary surgery[105, 164, 187]. This may motivate a more protracted time period for follow-up in patients receiving neoadjuvant treatment.

Treatment

LR in rectal cancer is an isolated tumour manifestation in 30–50% of patients in contrast to LR in colon cancer, which is commonly accompanied by DM[99, 105, 184]. Today, results for salvage surgery of LR in dedicated centres are improving[43, 99, 184, 244]. With optimal preoperative work up and patient selection, survival rates are around 30%. Early detection of LR before dissemination is a prerequisite; however, the majority of patients with LR will eventually die from their LR with severe morbidity. In a population-based Swedish report, surgery was performed in 40% of the patients with di-

Table 1. Data on oncological outcome for patients treated with major abdominal surgery (AR, APR and HA) in the Swedish Rectal Cancer Registry between 1995 and 2002 with 5-year follow-up.

		No. of patients	LR	DM	5-year cancer specific survival	5-year overall survival
Age (years)	<65	2657	8	25	73	65
	65-74	3072	8	22	70	57
	75-79	1706	7	21	68	47
	>80	1760	9	16	65	35
Gender	Male	5263	8	22	68	51
	Female	3932	8	21	71	56
Tumour height (cm)	Low: 0-5	2791	9	24	65	51
	Medium: 6-10	3453	8	20	70	54
	High: 11-15	2829	7	20	74	55
TNM stage	I	2088	3	7	92	76
	II	2956	7	16	81	63
	III	2903	12	37	59	44
	IV	1138	8	-	17	10
Surgery	AR	5373	7	20	75	60
	APR	2749	10	25	64	50
	HA	1073	10	20	51	28
Local radicality[a]	Yes	5968	7	20	79	62
	No	251	19	40	41	30
	Uncertain	181	14	34	46	31
Preoperative RT	No	4396	9	18	68	46
	Yes	4752	7	24	71	60
Preoperative CHT	No	8961	8	21	70	53
	Yes	127	16	35	51	38
Rectal washout[b]	No	1018	10	22	71	52
	Yes	4257	6	19	77	62
Perforation[c]	No	8285	8	21	71	55
	Yes	640	13	24	55	38
AL[b]	No	4867	7	20	76	61
	Yes	506	7	20	65	54
Postoperative RT	No	8108	7	22	72	56
	Yes	221	39	51	33	24
Postoperative CHT	No	6820	7	18	75	58
	Yes	1410	14	49	49	40

Values in columns are percentage and data in LR and DM columns are crude rates. AR, anterior resection; APR, abdominoperineal resection; HA, Hartmann's procedure; AL, anastomotic leakage; RT, radiotherapy; CHT, chemotherapy; LR, local recurrence; DM, distant metastasis. [a] According to the pathology reports for patients with tumours in TNM stages I-III. During 1995–2002 local radicality was registered in 5/6 oncological regions. [b] For AR. [c] Intraoperative incidental rectal perforation.

agnosed LR[184]. In 44% of the patients treated with surgery, the resection was curative; in this group, the 5-year overall survival was 57%. For the whole group of patients with LR, the 5-year overall survival was only 9%.

Distant metastasis

Definition

In the SRCR, DM is defined as the presence of tumour growth in any lymph node outside the pelvis, or in the ovary, liver, lung, peritoneum, bone, brain, or in any other organ as documented by clinical, radiological, or pathological examination or examination at surgery or autopsy.

Approximately 17% of the patients with rectal cancer have DM at diagnosis according to the SRCR[230]. The metastases are confined to the liver in 69% of the patients and to the lungs in 16%. Among patients with radical surgery for primary tumours in TNM stages I–III, DM will develop in 21% within five years[230]. The liver is the most common site (53%) followed by the lungs (46%).

Treatment

The prognosis for patients with DM at diagnosis as well as for those who develop DM is dismal. The 5-year survival in patients with untreated metastatic disease is less than 5%; however, using optimal preoperative staging and patient selection, dedicated centres have shown improving results in the treatment of metastatic disease with surgery alone or in combination with other treatments[205, 219, 220]. As for LR, only a small number of patients is suitable for surgery, and in only a proportion of these patients a curative resection is achievable. Approximately 20–30% of patients with liver metastases are potentially resectable[220]. The 5-year overall survival varies between 25–60%[219]. The lower figures are for all patients where surgery is performed, whereas the higher figures are for patients where curative surgery is achieved. Considering lung metastases, 10% of the diagnosed patients are candidates for surgery[205]. The reported 5-year overall survival has ranged from 25–65%[110, 205].

Survival

When analysing survival in rectal cancer, the high median age at diagnosis must be considered. The reported 5-year overall survival and the 5-year cancer-specific survival in the SRCR are 45% and 62%, respectively[182]. Compared to the 1970s and 1980s, these figures are in absolute numbers about 20% higher, reflecting the altered management of rectal cancer[20, 182, 232]. Today, in Sweden the prognosis is better for rectal cancer than colon cancer[20, 182, 232]. Other population-based reports show the same trends[37, 62, 167, 255]. Survival curves from the SRCR are shown in Figure 2.

Age

Rectal cancer is a disease of the elderly. The prognostic value of age is difficult to evaluate. Coexisting disease and decreased physical performance may withhold optimal neoadjuvant/adjuvant treatment from this group of patients, which might bias the outcome. Few studies investigate the impact of preoperative RT and adjuvant chemotherapy in the elderly, usually defined as patients >75 years[68, 210]. The results in the studies might also be influenced by insufficient follow–up in this group of patients. A study from the SRCR concluded that major abdominal surgery – i.e., anterior resection (AR), APR, and HA – was performed significantly less frequently and preoperative RT was significantly less used in Sweden for the elderly[119]. The relative survival was worse for the elderly, but there were no detectable differences in LR rate. Registry data from Norway confirmed the findings concerning LR rate and revealed a similar DM rate in elderly patients treated with cura-

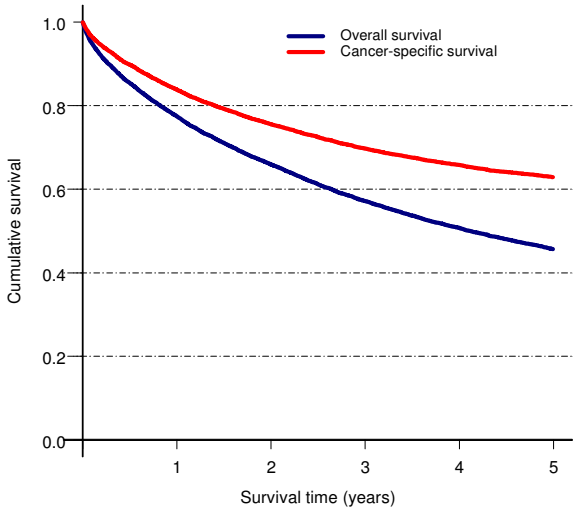


Figure 2. The 5-year overall and cancer-specific survival rate for all patients registered in the Swedish Rectal Cancer Registry, 1995-2002.

tive surgery compared to younger patients[64]. However, the relative survival among elderly treated with curative surgery was the same as in the younger patients. Obviously, overall survival is less for the elderly because of co-existing diseases[64, 68, 210].

Gender

Cancer-specific survival and overall survival are worse in males than females after surgery for rectal cancer[154, 156, 256]. The differences might be explained by the fact that males have a narrower pelvis than females, an anatomical limitation that makes surgery more difficult[156]. Gender-specific immune function is another possible mechanism[156, 256]. A Swedish study found that females in Sweden received preoperative RT less often than males, but they had better cancer-specific and overall survival[154]. Data on differences in LR rate between the genders are scarce in the literature. However, in the Swedish study, the LR rate was not different with respect to gender[154].

Preoperative staging

The preoperative local staging in rectal cancer includes rectal digital examination, rigid sigmoidoscopy, pelvic magnetic resonance imaging (MRI), and, when indicated, endorectal ultrasound (EUS). Chest and abdominal computed tomography (CT) is standard for staging of DM. Ultrasound of the liver and chest X-ray can be accepted. Ultrasound enhanced with contrast of the liver can complement CT when the findings are uncertain. Rigid sigmoidoscopy with biopsies is used for histopathologic ratification of an adenocarcinoma and assessment of the distance of the tumour from the anal verge. Rigid sigmoidoscopy cannot be correctly replaced by colonoscopy. Pelvic MRI is the most important investigation in the preoperative staging since it enables selection of patients in need of preoperative treatment[125, 160, 161, 224, 243]. Based on the assessment of the distance from the tumour to the circumferential resection margin (CRM), the patients are selected to no preop-

erative treatment, preoperative RT, or preoperative chemoradiotherapy. MRI can depict the extramural tumour spread with a mean difference of less than 0.5 mm with histopathological results as reference standards[161, 224, 243]. CRM involvement, commonly defined as tumour growth within 1 mm of the mesorectal fascia, is predicted with 92% sensitivity[160, 224, 243]. EUS is important in discriminating between T1- and T2-tumours as it is superior to MRI in assessing the mural invasion in the superficial bowel layers. However, MRI is superior for estimating deeper invasion[125]. For assessment of lymph nodes, all modalities (i.e., EUS, MRI, and CT) are insufficient, because the criteria for predicting lymph node involvement are unreliable. A size >8 mm has been defined as malignant, but this is not a reliable criterion as smaller lymph nodes have proven to be malignant. Other morphological features such as presence of mixed signal intensity within the lymph node or irregular borders due to capsule penetration have also proven unreliable[224, 243]. MRI with ultrasmall particles of iron oxide (USPIO) has shown promising results in identifying small tumour foci within mesorectal lymph nodes and might be useful in the future[224]. When the standard preoperative staging is uncertain concerning disseminated disease, positron emission tomography (PET) might provide additional information[243]. To exclude synchronous tumours and achieve a "clean" colon, colonoscopy should be performed, eventually substituted by CT colonography[174, 175].

Multidisciplinary team conference

During the last decades, multidisciplinary team (MDT) conferences have been established in Sweden. The conferences are structured meetings with surgeons, radiologists, pathologists, oncologists and special nurses. Each patient is individually discussed, pre- as well as postoperatively, and decisions concerning preoperative staging, treatment and follow-up are made. Thus, the aim of the MDT conferences is to tailor the optimal oncological treatment, neoadjuvant as well as adjuvant, and the optimal surgical procedure. Implementation of MDT conferences has been shown to select more patients to neoadjuvant treatment and to lower the rate of CRM involvement, which is an early surrogate marker for LR[36]. Improved survival has also been demonstrated[146]. Among patients with stage IV disease at diagnosis, more patients are referred to metastasis surgery after MDT discussion with a subsequent improval in survival[217].

Surgery

Surgery is the primary treatment of rectal cancer. Among patients registered in the SRCR between 1995 and 2003, surgery was performed in 90%[182]. Rectal cancer surgery can be performed as open major abdominal surgery – i.e., AR, APR, HA, or minimally invasive, local excision (LE) procedures. The most important aim is to achieve R0 resection to ensure cure and long-term survival. Local control to avoid LR is of utmost importance. The second aim is improvement or maintenance of the patient's quality of life. Preservation of normal defecation, bladder, and sexual functions are other important matters to consider. However, the oncological outcome is the main priority in rectal cancer surgery.

Surgical technique

The rectum is in its lateral and posterior parts surrounded by the mesorectum. The mesorectum is fatty tissue containing the lymph nodes that drain the rectum. The mesorectum is enclosed by the mesorectal fascia. In traditional rectal cancer surgery, dissection had been performed bluntly, and the importance of an intact mesorectum and adequate tumour resection margins ignored. In the early 1980s,

Heald *et al.,* used the earlier described TME technique[97]. In TME surgery, sharp dissection in the avascular plane surrounding the mesorectum down to the pelvic floor is performed. The mesorectal fascia is kept intact, and the hypogastric and parasymphatetic pelvic nerves are preserved. The entire mesorectum including the draining lymph nodes is resected and thereby a potential source for LR is eliminated. The superiority of the TME technique was later confirmed in histopathological studies by Quirke *et al.,*[197]. Today, TME is the gold standard in rectal cancer surgery worldwide. In Sweden, it was introduced nationally in the early 1990s through several workshops and training programmes[151, 182]. The superiority of the TME technique with or without neoadjuvant therapy in reducing the LR rate and increasing the survival has been demonstrated in several reports[98, 123, 187, 255].

Local excision

LE includes a variety of procedures such as transanal local resection (TAR), transanal endoscopic microsurgery (TEM), or the posterior approaches (ad modum Mason or Kraske)[142, 174]. In the SRCR, colonoscopic polypectomy and destruction by electrocautery are also registered as LE[230]. In the curative setting, LE is an acceptable procedure for T1N0M0-tumours without cytological or histological high-risk features such as poor histopathological differentiation, vascular or neural invasion, presence of mucinous histology, and tumour ulceration[142, 174]. Thus, a prerequisite for performing local surgery is thorough preoperative local staging with EUS. The full thickness techniques – TAR and TEM – are the methods of choice if the intention is cure. In TAR, specially developed instruments are used to expose the rectal mucosa. TAR is suited for lesions in the lower third of rectum. In TEM, the surgery is performed through a specially constructed proctoscope with an attached microscope. The TEM technique permits resection up to 20 cm from the anal verge. With thorough preoperative local staging and selection of patients, the oncological results after LE are the same as after major abdominal surgery[76, 142]. Other indications for LE are elderly patients or patients with comorbidity for whom a major abdominal resection is too traumatic with a high risk of morbidity or mortality. LE also plays a role in the palliative setting in patients with disseminated disease and a small primary tumour [142, 174]. In the SRCR, approximately 5% of the patients, including all indications, are operated with LE[182]. Combining chemoradiation with LE has been studied, but so far data are insufficient[142, 174].

Anterior resection

In AR, the bowel ends are immediately anastomosed after resection of the tumour-bearing segment, a technique that preserves the anal sphincter. In the beginning, this technique was reserved for high situated tumours, but with the introduction and development of stapling devices patients with tumours in the middle and lower thirds of rectum are also candidates for anastomosis. When used in low-situated tumours the procedure is named low anterior resection (LAR). In Sweden, 50% of the patients treated with surgery have an AR. Anastomotic leakage (AL) is complication specific for AR. Clinical leakage is detected in approximately 10% of patients after AR in Sweden[182]. A Swedish study has proven that a temporary, defunctioning stoma mitigates the consequences of an AL and significantly reduces the AL rate[155]. In Sweden, a temporary defunctioning stoma is recommended in LAR and usually a loop ileostomy is constructed[174, 175]. Another procedure-specific complication is the anterior resection syndrome. This syndrome consists of increased frequency, urgency, fragmentation of faeces and incontinence. It has been reported that 50–70% of the patients experience some sort of anorectal dysfunction after sphincter preserving surgery[174, 175].

Abdominoperineal resection

APR is the most technically demanding procedure for rectal cancer. The conventional APR starts with an abdominal dissection where the mesorectum is followed down to the pelvic floor and the top of the anal canal. The mesorectum is mobilized from the levator muscles. The sigmoid is transected, and an end-colostomy is constructed. The procedure is then completed from the perineum with excision of the anal canal, ischiorectal fat and lower portions of the levator muscles with the patient in the supine position. APR is indicated for tumours located within about 0–6 cm from the anal verge[174, 175]. However, if the tumour is not growing into the pelvic floor or the sphincter musculature, an anastomosis is possibly created. This requires a functionally continent sphincter. The decision to perform an APR depends on the preoperative evaluation, but sometimes the decision to perform an APR is made during the surgical performance when additional information is available. APR is performed in 25% of the surgically treated patients in Sweden, which is a somewhat higher figure than in other parts of Europe[182]. APR for low tumours has been associated with higher LR rates and poorer survival compared to AR for high tumours. This has been attributed to higher rates of CRM involvement and intraoperative perforations with the conventional APR technique, which may contribute to an inferior oncological outcome[6, 107, 171, 218, 251, 254]. APR with the conventional technique, as described above, often results in a waist on the specimen with a thin outer border consisting of the outer muscle layer of the rectal tube. The thin outer border constitutes the CRM with a subsequent high risk of involvement. The supine position of the patient limits the visualisation field during the perineal part of the procedure, which in addition to the thin border at the waist increases the risk for intraoperative perforation. APR by an extended posterior approach has been introduced to avoid the waist of the specimen and to lower the perforation rates[107]. The abdominal part of this approach is terminated at the upper borders of the levator muscles. The termination of the abdominal part at an earlier stage is the crucial detail of this approach. The dissection is continued until the insertions of the levator muscles on the pelvic sidewalls. Before the perineal part, the patient is turned to the prone jack-knife position. The visualisation is ensured for the surgeon and a cylindrical, thicker specimen is achieved as the levator muscles are attached to the specimen. Lower rates of CRM involvement and perforation as well as improved oncological outcome have been reported. Morbidity has not been found to be increased. However, long-term results still need to be assessed[107, 171, 251].

Hartmann's procedure

HA consists of a rectosigmoid resection without restoration of the bowel continuity. The proximal colon is fashioned as an end-colostomy, and the rectal stump, or sometimes the anal canal, is left as a pouch. SRCR data reveal that this approach is used in 10% of the patients treated by surgery[182]. HA is indicated as an alternative to AR in patients with pre-existing faecal incontinence, high risk for anastomotic complications, as well as poor medical condition with subsequent inability to manage an AL if such a complication should occur. In Sweden, the use of HA has increased[182]. The LR rate after HA is similar to the other major abdominal surgery procedures, but since HA is more frequent among the oldest patients with rectal cancer, a lower overall survival is seen[182].

Pelvic exenteration

In approximately 10% of the patients with rectal cancer, there is invasion to adjacent organs, which necessitates an extended surgical procedure to achieve R0 resection[71, 174, 183, 209, 264]. The

preoperative assessment and the MDT discussion are crucial. Preoperative chemoradiotherapy is mandatory, and the operating team must often include other surgical subspecialists than the colorectal surgeon. Intraoperative RT can also be used[71]. Depending on the extent of the tumour, the bladder, the internal genital organs (i.e., prostate/seminal vesicles or uterus, ovaries and/or vagina), and the distal sacrum are resected en bloc with the primary tumour. A total pelvic exenteriation is sometimes performed. R0 resection rates of 60%, LR rates of 40%, and 5-year overall survival of 40% have been reported from dedicated centres. The morbidity after this extensive surgery is high.

Other procedures

Other surgical procedures account for approximately 10% of the surgical procedures registered in the SRCR[182]. Other procedures are indicated in the palliative setting and include procedures without tumour resection such as exploratory laparotomy with or without stoma formation as well as various procedures with by-pass shunting of the bowels.

