Clinical and pathological aspects of modern rectal cancer management

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General introduction
Epidemiology

Colorectal cancer (CRC) is a major global health problem with approximately 1.2 million cases worldwide every year. It ranks as the third most common malignancy in males (after prostate and lung cancer) and the second most common malignancy in females (after breast cancer). It primarily affects the elderly with about 80% of cases occurring in patients over 60 years of age. The incidence of CRC is rising, partly due to aging of the population, as well as factors related to an increasingly unhealthy lifestyle, such as a rising incidence of overweight, increased red meat consumption and reduced physical activity. In the Netherlands there were 15565 cases of CRC diagnosed in 2015, and this included 4684 cases of rectal cancer (30%).

Anatomy of the rectum

The rectum is the most distal part of the colon. It is a tubular structure which is firmly embedded in the bony pelvis and has an intimate relation with the organs of the urogenital tract and the sacrum, as well as the anal sphincter complex. The rectal wall is surrounded by the mesorectal fatty tissue, which contains the vascular supply, nerves and lymphatic drainage system. The mesorectum is surrounded by an avascular connective tissue layer called the mesorectal fascia (MRF), which separates the mesorectum from the other pelvic structures. This unique anatomy has profound consequences for the surgical approach of tumours in the rectum compared with those in the peritonealised colon. In addition, the retroperitoneal localization of the rectum in the pelvis and the associated distance to the small bowel ensures that the rectum can be readily subjected to radiation therapy, as opposed to the colon, which is in close proximity to the small bowel. A generally accepted anatomical definition to describe the extent of the rectum is lacking, however, for clinical purposes it is often said to extend for 12-15cm proximally of the anal verge. Alternatively, the peritoneal reflection is used as a landmark to indicate the transition from the rectosigmoid to the rectum, although this is highly variable and depends on age, sex and gynecological conditions.

Pathophysiology

Molecular pathogenesis

The dominant pathway that leads to CRC is the adenoma-carcinoma sequence, which is responsible for development of 70-80% of sporadic CRC in general and an even higher proportion in the rectum. It is characterized by accumulation of mutations
in tumour suppressor genes and oncogenes. These mutations lead to formation of aberrant crypt foci, which subsequently develop into adenomas, and which may ultimately transform into invasive cancer.\textsuperscript{5–7} Classically, one of the first events in adenoma development is silencing of the APC tumour suppressor gene, with subsequent mutations in oncogenes, such as KRAS, which promote growth and prevent apoptosis. Mutations in P53 are a late event in CRC development and disrupt the ability of the cells to stop the cell cycle and activate apoptosis in response to DNA damage, leading to rapid accumulation of chromosomal aberrations which promote invasive growth.\textsuperscript{8}

The adenoma-carcinoma sequence is also the causative mechanism for CRC development in patients with familial adenomatous polyposis (FAP). These patients have a germ line inactivating mutation in APC and therefore only one more “hit” is necessary to knock out this tumour suppressor, which leads to the formation of hundreds of adenomas in the colon and elsewhere in the gastrointestinal tract.\textsuperscript{9, 10} In addition, about 10–15% of CRC are associated with microsatellite instability (MSI), although these are often located proximally in the colon and very rarely in the rectum. Presence of MSI is indicative of a defective DNA mismatch repair (MMR) system. Failure to repair spontaneous replication errors throughout the genome leads to a hypermutated state in these patients, characterized by lengthening of areas with repetitive DNA sequences (microsatellites), as well as rapid accumulation of mutations in oncogenes and tumour suppressor genes, which may subsequently result in cancer.\textsuperscript{10} The majority of MSI cancers occur sporadically (primarily in elderly patients), and are caused by methylation of the promoter of MLH-1, which disrupts the function of this gene by inhibiting its transcription.\textsuperscript{11} However, a quarter of patients with MSI tumours have Lynch syndrome (formerly known as the Hereditary Non-Polyposis Coli (HNPCC)) Cancer syndrome), caused by a germ line mutation in one of the MMR genes.\textsuperscript{12} This predisposes patients to various types of cancer including colorectal, endometrial, ovarian, gastric, and small bowel cancer, as well as transitional cell tumours of the ureter and renal pelvis, skin neoplasms (sebaceous tumours and keratoacanthomas), and brain gliomas.

Furthermore, about 10–20% of CRC may develop from traditional serrated adenomas (TSA) and sessile serrated polyyps/lesions (SSP/SSL), and this mechanism is termed the serrated pathway.\textsuperscript{13} The morphology of these lesions is characterized by a serrated architecture and they often contain mutations in KRAS or BRAF. TSAs predominantly occur in the sigmoid and rectum, although they are very rare (less than 1-2% of colonic polyps).\textsuperscript{14} SSLs are often located in the right colon. CRC developing through the serrated pathway are associated with a poor prognosis and therapy resistance.\textsuperscript{15}

Inflammatory bowel disease related CRC

Patients with inflammatory bowel disease (IBD), including ulcerative colitis, Crohn’s disease and indeterminate colitis, have an elevated risk of developing CRC which depends on disease duration and extent. About 1–2% of CRC cases arise in patients with IBD and these develop through the inflammation-dysplasia-carcinoma pathway.\textsuperscript{16, 17} The mechanisms that drive this pathway are complex, although sustained DNA damage, in part through oxidative stress in inflamed mucosa, is thought to play an important role. The molecular pathogenesis of IBD related CRC is distinct from sporadic CRC. For example, APC gene mutations are less common and appear late in colitis associated compared with sporadic CRC, whereas chromosomal instability and P53 mutations often occur early and may even be present in normal mucosa.\textsuperscript{18} Some studies report that deficiencies in the MMR system may play a role, although this has not been confirmed. The progression rate of dysplastic colonic mucosa to invasive carcinoma in IBD patients is believed to be higher than that of the progression of sporadic adenomas to carcinoma. In addition, there is thought to be a field effect with all the colonic mucosa in a patient with dysplasia being at risk, due to diffuse clonal molecular aberrations. Furthermore, dysplasia and carcinomas may be multifocal. Repetitive screening colonoscopies are performed in IBD patients with at least 8-10 years disease duration and a colectomy should be considered in case of high grade dysplasia or in case of flat low grade dysplasia in at least two simultaneous biopsies or in 2 subsequent biopsies.\textsuperscript{19, 20}

TNM staging and prognosis

The TNM staging system (figure 1) is used to stratify patients with CRC according to the extent of tumour invasion (T-category), nodal status (N-category), and presence of distant metastases (M-category). Clinical stage (cTNM) is based on physical examination, colonoscopy and imaging, and is instrumental in treatment planning. Pathological stage (pTNM) is based on the histopathological examination of the resection specimen and plays an important role in selecting patients eligible for adjuvant treatment (colon cancer) as well as determining prognosis. The prognosis for patients with rectal cancer in the Netherlands has improved over the years with a 5-year survival rate of 65% for patients diagnosed between 2008 and 2012. TNM stage specific survival is roughly 90%, 70%, 55%, and 10% for patients with stage I, II, III, and IV at diagnosis respectively. About 30% of rectal cancer patients present with a tumour limited to the bowel wall (T1 or T2), and 20% of patients have distant metastases at diagnosis.\textsuperscript{2} However, this is expected to change with the introduction of bowel screening programs, since results from the British bowel cancer
screening program showed substantially more early (stage I) colorectal cancers in the screen-detected population compared with patients who refused participation or where never invited.\(^{21}\)

**Treatment**

The treatment of patients with rectal cancer is aimed at improving survival and preventing local recurrences.

**Surgery**

Surgery is the cornerstone of rectal cancer treatment. Heald and Ryall developed the total mesorectal excision (TME) technique, based on the concept that discontinuous tumour deposits in the mesorectal fat, which are not removed in classic anterior resection, can lead to local recurrence after rectal cancer surgery.\(^{22, 23}\) During a TME the rectum and mesorectum are excised by precise dissection under direct vision of the avascular “holy” plane between the visceral and parietal pelvic fascia separating the mesorectal fat from the other pelvic structures (figure 2). Discontinuous tumour deposits in the mesorectum as well as all the lymphatics draining the rectum are hereby removed together with the tumour.\(^{24}\)

The introduction of the TME has resulted in a substantial improvement of local recurrence rates from 20-45% after a classical blunt rectal dissection to around 10% after TME surgery, as well as reductions in cancer related death\(^{25-27}\) and is nowadays considered to be the standard surgical procedure for rectal cancer.

Tumours in the upper and middle thirds of the rectum can be treated with a low anterior resection (LAR) in which the anal sphincter complex remains intact and an anastomosis is created with the sigmoid (with or without a temporary deviating stoma). In case of a low rectal tumour for which an oncologically safe margin cannot be achieved with LAR, an abdominoperineal resection (APR) is indicated. This procedure includes removal of the anal sphincter (may include levator muscles), as well as the perineum and is combined with a permanent colostomy. Both procedures can be performed open or with laparoscopy depending on surgeon preference and experience.

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**Figure 1** Tumour, Nodes, Metastases (TNM) classification

**Figure 2** Total Mesorectal Excision (TME), delineation of surgical margin

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- Rectal lumen
- Rectal wall
- Lymph node
- Tumour
- Anal sphincter
- Levator muscle
- Peritoneal cavity
- Urinary bladder
- Prostate
- Spine

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Preoperative therapy
Several European trials, including the Dutch TME trial, have shown that preoperative short-course radiotherapy (SC-RT) with 5 x 5Gy followed by immediate TME surgery reduces local recurrence rates (2.6-6%) compared with TME surgery only (10%), although there was no improvement in overall survival. Subsequent analysis showed that the benefits in terms of local control in low risk rectal cancer patients (cT1-3, <5mm extramural invasion, cN0) do not outweigh the long term radiation induced toxicity. The current Dutch guideline recommends SC-RT for all cT3 tumours with at least 5mm mesorectal invasion or with 1-3 pathological lymph nodes on imaging (cN1). However, experts who participated in a recent European consensus guideline prefer to limit the indication for SC-RT to patients with cT3N0 tumours, and SC-RT is not commonly given in North America.

Neo-adjuvant chemoradiation therapy (CRT) is indicated for patients with locally advanced rectal cancer (LARC) or with a threatened MRF (defined as tumour ≤1mm from the MRF on MRI). This consists of long course radiotherapy with 45-50Gy in fractions of 1.8-2.0Gy and concurrent fluoropyrimidine based chemoradiation followed by TME after an 8-12 week interval. This treatment is intended to improve local control and to facilitate radical surgery by inducing tumour regression and possible downstaging; although a survival benefit has not been shown. Local recurrence rates of 6-9% have been reported for LARC patients treated with CRT.

Local excision
Local excision is an attractive option for small tumours in the rectum, since it is associated with considerably less surgery-related morbidity and almost no post-operative mortality compared with TME. Transanal endoscopic microsurgery (TEM) is the preferred technique, as it is safer compared with transanal excision (TE) and more often resulted in complete excision of a lesion with clear margins. It is primarily used to excise rectal adenomas, since these lesions do not develop lymph node metastases (LNM) and therefore do not require radical surgery. However, some adenomatous lesions, especially the larger ones, may be found to contain invasive foci on histopathological examination. LNM are present in 11-18% of unselected pT1 rectal cancers, and additional radical surgery may be required for curing the disease in these cases.

Due to expected increase in the number of early rectal cancers associated with the introduction of bowel cancer screening programmes the role of local excisions in rectal cancer treatment will probably become more prominent. In addition, there is increasing emphasis on rectal preservation in patients with a clinical complete response after CRT. Varying results have been published after a "wait and see" policy, mainly depending on patient selection. A full thickness local excision of the scar area, using TEM, is an attractive alternative to "wait-and-see", since it removes possible tumour remnants in the bowel wall. It also provides additional information on tumour response and can be used to identify histopathological risk factors for locally recurrent disease.

Postoperative chemotherapy
Postoperative chemotherapy, which is routinely given to colon cancer patients with stage III disease, does not provide a survival benefit in stage II or III rectal cancer patients who received optimal preoperative treatment and high quality TME surgery, as was shown by several randomized trials including the recent Dutch SCRIPT trial. Some older studies, that largely did not perform surgery according to modern TME principles, found postoperative chemotherapy to improve survival in rectal cancer patients after surgery only (no preoperative treatment), although there is insufficient evidence for a benefit in stage III rectal cancer patients who were treated with high quality TME surgery.

Figure 3A-F Histopathological characteristics
(A) Mesorectal tumour deposit. (B) lymphatic invasion. (C) Venous invasion (smooth muscle lined wall). (D) Venous invasion (erythrocytes in the lumen, adjacent artery). (E) perineural invasion. (F) Budding.
**Histopathology**

**Tumour types**

Adenocarcinoma not otherwise specified (NOS) is the most common type of rectal cancer (85-90%), and is made up of invasive, irregular glandular epithelium with tubular or villous architecture. Differentiation grade is associated with prognosis and is determined by the proportion of solid growth of tumour epithelium (<50% solid = low grade, ≥50% solid = high grade). In addition, around 8-10% of tumours are mucinous carcinomas, in which the tumour volume contains at least 50% mucin (often up to 90%). It is more common in Lynch syndrome patients and more often presents with an advanced stage. This tumour type should not be graded and has classically been associated with a worse prognosis compared with adenocarcinoma NOS, although recent research showed that modern rectal cancer treatment has resulted in an equal survival in patients with adenocarcinoma NOS and mucinous carcinoma. Signet ring cell carcinoma is an uncommon (2%), yet highly aggressive variant in which at least 50% of tumour cells have signet ring cell morphology with intracytoplasmic mucus which pushes the nucleus to the periphery of the cell. Other tumour variants including medullary carcinomas, adenosquamous carcinoma, squamous cell carcinoma, small cell carcinoma, undifferentiated carcinoma and mixed carcinoid-adenocarcinoma are rare.

**Circumferential resection margin (CRM)**

TME surgery generates a pathological specimen with a circumferential resection margin (CRM) which involves the entire non-peritonealised surface of the specimen. CRM involvement, defined as a tumour distance of ≤1mm to the radial margin, is observed in 1-28% of patients with curatively operated rectal cancer, and is a powerful predictor of local recurrence (HR 2.7 [95%CI 1.72-4.35]), distant recurrence (HR 2.78 [95%CI 1.85-4.35]), and survival (HR 1.72 [95%CI 1.27-2.27]). After preoperative therapy the predictive value of CRM involvement for local recurrence is even stronger HR 6.3 [95%CI 3.7-16.7]), although an effect on distant recurrence and survival was not found.

**Number of lymph nodes**

Presence of LNM is a strong adverse prognostic factor in rectal cancer patients and is firmly integrated in staging. However, prognosis is also influenced by the total number of evaluated lymph nodes, with improved local recurrence and survival rates associated with a higher number of examined nodes in patients with stage II and III disease. The number of lymph nodes retrieved depends on many factors related to the patient (BMI, age, and gender), the tumour (size, differentiation grade, inflammatory reaction, localization), the surgeon (surgical technique, extent and quality of the resection), as well as the presence of preoperative therapy and the effort of the pathologist.

A minimum number of 10 or 12 lymph nodes are recommended depending on which guideline is cited. After preoperative therapy lymph node yield may be reduced by 7-53%.

**Mesorectal tumour deposits**

Tumour foci in the mesorectum, which are separated from the main tumour bulk, are called tumour deposits. A tumour deposit may represent a variety of causes, such as an overgrown LNM, vascular invasion, perineural growth or discontinuous tumour spread. How pathologists interpret these tumour deposits is crucial in staging.

**Other pathological characteristics**

A variety of other tumour related characteristics can be observed in histopathological specimens, some of which are routinely scored by pathologists. Lymphatic invasion is defined by some authors as “tumour cells in a space covered with endothelial cells in the absence of erythrocytes” and is associated with worse prognosis in patients with negative lymph nodes, although its relevance in patients with LNM is less clear. Venous invasion is “tumour within a smooth muscle-lined space or in an endothelial-lined space with additional fibrin clots, erythrocytes or both without erythrocyte extravasation into the surrounding tissue.” Extramural venous invasion is a well known indicator of poor prognosis and is especially associated with liver metastases.

Tumour budding or sprouting is the presence of de-differentiated single cells or clusters of up to 5 cells at the invasion front of a tumour. It is associated with presence of LNM and poor outcome, although integration in daily reporting has long been delayed, due to lack of agreement on how exactly it should be scored. Tumour expansion along nerves is called perineural invasion (PNI) and is reported to occur in 20.6% of rectal cancers. By definition tumour cells should surround at least a third of the nerve circumference. PNI is associated with increased local recurrence and worse disease free, cancer specific and overall survival.

Rectal cancers treated with chemoradiation therapy or long course radiation therapy show variable levels of tumour regression. In 8-24% of patients a pathological complete response (pCR) may be observed, which is associated with an excellent prognosis. Several methods, including 5-tier systems as described by Mandard and Dworak, and newer simplified 3-tier systems have been developed to further differentiate tumour response. Some studies report these classifications of TRG to be an independent prognosticator for survival, although others do not.
Outline of the thesis

This thesis discusses clinical and pathological aspects of rectal cancer with emphasis on the role of pathological evaluation of surgical specimens in improving diagnostics and treatment of this frequently occurring disease. In chapter 1 the literature is systematically reviewed to determine the clinical significance of macroscopic evaluation of surgical quality by pathologists. The prognostic value of the plane of resection achieved during rectal surgery, as well as various factors that may influence surgical quality are discussed. Histopathology also helps determine the optimal treatment type for specific patient groups. For example, in patients with early colorectal cancer a radical resection may constitute overtreatment in the absence of LNM. Chapter 2 systematically reviews the literature to identify histopathological factors that predict nodal involvement in pT1 colorectal cancer, which helps select patients who may be cured with a limited local excision. Interest in organ sparing approaches is also increasing in patients with substantial downstaging after preoperative treatment. The study in chapter 3 investigates predictors of residual nodal disease after long course CRT, which may help identify patients for whom a local excision could be safe. Tumour regression is associated with improved outcomes, although differences in methodology make it difficult to compare study results. Chapter 4 evaluates the association between tumour regression grading and outcome in a prospective cohort of uniformly treated and strictly defined LARC patients with central revision of histopathology. In chapter 5 the results of a population based study are described which compares TNM stage specific overall survival in rectal cancer patients treated either with preoperative CRT, SC-RT, or surgery only. Although the benefits of preoperative therapy have been demonstrated extensively in rectal cancer patients, clinicians are reluctant to offer this treatment to patients with IBD, due to fear of excessive side-effects. Therefore, the retrospective clinical study described in chapter 6 was conducted, which investigates preoperative therapy induced toxicity and postoperative complications in rectal cancer patients with IBD.

References


The importance of the pathologist’s role in assessment of the quality of the mesorectum

Systematic review

S.L. Bosch, I.D. Nagtegaal

Abstract

Total mesorectal excision (TME) is considered standard of care for rectal cancer treatment. Failure to remove the mesorectal fat envelope entirely may explain part of observed local and distant recurrences. Several studies suggest quality of the mesorectum after TME surgery as determined by pathological evaluation may influence prognosis. We aimed to determine the prognostic value of the plane of surgery as well as factors influencing the likelihood of a high quality specimen by reviewing the literature. A pooled meta-analysis of relevant outcome data was performed where appropriate. A muscularis propria resection plane was found to increase the risk of local recurrence (RR 2.72 [95% CI 1.36 to 5.44]) and overall recurrence (RR 2.00 [95% CI 1.17 to 3.42]) compared to an (intra)mesorectal plane. Plane of surgery is an important factor in rectal cancer treatment and the documentation by pathologists is essential for the improvement of TME quality and patient outcome.

Introduction

The development of total mesorectal excision (TME), introduced by Heald and Ryall in the early 1980s, is based on the notion that lateral mesorectal spread of small tumour foci, which are not removed in classic anterior resection, can lead to local recurrence after rectal cancer surgery.1,2 In a TME procedure the rectum and mesorectum are excised by precise dissection under direct vision of the avascular “holy” plane between the visceral and parietal pelvic fascia separating the mesorectal fat from the other pelvic structures.3 Discontinuous tumour deposits and possibly involved lymph nodes present in the mesorectum are hereby removed together with the tumour. The introduction of TME lead to the reduction of local recurrence rates from 20-45%,3 to around 10% with TME surgery alone, and to 2.4-6% after short-term neo-adjuvant radiotherapy.4-6 Predicting local recurrence by acknowledging the importance of lateral tumour spread led to the introduction of the circumferential resection margin (CRM). This margin, which comprises the entire non-peritonealised circumference of the resection specimen, has a relatively short, distally located anterior aspect, whereas posteriorly it has a triangular shape and runs up to the start of the sigmoid mesocolon.7 Currently, CRM involvement is considered to be one of the key factors in rectal cancer treatment. A large number of studies, pooled in a meta-analysis by Nagtegaal and Quirke and including over 17,500 patients, showed a CRM of ≤1 mm to be a strong predictor of local recurrence (HR 2.7 [95% CI 1.72 to 4.35]), distant recurrence (HR 2.78 [95% CI 1.85 to 4.35]) and survival (HR 1.72 [95% CI 1.27 to 2.27]). Moreover, after neo-adjuvant therapy, CRM involvement was found to be an even stronger predictor of local recurrence (HR 6.3 [95% CI 3.7 to 16.7]), but not distant recurrence and survival.8 However, local and distant recurrences may also develop in patients with an uninvolved CRM. The plane of resection created by the surgeon is another predictor of outcome that has been under investigation by pathologists for almost a decade, and which may explain part of the local recurrences in CRM negative patients. Several authors to date have included an evaluation of the plane of surgery in their protocol. However, these studies show considerable variation in population size, study design and results, making it difficult to appreciate the relevance of studied variables. It is the purpose of this article to critically review the current literature on the prognostic value of plane of surgery and the factors associated with achieving a satisfactory surgical specimen. A pooled meta-analysis of relevant outcome data will be performed where appropriate.
**Methods**

In this review the factors influencing the plane of surgery of a resection specimen after TME for rectal cancer and the prognostic value of this plane are evaluated. A PubMed search was performed using the keywords: “TME or total mesorectal excision” combined with “macroscopic evaluation, plane of surgery, quality of surgery or quality of mesorectum”. In addition, cross-referencing of relevant articles was performed. Only full-text articles available in English and including an assessment of the surgical quality of the mesorectum were considered. In case of obvious overlap between studies the study with the highest number of patients was included. There was still some possible overlap of patients in some of the remaining studies, therefore the total number of patients cannot be determined exactly, however, 18 studies containing published data of between 4399 and 4469 individual patients were used. Information on outcome was given in 9 of these studies (n=2405). Data was extracted and analyzed by a single investigator. For all studies in the pooled analysis the frequencies of mesorectal quality and number of events were available from the published text or tables. Relevant outcome measures are expressed as relative risks (RR) with 95% confidence intervals, and total effect sizes are calculated using Review Manager (RevMan) [Computer program]. Version 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011.

A summary of the articles, their methodology, and primary results is given in table 1.

**Quality of surgery: definitions**

In the CR07 trial protocol from January 1998 three grades of mesorectal surgical quality were introduced by Quirke et al. [P. Quirke, personal communication] (Table 2). We were the first to systematically describe the macroscopic quality of the mesorectum in rectal resection specimens from a large randomised clinical trial, and to correlate quality to outcome. We used the definitions as formulated in the CR07 protocol, but a specimen was called complete, nearly complete or incomplete, rather than good, moderate, or poor.

In more recent publications we and others prefer an even more descriptive evaluation of mesorectal quality based on surgical plane of resection. The circumferential resection margin is therefore said to be in the mesorectal plane (previously good/complete), the intramesorectal plane (previously moderate/nearly complete) or the muscularis propria plane (previously poor/incomplete) (Figure 1A + 1B).

An underlying reason for using descriptive rather than subjective qualifications is that this method does more right to the surgeon, since there is evidence, discussed later in this review, that other factors beside surgeon competence may explain an inadequate resection plane. Furthermore, in light of increasing demands for auditing of colorectal cancer treatment it is preferable to use objective terminology that is less likely to be misinterpreted by non-medical professionals and the public.

The studies described in this review generally use the definitions as mentioned in Table 2. One study uses modified definitions: an intact mesorectum is called complete, a mesorectum with injuries < 2 cm is incomplete, and a mesorectum with injuries >2cm is inadequate. Baik et al. [P. Quirke, personal communication] (25 patients with partial injury in the fascia propria of the rectum (less than 5 mm), thus of nearly complete grade). Differences in the use of definitions may partly explain variable results between studies.

Analogous to the plane of surgery of the mesorectal fat envelope, a comment can be made on the plane of surgery around the sphincter complex after an abdominoperineal resection (APR). To date, we published the only study to critically assess sphincter complex quality using the definitions in Table 2.

According to these definitions a specimen containing the levator ani muscle entirely is considered to be optimal, whereas the conventional APR specimen with the plane of resection on the sphincter complex is less than optimal, and defects in the muscularis propria of the sphincter or perforation into the lumen signify the worst grade.

As stated for the assessment of the mesorectum, the terminology for evaluating the sphincter area should be descriptive and objective.

**Incidence**

Twelve studies report frequencies of the different resection planes after open TME surgery on 3209 patients. The total percentage of mesorectal, intramesorectal and muscularis propria planes was 56.4%, 29.0% and 14.6% respectively.

There is substantial variation in achieved plane of resection between studies. The five studies reporting over 70% mesorectal plane of resection are all published after 2006. These studies are either performed in tertiary centres or specialised units or report results of an audited teaching programme.

Differences between studies may be related to the wide variation in methodology regarding patient selection, interpretation of definitions, study design, and surgeon or centre expertise. The time period in which the included patients were operated may influence the results because of growing awareness amongst surgeons of the importance of achieving a high quality TME. This is pointed out by Quirke et al. (2009) by reporting an improvement in plane of surgery achieved over the course of the trial.

Three studies stand out as having a high percentage of intramesorectal and muscularis propria planes. In our study on low rectal cancer we reported the surgical quality of APR specimens only, and this may explain the high percentage of muscularis propria

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The results reported by Leite et al. (2009) may be explained as a reflection of the individual performance of a single centre, whereas Leonard et al. (2010) describe an audit of the performance of 33 potential expert surgeons from multiple centres in Belgium. Surgeons in the latter study are candidate-TME-trainers, who agreed to an external audit of their consecutive TME cases to judge whether they could serve as an alternative to foreign TME experts in a national teaching programme. The fact that these are not recognized expert TME surgeons may explain a large part of the difference in achieved plane of resection with other studies. Interestingly, this study may actually give a more realistic view of average clinical practice than reports from trials by expert surgeons.
Chapter 1 Quality of the mesorectum

Surgeon experience

Variability between surgeons and centres regarding CRM involvement rates has been demonstrated repeatedly, and can also be expected regarding the achieved plane of surgery. In the previously mentioned national audit significant heterogeneity was demonstrated when comparing 33 surgeons. However, no difference was present in 2 smaller studies comparing consultants with supervised registrars.

Laparoscopic TME

Evidence that laparoscopic resection for rectal cancer is safe and has similar short-term and long-term oncological outcome as open surgery is accumulating. The effects of this procedure on mesorectal grade is described in 8 studies including 879 patients. The percentage of mesorectal, intramesorectal and muscularis propria planes was 61.8%, 23.7% and 14.6% respectively. From the 8 mentioned studies, 6 report mesorectal plane of resection in over 70% of cases. These studies are performed by experienced laparoscopy surgeons from specialised units, and include 4 single centre trials and one multicentre observational study.

As was observed for open surgery, the study by Leonard et al. (2010) shows a high percentage of intramesorectal (35.7%) and muscularis propria (48.2%) resection planes. A direct comparison between laparoscopic or open TME regarding achieved plane of surgery is made in 5 of the 8 articles, in three of those studies no difference was observed. One study found a better quality of surgery (as judged by the operating surgeon) in the laparoscopy arm, whereas in the national audit better results are reported for the open surgery arm. A meta-analysis showed no significant difference in plane of surgery for laparoscopic versus open TME (RR 1.31 [95% CI 0.93 to 1.84]).
Robotic-assisted TME

Robotic-assisted TME is an alternative for laparoscopy and the results of achieved planes have been studied in two study populations. Baek et al. (2010) report 84.2% mesorectal plane of surgery whereas Baik et al. (2009) compare laparoscopic and robotic-assisted TME in 113 consecutive cases reporting mesorectal plane in 75.4% and 92.9% respectively (p=0.033). These results need to be substantiated but seem to indicate that robotic-assisted TME can produce a good quality specimen.

Anterior resection versus abdominoperineal resection

Depending on the location of the tumour and the skills of the surgeon an anterior resection (AR) or abdominoperineal resection (APR) is performed. APRs tend to have higher local recurrence rates and worse survival than ARs. This can partly be explained by higher rates of CRM involvement and intraoperative perforation (IOP), which are related to the removal of less tissue at the level of the tumour in an APR.

As mentioned earlier the surgical quality of an APR can be evaluated at both the mesorectal as well as the sphincter level (tables 2A and 2B). In our study on quality of surgery in APRs we demonstrated a significant correlation between the surgical grades of the mesorectum and the sphincter (Pearson’s R=0.144, p=0.039).

Eight other studies compared mesorectal grades from AR and APR specimens after open TME. All studies except for Baik et al. (2008) report significantly less mesorectal and more muscularis propria planes in APR compared to AR specimens. The combined effect analysis showed RR 2.53 [95% CI 1.94 to 3.31] for achieving a muscularis propria plane after an APR compared to an AR. However, in a multifactorial analysis of 170 patients type of surgery was not an independent predictor of quality of surgery when compared to pathologic BMI, downstaging after chemoradiotherapy, and laparoscopic or open surgery.

Turnout distance to the anal verge is an important aspect in the decision to perform an APR. Five studies (n=997) described a significantly lower percentage of mesorectal and a higher percentage of muscularis propria resection planes in patients with tumours at <5cm from the anal verge compared to >5cm.

Neoadjuvant therapy

A number of clinical trials over the last 20 years have demonstrated the benefits of neoadjuvant therapy in rectal carcinoma.

The effect of radiotherapy and chemoradiotherapy on mesorectal quality was compared to no neoadjuvant therapy in six studies (n=2260). None of the studies showed a significant difference in plane of surgery achieved between the two groups. However, in one study a small subgroup of patients that did not show downstaging after long course CRT, had a higher incidence of muscularis propria plane of resection compared to patients who did show downstaging (p=0.0005 on multivariate analysis).

Other factors

Seven authors (n=2440) make a remark on the influence of tumour extent and presence of lymph node metastases on quality of surgery. No significant relation was found with T-stage, N-stage, TNM-stage or Dukes-stage.

Data about the correlation of plane of surgery and gender are confusing. In three studies with 437 patients no correlation was found. One study found body mass index (BMI) to show a nonlinear association with the probability of a muscularis propria plane of resection (p=0.003), indicating that both patients with a relatively high as well as those with a relatively low BMI are at risk. The authors state that on the one hand this indicates TME surgery is difficult in obese patients, and on the other hand little protective mesorectal fat increases the chance of accidental defects onto the muscularis propria. In contrast, Baik et al. (2008) found no significant influence of BMI, but points out that the lower range of BMI values found in an Asian compared to a Western population may explain the lack of significance in this study.

Age did not influence mesorectal quality in any of the studies.

Circumferential resection margin

Circumferential resection margin involvement is an important prognostic factor for the development of local recurrence, distant recurrence and survival in rectal cancer patients. It has been associated with advanced TNM-stage, large tumour size, low tumour position, abdominoperineal resection, an ulcerative or stenosing growth pattern, surgeon experience, and on histological examination an infiltrating margin, poor differentation, and vascular invasion.
The association of plane of surgery with CRM involvement has been investigated in 9 studies (n=2744). All except one of these show a significant association between achieving a muscularis propria plane of resection (combined with an intramesorectal plane in one study) and CRM involvement. The percentage of positive margins after a muscularis propria plane of resection ranges from 19% to 29% in the reviewed articles whereas after a mesorectal plane these percentages range from 1.6% to 14.6%.

Three studies showed a significant difference in the percentage of muscularis propria resection planes between CRM positive and CRM negative patients: respectively 44% versus 11% (p<0.001), 30.3% versus 7.9% (P=0.0001) and 43.6% versus 19.2% (P=0.006). Furthermore, 11.1% to 56.4% of patients with CRM involvement were found to have a mesorectal plane of excision indicating that a substantial part of CRM positivity can be explained by advanced tumour growth rather than suboptimal surgery.

**Prognosis**

**Local recurrence**

The prognostic value of plane of surgery after open TME was described in 6 studies (n=2174). Four of these report a significant effect of achieved plane of surgery on local recurrence rates in a multivariate analysis. Two studies combine the number of local recurrences in patients with a mesorectal and intramesorectal plane of resection and one study combines patients with an intramesorectal or muscularis propria plane. Therefore, two different graphs (figures 2A and 2B) are depicted showing prognostic significance of either a mesorectal or a muscularis propria plane versus the combination of the other two planes. In the combined effect analysis patients with either a muscularis propria plane of resection have a significantly higher risk of local recurrence compared to patients with a mesorectal or intramesorectal plane (RR 2.72 [95% CI 1.36 to 5.44]).

The combination of an intramesorectal and a muscularis propria plane of resection also significantly increases the risk of local recurrence compared to a mesorectal plane (RR 2.12 [95% CI 1.05 to 4.28]). Furthermore, subanalyses performed by Quirke et al. (2009) showed that patients who received neoadjuvant radiotherapy and had a mesorectal resection plane only developed local recurrence in 1% of cases compared to 10% of cases with a muscularis propria plane (HR 0.09 [95% CI 0.02 to 0.49]). Moreover, CRM negative patients showed a 4% versus 12% local recurrence rate for mesorectal and muscularis propria plane respectively (HR 0.33 [95% CI 0.15 to 0.74]), indicating clinical significance of quality of surgery in this group of patients.

**Overall recurrence**

Five studies (n=1887) report the effect of plane of resection after open TME on overall recurrence of which 3 show a significant difference. In 2 studies, the difference remains significant on multivariate analysis. In the meta-analysis the patients with a muscularis propria plane of resection had a significantly increased risk of overall recurrence compared to patients with a mesorectal or intramesorectal plane (RR 2.00 [95% CI 1.17 to 3.42]) (figure 3A). The comparison between the combined group of patients with an intramesorectal and a muscularis propria plane of resection and the patients with a mesorectal plane showed a trend towards significance (RR 1.84 [95% CI 0.94 to 3.61] Z=1.79 p=0.07) (figure 3B).

In one study CRM negative patients were found to have overall recurrence rates of 14.9% versus 28.6% (p=0.03) for mesorectal and intramesorectal versus muscularis propria plane respectively, indicating the relevance of an adequate resection plane in this subgroup as well.

**Overall survival**

Overall survival rates were only addressed in 2 studies (n=310). In our study (Nagtegaal et al. 2002) we found survival rates of 86% versus 76% (p<0.05) for mesorectal and intramesorectal planes versus a muscularis propria plane respectively, whereas Maslekar et al. (2006) did not find a significant difference.
Chapter 1 Quality of the mesorectum

Conclusion

We performed a meta-analysis of published data relating plane of surgery achieved after TME to patient outcome. The data consistently show that avoiding a muscularis propria plane of resection significantly reduces the risk of local recurrence and overall recurrence after TME surgery. Achieving an optimal (=mesorectal) plane of surgery also significantly improves local recurrence rates compared to a suboptimal (=intra-mesorectal or muscularis propria) plane, but for overall recurrence there is only a trend towards significance.

Worse local and overall recurrence rates after an intramesorectal or muscularis propria resection plane can partly be explained by CRM involvement. However, in most studies plane of surgery was a significant predictor of local recurrence in a multivariate analysis, and in CRM negative patients it is related to local recurrence as well, indicating an independent role for plane of surgery in rectal cancer treatment.

Many factors influence the plane of resection. Heterogeneity between surgeons indicates that the skill of the surgeon is an important factor.

Type of surgery has a significant effect with APR surgery showing an inferior plane of resection more often than AR, as well as surgery on tumours at a short distance from the anal verge. In patients with either a high or low BMI it is more difficult to achieve a mesorectal resection plane.

Results from studies comparing laparoscopic to open TME suggest that laparoscopy gives at least similar quality of mesorectum as open surgery when performed by experienced surgeons, whereas less experienced surgeons may generate inferior results. Results from robot-assisted TME studies are comparable to those for laparoscopy. It seems reasonable to suggest that laparoscopic and robot-assisted TME surgery should only be performed or supervised by surgeons well beyond the learning curve. Neoadjuvant therapy does not influence achieved plane of resection.

Plane of surgery is an important factor in the treatment of rectal cancer. Pathologists have the primary responsibility to comment on resection plane in pathology reports, however, surgeons need to be aware of its importance and have to ask their pathologists for the information if it is missing. A shared responsibility for the evaluation of the mesorectum is the best way to ensure accurate feedback on surgeon performance and improvement of TME quality as well as patient outcome. Furthermore, achieved plane of surgery should be an integral part of all rectal cancer studies and audits, and should preferably be reported according to the definitions cited in this article to enable adequate comparisons.
Chapter 1

Quality of the mesorectum

References

Predicting lymph node metastasis in pT1 colorectal cancer – a systematic review of risk factors providing rationale for therapy decisions

Systematic review

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Abstract

Background and objective: Population screening for colorectal cancer is expected to increase the number of pT1 colorectal cancers. Local excision is an attractive treatment option, but is only oncologically safe in the absence of lymph node metastasis (LNM). A systematic review of the predictive value of pathological risk factors for LNM in pT1 colorectal cancer was conducted to provide data for an evidence-based decision regarding follow-up or radical surgery after local excision.

Methods: PubMed was searched for reports on predictors of LNM in pT1 colorectal cancer. Published papers written in English containing at least 50 patients were included. Meta-analyses were performed using Review Manager 5.1.

Results: 17 studies were included totalling 3621 patients with available nodal status. The strongest independent predictors of LNM were lymphatic invasion (RR 5.2 [95% CI 4.0-6.8]), submucosal invasion ≥1mm (RR 5.2 [95% CI 1.8-15.4]), budding (RR 5.1 [95% CI 3.6-7.3]), and poor differentiation (RR 4.8 [95% CI 3.3-6.9]).

Limitations: Results could not be stratified according to location in colon or rectum. Very early tumours removed by polypectomy without surgical resection are not included in this meta-analysis. Included studies are primarily from Asian countries and results therefore need to be verified in Western populations.

Conclusion: The absence of lymphatic invasion, budding and poor differentiation is associated with low risk of lymph node metastases. Risk stratification models integrating these factors need to be investigated further.

Introduction

With the widespread introduction of population screening for colorectal cancer, the number of early colorectal cancers is expected to increase. In the past, approximately 25% of colorectal cancer cases presented with early disease (stage I) in which the primary tumour is limited to the submucosa (T1 tumours) or the bowel wall (T2 tumours). Local excision is an attractive option for early disease in both colon and rectal cancer, since it is associated with considerably less surgery-related morbidity and almost no post-operative mortality compared to colectomy and total mesorectal excision (TME), for which mortality rates of 1.9-6.5% (rectum) and 3.2-9.8% (colon) have been reported.

Clinically, local treatment is especially relevant for rectal cancer, since the consequences of TME surgery, which often results in a colostomy, sexual and urinary dysfunction and complaints of soiling and faecal incontinence, are greater than for colectomy. Moreover, the removal of tumours in the rectum, using transanal endoscopic microsurgery (TEM) and endoscopic submucosal dissection (ESD), is more effective than in the colon, where ESD is technically difficult and mainly polypectomy and endoscopic mucosal resection (EMR) are used. However, there is a considerable move towards organ preserving surgery for colon cancer as well, since techniques for local treatment in the colon are improving.

Local excision is generally reserved for T1 disease, since adequate removal of T2 tumours using ESD or EMR in the colon is not feasible and for rectal cancer local treatment of T2 lesions is reported to result in unacceptably high local recurrence rates and lower survival compared to radical resection.

Currently, patient related factors such as age and comorbidity are of primary importance when deciding whether or not to perform a radical resection for early colorectal cancer. For fit patients with T1 colorectal cancer the curative intent is absolute and a local excision can only be oncologically safe in the absence of lymph node metastasis (LNM). The overall incidence of LNM in T1 tumours is between 8-16% and several pathologic features of the primary tumour, such as poor differentiation, lymphatic or vascular invasion, and submucosal invasion depth, have been associated with its presence.

Patient selection through careful histological analysis of local excision specimens can therefore be very useful to avoid overtreatment and undertreatment. For rectal cancer, some of the mentioned characteristics are already included in national guidelines as indicators of high risk lesions necessitating additional radical surgery (e.g. American (National Cancer Institute), British, Japanese and Dutch national guidelines). However, despite the importance of high quality discrimination between low and high risk T1 cases, an adequate overview of the literature, quantifying the influence of the individual risk factors, is lacking. Although the described differences regarding...
treatment options and associated morbidity suggest the clinical relevance is currently greatest for rectal cancer, risk stratification is valuable for pT1 colon cancer as well. Unfortunately, a separate analysis is not feasible, since studies describing nodal involvement exclusively for pT1 rectal cancer are very scarce. Therefore, the current study provides a systematic review of the risk factors for the presence of LNM in pT1 colorectal cancer including meta-analyses where appropriate.

**Methods**

**Search strategy and selection criteria**

A comprehensive literature search for published studies was performed using the PubMed database from inception to May 25, 2011. The keywords used were “lymph nodes”, “lymph node metastasis”, “TEM” and “T1” combined with “colorectal cancer”. Additional searches were performed using manual cross-referencing. Only published studies written in English with at least 50 patients were included. Reports describing use of neo-adjuvant therapy (ypT) were not included. Radical resection was required to obtain a reliable lymph node status. The percentage or number of patients with nodal involvement, specified for presence and absence of a specific risk factor was required. Data from pT1 patients had to be reported separately. In case of possible overlap of data due to duplicate publications, only the article with the largest sample size was included.

**Measuring submucosal invasion depth**

Various methods to divide patients in a low or high risk group based on submucosal invasion depth are described in the literature. For sessile lesions a qualitative assessment according to Kudo et al. (1993) 37 is commonly used (sm1, sm2, and sm3: invasion into the most superficial, intermediate and deepest 1/3 of the submucosa respectively). This has been modified slightly into a semi-quantitative system by Kikuchi et al. (1995) 32 (sm1: invasion up to 0.2-0.3mm, sm2: intermediate invasion, sm3: invasion near the muscularis propria). A third method quantifies invasion depth (sm1: up to 0.5 mm, sm2: 0.5 to 1.0mm, sm3: beyond 1.0mm). 38 Alternatively, the invasion depth is measured and a cut-off value is defined distinguishing between superficial and deep submucosal invasion.

For the purpose of performing our meta-analysis the studies were divided into two groups: one group includes studies using a quantitative invasion depth and the other includes studies using qualitative or semi-quantitative invasion depth.

**Statistical analysis**

Data was extracted and analyzed by a single investigator (SLB). For all studies in the meta-analysis the frequencies of LNM per factor were available either from the text or from tables. Risk factors, incidence and events from the individual studies were entered into Review Manager 5.1 (RevMan, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). If a factor was reported in at least 3 studies with comparable methodology, a meta-analysis was performed to summarize its prognostic effect in terms of a relative risk with 95%-confidence interval. A random effects model with Mantel-Haenszel weighting was used. Heterogeneity was assessed using a $\chi^2$ test for heterogeneity with a $P$-value of <0.10 taken to reflect the presence of significant heterogeneity. The $I^2$ statistic was calculated to quantify the degree of heterogeneity.

Publication bias was assessed by inspection of the funnel plot by an experienced statistician (ST). Data presented as pooled estimates do not account for heterogeneity between studies and are reported for explorative purposes only.

**Results**

**Study selection and inclusion**

The initial search returned a total of 43 studies (figure 1). Following review, 32 potentially relevant studies were identified as eligible of which 12 were excluded for duplicate series of patients, and 2 32,42 were excluded since they did not report separate data for patients with a radical resection and patients with local excision only. In 3 studies 39-41 patients who underwent local excision only were reported separately and only the patients who received radical resection were included in our analyses. Another study was excluded, because of a major discrepancy between data reported in text and tables.43 Repeated attempts to contact the authors by email to clarify this issue were unsuccessful. Therefore, we assumed the data from this study were unreliable.

The 17 studies selected for this review 28,38-41,44-55 included a total of 3621 patients with pT1 tumours and available nodal status after radical resection. The median number of patients per study was 140 (range: 65-865). Table 1 outlines the characteristics of these studies. A number of 1544 were rectal carcinomas (41.3%), 2056 were colon carcinomas (54.9%) and in 141 cases no distinction was made between colon and rectal carcinomas (3.8%). The incidence of LNM was 11.4% (414/3621). Of the included studies 2 were performed prospectively and 15 retrospectively.

**Publication bias and heterogeneity**

Inspection of the funnel plots (Supplemental content 1) did not reveal asymmetry, therefore there was no indication for publication bias. However, the funnel plot
analysis was limited in many cases by the low number of studies. Forest plots (Supplemental content 2 to 7) were checked for consistency of the effects. There was only quantitative heterogeneity.

Factors predicting LNM
Factors that were investigated in at least 3 studies with comparable methodology were included in our meta-analyses. These factors are depicted in figure 2 and include tumour location, lymphatic, vascular and lymphovascular invasion, submucosal invasion depth (based on sm-levels, cut-off value 1mm and cut-off value 2mm), width of submucosal invasion (cut-off 5mm), histological differentiation grade (high grade vs. low grade), budding, and poor differentiation at the invasion front. Forest plots generated for the analysis of each individual risk factor are included in supplemental content 2 to 7.

Table 2 provides the data for the dichotomous risk factors with total number of patients available for each analysis, the calculated relative risks (RR) for presence of LNM, and the level of heterogeneity. The data for submucosal invasion depth based on the three-tiered sm-levels (see method section) are shown in table 3. For all relevant factors the corresponding sensitivity, specificity, positive predictive value and negative predictive value are included in table 4. The table in Supplemental content 8 shows the results of the multivariable analyses in the different studies. For submucosal invasion depth the methodology differs considerably between the studies.
Chapter 2 Predicting LNM in pT1 colorectal cancer

Lymphatic, vascular and lymphovascular invasion

Most studies included lymphatic or vascular (sometimes called venous) invasion either as separate variables or gathered under the heading of lymphovascular invasion. Definitions were not often provided, with the exception of Tsuruta et al. (2000) and Wang et al. (2005) who define lymphatic invasion as tumour cells in a space covered with endothelial cells in the absence of erythrocytes. Blood vessel invasion was defined as tumour within a smooth muscle-lined space or in an endothelial-lined space with additional fibrin clots, erythrocytes or both without erythrocyte extra-vasation into the surrounding tissue (Wang, 2005). Lymphovascular invasion has been defined by Okabe et al. (2004) as the presence of tumour cells within an epithelium-lined channel thought to represent either a lymphatic vessel or a blood vessel. Additional staining techniques such as Victoria blue and Elastica von Gieson (EVG) or immunohistochemical stains like D2-40, LYVE1, vWF, and CD34 have been used by some authors.

Figure 2 Forest plot summarizing effect sizes of analyzed risk factors

Table 2 Dichotomous variables: results of the meta-analyses

<table>
<thead>
<tr>
<th>Factor</th>
<th>Studies (N)</th>
<th>references</th>
<th>Patients (N)</th>
<th>Low risk group</th>
<th>LNM+/total</th>
<th>LNM (%)</th>
<th>High risk group</th>
<th>LNM+/total</th>
<th>LNM+ (%)</th>
<th>RR (95% CI)</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour location</td>
<td>10</td>
<td>[28, 38, 45-47, 49-51, 53, 55]</td>
<td>2722</td>
<td>Colon</td>
<td>169/1699</td>
<td>9.9</td>
<td>Rectum</td>
<td>141/1023</td>
<td>13.8</td>
<td>1.4 (1.1-1.7)</td>
<td>χ² = 8.12, p = 0.52, I²: 0%</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>10</td>
<td>[28, 44-48, 50, 53-55]</td>
<td>1931</td>
<td>neg</td>
<td>71/1324</td>
<td>5.4</td>
<td>pos</td>
<td>162/807</td>
<td>26.7</td>
<td>5.2 (4.0-6.8)</td>
<td>χ² = 8.74, p = 0.46, I²: 0%</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>10</td>
<td>[28, 44-48, 50, 53-55]</td>
<td>1931</td>
<td>neg</td>
<td>154/1552</td>
<td>9.9</td>
<td>pos</td>
<td>79/379</td>
<td>20.8</td>
<td>2.2 (1.4-3.2)</td>
<td>χ² = 17.32, p = 0.04, I²: 48%</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>5</td>
<td>[28, 41, 49, 51, 53]</td>
<td>1332</td>
<td>neg</td>
<td>75/1053</td>
<td>7.1</td>
<td>pos</td>
<td>73/332</td>
<td>22.0</td>
<td>3.9 (2.7-5.6)</td>
<td>χ² = 4.30, p = 0.37, I²: 7%</td>
</tr>
<tr>
<td>Submucosal invasion depth</td>
<td>5</td>
<td>[38, 41, 47, 48, 51]</td>
<td>1835</td>
<td>&lt;1mm</td>
<td>6/405</td>
<td>1.5</td>
<td>≥1mm</td>
<td>176/1430</td>
<td>12.3</td>
<td>5.2 (1.8-15.4)</td>
<td>χ² = 6.26, p = 0.18, I²: 36%</td>
</tr>
<tr>
<td>Submucosal invasion depth</td>
<td>3</td>
<td>[41, 47, 51]</td>
<td>1463</td>
<td>&lt;2mm</td>
<td>25/464</td>
<td>5.4</td>
<td>≥2mm</td>
<td>133/999</td>
<td>13.3</td>
<td>2.4 (1.6-3.7)</td>
<td>χ² = 1.55, p = 0.46, I²: 0%</td>
</tr>
<tr>
<td>Submucosal invasion width</td>
<td>3</td>
<td>[41, 51, 53]</td>
<td>620</td>
<td>&lt;5mm</td>
<td>12/213</td>
<td>5.6</td>
<td>≥5mm</td>
<td>69/407</td>
<td>16.9</td>
<td>2.7 (1.4-5.6)</td>
<td>χ² = 2.40, p = 0.30, I²: 7%</td>
</tr>
<tr>
<td>High vs. low grade histology</td>
<td>13</td>
<td>[28, 38, 39, 41, 44, 45, 47, 48, 51-55]</td>
<td>2847</td>
<td>Low grade</td>
<td>229/2578</td>
<td>8.9</td>
<td>High grade</td>
<td>66/289</td>
<td>24.5</td>
<td>4.8 (3.3-6.9)</td>
<td>χ² = 16.04, p = 0.19, I²: 25%</td>
</tr>
<tr>
<td>Budding</td>
<td>7</td>
<td>[41, 44, 46, 47, 51, 54, 55]</td>
<td>1991</td>
<td>neg</td>
<td>59/1173</td>
<td>5.0</td>
<td>pos</td>
<td>174/818</td>
<td>21.3</td>
<td>5.1 (3.6-7.3)</td>
<td>χ² = 7.54, p = 0.27, I²: 20%</td>
</tr>
<tr>
<td>Poor differentiation at</td>
<td>4</td>
<td>[45-47, 51]</td>
<td>1307</td>
<td>neg</td>
<td>111/1083</td>
<td>10.2</td>
<td>pos</td>
<td>48/250</td>
<td>19.2</td>
<td>2.5 (1.8-3.5)</td>
<td>χ² = 0.04, p = 1.00, I²: 0%</td>
</tr>
</tbody>
</table>

*This result is mainly based on one large study [47], which contributes 69.6% of the weight in this analysis.

LNM: lymph node metastasis, neg: negative, pos: positive
Lymphatic invasion was the most powerful predictor of LNM emerging from the meta-analyses (RR 5.2 [95% CI 3.7-7.3]). Multivariable analyses also provide solid evidence for an independent effect. It is important to report lymphatic invasion and vascular/venous invasion separately, since vascular invasion is a much weaker predictor of LNM (RR 2.2 [95% CI 1.4-3.2]). Combining the two factors as lymphovascular invasion (LVI) logically generates an intermediate relative risk (RR 3.9 [95% CI 2.7-5.6]), which is less informative. Additional staining techniques increase inter-observer agreement for lymphatic invasion from fair in HE stained slides ($\kappa=0.30$) to moderate ($\kappa=0.56$) in D2-40 stained slides, and for vascular invasion from considerable for HE slides ($\kappa=0.10$) with marked improvement after EVG staining ($\kappa=0.48$).58

Tumour budding
Budding, which is also called “sprouting” or “single cell infiltration”, is reported in 7 studies (41,44,46,47,51,54,55) totalling 1991 patients. The various authors do not use a uniform definition, however budding is usually described as foci of isolated cancer cells or clusters of less than 5 cancer cells at the invasive front of the lesion. Ueno et al. (2004) regard 5 or more of these foci in a microscopic field at 200x magnification as positive, whereas Ishikawa et al. (2008) require more than 4 foci at a magnification of 400x. Other

### Table 3 Sm-levels and nodal involvement

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Low risk / LNM+</th>
<th>High risk / LNM+</th>
<th>RR (95% CI)</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>sm1 vs. sm2</td>
<td>6/174</td>
<td>3/4</td>
<td>2.4 (0.9-6.1)</td>
<td>$\chi^2=0.01, p=0.9, I^2=0%$</td>
</tr>
<tr>
<td>sm2 vs. sm3</td>
<td>2/1000</td>
<td>8/5</td>
<td>2.7 (0.6-11.2)</td>
<td>$\chi^2=0.01, p=0.9, I^2=0%$</td>
</tr>
<tr>
<td>sm1 vs. sm3</td>
<td>1/174</td>
<td>3/4</td>
<td>4.8 (1.5-16.2)</td>
<td>$\chi^2=0.01, p=0.9, I^2=0%$</td>
</tr>
<tr>
<td>sm2/3 vs. sm3</td>
<td>23/174</td>
<td>6.1</td>
<td>3.6 (1.3-9.8)</td>
<td>$\chi^2=0.01, p=0.9, I^2=0%$</td>
</tr>
<tr>
<td>sm1 vs. sm2/3</td>
<td>23/174</td>
<td>6.1</td>
<td>3.3 (1.8-6.2)</td>
<td>$\chi^2=0.01, p=0.9, I^2=0%$</td>
</tr>
</tbody>
</table>

Data is presented as pooled estimates and relative risk with associated heterogeneity. Data is extracted from 4 studies (refs. [49,52,54,55]) with a total of 635 patients. LNM: lymph node metastasis, RR: relative risk.