Tumour height

Tumour height may influence the oncological outcome with worse outcome in low situated tumours; however, the evidence is not clear cut. Different definitions of the levels contribute to the difficulties in evaluating the existing data. One issue is the comparison of rectal cancer to colon cancer. An older study compared tumours in the upper rectum (10–15 cm) with tumours in the sigmoid colon and in the lower rectum (<10 cm) and found that the oncological outcome of tumours in the upper rectum was similar to tumours in the sigmoid colon concerning tumour recurrence and survival[144]. Based on the finding that tumour-spread in the longitudinal direction is extremely rare (see section on resection margins), the partial mesorectal excision (PME) technique has been accepted for the high situated rectal cancers[144, 174, 175]. PME is transection of the mesorectum and the bowel wall 5 cm below the tumour without performing a TME. However, a study from Sweden on the sites of LR after R0 surgery revealed that PME for tumours in the upper third of rectum might be associated with an increased LR risk due to LR emanating from tumour deposits in residual mesorectum[231]. In addition, a recent study comparing tumours in the sigmoid colon with tumours in the upper and middle rectum (5.1–10 cm) found that tumours in the upper rectum behaved more like tumours in the middle rectum than in the sigmoid colon[206]. A less favourable oncological outcome was stated for patients with tumours in the upper rectum than in the sigmoid colon. The study concluded that tumours in the upper rectum might benefit of more aggressive therapy than primary resection followed by adjuvant therapy in selected cases.

The other issue is the differences between tumours at the different rectal levels. Low tumours have been correlated to higher LR rate and worse survival[115], but studies adjusting for several covarieties have shown the opposite[138, 254]. These studies have suggested that the surgical technique might be inferior for low situated tumours, but with optimal management the outcomes might not be worse for low situated tumours. As stated in the section on RT, the importance of RT at the different heights is also under debate. A thorough measure of the tumour height is of utmost importance in the clinical setting, since it guides in the decision-making when choosing surgical technique, PME or TME, as well as neoadjuvant treatment.

High *vs.* low tie

There is no consensus whether the inferior mesenterior artery (IMA) should be ligated at its aortic origin (high tie) or if the superior rectal artery (SRA) should be ligated below the

origin of the left colic artery (low tie) during rectal cancer surgery. Advocates of the high tie technique mean that it improves survival and the accuracy of tumour staging as it implicates the resection of the apical lymph nodes along IMA. Advocates of the low tie technique believe that high tie compromises the blood flow to the proximal limb of the anastomosis or the colostomy and increases the risk for autonomous nerve damage. Two recently published reviews came up with the same results, but with opposite conclusions[136, 235]. None of the reviews found any evidence for either of the strategies. However, Titu *et al.,*[235] concluded that high tie should be used, whereas Lange *et al.,*[136] concluded that low tie should be used. The national Swedish guidelines presently recommend low tie[175].

Surgical lateral lymph node retrieval

Considering the management of lateral pelvic side wall lymph nodes, there are different approaches in the West *vs.* in Japan and in some institutions in the USA. These lymph nodes are not removed by the TME procedure. The Western approach has been preoperative RT or chemoradiotherapy if clinical suspicion of lateral lymph node involvement exists. In Japan, extensive lateral lymph node dissection has been used. The lateral lymph node dissection is associated with very high morbidity, i.e., impotence and bladder dysfunction. The oncological outcome has been equal, but results are difficult to compare due to differences in definitions and in patient groups[133, 266]. For the time being, extensive lateral lymph node dissection is not recommended in the national Swedish guidelines[174].

Laparoscopic and robotic-assisted resection for rectal cancer

Compared to colon cancer data on laparoscopic surgery for rectal cancer are scarce. However, to date the early complications seem to be less and the oncological outcome seems comparable to open surgery. Long-term data are missing, but there are several ongoing trials. Based on current knowledge, laparoscopic rectal cancer surgery cannot be recommended outside trials[207]. A development of the laparoscopic technique is robotic-assisted surgery, which has been practised for rectal cancer. However, here the data are even more scarce than for laparascopic surgery, making this approach strictly investigational[252].

Volume

Studies of the impact of the surgeon as well as the hospital caseload/volume on the oncological outcome have been contradictory[18, 212]. The studies are heterogeneous, and the major drawback is the lack of a uniform definition of high volume. A meta-analysis concluded that the surgeon's volume did not affect survival, but the surgeon's education and experience (i.e., subspecialization in colorectal surgery) did, and the high volume hospitals were associated with improved survival[112]. Martling *et al.,* reported that LR rate and cancer-specific survival in the Stockholm region was significantly better for high volume surgeons[153]. A study from the SRCR detected a lower LR rate in the non-irradiated subgroup managed at high-volume hospitals, but no influence on survival was found according to the volume of the hospital[130].

Intraluminal malignant cells

The occurrence of viable, exfoliated intraluminal malignant cells during surgery in patients with rectal cancer has been demonstrated[72, 221, 241]. These cells have the potential to grow and metastasise. They are considered a potential source of LR by implantation at the anastomotic site during stapling or through pelvic seeding from leakage of intraluminal contents[83]. Such seeding of malignant cells may occur from the

rectal stump, open stump when using the double purse string technique or when puncturing the rectal stump with the trocar when using the cross staple technique[117,221], or from the defect in the bowel after an intraoperative incidental perforation[65,195,203,222,271] as well as after a postoperative anastomotic leakage[60,162,189].

Rectal washout

Rectal washout – peroperative irrigation of the rectum after cross-clamping below the tumour and before transection during AR or HA – has been proposed to eliminate the viable, exfoliated intraluminal malignant cells thereby reducing the risk of LR. Conducted studies have failed to reach a definitive conclusion[3,117,233]. In the SRCR, 80% of the patients treated with AR and 42% of the patients treated with HA were reported to have rectal washout[182]. The LR rate was significantly higher after AR without rather than with rectal washout, a difference that was not found for HA. Data concerning the importance of rectal washout when performing an APR are scarce. In Sweden approximately 15% of the patients operated with APR have rectal washout, which is indicated to lower the LR rate[230]. In a literature search performed by Constantinides *et al.,* no benefit of rectal washout was seen in terms of reducing the LR rate[50]. However, they found that the available data for drawing of a definitive conclusion were poor. Until a randomised, controlled trial that has evaluated the effect of rectal washout is conducted, they recommend rectal washout to be performed since it is risk-free and does not significantly lengthen the operative time. These two arguments in favour of rectal washout might, as Cohen stated[46], make it unlikely to evaluate the impact of rectal washout in a randomised trial, also the great number of patients needed to be included holds against the conduction of such a trial[50]. In a recent evaluation of the importance of rectal washout on the LR rate after AR from the SRCR, a significant impact in favour of rectal washout was seen (Kodeda *et al., Br J Surg,* In press). Other unanswered questions address the impact of rectal washout on the different major abdominal surgical procedures, what solution to use, and what amount of solution to use. The national Swedish guidelines recommend rectal washout when performing AR[175].

Intraoperative perforation

Incidental perforation in rectal cancer surgery (i.e., unintended perforation of the rectum during the course of surgical resection) is considered to affect the oncological outcome adversely. Incidental perforations are more common after APR than other resections[6,65,171,218,271], and perforations during the perineal part of the procedure is more common than during the abdominal[195]. An increased LR rate[65,195,203,222,271] as well as an increased overall recurrence (OAR) rate[203] have been reported in previous studies. Moreover, a reduced 5-year overall survival is also reported after incidental perforations[65,195,203,222,271]. Only one small study could not detect any difference in the LR rate or any impact on the 5-year overall survival between patients with and without incidental perforation[121]. We have not been able to find any data in the literature on the impact of incidental perforation on the DM rate or the 5-year cancer-specific survival. A majority of the few studies that address the impact of incidental perforation on the oncological outcome was performed before the introduction of modern treatment strategies for rectal cancer. This must be considered when the results are evaluated. Some studies indicate that only perforations in the tumour influence the oncological outcome, whereas perforations in other rectal parts do not; however, the evidence is not solid[222,271].

Anastomotic leakage

AL has been suggested to enhance the omnipresent, self-limiting systemic inflammatory

response in the postoperative period, which in turn affects the immunity and facilitates the implantation of malignant cells, eventually resulting in an increased LR risk[157, 159]. The issue of the impact of AL on the oncological outcome is well studied in the literature, but the results are contradictory. An increased LR rate has been reported[4, 12, 29, 45, 60, 79, 139, 162, 189, 196], although other studies have not detected such an increase[15, 58, 66, 120, 124, 140]. The same holds for the impact of AL on the DM rate with some authors reporting an increased risk[4, 45, 139], but others have not been able to confirm this finding[15, 29, 79]. In five papers, the rates of LR and DM have been analysed as one entity, the OAR rate, which was reported to be higher after AL[4, 6, 45, 120, 139]. The reported overall survival after AL has been worse in some studies[58, 60, 120, 157, 247] but not in others[66, 140, 189]. The cancer-specific survival has been demonstrated to be reduced after AL[4, 60, 79, 120, 157, 162, 189, 247], but also to be unaffected[58, 140, 196]. Existing studies are difficult to compare as the definition of AL varies as well as the patient selection, the surgical technique, and the use of neoadjuvant/adjuvant treatment. However, in the latest and largest studies reflecting modern treatment strategies, a negative impact on the oncological outcome has not been demonstrated[58, 66]. Data on the late occurring AL are scarce in the literature. A few studies have reported a negative impact on the oncological outcome after radiological AL[4, 12, 247], but the conclusions are based on few patients. In the SRCR, clinical AL within 30 postoperative days is registered on the primary registration form, and later occurring AL is registered on the follow-up registration form[230]. Unfortunately, late AL is not registered as a separate entity, but altogether with other anastomotic late occurring complications. Postoperative testing of the anastomosis is performed in Sweden when there is clinical suspicion of AL, but this is not done routinely. Radiological AL is not registered in the SRCR. However, even if data on the oncological outcome after AL are contradictory, the morbidity after AL is high with certainty.

Radiotherapy

In addition to surgery, RT has proven to reduce the LR rate and to increase the overall survival rate[38, 47, 75, 104, 178, 262]. Several studies of neoadjuvant/adjuvant RT in rectal cancer have been conducted in northern Europe including four major important trials in Sweden. In the Stockholm I/Malmö trial, short-term preoperative RT (25Gy/5d) *vs*. surgery alone was evaluated[41]. The Swedish Rectal Cancer Trial (SRCT)[178] and the Stockholm II trial[152] studied the same question. However, there was a changed field-technique (from two-field to three- or four-field), a lowered border of the field to the L4 vertebra, as well as an upper age limit of 80 years for inclusion in the trials. The Stockholm II trial was in part coordinated with the SRCT. In several of the early studies, RT was studied together with the conventional, blunt, suboptimal surgery as performed before the introduction of TME surgery. However, the value of preoperative RT in reducing the LR rate has been proven also in combination with TME surgery in the Dutch multicentre TME trial[122, 187]. In this trial, patients were randomised between TME surgery with or without preoperative RT (25Gy/5d). In general, a relative reduction of the LR rate with 50–70% is observed when preoperative RT is added to surgery[84]. The relative reduction of the LR rate is of the same magnitude with TME surgery as with non-TME surgery. The absolute numbers of patients developing LR after TME surgery alone are few, so any impact on the overall survival has not yet been possible to detect when studying RT in combination with TME surgery[187].

In Sweden, approximately 50% of the patients with rectal cancer are treated with preoperative RT[182, 230]. As stated in the section above on preoperative staging, MRI is crucial in the local staging of the tumour. Based on

the MRI findings, the tumours are classified as "good", "bad" or "ugly"[25,175,224]. The recommended national Swedish guidelines are based on this classification when deciding whether the patient should be treated with preoperative RT and the strategy to be used (Fig. 4). Despite its favourable impact on the oncological outcome, some controversies still exist in the role of RT in rectal cancer treatment: pre- *vs*. postoperative treatment, the fractionation and timing of surgery, as well as the impact of RT on different tumour heights.

Preoperative *vs.* postoperative radiotherapy

One issue is whether the RT should be delivered pre-or postoperatively. In northern Europe, preoperative treatment has been advocated. The most common strategy in northern Europe has been short-term preoperative RT (25Gy/5d) with immediate surgery the following week. In other parts of the world, selective postoperative RT to high-risk patients of LR with 40–60Gy/5–8 weeks has been used[84, 213]. A systematic review concluded that short-term preoperative RT was superior to postoperative RT in reducing the LR rate as well as improving survival[84]. In the Uppsala trial[77], short-term preoperative RT was compared to postoperative RT (60Gy/8 weeks). A German study compared preoperative chemoradiotherapy (50,4Gy/28d+5FU) with postoperative chemoradiotherapy (50,4Gy/28d+5FU) in patients with locally advanced rectal cancer, defined as clinical stage T3 or T4 or node-positive disease[213]. Pre- *vs*. postoperative RT was also recently studied in the CR07 study in which short-term preoperative RT 25Gy/5d was compared to selective postoperative chemoradiotherapy 45Gy/25d+5FU for patients with involved CRM[216]. The argument for the selective postoperative RT approach is that only high-risk patients of LR are treated with subsequent avoidance of overtreatment and potential side effects. These later studies have reported superior results in reducing the LR rate for pre- *vs*. postoperative RT, although any improved impact on overall survival has not been found. Reduced toxicity and better compliance has been stated after preoperative RT in these studies.

Fractionation and timing of surgery

Another matter is the optimal fractionation and the optimal timing of surgery. Today, two fractionations with different timing of the surgery are used in Sweden, short-term (25Gy/5d) preoperative RT with immediate surgery (within 7 days) and long-term (50Gy/25d) preoperative RT with delayed surgery (after 6–8 weeks). The long-term fractionation is often used in combination with radiosensitizising chemotherapy. The aims of the fractionations are different. The short-term fractionation aims at improving local control by eliminating potentially viable tumour cells in the pelvis – i.e., in the mesorectal lymph nodes or in the lymph nodes along the lateral pelvic side walls – in an otherwise primary resectable tumour, the intermediate "bad" group (Fig. 4). The long-term fractionation aims at downsizing and thus downstaging to facilitate resection of a primary locally irresectable tumour, the advanced "ugly" group (Fig. 4). In the SRCR, approximately 10% of the patients that receive preoperative RT are treated with the long-term course[230]. The effect of short-term *vs*. long-term preoperative RT has been evaluated in a Polish study[34]. In this study, patients with clinical resectable T3 or T4 cancer were randomized to either short-term (25Gy/5d) preoperative RT with immediate TME surgery or chemoradiotherapy (50,4Gy/28d+5FU/leucovorin) with delayed surgery after 4–6 weeks. No difference in LR rate, overall survival or late toxicity was detected. The optimal fraction of RT as well as the optimal timing of surgery, another matter on which the data are scarce, is studied in the ongoing Stockholm III

trial[190]. The patients are randomised to short-term RT (25Gy/5d) with surgery within one week, short-term RT (25Gy/5d) with surgery after 6 weeks, or long-term RT (50Gy/25d) with surgery after 6–8 weeks.

Preoperative chemoradiotherapy has been reported to lead to complete clinical response or complete pathological response in 10–30% of patients[93, 243]. Complete clinical response is defined as absence of any residual scar, mass or ulcer after clinical and radiological assessment. The definition includes relief of symptoms and negative results on digital rectal examination, CT, or EUS as well as measurements of CEA. The definition of complete pathologic response, pT0N0M0, is absence of viable tumour cells after full pathologic examination of the resected specimen. In the highly selected group of patients with complete clinical response, a strategy of close observation without surgery has been demonstrated to be possible with long-term results comparable to preoperative chemoradiotherapy combined with surgery[93, 243].

Radiotherapy and tumour height

The effect of preoperative RT for rectal cancer at different tumour heights above the anal verge is debated. In Sweden, fewer tumours in the upper third of rectum have been treated with RT than in the other parts because RT has been thought to be less important in the upper third[230]. However, significantly reduced LR rate after RT on all heights has been reported from the Stckholm II trial[106], as well as from the recently published CR07 trial[216]. In the 13-year follow-up analysis of the SRCT, it was demonstrated that preoperative RT reduced the number of LR at all three tumour heights, although the difference was not statistically significant for tumours in the upper third of the rectum[75]. However, the reduction in the upper third as well as in the lower was not found in the Dutch TME trial[122, 187]. After median follow-up of six years, a significant reduction of LR after preoperative RT was observed only for tumours located in the middle third, but not in the upper or the lower third of the rectum[187]. Peeters *et al.,* suggested that RT might be of less importance if proper TME surgery is performed in the upper third. They also hypothesised that there might be more patients with tumours in the lower third with involved CRM in the Dutch study compared to SRCT due to an inferior APR technique in the Dutch study. RT does not compensate for involved CRM. Unfortunately, the SRCT does not have data on CRM involvement so the hypothesis cannot be verified. The authors of the TME study also pointed out that the support for their results on the effect on different levels were based on subgroup analyses with rather small subgroups, which was a limitation.

Postoperative complications and side effects associated with radiotherapy

In the early Stockholm I/Malmö trial, an increased postoperative mortality, mainly due to cardiovascular deaths in irradiated patients >75 years, was observed. This outweighed the potential positive effect on the overall survival of RT in this group[41]. Consequently, in the following studies the irradiated volumes were reduced, three- or four-field technique introduced, the upper age limit lowered, and the patient's general condition considered. The mortality has been reduced, but even with modern irradiation techniques and improved patient selection, the potential acute as well as late adverse effects are substantial. The impact of RT on intraoperative adverse events, postoperative complications, inhospital mortality, and postoperative mortality is also debated. The use of RT must be balanced against all these matters.

Acute side effects evolve during the treatment or within three months after treatment. Acute side effects include fatigue, skin reac-

tions (erythema), gastrointestinal (nausea, diarrhoea), genitourinary (cystitis), and neurological complications (lumbosacral plexopathy)[78, 147, 177]. The acute side effects are usually self-limiting and do not require intervention. The incidence of acute side effects has been reported to be higher after postoperative RT than preoperative[213]. Differences in acute side effects have not been observed when short-term preoperative and long-term preoperative RT are compared[33].

The late side effects after RT are less well documented, but data are accumulating[23]. Many late side effects come from the gastrointestinal tract. Anal dysfunction with incontinence, diarrhoea, bleeding, abdominal pain and small bowel obstruction are reported with increased incidences in irradiated patients[22–24, 186, 192]. Urinary dysfunction with incontinence[31, 193] as well as impaired sexual function in both genders[23] are documented after RT. Increased cardiovascular morbidity was seen in the long-term follow-up of the Stockholm I/Malmö and Stockholm II trials[193]. In the long-term follow-up of patients included in the Uppsala trial and SRCT, the risk of secondary malignancies was almost doubled in irradiated patients, and the risk was mainly related to malignancies in organs within or adjacent to the irradiated volume[21]. Pollack *et al.,* reported that the risk of anal dysfunction was increased when short-term preoperative RT were combined with TME surgery *vs*. short-term preoperative RT and non-TME surgery[194]. To some extent, this was explained by the fact that patients treated with TME surgery had lower anastomoses.

The late side effects have been reported to be more common after postoperative RT than preoperative RT[77, 213]. Differences in late side effects have not been observed when short-term preoperative and long-term preoperative RT have been compared[34]. In the TME trial, there was no difference between irradiated and non-irradiated patients concerning intraoperative adverse events, but the overall postoperative complication rate was significantly higher in the irradiated group[147]. However, this was due to a higher perineal wound complication rate in irradiated patients treated by APR. The incidences of other complications including the serious complications AL, abscess-formation, abdominal wound dehiscence and ileus were similar in the groups. Furthermore, the inhospital as well as the postoperative mortality were similar in the groups. There were no differences in complication rates, inhospital mortality or postoperative mortality in the studies from Germany[213] and Poland[33], but in the first report from the ongoing Stockholm III trial a tendency towards higher postoperative complication rate is seen in the group randomised to short-term RT with surgery within one week[190].