### Table 4 Sensitivity, specificity, positive predictive value and negative predictive value for the identified histological risk factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour location in rectum</td>
<td>45.5</td>
<td>63.4</td>
<td>13.8</td>
<td>90.1</td>
</tr>
<tr>
<td>Lymphatic invasion +</td>
<td>69.5</td>
<td>73.8</td>
<td>26.7</td>
<td>94.6</td>
</tr>
<tr>
<td>Vascular invasion +</td>
<td>33.9</td>
<td>82.3</td>
<td>20.8</td>
<td>90.1</td>
</tr>
<tr>
<td>Lymphovascular invasion +</td>
<td>49.3</td>
<td>79.1</td>
<td>22.0</td>
<td>92.8</td>
</tr>
<tr>
<td>Submucosal invasion depth ≥1mm</td>
<td>96.7</td>
<td>24.1</td>
<td>12.3</td>
<td>98.5</td>
</tr>
<tr>
<td>Submucosal invasion depth ≥2mm</td>
<td>84.2</td>
<td>33.6</td>
<td>13.3</td>
<td>94.6</td>
</tr>
<tr>
<td>Submucosal width of invasion ≥5mm</td>
<td>85.2</td>
<td>37.3</td>
<td>17.0</td>
<td>94.4</td>
</tr>
<tr>
<td>High grade histology</td>
<td>22.4</td>
<td>92.0</td>
<td>24.5</td>
<td>91.1</td>
</tr>
<tr>
<td>Budding</td>
<td>74.7</td>
<td>63.4</td>
<td>21.3</td>
<td>95.0</td>
</tr>
<tr>
<td>Poor differentiation at invasive front</td>
<td>30.2</td>
<td>82.8</td>
<td>19.2</td>
<td>89.8</td>
</tr>
<tr>
<td>sm2/3 (vs. sm1)</td>
<td>92.7</td>
<td>30.4</td>
<td>16.8</td>
<td>96.6</td>
</tr>
<tr>
<td>sm3 (vs. sm1/2)</td>
<td>72.0</td>
<td>63.5</td>
<td>22.6</td>
<td>93.9</td>
</tr>
</tbody>
</table>

PPV: positive predictive value, NPV: negative predictive value
authors do not provide a cut-off value. Interestingly, the use of various definitions and cut-off values did not result in significant heterogeneity in the meta-analysis \((\chi^2 = 7.54, p = 0.27, I^2 = 20\%), \text{table 2}\). The relative risk is strongly increased \((RR 5.1 [95\% CI 3.6-7.3])\), and 5 of 6 studies show an independent predictive value.

**Submucosal invasion depth: qualitative or quantitative measurement**

In this meta-analyses, studies evaluating submucosal invasion depth for sessile lesions were divided into two groups consisting of the ones applying a qualitative or semi-quantitative definition \(19,52,54,55\) and those applying a strictly quantitative definition for submucosal invasion \(38,41,47,48,50,51\). Although the studies by Masaki et al. (2006) and Yamamoto et al. (2004) use the designations sm1, sm2 and sm3 we included them in the quantitative group, since they define sm-levels according to a specific invasion depth in mm. In both groups, submucosal invasion depth is strongly associated with risk of LNM. Increasing semi-quantitatively determined invasion depth is associated with increased risk of lymph node metastases (table 3, sm1/2 versus sm3 \(RR 3.3 [95\% CI 1.8-6.2]\) and for sm1 vs. sm2/3 \(RR 3.6 [95\% CI 1.3-9.8]\)). However, an independent value was only shown in 1 of 3 multivariable tests (sm1 vs. sm3, Nascimbeni et al. 2002). In our meta-analysis there was a significant difference between sm2 and sm3 \(RR 2.7 [95\% CI 1.6-4.4]\), but only a trend for sm1 versus sm2 \(RR 2.4 [95\% CI 0.9-6.1]\), \(p=0.08\).

For specimens lacking a muscularis propria layer, quantitative measurement of the invasion depth from the muscularis mucosa to the deepest part of invasion is an alternative. An invasion depth of more than 1mm in the submucosa shows a strong increase in relative risk for LNM \(RR 5.2 [95\% CI 1.8-15.4]\), and is an independent predictive factor in 2 out of 3 multivariable analyses.

**Additional histological factors**

Several additional histological factors have been evaluated, however, a meta-analysis was not justified in these cases because of a small number of studies, use of varying definitions and classifications or lack of relevant data. These factors include submucosal invasion depth according to Haggitt levels for polyposid lesions, tumour size, histological tumour type, presence of inflammatory infiltrate, growth pattern, a cribriform subtype, microvessel density, and macroscopic tumour type.

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**Discussion**

Published data of 3621 patients with pT1 colorectal carcinoma and available nodal status following radical resection were included in this systematic review. The tumour related factors that showed the strongest independent predictive value for LNM are lymphatic invasion, budding, poor histological differentiation, and a submucosal invasion depth \(>1\) mm. Since a curative intent for all fit patients with a pT1 colorectal cancer is the most important principle, focus should be on selecting patients, who have a very low risk and can safely be spared radical surgery. Several issues need to be solved before such selection procedure can safely be performed. Standards should be set for histological characteristics in order to improve reproducibility. Appropriate cut-off levels for several factors should be established and risk stratification models applying a combination of risk factors should be evaluated to establish the optimal combination of predictive factors. Standardization starts with the use of specific definitions. The results for the predictive value of lymphatic invasion illustrate this. While lymphatic invasion is a strong and reproducible predictive factor for LNM, especially when determined by specific antibody staining, the combination with vascular invasion results in an intermediate relative risk, which is not informative enough for clinical treatment decisions. Similarly, histological differentiation is well known for its inter-observer variation, although the current classification in which low grade and high grade tumours are distinguished, has improved reproducibility. In the current study, poorly differentiated or high grade carcinoma is indeed a strong predictor of LNM, with confirmation in four out of ten multivariable analyses. Budding is a relatively new and not routinely reported risk factor that consistently shows a strong association with the presence of LNM. However, many different definitions are used throughout the literature and there is limited evidence for reproducibility. However, the strong result from the meta-analysis, which lacked significant heterogeneity, indicates that budding, evaluated by any method, is still a powerful marker for LNM.

Determination of cut-off levels is especially important in the determination of submucosal invasion depth. While semi-quantitative methods have proven to be useful in subsets of tumours in the literature, their value in daily practice might be more limited. Especially in endoscopically resected specimens the muscularis propria is often missing and the involved proportion of the submucosal layer is therefore hard to estimate. Quantitative measurement of the invasion depth from the muscularis mucosa to the deepest part of invasion is a more feasible method, although the muscularis mucosa may not always be identifiable due to tumour overgrowth. On the other hand, an invasion depth of more than 1mm in the submucosa shows a strong increase in relative risk for LNM \(RR 5.2 [95\% CI 1.8-15.4]\),
and is an independent predictive factor in 2 out of 3 multivariable analyses, suggesting it could be a helpful tool for risk stratification. Indeed, assuming the pooled data are representative for clinical practice, a 1mm cut-off point would assign LNM positive patients to the high risk group with a sensitivity of 96.7%. However, this would be at the expense of a low specificity (24.1%) resulting in a high number of patients undergoing unnecessary surgery (false positives). A 1mm cut-off may therefore not be the optimal method for risk stratification.

As becomes clear from table 4 no single predictor discussed in this review allows an optimal selection of low-risk patients by itself, since they are either not sensitive or not specific enough. It therefore seems sensible to investigate the potential of combining risk factors in algorithms to identify low risk patients.

Ueno et al. (2004) investigated several combinations of risk factors and distilled a low risk group, defined by absence of unfavourable grade, lymphovascular invasion, and budding, that contained 55% of patients and is associated with only 0.7% nodal involvement. The addition of submucosal depth of invasion ≥2mm as a high risk factor eliminated nodal involvement in the low risk group, but also decreased the percentage of low risk patients to 32.3%.

In a paper by Nakadoi et al. (2011) the authors employed the high risk factors described in the 2010 guidelines of the Japanese Society for Cancer of the Colon and Rectum (JSCCR), which are very similar to the ones used by Ueno et al. (2004) and found that when a tumour was low risk (well/moderately differentiated, no vascular invasion or tumour budding) the incidence of LNM was only 1.2% with 49.9% of patients assigned to the low risk group.

A limitation of the current study is that we were not able to investigate those combinations of factors. Another inevitable source of bias is that this systematic review only includes tumours of patients who underwent radical surgery. There is a population of early T1 cancers removed by polypectomy without radical surgery, which are not considered in our analysis as the definitive lymph node status is not available. We realise that this is especially relevant for pedunculated lesions, which undergo local excision more frequently (Ueno et al), and are known to have a low risk of LNM. Future studies should take this into account.

Furthermore, the majority of papers included in this study originated from Asian countries in which pathological workup may be more extensive, which might influence results. Validation of the currently identified risk factors in Western populations is necessary.

In conclusion, several factors can be identified in pT1 tumours that predict the presence of LNM. Lymphatic invasion, budding, and poor histological differentiation are the strongest predictors of LNM that also show a consistent independent predictive value in multivariable analyses. Their absence indicates a low risk of LNM and may justify a conservative policy. Future studies should investigate all of the above mentioned factors, including invasion depth in mm, and aim to standardize the detection of these powerful markers, preferably using immunohistochemical staining techniques. These recommendations may lead to the development of a validated model incorporating various risk factors for the prediction of LNM, which may help to select patients who can be spared radical resection, and such a model may thereby prevent unnecessary surgery without compromising oncological safety.
Chapter 2 Predicting LNM in pT1 colorectal cancer


Supplemental content

Funnal plots for the assessment of publication bias.
A: Sm1 vs. sm2. B: Sm1 vs. sm3. C: Sm2 vs. sm3. D: Sm1 vs. sm3 vs. sm5. E: Sm1 vs. sm5. F. Male vs. female. G: Ly+ vs. Ly-. H: V+ vs. V-. I: Ly+ vs. Ly-. J: Budding vs. no budding. K: Low grade histology vs. high grade histology. L: Well diff. vs. mod. diff. M: poor diff. Inv. fr. vs. no poor diff. Inv. Fr. N: sm. depth <1mm vs. >1mm. O: sm. depth <1mm vs. >2mm. P: sm. width <5mm vs. >5mm. Q: Location in colon vs. rectum.

Supplemental content 1
Chapter 2 Predicting LNM in pT1 colorectal cancer

### Supplemental content 2

**Forest plot depicting risk of nodal involvement according to tumor location (colon vs. rectum).**

- **Recanal Colon Risk Ratio**
  - Study or Subgroup
    - Osaki 2004
      - Events: 8
      - Total: 176
      - Weight: 27
      - Risk Ratio: 1.42 (95% CI: 1.34, 1.50)
    - Yamamoto 2006
      - Events: 5
      - Total: 20
      - Weight: 5
      - Risk Ratio: 1.45 (95% CI: 1.34, 1.57)
    - Kaminuma 1997
      - Events: 10
      - Total: 126
      - Weight: 10
      - Risk Ratio: 1.48 (95% CI: 1.36, 1.60)
    - Suzuki 2003
      - Events: 10
      - Total: 117
      - Weight: 10
      - Risk Ratio: 1.52 (95% CI: 1.41, 1.64)
    - Oh et al. 2004
      - Events: 10
      - Total: 162
      - Weight: 10
      - Risk Ratio: 1.49 (95% CI: 1.35, 1.64)
    - Nasu et al. 2002
      - Events: 10
      - Total: 178
      - Weight: 10
      - Risk Ratio: 1.38 (95% CI: 1.28, 1.50)
  - **Recanal Rectum Risk Ratio**
  - Study or Subgroup
    - Osaki 2004
      - Events: 46
      - Total: 145
      - Weight: 46
      - Risk Ratio: 1.43 (95% CI: 1.35, 1.52)
    - Yamamoto 2006
      - Events: 4
      - Total: 26
      - Weight: 4
      - Risk Ratio: 1.47 (95% CI: 1.31, 1.66)
    - Kaminuma 1997
      - Events: 5
      - Total: 66
      - Weight: 5
      - Risk Ratio: 1.50 (95% CI: 1.37, 1.65)
    - Suzuki 2003
      - Events: 10
      - Total: 112
      - Weight: 10
      - Risk Ratio: 1.52 (95% CI: 1.41, 1.65)
    - Oh et al. 2004
      - Events: 10
      - Total: 162
      - Weight: 10
      - Risk Ratio: 1.49 (95% CI: 1.35, 1.64)
    - Nasu et al. 2002
      - Events: 10
      - Total: 178
      - Weight: 10
      - Risk Ratio: 1.38 (95% CI: 1.28, 1.50)

  - **Total events**: 141 (in colon) + 109 (in rectum) = 250
  - **Total risk ratio**: 1.37 (95% CI: 1.11, 1.69)

**Forest plot depicting the relation between risk of nodal involvement and lymphatic invasion.**

- **LNM: lymph node metastasis, ly = lymphatic invasion**
- **Study or Subgroup**
  - Epicentro 2004
    - Events: 6
    - Total: 112
    - Weight: 6
    - Risk Ratio: 1.32 (95% CI: 1.21, 1.44)
    - Test for overall effect: Z = 2.88 (p = 0.004)
    - More LNM in colon
  - **Study or Subgroup**
    - Weng 2005
      - Events: 7
      - Total: 129
      - Weight: 7
      - Risk Ratio: 1.40 (95% CI: 1.27, 1.55)
      - Test for overall effect: Z = 3.19 (p = 0.001)
    - Kaminuma 1997
      - Events: 10
      - Total: 117
      - Weight: 10
      - Risk Ratio: 1.45 (95% CI: 1.32, 1.59)
    - Kaminuma 2004
      - Events: 23
      - Total: 297
      - Weight: 23
      - Risk Ratio: 1.50 (95% CI: 1.39, 1.61)
  - **Study or Subgroup**
    - Imai 2009
      - Events: 13
      - Total: 79
      - Weight: 13
      - Risk Ratio: 1.53 (95% CI: 1.33, 1.74)
    - Tournoi 1999
      - Events: 13
      - Total: 87
      - Weight: 13
      - Risk Ratio: 1.60 (95% CI: 1.42, 1.80)
    - Ishihara 2008
      - Events: 18
      - Total: 100
      - Weight: 18
      - Risk Ratio: 1.57 (95% CI: 1.41, 1.74)
  - **Study or Subgroup**
    - Oh et al. 2004
      - Events: 13
      - Total: 108
      - Weight: 13
      - Risk Ratio: 1.58 (95% CI: 1.41, 1.77)
    - Ishihara 2008
      - Events: 11
      - Total: 65
      - Weight: 11
      - Risk Ratio: 1.60 (95% CI: 1.41, 1.82)
    - Ishihara 2008
      - Events: 11
      - Total: 65
      - Weight: 11
      - Risk Ratio: 1.60 (95% CI: 1.41, 1.82)
  - **Study or Subgroup**
    - Susa et al. 2003
      - Events: 10
      - Total: 60
      - Weight: 10
      - Risk Ratio: 1.69 (95% CI: 1.51, 1.90)
    - Susa et al. 2003
      - Events: 10
      - Total: 60
      - Weight: 10
      - Risk Ratio: 1.69 (95% CI: 1.51, 1.90)

- **Total events**: 192 (in colon) + 71 (in rectum) = 263
- **Total risk ratio**: 1.60 (95% CI: 1.44, 1.76)

**Forest plot depicting the relation between risk of nodal involvement and vascular invasion.**

- **LNM: lymph node metastasis, V = vascular invasion**
- **Study or Subgroup**
  - Epicentro 2004
    - Events: 6
    - Total: 112
    - Weight: 6
    - Risk Ratio: 1.34 (95% CI: 1.21, 1.50)
    - More LNM with ly = More LNM with V
  - **Study or Subgroup**
    - Weng 2005
      - Events: 7
      - Total: 129
      - Weight: 7
      - Risk Ratio: 1.40 (95% CI: 1.27, 1.55)
      - Test for overall effect: Z = 3.19 (p = 0.001)
    - Imai 2009
      - Events: 13
      - Total: 79
      - Weight: 13
      - Risk Ratio: 1.53 (95% CI: 1.33, 1.74)
  - **Study or Subgroup**
    - Tournoi 1999
      - Events: 13
      - Total: 87
      - Weight: 13
      - Risk Ratio: 1.60 (95% CI: 1.42, 1.80)
    - Ishihara 2008
      - Events: 11
      - Total: 65
      - Weight: 11
      - Risk Ratio: 1.60 (95% CI: 1.41, 1.82)
    - Ishihara 2008
      - Events: 11
      - Total: 65
      - Weight: 11
      - Risk Ratio: 1.60 (95% CI: 1.41, 1.82)
  - **Study or Subgroup**
    - Susa et al. 2003
      - Events: 10
      - Total: 60
      - Weight: 10
      - Risk Ratio: 1.69 (95% CI: 1.51, 1.90)
    - Susa et al. 2003
      - Events: 10
      - Total: 60
      - Weight: 10
      - Risk Ratio: 1.69 (95% CI: 1.51, 1.90)

- **Total events**: 192 (in colon) + 71 (in rectum) = 263
- **Total risk ratio**: 1.60 (95% CI: 1.44, 1.76)

**Forest plot depicting the relation between risk of nodal involvement and lymphovascular invasion.**

- **LNM: lymph node metastasis, LVI = lymphovascular invasion**
- **Study or Subgroup**
  - Ueno 2004
    - Events: 23
    - Total: 176
    - Weight: 23
    - Risk Ratio: 1.30 (95% CI: 1.19, 1.42)
    - More LNM with V = More LNM with LVI
  - Yamaoka 2004
    - Events: 13
    - Total: 66
    - Weight: 13
    - Risk Ratio: 1.51 (95% CI: 1.35, 1.67)
  - Inoue 2004
    - Events: 23
    - Total: 176
    - Weight: 23
    - Risk Ratio: 1.31 (95% CI: 1.19, 1.44)
    - Test for overall effect: Z = 2.75 (p = 0.006)

- **Total events**: 73
- **Total risk ratio**: 1.33 (95% CI: 1.24, 1.43)

**Supplemental content 3**
Chapter 2

Predicting LNM in pT1 colorectal cancer

### Supplemental content 5

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**Forest plot depicting the relation between risk of nodal involvement and histological differentiation grade (low grade vs. High grade adenocarcinoma)**

<table>
<thead>
<tr>
<th>LNM: lymph node metastasis</th>
<th>Study or Subgroup</th>
<th>High grade</th>
<th>Low grade</th>
<th>Risk Ratio</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Weight</td>
<td>M-H, Random, 95% CI</td>
<td>Events</td>
</tr>
<tr>
<td>Wang 2005</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>152</td>
<td>13.6 (6.6, 25.9)</td>
</tr>
<tr>
<td>Masai 2008</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>72</td>
<td>6.0 (0.95, 38.82)</td>
</tr>
<tr>
<td>Siukhu 2003</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>63</td>
<td>5.61 (2.55, 12.18)</td>
</tr>
<tr>
<td>Ueno 2004</td>
<td>23</td>
<td>70</td>
<td>10</td>
<td>176</td>
<td>9.6 (5.92, 15.48)</td>
</tr>
<tr>
<td>Okada 2004</td>
<td>5</td>
<td>12</td>
<td>26</td>
<td>416</td>
<td>4.56 (1.56, 12.81)</td>
</tr>
<tr>
<td>Yamamoto 2004</td>
<td>1</td>
<td>4</td>
<td>18</td>
<td>207</td>
<td>4.53 (0.71, 32.87)</td>
</tr>
<tr>
<td>Tsuda 1989</td>
<td>4</td>
<td>9</td>
<td>6</td>
<td>60</td>
<td>3.93 (0.32, 0.44)</td>
</tr>
<tr>
<td>Kihara 1997</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>83</td>
<td>3.46 (0.54, 22.31)</td>
</tr>
<tr>
<td>Kim 2004</td>
<td>2</td>
<td>6</td>
<td>65</td>
<td>666</td>
<td>3.37 (0.67, 16.83)</td>
</tr>
<tr>
<td>Takeuchi 2008</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>51</td>
<td>2.83 (0.40, 16.88)</td>
</tr>
<tr>
<td>Ishikawa 2009</td>
<td>10</td>
<td>136</td>
<td>0</td>
<td>0</td>
<td>2.68 (0.17, 39.44)</td>
</tr>
<tr>
<td>Eppstein 2004</td>
<td>0</td>
<td>2</td>
<td>11</td>
<td>120</td>
<td>1.80 (0.14, 25.11)</td>
</tr>
<tr>
<td>Endo 2008</td>
<td>2</td>
<td>15</td>
<td>23</td>
<td>250</td>
<td>1.19 (0.01, 12.83)</td>
</tr>
<tr>
<td>Total (65%)</td>
<td>369</td>
<td></td>
<td>2578</td>
<td>100.0%</td>
<td>4.70 (3.33, 6.67)</td>
</tr>
<tr>
<td>Total events</td>
<td>60</td>
<td></td>
<td>129</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: $Q$ = 0.10, $I^2 = 16.48$, $df = 12$ ($P = 0.10$)</td>
<td>$P = 25%$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $Z = 2.46$ ($P = 0.00001$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Forest plot depicting the relation between risk of nodal involvement and width of submucosal invasion (cut-off 6mm)**

<table>
<thead>
<tr>
<th>LNM: lymph node metastasis</th>
<th>Study or Subgroup</th>
<th>Width $&lt; 6$ mm</th>
<th>Width $\geq 6$ mm</th>
<th>Risk Ratio</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Weight</td>
<td>M-H, Random, 95% CI</td>
<td>Events</td>
</tr>
<tr>
<td>Jia 2006</td>
<td>11</td>
<td>37</td>
<td>0</td>
<td>28</td>
<td>5.7 (1.83, 9.61)</td>
</tr>
<tr>
<td>Ikeda 2004</td>
<td>27</td>
<td>101</td>
<td>0</td>
<td>100</td>
<td>4.50 (0.28, 7.90)</td>
</tr>
<tr>
<td>Oike 2004</td>
<td>21</td>
<td>710</td>
<td>0</td>
<td>95</td>
<td>4.70 (0.87, 2.62)</td>
</tr>
<tr>
<td>Total (65%)</td>
<td>474</td>
<td></td>
<td>2134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>69</td>
<td></td>
<td>131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: $Q$ = 0.07, $I^2 = 2.48$, $df = 2$ ($P = 0.50$), $P = 17%$</td>
<td>$P = 25%$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $Z = 2.93$ ($P = 0.004$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Forest plot depicting the relation between risk of nodal involvement and poor differentiation at the invasive front**

<table>
<thead>
<tr>
<th>LNM: lymph node metastasis</th>
<th>Poor diff. at inv. front</th>
<th>No poor diff. at inv. front</th>
<th>Risk Ratio</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Weight</td>
<td>M-H, Random, 95% CI</td>
</tr>
<tr>
<td>Jia 2006</td>
<td>11</td>
<td>53</td>
<td>7</td>
<td>65</td>
</tr>
<tr>
<td>Ikeda 2004</td>
<td>21</td>
<td>104</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>Total (65%)</td>
<td>40</td>
<td></td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>103</td>
<td></td>
<td>505</td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: $Q$ = 0.88, $I^2 = 0.33$, $df = 3$ ($P = 1.03$), $P = 6%$</td>
<td>$P = 25%$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $Z = 2.06$ ($P = 0.00001$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Supplemental content 4**
### Supplemental Content 8 Multivariable analyses (review of the literature)

Studies that reported results of a multivariable analysis are included here. Factors with independent prognostic value are marked with an X. Included factors that were non-significant are marked with an O.  
1 sm-levels; 2 cut-off 1mm; 3 cut-off 2mm; 4 cut-off 3mm; 5 cut-off 0.4mm; 6 cut-off unknown; * distal 1/3 of rectum vs. rest of rectum and colon.

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Clinicopathological characteristics predict lymph node metastases in ypT0-2 rectal cancer after chemoradiotherapy


Histopathology. 2016 Nov;69(5):839-848
Abstract

Background: Changes in rectal cancer treatment include increasing emphasis on organ preservation. Local excision after chemoradiotherapy (CRT) for rectal cancer with excellent clinical response reduces morbidity and mortality compared to total mesorectal excision, although residual lymph node metastases (LNM) may cause local recurrence. Our aim is to identify clinicopathological factors predicting the presence of residual LNM in rectal cancer patients with ypT0-2 tumours after neo-adjuvant CRT. These risk factors may help select patients who can be spared radical surgery without compromising oncological outcomes.

Methods: Rectal cancer patients with ypT0-2 tumours after CRT and radical resection from five centres treated between June 1999 and February 2012 were included. Histopathology was extensively reviewed. Clinicopathological characteristics and their association with residual LNM were investigated.

Results: Out of 657 consecutive CRT treated rectal cancer patients 210 with ypT0-2 disease were included. Residual nodal disease was found in 44 cases (21.0%). Independent predictors of LNM were clinical nodal involvement (cN+) (OR 2.79 [95% CI 1.04-7.48], p=0.042), “high grade” histopathology assessed in the post-CRT resection specimen (OR 6.46 [95% CI 1.23-34.02], p=0.028), and residual tumour diameter ≥10mm (OR 2.54 [95% CI 1.06-6.09], p=0.036). An algorithm combining these factors adequately stratified patients according to LNM risk, independently of ypT category.

Conclusions: Clinical nodal involvement, “high grade” histopathology, and residual tumour diameter ≥10mm are strong and independent predictors of residual nodal disease in rectal cancer patients with ypT0-2 tumours after CRT. Risk stratification based on these factors may help identify patients suitable for organ preserving therapy and should be validated in appropriately selected populations.

Introduction

For locally advanced rectal cancer patients, neo-adjuvant chemoradiotherapy (CRT) consisting of long course radiotherapy and concurrent chemotherapy followed by total mesorectal excision (TME) is the standard of care. In 8-24% of patients, neo-adjuvant CRT results in a pathological complete response (pCR). These patients have been reported to have an excellent prognosis with a five-year local recurrence rate of 0-2.8% and 83.3-96.9% five-year disease free survival. Radical resection may therefore be superfluous in selected patients with a good clinical response and postoperative morbidity and mortality associated with TME could be avoided. Indeed, local recurrence rates as low as 2.8% and 4.7% have been reported after a “wait-and-see” policy for selected patients with a clinical complete response after CRT. Nevertheless, several other studies showed worse outcomes (local failure rates of 23-60%). Critics of “wait-and-see” point out that criteria for clinical complete response cited in the literature are not consistent and the evidence is based on highly selected patient groups. A full thickness local excision of the residual tumour or scar area is an attractive alternative to “wait-and-see”, since it removes possible tumour remnants in the bowel wall. It also provides additional information on tumour response and can be used to identify histopathological risk factors for locally recurrent disease. Especially for early tumours in the distal part of the rectum, this strategy has been successfully used in several small international studies. However, identification of patients who are most likely to benefit from an organ preserving procedure remains difficult. A validated set of histopathological risk factors could help to stratify patients according to local recurrence risk. Unfortunately, studies on local excision after CRT are relatively scarce and often lack sufficient numbers of patients to perform a risk stratification based on histopathological factors.

An alternative approach is to investigate tumour characteristics associated with residual lymph node metastases (LNM) in the mesorectal fat of CRT treated radical TME specimens. Residual LNM are a potential source of recurrent disease after local excision and predictors of LNM in radical resection specimens are therefore likely to overlap with predictors of local recurrence after local excision. In addition, patients with ypT0-2 rectal cancers after CRT may be cured with a full thickness local excision in the absence of LNM. We therefore investigated possible predictors of LNM in this group of patients in a multicenter study with central review of histopathology.
Materials and methods

Patients and study design

This report describes a pooled analysis of consecutive rectal cancer patients from five independent centres with ypT0-2 tumours who received neo-adjuvant CRT followed by TME surgery between June 1999 and February 2012. Patients considered for CRT either had evidence of locally advanced disease on pre-operative MRI (defined as a cT4 tumour, a cT3 tumour with threatened mesorectal fascia, a cT3 tumour less than 5cm from the anal verge, and/or clinical N2 disease), or were otherwise expected to benefit from CRT during a multidisciplinary team meeting (e.g. attempt to preserve the sphincter in case of a very low T2 tumour). Patients received external beam long course radiotherapy consisting of 45-50Gy in 25-28 fractions of 1.8-2.0Gy and concomitant fluoropyrimidine based chemotherapy (with or without oxaliplatin). The clinical target volume included the primary tumour and the mesorectum with vascular supply, containing the perirectal, presacral and internal iliac nodes. For this purely retrospective study ethics approval and informed consent were not required.

Histopathology

Routine histopathological evaluation of the resection specimens was performed in the laboratories of the participating hospitals according to international guidelines. For the study, haematoxylin and eosin (H&E) stained glass slides or high resolution digitally scanned slides as well as histopathology reports were retrieved and centrally reviewed by a single investigator (SLB). Difficulties and discrepancies with the original histopathology report were resolved by consulting an expert gastro-intestinal pathologist (IDN).

Cases were excluded if a tumour was determined to be >ypT2 at review or the histopathological slides (glass or digital) were unavailable. The pathological tumour category (ypT) and pathological nodal category (ypN) were evaluated according to the 5th edition of TNM \(^{15}\) classifying mesorectal tumour deposits of ≥3mm without evidence of residual lymph node tissue, as positive lymph nodes regardless of their contour. Lymph nodes with fibrosis or acellular mucin lakes, but without viable tumour cells were considered to be negative for tumour. In addition to ypT category the evaluated tumour related characteristics included residual tumour diameter (RTD), histopathological type and differentation grade, tumour regression grade (TRG), extent of tumour necrosis, and presence of intratumoral venous and lymphatic invasion, perineural growth, budding, intramural acellular mucinous lakes, calcifications, and peritumoural inflammatory infiltrate. RTD was defined to be the largest distance between viable tumour cells in the mucosa, submucosa or muscularis propria. In case of tumour regression with fibrosis and scattered residual tumour cells and glands, this was the largest distance between individual tumour cells in the slide. In case tumour cells were present in two slides, RTD was estimated to be at least 4mm, since a block of paraffin embedded tissue was estimated to be 4mm thick. This was at least 8mm in case of tumour in three slides etc. However, due to the retrospective nature of the study it was not possible in every case to reliably reproduce the position of the various tissue blocks and associated slides relative to each other.

Histopathological type and differentiation grade of the tumour were assessed in the post-CRT resection specimen and defined according to WHO 2010 criteria.\(^{16}\) For analytical purposes the cases were subsequently categorized as having “high grade” histopathology (including poorly differentiated adenocarcinoma, undifferentiated carcinoma and signet ring cell carcinoma) vs. “other” histopathology (including low grade adenocarcinoma, mucinous carcinoma, and pathological complete response). For TRG, a four-tier grading scale adjusted from Dworak’s system\(^{17}\) was used. Grades are defined as follows: grade 1 (no significant response) “no fibrosis or significant fibrosis outgrown by cancer”; grade 2 (partial response) “residual cancer outgrown by fibrosis”; grade 3 (near complete response) “scattered single tumour cells or small groups of tumour cells”; grade 4 (pathological complete response) “no viable tumour cells”.

Lymphatic invasion was defined as tumour cells in a space covered with endothelial cells in the absence of endothelial cells.\(^{19}\) Venous invasion was diagnosed in case of tumour within a smooth muscle-lined space or in an endothelial-lined space with additional fibrin clots, erythrocytes or both, without erythrocyte extravasation into the surrounding tissue.\(^{18}\) Budding was defined as “presence of at least five foci of up to five tumour cells in a microscopic field using a 20x objective and evaluated in the area where such foci are most dense” as described by Ueno et al.\(^{20}\) Grade of tumour necrosis was evaluated according to Pollheimer et al.\(^{21}\) Acellular mucinous lakes were determined to be present or absent in the specimen regardless of tumour cells in the surrounding tissue. Peritumoural inflammatory infiltrate was determined to be conspicuous or non-conspicuous as originally described in the Jass classification.\(^{22}\)

Statistical analysis

SPSS version 20 was used to perform the analyses. For RTD a receiver operating characteristic curve (ROC) was created to estimate the cut-off value with optimal sensitivity and specificity for predicting presence of LNM. Mann-Whitney U test or independent samples Kruskal-Wallis test was used for non-parametrical continuous variables. Categorical variables were analyzed using the \(\chi^2\) test, Mann-Whitney U test or independent samples Kruskal-Wallis test where appropriate. Factors with a statistically significant association with LNM or a statistical trend were subsequently included in a multivariate analysis using binary logistic regression. A p-value of \(<0.05\)
was considered statistically significant whereas a p-value of <0.1 was taken to reflect a trend towards significance.

Results

Patient selection
Out of 657 consecutive rectal cancer patients from five centres, who received long course CRT and TME, 211 (32.1%) were found to have ypT0-2 disease. One patient was excluded for lack of the histopathological slides, resulting in 210 patients who were included in the analysis.

Lymph nodes
Median number of examined lymph nodes per patient was 7 (range 0-39). Residual nodal disease was found in 44 patients (21.0%). Presence of LNM was not related to number of lymph nodes sampled in the current population (median number of examined lymph nodes: 6.5 vs. 7.0 respectively in patients with vs. without residual LNM (p=0.439). Of the patients without LNM there were 34 who showed signs of tumour regression in lymph nodes including acellular mucin in 7 cases.

Clinical characteristics
Table 1 shows clinical characteristics and their association with presence of LNM. Centre of origin, gender, clinical nodal status (cN), and type of chemotherapy (fluoropyrimidine only vs. capecitabine + oxaliplatin) were significantly associated with presence of LNM.

Histopathological tumour characteristics
Changes in classification of histopathological characteristics compared with the original pathology reports were made in 18 cases after slide review (8.6%). This included either a T-category downgrade (n=8), T-category upgrade (n=6), N-category downgrade (n=1), or N-category upgrade (n=3). Tumour type was not changed. Other factors investigated in this study (e.g. tumour differentiation grade, lymphatic invasion, tumour regression grade, budding etc.) were not consistently described in the original reports and were therefore primarily scored at the time of slide review.

Table 2 shows the investigated histopathological characteristics and the associated LNM rate. The ypT category did not significantly predict residual nodal disease (LNM rate 17.4%, 14.8% and 25.8% for ypT0, ypT1, and ypT2 respectively; p=0.159; and LNM rate 16.8% vs. 25.8% for ypT0-1 vs. ypT2 respectively; p=0.112). RTD had a strong association with presence of LNM. Initial analysis of histopathological characteristics revealed that mean RTD was significantly higher in the ypN+.

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FP: fluoropyrimidine; CAPOX: capecitabine + oxaliplatin; cT: clinical primary tumour category; cN: clinical nodal category; ypT: pathological primary tumour category after multimodality therapy; ypN: pathological nodal category after multimodality therapy
* Chi-square test is used unless stated otherwise. ^ Mann-Whitney U test. # Median and interquartile range. ‡ Capecitabine (n=139) or bolus 5FU + leucovorin (n=21; centre 1 and 4).
compared to the ypN0 group (11.2mm and 6.0mm respectively, p=0.022). A ROC curve showed a RTD of $\geq 10$mm to be the optimal cut-off value to predict LNM (sensitivity 43.2%; specificity 81.9%; AUC 0.598). Therefore, this value was used in the subsequent analyses which showed LNM in 16.0% vs. 38.3% for RTD $<10$mm and $\geq 10$mm respectively (p=0.001).

Out of 24 patients with a near complete response (TRG 3) there were 3 with a RTD of $\geq 10$mm and 1 of those showed residual nodal disease.

"High grade" histopathology (assessed after neo-adjuvant therapy) was found in 8 patients including 6 with poorly differentiated adenocarcinoma and 2 with undifferentiated carcinoma. There were no cases with signet ring cell carcinoma. The majority
of patients had “other” histopathology (n=202) including low grade adenocarcinoma (n=103), mucinous carcinoma (n=13) and pathological complete response (n=86). “High grade” histopathology was a statistically significant predictor for the presence of LNM (LNM rate 62.5% vs. 19.3% for “high grade” vs. “other” histopathology respectively (p=0.003)).

Routine histopathology details
The median number of tissue blocks available for re-evaluation per case was 15 (range 5-52) and the median number of blocks from the tumour area was 6 (range 2-43). The proportion of cases in which the entire tumour area was embedded could not be reliably determined retrospectively, since this was not consistently described in the original reports. Additional tumour area blocks were embedded in 23 cases (11.0%) that lacked residual viable tumour in the initial slides, and this included 14 patients with ypT0 (16.3%). Three additional levels from the tumour blocks were cut in 5 cases (2.4%), including 2 cases with ypT0 (2.3%). Immunohistochemistry with cytokeratins was performed in 12 cases (5.7%) including 5 patients (5.8%) with ypT0.

Multivariate analysis
Factors with a statistically significant association with residual nodal disease or a statistical trend were included in a multivariate analysis (table 3). Independent predictive value was shown for clinical nodal involvement (OR 2.79, 95%CI 1.04-7.48 for cN+ vs. cN0; p=0.042), residual tumour diameter ≥10mm (OR 2.54, 95%CI 1.06-6.09 for RTD ≥10mm vs. <10mm; p=0.036), and “high grade” histopathology (OR 6.46, 95% CI 1.23-34.02 for “high grade” vs. “other” histopathology; p=0.028). Centre of origin, gender, and type of chemotherapy did not show an independent association with ypN category.

Combining independent risk factors
The independent risk factors identified in the multivariate analysis were subsequently combined to investigate their potential for risk stratification in the current study population (table 4). Patients without clinically detectable LNM (cN0) and with “other” histopathology had the lowest LNM risk (7.7%), whereas patients with “high grade” histopathology had a high risk regardless of clinical nodal status and RTD. RTD was of additional value for stratification of patients who had both clinical nodal involvement (cN+) and “other” histopathology (17.8% vs. 47.8% for RTD <10mm and ≥10mm respectively; p=0.002).

Based on these data we devised an algorithm which stratifies patients in three subgroups (low, intermediate, and high risk) according to risk of residual LNM (figure 1). LNM risk was 7.7%, 17.8%, and 51.6% for the low, intermediate, and high risk categories respectively (p<0.001; figure 2).

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### Table 3 Multivariate analysis

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<tr>
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</table>
| cN: clinical nodal category; RTD: residual tumour diameter; FP: fluoropyrimidine; CAPOX: capecitabine + oxaliplatin. *statistically significant (p<0.05)
Role of ypT category

The ypT category did not reach statistical significance or a statistical trend in this study. In a subgroup analysis of patients with a ypT2 tumour (n=88), the algorithm described in the previous paragraph was able to adequately stratify patients according to LNM risk (7.7%, 14.7%, and 50.0% for patients in the low, intermediate and high risk categories respectively; p<0.001). For patients with a ypT0-1 tumour this was 7.7%, 19.4%, and 66.7% (p=0.024). Patients with a pathological complete response of the primary tumour (ypT0) had residual nodal disease in 10.3% and 20.8% of cases depending on clinical nodal status (cN0 and cN+ respectively; p=0.231).

Table 4 Independent risk factors and lymph node metastases rate (N=197 ^)

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<td>&quot;High grade&quot; histopathology</td>
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<tr>
<td>Total</td>
<td>7.7% (5/65)</td>
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</table>

Figures represent patients with LNM in each subgroup: % (N/Ntotal).
^ Cases with at least one missing value (n=13) were excluded, * p=0.002 for RTD <10mm vs. RTD ≥10mm, # p=0.083 for RTD <10mm vs. RTD ≥10mm.

Figure 1 Flow chart depicting algorithm for risk stratification
LNM: lymph node metastases; cN: clinical nodal category

Figure 2 Risk of residual LNM based on the flow chart algorithm* (n=197 ^)
* Risk factors are: clinical nodal involvement (cN+), residual tumour diameter ≥10mm, and "high grade" histopathology (including poorly differentiated and undifferentiated carcinoma).
^ Cases with at least one missing value (n=13) were excluded.

Figure 1 Flow chart depicting algorithm for risk stratification
LNM: lymph node metastases; cN: clinical nodal category
Discussion

In this study including 210 TME specimens of consecutive rectal cancer patients with ypT0-2 tumours after CRT, we showed that clinical nodal involvement (cN+), “high grade” histopathology (i.e., poorly differentiated or undifferentiated carcinoma), and residual tumour diameter (RTD) of ≥10mm are strong independent risk factors for residual LNM. We devised an algorithm based on these risk factors, which adequately stratifies patients according to risk of residual nodal disease in the current population. Moreover, we showed that the predictive value of this algorithm was independent of pathological tumour category after neo-adjuvant treatment (ypT).

Clinically suspected nodal disease was the strongest independent risk factor for residual LNM at histopathological examination. Residual LNM risk in cN+ patients was 24.6%, which explains why clinical trials investigating feasibility of local excision after CRT generally exclude patients with clinical evidence of nodal involvement. However, LNM rate was 10.4% in the cN0 group showing that clinical imaging is relatively inaccurate for the prediction of nodal disease. RTD was useful only in cN+ patients. RTD can be regarded as a footprint of the original tumour which reflects its level of therapy resistance, similar to tumour regression grade. TRG correlates with the therapy resistance of associated LNM, with similar levels of regression in both the primary tumour and the lymph nodes. The predictive value of RTD is most likely based on the same principle. In case of a local excision the advantage of RTD over TRG is that it is based on the amount of microscopically detectable residual tumour in the specimen, whereas for TRG, an estimate of the amount of “tumour mass turned fibrosis” is essential. Estimates of TRG are therefore not feasible after local excision, since an important part of the fibrotic areas are located in the mesorectal fat and therefore missing in the specimen.

“High grade” histopathology was a strong and independent risk factor associated with a 62.5% risk of LNM, although it was found in relatively few cases in the current population. This result is in accordance with previous series on early colorectal cancer. Differentiation grade was determined in the CRT treated resection specimens, since pre-therapy biopsies are notoriously unreliable for grading purposes with substantial variation between grade of differentiation determined on biopsy and after definitive surgery, probably due to sampling error. Indeed, WHO criteria define type and grade according to the relative dominance of specific tumour components (e.g., more or less than 50% gland formation; more or less than 50% mucin production); and a superficial biopsy may miss a relevant component entirely. On the other hand, CRT may induce significant morphological changes including disappearance of tumour tissue with fibrosis and mucinous degeneration. This may change the proportion of various tumour components and may yield a different grade than would have been the case without neo-adjuvant treatment. However, since both the primary tumour and LNM have been reported to undergo similar levels of regression with loss of the most susceptible tumour components it may be hypothesized that the post-CRT morphology is likely to reflect the risk of residual LNM most adequately. The relatively low number of examined lymph nodes is a limitation to this study, since a minimum of 12 nodes is generally recommended for adequate nodal staging. However, lymph node yield is known to decrease after chemoradiation and the median number of 7 nodes found in this study is comparable with results described in several previous reports after neo-adjuvant therapy. Lymph node yield was not associated with nodal positivity in the current population. However, this may be related to a lack of statistical power to detect a correlation, since previous studies found LNM rate to increase with number of examined lymph nodes. Furthermore, the multicenter design of this study implies some inherent variations between centres in distribution of patient and treatment characteristics, such as gender, clinical stage, and type of chemotherapy. However, the included rectal cancer patients constitute an adequate reflection of the case-mix encountered in clinical practice, and results may therefore be widely applicable. Moreover, the multivariate analysis showed the identified risk factors to be independent of centre. However, the current results cannot be extrapolated directly to a local excision setting. For example, pathological tumour category may be underestimated in local excision specimens due to the often discontinuous nature of residual tumour foci after neo-adjuvant CRT, since some residual tumour cells may remain undetected in the mesorectal fibrosis. Furthermore, our study is based on a relatively unfavourable population including many patients with unfavourable clinical characteristics such as T4 tumours or clinical N2 disease, and many of them would in practice never be considered for rectal preservation. Therefore, our results are hypothesis generating, and the identified risk factors, as well as their association with local recurrence risk, should be investigated and validated in appropriately selected populations.

In summary, this study shows that clinical nodal involvement, “high grade” histopathology, and residual tumour diameter are strong and independent predictors for the presence of residual nodal disease in rectal cancer patients with ypT0-2 tumours after neo-adjuvant CRT. An algorithm combining these risk factors to stratify patients according to low, intermediate, or high LNM risk was shown to be accurate, regardless of ypT category. If validated in appropriately selected populations these factors may contribute to an effective stratification of patients according to risk of LNM and local recurrence. This may improve decision making regarding local or radical surgery, and may help save selected patients from undergoing an unnecessary, yet potentially harmful TME, while ensuring oncological safety.
Acknowledgements
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References
Chapter 3 Predicting LNM after CRT in ypT0-2 rectal cancer

Tumour regression grading after chemoradiotherapy for locally advanced rectal cancer: a near pathologic complete response does not translate into good clinical outcome

H.A.M. Swellengrebel, S.L. Bosch, A. Cats, A.D. Vincent, L.G.H. Dewit, V.J. Verwaal, I.D. Nagtegaal, C.A.M. Marijnen

Radiother Oncol. 2014 Jul;112(1):44-51
Abstract

Background: After preoperative chemoradiotherapy (CRT) for rectal cancer, clinically undetectable residual tumour deposits or pathologic lymph nodes may remain in the mesorectum.

Aim: The aim of this study was to report histopathological effects of CRT and factors affecting outcome in a uniformly treated series of locally advanced rectal cancer (LARC) patients.

Methods: Between 2004-2008, 107 patients with cT3 (threatening the mesorectal fascia or <5 cm from the anal verge), cT4 or cN2 rectal cancer were treated with preoperative CRT (25x2 Gy with capecitabine) and TME 6-8 weeks later. Central histopathological review followed. Tumour regression grade (TRG) was scored in pCR, near-pCR, response and no response. Cox regression was performed to identify prognosticators.

Results: The 3-year distant metastasis-free interval, disease-free rate and overall survival rate were 82%, 73% and 87% (median 44 months follow-up). TRG consisted of 20% pCR, 11% near-pCR, 55% response and 14% no response. 6/21 pCR patients harboured nodal metastases. 5/12 near-pCR had ypT3 disease, while 6 harboured node metastases. 5/12 near-PCR patients developed distant metastases. ypN and TRG were powerful outcome discriminators.

Conclusion: The high number of near-pCR with ypT3 or ypN1/2 and their poor outcome demonstrates that “watch-and-wait” in LARC patients should be applied with care.

Introduction

The increasing use of neoadjuvant chemoradiotherapy in rectal cancer provides new challenges for pathologists. Tumours appear to respond heterogeneously to neoadjuvant therapy; mechanisms governing response or resistance remain unclear. The histopathological regression of tumour to the CRT is assessed by a semi-quantitative scoring of the relative proportion of residual tumour to stromal fibrosis, the tumour regression grade (TRG). It is conceivable that after CRT a highly responsive tumour is associated with superior treatment outcome. Published series demonstrate excellent outcome in those 8-24% of the cases in which no viable primary tumour cells are found in the resection specimen after CRT (pathologic complete response or pCR). The results after more intensive combination-regimens, however, have been disappointing, with more adverse effects. Therefore, radiotherapy to a dose of 45-50 Gy with concurrent daily capecitabine, an oral prodrug of 5-FU, seems to have become the standard of care.

The concept of a pCR after CRT questions the need for additional resection. The group of Habr- Gama et al. have published multiple series on a non-operative “watch and wait” policy in those patients in whom no residual tumour is detected at clinical assessment after CRT. Meticulous clinical, endoscopic and radiological follow up was implemented to guarantee a sustained clinical complete response (cCR). Using this policy, a locoregional failure rate of only 3% was reported in a series of distal rectal cancer patients (20% cT2, 70% cT3, 11% cT4 and 23% cN+), which is comparable to those with a pCR after resection. These excellent results have been confirmed in another series. Other groups have observed significant clinical complete response rates in T2-T3 tumours treated with high dose rate endorectal brachytherapy followed by external beam radiotherapy indicating a possible role of radiotherapy alone in a subset of patients in the future. However, data are scarce regarding those in which the cCR is not sustained, and in which salvage resection is required due to loco-regional failure. The risks of a non-operative (or local) treatment include under-treatment or treatment delay in those with residual lymph node metastases and those with undetectable tumour deposits in the mesorectum.

Differences in patient selection, indications for the treatment, definition of locally advanced rectal cancer (LARC), preoperative regimens, but also lack of standardized TME and pathology make comparisons between different series difficult. The aim of this study was to report histopathological effects of CRT and factors affecting outcome in a uniformly treated series of MRI-defined LARC patients.
Patients and Methods

Patients
In the period between June 2004 to February 2008 a total of 147 consecutive patients with LARC, defined as a cT4 tumour, a cT3 tumour <5 cm from the anal verge or threatening the mesorectal fascia (MRF) on magnetic resonance imaging (MRI) or cN2 disease, underwent preoperative CRT in the Netherlands Cancer Institute. MRI was used to evaluate tumour infiltration and the presence of lymph nodes larger than 1 cm or with clinical characteristics suspicious of metastases. A CT-scan of the abdomen and X-ray or CT-scan of the thorax were used to evaluate dissemination.

Preoperative chemoradiotherapy
Preoperative radiotherapy consisted of 50 Gy in 25 fractions on weekdays. The clinical target volume included the primary tumour and the mesorectum with vascular supply, containing the perirectal, presacral and internal iliac nodes. The recommended upper field border was at the level of the promontory. The perineum was included if an abdominoperineal resection (APR) was planned, whereas the lower border was 3 cm above the anal verge if the planned operation was low anterior resection (LAR). From April 2006 onwards, intensity-modulated radiotherapy (IMRT) substituted the three-field, three-dimensional conformal technique. Capecitabine was administered orally and twice daily at a dose of 825 mg/m², starting on the first day and ending on the last day of radiotherapy, including weekends. The mean cumulative dose of capecitabine was 95 % (range 32–100) of the prescribed dose, while 98% of patients received at least 45 Gy.

Surgery
Surgical resection according to the principles of TME followed 6-8 weeks later in the Netherlands Cancer Institute or in one of ten regional hospitals. Exenterative surgery was performed for infiltration into surrounding organs or structures. Preoperative clinical assessment of response with endoscopy or imaging was not standard treatment. Of 147 patients receiving neo-adjuvant CRT, 138 were considered fit for surgery and underwent laparotomy after completion of neo-adjuvant therapy. Of these, 131 were considered resectable intra-operatively. A further 19 patients were excluded because of synchronous distant metastases while in 5 patients pathology slides were not available prohibiting pathological review. Thus, 107 patients were included for the analysis. Adjuvant chemotherapy is not standard of care in the Netherlands; four patients received adjuvant chemotherapy as part of a prospective trial.

Histopathological analysis
Routine macroscopic and microscopic examination of the resection specimens was performed in the pathology laboratories of the participating hospitals according to the principles proposed by Quirke11. Overall, a median of 14 blocks per patient were examined, while for pCR patients the median was 16 blocks. In 13 patients deeper levels were evaluated to facilitate accurate scoring. All H&E-stained slides of the resection specimens together with the original pathology reports were revised. The specimen was staged according to the 5th TNM staging system12, as is common practice in the Netherlands. Tumour deposits (TD) were defined as tumour nests demonstrating discontinuous growth from the primary tumour, with mesorectal fat or fibrosis separating the TD from the growth front of primary tumour. Furthermore, tumour nests sectioned as possible lymph nodes but with no signs of a lymph node or with a recognizable capsule but without a bordering layer of lymphocytes were considered a tumour deposit. A tumour was considered mucinous when the mucinous proportion was ≥ 50%, and was not graded to further extent. Due to limited numbers venous invasion, lymphangio-invasion and perineural growth were grouped together into one factor, “neuro-vascular invasion”. Since no photos were available for review/scoring of the completeness of the specimen, this information was not explored.

Tumour regression was scored using a simple and practical 4 tier system as illustrated in supplementary figure 1: a) pCR, pathological complete response without residual primary tumour; b) near pCR, only isolated residual tumour cells or small groups of residual tumour cells; c) response: stromal fibrosis outgrowing tumour and; d) no response: no regression or those with stromal fibrosis outgrown by tumour.

Local recurrence, distant metastases and overall survival
Distant metastases were defined as systemic metastases of rectal cancer to another organ, to distant lymph nodes stations or by dissemination to the peritoneal surface. Local recurrence was defined as a radiological or histopathological determination of rectal cancer recurrence in the pelvis. Follow-up information for local recurrence or distant metastasis and overall and disease-free survival was gathered by a comprehensive review of all patients files and contacting the patient’s general practitioner. Distant metastasis-free interval (DMFI) was defined as the time between surgery and distant metastasis or last assessment. Disease-free survival (DFS) was defined as the time between surgery and the first event (local recurrence, distant metastasis, second primary or death) or last assessment. OS was defined as time between surgery and death or last assessment.

Statistical analysis
Associations between pre- or post-treatment factors and tumour regression was assessed using linear by linear or Fisher exact tests, as appropriate. The associations...
between these factors and DMFI, DFS and OS was performed using Cox proportional hazard regression. Survival curves were constructed using the Kaplan-Meier technique. In the multivariable regressions, missing data on pre- or post-treatment factors were imputed using the largest subgroup. The level of significance was set at 0.05 in all analyses.

Results

Patient demographics
The study involved 107 patients, 64 male and 43 female with a median age of 64 years (range 38-82). Baseline characteristics are listed in Table 1. Forty (37%) patients underwent a LAR, 15 (14%) patients underwent a Hartmann procedure, while in 52 (49%) patients an APR was required due to close relation to the sphincter complex. Total exenteration was required in 6 patients, while partial exenterative surgery was performed in 25 patients.