Sphincter preservation

Long-term preoperative RT, with or without chemotherapy, is hypothesised to increase the rate of sphincter preserving surgery in low situated tumours by tumour shrinkage making anastomosing possible, but the data supporting this are uncertain. Among patients in the German study, that the surgeon before randomisation to pre- or postoperative RT thought would need an APR, preoperative RT more than doubled the rate of sphincter preserving surgery[213]. In the Polish study, there was no benefit in sphincter preservation when short-term RT or long-term chemoradiotherapy were compared[32]. In a systematic review by the same Polish group, no significant effect of preoperative RT on the rate of sphincter preservation was found[35].

Chemotherapy

Chemotherapy is a systemic therapy aiming at eliminating the potential risk of micrometastasis from circulating CRC cells[81]. The most commonly used chemotherapeutic agent in rectal cancer has been 5-fluorouracil (FU)/

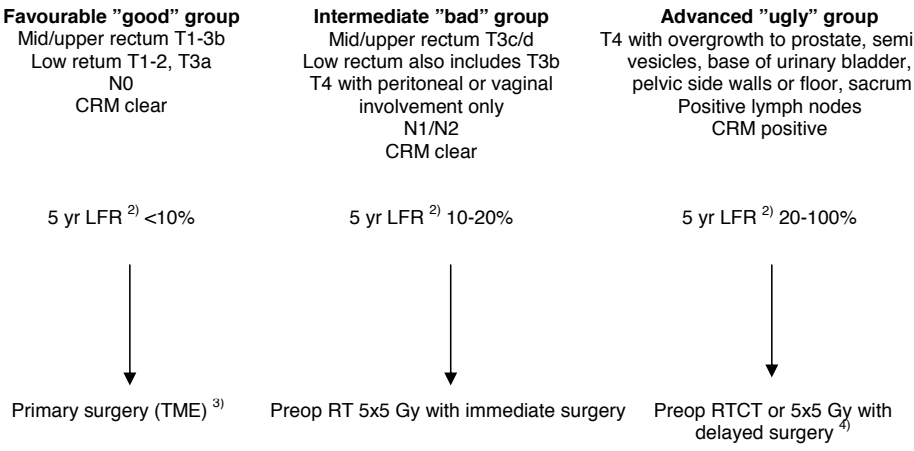
leucovorin. The enzyme thymidylate synthase (TS) and therefore DNA synthesis is inhibited by 5-FU. Leucovorin stabilizes the binding of 5-FU to TS. Newer drugs that have been introduced in clinical practise are capecitabine, irinotecan and oxaliplatin. Capecitabine is an oral fluoropyrimidine. Irinotecan is a topoisomerase inhibitor that blocks DNA repair. Oxaliplatin is an inhibitor of DNA replication and possibly a down-regulator of TS[8, 81, 180].

Neoadjuvant chemotherapy

According to Blomqvist *et al.,* in patients with tumours belonging to the advanced "ugly" group (Fig. 3) the addition of chemotherapy to long-term preoperative RT has proven to improve local control, but this has not been proven to influence survival[25, 27, 86]. The national Swedish guidelines recommend the use of chemotherapy in this setting as outlined in Figure 3.

Adjuvant chemotherapy

In colon cancer, adjuvant chemotherapy has proven to increase the overall survival in absolute numbers with approximately 5% in high-risk, e.g., poor histopathologic differentiation grade, venous- or lymphatic invasion or CRM involvement, TNM stage II patients and 10% in TNM stage III patients. Most studies have been based on six months of therapy with 5-FU/leucovorin. Adjuvant chemotherapy is less studied in rectal cancer than colon cancer, and the results from colon cancer have not been reproduced in rectal cancer[8, 85, 180, 200, 261]. However, in many countries the results from colon cancer have been extrapolated to rectal cancer, and adjuvant chemotherapy is adminis-



1) The algorithm does not primarily address the risk of systemic disease, although this risk also increases with the presence of many of "the risk factors", however, not necessarily parallel to the local failure rate (LFR).
2) Calculated in the group of patients planned for surgery, i.e., irrespective of the surgical outcome. The figures are valid if the surgeon is an experienced rectal cancer surgeon and no pre-treatment is given.
3) A local procedure is possible in a few (chiefly pT1, sm1+2, N0).
4) RTCT means radiochemotherapy to 50.4 GY in1.8 fractions with fluorouracil. Preop RT 5x5 Gy with delayed surgery is used in patients not fit for RTCT. The relative antitumour efficacy of conventionally fractioned RT or the short-course schedule is not known with any greater certainty.

Figure 3. MRI-directed preoperative evaluation[25]. Reproduced with the permission of the publisher.

tered based on the same recommendations in both patient categories. In the national Swedish guidelines, adjuvant chemotherapy is not recommended for patients with rectal cancer outside clinical trials[174, 175]. Despite this, according to the SRCR, a small group of patients, <5%, in TNM stage II <75 years and a large portion, 35%, of patients in TNM stage III <75 years have received adjuvant chemotherapy[230].

Palliative chemotherapy

In the palliative setting, the use of chemotherapy has improved the median survival from six months to two years[17, 81]. In addition, most studies are performed with the use of 5-FU/leucovorin based strategies[81, 220], and despite the development of new drugs, 5-FU/leucovorin still is the most used first line therapy. However, different combination therapies with 5-FU/leucovorin and irinotecan or oxaliplatin are becoming more common[81, 220, 261]. In addition to the development of new cytotoxic drugs, monoclonal antibodies against proteins thought to be of importance in the proliferation of malignant cells have been developed[81, 258, 261]. Bevacizumab is a monoclonal antibody against the vascular endothelial growth factor (VEGF), and thus it is an anti-angiogenesis factor. Cetuximab and panitumumab are monoclonal antibodies that inhibit the epidermal growth factor receptor (EGFR), a transmembrane glycoprotein interacting with a variety of intracellular signalling pathways. The monoclonal antibodies have in combinations with the newer chemotherapeutic agents proven to further improve survival in the palliative setting.

Follow-up

The aim of postoperative follow-up of patients after curative surgery for rectal cancer is to detect LR or DM at a stage when curative intervention is possible. The patients to be followed-up should also tolerate these interventions, surgical as well as oncological, otherwise the follow-up is of no value. The value of follow-up to increase survival has been questioned. However, a Cochrane systematic review in 2008 including data for 2141 patients from eight studies after curative surgery for CRC concluded that there was a significant overall survival benefit for patients with intensified follow-up[116]. Other studies[1, 236, 257], in addition to this review, have shown an absolute survival benefit of approximately 10% with intensified follow-up strategy. In the Cochrane systematic review, there was no difference in the incidence of recurrence in the group with intensified follow-up *vs*. in the group with non-intensive follow-up, but significantly more surgical procedures were performed among patients in the intensively followed group. This indicates earlier detection of recurrences and better survival due to curative treatment of recurrences in this group. The included studies were heterogeneous with varying definitions of high frequency follow-up, follow-up investigations and length of follow-up. The number of included patients was small and the studies included patients from varying periods. Thus, to draw any firm conclusions from the analysis were difficult.

Intensity and duration of follow-up as well as what investigations to perform are still controversial. In existing studies, most recurrences are detected within the first two to three years from primary surgery, but modern neoadjuvant/adjuvant treatment has been indicated to postpone the recurrences thus motivating a longer follow-up[105, 164, 187]. Controversies also exist in what investigations to use since some studies have followed the patients with blood samples and others with imaging. To address these issues, several multicentre randomized trials are ongoing. The GILDA trial in Italy, the FACS trial in the UK and the COLOFOL trial in Denmark, Sweden, Poland, the UK, the Netherlands and Uruguay plan to include far greater numbers of patients than the ear-

lier studies. By this and by using well-defined study protocols, the issues concerning follow-up will hopefully be resolved[90, 116, 174, 175, 257].

During the period studied in this thesis, there was no standard national follow-up strategy in Sweden, but each patient was followed according to each hospital's routine. Today, the national Swedish guidelines recommend inclusion in the COLOFOL study[175]. If the patients are not included in the COLOFOL trial, follow-up according to the low intense arm of this study is recommended as well as colonoscopy every fifth year until the age of 75 after curative surgery.

Pathology

The pathologist is an important member of the MDT team. Correct handling of the surgical specimen and macro- as well as microscopic examination is of utmost importance. In the same manner as workshops have been held to teach the TME technique, workshops have been held in CRC pathology[198, 243]. The pathologist should classify the tumour histologically, state the TNM stage, as well as identify the completeness of the surgery[174, 175, 198, 243]. This information is of prognostic value since the information is crucial in the decision-making regarding adjuvant treatment. For the surgeon, the pathology report serves as an audit of the quality of the surgery. For the radiologist, it is a feedback on the accuracy of the CRM assessment on the preoperative MRI. The macro- and the microscopic evaluation should be in an ordered manner. Photography of the macroscopic specimen as well as the specimen slices is recommended. The use of a standardized pro forma for reporting CRC resection specimens has been reported to improve the quality of histopathological reporting[11], and this is recommended in the national Swedish guidelines[174,175]. In the macroscopic evaluation, the surfaces should be examined to record any perforation and the plane of surgery. An intact mesorectum is of prognostic importance. The specimen is opened anteriorly, except for the area of the tumour, to allow CRM assessment. After formalin-fixation, the specimen is described and then the tumour including 2 cm below and above is sliced in 3–5 mm thick slices. The CRM is evaluated on these slices. Additional blocks are then taken from the area with the closest distance between the tumour and the CRM and from other areas outside the muscularis propria to confirm the presence or absence of extramural venous invasion. Accurate nodal staging is very important in the selection of patients to adjuvant treatment (see below), and here the pathologist's task is to find a sufficient number. The pathologist also gives the oncologist feedback by evaluating the tumour regression. Several grading systems for grading of tumour response after preoperative chemoradiotherapy exist[243]. A system described by Dworak *et al.,*[59] (Table 2) is one of the most common systems used.

Histopathologic classification

Rectal cancer is histopathologically classified according to the internationally accepted histologic classification proposed by the World Health Organization[48,174,175] as shown in Table 3. Adenocarcinoma is the dominating type followed by mucinous (colloid) adenocarcinoma and signet-ring cell carcinoma. Signet-ring cell carcinoma and small-cell (oat cell) carcinoma are prognostically unfavourable and medullary carcinoma prognostically favourable according to current knowledge. The prognostic significance of mucinous (colloid) adenocarcinoma is controversial since it has been linked with adverse outcome, but when it is associated with microsatellite instability (MSI) it has been prognostically favourable.

Histopathologic grading

Based on microscopic features, rectal tumours have been graded by a number of different

Table 2. Grading of tumour response[59].

Grade	
0	No regression
1	Dominant tumour mass with obvious fibrosis and/or vasculopathy
2	Dominantly fibrotic changes with few tumour cells or groups (easy to find)
3	Very few (difficult to find microscopically) tumour cells in fibrotic tissue with or without mucous substance
4	No tumour cells, only fibrotic mass (total regression or response)

grading schemes. The most common is shown in Table 4.

Due to difficulties in discriminating between well and moderately differentiated carcinoma, a new system based on the proportion of gland formation by the tumour (>50% or <50% gland formation) has been implemented. Well and moderately differentiated tumours should be classified as low differentiation grade and poorly or undifferentiated tumours as high grade. This is thought to facilitate the grading and increase the reproducibility. This classification is also recommended in the national Swedish guidelines[175]. Histopathologic grade has in several studies proven to be a stage-independent prognostic factor and especially high grade has proven to impact negatively the oncological outcome[48, 54].

Tumour, Node, Metastasis staging

The most common staging system for rectal cancer is the TNM (Tumour, Node, Metastasis) staging system (Table 5 and 8). The system is developed in collaboration between the Union International Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC)[88, 227]. The TNM stage is based on the anatomical extent of the disease. The TNM system is continuously revised; in 2010 the 7th revision was published. In the beginning, staging in the SRCR was by Dukes' classification

Table 3. World Health Organization Classification of Colorectal Carcinoma[48] .

Adenocarcinoma
Medullary carcinoma
Mucinous (colloid) adenocarcinoma (>50% mucinous)
Signet-ring cell carcinoma (<50% signet-ring cells)
Squamous cell (epidermoid) carcinoma
Adenosquamous carcinoma
Small-cell (oat cell) carcinoma
Undifferentiated carcinoma
Others

Table 4. Histopathologic grade.

Differentiation grade	
GX	Grade cannot be assessed
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated
G4	Undifferentiated

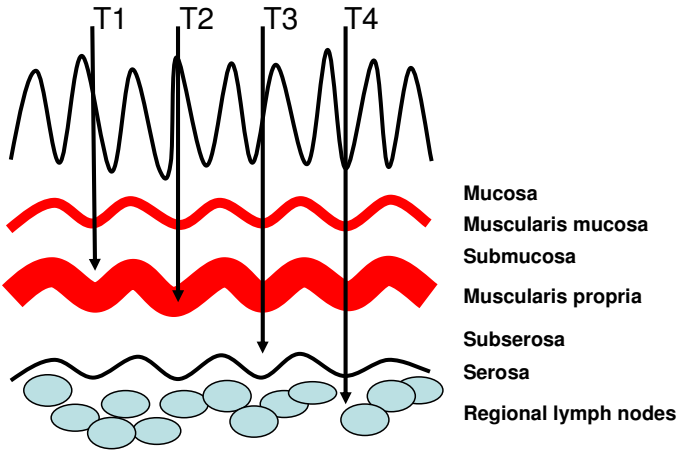


Figure 4. The layers of the bowel wall and the T stages.

but later changed to the TNM classification according to the 5th edition[182,225]. In the SRCR, the staging is still done according to this edition to facilitate comparisons over time.

T stage

The T stage describes the depth of invasion through the layers of the bowel wall of the primary tumour (Fig 4). Subdivision of the T4 stage was included in the 7th edition of the TNM manual (Table 5). This subdivision can be a matter of confusion for clinicians since a subdivision of T4 stage was recommended in a supplement to the 6th edition of the TNM manual[259]. This subdivision was adopted in clinical practise and in Sweden it is also used in the SRCR[49,175,230]. In this subdivision, T4a represented extension into adjacent organs or structures, and T4b was tumour perforation of the visceral peritoneum. In the 7th edition, the T4 stage subdivision is quite the opposite. Apart from the subdivision of the T4 stage proposed in the TNM system, T1 and T3 stages can also be subdivided. The T1 stage is subdivided according to the extent of the invasion in the submucosa (Table 6)[131,173,176]. The risk of lymph node metastasis increases with increasing sm subclass and has been reported to be as much as 20% in sm3[173]. Table 7 shows the subclassification of the T3 stage[259]. Also, this classification is of prognostic value with worse prognosis with increasing depth of invasion[91].

N stage

The N stage describes the spread to regional, perirectal lymph nodes and the number of involved nodes. The N stage is subdivided as shown in Table 5.

M stage

The M stage describes the occurrence of distant metastases including metastases in non-regional lymph nodes. M1 disease is tumour growth in any distant organ, any non-regional lymph node as well as peritoneal carcinomatosis and positive peritoneal fluid cytology. In the 7th edition, subdivision of the M stage was added in the TNM staging system (Table 5).

The T, N, and M stage are combined into the group TNM stage (Table 8). There is a

Table 5. TNM classification[225-227].

5th and 6th edition		7th edition	
TX	Primary tumour cannot be assessed	TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour	T0	No evidence of primary tumour
Tis	Carcinoma *in situ*: intraepithelial or invasion of lamina propria	Tis	Carcinoma *in situ*: intraepithelial or invasion of lamina propria
T1	Tumour invades submucosa	T1	Tumour invades submucosa
T2	Tumour invades muscularis propria	T2	Tumour invades muscularis propria
T3	Tumour invades through muscularis propria into subserosa or into non-peritonealized pericolic or perirectal tissues	T3	Tumour invades through muscularis propria into subserosa or into non-peritonealized pericolic or perirectal tissues
T4	Tumour directly invades other organs or structures and/or perforates visceral peritoneum	T4	Tumour directly invades other organs or structures and/or perforates visceral peritoneum
		T4a	Tumour perforates visceral peritoneum
		T4b	Tumour directly invades other organs or structures
NX	Regional lymph nodes cannot be assessed	NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis	N0	No regional lymph node metastasis
N1	Metastasis in 1 to 3 regional lymph nodes	N1	Metastasis in 1 to 3 regional lymph nodes
		N1a	Metastasis in 1 regional lymph node
		N1b	Metastasis in 2-3 regional lymph nodes
		N1c	Tumour deposit(s), i.e. satellites, in the subserosa, or in non-peritonealized pericolic or perirectal soft tissue without regional lymph node metastasis
N2	Metastasis in 4 or more regional lymph nodes	N2	Metastasis in 4 or more regional lymph nodes
		N2a	Metastasis in 4-6 regional lymph nodes
		N2b	Metastasis in 7 or more regional lymph nodes
MX	Distant metastasis cannot be assessed	MX	Distant metastasis cannot be assessed
M0	No distant metastasis	M0	No distant metastasis
M1	Distant metastasis	M1	Distant metastasis
		M1a	Metastasis confined to one organ [liver, lung, ovary, non-regional lymph node(s)]
		M1b	Metastasis in more than one organ or the peritoneum

Table 6. Classification of submucosal invasion[131].

Subclass	
Sm1	Invasion into the upper third of the submucosa
Sm2	Invasion into the middle third of the submucosa
Sm3	Invasion into the lower third of the submucosa

Table 7. Subclassification of the T3 stage[259].

Subclass	
T3a – minimal invasion	<1 mm beyond the border of the muscularis propria
T3b – slight invasion	1–5 mm beyond the border of the muscularis propria
T3c – moderate invasion	>5–15 mm beyond the border of the muscularis propria
T3d – extensive invasion	>15 mm beyond the border of the muscularis propria

Table 8. TNM stage grouping[225-227].

5th and 6th edition		7th edition	
Stage 0	Tis, N0, M0	Stage 0	Tis, N0, M0
Stage I	T1–2, N0, M0	Stage I	T1–2, N0, M0
Stage II	T3–4, N0, M0	Stage II	T3–4, N0, M0
Stage IIA	T3, N0, M0	Stage IIA	T3, N0, M0
Stage IIB	T4, N0, M0	Stage IIB	T4a, N0, M0
		Stage IIC	T4b, N0, M0
Stage III	Any T, N 1–2, M0	Stage III	Any T, N1–2, M0
Stage IIIA	T1–2, N1, M0	Stage IIIA	T1–2, N1, M0
			T1, N2a, M0
Stage IIIB	T3–4, N1, M0	Stage IIIB	T3–4a, N1, M0
			T2–3, N2a, M0
			T1–2, N2b, M0
Stage IIIC	Any T, N2, M0	Stage IIIC	T4a, N2a, M0
			T3–4a, N2b, M0
			T4b, N1–2, M0
Stage IV	Any T, Any N, M1	Stage IVA	Any T, Any N, M1a
		Stage IVB	Any T, Any N, M1b

clinical as well as a pathological TNM classification. Clinical classification (cTNM) is predominantly based on radiological imaging before treatment and is indicated by the prefix "c". The pathological classification (pTNM) is based on pathological examination of the resected tumour specimen indicated with the prefix "p". In clinical practise, the T and N stage are based on the definitive pathological examination, whereas the M stage is mainly determined by the radiological examination or the perioperative findings. Therefore, the TNM stage used in clinical practise is a mixture of clinical and pathological classification. A third prefix ("y") is also used when a tumour is staged after given neoadjuvant treatment.