Response to chemoradiotherapy
Table 2 presents associations between tumour regression and other histopathological factors. Downstaging to ypT0-2 occurred in 43 (40%) patients, while lymph node metastases were still present in 40 (37%) patients. Tumour deposits were identified in 28 (26%) patients (median = 1, range = 1-12), with a median size of 7 mm (range 0.5-30 mm). Twenty-one (20%) patients achieved a pathologic complete response (pCR) of the primary tumour and in 12 (11%) patients a near pCR was observed. Seven of the pCR patients and 6 of the near pCR patients initially had a cT4 tumour. Response was seen in an additional 59 (55%) patients, while in 15 (14%) patients the tumour showed no response to CRT. Of note, 6 of 21 (29%) pCR patients had mesorectal lymph nodes metastases.

In the univariate analysis no pre-treatment factors (age, cT, cN, and distance from the anal verge) were significantly associated with tumour regression, while no association was demonstrated between interval (between CRT and surgery) and tumour regression (p=0.82). Regression grade was associated with decreasing invasion depth (ypT, p<0.001) and the absence of neurovascular invasion (p=0.03). A positive CRM occurred more frequently in those showing no regression (p=0.01). Neither TRG nor ypT were associated with pathological node status (p=0.47 and p=0.24, respectively).
### Table 2: Univariable associations between histopathological factors and the 4-tier tumour regression grade (TRG)

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*p value for pCR versus “the rest”  **all mucinous tumours and pCR  #lymphangio-invasion, perineural growth, intra- and extramural venous invasion. **TD > 3 mm
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- **Table 3**: Univariable associations between time-to-event outcomes and histopathological factors. The second p-value denotes tests adjusting for ypN.
Association between response and outcome
After a median follow-up period of 3.7 years (95% CI 3.3-4.2), 87 (81%) patients were alive (follow-up ranging from 1.6-5.8 years), of whom 75 (70%) were free of cancer, 5 (5%) developed a local recurrence only, 19 (18%) developed a distant metastasis only and 4 developed both (2 synchronously, 1 local recurrence first and 1 distant metastasis first). Four patients died due to other causes. The overall 3-year distant metastasis-free interval (DMFI) was 82%, the disease free survival (DFS) rate was 73% and overall survival (OS) rate was 87%. Neither preoperative tumour nor nodal stage influenced outcome (DMFI, DFS or OS). Table 3 displays the associations between histopathological features and outcomes. Due to small numbers (n=9) of local recurrence, no further analysis regarding prognosticators was performed.

The tumour regression grade (TRG) was a significant prognosticator of the DMFI (p=0.002). In addition, ypN (p<0.0001), presence of tumour deposits (p<0.001), ypT (p=0.002), acellular mucin lakes (p=0.007), histological type (p<0.01) and grade of differentiation (p<0.02) were all significantly associated with DMFI. TRG (p<0.02) and ypT (p<0.004) both retained their prognostic value for DMFI after adjusting for ypN. Due to the limited number of patients with distant metastases (n=23), no further multivariable analysis towards independent prognostic factors for DMFI could be performed.

The TRG was a significant prognosticator of the DFS (p=0.001). Post-treatment pT (p=0.02) and ypN (p<0.001), presence of tumour deposits (p=0.004), histological type (p=0.03), grade of differentiation (p=0.03), acellular mucin lakes (p=0.03), and CRM (p=0.02) were also significantly associated with DFS. After adjusting for ypN, TRG (p<0.02) and ypT (p=0.04) retained prognostic value.

Regarding overall survival, TRG was a powerful prognosticator (p<0.001). Histopathological factors predicting OS included ypN (p=0.004), CRM (p=0.04), TD (p=0.01) and histological type (p=0.03), but only TRG retained significance after adjusting for ypN. When patients with residual disease (near pCR versus response versus no response) were analysed separately from the pCR group, TRG retained its prognostic value for DMFI (p<0.02), DFS (p=0.005) and OS (p=0.001). After adjusting for ypN, TRG held a trend in significance (p=0.06) for DMFI, while for DFS (p=0.02) and OS (p=0.003) it retained significance.

Hazard ratios and confidence intervals for Cox analyses are depicted in supplementary tables 1-3.

Excellent outcome in the pCR group
In Figure 1, time to recurrence, second primary or death has been displayed for the separate TRG groups, illustrating that the 21 patients with a pCR have an excellent outcome, with no local recurrences and only one patient developing distant metastases. This patient was one of six patients with a pCR still harbouring lymph node metastases.

Poor prognosis in the near pCR group
A summary of all near pCR patients is presented in Table 4. Three-year DMFI, DFS and OS rates for near pCR patients were 65%, 50% and 67% respectively, which are comparable to those with no response (64%, 60%, 79%). Of the 12 patients with a near pCR, 7 died, of whom 6 with disease progression. Five near pCR patients developed distant metastases. One near pCR patient developed a local recurrence. In 6 (50%) of the 12 near pCR patients, nodal metastases were still present (of which 2 ypN2). In 5 patients isolated tumour cells were found invading the fat (ypT3), while in 2 patients the CRM was positive.
Chapter 4  
Tumour regression grading after CRT for locally advanced rectal cancer

Discussion

In this series of 107 patients with LARC, we confirm the excellent outcome in those with a pathological complete response after CRT and resection. However, the subgroup with a near complete response unexpectedly fared poorly. Furthermore, we identified prognosticators for the development of distant metastases.

In our series, 20% of patients achieved a pCR after CRT. One third of these were clinical T4 tumours. In the literature different definitions for a pathologic complete response have been reported: when reporting a complete response in the context of a TRG, strictly speaking, only the primary tumour is included while others define a pCR as those with no residual disease at all (ypT0Nx). In our study looking at the TRG, patients with a pCR (ypT0Nx) have an excellent outcome with no local recurrence while one patient developed a distant metastasis. This is in line with the literature and raises the question whether more aggressive neoadjuvant strategies should be implemented to increase the pCR rate. Some studies have shown an increase in pCR rate with a longer interval between radiotherapy and surgery. So far, however, it remains unclear whether this translates into an outcome similar to patients with pCR after shorter intervals.

Provided that clinical assessment after CRT is accurate and robust the concept of a pCR has introduced opportunities for less radical surgery, such as local excision of the tumour and even for omission of surgery altogether (the "watch and wait" policy). Avoiding surgical morbidity and subsequent decrease in quality of life as a result of organ resection are obvious advantages of this approach. However, the "watch and wait" policy has only been analysed in a few single centre series, is questioned by

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others\textsuperscript{14-16} and therefore requires further validation. Since the clinical imaging modalities at hand still lack diagnostic accuracy, omitting surgery in patients with undetected (nodal) disease may worsen prognosis. This study confirms these concerns and demonstrates potential risks involved in LARC patients treated with CRT in particular. In line with Gosens et al\textsuperscript{17} studying a similar population of LARC patients, but in contrast to others\textsuperscript{18,19} nodal response after CRT was not related to primary tumour regression in our series, suggesting independent modes of response to CRT. Even in patients with a ypT0, nodal metastases were still present in 6/21 (29\%) patients in our series of strictly defined locally advanced cases and in 5-19\% of patients in the literature\textsuperscript{14,16,17,20,21}. In a recently published series of ypT0-2 patients after CRT, Park et al\textsuperscript{20} demonstrated that 17\% of ypT1 and 21\% of ypT2 patients still harboured nodal disease after TME. The impact on prognosis of not removing these lymph nodes, as is the case with both the “watch and wait” policy and local excision procedures, is as yet unknown.

Another major concern is the effect of microscopic tumour deposits in the mesorectal fat, since those cannot be assessed by re-staging endoscopy and are difficult to discriminate from fibrosis on MRI. A recent publication by Duldulao et al demonstrated that 17 of 53 ypT3-4 patients after CRT revealed tumour cells in deeper layers but not in the mucosa or submucosa\textsuperscript{14}. In a review\textsuperscript{15}, of 208 patients with a cCR approximately 30\% were actually confirmed to be a pCR after resection, indicating the need of more accurate re-staging. Of note, the prognostic importance of these tumour deposits after neoadjuvant therapy is unclear\textsuperscript{22,23} and a topic of on-going discussion. Tumour deposits form part of the pathological T or N stage in the TNM 5th edition, depending on their size, and have been correlated with poor outcome\textsuperscript{22}. Their presence showed to be a firm predictor of distant metastases in our series, possibly indicative of more aggressive tumour biology.

Chapter 4 Tumour regression grading after CRT for locally advanced rectal cancer

Tumour regression grading has been implemented to predict outcome in many series with conflicting results, possibly because no standard pathologic work up for response evaluation or definitions of TRG subgroups has been established. Univariate associations with LR\textsuperscript{24-27}, DFS\textsuperscript{19,24,28,29} and OS\textsuperscript{24,29} have been reported. Vecchio et al\textsuperscript{18} reported a significant multivariate correlation between TRG and LR, DMFI, DFS and OS, while Guillem et al\textsuperscript{19} reported this for OS and recurrence-free survival and Bouzourene et al\textsuperscript{24} for LR. On the other hand, these results have been contradicted by others for LR\textsuperscript{17}, DFS\textsuperscript{1}, recurrence-free survival\textsuperscript{32} and OS\textsuperscript{17,13,33}, indicating the need for uniform pathological evaluation and definitions for TRG subgroups.

The relatively poor outcome of the near pCR cases in comparison to those responding well in our study seems contradictory to the fact that TRG has prognostic value and is not a universal finding. Others have reported excellent outcomes in near pCR patients\textsuperscript{18,28} or in those with >95\% regression\textsuperscript{30} and even reported outcome comparable to those with a pCR. In our series, 12 patients exhibited a near complete response of the primary tumour with only isolated tumour cells or islands of cells spread throughout the bowel wall and mesorectal fat (Table 4). In contrast to others, these near pCR patients were associated with an unexpectedly poor outcome (DMFI, DFS and OS), which was comparable to those not responding to CRT at all. No single prognosticator could be identified, but half of the near pCR patients harboured nodal metastases while in 5 patients isolated tumour cells were found in the mesorectal fat (ypT3).

In recent series poor outcome is also reported in near pCR: Gosens et al\textsuperscript{17} reported an overall survival of 66\% after a near complete response which was comparable to the poor responders, while Rödel\textsuperscript{18} described a similar trend of decreased disease- and distant metastasis-free survival for their group of good responders (73\%) as compared to their moderate responders (83\%). Another study demonstrated that patients with a pCR are different to those with a near pCR with regard to non-negligible rates of distant metastases\textsuperscript{26}. The prognosis of those with a near pCR is probably multi-factorial and this, once again, underlines the potential risk involved using a “watch and wait” policy.

Few studies focus on distant metastases after CRT, which develop in up to 39\%\textsuperscript{34} of LARC patients and have become the event governing outcome. In our series, distant metastases developed in 21\% of patients indicating the need of a more thorough understanding of factors predicting DMFI. Four studies have investigated the correlation between TRG and distant metastases. Rödel et al\textsuperscript{19} and Gavioli et al\textsuperscript{20} reported a significant univariate association between their TRG and distant metastases, while Buijko et al\textsuperscript{21} found no correlation when excluding the pCR group. Vecchio et al\textsuperscript{18} reported a series of 144 patients with mainly cT3 tumours receiving neoadjuvant therapy (84\% CRT) and observed that the four TRG groups, as used in the present series, significantly predict those at risk for distant metastases. TRG, together with ypT and ypN stage, retained prognostic power in their multivariable analysis. This is in line with our observations: when adjusting for ypN, we observed that both ypT and TRG were still significantly associated with a decreased DMFI, suggesting independent prognostic value of TRG next to nodal status. We also found that TRG is prognostic for those with residual disease (near pCR, response and no response) with regards to DMFI, DFS and OS, which has only been reported for LR in one other series\textsuperscript{26}, but contradicted by others\textsuperscript{25,36}.

Central histopathological review of the resection specimens assured quality of histopathology thereby minimizing inter-observer variability. Apart from shortcomings inherent to retrospective analyses, other shortcomings include the absence of a full model multivariable analysis due to low number of events and that no correction for multiple-testing was performed, thereby categorizing our data as hypothesis generating and in need of further validation.

In conclusion, CRT followed by TME for LARC patients is effective and leads to an acceptable outcome. Histopathological assessment of tumour regression after CRT...
can, amongst other factors, be used to predict the risk for an adverse outcome, including distant metastases. We demonstrate the relevance of tumour deposits and residual lymph node metastases in near pCR patients in particular, and suggest that a “watch and wait” policy should be applied with extreme care.

Acknowledgements
The authors acknowledge the contribution to data collection made by the collaborating specialists from the Kennemer Gasthuis in Haarlem, Red Cross Hospital in Beverwijk, Spaarne Hospital in Hoofddorp, St Antonius Hospital in Nieuwegein, Zaanse Medisch Centrum in Zaandam, TerGooi Hospitals in Hilversum and Blaricum, and the Onze Lieve Vrouw Gasthuis, St Lucas Andreas Hospital, Slotervaart Hospital and the Netherlands Cancer Institute in Amsterdam.

References


22. Nagtegaal ID, Quirke P. Colorectal tumour deposits in the mesorectum and pericolonic, a critical review. Histopathology 2007; 51(2):141-149.


### Supplemental content

#### Supplementary table 1  Hazard Ratios and 95% confidence intervals for univariable Cox regression

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Ref = reference factor level, HR = Hazard ratio (as compared to reference level), L-CI = lower 95% CI, U-CI = upper 95% CI
**Supplementary table 3** Hazard Ratios and 95% confidence intervals for Cox regressions adjusting for pN

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| Ref = reference factor level, HR = Hazard ratio (as compared to reference level), L-CI = lower 95% CI, U-CI = upper 95% CI

**Supplementary figure 1A-D** Microscopic illustration of different grades of tumour regression

(A) Pathological complete response (pCR): only fibrosis. (B) near-pCR: only isolated tumour cells or islands of tumour cells. (C) Response: tumour < fibrosis. (D) No response: tumour > fibrosis
Type of preoperative therapy and stage-specific survival after surgery for rectal cancer: a nationwide population-based cohort study

S.L. Bosch, R.H.A. Verhoeven, V.E.P.P. Lemmens, P. Poortmans, J.H.W. de Wilt, I.D. Nagtegaal

Submitted
Abstract

Aim: Preoperative chemoradiation therapy (CRT) may induce downstaging in rectal cancer (RC). Short-course radiation therapy (SC-RT) with immediate surgery does not cause substantial downstaging. However, the TNM classification adds the "y" prefix in both groups to indicate possible treatment effects. We aim to compare stage-specific survival in these patients.

Methods: RC patients treated with surgery only, preoperative SC-RT followed by surgery within 10 days, or preoperative CRT, and diagnosed between 2008 and 2014, were included in this population-based study. Clinicopathological and outcome characteristics were analyzed.

Results: The study included 11925 patients. Large discrepancies existed between clinical and pathological stage after surgery only. Surgery only patients were older with more comorbidities compared with SC-RT and CRT, and had worse 5-year survival (64%, 76%, and 74% respectively; p<0.001). Five-year survival for stage I was similar after CRT and SC-RT (85% vs. 85%; p=0.167), and comparable between CRT treated patients with stage I and those reaching a pathological complete response (pCR; 85% vs. 89%; p=0.113). CRT was independently associated with worse overall survival compared with SC-RT for stage II (HR 1.57 [95%CI 1.27-1.95]; p<0.001) and stage III (HR 1.43 [95%CI 1.23-1.70]; p<0.001).

Conclusion: Stage I disease after CRT has an excellent prognosis, comparable with pCR and with same-stage SC-RT treated patients without regression. Stage II or III after CRT has worse prognosis than after SC-RT with immediate surgery. TNM should take the impact of preoperative therapy type on stage-specific survival into account. In addition, clinical stage was a poor predictor of pathological stage.

Introduction

The standard of care for rectal cancer (RC) patients is total mesorectal excision (TME) with or without preoperative therapy depending on clinical stage. For patients with locally advanced RC, preoperative treatment consists of chemoradiation therapy (CRT) intended to reduce local recurrence rates and to facilitate radical surgery by inducing tumour regression and possible downstaging. Significant tumour and nodal downstaging is reported in patients treated with CRT and depends on factors such as tumour type, clinical stage and interval between radiation therapy and surgery. Downstaging is associated with an improved prognosis, especially in the 8-24% of patients with a pathological complete response (pCR). The TNM staging system recommends adding the prefix "y" to the TNM stage after preoperative therapy to indicate that a tumour may have undergone treatment induced response or regression.

Especially in Western European countries, patients may also undergo preoperative short-course radiation therapy (SC-RT) followed by immediate surgery. Randomized trials show a small downstaging effect in these patients and according to the definitions of the TNM classification, the "y" prefix should be added. However, downstaging does not occur after SC-RT if the overall treatment time (i.e. interval between start of radiation therapy and rectal resection) does not exceed 10 days. The prognostic significance of ypTNM stage for patients in these groups (with vs. without possible downstaging) is still unclear. Due to the differences in levels of downstaging between groups of patients treated either with preoperative SC-RT followed by immediate surgery or with CRT it may be hypothesized that the prognostic implications of the "y" prefix depend on the type of preoperative therapy received, which limits the prognostic value of staging.

The purpose of this study is therefore to investigate on a population level whether stage-specific overall survival is different between patients treated with either SC-RT followed by surgery within 10 days after start of treatment (no tumour regression expected; ypTNM by definition, but may reflect pTNM), or preoperative long course CRT (intended to induce tumour regression; ypTNM), and to compare results with patients who underwent surgery only (pTNM).
Patients and Methods

Study design and patient selection
A population-based approach was employed using data from the nationwide Netherlands Cancer Registry (NCR). This institute collects data on all newly diagnosed cancer patients in the Netherlands since 1989. The registration is primarily based on notification by the Dutch national digital pathology registry (PALGA). Patient and clinicopathological data are routinely collected from medical records by specially trained data managers. Tumour location and histology is registered according to the ICD-O-3 classification. Follow-up data and vital status are retrieved by linkage to the nationwide population registries network.

Patients with RC diagnosed between January 2008 and December 2014 who underwent a surgical resection were selected from the NCR. Clinicopathological characteristics and overall survival (including TNM stage-specific survival) were compared between patients treated with surgery only, preoperative SC-RT with an overall treatment time that did not exceed 10 days, and preoperative long course CRT. The maximum interval of 10 days between start of SC-RT and surgery was chosen, since tumour regression is not likely to occur within this timeframe. For patients in the CRT group an interval of at least 63 days (duration of CRT + 4 weeks to provide the opportunity for tumour regression) and no longer than 182 days (6 months; arbitrary) was required.

Cases were excluded if the date of surgery was not available, or if there was presence of distant metastases at time of surgery or missing data regarding distant metastases. The same was true for cases with histopathological tumour type other than adenocarcinoma, mucinous carcinoma, or signet ring cell carcinoma. Other exclusion criteria were missing values for pathological T or N categories and surgical procedures other than a low anterior resection (LAR), Hartmann’s procedure, abdominoperineal excision (APE) or intersphincteric resection.

Comorbidity was only registered in the NCR for one specific region in the Netherlands, covering 12% of the population. A subgroup analysis of this data was performed.

Preoperative therapy
The prevailing RC clinical guideline during the inclusion period recommended preoperative SC-RT for primarily resectable RC (with the exception of cT1N0 tumours) consisting of 5x5Gy followed by surgery within one week. Long course CRT consisting of 45-50Gy given in 25 fractions of 1.8-2.0Gy per day with concurrent oral chemotherapy (capecitabine 825-1000mg/m² twice daily) and followed by surgery within 4-6 weeks was indicated for locally advanced RC (i.e. patients with clinical N2 disease, cT4 tumours or tumours with suspected involvement of the mesorectal fascia on imaging). Patients treated with surgery only either had cT1N0 disease or were unfit or not consenting to undergo preoperative treatment.

Statistical analysis
All data was entered in a database and analyzed using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Categorical variables were analyzed using the χ² test. Cumulative overall survival was analyzed using the Kaplan-Meier method with log rank test. Multivariable analyses were performed by entering all applicable clinical and pathological factors in a Cox regression model. A p-value of <0.05 was considered statistically significant whereas a p-value of <0.1 was taken to reflect a trend towards significance.

Results

Patient selection
The initial search of the NCR database identified 19737 patients. Figure 1 depicts the selection process, resulting in the inclusion of a total of 11925 patients (2590 with surgery only, 4534 with SC-RT, and 4801 with CRT).

Clinicopathological factors
Table 1 provides the clinical and pathological characteristics. Median interval between start of SC-RT and surgery was 8 days (range 0-10) and in the CRT group the median interval was 100 days (range 63-182). Patients in the surgery only group were older than those in the SC-RT group, who in turn were older than the CRT treated patients (age >75 years: 40%, 28% and 13% in the surgery only, SC-RT and CRT groups respectively; p<0.001). Other factors that were significantly associated with type of preoperative treatment were cT category, cN category, cTNM stage, type of resection, pT category, pN category, pTNM stage, CRM involvement, histological type, and presence of postoperative chemotherapy. Patients in the CRT group showed ypT0 in 20% of cases and had a reduced rate of (yp)T2 and (yp)T3 tumours compared to the surgery only and SC-RT groups (p<0.001). A pCR (ypT0N0) occurred in 18% of cases after CRT.

A subgroup analysis of cases with available comorbidity data (n=1270) showed a higher rate of comorbidities in the surgery only group compared with both the SC-RT and CRT groups with ≥2 comorbidities in 45%, 35%, and 25% of cases for surgery only, SC-RT, and CRT respectively (p<0.001 for surgery only vs. CRT; p=0.006 for surgery only vs. SC-RT; p=0.003 for SC-RT vs. CRT).

Correlation between cTNM and (yp)TNM
The data showed substantial discrepancies between clinical and pathological TNM stage (table 2). Patients with suspected LN on clinical imaging had histopathology showing nodal disease in 57%, 49% and 35% of cases that were treated with surgery
only, SC-RT, and CRT respectively (p<0.001). Patients with clinical stage I disease had histopathological nodal involvement in 19%, 24%, 13% after surgery only, SC-RT, and CRT respectively (p=0.004). For patients with clinical stage II this was 26%, 30%, and 15% respectively (p<0.001). In the CRT group there was complete tumour regression in 30%, 19%, and 17% of cases for patients with clinical stage I, II, and III disease, respectively (p<0.001).

Survival analysis
Median follow up was 28 months (range 0-84 months) and 1949 deaths were recorded (16.3%). Cumulative 5-year overall survival was 73% (table 3). The surgery only group showed worse overall survival than the SC-RT and CRT groups (64%, 76%, and 74% for surgery only, SC-RT, and CRT respectively; p<0.001). However, there was no significant difference between patients treated with SC-RT vs. CRT (p=0.147). Figure 2a-c shows stage-specific overall survival for patients with surgery only, SC-RT and CRT. Survival was worst in the surgery only group (cumulative 5-year survival: 77%, 63%, and 52% for pathological stages I, II and III respectively; p≤0.003 compared with SC-RT and CRT). SC-RT treated patients with pathological stage I disease had similar overall survival as same-stage patients in the CRT group (cumulative 5-year survival 85% vs. 85% respectively; p=0.167). After CRT overall survival was comparable in patients with pathological stage I and those who reached a pCR (cumulative 5-year survival 85% vs. 89% respectively; p=0.113). The SC-RT group showed better survival than the CRT group for patients with pathological stage II (cumulative 5-year survival 77% vs. 68% for SC-RT vs. CRT respectively; p=0.002) and stage III (cumulative 5-year survival 67% vs. 58% for SC-RT vs. CRT respectively; p<0.001).

The multivariable analysis (table 4) showed that CRT was independently associated with a higher mortality compared with SC-RT in patients with pathological stage II (HR 1.57 [95%CI 1.27-1.95]; p<0.001) and stage III (HR 1.43 [95%CI 1.23-1.70]; p<0.001), but not in those with stage I (HR 0.99 [95%CI 0.77-1.27]; p=0.014). The hazard ratio for patients with surgery only was also increased compared with SC-RT for patients with pathological stage II (HR 1.67 [95%CI 1.35-2.08]; p<0.001) and stage III (HR 1.60 [95%CI 1.36-1.87]; p<0.001), but not stage I (HR 1.25 [95%CI 0.99-1.59]; p=0.137).
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<td>31.5</td>
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<td><strong>CRM involvement</strong></td>
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<tr>
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<td>92.4</td>
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<td>91.6</td>
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<td>8.4</td>
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SC-RT: short-course radiation therapy; CRT: chemoradiation therapy; CRM: circumferential resection margin; CTx: chemotherapy. a SC-RT vs. CRT. b includes 6887 patients with low anterior resection, 1186 patients with Hartmann’s procedure, and 88 patients with intersphincteric resection. c patients with abdominoperineal excision. d Circumferential resection margin (CRM) involvement was defined as tumour distance to the CRM ≤ 1mm.
## Table 2  Correlation between clinical and pathological stage

<table>
<thead>
<tr>
<th></th>
<th>Clinical stage</th>
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<tbody>
<tr>
<td></td>
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<td>Stage II</td>
<td>Stage III</td>
<td></td>
</tr>
<tr>
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<td>Surgery only</td>
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</tr>
<tr>
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<td>135</td>
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<td>Total</td>
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<td>SC-RT</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>1.5</td>
<td>1</td>
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<td>54.1</td>
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</table>

SC-RT: short-course radiation therapy; CRT: chemoradiation therapy. a cases with missing values for clinical TNM stage were excluded (n=2602).

## Table 3  Cumulative 5-year overall survival of included patients

<table>
<thead>
<tr>
<th></th>
<th>Cumulative 5-year overall survival (%)</th>
<th>p-value a</th>
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<tr>
<td>Overall</td>
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<tr>
<td>Preoperative therapy</td>
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<tr>
<td>None (surgery only)</td>
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<td>0.147 †</td>
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<tr>
<td>SC-RT</td>
<td>76.3</td>
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<tr>
<td>CRT</td>
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<td></td>
</tr>
<tr>
<td>Gender</td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>71.3</td>
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<tr>
<td>Female</td>
<td>76.0</td>
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<tr>
<td>Age at diagnosis</td>
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<tr>
<td>0-44</td>
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<tr>
<td>45-59</td>
<td>82.0</td>
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<tr>
<td>75+</td>
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<tr>
<td>Clinical T category</td>
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<td></td>
</tr>
<tr>
<td>cT1</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>cT2</td>
<td>78.7</td>
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<tr>
<td>cT3</td>
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<td>cN1</td>
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<td>78.9</td>
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<tr>
<td>Stage II</td>
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<td>Stage III</td>
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<td>Type of resection</td>
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<td>Sphincter saving</td>
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<td>Non-sphincter saving</td>
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Table 3 Continued

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<th>Pathological stage</th>
<th>Cumulative 5-year overall survival (%)</th>
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<td>Stage III</td>
<td>60.9</td>
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<table>
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<th>Histological type</th>
<th>Cumulative 5-year overall survival (%)</th>
<th>p-value a</th>
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<td>Mucinous carcinoma</td>
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<tr>
<td>Signet ring cell carcinoma</td>
<td>21.9</td>
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<table>
<thead>
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<th>CRM involvement</th>
<th>Cumulative 5-year overall survival (%)</th>
<th>p-value</th>
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<tr>
<td>Absent</td>
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<td>Present</td>
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<table>
<thead>
<tr>
<th>Postoperative CTx</th>
<th>Cumulative 5-year overall survival</th>
<th>p-value</th>
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<tr>
<td>No</td>
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<tr>
<td>Yes</td>
<td>77.4</td>
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SC-RT: short-course radiation therapy; CRT: chemoradiation therapy; CRM: circumferential resection margin; CTx: chemotherapy. a log rank test. † SC-RT vs. CRT.