The group TNM stage is the most important prognostic factor of the oncological outcome in rectal cancer[48]. In SRCR, approximately 21% belongs to TNM stage I at diagnosis, 26% to TNM stage II, 25% to TNM stage III, and 17% to TNM stage IV. In 11% of the cases, data on the TNM stage are not stated[230]. The risk of LR and DM increases with TNM stage in TNM stages I–III, and survival rate is worse with higher TNM stage[49, 174, 182]. However, there is great variation of the oncological outcome within each stage group. This has motivated further subgrouping. The importance and considerations of this subgrouping is increasing in clinical practise. The T and N stage are independent prognostic factors with worse prognosis with more advanced stage, and the interaction between the T stage and N stage is complex[49, 89, 91, 176].

The number of examined lymph nodes is an important prognostic factor with better outcome within each stage the more nodes examined[61, 91]. In Sweden, the recommended minimum number of examined lymph nodes is 12[174, 175], a value that is in line with the recommendations from the UICC and AJCC[88, 227]. The ratio of metastatic to harvested lymph nodes has also been proven to have prognostic impact with worse prognosis with increasing ratio[128, 188].

In the 5th TNM manual, mesorectal tumour deposits (satellites) – macroscopic or microscopic tumour nests or nodules found without histological evidence of residual normal lymph node >3 mm in diameter – were classified as N-disease, whereas similar findings ≤3 mm in diameter was classified in the T3 category as a discontinuous extramural extension of the tumour[225]. In the 6th edition, a discrete extramural tumour deposit without histological evidence of residual lymph node in the nodule with smooth contours, irrespective of size, was included in the N category as a positive lymph node[226]. If the contours of the nodule were irregular, it should be classified in the T category. It should also be coded as V1 (microscopic venous invasion) or V2, if it was grossly evident since it is likely to represent venous invasion. Finally, in the 7th edition[227], the mesorectal tumour deposits are considered as discontinuous spread, venous invasion with extravascular spread (V1/V2), or a totally replaced lymph node. If the deposits were found with lesions that otherwise would be classified as T1 or T2, the classification is not changed but the deposit(s) is recorded as N1c (Table 5). If a deposit is considered by the pathologist to be a totally replaced lymph node (most commonly with a smooth contour), it should be recorded as a positive lymph node and not a satellite. Each such deposit should be counted separately as a lymph node in the final N determination.

Today, the importance of micrometastases and isolated tumour cells (ITC) is under debate. Micrometastases are small deposits of metastatic tumour that measure >0.2 mm but ≤2 mm in diameter. Micrometastases should be noted as either N1 (mi) or M1 (mi) in the pathology report. However, based on available data, the search for ITC or micrometases is not recommended in clinical practise[49, 269]. ITC are small numbers of tumour cells detected only by special techniques – immunohistochemistry (IHC) or polymerase chain reaction (PCR) – or seen histologically but measuring

≤0.2 mm. ITC can be found within lymph nodes or at distant sites. Since their biological importance is unsure, ITC are classified as N0 or M0[49, 269].

That the TNM stage system is continuously revised is one of its advantages, but also one of its disadvantages. Revision might cause stage migration, making it hard to compare outcomes in newer studies with older studies[199]. The TNM staging system only takes into account the anatomic extent of the disease, while biological properties of the tumour are not incorporated, an accounting that can be seen as a drawback[49]. The prognosis after treatment for tumours in TNM stage IV varies considerably depending on the number and location of the metastases. The system has also been criticized as M stage in the earlier version because it only told whether metastases were present or not without considering the number or the location. However, in the 7th edition, the M1 stage is subdivided according to the number of affected organs[227].

Other stage-independent prognostic factors

Although not established in clinical practise, the prognostic value of other tissue-based factors independent of stage has been proven[49, 56, 272]. A problem when assessing the impact of such factors is the lack of standardization, making the results from different studies difficult to compare. There is an interobserver variability among pathologists, the histopathologic criteria varies and different visualisation techniques (i.e., conventional hematoxylin-eosin staining or IHC) with different sensitivity and specificity are used[49].

The presence of tumour within a space lined by endothelial cells and smooth muscle or elastic fibres is defined as venous invasion[56]. It can be classified as either intramural venous invasion (IMVI) or extramural venous invasion (EMVI), i.e., venous invasion outside the muscularis propria within the surrounding mesorectal fat. Its independent prognostic association with increased risk of LR, DM and reduced survival is established[49, 56, 224, 272].

The presence of tumour in an endothelial lined space, but no smooth muscle or elastic fibres, either in the bowel wall or in the mesorectal fat is defined as lymphatic invasion[56]. Lymphatic invasion has been proven to have independent negative impact on the risk of LR, DM and reduced survival[49, 56, 224, 272].

Tumour cells detected along or around a nerve within the perineural space is the definition of perineural invasion. This feature has also been reported as an independent risk factor of LR, DM and reduced survival[48, 80, 239].

The tumour border could be characterized as either pushing (expanding) or infiltrating. The pushing border is rather well-circumscribed, whereas at the infiltrating border, the normal tissue is dissected by the tumour, and the boundary between tumour and normal tissue is lost[48, 114, 273]. The infiltrating growth pattern has been associated to adverse prognostic impact with increased rates of LR and DM and reduced survival[48, 114, 273].

Tumour budding is single cells or small clusters of undifferentiated cancer cells just ahead of the invasive front of the tumour. Tumour budding is suggested to be the first histologic event in tumour cell migration and invasion[48, 273]. Tumour budding is linked to increased risk of LR and DM as well as reduced survival[48, 240, 273].

A high rate of cells involved in the host immunologic response to the tumour (i.e., lymphocytes, mast cells, macrophages and neutrophils in the tumour or the peritumoural tissue) has shown to be a favourable prognostic feature[48, 114, 273].

Resection margins

Circumferential resection margin

The CRM is the minimal distance from the outermost part of the tumour or malignant

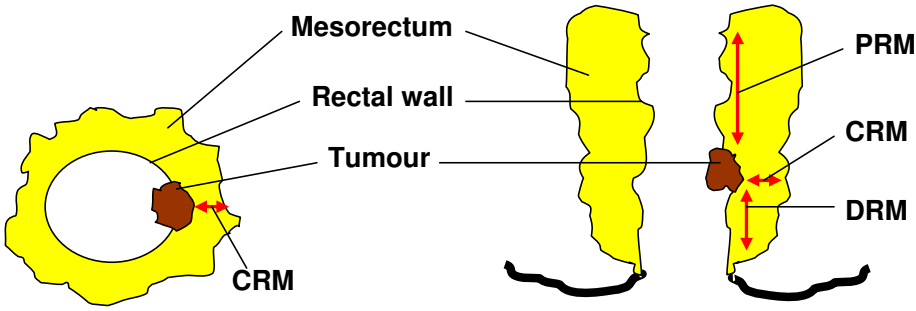


Figure 5. Illustration of the resection margins. Left illustration horizontal section of the lower rectum beneath the peritoneal reflection. Right illustration frontal section. PRM, proximal resection margin; CRM, circumferential resection margin; DRM, distal resection margin.

tissue to the non-peritonealized surface of the resection specimen created by dissection of the subperitoneal aspect at surgery (Fig. 5). The lateral, radial or mesorectal resection margins are synonymous with the CRM. Apart from continuous spread of the primary tumour, lymph node metastases, discontinuous tumour deposits, venous or lymphatic invasion as well as perineural tumour spread are considered when measuring the CRM[102]. Involvement of the CRM has a significant negative impact on LR rate, DM rate, as well as cancer-specific and overall survival[10, 14, 87, 148, 170, 172, 197, 234, 253]. The CRM can be used as an immediate prognostic surrogate marker when assessing the risk of LR and reduced survival[19]. In the majority of studies, the cut off value for CRM-positive (CRM+) has been the presence of tumour/malignant tissue at the CRM or a minimal distance between tumour and CRM ≤1 mm, whereas the cut off value for CRM-negative (CRM-) has been a minimal distance >1 mm between tumour and CRM[87, 102]. However, some studies including fewer patients have raised the question if a margin of 2 mm is a more relevant cut off value[14, 170, 234]. Today, it is stated that the prognosis is better the larger the distance of the tumour/malignant tissue from the CRM is, but the exact cut off value remains uncertain[87, 172, 253]. Still, the evidence is strongest for CRM+ defined as tumour/malignant tissue ≤1 mm of the CRM, but it is been recommended to report the exact margin distances instead of CRM+ or CRM-[170, 234]. In the SRCR, the exact distance (within a tenth of a mm) is now registered[230], and the national Swedish guidelines recommend a cut off value of 2 mm[175]. It has been established that preoperative short-term RT as well as adjuvant chemoradiotherapy cannot compensate for involved CRM[10, 147, 216].

Distal resection margin

Involvement of the distal resection margin (DRM) (Fig. 4) is also a risk factor of LR, DM and reduced survival[97, 185, 215]. Distal spread can be either intramural or mesorectal and can occur by the same mechanisms as the circumferential spread. Distal spread below the tumour occurs in about 25% of patients[185]. Mesorectal spread has been reported to be more common than intramural spread[215, 237, 270]and more extensive[97, 185, 237, 270]. Heald *et al.,* reported mesorectal microscopic deposits as far as 4 cm below the tumour[97]. However, only 10% of

cases with distal spread demonstrate spread beyond 1 cm[185]. Studies have demonstrated that tumours with distal spread >1 cm usually are associated with an advanced stage at diagnosis, high grade histopathology, and a high presence of lymphatic and perineural invasion. Subsequently, the prognosis is dismal, and the patients are likely to die from metastatic disease[185]. This implies that distal spread might be regarded as representing systemic spread rather than a regional lesion[270]. The optimal DRM is still undefined. Until the 1980s, a DRM of 5 cm was required, which thereafter was changed to 2 cm[208]. With the development of stapling devices and the possibility of sphincter-saving procedures for low tumours, there are several studies that have reported excellent results with DRM below 2 cm[141, 168, 208]. Studies have also shown that the length of the DRM is even less important in patients that have received preoperative chemoradiotherapy[185]. As stated in the section on surgery for high situated tumours, a PME procedure has been proven to be adequate[145]. Thus, in the national Sweden guidelines a PME with 5 cm DRM is recommended for high situated tumours, a DRM of 1 cm with an intact mesorectum is recommended for middle- and low-situated highly differentiated tumours, and a longer margin is recommended for poorly differentiated tumours[174, 175].

Proximal resection margin

Spread in the proximal direction can occur in the same way as in the distal. However, since it is extremely rare not to achieve a proximal resection margin (PRM) (Fig. 4) of at least 5 cm when performing rectal cancer surgery, PRM involvement is not a clinical problem; therefore, this has not been examined in detail[49].

Table 9. Residual (R) Tumour Classification[100].

RX	Presence of residual tumour cannot be assessed
R0	No residual tumour
R1	Microscopic residual tumour
R2	Macroscopic residual tumour

Residual tumour classification

In 1987, the UICC adopted the Residual (R) Tumour Classification[100] denoting the absence or presence of residual tumour after treatment (Table 9). Thus, the R classification supplements the TNM classification, which describes the anatomical extent of the tumour without considering treatment. In the strict definition of the R classification, the residual tumour status is considered in the area of the primary tumour as well as in distant sites. However, some apply the R classification only to the primary tumour and its local or regional extent. This is a matter of confusion, and the specific application should be noted when using the R classification[227]. The R classification reflects the effects of therapy, guides in the decision of further therapeutic interventions, and predicts prognosis. The prognostic importance of the classification has been shown by Hermanek *et al.,*[101]. Significant lower LR rate and increased survival rate were found after R0 *vs.* R1 and R2 resections. In fact, an acceptable long-term survival can only be expected after R0 resections. There is confusion between the different definitions of the R classification and the CRM status. CRM negativity is not the same as R0 and CRM positivity is not the same as R1 or R2. In the R classification, both the CRM and DRM are considered as well the presence of DM. Wittekind *et al.,* consider the R classification and the CRM status as complementary and have proposed an Expanded Residual Tumour Classification (Table 10) where the R classification and CRM status are incorporated[260]. This type of classification should eliminate any confusion of the different definitions.

Table 10. Proposed Expanded Residual (R) Tumour Classification[260].

RX	Presence of residual tumour cannot be assessed
R0 >1 mm	No residual tumour, minimal distance between tumour and resection margin; margin >1 mm
R1 ≤1 mm	No residual tumour, minimal distance between tumour and resection margin; margin ≤1 mm
R1-dir	Microscopic residual tumour, tumour directly at the resection margin (tumour transected)
R2a	Local macroscopic residual tumour
R2b	Distant macroscopic residual tumour
R2c	Macroscopic residual tumour in both sites

Tumour biology

Tumourigenesis

CRC is one of the genetically most well-described cancers. In a stepwise order during the cancer development, multiple alterations of the cancer cell genome accumulate and the cell dysplasia gradually increases. In CRC, there is a continuous progression from normal epithelium to aberrant crypt foci (ACF), early adenoma, late adenoma, cancer, and finally metastasis. The accumulation of genetic alterations in various genes and morphological progression from normal epithelium to CRC (the adenoma-carcinoma sequence) was initially described by Fearon and Vogelstein (Fig. 6)[69, 245]. Approximately 10% of the adenomas progress to CRC; this progression takes between 10 and 15 years[245]. Although the alterations commonly occur in a preferred order, the total amount of alterations is most crucial in the tumour progression[69]. Today, it is considered that at least seven alterations are required for the progression from normal epithelium to CRC[69, 211]. The adenoma-carcinoma sequence is the result of a clonal expansion of cells, which have acquired a selective growth advantage, making them able to outgrow surrounding cells. This is enabled by alterations in genes that control cellular proliferation, differentiation, and programmed cell death (apoptosis). The genes are usually divided into three categories: (proto)oncogenes, tumour suppressor genes, and DNA mismatch repair (MMR) genes[26, 69, 94, 149, 211, 245].

A (proto)oncogene is a gene which activation will result in cellular proliferation, differentiation, or inhibition of apoptosis mediated by the protein gene product. Tumourigenesis is directly promoted. Activation of the (proto)oncogene turns it into an oncogene resulting in a gain of function. Oncogenes act in a dominant manner as alteration in one allele is sufficient for their function. One of the best characterized oncogenes in CRC is *KRAS*. A tumour suppressor gene is a gene that is involved in the normal cell homeostasis by repressing cell proliferation and promoting cell differentiation. Thereby, it opposes the malignant phenotype. Alteration of a tumour suppressor gene results in a loss of function. They act in a recessive manner as both alleles have to be inactivated before their function is lost. Common tumour suppressor genes involved in colorectal tumourigenesis are *APC, TP53*, and *SMAD4*. Our DNA is continuously exposed to damage and MMR genes represent one group of genes involved in the mechanisms for repair. The MMR genes maintain the stability and integrity of the genome and prevent the manifestation of potentially tu-

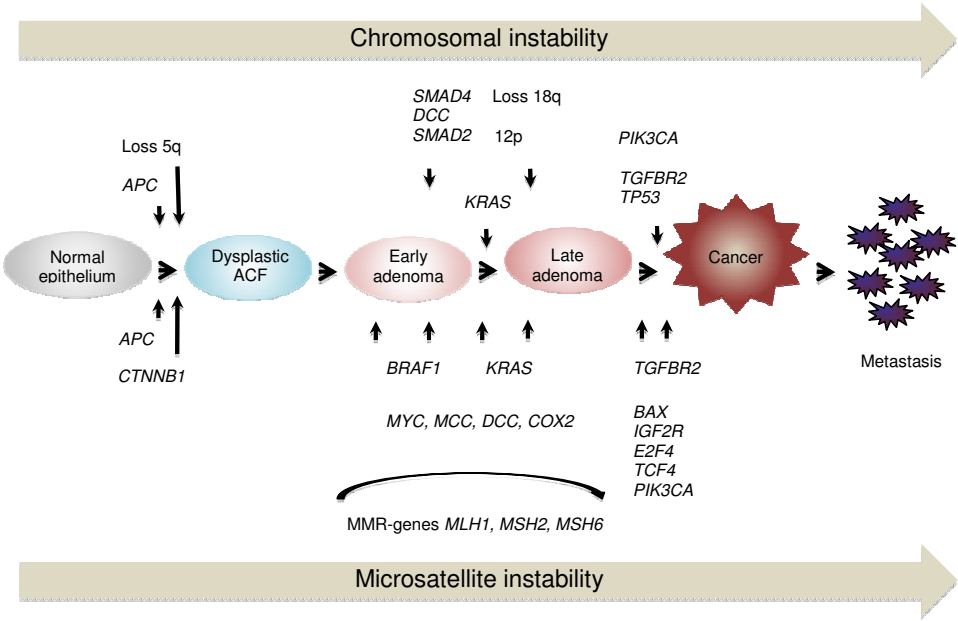


Figure 6. The adenoma-carcinoma sequence[69]. The figure is reproduced by permission of Anna Isinger Ekstrand.

mourigenic mutations. Inactivation of these genes promotes tumourigenesis through increased mutation rates. The most common altered MMR genes in CRC are *MLH1, MLH3, MSH2*, and *MSH6*.

Since the original description of the adenoma-carcinoma sequence, important data concerning the molecular pathogenesis of CRC have accumulated. More genes than the initially described *APC, KRAS*, and *TP53* have gained attention, and a heterogeneous pattern of mutations has been detected. The key principles of the adenoma-carcinoma sequence are of importance for the understanding of CRC tumourigenesis, but the accumulated data support that there are multiple alternative genetic pathways leading to CRC[211, 223]. Three major pathways have been described: the chromosomal instability (CIN), the microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP) pathways. The first pathway includes approximately 85% of all CRC, whereas the last two include the remaining 15%.

The chromosomal instability pathway

The term CIN refers to an accelerated rate of gains and losses of whole or large portions of chromosomes, resulting in karyotypic variability from cell to cell. CIN tumours are preferably located in the distal colon and show classical morphology[143]. The CIN pathway follows the originally described adenoma-carcinoma sequence, with inactivation of the *APC* gene at initiation, followed by activation of *KRAS* and subsequent alterations in *TGF-β, PIK3C* and *TP53* pathways[149, 211].