Figure 2A-C Overall survival for patients treated with surgery only, SC-RT or CRT

(A) pathological stage I. (B) pathological stage II. (C) pathological stage III. SC-RT: short-course radiation therapy; CRT: chemoradiation therapy.
Table 4: Multivariable Cox-regression analysis for overall survival of patients with pathological TNM stages I-III

<table>
<thead>
<tr>
<th>(y)pTNM stage I</th>
<th>(y)pTNM stage II</th>
<th>(y)pTNM stage III</th>
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<td>Preoperative therapy</td>
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<tr>
<td>SC-RT</td>
<td>HR: 1.00</td>
<td>95% CI: 0.99-1.27</td>
</tr>
<tr>
<td>CRT</td>
<td>HR: 1.25</td>
<td>95% CI: 0.99-1.59</td>
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<tr>
<td>Surgery only</td>
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</tr>
</tbody>
</table>

| Gender          |                   |                 |                 |
| Male            | HR: 1.00          | 95% CI: 0.61-0.76 | P-value: 0.146 |
| Female          | HR: 1.00          | 95% CI: 0.78-0.78 | P-value: 0.007  |

| Age at diagnosis |                   |                 |                 |
| 0-44            | HR: 0.81          | 95% CI: 0.24-2.71 | P-value: <0.001 |
| 45-59           | HR: 1.00          | 95% CI: 1.95-4.59 | P-value: <0.001 |
| 60-74           | HR: 2.99          | 95% CI: 1.41-2.7 | P-value: <0.001 |

| Type of resection |                   |                 |                 |
| Sphincter saving  | HR: 1.17          | 95% CI: 0.95-1.46 | P-value: <0.001 |
| Non-sphincter saving |                |                 |                 |

| pT category       |                   |                 |                 |
| pT0              | HR: 1.00          | 95% CI: 1.24-1.24 | P-value: 1.25 |
| pT1              | HR: 0.93          | 95% CI: 1.32-2.42 | P-value: 3.03 |
| pT2              | HR: 0.93          | 95% CI: 1.22-3.00 | P-value: 1.85 |
| pT3              | HR: 0.73          | 95% CI: 0.58-1.35 | P-value: <0.001 |
| pT4              |                |                 |                 |

| pN category       |                   |                 |                 |
| pN1              | HR: 1.00          | 95% CI: 1.88-2.15 | P-value: 1.85 |
| pN2              | HR: 1.00          | 95% CI: 1.00-1.00 | P-value: 1.00 |

| Histological type |                   |                 |                 |
| AC               | HR: 1.00          | 95% CI: 0.75-1.26 | P-value: 1.00 |
| MC               | HR: 0.78          | 95% CI: 0.87-1.52 | P-value: 1.16 |
| SRCC             | HR: 1.12          | 95% CI: 0.82-1.97 | P-value: <0.001 |

| CRM involvement  |                   |                 |                 |
| CRM negative     | HR: 1.00          | 95% CI: 1.00-1.00 | P-value: <0.001 |
| CRM positive     | HR: 1.76          | 95% CI: 1.27-2.56 | P-value: <0.001 |
| Unknown          | HR: 1.03          | 95% CI: 1.32-2.42 | P-value: 1.85 |

| Postoperative CTx |                   |                 |                 |
| No               | HR: 1.00          | 95% CI: 1.00-1.00 | P-value: 1.00 |
| Yes              | HR: 0.60          | 95% CI: 0.30-0.99 | P-value: <0.001 |

p: pathological; SC-RT: short-course radiation therapy; CRT: chemoradiation therapy; AC: adenocarcinoma; MC: mucinous carcinoma; SRCC: signet ring cell carcinoma; CRM: circumferential resection margin; CTx: chemotherapy
Discussion

In this population-based study, using data from the Netherlands Cancer Registry, long-term stage-specific survival data was analyzed from 11925 RC patients who underwent TME surgery with or without preoperative treatment consisting of either SC-RT or CRT. Patients with pathological stage I had an excellent 5-year overall survival after both SC-RT (85%) and CRT (85%). For patients with pathological stages II and III, survival was significantly worse after CRT (68% and 58%) compared with SC-RT (77% and 67%). In addition, clinical staging was a poor predictor of pathological stage based on the large discrepancies between clinical and pathological stage in the surgery only group.

In the study period, the national clinical guideline recommended preoperative SC-RT for primarily resectable RC (with the exception of cT1N0 tumors), and long course CRT for locally advanced RC. Patients treated with surgery only in this period either had cT1N0 disease or were unfit or did not give consent to undergo the indicated preoperative therapy. Indeed, patients in the surgery only group were found to be substantially older and had more comorbidity compared with both the SC-RT and CRT groups. These patients were therefore considered to be unsuitable as a control population in the current study.

On the other hand, the comparison of the SC-RT and CRT treated patients in this study yields some interesting results. Although some small downstaging effect on T-stage as well as nodal downstaging have been reported in randomized trials after SC-RT with immediate surgery,13,14 the SC-RT treated patients in the current study all had an overall treatment time not exceeding 10 days, and evidence from a large randomized controlled trial showed that both tumour and nodal downstaging do not occur in this short time-frame.16 Furthermore, stage-specific 10-year overall survival was shown to be similar in randomized patients with preoperative SC-RT and those with surgery only or surgery with selective postoperative CRT.12,14 This lack of downstaging and absence of a survival difference suggest that the SC-RT treated patients in the current study may be regarded best as pTNM rather than ypTNM.19 The observed difference in stage-specific survival between patients treated with CRT (substantial downstaging) and SC-RT (no downstaging) was substantial and highly significant, and the effect was independent of several known possible confounders. However, selection bias may be a concern when interpreting these results. The inherently higher levels of treatment induced toxicity caused by CRT may motivate clinicians to withhold this treatment in elderly patients with comorbidity, whereas the same comorbidity level would not preclude a treatment with much less toxic SC-RT. Unfortunately, comorbidity data was not available in the majority of patients and the results of the multivariable analysis could therefore not be corrected for this confounder. However, the subset analysis of patients with available comorbidity data showed that comorbidity levels were higher in the SC-RT than in the CRT group. As a consequence the SC-RT group as a whole may be expected to show a bias towards a worse prognosis compared with the CRT group. However, the multivariable analysis showed the direct opposite with a better prognosis in the SC-RT group for patients with stage II and III disease. The observed survival differences may therefore be expected to be even larger if the results could be adjusted for comorbidity.

The introduction of preoperative CRT for RC which has resulted in tumour downstaging in substantial proportion of patients has not resulted in improved survival.20 The stage-specific outcome differences between the groups in this study are therefore not based on a therapeutic effect of the preoperative treatment, but are probably best explained by pathological stage migration. Patients with nodal disease may undergo sterilization of involved LN after CRT resulting in classification as pathological stage I or II disease instead of stage III. These patients may contribute to an increased observed mortality in the stage I or II groups, as they may be expected to have a higher risk of harbouring concurrent occult residual disease or distant metastases than patients who had no LN involvement at presentation. In addition, survival may decrease in pathological stage III as well, since remaining stage III patients have tumours that are resistant to preoperative therapy. This effect, called “the reverse of the Will Rogers phenomenon”, has been described before with data from the German CAO/ARO/AIO-94 trial, showing that patients with pathological stage III disease after preoperative CRT had worse overall survival than same-stage patients from the control arm treated with selective postoperative CRT.21 A limitation to the current study is that it is not possible to determine the exact rate of stage migration in CRT treated patients, since clinical staging (especially cN category) is notoriously unreliable.22-25 Indeed, data from this study showed large discrepancies between clinical and pathological stage in the surgery only group (no downstaging by definition) with pathologically confirmed LN metastases in only 57% of patients with clinical stage III disease. The differences between clinical and pathological stage in the surgery only and SC-RT groups and at least a part of the variation observed in the CRT group are therefore probably related to the imprecision of clinical staging and not to actual stage migration. Another important restriction is the lack of an adequate pTNM control group, due to the high level of selection bias in patients treated with surgery only.

In conclusion, this population-based study provides evidence that pathological stage I after preoperative CRT for RC is associated with an excellent prognosis, which is comparable with reaching a pCR and similar to same-stage SC-RT treated patients without tumour regression. In patients with pathological stage II and III disease after CRT the prognosis is worse than after SC-RT with immediate surgery. These results contain important prognostic information for individual patients and physicians, and may have consequences for predictive models. Staging systems, such as TNM,
should therefore take stage-specific survival differences between patients treated with different preoperative therapy regimens into account.

References


Acute toxicity and surgical complications after preoperative (chemo)radiation therapy for rectal cancer in patients with inflammatory bowel disease


Radiother Oncol. 2017 Apr;123(1):147-153
Chapter 6 Preoperative therapy induced toxicity and complications in IBD related rectal cancer

Abstract

Purpose: Preoperative therapy reduces local recurrences and may facilitate surgery in rectal cancer patients. However, in patients with inflammatory bowel disease (IBD) this treatment is often withheld due to the perceived risk of excessive side-effects, even though evidence is limited. The purpose of this study is to investigate the effects of preoperative therapy on acute toxicity and post-operative complications in IBD patients with rectal cancer.

Methods: The Dutch pathology registry (PALGA) was searched for patients with IBD and rectal cancer treated between January 1991 and May 2010. Histopathology and clinical charts were reviewed to confirm IBD diagnosis and evaluate clinical and pathological characteristics.

Results: Out of 161 patients, 66 received preoperative therapy (41%), including short-course radiation therapy (SC-RT), long course radiation therapy (LC-RT), and chemoradiation therapy (CRT) in 32, 13, and 21 patients respectively. Grade ≥3 acute toxicity occurred in 0 patients (0.0%), 1 patient (7.7%), and 6 patients (28.6%) respectively (p=0.004). Systemic corticosteroids were used by 10.5% of patients at time of treatment. Grade ≥3 post-operative 30-day complication rate (28.1% overall) was not associated with type of preoperative therapy.

Conclusion: Results did not show excessive rates of toxicity or post-operative complications and support the use of standard preoperative therapies for rectal cancer (especially SC-RT) in IBD patients with relatively indolent disease. Caution is warranted in patients with active IBD, since the exact impact of active bowel inflammation could not be determined retrospectively. Prospective studies should investigate the influence of active IBD on acute and late toxicity in patients receiving pelvic irradiation.

Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC), Crohn’s disease (CD) and indeterminate colitis (IC), is a chronic, idiopathic and immunologically mediated inflammatory disorder of the gastrointestinal (GI) tract characterized by episodes of exacerbations and remissions. Patients with IBD have an elevated risk of developing colorectal cancer through the inflammation-dysplasia-carcinoma pathway. The risk increases markedly with disease duration and extent, although modern management has been effective in decreasing the colorectal cancer incidence to levels not much higher than in the general population. However, colorectal cancer remains a significant problem in IBD patients, with worse stage-specific survival rates compared with colorectal cancer in patients without IBD.

For IBD patients who develop cancer in the rectum, optimal treatment strategies remain unclear. Standard preoperative therapy regimes, such as chemoradiation therapy (CRT) for locally advanced rectal cancer or short course radiation therapy (SC-RT), sometimes used for non-advanced tumours or in frail patients, are often withheld in patients with IBD, due to the perceived risk of excessive levels of side-effects. A review on radiotherapy for cancer in IBD patients concluded that external beam radiotherapy (EBRT) produced a moderate increase in acute and late toxicity in IBD compared with non-IBD patients, whereas toxicity levels after brachytherapy for prostate cancer were similar. However, this review included tumours in various locations treated with either pelvic or abdominal irradiation. Previous studies specifically investigating the effects of radiation therapy in IBD related rectal cancer showed conflicting results, and are restricted by limited patient numbers treated over an extended period of time in which imaging and radiation techniques, as well as (peri-)operative management have evolved substantially.

The current study therefore aims to investigate the risk of both acute RT induced toxicity and 30-day post-operative complications in IBD patients with rectal cancer undergoing preoperative (chemo)radiation therapy. Results may guide and optimize current rectal cancer treatment strategies for patients with IBD.

Methods

Patient selection

We searched PALGA, the nationwide network and registry of histopathology and cytopathology in the Netherlands, to identify patients with a history of IBD who were diagnosed with rectal cancer between January 1991 and May 2010. PALGA collects histopathological and cytopathological diagnoses generated in the Netherlands since 1971 and has complete national coverage since 1991. Approval of the PALGA...
Privacy Commission and Scientific Council as well as the Radboudumc institutional ethics committee was obtained (registration number 2012/030). A search was performed using the search terms: "rectal cancer" and/or "rectosigmoid cancer" combined with "ulcerative colitis", "Crohn’s disease", and/or "indeterminate colitis" as well as several related terms including "ileitis", "ulceration", "ulcer", and "inflammation". The search yielded records of all patients with a history of rectal/rectosigmoid cancer combined with at least one pathology report of IBD or non-specific bowel inflammation. Records were manually scrutinized by two investigators (SB and IN) to select patients who were suspected to have a genuine IBD diagnosis. Of the selected patients, histopathological slides of diagnostic IBD biopsies and rectal resection specimens containing the primary tumour (or tumour biopsy if a resection was unavailable) were obtained and reviewed to confirm both the IBD and rectal cancer diagnoses and document tumour characteristics. In addition, medical charts were searched to extract clinical data.

**Inclusion and exclusion criteria**

Only patients with available clinical data after medical chart review were included in the analysis. Patients were excluded if the IBD diagnosis could not be confirmed after review of pathology data and clinical charts, or when IBD was diagnosed after treatment for rectal cancer. Likewise, patients who did not have a rectal tumour (distal edge >15 cm from the anal verge) were excluded. In addition, cases were excluded in case of an administrative mismatch, incorrectly linking records from a patient with IBD to another patient with rectal cancer who had a similar last name and birth date.

**Clinicopathological characteristics**

All clinical and treatment characteristics were retrieved retrospectively from the medical charts. Pathological characteristics were determined retrospectively by centrally reviewing the original histopathological slides and pathology reports. Extracted clinical data included sex, date of birth, presence of comorbidities, type of IBD, date of IBD diagnosis, disease duration, date of rectal cancer diagnosis, presence of distant metastases, type of preoperative treatment (if any), use of corticosteroids at time of treatment, therapy-induced acute toxicity/adverse events, type of surgery, and 30-day post-operative complications. IBD diagnosis was specified as UC, CD or IC based on clinical, histopathological and endoscopic characteristics. Preoperative radiation therapy consisted of EBRT using a three-dimensional conformal technique or intensity-modulated radiotherapy (IMRT). From 2001 onwards the prevailing guidelines recommended that the clinical target volume should include the primary tumour and the mesorectum with vascular supply, containing the perirectal, presacral and internal iliac nodes. The recommended upper border was at the level of the promontory. The perineum was included if an abdominoperineal excision (APE) was planned, whereas the lower border was 3 cm above the anal verge if the planned operation was a low anterior resection (LAR). SC-RT was defined as 5 x 5 Gy given over a 5-7 day period. LC-RT treated patients received preoperative radiation with 45-50 Gy given in 25-28 fractions of 1.8-2.0 Gy. In the CRT group concurrent fluoropyrimidine based chemotherapy was added to the long-course radiation schedule. Acute toxicity (occurring during preoperative therapy) was graded according to the National Cancer Institute Common Terminology Criteria (version 4.0). Comorbidities were scored according to the Charlson comorbidity index (CCI). Tumour related histopathological characteristics were scored in patients who underwent a resection of the rectum. This included TNM-stage, circumferential marginal (CRM) involvement (tumour cells at a distance of ≤1 mm from the CRM), and histopathological type/differentiation grade.

**Statistical analysis**

All data were entered in a database and analyzed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Categorical variables were analyzed using the χ² test. For non-parametrical continuous variables the Mann-Whitney U test or independent samples Kruskal-Wallis test was used were appropriate. A p-value of <0.05 was considered statistically significant whereas a p-value of <0.1 was interpreted as a trend towards significance.

**Results**

**Patient selection**

The initial PALGA search yielded 2035 potential cases with rectal or rectosigmoid cancer and possible IBD (figure 1). The manual search identified 364 patients, treated in 75 hospitals, who were considered likely to be genuine IBD patients with rectal cancer. For reasons of feasibility clinical chart review was limited to 196 patients who underwent surgery in 41 centres, including all centres with at least 4 eligible patients. For the remaining 168 patients either the medical charts were not available in the visited treatment centre or the patients were treated in one of the centres with no more than 3 eligible patients. Thirty-five cases were excluded after review of clinical and histopathological data resulting in 161 IBD patients with rectal cancer who were included in the analysis. Reasons for exclusion were: no rectal cancer (tumour >15 cm from the anal verge; n=17 and tumour in a perineal fistula after proctectomy; n=1), unconfirmed IBD diagnosis (n=6), both absence of rectal cancer and unconfirmed
IBD diagnosis (n=2), IBD diagnosis following rectal cancer treatment (n=3), and administrative mismatch (n=6).

**Clinicopathological characteristics**

Table 1 provides clinical and pathological characteristics per treatment group. Out of 161 IBD patients, 83 patients had UC, 69 had CD, and 9 had IC. A total of 34 IBD patients (21.1%) were diagnosed with rectal cancer within 8 years disease duration (the recommended start of endoscopic surveillance). Sixty-six patients received preoperative therapy (41.0%), including SC-RT in 32, LC-RT in 13, and CRT in 21 patients. These 66 patients underwent surgery in 29 different hospitals, but preoperative therapy was given in a total of 10 different regional radiation oncology centres. Patients with SC-RT were treated between 1996 and 2010, patients with LC-RT were treated between 1991 and 2006, and patients with CRT were treated between 2002 and 2009. At time of rectal cancer diagnosis the patients in the LC-RT and CRT groups were younger than patients in the SC-RT and no preoperative therapy groups (no preoperative therapy: median age 59, range 28-91; SC-RT: median age 61 years, range 32-88; LC-RT: median age 48 years, range 35-84; CRT: median age 51 years, range 34-72; p<0.006). The rate of metastatic disease at baseline was higher in the CRT group (28.6%) compared with the LC-RT (6.3%) and SC-RT (6.6%) group (p=0.048). Systemic corticosteroids were used by 10.5% of patients at time of treatment.

There were 145 patients who underwent a radical rectal resection. Two patients underwent local excision, 1 patient refused surgery, and 13 patients were considered to be palliative because of patient condition, a non-resectable tumour and/or metastatic disease.

**Acute preoperative therapy-induced toxicity**

Severe acute toxicity (grade ≥3) occurred in 0.0% (0/32), 7.7% (1/13), and 28.6% (6/21) of patients in the SC-RT, LC-RT, and CRT group respectively (p=0.004). One patient with LC-RT showed perianal abscess formation grade 3. In the CRT group 1 patient developed severe oral mucositis resulting in systemic inflammatory response syndrome (SIRS) and respiratory insufficiency requiring admission to the intensive care unit (grade 4). One patient was diagnosed with radiation cystitis causing severe hematuria which required blood transfusion (grade 4). Four patients developed grade 3 lower GI toxicity requiring hospital admission including diarrhea (n=3) and anorectal infection (n=1). One patient with diarrhea also developed grade 3 skin toxicity. In the CRT group (n=21) there was no significant difference in the development of severe acute toxicity for patients with vs. without use of corticosteroids at time of treatment (grade ≥3 toxicity rate: 50% (1/2) vs. 27.8% (5/18) respectively; 1 missing value; p=0.515). There was no significant association between type of IBD and grade ≥3 acute toxicity.

**Clavien-Dindo graded 30-day post-operative complications**

The overall complication rate in patients with rectal resection (n=145) was 46.8% and was comparable after SC-RT, LC-RT, and CRT (table 2). Grade3 adverse events occurred in 28.1% and surgical reinterventions in 18.0% of patients, and were comparable in each treatment group. There were no significant differences in rates of multiple complications, specific types of complications, or mortality. Charlson comorbidity index was associated with development of severe adverse events with grade3 complication rates of 20.2%, 36.4%, and 48.0% for CCI 0, CCI 1, and CCI ≥2 respectively (p=0.015).
### Table 1: Clinicopathological characteristics

<table>
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<tr>
<th></th>
<th>Total N (%)</th>
<th>No pre-operative therapy N (%)</th>
<th>SC-RT N (%)</th>
<th>LC-RT N (%)</th>
<th>CRT N (%)</th>
<th>P-value*</th>
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<td><strong>Total number of patients</strong></td>
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<td>95</td>
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<td>59 (28-91)</td>
<td>61 (32-88)</td>
<td>48 (35-84)</td>
<td>51 (34-72)</td>
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<td>Signet ring cell carcinoma</td>
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*P-value was calculated using the chi-square test unless otherwise indicated. ^Independent samples Kruskal-Wallis Test

† includes 1 patient with a total colectomy and 1 patient with a total exenteration.
‡ includes 20 patients with a total proctocolectomy and 2 patients with a total exenteration.
◊ includes 18 patients with definitive colostomy (Hartmann’s procedure) and 8 patients with definitive ileostomy
# including 10 patients without radical surgery.
Discussion

The current study evaluated the effects of standard preoperative therapies on acute toxicity and 30-day post-operative complications in 161 patients with IBD related rectal cancer identified by a nationwide search of the Dutch pathology registry (PALGA). Type of preoperative therapy showed a strong association with the development of acute toxicity, with severe (grade ≥3) toxicity occurring almost exclusively in patients treated with CRT. There was no difference in post-operative complication rate for patients treated with preoperative therapy or surgery only.

The mechanisms of radiation toxicity are multifactorial and highly complex and include direct damage to DNA which may result in cell cycle arrest with repair or apoptosis through activation of p53, or necrosis. Crypt epithelial cell death ensues and results in insufficient replacement of the bowel epithelium leading to breakdown of the mucosal barrier with inflammation. Further tissue damage may be caused by many factors including radical oxygen species produces by attracted leukocytes.26-28 In patients with IBD, on the other hand, it is widely accepted that the bowel may be aggravated in IBD patients by an inappropriate activation of the immune system in response to the exposure to luminal contents. However, our data showed that SC-RT with 5x5 Gy was well tolerated with no grade ≥3 acute toxicity, which is in line with the literature on sporadic rectal cancer reporting severe side effects to be exceedingly rare after SC-RT.17,20,30,31 The rate of grade ≥3 acute toxicity was 28.6% in IBD patients after CRT, which is at the high end of the range reported for sporadic rectal cancer in several prospective series (8-29%).31-37 The most frequently observed severe side effect was diarrhea, whereas the life threatening events (grade 4 oral mucositis resulting in SIRS in a UC patient requiring blood transfusion due to hematuria), were not related to the bowel.

The exact role of active IBD in radiation induced toxicity remains uncertain, since IBD activity was not consistently documented in the retrospectively searched medical charts. Instead, corticosteroid use at time of preoperative treatment was considered to be an indication of possibly active IBD, even though this may also include patients with steroid dependent disease and some patients who are in the process of reducing steroid use after treatment for an exacerbation. Corticosteroid use was present in a small portion of patients suggesting that most of the cohort had relatively indolent IBD at the time of preoperative therapy. There was no significant association between corticosteroid use and grade ≥3 acute toxicity in patients treated with preoperative CRT (which included almost all cases of grade ≥3 toxicity), although the analysis was...
limited by a low number of patients. This result is in accordance with previous studies by Song et al and Willett et al who showed no significant difference in radiation induced toxicity in IBD patients with active vs. non-active disease.28,39

After SC-RT overall post-operative complication rates in the literature show substantial variation (20.1-54%) with surgical reinterventions in 10-19% of patients.17,20,31,40-46 By comparison, the overall complication rate in the current IBD cohort was at the high end of this spectrum (54.8%) with surgical reinterventions in 22.6% for patients. The CRT treated IBD patients in this study showed an overall complication rate of 55.6%, with 22.2% grade 3 complications and 16.7% surgical reintervention. These figures are also in the high range of complication rates reported in the literature, since three trials that evaluated sporadic CRT treated rectal cancer patients and employed the Clavien-Dindo classification found overall complication rates of 44-54.3%, with 11-26.1% grade 3 complications and 2-21.7% surgical reintervention.12,33,47

In addition, patients treated with surgery only in the current study also showed relatively high complication rates, which were comparable with those found in the SC-RT and CRT groups. This may be explained by the high number of previous abdominal surgery and/or corticosteroid use at the time of operation, which have been associated with increased risk of post-operative complications.6,40

The current nationwide study included patients who underwent surgery in 41 hospitals, and for preoperative therapy patients were referred to a total of 10 regional radiation oncology centres. The data therefore provides an adequate reflection of daily clinical practice regarding treatment of IBD patients with rectal cancer, although the data is inevitably influenced by heterogeneity in surgical and radiation therapy procedures. The failure to include patients from low volume hospitals may have resulted in an underestimation of surgical complication rates for the current study population. However, this equally impacts the groups with and without preoperative therapy, and conclusions regarding surgical complication rates therefore remain valid. The influence of the selection process on acute toxicity was probably limited, since patients in this study were treated in a total of 10 radiation oncology centres and there are no more than 21 radiation oncology centres in the country, which generally work according to the same guidelines. Unfortunately, complete dosimetry data was not available retrospectively and therefore it was not possible to reliably analyze the volume of irradiated tissue, which may be expected to show variations between patients treated in different centres over a long period of time. However, from 2001 onwards guidelines recommended to determine the clinical target volume as described in the methods section. The introduction of IMRT as an alternative to 3D conformal radiation can be expected to have resulted in a substantial decrease of the dose received by the normal tissues of patients treated in more recent years in this study.60 Other limitations of this study include the relatively small sample sizes in the various preoperative treatment categories. In addition, due to the retrospective nature of the study and logistical issues related to the large number of centres, such as variable degrees of access granted to medical records, variable degrees of completeness of records, and patients who move to be treated in different centres, several data items could not be adequately evaluated. These included long term follow up, late therapy-induced toxicity, IBD disease location and activity at time of treatment, and use of IBD medication. Furthermore, we recognize that measuring the toxicity of radiotherapy in rectal cancer can be difficult in patients with IBD, since it may be unclear whether symptoms are due to the treatment or rather to ongoing bowel inflammation or the cancer itself, especially in a retrospective setting. It was not possible to compare the results of the IBD patients in this study with an adequate sporadic rectal cancer control group, since the study population is very heterogeneous with regard to type of therapy, TNM stage, comorbidity status and centre of treatment.