APC and CTNNB1

Inactivation of the tumour suppressor *APC*

gene, located on chromosome 5, is considered as the initiating step in the adenoma-carcinoma sequence. The *APC* gene is referred to as the "gatekeeper gene" of CRC. *APC* mutations are present already in the ACF. Germline mutations in the *APC* gene give rise to the inherited syndrome FAP. *APC* interacts with the (proto)oncogene *β-catenin (CTNNB1)* and modulates activity in the Wingless/Wnt signalling pathway and in E-cadherin mediated cell adhesion. In the absence of APC, activation of the Wnt signalling pathway leads to translocation of β-catenin into the nucleus, and transcription of genes involved in proliferation, invasion, apoptosis, and cell cycle progression. Apart from its role in the Wnt signal transduction pathway, β-catenin also forms complexes with the adhesion molecule E-cadherin, resulting in enhanced adhesiveness of the cells. Both these actions are inhibited by the presence of APC since it forms complexes with β-catenin, making it accessible for degradation. An alternative way of activation of the Wnt signalling pathway by gain of function mutations in the *CTNNB1* gene has been found in CRC with intact *APC* gene. Activation of the Wnt signalling pathway is thereby achieved by increased activation of *CTNNB1* in the presence of APC[7, 149, 211, 223].

KRAS

Activation of *KRAS*, located on chromosome 12, is associated with the growth of the adenoma. *KRAS* regulates multiple cellular functions through well-described pathways, i.e., cell growth, differentiation, proliferation, cell motility, cytoskeleton organization, cell cycle progression, cell survival, and apoptosis[149, 211, 223].

Loss of chromosome 18q

Loss of heterozygosity (LOH) at chromosome 18q is common in late stages of the adenoma-carcinoma sequence, presumably leading to further growth and progression. Loss of the tumour suppressor gene *deleted in colorectal carcinoma (DCC)* has been found in CRC. The *DCC* gene encodes a protein involved in apoptosis, and cell cycle arrest as well as cell-cell and cell-extracellular matrix (ECM) interactions. However, the role of *DCC* in CRC pathogenesis has been questioned since mutation of the gene is a rare finding in human CRC. Other tumour suppressor genes located on 18q are *SMAD2* and *SMAD4* which regulate cell growth, differentiation, and apoptosis. However, inactivation of the *SMAD2* and *SMAD4* genes are rare in CRC, so their importance also remains elusive[149, 211].

TP53

The tumour suppressor *TP53* gene, on chromosome 17, has been named "the guardian of the genome", and *TP53* dysfunction is the most frequently described alteration in human cancers. The inactivation of *TP53* mediates the transition from adenoma to carcinoma. The *TP53* protein controls transcription of multiple genes involved in DNA metabolism, apoptosis, cell cycle regulation, senescence, angiogenesis, immune response, cell differentiation, motility, and migration. In the normal cell, *TP53* is a tumour suppressor and a coordinator of cellular responses to stress. DNA damage, aberrant proliferative signals, and oxidative stress activate the *TP53* gene. In CRC tumourigenesis, its inactivation is linked to the transition from adenoma to carcinoma. In the very early phase of the adenoma-carcinoma sequence, inactivation of *TP53* is a rare event, indicating that *TP53* has a role in tumour progression and not initiation[149, 211, 223, 246].

The microsatellite instability pathway

MSI is a hypermutable phenotype due to a defective function of the MMR system. It is characterized by multiple replication errors at so called microsatellites. Microsatellites are DNA sequences composed of tandem repeats one to six nucleotides long, usually located in non-coding regions scattered throughout the

genome. During DNA replication, slippage of the DNA polymerase is common during replication of long repetitive DNA sequences, such as microsatellites. Normally this is repaired by the MMR system. With a non-functioning system, these errors are not repaired. The mutation is propagated resulting in a truncated, non-functional protein and in genomic instability. The MSI tumours are commonly located in the right colon, are poorly differentiated with a high mucinous component, are surrounded by a greater number of activated/cytotoxic tumour-infiltrating lymphocytes, and are diploid. Germline mutations in one of the genes coding for the proteins in the MMR system is the cause of the most common hereditary CRC syndrome, HNPCC. The *MSH2* (chromosome 2), *MLH1* (chromosome 3), *MSH6* (chromosome 2), and *PMS2* (chromosome 7) are different genes that are inactivated in this syndrome[26, 149, 211].

CpG island methylator phenotype pathway

However, 75–80% of the MSI tumours do not have a germline mutation in one of the genes in the MMR system. These CRC are not inherited, but arise through sporadic methylation-induced silencing of the *MLH1* gene. Many genes have promoters embedded in clusters of cytosine-guanosine residues called CpG islands. DNA methyltransferases can methylate cytosines in these regions. By methylation the gene is permanently silenced. The characteristic features of the CIMP pathway are biallelic methylation of the *MLH1* promoter, absence of *MLH1* and *PMS2* proteins, frequent mutation in *BRAF*, and the tumour cells are diploid. Familial clustering is missing and the patients are older than patients with HNPCC. Thus, both the MSI and CIMP pathways are consequences of a defective MMR system, but through different mechanisms[26, 149, 211].

Tumour markers

Prognostic and predictive tumour markers in serum and tissue

The potential prognostic or predictive value of several specific tumour-associated proteins (tumour markers), characteristic of particular cellular events in tumour tissue have been studied in CRC. The prognostic markers serve as identifiers of patients at risk of a specific outcome, such as tumour recurrence or death. The prognostic markers have no function in the choice of a specific therapy. The predictive markers serve to predict the efficacy or benefit of a specific therapy and may thereby be used to guide the choice of therapy. Despite extensive research, still no single marker or combinations of markers have provided any unequivocal prognostic or predictive information in CRC. Various techniques – IHC or PCR – have been used in the analyses. Information on rectal cancer as one entity is scarce, since in most studies colon and rectal cancer are analysed together. The studies are often hampered by insufficient clinical data of the tumour material and lack of standardization of methods. Conclusions from and comparison of the studies are difficult due to these drawbacks.

Serological markers

The carcinoembryonic antigen (CEA) is a serum glycoprotein and a member of the immunoglobulin superfamily. CEA is an adhesion molecule promoting aggregation of CRC cells and thereby facilitating metastasis. CEA is elevated in 85% of patients with CRC. Elevated CEA at diagnosis is an indicator of poor prognosis. Postoperatively CEA levels should return to normal within six weeks. Elevated levels in the postoperative period suggest remaining or recurrent tumour[13, 57, 228]. Carbohydrate antigen 19–9 (CA19–9) and 242 (CA 242) are measures of tumour-associated mucin

and have been correlated to poor prognosis[13, 57, 228]. The same holds for elevated preoperative levels of the glycoprotein, tissue inhibitor of metalloproteinase type 1 (TIMP-1) that inhibits matrix metalloproteinases, promotes cell proliferation, and inhibits apoptosis[57, 228]. The markers – tissue polypeptide antigen (TPA), which corresponds to proteolytic fragments of the cytokeratins 8, 18, and 19 from epithelial cells and specific tissue polypeptide antigen (TPS), which indicates a soluble fragment of cytokeratine 18[40, 144, 166] – have also been found to indicate worse prognosis. However, the results have been contradictory and the sensitivity of the markers is low. Presently CEA is the only one of the serological markers that is used in clinical practise[174, 175].

Tissue markers

The prognostic value of *APC/β-catenin* alterations remains uncertain. Because alterations in *APC/β-catenin* in CRC are common, they are difficult to evaluate. Overexpressed *β*-catenin in general does not seem to be a prognostic indicator, but determining the cellular location of overexpressed *β*-catenin might be. Reduction of membranous staining and lack of cytoplasmatic staining have been correlated to increased risk of DM. Nuclear accumulation of *β*-catenin has been correlated to worse survival. However, there are studies contradicting these results[74, 149, 169, 248].

Ki-67 is a proliferation antigen present in all phases of the cell cycle, except for the resting cells in G0. Determination of Ki-67 correlates to the "growth fraction" of cancer cells as well as normal cells. In several tumours, Ki-67 has been of prognostic value, but in CRC the findings have been opposing, with both a low and a high Ki-67 expression linked to worse survival[13, 211].

EGFR is a transmembrane receptor tyrosine kinase, which can be activated by ligand-dependent, ligand-independent, and overexpression mechanisms. EGFR activates several pathways involved in proliferation, differentiation, invasion, DNA repair, angiogenesis and apoptosis. EGFR is the target for the monoclonal EGFR-specific antibodies as well as tyrosine kinase inhibitors. High EGFR expression has been correlated to less benefit of preoperative RT and poor survival[9, 16, 258].

The results from studies addressing the importance of *KRAS* mutation for recurrence and death are contradictory. However, *KRAS* mutation is of clinical predictive importance since it has been found that tumours with *KRAS* mutations do not respond to therapy with EGFR inhibitors, since *KRAS* mutations lead to constitutive downstream activation of EGFR signalling. *KRAS* mutation status is now used in clinical practise as a predictive marker for treatment with EGFR inhibitors[13, 57, 149, 175, 211, 228, 248]. *BRAF* acts downstream of *KRAS*. Mutations in *BRAF* have also been found to result in resistance to EGRF inhibitors[149, 248].

The results are inconsistent with respect to the prognostic role of p53: some studies report a higher risk of death with *TP53* mutation and others do not. Patients with *TP53* mutant tumours have been reported to not have a survival benefit from 5-FU based adjuvant chemotherapy, whereas those without mutations did. However, other reports have opposed this, so p53 is not recommended as a predictive marker in 5-FU based therapy[5, 13, 57, 211, 228, 248]. *Bcl-2* is a (proto)oncogene that encodes an intracellular membrane protein inhibiting apoptosis. Bcl-2 is thought to be inhibited by p53. Reports on the correlation between overexpression of Bcl-2 and survival are also conflicting[13].

LOH of the long arm of chromosome 18 has been suggested to correlate with poor survival, but there are contradicting reports. Less favourable outcome after 5-FU based adjuvant chemotherapy has been reported. *DCC* was the first of the CRC-associated genes described on 18q. In addition, the genes *SMAD2* and *SMAD4* are located there. Therefore, the lost region might contain these two genes as

well as *DCC*. Loss of any one of these genes might be of prognostic or predictive value[5, 149, 211, 228, 248].

MSI has been correlated to improved survival. Several studies have also proven that patients with tumours with MSI do not benefit from 5-FU based adjuvant chemotherapy. The prognostic and predictive value of MSI holds for both germline mutations in the MMR, as well as *MLH1* methylation-associated silencing[13, 26, 57, 211, 228, 248].

VEGF is a proangiogenic factor. VEGF regulates normal and pathologic angiogenesis. It promotes endothelial cell growth, migration, differentiation, and vascular permeability. Monoclonal VEGF-specific antibodies have become an important adjunct in the modern therapeutic arsenal. High VEGF expression is an indicator of poor survival, but does not predict response to anti-VEGF treatment[13, 55, 258].

To be able to invade and metastasise, cancer cells need to break down the surrounding ECM. Matrix metalloproteinases (MMPs) are a family of metalloenzymes that can do this. High expression of MMP has been correlated to reduced survival in some reports, but other reports have not been able to reproduce this[13, 214, 274].

The urokinase plasminogen activator (uPA) is a member of the serine protease family and binds to a specific cell surface receptor (uPAR). The enzyme uPA is also involved in ECM degradation and a key component in cancer cell migration, invasion, and metastasis. The plasminogen activator inhibitor-1 (PAI-1) controls uPA. High levels of both uPA and PAI-1 have been correlated to worse survival[137, 165, 228].

In DNA replication, TS is an essential enzyme. TS is the target for 5-FU. Increased levels of TS are associated with resistance to 5-FU. In addition, high TS levels have been correlated to poor survival, but the results are also conflicting[5, 13, 57, 126,228, 248].

Ezrin

Ezrin (cytovillin, p81, 80K), the product of the *Vil2* gene on chromosome 6, is a protein belonging to the ezrin/radixin/moesin (ERM) family. The primary function of ezrin is to link the plasma membrane to the actin-based cytoskeleton and to stabilize this linkage. In addition, ezrin participates in the organization of the distribution of several membrane receptors and the signalling by these receptors[30, 70, 103, 109, 158, 242, 268].

In vivo, epithelial and mesothelial cells express ezrin. In normal cells, ezrin is usually located under the apical plasma membrane, whereas in cancer cells translocation to the cytoplasm or the complete apical membrane is common. Ezrin is synthesized in an inactive form in which the N-terminal domain binds the C-terminal domain. Thereby, the domains are mutually blocking the ability to bind to other molecules. Activation is achieved in a two-step model by a combination of phospholipid binding and phosphorylation. Upon activation, the free N-terminal domain binds to membrane proteins, and the free C-terminal domain binds to actin[28, 30, 70, 103, 109, 158, 179, 242, 265, 268]. It is debated whether measure of phosphorylated and active ezrin is a more valuable predictor of clinical biology than measure of total ezrin. Since the antibodies for phosphorylated ezrin might cross react with phosphorylated forms of the other ERM proteins, most investigators have chosen to analyse total ezrin[30, 51].

Ezrin has been found to be involved in several crucial events in cell homeostasis, e.g., proliferation, cell-cell communication, cell-ECM communication, motility, differentiation, and apoptosis. The central role of ezrin in all these events makes ezrin a key factor in tumour development and metastasis. Considerable knowledge of the role of ezrin has gathered recently, but still much of its role remains to be elucidated[30, 67, 70, 103, 109, 132, 135, 242, 268].

Ezrin expression has been analysed in cell

lines and tumour tissue from various solid cancers such as oesophageal[263], pancreatic[51], breast[30], melanoma[111], lung[30], glioma[238], ovarian[134], prostate[30], and sarcoma[30, 127, 250]. High ezrin expression has been correlated to aggressive biological behaviour with correlations to reduced time to LR, increased DM rate, poor survival, and chemotherapy resistance[28, 30, 51, 111, 127, 134, 238, 250, 263].

In CRC cell lines, the role of ezrin in cell-cell contact, cell-ECM adhesion, and motility has been demonstrated, as well as a possible interaction with E-cadherin and β-catenin[82, 103]. Ezrin has recently been analysed in CRC tumour tissue samples[63, 82, 249, 265], indicating the importance of ezrin for tumour progression, development of lymph node metastasis and DM, and subsequent reduced survival. In the study by Yan *et al.,* that included 86 patients with rectal cancer, the ezrin expression was higher in CRC than in normal epithelium[265]. In addition, it was significantly higher among patients that developed DM. Moreover, increased ezrin expression was also correlated to worse survival. Furthermore, translocation of ezrin to the complete apical membrane in contrast to cytoplasmic staining revealed membrane translocation to be associated with increased risk of DM and worse survival[265]. However, data for rectal cancer tumours were not separately analysed in the study. The association of membrane translocation of ezrin and metastatic potential has also been demonstrated in CRC cell lines[179]. In a study by Wang *et al.,* ezrin expression was analysed in 80 CRC and 22 normal colorectal epithelium specimens without stating the number of rectal cancer specimens[249]. It was concluded that ezrin expression, as found by Yan *et al.,*[265], was significantly higher in CRC than normal epithelium and that high ezrin expression was closely related to poorer degree of tumour differentiation, higher extent of lymph node involvement, and more advanced Dukes' stage. Neither clinical nor histopathological features could be correlated to ezrin expression in the study by Yan *et al.,*[265]. Elzagheid *et al.,* analysed ezrin expression in 74 patients with advanced CRC where the majority had DM at diagnosis and the remaining later developed DM[63]. However, this study included only 14 patients with rectal cancer. Among patients with high ezrin expressing tumours in TNM stages II–III, a shorter disease-specific survival was observed; similarly, patients with high ezrin expressing tumours in TNM stage IV also had a shorter survival. Elzagheid *et al.,* also observed a more intense expression in the cancer cells at the invasive front, and the cells also had morphologic characteristics of "budding cells"[63]. A more intense staining at the invasive front has also been found by Gavert *et al.,*[82].

The predictive role of ezrin is even less documented than the prognostic role. However, ezrin has been associated to resistance to chemotherapy[28]. One suggested mechanism for resistance to chemotherapy is the connection of ezrin to P-glucoprotein, a major mediator of multidrug resistance encoded by the *MDR*-gene[28]. The study by Elzagheid *et al.,* in which all patients received chemotherapy, showed a declining trend in response to 5-FU treatment in parallel with increasing expression of ezrin[63]. In addition, a potential role of ezrin in platinum-based chemotherapy has also been described in CRC cell lines[204, 267]. The protein expression of ezrin was increased with the duration of oxaliplatin treatment[267], and ezrin involvement in cisplatin-induced apoptosis has also been demonstrated[204]. Although the data are scarce, it suggests a possible future predictive role of ezrin and possibly ezrin or factors regulating ezrin activation as potential targets of newer therapeutics.

Immunohistochemistry

IHC is the demonstration of antigens (proteins) within a tissue section by means of specific antibodies. The antigen-antibody binding is visualized by a marker such as a fluorescent dye or an enzyme able to catalyse a

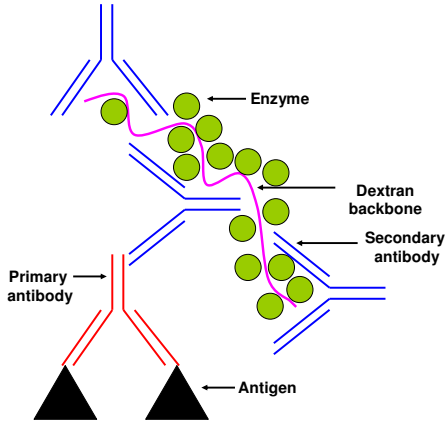


Figure 7. Principle for immmunohistochemistry – indirect polymer method.

colour-producing reaction. The technique is widely used for localization of tumour markers[201]. First, the formalin-fixed paraffin-embedded tumour tissue is deparaffinized and rehydrated. Formalin-fixation and paraffin-embedding of the tumour tissue might have caused alterations of the antigens; this is why a step is needed where the antigens are restored: this step is called unmasking. It can be achieved either by the use of heat (Heat Induced Epitope Retrieval) or enzyme digestion (Proteolytic Induced Epitope Retrieval). There are two IHC strategies, the direct method or the indirect method. The direct method is a one-step method and involves a labelled antibody reacting directly with the antigen in the tumour tissue. The method is simple and rapid. However, due to little signal amplification, the sensitivity can be low. The indirect method uses an unlabeled primary antibody that reacts with the antigen and a secondary antibody that reacts with the primary antibody. The sensitivity of this method is greater due to signal amplification through several secondary antibody reactions with different antigenic sites on the primary antibody. The indirect method is the most commonly used. The secondary antibody can be labelled with a fluorescent dye (indirect immunofluorescence method) or an enzyme (indirect immunoscence method). The enzyme then reacts with a substance (chromogen) that upon oxidation by the enzyme produces staining at the site of the antigen. The techniques are continuously evolving, striving to increase sensitivity. One variant of indirect IHC is illustrated in Figure 7. In this polymer-based IHC method, multiple enzyme molecules and secondary antibodies are attached to a dextran backbone[202]. IHC analysis in tumour samples stored over 50 years has been possible to perform[39].