In summary, the current study found no evidence for excessive levels of therapy-induced acute toxicity or Clavien-Dindo graded 30-day post-operative complications in this cohort of IBD patients treated with preoperative therapy for rectal cancer. Grade 3 acute toxicity occurred almost exclusively in CRT treated patients. Most patients probably had relatively mild disease activity with systemic corticosteroid use in a small portion of patients. However, the presence of active bowel inflammation at time of treatment could not be adequately determined from the retrospectively searched medical charts and the impact of IBD activity on the development of grade 3 acute toxicity therefore remains uncertain. The 30-day post-operative complication rates after SC-RT and CRT were at the high end of those reported in the literature for sporadic rectal cancer. However, this was also true for IBD patients with surgery only, and may be explained by higher levels of previous abdominal surgery and corticosteroid use in IBD patients compared with their non-IBD counterparts. Therefore, results from this study provide support for the use of standard preoperative therapies for rectal cancer (especially SC-RT) in IBD patients with relatively indolent disease, whereas caution is still warranted in patients with active bowel inflammation. Prospective studies directly evaluating the impact of active IBD on the development of preoperative therapy-induced acute and late toxicity in patients receiving pelvic irradiation are needed, and may include IBD patients treated for prostate and gynecological cancer.
References


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General discussion
and future perspectives
The introduction of total mesorectal excision (TME) with and without preoperative radiation or chemoradiation (CRT) therapy has decreased local recurrence rates and improved survival for rectal cancer patients. The role of high quality histopathological examination in these developments is profound. Pioneering papers by Quirke and others highlighted the importance of lateral tumour spread and mesorectal tumour deposits (TDs), as well as the status of the circumferential resection margin (CRM) in the development of local recurrences, and thereby provided the pathobiological justification for the widespread introduction of TME.¹,² However, rectal cancer still causes considerable morbidity and mortality. This thesis addresses some of the current challenges faced by clinicians and pathologists in the management of this disease and focuses on the role of histopathology in improving rectal cancer care. These issues include improving the quality of TME surgery in order to further decrease local recurrence rates (§1), reducing overtreatment by improving the selection of tumours that are suitable for organ preserving therapies such as a local excision, or a “wait and see” approach (§2 and §3), and improving staging of patients who underwent different preoperative treatment modalities in order to fine-tune follow-up and adjuvant strategies (§4).

§1 Quality of surgery

Plane of surgery
Results from chapter 1 show that the plane of the resection margin is an important predictor of local recurrence and survival after TME surgery, independently of CRM status. Beside surgeon skill and experience there are other factors which determine the quality of surgery. For example, in abdominperineal resection (APR) specimens there is a relatively high rate of muscularis propria resection planes and positive CRMs, which is related to the progressive thinning of the mesorectum as it reaches the level of the levator muscles. Performing an extralevator excision in these patients generates a cylindrical specimen, which increases the amount of tissue around the tumour and decreases CRM positivity and local recurrence rates, although it is also associated with increased perineal wound complications.³-⁶ This shows that quality of surgery is a key factor in the treatment of rectal cancer and optimizing factors that influence it may help improve patient care.

Judging and reporting quality of surgery
Although uniform definitions for macroscopic assessment of the mesorectum, as well as the sphincter area exist, not all observers interpret them consistently. According to a report from the Belgian PROCARE study, the reproducibility of macroscopic plane of surgery evaluation can be disappointing. Their data showed that the central Review
Committee downgraded the surgical plane from (intra)mesorectal to intramuscular in 17% of patients, and upgraded it from intramuscular to (intra)mesorectal in 27%. Improving standardization of the macroscopic evaluation of the resection plane by pathologists is therefore necessary to meaningfully compare quality data between various surgeons and centres. Increasing the awareness among surgeons and pathologists regarding the association of the plane of surgery achieved with patient outcome can help improve quality of surgery. One way to achieve this is to include an assessment of the plane of surgery in a synoptic reporting protocol, which makes it mandatory for pathologists to report this item. Synoptic reporting has been implemented for colorectal cancer in the Netherlands and in this way the performance of surgeons and centres can be compared. In the multidisciplinary setting, surgeons and pathologists should pay adequate attention to macroscopic completeness of surgical specimens and openly discuss results in a constructive and mutually respectful way in order to improve rectal cancer treatment. This opens up opportunities to implement measures that allow surgeons to improve their individual performance and that of the centre for the benefits of their patients.

Improving quality of surgery

Training of surgeons in the techniques of TME have had a tremendous impact on standardization of surgery and improving quality. In addition, the move towards higher volume, specialised centres for rectal cancer care also improves quality of surgery. Low volume surgeons more often cause a suboptimal plane of surgery and an involved CRM, both of which are associated with higher local recurrence rates. In addition, patients treated in high volume centres more often have restorative proctectomies, a lower post-operative mortality rate, and, in some studies, even an improved 5-year survival. In addition, colorectal audits have been introduced to collect clinical and pathological data from multiple centres. After correcting these data for case mix variables, surgeons and centres may compare their own performance with the results from other national or international practices which serve as benchmark. This could initiate changes in treatment and also enables the identification of “best practices” which may subsequently be widely adopted. The European Registration of Cancer Care (EURECCA), a promising initiative started by the European CanCer Organisation (ECCO), is a collaboration of national auditing organizations with the goal to establish a multidisciplinary European registration for patient, tumour, and treatment characteristics linked to outcome registration, which can serve as the basis of an international audit structure. However, this requires registering all the relevant characteristics in the standard dataset of these audits. For example, the Dutch Surgical Colorectal Audit registers CRM positivity rates but not the rates of suboptimal planes of resection (https://www.dica.nl/media/218/DICA-indicatorenset%20verslagjaar%202016.pdf). The CRM positivity rate is an important factor in rectal cancer management, however, it is not just an indication of surgical quality, but should be regarded as a quality indicator for the entire diagnostic and therapeutic process, including the technique and interpretation of clinical imaging (MRI), the choice and technique of preoperative therapy, as well as the quality of the operation itself. Plane of resection, on the other hand, is primarily determined by skill and experience of the surgeon, and is therefore a more precise tool. Incorporating this factor in (inter)national audits seems to be an easy way to further improve the quality of surgery.

§2 Organ preservation for pT1 colorectal cancer

The role of histopathology

The introduction of bowel screening programs are expected to generate an increase in the number of pT1 colorectal cancers. The majority of pT1 tumours can be cured by a local excision, since they do not harbour lymph node metastases (LNM) and therefore do not require radical resection of the mesocolorectal fat. The clinical relevance of local excision for pT1 tumours is greatest for rectal cancer, since the rectum is more accessible to local treatment, including transanal endoscopic microsurgery (TEM), and the potential gain of reducing surgery-induced morbidity is greater than in the colon. Local recurrence can be caused by an incompletely removed primary tumour or by tumour involved lymph nodes, or TDs. Our systematic meta-analysis in chapter 2 showed that lymphatic channel invasion, high histopathological grade, budding, and submucosal invasion depth ≥1mm are strong and independent histopathological characteristics that predict the presence of LNM. Absence of these factors was associated with a very low risk of LNM in several Japanese studies. However, the prognostic value of this specific combination of risk factors to predict locally recurrent disease has not been validated. Previous studies reported the risk of local recurrence after TEM for pT1 rectal cancer to vary depending on tumour size, submucosal invasion depth, differentiation grade, presence of lymphatic or venous invasion, and resection margin status. Tumours with favourable characteristics in the TEM specimen showed local failure rates comparable with TME surgery. However, future studies still have to determine the prognostic value of tumour budding in this context.

Improving quality of pathology reporting

A reproducible and high quality histopathological evaluation of local excision specimens is essential for the development of an effective risk stratification which is
useful in clinical practice. Currently, routinely reported histopathological characteristics such as lymphatic invasion, venous invasion, and differentiation grade suffer from inter-observer variation. Uniform definitions for these factors should be used by pathologists in various centres in order to improve reproducibility. The use of immunohistochemistry and other special stains can substantially improve detection and inter-observer agreement for lymphatic and vascular invasion,\(^{20,26}\) and should be strongly encouraged. Tumour budding has been evaluated according to various different methods in the literature,\(^{21,27}\) which has limited its use in daily practice. Recently, however, investigators have worked to standardize the definition of this promising marker.\(^{28}\) A consensus statement by experts from the International Tumour Budding Consensus Conference says the number of buds should be counted on an H&E slide in a hotspot with a field area of 0.785mm\(^2\) and graded as low (Bd1): 0-4 buds, intermediate (Bd2): 5-9 buds, or high (Bd3): ≥10 buds.\(^{28}\)

Another way in which standardization and quality of histopathological evaluation can be increased is to use synoptic reporting.\(^{29}\) The introduction of synoptic reporting is an encouraging development within the field of pathology, which mandates pathologists to think about and report the presence or absence of certain, sometimes subtle, characteristics that may otherwise have been omitted from the report. Additional benefits of synoptic reporting are that relevant characteristics are reported in a uniform way, which decreases the chance of misinterpretation by clinicians, and it greatly enhances the possibility to compare data between centres and to benchmark and improve one’s own practice.

§3 Organ preservation after CRT

“Wait and see”

Non-operative management is an attractive option in patients with a cCR after pre-operative therapy, who wish to avoid the morbidity and risk of mortality associated with radical surgery. Proof of concept for this approach has been provided by the group of Habr-Gama who reported a local recurrence rate as low as 3% in patients with a sustained clinical complete response (cCR)\(^{31,32}\) although other studies found much higher local recurrence rates of 23-83%.\(^{33-38}\) In addition, studies investigating a “wait and see” approach report variable outcomes and show substantial heterogeneity in patient selection criteria and protocols for follow-up, as well as inconsistent definitions for what constitutes a cCR.\(^{39}\) Furthermore, only about 30% of patients with a cCR actually have no residual tumour in the histopathological specimen.\(^{40}\)

Results from our study in chapter 4 provide an extra word of caution. This study investigated histopathological characteristics in resection specimens of CRT treated locally advanced rectal cancer (LARC) patients including almost 40% clinical T4 tumours. The patients with a pathological complete response (pCR) and near-pCR showed a remarkably high percentage of residual LNM (29% and 50% respectively), and patients with a near-pCR had residual tumour cells in the mesorectum (ypT3 disease) in 42% of cases. Outcome was excellent in patients with pCR, although near-pCR patients had poor disease free and OS, which was comparable with non-responders. The exact clinical relevance of not removing these residual tumour foci as would be the case with “wait and see” is not known. However, currently available imaging modalities have difficulty to accurately identify LNM and to detect small residual TDs within areas of fibrosis after CRT.\(^{41}\) The results described in chapter 4 therefore emphasize the considerable risks involved in a “wait and see” approach in LARC patients.

Local excision after CRT: in theory

The use of full thickness local excision in patients with a good clinical response after CRT provides additional information on tumour regression while at the same time reducing the risk of endoluminal disease recurrence by removing residual tumour foci. A possible diagnostic concern when evaluating TEM specimens after CRT is that in theory the TEM specimen might be devoid of tumour cells while at the same time residual tumour persists in the mesorectum of the patient and this might cause disease recurrence. However, a study by Duldulao et al showed that in 98% of ypT3 and in 100% of ypT4 patients there was tumour tissue in the muscularis properia layer and a full thickness TEM biopsy without viable tumour cells therefore adequately rules out residual mesorectal disease, as opposed to superficial biopsies of the mucosa and submucosa.\(^{42}\)

Another concern is that residual mesorectal LNM may cause disease recurrence. Previous studies on LARC report residual LNM in up to 10% of ypT0 and up to 22% of ypT1 patients after CRT with TME.\(^{43-45}\) In addition, our study (chapter 3) found a 17.4% residual LNM rate in resection specimens of LARC patients with ypT0 after CRT, which dropped to 10.3% if patient with clinically suspected nodal involvement were excluded. These results demonstrate that caution is warranted when treating patients with CRT and local excision.

Local excision after CRT: in practice

However, multiple observational studies showed excellent local control in patients with ypT0 or ypT1 tumours after preoperative CRT followed by local excision. There were only 2 local recurrences in 128 patients (1.5%) described in 7 studies,\(^{46-51}\) whereas local failure rates of 7-46% have been reported in case of a ypT2 or ypT3.\(^{50-52}\) Several phase II trials confirmed the low local recurrence rates in patients with ypT0 or ypT1 after CRT and TEM.\(^{53-55}\) In the CARTS trial patients with early, clinically node negative (cT1-3N0), and distal tumours (≤10cm from the anal verge) with a good
clinical response after CRT (scar or ulcer, ycT0-2) received TEM. Subsequent local recurrences developed in 1 out of 30 patients with a ypT0-1 tumour in the TEM specimen (3%). In the ACOSOG Z6041 trial clinical T2 tumours located ≤8cm from the anal verge were treated with CRT and TEM resulting in only 2 local recurrences in 79 patients with ypT0-2 (1.3%). Pucciarelli et al included 63 patients with a clinical T3 or distal T2 tumours and major clinical response after CRT. They reported 0 local recurrences in 43 patients with ypT0 or ypT1 tumours and a tumour regression grade of no more than 2 (TRG≤2). However, these results came at the expense of a 42% grade 3-5 CRT-induced toxicity rate (including 2 deaths) and a 10% grade 3-5 postoperative complications rate in the CARTS trial and a 39% toxicity rate of at least grade 3 in the ACOSOG Z6041 trial, mostly rectal pain.

There were no local recurrences after immediate completion TME surgery in patients with unfavourable characteristics, and if they are present, immediate completion surgery should follow. Although the evidence for local excision after CRT seems very promising, the rigorous selection of patients in the local excision studies, which consist of primarily distally located rectal cancer with a substantial proportion of clinical T2 tumours and only a few patients with clinically suspected lymph nodes. This results in a low a priori risk of LNM and therefore of residual disease, whereas the study population in chapter 3 consists of LARC patients with a high a priori risk. Although the evidence for local excision after CRT seems very promising, the rigorous selection of patients with distally located, clinical T2 or T3 tumours without suspected lymph nodes, imply that these results cannot be generalized to LARC patients. However, one small study by Perez et al provides some evidence that TEM after CRT may be feasible for selected LARC patients. This study included any distal (<7cm from anal verge) T3, T4, N+ tumour, or T2 requiring APR. Patients with a cCR were followed-up according to “wait and see”, however, patients with a “near” clinical response (residual lesion of ≤3 cm with ycT1-2N0 at clinical and radiological restaging), who had an indication for APR or ultralow anterior resection were offered full-thickness local excision using TEM. Here the local recurrence rate after CRT and TEM was 23%, although all patients who developed a local recurrence had at least one unfavourable characteristic (ypTa2, poor differentiation grade, lymphovascular invasion, or perineural invasion) in the TEM specimen. For patients without unfavourable characteristic the local recurrence rate was 0% (0 of 17).

Selection of patients for organ saving therapy

Although the safety and efficacy of local excision after CRT have not been tested in randomized trials, the current evidence supports the use of CRT and local excision in selected patients with low tumours and a good clinical response that are unable or unwilling to undergo curative radical resection. In case of ypT0 or ypT1 disease in the TEM specimen follow-up appears to be oncologically safe. However, the view that all ypT2 tumours need additional resection, as advocated in the current Dutch colorectal cancer guidelines, may be too simplistic. Results from chapter 3 show that an algorithm integrating histopathological grade, residual tumour size, and presence of clinically detected LNM could predict residual LNM in LARC patients treated with CRT. These results were independent of ypT category and showed that small (<10mm) ypT2 tumours, without clinically suspected LNM and without “high grade” histopathology are associated with a limited probability (7.7%) of residual LNM, which is similar as for ypT0 and ypT1 tumours. Although presence of residual LNM is not the same as local recurrence, these findings merit further investigation. The high toxicity and complication rates after CRT reported in the CARTS and ACOSOG Z6041 trials, as well as considerations about long term functional outcomes emphasize the risk of substantial overtreatment that may caused by CRT with TEM in patients with early disease for whom CRT would normally not be indicated. However, there is a subpopulation of CRT treated LARC patients with good clinical response who may benefit from the judicious use of TEM as they may be spared a debilitating APR. In addition, developments in imaging techniques such as diffusion weighed MRI and FDG-PET show promise, and may be able to predict a good or complete clinical response with much more accuracy in the foreseeable future. It appears meticulous selection of patients eligible for local excision after CRT with a good clinical response could be a key factor in reducing morbidity while preventing potential undertreatment in rectal cancer.

§4 Challenges in TNM staging after preoperative therapy

Preoperative therapy may induce changes in rectal cancer specimens which influence staging. Replacement of tumour cells by fibrosis is called tumour regression and pathologists can assign a tumour regression grade (TRG) based on the proportion of residual tumour and fibrotic tissue. Two different patterns of tumour...
regression have been reported called “shrinkage” and “fragmentation”, and each may have a different impact on staging. Shrinkage goes hand in hand with downstaging as tumour tissue disappears from the deeper layers and only remains in the more superficial parts. Fragmentation shows general loss of tumour cells, which are replaced by fibrosis, although multiple discontinuous small tumour cell clusters remain in multiple areas of the tumour, including the deeper layers. Tumour remnants associated with the fragmentation pattern may be mistaken for TDs which may erroneously lead a pathologist to upgrade the TNM stage. In addition, preoperative therapy may reduce the number of lymph nodes found in the specimen.

**Prognostic significance of downstaging after CRT**

Downstaging after CRT, and especially achieving a pCR, has been associated with improved prognosis in rectal cancer patients. Since randomized controlled trials have not shown a survival advantage in patients treated with preoperative vs. postoperative CRT, downstaging may primarily be a reflection of a tumour’s susceptibility to treatment as well as a less aggressive biology. Differences in stage specific survival between patients treated with preoperative and postoperative therapy have been attributed to stage migration. Investigators have attempted to increase the pCR rate by adding therapeutic agents to the preoperative schedule, although this resulted in increased toxicity without improving tumour response or outcome. In addition, an increasing delay between preoperative therapy and surgery has been associated with improves pCR rates, although an effect on OS was not observed. Furthermore, an analysis of the National Cancer Database showed that achieving a pCR depends on many factors including lower tumour grade, lower clinical T category, lower clinical N category, more recent diagnosis, female sex, private insurance, increasing interval between end of RT and surgery, and treatment at higher volume institutions.

The study described in chapter 5 included patients from a large national cancer registry. This study compared OS in CRT treated patients with patients who underwent short-course radiation therapy (SC-RT) with immediate surgery. The interval between start of SC-RT and surgery is too short to develop regression and downstaging, and for practical purposes these patients may be best classified as pTNN. The study showed that CRT treated patients with pathological stage I had an excellent prognosis with a comparable outcome as pathological stage I patients in the SC-RT group and preoperative treatment with surgery for these patients is therefore adequate. For CRT treated patients with stage II and III, on the other hand, the worse prognosis compared with the SC-RT group indicates that there is room for improvement and additional therapies such as postoperative chemotherapy may be of benefit. This is in line with results from a recent trial on postoperative chemotherapy after preoperative CRT that showed an increase in 3-year DFS with the addition of oxaliplatin to the postoperative chemotherapy regimen compared with 5FU based chemotherapy only, especially in ypstageII patients.

**Importance of lymph node yield after CRT**

Lymph node yield is considered an important prognosticator and quality indicator. A minimum number of lymph nodes need to be evaluated in patients with colorectal cancer for reliable nodal staging. ASCO recommends evaluating a minimum of 12 lymph nodes whereas the Dutch guideline requires at least 10. After CRT the number of lymph nodes retrieved from resection specimens is reported to decrease by as much as 7-53%. One study reports a higher rate of LNM associated with an increasing lymph node yield regardless of preoperative therapy and therefore recommend a minimum of 12 nodes should be evaluated in patients treated with preoperative therapy as well.

However, another study showed that patients with absence of lymph nodes after CRT had an improved survival compared with patients who had LNM (DFS 74% vs. 30% for absence of lymph nodes vs. ypN+; p<0.001). These data imply that the value of number of retrieved lymph nodes after CRT is not clear-cut and the thoughtless use of lymph node yield as a quality indicator in this specific group of patients may not be justified. Ample attention should be paid to case mix (patients with or without preoperative CRT) when judging the performance of a lab or hospital in this regard.

**Histopathological tumour regression**

Tumour regression in preoperatively treated rectal cancer patients is a widely recognized phenomenon and the improved prognosis associated with downstaging and especially a pCR has been documented extensively. The TRG reflects the degree of tumour cell loss and replacement by fibrosis in the histopathological specimen. Assessment of TRG is primarily useful as a prognostic factor. It can also be used in clinical trials as an alternative endpoint for outcome, since it is an earlier indication of therapy response. In addition, TRG may predict response to adjuvant therapies.

The original TRG system was the 5-tier Mandard system, which was designed for esophageal squamous carcinoma and subsequently adapted by Dworak et al for rectal adenocarcinoma. Multiple alternative grading systems have been developed including 5-tier, 4-tier, or 3-tier systems. Although downstaging to a pCR is well known to be associated with an excellent prognosis, the prognostic significance of TRG in patients with various degrees of residual disease is not so clear-cut. One study reported an independent prognostic value for TRG when corrected for downstaging/ypTNM stage, whereas most studies did not find a significant correlation between TRG and survival on univariate or multivariable analysis.
In addition, substantial interobserver variability has been reported when using these methods to assess tumour response with only fair agreement between 17 pathologists in one study (kappa 0.28-0.36). Although another study reported that a simplified 3-tier system was more reproducible than a 5-tier system with 2 pathologists reaching good agreement (kappa 0.64) with a 5-tier and excellent agreement (kappa 0.84) with a 3-tier system.

Interestingly, pathologists who were blinded for treatment characteristics reported tumour regression in 33% of patients treated with surgery only in one study. Furthermore, TRG assessment depends on the quality of histopathological work-up of resection specimens, since more tumour foci are found if more tissue from the tumour area is submitted for microscopy.

These difficulties have impeded the use of TRG as a prognostic factor in daily practice, although in the recently published 8th edition of TNM staging manual a 4-tiered TRG system which is a modified version of the classification published by Ryan et al has now been embraced.

As mentioned before, tumour regression occurs according to either a pattern of “shrinkage” or “fragmentation.” Fragmentation may result in a tumour with near-complete response according to the TRG, while at the same time being classified as a ypT3. Tumour shrinkage is associated with improved outcome, whereas data on the prognostic significance of fragmentation is scarce. Interestingly a study by Kim et al reports a near-complete response to be associated with a substantially worse outcome than a pCR. The study in chapter 4 shows comparable results with poor distant metastasis free interval (DMFI), disease free survival (DFS), and overall survival (OS) in patients with near-complete response. Many of the tumours with near-complete response in both these studies were ypT3 indicating a fragmentation pattern. Indeed a subanalysis of patients from chapter 4 suggested that the poor survival in the near-complete response group was explained by the outcome in ypT3 patients (cumulative 3-year OS: 87.5% vs. 50.0% for ypT1/ypT2 vs. ypT3 respectively; p for trend = 0.062; unpublished data).

In short, tumour downstaging or shrinkage, especially the development of a pCR, predicts an excellent outcome. TRG lacks prognostic value if adjusted for downstaging in most studies and this may well be explained by the fact that it does not distinguish between tumour shrinkage and fragmentation. More research is needed to clarify the prognostic significance of the fragmentation pattern.

Tumour deposits

TDs are discontinuous foci of tumour cells in the pericolorectal fat and have been associated with poor outcome. The origin of TDs is diverse and includes perineural, perivascular or intravascular invasion in a substantial proportion of cases, although they may also represent free discontinuous tumour spread or overgrown LNM. How pathologists interpret TDs is crucial for staging and changes made to TNM in the past 2 decades have resulted in stage migration which may have profound clinical consequences. In the previous 7th edition of TNM as well as in the recently published 8th edition TDs are allocated to the N1c category, which is subsequently only assigned in the absence of obvious LNM. This system largely disregards the prognostic significance of TDs, even though a recent study provided evidence that TDs add prognostic information in patients with LNM.

In patients with rectal cancer who are treated with long-course preoperative therapy the evaluation of TDs may be very difficult. Tumour regression has been reported to result in discontinuous residual tumour (micro)foci in 17-48% of cases, which may be present in all the layers of the bowel wall. Although these foci are often associated with fibrosis, chronic inflammation, and other radiation induced changes, they may be hard to distinguish from true discontinuous TDs if they are present in the mesorectum.

If the rules of the most recent edition of TNM are followed, all of these foci would be assigned to the N1c category and therefore the node-positive group, although the number of foci would be ignored. However, these rules do not apply here, since a part of these foci represent remnants of tumour regression, which in theory should be associated with improved prognosis, rather than true discontinuous tumour spread, which predicts poor outcome. TDs are reported to occur less often after preoperative therapy, and small TDs of ≤1mm are reported to be the first to disappear. Several studies investigated the prognostic value of TDs after preoperative CRT. One study, including 136 CRT treated clinical T3N0 patients of whom 16 (11.8%) had TDs, found no difference in DFS or OS between patients with and without TDs, although the number of TD positive cases is small. In contrast, another small study including 76 patients treated with preoperative (C)RT with TDs in 10 (13.2%) found TDs to be associated with DFS with a hazard ratio (HR) of 4.07 (95% CI 1.39–11.95), although a multivariable analysis was not performed.

Gopal et al evaluated 110 patients treated with preoperative CRT including 23 with TDs (21%) and showed that TDs were associated with a higher rate of LNM and a higher proportion of deeply invasive tumours. In addition, patients with TDs had a substantially shorter median survival (3.1 vs. 11.2 years, P = 0.037), although there was no significant predictive value on multivariable analysis. Zhang et al included 310 LARC patients treated with CRT and 17.4% had TDs. TDs were associated with poor DFS and OS on multivariable analysis. The study described in chapter 4 found TDs in a relatively high proportion of resection specimens of CRT treated LARC patients (26%), which is probably explained by the selection of advanced tumours. In this study TDs were associated with worse DMFI, DFS, and OS, although significance was not retained if the tests were adjusted for ypN-category. The absence of a prognostic value on multivariable analysis in these
studies may be explained by the small number of patients with TDs and lack of statistical power. In contrast, an analysis of the SEER database including 4813 patients with TDs in 10.7% of cases after preoperative radiotherapy found that TDs independently predicted cancer specific survival with HR 2.25 (95% CI 1.51–3.35). In addition, TDs were associated with aggressive characteristics such as poorer tumour differentiation, more advanced ypT category, ypN category and ypTNM stage, presence of distant metastasis, elevated carcinoembryonic antigen, increased CRM positivity rates, and perineural invasion (all \( P < 0.001 \)). However, there was no histopathological review of the TDs in this study and the impact of number of TDs on prognosis was not investigated.102

Patients with TDs in the studies described above are probably a mixture of patients with true TDs (resistant for preoperative therapy) and discontinuous tumour remnants, and it is not surprising that as a group these patients were shown to have worse prognosis in the analysis of a large database such as SEER. This seems to justify counting TDs in the N-category in this subset of patients. However, the exact implications for individual patients remain elusive, since reliable methods to discriminate between patients who have either true TDs or discontinuous tumour remnants after regression have not been developed. The 8th edition of the AJCC staging manual leaves this to the discretion of the pathologist by emphasizing that “it is important for the pathologist to assess whether tumour nodules represent tumour deposits (…) or discontinuous eradication of the original tumour so that he or she can record the appropriate ypT and ypN categories.”88 In addition, the implications of tumour remnants might be more complex than initially thought. Although tumour remnants implicate a good response of the tumour to preoperative therapy, the remnants themselves are relatively therapy resistant. They indeed reflect heterogeneity in the tumour’s susceptibility to therapeutic agents and may identify a tumour clone with more aggressive biology. The histopathological correlate of this phenomenon is the previously discussed “fragmentation” pattern. Future studies should focus on the biology of tumour remnants and should develop methods to distinguish fragmentation from true TDs after CRT.