Tissue microarray

IHC analysis of tumour markers on whole tissue sections is labour intensive, time consuming, and tissue consuming. IHC analysis with TMA technique is an alternative well-established method[129]. In the TMA technique, tissue cores, usually with a diameter of 0.6 mm, are obtained using a hollow needle from a formalin-fixed paraffin-embedded tumour sample (donor block). Tissue cores can be taken from regions of interest, and preoperative as

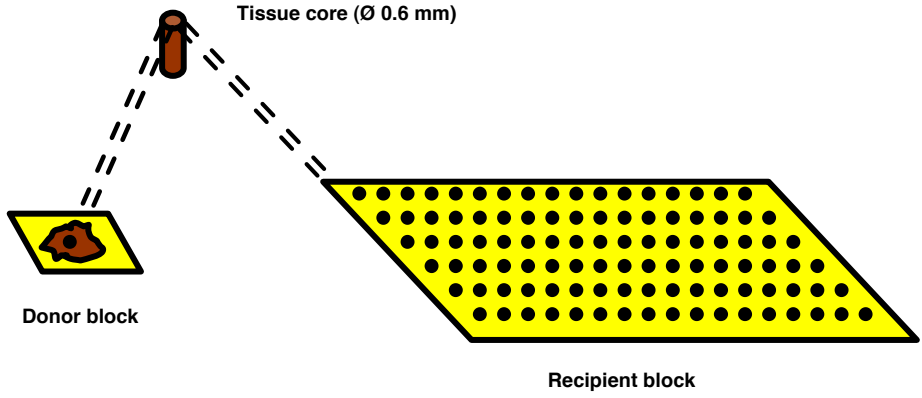


Figure 8. Principle for construction of TMA block.

well as operative specimens can be used. The tissue cores are then inserted and re-embedded in a recipient block in a precisely spaced array pattern (Fig. 8). Sections from this recipient block are then cut with a microtome and mounted on a microscope glass slide. Thus, each slide can contain up to 1000 tissue cores from multiple tumours. To make the reading of the slides more comfortable, usually 100–300 cores are inserted. The slide can then be analysed by any method of standard histology such as IHC. Depending on the height of the tissue core, which depends on the thickness of the donor block, the recipient block can be cut into a various number of sections. Up to 300 sections can be achieved. Each section, containing tissue cores from a number of samples, can then be analysed for a tumour-associated protein with IHC. Thus, analysis of several markers in a great number of tumours is facilitated, and the technique takes less time and preserves more tissue. The technique has been evaluated for rectal cancer[73]. In TMA, only a small sample of the tumour is analysed; this is a limitation since tumour heterogeneity is common. TMA also has technical limitations such as non-uniform staining, unrepresentative material due to necrosis or benign tissue within the specimen, and loss of tissue cores during array construction or folding after sectioning of the recipient tissue block[39, 73]. Commonly, 10–15% of the sections are reported to be lost when using the TMA technique[39]. By taking three tissue cores from each tumour, the loss of material is minimized. In addition, if they are taken from three different areas of the tumour, the problem with tumour heterogeneity is overcome. However, of utmost importance is accurate sampling from histologically representative regions of the specimen[108, 129]. By considering the above-mentioned drawbacks, comparable results to whole tissue sections have been obtained[39, 73, 108, 129].

Aims

Paper I

- To use the unvalidated, prospectively registered data in the SRCR to further analyse the 5-year LR rate, the 5-year overall survival rate, and the 5-year cancer-specific survival rate.

- To use the unvalidated, prospectively registered data in the SRCR to identify potential risk factors of LR.

- To analyse the subgroup of patients with registered LR in a descriptive manner using additional data from original medical records.

- To validate the variable LR and other variables in the subgroup of patients with registered LR in the SRCR by comparison to original medical records.

Paper II

- To analyse the impact of AL after AR in patients with tumours in TNM stages I–III and R0 surgery on the rates of LR, DM, and OAR as well as overall and cancer-specific survival in a validated subgroup of patients with registered AL after AR compared to patients without AL selected from the SRCR.

- To validate the variable AL and other variables in a subgroup of patients with registered AL after AR and patients without AL selected from the SRCR.

Paper III

- To analyse the impact of incidental perforation in patients with tumours in TNM stages I–III and R0 surgery on the rates of LR, DM, and OAR as well as overall and cancer-specific survival in a validated subgroup of patients with registered perforation at major abdominal surgery compared to patients without registered perforation selected from the SRCR.

- To validate the variable perforation and other variables in a subgroup of patients with registered perforation at major abdominal surgery and patients without registered perforation selected from the SRCR.

Paper IV

- To evaluate the prognostic value of the ezrin expression in a well-defined cohort of patients with rectal cancer that developed isolated LR or LR in combination with DM within five years of R0 major abdominal surgery.

Patients and methods

Patients

The cohort of studied patients in this thesis includes all patients (*n*=4153) with newly diagnosed rectal cancer registered in the SRCR between 1 January 1995 and 31 December 1997. Thirteen patients (0.3%) were lost to follow-up. The selection of patients from the cohort in Papers I–IV is illustrated in Figure 9. Surgery was performed in 3872/4153 (93%) patients. Baseline characteristics of this group of patients as well as the tumours and the treatment are listed in Table 11. Preoperative RT was administered to 2135/3872 (55%) patients treated with surgery. Major abdominal surgery was performed in 3196/3872 (83%) patients, LE was chosen in 276 (7%) patients, and exploratory laparotomy only or with a stoma formation was carried out in 400 (10%) patients. Rectal washout was performed in 1573/2183 (72%) patients having AR or HA. Incidental perforation was registered in 208/3196 (6%) patients treated with major abdominal surgery. Local radicality was achieved in 2959/3872 (76%) patients in the cohort with no significant differences according to the tumour height (0–5 cm: 76%, 6–10 cm: 78%, 11–15 cm: 77%). AL was registered in 172/1977 (9%) patients after AR.

In Paper I, the whole cohort (*n*=4153) was studied. For the patients with registered LR (*n*=326), subgroup analysis was performed. In Paper II, all patients with registered AL (*n*=172) after AR (*n*=1977) were selected from the cohort. One control for each patient with registered AL was selected randomly among patients that had undergone AR in the cohort, but without registered AL. Thus, 344 patients were included in Paper II. In Paper III, all patients with a registered incidental perforation (*n*=208) at major abdominal surgery (*n*=3196) were selected from the cohort. Controls were selected randomly among patients that had undergone major abdominal surgery in the cohort, but without registered incidental perforation. The number of controls was

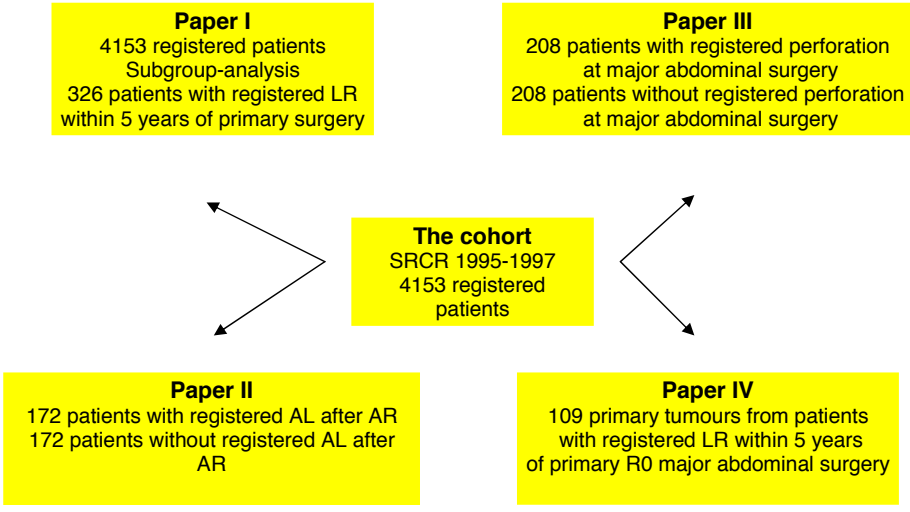


Figure 9. The selection of patients from the cohort to Paper I-IV.

Table 11. Patient, tumour, and treatment characteristics for patients registered in the Swedish Rectal Cancer Registry, 1995-1997 treated with surgery.

		Patients treated with surgery (*n*=3872)
Age (years) at primary surgery		72 (21–95)*
Gender	M	2191 (57)
	F	1681 (43)
Tumour height (cm)	Low: 0–5	1218 (31)
	Medium: 6–10	1437 (37)
	High: 11–15	1129 (29)
	Unknown	88 (2)
TNM-stage	I	892 (23)
	II	1110 (29)
	III	1057 (27)
	IV	574 (15)
	Unknown	239 (6)
Preoperative radiotherapy	No	2135 (55)
	Yes	1677 (43)
	Unknown	60 (2)
Preoperative chemotherapy	No	3751 (97)
	Yes	45 (1)
	Unknown	76 (2)
Surgery	AR	1977 (51)
	APR	1013 (26)
	HA	206 (5)
	LE	276 (7)
	Other procedure[a]	400 (10)
Local radicality	Radical	2959 (76)
	Uncertain/non-radical	808 (21)
	Unknown	105 (3)
Rectal washout[b]	No	527 (24)
	Yes	1573 (72)
	Unknown	83 (2)
Rectal perforation[c]	No	2810 (88)
	Yes	208 (6)
	Unknown	178 (6)
Anastomotic leakage[d]	No	1805 (91)
	Yes	172 (9)

Values in parentheses are percentages, unless * where it is range. [a]Exploratory laparotomy with or without stoma formation. [b]Studied for AR and HA. [c]Studied for AR, APR and HA. [d]Studied for AR. AR, anterior resection; HA, Hartmann's procedure; APR, abdominoperineal resection; LE, local excision.

the same as the number of cases. Thus 416 patients were included in Paper III. In Paper IV, patients treated with R0 major abdominal surgery for tumours in TNM stages I–III and who developed LR within 5 years were identified in the cohort (*n*=174). Tumour tissue blocks from 109 primary rectal cancers were possible to retrieve. Thirty-two patients had been treated with preoperative RT, and 77 patients were treated with surgery alone.

Methods

Paper I–III

In Paper I, the analysis of the whole cohort was performed by the use of unvalidated SRCR data, primary as well as follow-up data. The 5-year LR rate and the 5-year overall as well as cancer specific survival were analysed. LR risk factors were analysed with multivariate methods. For the subgroup-analysis of patients with registered LR within 5 years of primary surgery, the data in the SRCR database were validated by comparison to the original medical records. Additional data were also extracted from the original medical records. The subgroup was analysed in a descriptive manner. Time to development of LR and survival was analysed in this group. In Paper II and III, SRCR data were validated and additional data extracted in the same manner as for the subgroup of patients with registered LR in Paper I. In Paper II, differences between patients with AL after AR *vs*. controls, in LR and DM rate, as well as overall and cancer-specific survival at 5-year follow up were analysed with multivariate methods. In Paper III, differences between patients with incidental perforation *vs*. controls in oncological outcome were analysed in the same manner as in Paper II. In Paper IV, patients were defined in the subgroup of patients with registered LR in Paper I. Therefore, data on the patients, tumours, and treatment were validated and supplemented in Paper I. In Table 12, variables in the SRCR that were validated by comparison to original medical records are listed. In Table 13, the additional data not included in the SRCR but extracted from original medical records are listed.

The number of patients in Paper I–III

Table 12. Validated variables in the Swedish Rectal Cancer Registry.

Primary registration form	Follow-up form
Tumour height	LR
Preoperative RT	Date of LR
Preoperative chemotherapy	DM
Surgery performed or not	Date of DM
Date of surgery	Location of DM
Type of surgery	
Local radicality according to the pathologist	
Local radicality according to the surgeon	
Rectal washout	
Rectal perforation	
AL	
TNM stage	

RT, radiotherapy; AL, anastomotic leakage, LR, local recurrence; DM, distant metastasis.

Table 13. Additional variables not included in the Swedish Rectal Cancer Registry.

Tumour related	Treatment related	Postoperative period related	Pathology related	Follow-up related
Macroscopic type[a]	Radiation dose[c]	Time to AL	Differentiation[g]	Location of LR
Position[b]	TME or not	Site of AL	T stage	Diagnostic modality of LR
	Anastomosis type	Management of AL[f]	N stage	
	Moment of perforation[d]		No. of examined lymph nodes	
	Site of perforation[e]		No. of positive lymph nodes	
	Faecal contamination		CRM (mm)	
	Abdominal drainage		DRM (mm)	
	Diverting stoma		R classification	

AL, anastomotic leakage; CRM, circumferential resection margin; DRM, distal resection margin; LR, local recurrence. [a]Non-annular or annular. [b]For non-annular tumours; anterior, posterior or lateral. [c]25 or 50 Gy. [d]For abdominoperineal resection, abdominal or perineal phase. [e]In the tumour or in another part of rectum. [f]conservative *vs.* surgical treatment and in case of surgery, surgical procedure performed. [g]Tumour differentiation grade: well, moderate or poor.

Table 14. Missing medical records in retrieval of additional data.

	Paper I	Paper II		Paper III		Total
	Patients with LR (*n*=326)	Cases (*n*=172)	Controls (*n*=172)	Cases (*n*=208)	Controls (*n*=208)	Patients (*n*=1086)
Complete medical record	3	0	2	4	5	14
Sections of medical record	8	4	4	5	4	25

LR, local recurrence.

where retrieval of additional data from original medical records was unsuccessful is listed in Table 14. When requested data were not possible to retrieve, the data in the SRCR were used in the analyses.

Paper IV

Patients and specimens

Tumour blocks from 109 patients were retrieved. The median age of the 109 patients (59 men, 50 women) at diagnosis of the primary tumour was 73 (37–87) years. Ten (9%) tumours belonged to TNM stage I, 32 (29%) to TNM stage II, and 67 (62%) to TNM stage III. TMAs were constructed using three core biopsies from each tumour. Diagnostic tumour biopsies were used from the 32 patients treated with RT, whereas the operative specimens were used from the 77 patients who were treated with surgery alone. For the TMAs, representative tumour areas were selected (FJ and

GL), and 0.6-mm core needle biopsies were obtained using a manual arrayer (Beecher Instruments, Sun Prairie, Wisconsin, USA).

Immunohistochemistry

Fresh 4-µm sections from TMA blocks transferred to glass slides (DAKO ChemMate Capillary Gap Microscope Slides, 75 mm, DAKO A/S, Glostrup, Denmark) were deparaffinised, rehydrated, and then pre-treated in Tris-EDTA-buffer S2367 (pH 9) (DAKO A/S, Glostrup, Denmark) in a pressure cooker for 15–20 minutes for antigen retrieval. An automated immunostainer (TechMate® 500Plus, DAKO, A/S, Glostrup, Denmark) was used for the staining procedure with DAKO REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse (DAKO A/S, Glostrup, Denmark) based on an indirect polymer method. The primary antibody murine monoclonal IgG to human ezrin, clone 3C12, mouse ascites fluid (Sigma®, St Louis, Missouri, USA) was diluted 1:5000. After counterstaining with hematoxylin, the slides were dehydrated in ascending concentrations of ethanol and mounted. For 5/109 (5%) tumours, the material was lost during preparation.

Immunoreactivity scoring

All slides were evaluated for cytoplasmatic staining by two of the investigators (FJ and MN) in an open discussion without knowledge of the clinical data. The immunostaining was graded into four categories – (negative); 1+ (weak); 2+ (moderate); and 3+ (intense). For statistical analysis, the categories were dichotomized. Weak-moderate staining was considered low ezrin expression; intense staining was considered high ezrin expression.

Statistics

Paper I–III

Data were analysed with the use of SPSS® version 15.0.0 for Windows® (SPSS, Chicago, Illinois, USA) statistical software, and figures were made in S-PLUS® version 6.0.2 for Windows® (Insightful Corporation, Seattle, Washington, USA). In Paper I, odds ratios in the multivariate analysis were calculated using multivariate logistic regression and adjusting for several covariates. In Paper II and III, relative risks in the multivariate analysis were calculated using Cox regression. *P*-values in results were calculated using the *t*-test and X^2-test. The Kaplan-Meier method was used in SPSS to calculate coordinates, and these were then used in S-PLUS to make the survival curves. All tests are two-sided and *P*-values <0.05 were considered statistically significant.

Paper IV

Data were analysed using SPSS® version 15.0.0 for Windows® (SPSS, Chicago, Illinois, USA). The X^2-test, X^2-test for trend, Mann-Whitney U test, and Fisher's exact test were used for group comparisons when appropriate. Curves for time to diagnosis of LR and DM were calculated according to the Kaplan-Meier method, and the log-rank test was used for comparison between the groups. A trend version of the log-rank test was used for comparison of prognosis in three ordered groups. Cox regression analysis was used for multivariate analysis of time to development of LR. The underlying proportional hazards assumptions were checked graphically. Stata/SE 11.0 for Windows (StataCorp LP, College Station, Texas, USA) was used for multivariate analysis and to draw the curves in Figure 1. All tests are two-sided and *P*-values <0.05 were considered statistically significant.

Ethics

The studies were approved by the Regional ethical review boards.

Results

Paper I

Unvalidated SRCR data

In the cohort, 326 LR were registered within five years from primary surgery giving a crude 5-year LR rate of 8%. The 5-year overall survival rate was 45% and the 5-year cancer specific survival rate was 62%. The 5-year overall survival rates for patients with LR or without LR were 18 and 51%, and the 5-year cancer specific survival rates were 27 and 68%, respectively. Identified risk factors of LR in the performed multivariate analysis were tumour height below 5 cm from the anal verge, no preoperative RT, rectal perforation, TNM-stages II/III, and an uncertain or non-radical surgical procedure as indicated by the status of the CRM. After R0 major abdominal surgery for tumours in TNM-stages I–III, the LR rate was 10% (81/812) for the tumours at the height of 0–5 cm, 8% (76/993) at 6–10 cm, and 6% (47/798) at 11–15 cm. There was a significant reduction in the number of LR after preoperative RT irrespective of the tumour height: 0–5 cm: OR 0.50 (0.30–0.83), 6–10 cm: OR 0.42 (0.25–0.71), and 11–15 cm: OR 0.29 (0.13–0.64).

In the initial multivariate analysis, rectal washout and AL did not have any significant impact on the LR rate. The potential impact of rectal washout, rectal perforation, and AL on the development of LR according to whether preoperative RT had been given or not was further analysed. Rectal washout decreased the risk of LR in the patients who had not received preoperative RT. The decrease was almost statistically significant [OR 0.65 (0.43–1.00)]. Rectal perforation increased the risk of LR in the patients who had not received preoperative RT, this finding was statistically significant [OR 2.50 (1.48–4.24)], a finding that was not the case in patients who had received preoperative RT [OR 1.55 (0.82–2.95)]. Thus, the observed increase in risk of LR in the initial multivariate analysis was due to the impact of rectal perforation in the group who had not received preoperative RT. After AL, the risk of developing LR had no connection to whether preoperative RT had been given or not. Rectal washout did not statistically significantly reduce the number of LR if a rectal perforation occurred [OR 0.84 (0.36–1.93)].

Validated data for patients with registered LR within five years of primary surgery

The validation of the SRCR data excluded 35/326 (11%) patients with registered LR within five years of primary surgery (Table 15). In 30/291(10%) of the validated patients, the primary surgical procedure was LE. In this group, various local procedures had been performed, and data on local stage, local radicality, and TNM stage were uncertain. This group was not further analysed. Thus, 261 patients developed LR within five years of major abdominal surgery. Preoperative RT was administered to 102/261 (39%) patients, and the majority, 90/102 (88%), received a short-term 25Gy/5d course. R0 resection was achieved in 202/261 (77%) patients and of these 202 patients, 174 (86%) belonged to TNM stages I–III.

LR was an isolated tumour manifestation in 103/261 (39%) patients. In 157/261 (60%) patients, the diagnosis of LR was made by cytopathology or histopathology, in 62 (24%) by radiology (CT, MRI or ultrasonography), and in the rest by other methods. In 212/261 (81%) of the patients, the LR was confined to one site, and in the remaining there was a combination of different sites. In the 212 patients with LR at one site, 128 (60%) were in the lesser pelvis, 50 (24%) at the anastomotic site, 19 (9%) in the perineum, four (2%) were located perirectally, and 11 (5%) at another site. The time to diagnosis of LR for the pa-

tients who had received preoperative RT was prolonged. This prolongation was most obvious for the subgroup of patients who had R0 major abdominal surgery. After three years, when almost 90% of the LR patients were diagnosed, there was still a delay for the RT group, but the subgroup with R0 resections no longer differed from other irradiated patients. DM were present at diagnosis of the primary tumour in 27/261 (10%) patients or developed within five years of follow-up in 131 (55%) patients belonging to TNM stages I–III. After R0 major abdominal surgery for tumours in TNM stages I–III, there was an insignificantly survival benefit for patients treated with preoperative RT (P=0.18) when survival was calculated from primary surgery. However, when the survival time was estimated from the time of the occurrence of LR, there was no survival benefit observed in the group receiving preoperative RT.

Paper II

After validation of the SRCR data (Table 15), 250 patients with R0 resections in TNM stages I–III of the originally 344 selected patients remained for the final analysis: 114 with AL and 136 controls.

There were only minor differences in baseline characteristics for included patients, tumours, and treatment between the groups. Significantly more patients with AL than controls had TME surgery (P=0.008). The presence of intraoperative faecal contamination tended to be more frequent in patients developing AL than in controls (P=0.085). In addition, the mean age tended to be higher for the patients with AL than for the controls (P=0.053). Preoperative RT was administered to 63/114 (55%) patients with AL and 72/136 (53%) controls. All but one patient among the patients with AL received a short-term 25Gy/5d course.

Anastomotic leakage

The median postoperative day for diagnosis of AL was day 12 (range 3–30). In 52/114 (46%) patients, the AL was detected within 10 days of primary surgery. In 78/114 (68%) patients, the AL was treated by surgery; a conservative approach was adopted in the rest. Among patients treated by surgery, 41/78 (53%) patients had a diverting stoma only, and 37 had another surgical procedure. Surgery was more common for early-detected AL, but the difference was not statistically significant when different time intervals of time from primary surgery to the detection of AL were compared.

Tumour recurrence

Within five years of primary surgery, a total number of 21 patients developed LR, yielding a cumulative incidence of 8%. They were evenly distributed with nine (8%) among patients with AL and 12 (9%) among controls (P=0.97). In 51 (20%) patients, DM was diagnosed without any statistical difference between the groups: 20 (18%) among the patients with AL and 31 (23%) among the controls (P=0.37). In all, OAR occurred in 60 (24%) patients: 22 (19%) of the patients with AL and 38 (28%) of the controls (P=0.15). Cox regression analysis on the impact of the time from primary surgery to the detection of the AL on the LR, DM, or OAR rates could not reveal any difference whether the AL occurred early or late. There was no difference in the LR, DM, or OAR rates irrespective whether the AL was managed by surgery or conservatively. Among the patients with AL and the controls that developed LR, DM, or OAR, the rates that had received preoperative RT or rectal washout were the same (data not shown).

Survival

The overall postoperative mortality was six (2%) patients: four patients with AL and two

controls. The 5-year overall survival rate for patients with AL was 63% and for controls 66% (P=0.38). The 5-year cancer-specific survival rate was 79% for patients with AL and 77% for controls (P=0.50). The 5-year overall as well as the 5-year cancer specific survival curves indicated worse survival for patients with AL during the initial postoperative years.

Univariate and multivariate analyses of potential risk factors of tumour recurrence and survival

Univariate analysis of potential risk factors (age, AL, gender, tumour height, TNM stage, preoperative RT, rectal washout, and TME surgery) of LR, DM, OAR, and reduced 5-year overall and 5-year cancer-specific survival was performed (data not shown). Multivariate analysis was performed for the potential risk factors with a P-value ≤0.10 in the univariate analysis. In the multivariate analysis, the relative risk of LR after AL was 1.24 (CI 95%: 0.50–3.09, P=0.65), DM was 0.86 (CI 95%: 0.49–1.52, P=0.61), OAR was 0.72 (CI 95%: 0.42–1.24, P=0.24), reduced 5-year overall survival was 1.46 (CI 95%: 0.93–2.30, P=0.10), and 5-year cancer-specific survival was 1.29 (CI 95%: 0.70–2.38, P=0.42). Thus, AL was not an independent risk factor of LR, DM, OAR, or reduced 5-year overall as well as 5-year cancer-specific survival.

Paper III

After validation of the SRCR data (Table 15), 273 patients with R0 resections for tumours in TNM stages I–III of the originally 416 selected patients remained for the final analysis: 118 with incidental perforation and 155 controls. The two groups were well balanced concerning patient, tumour, and treatment characteristics. However, the group with perforations included significantly more low situated tumours (P<0.001), and intraoperative faecal contamination was significantly more common in this group (P<0.001). In addition, the type of surgery – there were more APRs among the patients with perforation – was significantly different (P<0.001). Preoperative RT was administered to 65/118 (55%) patients with perforation and 85/155 (55%) controls. All but three patients among the patients with perforation received a short-term 25Gy/5d course.

Perforations

Among patients with incidental perforation, 46/118 (39%) perforations occurred in the tumour, 58/118 (49%) in another part of the rectum, 3/118 (3%) had a combination, and for 11/118 (9%) patients data on the site of perforation were unavailable. Among patients with APR and perforation, 24/75 (32%) occurred during the abdominal phase of the surgical procedure, 43/75 (57%) during the perineal phase, and for 8/75 (11%) patients it was not stated in the operation notes when the perforation occurred.

Tumour recurrence

Within five years of primary surgery, a total of 35 patients developed LR, yielding a cumulative incidence of 13%. Significantly more LR were registered among patients with perforation than among controls, 23 (20%) *vs*. 12 (8%) (P=0.007). Metachronous DM was diagnosed in 65/273 (24%) patients, but there was no significant difference between groups with 32 (27%) among the patients with perforation and 33 (21%) among the controls (P=0.33). Together, this gives an OAR rate of 79/273 (29%) in the study. OAR tended to be more common among patients with perforation than controls, 41 (35%) *vs*. 38 (25%), but the difference was not statistically significant (P=0.087). LR tended to be more common among patients with perforation in the tumour, 13/46 (28%), than among patients with perforation in another part of the rectum,

Table 15. Excluded patients after validation, Paper I-III.

Reasons for exclusion	Paper I	Paper II		Paper III	
	Patients with LR (*n*=326)	Cases (*n*=172)	Controls (*n*=172)	Cases (*n*=208)	Controls (*n*=208)
No primary rectal cancer					
High grade dysplasia	3		1	1	3
Histopathology not adenocarcinoma	4			1	
Colon cancer registered as rectal cancer	2				3
LR from colon cancer	1				
LR registered as primary rectal cancer	6			3	
Incorrect registration					
DM registered as LR	9				
Primary surgery without tumour resection	3				
No surgery performed	2				
False registration of LR	5				
Not AL in colorectal anastomosis		6			
Radiological AL		2			
Late AL*		20			
Not registered AL			8		
Other operation than AR			5		
Preoperative perforation				9	
Perforation of small bowel				1	
False registration of perforation				11	
Perforation not registered					11
Total	35 (11)	28 (16)	14 (8)	26 (12)	17 (8)

Values in parenthesis are percentage. *AL diagnosed >30 days postoperatively. LR, local recurrence; DM, distant metastasis; AL, anastomotic leakage; AR, anterior resection.

8/46 (14%) (*P*=0.11). Concerning DM and OAR, no difference was seen irrespective of where the perforation occurred.

Survival

The overall postoperative mortality was six (2%) patients: two patients with perforation and four controls. The 5-year overall survival rate for patients with perforation was 44% and for controls 64% (*P*=0.002). The 5-year cancer-specific survival rate was 66% for patients with perforation and 80% for controls (*P*=0.026).

Univariate and multivariate analyses of potential risk factors of tumour recurrence and survival

Univariate analysis of potential risk factors (age, gender, tumour height, preoperative RT, surgical procedure, TME surgery, perforation, intraoperative faecal contamination, rectal washout, tumour grade, TNM stage, and T stage) of LR, DM, OAR, and reduced 5-year overall or 5-year cancer-specific survival was performed (data not shown). Multivariate analysis was performed for the potential risk factors with a *P*-value ≤0.10 in the univari-

ate analysis. In the multivariate analysis, the relative risk for LR after perforation was 2.52 (CI 95%: 1.12–5.69, *P*=0.026), for DM was 1.56 (CI 95%: 0.93–2.59, *P*=0.091), for OAR was 1.85 (CI 95%: 1.09–3.14, *P*=0.022), for reduced 5-year overall survival was 1.69 (CI 95%: 1.09–2.63, *P*=0.020), and for 5-year cancer-specific survival was 2.07 (CI 95%: 1.18–3.64, *P*=0.011). Thus, perforation was an independent risk factor of LR, DM, OAR, or reduced 5-year overall as well as 5-year cancer-specific survival.

Paper IV

Ezrin expression

In total, tumours from 104/109 (95%) patients were successfully analysed. Ezrin expression was weak in 18/104 (17%) tumours, moderate in 64/104 (62%), and intense in 22/104 (21%). In 26 patients, tissue from the diagnostic biopsy as well as the irradiated operative specimen was available, and these cases were used for comparative analyses of ezrin staining before/after RT, but the low number of analysed patients did not allow for any conclusions. In order to exclude bias from assessment of different tumour areas and to check for intratumoural heterogeneity, the luminal as well as the invasive tumour front was stained using whole tumour blocks from 25 cases. These stainings were selected to represent five tumours with weak, ten with moderate, and ten with intense staining, all with moderate tumour differentiation. Only 3/25 (12%) tumours showed a heterogeneous expression, and only 4/25 (16%) tumours presented a discrepancy between the TMA and the whole tissue expressions.

Patient, tumour, and treatment characteristics

When comparing patient, tumour, and treatment characteristics between subgroups with dichotomized low and high ezrin expression, most variables were balanced. High ezrin expression tended to be more common among patients with both LR and DM, 16/59 (27%), compared to patients with isolated LR, 6/45 (13%) (*P*=0.088).

Time to development of LR and DM

The median time from primary surgery to diagnosis of LR for all patients was 548 (98–1673) days. The time to LR was significantly shorter in patients with high *vs*. low ezrin expression (*P*=0.0004) with median time of 316 (range 98–961) days *vs*. 621 (range 127–1673) days. In order to strengthen the data, trend analysis was performed for the categories weak, moderate, and intense expression, and confirming significant impact from increasing ezrin expression (*P*=0.0001). Multivariate analysis of potential risk factors (high ezrin expression, age, gender, TNM stage, tumour differentiation, pre- and postoperative RT, as well as pre- and postoperative chemotherapy) for shorter time to development of LR was performed (data not shown). High ezrin expression was the only factor that had any significant impact on the time from primary surgery until diagnosis of LR [HR 2.4 (CI 95: 1.3–4.2, *P*=0.003)]. Ezrin expression had no impact on the time from primary surgery to diagnosis of DM.

Survival

The 5-year overall and cancer-specific survival rates from primary surgery were compared in groups, but significant differences were not demonstrated (data not shown). Overall and cancer-specific survival from diagnosis of LR and DM were analysed without detection of significant differences between the high and low expression groups (data not shown).

General discussion

Advances in the management of rectal cancer patients have reduced the LR rate and improved survival. To improve the outcome, it is essential to further identify prognostic and predictive factors related to the patient, the treatment, and the tumour in purpose to select patients to optimal, personalised treatment and follow-up strategy.

Management of tumours at different heights, especially in the upper third of the rectum, concerning the use of preoperative RT and the appropriate surgical technique is controversial. LR rates and survival similar to sigmoid colon tumours have been demonstrated for tumours in the upper third. PME without preoperative RT has been advocated for tumours in this part of the rectum[145]. This strategy is presently the national recommendation in Sweden[174, 175]. However, recent data have revealed that the oncological outcome for these patients is more similar to the outcome for patients with tumours in the middle third of the rectum than the sigmoid colon[206]. Moreover, PME for tumours in the upper third might be associated with increased LR risk due to LR emanating from tumour deposits in mesorectal remnants[231]. In Paper I, exploring non-validated data from a 3-year cohort in the SRCR, we demonstrated a clear benefit of preoperative RT in reducing the LR rate irrespective of the tumour height, a finding that has been indicated in other reports as well[75, 106, 216]. However, in the follow-up analysis of the Dutch TME study, a significant reduction of LR after preoperative RT was observed only for tumours in the middle third but not in the upper or the lower third of the rectum[187]. In that trial, TME was performed even for tumours in the upper part, which might indicate less impact of preoperative RT after proper TME surgery. Concerning the results in the lower rectum, it was hypothesised that an inferior APR technique had been practised with a subsequent high rate of CRM involvement, which cannot be compensated for by RT. However, it must be considered that the results in all referred reports are based on analyses of subgroups. In addition, the patients in the studies are not stratified according to tumour height and the method for measurement of tumour height is not standardized. Based on the present findings, we recommend that patients with tumours in the upper third of the rectum similarly to patients with low- or midrectal tumours are subjected to a thorough discussion on an MRI-based MDT conference in which the patient's condition as well as the potential adverse effects of RT are balanced against the benefits before a decision is made about neoadjuvant RT. The appropriateness of PME or TME in combination with neoadjuvant RT for tumours in the upper third of the rectum in terms of risk of LR needs to be further explored.

Although it is demonstrated that viable, exfoliated malignant cells are harboured in the bowel lumen, the importance of such cells for the development of LR remains controversial[72, 221, 241]. It has been hypothesised that rectal washout eliminates intraluminal cells and thereby reduces the risk of LR. In turn, AL as well as intraoperative perforation may cause a seeding of tumour cells in the pelvic cavity, a phenomenon that may encourage implantation and a subsequent development of LR. The importance of rectal washout and AL are debated, whereas the studies concerning the impact of perforation have been more uniform in their conclusions[4, 12, 15, 29, 45, 50, 58, 60, 65, 66, 79, 120, 124, 139, 140, 162, 189, 195, 196, 203, 222, 271].

In Paper I, neither rectal washout nor AL were demonstrated to have any significant impact on the risk of LR. However, when we further analysed the potential impact of rectal washout, intraoperative perforation, and AL on the development of LR according to whether preoperative RT had been given or not, rectal perforation significantly increased the LR rate in non-irradiated patients, but had no significant impact in irradiated patients.

A borderline significance was found for rectal washout in decreasing the LR rate in non-irradiated patients, but no impact was demonstrated in irradiated patients. Our findings indicate a dual role of neoadjuvant RT in reducing the LR rate. Not only does neoadjuvant RT affect lateral margins and preoperative pelvic tumour cell dissemination, but preoperative RT might also be of importance for eradication of free vital intraluminal tumour cells. AL had no impact on the development of LR irrespective of whether preoperative RT had been given or not. According to our findings in Paper I, we recommend rectal washout before transection of the rectum in order to prevent LR when performing AR or HA for rectal cancer. However, there are no data available on what solution to use and what amount of solution to use in achieving optimal washout and tumoricidal effect. Therefore, analysis of rectal washout fluid for the presence and characterization of rectal cancer cells needs further studies. Evaluation of the importance of rectal washout when performing APR is also warranted.

The contradictory results in previous studies and our analysis of unvalidated SRCR data on the oncological outcome of clinical AL after AR made us to analyse this further. This study was performed on a selected patient group with R0 surgery for tumours in TNM stages I–III using validated SRCR data and additional data from the original medical records. We could not detect any significant impact of AL on the rates of LR, DM, OAR, as well as the 5-year overall and cancer-specific survival. The four largest recent series of patients that studied the impact of AL on the oncological outcome demonstrate similar findings[15, 58, 66, 120]. On the other hand, a number of studies have come to the opposite conclusion[4, 12, 29, 45, 60, 79, 139, 162, 189, 196, 247]. However, many of the studies reporting a worse oncological outcome are old and include only a few patients with AL. To our knowledge, considering the number of studied patients with AL, our study is the fifth largest. In addition, a great proportion of included patients in our study had received neoadjuvant treatment, and TME surgery including rectal washout was almost mandatory. Thus, the patients were managed according to modern principles. Remarkably, in our study we found a reduced survival, overall as well as cancer-specific, during the first years in patients with AL even after correction for deaths within 30 days of surgery. Thus, AL results in excessive death more than 30 days postoperatively. A tendency to higher age and thereby deteriorated state of health in the AL group might be contributing. AL is suggested to compromise the patient's immunity and enhance as well as prolong the postoperative systemic inflammatory response in the postoperative period[157, 159]. One might assume that the compromised immunity makes the patients more vulnerable for a period of more than 30 days after surgery, which influences the survival negatively. We also found a tendency that among patients who developed tumour recurrence, the recurrences were detected earlier in the group with AL. Since most recurrences will lead to death, this might also have contributed to the excess mortality during the first years in the AL group. The high rate of neoadjuvant therapy and the use of rectal washout in Paper II might have had an impact on the results. One might also assume that the time of the occurrence of the AL is crucial. It might be that the number of exfoliated, viable intraluminal tumour cells decreases with time after surgery. The median day for diagnosis of AL in our study was day 12, which is later than what is generally reported. We found no impact on the oncological outcome irrespective whether the AL occurred early or late within the period of the first 30 postoperative days. However, the number of AL in our study was too small to answer this question. The impact of AL occurring more than 30 days postoperatively on the oncological outcome is not well documented, and unfortunately the SRCR do not enable this to be studied since late oc-

curring AL are not registered separately, but together with other anastomotic complications. A few small studies have also included radiological AL in their risk assessment on the oncological outcome and have found a negative impact of radiological AL[4, 12, 247]. We conclude that AL results in worse survival in the immediate postoperative period, whereas in the long-term perspective AL does not have any negative impact on the oncological outcome. According to these findings, AL does not motivate adjuvant treatment or more intense follow-up.

In Paper I, incidental perforation was a risk factor of worse oncological outcome, which has been reported in a few studies[65, 195, 203, 222, 271]. However, in the majority of the previous studies, conventional blunt surgery was performed and neoadjuvant therapy was not administered. The observed impact of perforation in Paper I was most pronounced in non-irradiated patients. In a similar way as in Paper II, we decided to analyse the impact of perforation in a validated patient group with R0 surgery for tumours in TNM stages I–III. We found incidental perforation to be an independent risk factor of LR, OAR, and reduced 5-year overall as well as 5-year cancer-specific survival. No impact was seen on the DM rate, probably reflecting different origins of LR and DM. LR might emanate from local implantation in the pelvic cavity of cancer cells seeded from the perforation, whereas DM occurs after implantation of circulating cancer cells in the lymphatics or the blood. Contrary to AL, where the leakage occurs several days after surgery, the leakage at the perforation occurs during surgery. In Paper I and II, we hypothesised that the time for the occurrence of the seeding of tumour cells from the bowel lumen was crucial for the impact on the oncological outcome. We believe that the results in Paper III support this hypothesis. Although a significant impact on the oncological outcome apart from the DM rate was found in our study, it was less pronounced than in earlier reports. Perhaps, the high quality of the surgery, the frequent administration of preoperative RT, and the high use of rectal washout contributed. In the analysis in Paper III, preoperative RT and rectal washout significantly reduced the LR rate (data not shown). However, the smaller number of patients with LR included in Paper III did not allow further analysis of the in Paper I observed different impact of incidental perforation in non-irradiated *vs*. irradiated patients. In line with earlier studies[222, 271], perforation in the tumour was indicated in our study to have a more pronounced impact on the LR rate than perforations elsewhere in the rectum, but firm conclusions could not be drawn. This issue needs further analyses. The information on the site of perforation in our study was from the additional data extracted from original medical records. Until 2007, the site of perforation was not registered in the SRCR. Thus, when enough data are encountered in the SRCR, the issue on the importance of the site of the perforation on the oncological outcome might be properly analysed. We also confirmed that perforation is more common after APR than after other major abdominal procedures and that the perforation most commonly occurs during the perineal phase of the APR[65, 171, 195, 218, 271]. Certainly, this is explained by an inferior technique when performing APR, which motivates adoption of the new approach, the extended posterior approach, to optimize the APR technique and thereby the oncological outcome[6, 107, 171, 218, 251, 254]. Although a less pronounced impact on the oncological outcome was found in our study, incidental perforation still had a significant negative impact. The importance of preoperative RT and rectal washout is also confirmed. After incidental perforation, thorough consideration should be taken when discussing potential adjuvant treatment and follow-up strategy.

Both Paper II and III are hampered by the relative rarity of AL, incidental perforation, and tumour recurrence, making the conclu-

sions based on analysis from rather small subgroups. However, this drawback is universal for all studies addressing the impact of AL and incidental perforation on the onclogical outcome. The decision not to match the controls when selecting the control groups can also be questioned. In Paper II and III, the groups of patients with AL or incidental perforation and the respective control groups were well balanced concerning patient, tumour, and treatment characteristics. In addition, we used multivariate methods with adjustment for several covariates in both studies motivating our choice of not matching the controls. The number of selected controls could also be discussed. Because the retrieval and extraction of data from original medical records are labour intensive and time consuming, we chose a number where the workload was reasonable and realistic to achieve without missing too much data.

In Paper I, we found that 90% of the LR occurred within three years of primary surgery, and the time to diagnosis of LR was prolonged among patients who had received preoperative RT, which is in line with earlier studies[2, 105, 187]. Recently Merkel *et al.,* in a systematic review and meta-analysis concluded that approximately 25% of the LR occurred later than five years from primary surgery in patients treated with long-term preoperative RT or chemoradiotherapy, whereas in patients treated with surgery alone <10% of the LR occurred later than five years from primary surgery[164]. For patients with short-term preoperative RT, conclusions could not be made. They recommended a minimal follow-up regarding local control of five years after surgery alone and seven to eight years after preoperative long-term therapy. For the period studied in Paper I, the majority of patients who received preoperative RT received short-term RT and the follow-up was five years. To update the data with ten-year follow-up data would add important information to the study by Merkel *et al.,* concerning the occurrence of LR after five years in patients treated with preoperative RT. Possibly, certain patients in need of extended follow-up would be identified. However, whether an extended follow-up period would improve survival remains to be clarified. Almost 40% of the diagnosed LRs in our study were isolated without signs of disseminated disease and one-third were confined to the anastomotic site or located perirectally. This group with isolated, contained LR might with optimal management at dedicated centres be curable[43, 99, 184]. Our findings (as well as the findings in other studies) concerning the impact of RT on the time to LR and the fact that a significant proportion of patients with LR might be curable indicate that subgroups of patients might benefit from intense and prolonged follow-up. However, these findings highlight the need for follow-up studies such as the ongoing GILDA, FACS, or COLOFOL for greater understanding[90, 116, 174, 175, 257]. Nevertheless, alertness of late occurring LR and awareness that 40% of LR are isolated recurrences are of utmost importance when screening for LR in a potentially curable stage.

As outlined in Table 15, erroneous registration was found in approximately 10% of the patients concerning the variables LR, AL, and incidental perforation. However, for other validated variables (Table 12) erroneous registration was found in 0–5% of the patients (unpublished data). Studies from large population-based quality registries have become more common and important in medical research. However, for the results to be reliable, high completeness and validity of the registered variables are prerequisites. It has been suggested that a completeness of >95% of cases that are intended to be registered and a validity of <5% missing/erroneous registrations of each variable combined with not >10% missing registrations of any occurrence will guarantee reliable results[92]. The erroneous registration of the variables LR, AL, and perforation was unexpectedly high in our study especially since an earlier validation of five vari-

ables (type of surgery, incidental perforation, AL, postoperative mortality, and TNM stage) had shown validity with <5% discrepancy[182]. However, our studied cohort was from the first years of the SRCR when registration routines were not settled, which certainly could have yielded the higher figures in our validation. LR, AL, and incidental perforation might also be parameters that are more difficult to register than others due to unclear definitions, i.e., early *vs*. late AL, clinical *vs*. radiological AL, and preoperative perforation *vs*. intraoperative incidental perforation. Supporting this is our finding of higher validity for the other validated variables. Since the first years of the SRCR, the registration has been improved and the definitions more distinctly formulated, why one might assume a higher validity today. In addition, our validation proved the good order in the keeping of medical records in the Swedish healthcare system since only for a few patients the original medical records were not possible to retrieve (Table 14). Based on our findings in Paper I–III, we conclude that the validity of the SRCR is good, making it possible to draw reliable conclusions from SRCR data.

Despite improvements in staging, use of neoadjuvant treatment, and surgery, patients still develop tumour recurrence after R0 surgery. This might indicate that there are subgroups of tumours with a more aggressive biological behaviour than others making them more prone to recur. Several tumour markers have been studied in CRC, but few have proven to be of prognostic or predictive value in clinical practise. In Paper IV, we found that ezrin, the membrane-cytoskeleton linker protein suggested to be a marker of poor prognosis in several malignancies[30, 127, 250], was expressed in all primary tumours from patients with rectal cancer that developed isolated LR or LR in combination with DM within five years of primary surgery. In addition, high ezrin expression was compared to low ezrin expression correlated to earlier occurrence of LR. High ezrin expression also tended to be more common in tumours among patients that developed LR in combination with DM than among patients with isolated LR. Our study indicates a linkage of high ezrin expression and aggressive biological behaviour, which has been found in CRC cell lines[82, 103] and tumour tissue[63, 82, 249,265]. To our knowledge, ezrin expression has not been studied in rectal cancer separately. High ezrin expression has been correlated to poor histopathologic differentiation grade, lymph node involvement, and advanced TNM stage[249]. As Yan *et al.*,[265], we could not confirm this. We could neither detect any survival benefit in the group of patients with low ezrin expressing tumours as found by Elzagheid *et al.*,[63]. However, the number of patients in our study and the number of patients with rectal cancer in the Finnish study is rather small for survival analysis. The indication of a predictive role of ezrin as an indicator of resistance to chemotherapy is an interesting field to explore[28, 63, 204, 267]. Concerning our material where all tumours expressed ezrin the importance of high or low expression in predicting response to chemotherapy needs to be clarified. Future decisions to use neoadjuvant/adjuvant treatment as well as choice of follow-up strategy will likely be based on several modalities, including molecular tumour profiling. Our findings in a series of patients treated with R0 major abdominal surgery who all developed isolated LR or LR in combination with DM suggest that ezrin is a promising candidate. However, the findings need validation in large and independent series of rectal cancer.

In our studies from a 3-year validated national cohort of rectal cancer patients with 5-year follow-up, we have identified some important risk factors of poor oncological outcome related to the treatment and the tumour. These risk factors might be considered when selecting patients to optimal, personalised treatment and follow-up strategy.

Conclusions

- Preoperative radiotherapy significantly reduces the local recurrence rate irrespective of the tumour height.
- Rectal washout before transection of the bowel is recommended.
- Local recurrence in patients treated with preoperative radiotherapy tends to occur later than in non-irradiated patients.
- Local recurrence is often an isolated tumour manifestation.
- Anastomotic leakage is not a risk factor of local recurrence, distant metastasis, overall recurrence, or reduced 5-year overall as well as 5-year cancer-specific survival.
- Incidental perforation is a risk factor of local recurrence, overall recurrence, and reduced 5-year overall as well 5-year cancer-specific survival.
- The validity of variables registered in the Swedish Rectal Cancer Registry is good.
- Ezrin is indicated to be a tumour marker of prognostic value.

Summary in Swedish (Sammanfattning på svenska)

I Sverige diagnosticeras årligen ca 2000 personer med ändtarmscancer (rektalcancer). Sjukdomen är något vanligare bland män än kvinnor. Framför allt drabbas individer över 70 år. I ungefär 20% av fallen är sjukdomen ärftlig. Den botande behandlingen är kirurgi. Under 70- och 80-talen drabbades 30–70% av patienter som genomgått botande kirurgi av lokalrecidiv (LR), dvs tumöråterfall lokalt i lilla bäckenet, och den cancerspecifika 5-årsöverlevnaden uppgick till endast 30–40%. Stora framsteg i behandlingen av patienter med ändtarmscancer under de senaste två decennierna har medfört att färre än 10% drabbas av LR, och den cancerspecifika 5-årsöverlevnaden har stigit till omkring 60%. Uppkomsten av fjärrmetastaser efter kirurgi, dvs tumöråterfall i andra organ utanför lilla bäckenet, har dock legat relativt konstant omkring 20–25%. Standardiserad preoperativ utredning, diskussion av enskilda patienter på multidisciplinära (MDT) konferenser, införande av behandling med preoperativ strålning (RT) och cytostatika, centralisering av kirurgin samt ändrad kirurgisk teknik (sk TME-teknik) är förändringar som införts och som man bedömer vara förklaringen till de förbättrade resultaten vid ändtarmscancer.

LR efter potentiellt botande kirurgi för ändtarmscancer är sällan botbart, och medför hög sjuklighet samt ökad cancerrelaterad död. Kunskapen om prognostiska faktorer, dvs. faktorer som kan förutsäga risken för LR, fjärrmetastaser och cancerspecifik död, är bristfällig. Dessa faktorer kan vara relaterade till patienten, behandlingen eller till egenskaper hos tumören i sig. För att förbättra resultaten vid ändtarmscancer ytterligare och för att kunna individualisera omhändertagandet av den enskilde patienten krävs förbättrad kartläggning av prognostiska faktorer. Genom att identifiera patienter med hög risk för tumöråterfall, och därmed ökad risk för cancerspecifik död, kan dessa patienter erbjudas tilläggsbehandling i form av RT eller cytostatikabehandling, samt en intensivare uppföljning för upptäckt av återfall i ett tidigt och potentiellt botbart skede. Patienter som inte har nytta av tilläggsbehandling eller intensiv uppföljning, dvs patienter med låg risk för tumöråterfall, kan i sin tur besparas onödig tilläggsbehandling och uppföljning.

På Socialstyrelsens initiativ bildades 1995 det Svenska rektalcancerregistret (SRCR). Registrets syfte var att höja kvaliteten på omhändertagandet av patienter med ändtarmscancer. Alla patienter med nydiagnosticerad ändtarmscancer i Sverige registreras i SRCR. Många uppgifter om kirurgisk teknik, kirurgiska komplikationer, tilläggsbehandling, senkomplikationer samt tumöråterfall (LR och fjärrmetastaser), och cancerspecifik död återfinns i registret.

Denna avhandling identifierar potentiella riskfaktorer för tumöråterfall och cancerspecifik död i en grupp patienter registrerade i SRCR mellan januari 1995 och december 1997 (n=4153) med inrapporterad 5-årsuppföljning. Riskfaktorer relaterade till kirurgi samt tumörbiologi studerades. Uppgifter i SRCR användes, och för några patientgrupper inhämtades data från journaler för tilläggsdata och kvalitetsgranskning av uppgifter inrapporterade till SRCR. För patienter som drabbats av LR rekvirerades vävnadsmaterial från modertumören för tumörbiologiska studier.

Delarbete I

I delarbete I analyserades förekomsten av LR och överlevnaden i hela patientgruppen (n=4153) med hjälp av SRCR data. För patienter med registrerat LR framtogs tilläggsdata och registrerade SRCR data granskades. LR drabbade 8% av de registrerade patienterna. Totalöverlevnaden efter fem år var 45% och den cancerspecifika överlevnaden 62%.

Resultaten avseende förekomsten av LR och överlevnadsdata är jämförbara med siffror rapporterade ifrån andra populationsbaserade register. Signifikanta riskfaktorer för LR var lågt sittande tumör, ej given preoperativ RT, accidentell ändtarmsperforation vid kirurgi, TNM stadier II/III samt icke-radikal eller osäkert radikal kirurgi. Det viktigaste fyndet var att preoperativ RT medförde en signifikant riskreduktion för LR oavsett tumörnivå i ändtarmen. Nyttan av preoperativ RT, framför allt för tumörer i den övre tredjedelen av ändtarmen, har varit omdebatterad och resultaten i befintliga studier motstridiga. Patienter med ändtarmsperforation i samband med kirurgi och som inte fått preoperativ RT hade säkerställd högre risk att drabbas av LR än strålade patienter. Sköljning av ändtarmen före delning av tarmen hos de patienter som opererades med främre resektion (AR) eller Hartmanns operation (HA) medförde en statistiskt nästan säkerställd reduktion av risken att utveckla LR. Subgruppsanalys av patienter med LR visade att preoperativ RT förlängde tiden till diagnos av LR men påverkade inte överlevnaden i denna grupp. Andra studier har indikerat att preoperativ RT förlänger tiden till diagnos av LR, vilket skulle kunna motivera uppföljning av strålade patienter under ett längre tidsintervall. LR var en isolerad tumörmanifestation hos cirka 40% av patienter med LR. Detta är viktig kunskap då möjligheten till botande behandling av LR är högre för isolerat LR än LR kombinerat med fjärrmetastaser. Felregistrering av variabeln LR upptäcktes hos 11% av patienterna med LR, vilket är en acceptabel siffra. Slutsatser från delarbetet är att preoperativ RT bör övervägas noga även vid tumörer i ändtarmens övre tredjedel, att accidentell intraoperativ ändtarmsperforation bör undvikas, att peroperativ sköljning av ändtarmen bör ske före delning av tarmen vid AR och HA och att patienter som fått RT innan kirurgi bör följas upp under en längre tid än ostrålade patienter.

Delarbete II

Anastomosläckage (AL), dvs läckage i tarmskarven efter AR, har ansetts vara en möjlig riskfaktor för LR och därmed försämrad överlevnad. Data i befintliga studier är emellertid motstridiga. I delarbete II identifierades samtliga patienter (*n*=172) som drabbats av AL efter AR i gruppen av patienter registrerade i SRCR under 1995-1997, och en kontrollgrupp (*n*=172) bestående av patienter som opererats med AR men som inte drabbats av AL selekterades. Tilläggsdata framtogs och SRCR data granskades för samtliga inkluderade patienter. Analys av det onkologiska utfallet efter 5-årsuppföljning, dvs förekomsten av LR och fjärrmetastaser samt 5-årsöverlevnad (total respektive cancerspecifik), analyserades i gruppen som genomgått potentiellt botande kirurgi, dvs R0-resektion och TNM stadier I–III (*n*=250; 114 patienter med AL respektive 136 kontroller). AL medförde en försämrad överlevnad i det postoperativa förloppet, men hade ingen betydelse för det onkologiska utfallet på sikt. Hos 12% av patienterna var variabeln AL felaktigt registrerad. AL påverkar inte det onkologiska resultatet och motiverar därför inte onkologisk tilläggsbehandling respektive intensifierad eller förlängd uppföljning.

Delarbete III

Accidentell intraoperativ ändtarmsperforation är ytterligare en kirurgirelaterad faktor som i ett fåtal studier, varav majoriteten genomfördes innan införandet av preoperativ RT och TME-kirurgi, visats kunna medföra ett försämrat onkologiskt resultat. I delarbete III identifierades samtliga patienter (*n*=208), där ändtarmsperforation inträffat i samband med resektionskirurgi, dvs AR, rektumamputation (APR) och HA, i gruppen av patienter registrerade i SRCR under 1995–1997 och en kontrollgrupp (*n*=208) bestående av patienter som opererats med resektionskirurgi men

utan att ändtarmsperforation registrerats selekterades. Tilläggsdata framtogs och SRCR data granskades för samtliga patienter. Analys av det onkologiska utfallet efter 5-årsuppföljning analyserades i gruppen som genomgått potentiellt botande kirurgi (*n*=273; 114 patienter med ändtarmsperforation respektive 136 kontroller). En statistiskt säkerställd ökad risk för LR och försämrad 5-årsöverlevnad, total respektive cancerspecifik, påvisades efter ändtarmsperforation. Någon skillnad i risken att utveckla fjärrmetastaser kunde inte påvisas. Ändtarmsperforation var vanligare i samband med APR, och flest perforationer uppstod under den perineala fasen av detta ingrep i enlighet med tidigare studier. En tendens till större riskökning för LR då perforationen inträffat i tumören jämfört med annan del av ändtarmen kunde påvisas, men denna observation var inte statistiskt säkerställd. Variabeln accidentell intraoperativ ändtarmsperforation var felregistrerad hos 10% av patienterna. Studien visade att en accidentell intraoperativ ändtarmsperforation är en betydande riskfaktor för ett försämrat onkologiskt resultat. Detta bör beaktas då postoperativ tilläggsbehandling och uppföljningsstrategi diskuteras. Resultaten understryker vikten av en optimal kirurgisk teknik, ffa under den perineala fasen i samband med APR.

Delarbete IV

Trots optimal tilläggsbehandling och botande kirurgi utan kvarlämnande av tumörvävnad drabbas patienter ändå av tumöråterfall. Detta talar för att det finns undergrupper av tumörer som är biologiskt mer aggressiva än andra. En association mellan högt uttryck av proteinet ezrin, som bla är viktigt för flera funktioner i cellernas membran, och dålig prognos har påvisats i flera tumörtyper. I delarbete IV analyserades uttrycket av ezrin med immunohistokemisk teknik i 104 primärtumörer från patienter med ändtarmscancer som utvecklat isolerat LR eller LR i kombination med fjärrmetastaser inom fem år från operationen. Alla tumörer uttryckte ezrin. LR upptäcktes signifikant tidigare hos patienter med tumörer med ett högt uttryck av ezrin jämfört med ett lågt uttryck. En tendens till att högt uttryck av ezrin var vanligare i tumörer hos patienter som utvecklade LR kombinerat med fjärrmetastaser jämfört med isolerat LR konstaterades. Fynden indikerar att ezrin kan ha prognostisk betydelse vid ändtarmscancer, men detta måste valideras i ett större material och jämföras med en kontrollgrupp med ändtarmscancerpatienter som inte drabbats av tumöråterfall efter kirurgi.

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