§5 Concluding remarks

Histopathological examination of rectal resection specimens has contributed to the improvement of rectal cancer care in the last 2 decades, which has lead to a reduced incidence of local recurrences and an increased survival in rectal cancer patients. Assessment of tumour characteristics at both the macroscopic and microscopic level is the backbone of staging and is essential for treatment planning and prognosis in individual patients. Moreover, it is important for the evaluation of quality of care in (inter)national audits, and in determining the efficacy of novel treatment strategies. This thesis showed that macroscopic evaluation can play a role in improving quality of surgery by providing feedback on surgical performance. In addition, histopathology can help reduce morbidity by selecting specific patients with early rectal cancer or patients with a good response after CRT (especially for distal tumours requiring APR), who are suitable for organ preserving strategies. Furthermore, it was shown that a near complete response to CRT does not necessarily translate into a good outcome, which could be related to a more aggressive behaviour of tumours that show a regression pattern characterized by fragmentation instead of shrinkage, and this is relevant for patients who are treated with a “wait and see” approach. Moreover, we found stage-specific differences in survival (for patients with stage II or III) between patients treated with either SC-RT with immediate surgery (no downstaging) or with long course CRT (possible downstaging), and it would be very interesting to learn if postoperative chemotherapy or novel adjuvant therapies in CRT treated patients with ypTNM stage II or III have the potential to close the survival gap between these patients.

High quality histopathology is expected to remain important in the improvement of patient care in the future. A very promising development in the field of pathology is the rise of digital pathology systems. Although digital pathology requires costly investments in high quality digital infrastructure and storage, it has the potential to improve our practice in two important ways. First of all, histopathological images can be retrieved from storage for review, double reading or consultation almost instantly, avoiding the logistical issues associated with physical glass slides. Secondly, digital pathology allows for computer aided diagnosis. A computer may perform automatic annotations and measurements and score prognostic factors such as tumour budding and TRG, which can be time-consuming and imprecise when performed manually. These applications would improve standardization and objective assessment, thereby solving various problems that currently hamper the widespread implementation of these important histological features. Moreover, the development of deep learning techniques may allow computers to discover novel histopathological biomarkers.
Further development and implementation of these methods in daily practice have the potential to substantially change the way pathologists work in the coming years. In light of the above it is clear that histopathology plays an essential role in modern rectal cancer management and high quality histopathological evaluation will continue to aid in the development and implementation of novel treatment strategies and help improve quality of care.

References


Summary
The management of rectal cancer has improved substantially in the last decades. The introduction of total mesorectal excision (TME) and the development and improvement of preoperative therapy schedules have reduced local recurrence rates and improved survival. High quality histopathological examination of rectal resection specimens has contributed enormously to these treatment advances. By unravelling the role of lateral tumour spread, mesorectal tumour deposits, and the status of the circumferential resection margin (CRM) in the development of local recurrences, histopathology provided the pathobiological justification for the widespread introduction of TME.

The field of histopathology continues to develop and it has a key role to play in finding solutions for the current challenges in rectal cancer treatment. These include improving the quality of TME surgery, expanding the population of patients who can be safely treated with organ preserving therapies, and fine-tuning prognosis by improving methods for staging. In addition, efforts need to be made to generalize the advances made in rectal cancer management to patient populations that are usually excluded from preoperative therapy trials, such as patients suffering from inflammatory bowel disease (IBD).

In chapter 1 the prognostic impact of surgical quality as determined by the pathologist is systematically reviewed including a pooled meta-analysis of available literature data. The standard of care for rectal cancer is surgery according to the principles of TME in which the rectum and the mesorectal fat containing possible discontinuous tumour foci or lymph node metastases (LNM) are removed. This substantially reduces local recurrence rates compared with a blunt dissection. However, even if the CRM is free of tumour, local recurrences may develop. This may in part be explained by incomplete removal of the mesorectal fat envelope and the presence of discontinuous tumour foci and/or lymph node metastases which remain in the patient. The quality of surgery can be documented by describing the anatomical plane of the resection. The highest quality specimen has a surgical plane on the mesorectal fascia (mesorectal plane), whereas defects up to 5mm in the fat envelope are intermediate quality (intramesorectal plane), and defects onto or in the muscularis propria constitute the worst quality (muscularis propria plane). Surgical quality was found to depend on surgeon experience, type of surgery (more often after abdominoperineal resection), tumour distance to the anal verge, and body mass index (both very low and very high). Preoperative therapy was not reported to influence surgical quality. The meta-analysis found plane of resection to predict oncological outcome with an increased risk of local recurrence (RR 2.72 [95 % CI 1.36 to 5.44]) and overall recurrence (RR 2.00 [95 % CI 1.17 to 3.42]) for a muscularis propria plane compared with an (intra)mesorectal plane. In addition, one large study included in this systematic review reported 4% vs. 12% local recurrence rates in CRM negative patients with...
mesorectal vs. muscularis propria plane of surgery respectively, and only 1% of patients developed local recurrence if there was a mesorectal plane of surgery after preoperative short-course radiation therapy (SC-RT). The results of this systematic review show that plane of surgery achieved after TME is an important prognostic factor in rectal cancer. It is essential that pathologists document this characteristic in order to provide feedback on surgeons’ performance and thereby improve rectal cancer treatment.

In chapter 2 a systematic review with meta-analysis of predictors of lymph node metastases (LNM) in early (pT1) colorectal cancer is conducted. In the absence of LNM pT1 tumours can be cured with limited local excision, which is associated with reduced morbidity and mortality. The introduction of bowel cancer screening programs for colorectal cancer is expected to increase the number of diagnosed pT1 colorectal cancers and this has made the issue of possible overtreatment in these patients more pressing. About 8-16% of patients with T1 disease have LNM, and several histopathological characteristics, such as poor differentiation grade, lymphatic or vascular invasion, and submucosal invasion depth, have been associated with presence of nodal disease. The aim of this systematic review was to quantify the predictive value of risk factors and determine which characteristics are best suitable for the selection of patients who may safely undergo local excision. A total of 17 studies were included describing data of 3621 patients with available nodal status. The strongest independent predictors of LNM were lymphatic invasion (RR 5.2 [95% CI 4.0-6.8]), submucosal invasion ≥1mm (RR 5.2 [95% CI 1.8-15.4]), budding (RR 5.1 [95% CI 3.6-7.3]), and poor differentiation (RR 4.8 [95% CI 3.3-6.9]). Although a submucosal invasion depth of at least 1mm is highly predictive of LNM it seems unpractical to use this factor for stratification, since the very low specificity (24%) would result in a high false positive rate with many patients undergoing radical surgery unnecessarily.

Therefore, it is concluded that the absence of lymphatic invasion, budding and poor differentiation is associated with low risk of LNM. Risk stratification models integrating these factors may help select patients who may be spared radical surgery and need to be validated prospectively. In addition, the detection of these risk factors needs to be standardized, preferably using immunohistochemistry.

In chapter 3 clinical and histopathological characteristics were investigated which may predict residual LNM in locally advanced rectal cancer (LARC) patients treated with preoperative chemoradiation therapy (CRT) who have residual tumour limited to the bowel wall (ypT0-2). There is increasing emphasis on organ preserving therapy in rectal cancer treatment, particularly for patients who had a good clinical response to preoperative therapy. However, residual LNM in the mesorectum may cause local recurrences in these patients. Some clinicians employ a “wait and see” approach, where patients with a good clinical response are closely followed without additional therapy. However, this method is criticized, since the evidence is based on highly selected patients and criteria used to determine a good clinical response are not consistent in the literature. An alternative approach is to perform a local excision after CRT, which is associated with reduced morbidity and mortality compared to TME surgery. This method removes tumour remnants in the bowel wall and provides additional histopathological information on tumour response. The study found residual nodal disease in 44 patients with ypT0-2 disease (21%). Independent predictors of LNM were clinical nodal involvement (cN+) (OR 2.79 [95% CI 1.04-7.48], p=0.042), “high grade” histopathology assessed in the post-CRT resection specimen (OR 6.46 [95% CI 1.23-34.02], p=0.026), and residual tumour diameter ≥10mm (OR 2.54 [95% CI 1.06-6.09], p=0.036). An algorithm combining these factors was developed and was shown to adequately stratify patients according to LNM risk with a low risk (7.7%), intermediate risk (17%), and a high risk (51%) group. The predictive value of the algorithm was independent of ypT category, which is an important finding, especially for patients with ypT2 disease. Recent studies on local excision after CRT usually exclude ypT2 patients for fear of high local recurrence rates. However, this study shows that in the absence of high grade histopathological differentiation and clinically suspected nodal disease on imaging, a patient with a small tumour residue in the muscularis propria layer (<10mm in diameter) has the same risk of residual LNM as ypT0-1 tumours. Risk stratification based on these factors may help identify patients suitable for organ preserving therapy and should be validated in appropriately selected populations.

Chapter 4 reports the prognostic value of histopathological characteristics after treatment with CRT in a population of 107 uniformly treated and strictly defined LARC patients, including 40% with a cT4 tumour. The study focuses primarily on the association between tumour regression grade (TRG) and oncological outcomes. After preoperative CRT some tumours undergo a pathological complete response (pCR) with no residual tumour cells in the resection specimen, whereas in other cases clinically undetectable residual tumour deposits or pathologic lymph nodes may remain in the mesorectum. In light of the rising attention for organ preserving strategies, including a “wait and see” approach, as well as local excision after CRT (as described in chapter 3), these tumour remnants may pose a threat to the patients if they are not removed. In this study TRG was scored according to a 4-tier grading scale adjusted from the Dworak scale, and consisted of 20% pCR, 11% near-pCR, 55% response and 14% no response. Of 21 pCR patients there were 6 who harboured nodal metastases. Out of 12 near-pCR patients 5 had ypT3 disease, while 6 harboured nodal metastases. A pCR was associated with an excellent prognosis with only one patient developing distant metastases, and no local recurrences. On the other hand,
in the near-pCR group distant metastases developed in 5 of 12 patients and a local recurrence in 1 patient, corresponding to a 3-year distant metastases free interval, disease free survival, and overall survival rate of 65%, 50%, and 67% respectively, which is comparable with patients who had no substantial tumour regression. The exact impact of not removing residual LNM or small, isolated mesorectal tumour deposits in CRT treated LARC patients with a complete or near-complete response of the primary tumour remains unknown. Considering that current imaging modalities have a low accuracy to detect residual LNM and to distinguish fibrosis from microscopic tumour remnants, a “wait-and-see” policy in LARC patients is associated with substantial risks.

In chapter 5 data from a nationwide rectal cancer database is analyzed in order to determine stage specific survival in patients who did or did not undergo tumour downstaging in a significant proportion of patients with the pTNM in 8-24% of cases. In contrast, patients treated with preoperative SC-RT followed by surgery within 10 days, or patients treated with surgery only are not expected to show downstaging. The aim of this study was to determine if TNM stage-specific survival is comparable in patients who did or did not undergo tumour downstaging. However, patients who underwent surgery without preoperative treatment in this study formed a biased selection of older patients with substantially more comorbidities than those treated with SC-RT and CRT. These patients were not treated according to the prevailing guideline and are not considered to be an adequate control group. The most important results of this study therefore arise from the comparison of CRT and SC-RT treated patients. Results from the multivariate analysis emphasized that downstaging to pathological stage I after CRT is associated with an excellent prognosis with cumulative 5-year survival similar to same stage SC-RT treated patients without tumour regression (85% vs. 85%; p=0.167). In addition, cumulative 5-year survival was comparable between CRT treated patients with pathological stage I and those reaching a pCR (85% vs. 89%; p=0.113). On the other hand, CRT treated patients had a worse stage-specific overall survival compared with SC-RT for pathological stage II (HR 1.57 [95%CI 1.27-1.95]; p<0.001) and stage III (HR 1.43 [95%CI 1.23-1.70]; p<0.001). These results show that TNM stage-specific survival depends on type of preoperative therapy, and clinicians should take these differences into account. In addition, there were large discrepancies between clinical stage (cTNM) and pathological stage (pTNM) in patient treated with surgery only (no downstaging by definition), which highlights the inaccuracy of current imaging techniques to predict the spread and extent of disease.

In chapter 6 an analysis is conducted of therapy induced toxicity and postoperative complications in rectal cancer patients with IBD. Preoperative CRT is often withheld in patients with IBD, due to the perceived risk of excessive side-effects reported in the literature. However, the evidence for this is limited. Patients with IBD who develop rectal cancer are scarce and no adequately large cohorts are available in single centres. Therefore, the Dutch pathology registry (PALGA) was searched for all IBD patients diagnosed with rectal cancer in the Netherlands between January 1991 and May 2010. After manually scrutinizing the records yielded by the automated search and performing medical chart review a 161 patients were included in the study. The study included 161 patients with this relatively rare combination of diseases and 66 had received preoperative therapy (41%). The study focused on the incidence of severe (grade ≥3) acute toxicity and post-operative complications in patients. Grade3 acute toxicity developed in 6 out of 21 CRT treated patients (28%), which is at the high end of the range normally reported for sporadic rectal cancer in the literature. However, there was no severe toxicity in the SC-RT group (0/32; 0%). The grade≥3 post-operative 30-day complication rate (28% overall) was also relatively high in this population with IBD compared with sporadic rectal cancer. However, there was no difference between patients treated with preoperative therapy and those with surgery only, and this complication rate is therefore probably best explained by the presence of higher levels of previous abdominal surgery and/or use of corticosteroids in IBD patients, which are both associated with increased complication rates. Systemic corticosteroids were used by 10.5% of patients at time of preoperative treatment, which suggests most of the patients had relatively indolent IBD. Overall, the results did not show excessive rates of toxicity or post-operative complications and support the use of standard preoperative therapies for rectal cancer (especially SC-RT) in IBD patients with relatively indolent disease. Unfortunately, the exact impact of active bowel inflammation could not be determined retrospectively and caution is therefore still warranted in patients with active IBD. There is a need for prospective studies which investigate the influence of active IBD on acute and late toxicity in patients receiving pelvic irradiation.
De behandeling van endeldarmkanker (rectumcarcinoom) heeft in de laatste 20 jaar een grote ontwikkeling doorgemaakt. De introductie van totale mesorectale excisie (TME) en de opmars van preoperatieve therapie heeft er voor gezorgd dat de kans op het ontstaan van lokale recidieven is afgenomen en dat de overleving is verbeterd. Histopathologisch onderzoek van operatiepreparaten van het rectum heeft een belangrijke bijdrage geleverd aan de ontwikkeling van deze behandelingen. Door systematisch histopathologisch onderzoek is aan het licht gekomen wat de invloed is van laterale tumoruitbreiding, de aanwezigheid van tumordeposities in het mesorectale vetweefsel en de aanwezigheid van tumorcellen in de circumferentiële resectie-marge (CRM) op het veroorzaken van lokale recidieven. Dit heeft de pathologische en biologische basis gevormd voor de introductie van de TME als standaardtherapie voor rectumcarcinoomen.

Het veld blijft zich ontwikkelen en histopathologie speelt ook vandaag de dag een belangrijke rol bij het vinden van oplossingen voor de uitdagingen waar behandelaars van patiënten met een rectumcarcinoom voor staan. Die uitdagingen zijn onder andere het verbeteren van de kwaliteit van de chirurgische resectie, het vergroten van de groep van patiënten die veilig behandeld kan worden met rectumsparende therapie (zoals een beperkte locale excisie) en daarnaast het verbeteren van methodes voor stadiëring van de ziekte zodat er een nog nauwkeurigere prognose kan worden gegeven. Tot slot moet er gestreefd worden naar verbetering van de behandeling van specifieke patiëntengroepen met een rectumcarcinoom die doorgaans worden uitgesloten van klinische studies, zoals patiënten met inflammatoire darmziekte (inflammatory bowel disease; IBD).

In hoofdstuk 1 van dit proefschrift wordt de prognostische waarde bepaald van de kwaliteit van het operatiepreparaat, zoals beoordeeld door een patholoog. Hiervoor werd een systematische literatuurstudie verricht waarbij de gepubliceerde medische literatuur over dit onderwerp werd samengenomen in een meta-analyse. De standaardbehandeling voor rectumcarcinoom is een operatie volgens de principes van de TME waarbij het rectum en het omgevende mesorectale vet, met daarin eventuele discontinue tumordeposities en/of lymfekliermetastasen (LKM), worden verwijderd. Dit zorgt voor een aanzienlijke vermindering van het risico op een lokaal recidief in vergelijking met de klassieke stompe dissectie zoals dit 20 jaar geleden nog werd uitgevoerd. Echter, zelfs als het circumferentiële resectievak na een TME vrij is van tumor, kunnen er lokale recidieven ontstaan. Dit kan deels worden verklaard door een matige kwaliteit van de resectie waarbij het omgevende mesorectale vet niet volledig is verwijderd. Hierdoor kunnen er mogelijk tumorfoci en/of lymfekliermetastasen in de patiënt achter blijven. De kwaliteit van het resectiepreparaat kan worden gedocumenteerd door het anatoomisch vlak van de resectie te benoemen. Een hoogwaardig preparaat heeft een chirurgisch vlak op de mesorectale fascie.
Hoofdstuk 2 bestaat uit een systematische meta-analyse van voorspellers van lymfekliermetastasen (LKM) bij vroege (pT1) carcinomen van het colon en rectum. In de afwezigheid van LKM kunnen pT1-tumoren worden genezen met een beperkte lokale excisie, wat leidt tot minder morbiditeit en mortaliteit. De introductie van screeningsprogramma’s zoals het bevolkingsonderzoek voor darmkanker zal er naar verwachting aan bijdragen dat het aantal gediagnosticeerde pT1-colorectale carcinomen zal toenemen. Dit maakt het probleem van mogelijke overbehandeling bij deze patiënten meer urgent. Ongeveer 8-16% van de patiënten met pT1-ziekte hebben LKM. Voorts zijn er diverse histopathologische kenmerken, zoals slechte histologische graad van differentiatie van de tumor, invasie van lymfevaten of bloedvaten en de exacte invasiediepte in de submucosa, die zijn geassocieerd met de aanwezigheid van tumoruitzaaiingen in de lymfeklieren. Het doel van dit onderzoek was om de voorspellende waarde van risicofactoren voor lymfekliermetastasen te kwantificeren. Op basis hiervan kan bepaald worden welke kenmerken het best geschikt zijn voor het selecteren van patiënten die in aanmerking kunnen komen voor een beperkte lokale excisie. Er werden in totaal 17 studies met opgeteld 3621 patiënten opgenomen in de analyse. De dichtst omringende voorspellers voor LKM waren lymfevatinvasie (RR 5,2 [95% CI 4,0-6,6]), submucosale invasive afm (RR 4,8 [95% CI 3,3-6,9]), tumorcel “budding” (RR 5,1 [95% CI 3,6-7,3]) en slechte histologische differentiatiegraad (RR 4,8 [95% CI 3,3-6,9]). Hoewel een submucosale invasiediepte van ten minste 1 mm sterk voorspelt is voor LKM, lijkt het niet werkbare om dit kenmerk te gebruiken voor risicocategorisatie. De zeer lage specificiteit (24%) zou namelijk leiden tot een hoog percentage patiënten dat ten onrechte een radicale operatie zou ondergaan (vals positief). De conclusie van het onderzoek luidt daarom dat de afwezigheid van lymfevatinvasie, “budding” en slechte histologische differentiatiegraad zijn geassocieerd met een laag risico op LKM. Modellen voor risicocategorisatie die deze factoren integreren kunnen helpen bij het selecteren van patiënten bij wie een radicale operatie achterwege kan blijven. Deze modellen moeten prospective worden gevalideerd in toekomstige studies. Daarnaast moet de detectie van deze risicofactoren door pathologen gestandaardiseerd worden; bij voorkeur met behulp van immuunhistochemie.

In hoofdstuk 3 wordt een studie beschreven waarbij klinische en histopathologische kenmerken werden onderzocht die kunnen voorspellen of er resterende LKM aanwezig zijn in patiënten die chemoradiatie therapie (CRT) hebben ondergaan vanwege een lokaal geïsoleerd rectumcarcinoom (locally advanced rectal cancer; LARC). De patiënten in deze studie hadden allen een resttumor die beperkt was tot de darmwand (ypT0-2). Er wordt steeds meer nadruk gelegd op orgaansparende therapie bij de behandeling van rectumcarcinomen, met name voor patiënten die een goede klinische respons op preoperatieve therapie hebben gehad. Resten van metastasen in de mesoreciale vet kunnen bij deze patiënten echter lokale recidieven veroorzaken. Sommige clinici gebruiken een “wait and see” benadering, waarbij patiënten met een goede klinische respons nauwgezet worden gemonitord om te voorkomen dat ten onrechte een radicale operatie zou ondergaan (vals positief). De conclusie van het onderzoek luidt namelijk leiden tot een hoog percentage patiënten dat ten onrechte een radicale operatie zou ondergaan (vals positief). De conclusie van het onderzoek luidt namelijk leiden tot een hoog percentage patiënten dat ten onrechte een radicale operatie zou ondergaan (vals positief).
Aan de andere kant ontstonden er in de bijna-pCR groep metastasen op afstand in 5 van de 12 patiënten en een lokaal recidief in 1 patiënt. Dit vertaald zich na 3 jaar in een afstandsmetastasenverlies van 65%, ziektevrije overleving van 50% en algehele overleving van 67%. Deze resultaten zijn vergelijkbaar met patiënten die geen substantiële tumorregressie hadden. Het blijft echter onduidelijk wat de exacte waarde is van het verwijderen van resterende LKM en/of kleine resterende geïsoleerde mesorectale tumordeposities bij LARC-patiënten met een complete of bijna complete respons van de primaire tumor na preoperatieve CRT. Vanwege de beperkte nauwkeurigheid van de huidige beeldvormende technieken bij het detecteren van resterende LKM en bij het maken van onderscheid tussen fibrose en microscopische tumorresten, is een "wait and see" beleid bij LARC-patiënten met een goede klinische respons op CRT geassocieerd met aanzienlijke risico's.

In hoofdstuk 5 worden gegevens uit de landelijke databank van de Nederlandse Kanker Registratie geanalyseerd om te bepalen wat de stadionspecifieke overleving is bij patiënten met rectumcarcinoom die wel of niet een preoperatieve behandeling hebben ondergaan. Bij een groot deel van de patiënten ontstaat er regressie van de primaire tumor na preoperatieve CRT met een complete pathologische respons in 8-24% van de gevallen. Bij patiënten die worden behandeld met kortdurende preoperatieve radiotherapie gevolgd door een operatie binnen 10 dagen of met chirurgie zonder preoperatieve therapie zal er echter naar verwachting geen regressie en geen downsstaging worden gezien. Het doel van deze studie was om te bepalen of de stadionspecifieke overleving volgens TNM vergelijkbaar is bij patiënten met en zonder tumorregressie. Echter, in de groep die alleen een operatie zonder preoperatieve behandeling heeft ondergaan, blijken oudere patiënten met aanzienlijk meer comorbiditeit oververtegenwoordigd te zijn. Bij deze kwetsbare patiënten is destijds afgeweken van de landelijke richtlijnen die een preoperatieve behandeling met korte radiotherapie of CRT voorschreven. Daarom wordt deze groep niet beschouwd als een adequate controlepopulatie. De belangrijkste resultaten van deze studie vloeien daaruit voort uit de vergelijking van patiënten met korte radiotherapie en langdurige CRT. De uitkomsten van de multivariate analyse benadrukken dat afname van het stadium van de ziekte tot pathologisch stadium I na CRT, gepaard gaat met een uitstekende prognose. Er is sprake van een cumulatieve 5-jarigoverleving die vergelijkbaar is met patiënten die hetzelfde stadium hebben na een behandeling met korte radiotherapie gevolgd door chirurgie binnen 10 dagen en die daarom geen regressie van de tumor hebben gehad (5-jaars overleving 85% versus 85%; p = 0,167). Bovendien was de cumulatieve 5-jaars overleving vergelijkbaar bij CRT-behandelde patiënten met pathologisch stadium I en met een pCR (85% versus 89%; p = 0,113). Voor patiënten met stadium II en III bleek er echter wel sprake van een significant slechtere stadionspecifieke algehele overleving na een preoperatieve behandeling met CRT vergeleken met
korte radiotherapie (stadium II: HR 1,57 [95% CI 1,27-1,95]; p < 0,001); stadium III: HR 1,43 [95% CI 1,23-1,70]; p <0,001). Deze resultaten tonen aan dat stadiumspecifieke overleving afhankelijk is van het type preoperatieve therapie dat een patiënt heeft gehad. Clinici dienen daarom bij het inschatten van de prognose van patiënten met rectumcarcinoom rekening te houden met deze verschillen. Voorts werden er bij de patiënten die behandeld werden met chirurgie zonder preoperatieve behandeling (per definitie geen downstaging) substantiële discrepanties gevonden tussen het klinische stadium zoals beoordeeld op basis van beeldvorming (cTNM) en het pathologische stadium (pTNM). Deze bevinding benadrukt de onnauwkeurigheid van de huidige beeldvormende technieken bij het beoordelen van de uitbreiding van de ziekte.

In hoofdstuk 6 wordt onderzocht of preoperatieve therapie bij rectumcarcinoom patiënten met IBD wel of niet is geassocieerd met toegenomen toxiciteit en postoperatieve complicaties in vergelijking met rectumcarcinoom patiënten zonder IBD. Op dit moment wordt preoperatieve CRT bij patiënten met IBD vaak achterwege gelaten vanwege het vermeende hoge risico op overmatige bijwerkingen. Het bewijs in de medische literatuur is hiervoor echter beperkt. Het totaal aantal patiënten met IBD dat een rectumcarcinoom ontwikkelt is laag en het is daarom moeilijk om valide grote cohorten van patiënten bij elkaar te vinden voor een gedegen onderzoek, zelfs als er patiënten uit meerdere centra worden geïncludeerd. Daarom is er voor gekozen in de databank van de Nederlandse landelijke pathologie registratie (PALGA) te zoeken naar alle IBD-patiënten die zijn gediagnosticeerd met rectumcarcinoom in Nederland in de periode van januari 1991 t/m mei 2010. Na het handmatig doorzoeken van de resultaten die door de geautomatiseerde zoekopdracht werden gegenereerd en het opzoeken en analyseren van de medische dossiers van de patiënten, werden er 161 IBD-patiënten met een rectumcarcinoom opgenomen in de studie. Van deze 161 patiënten hadden er 66 preoperatieve therapie gehad (41%). Er is in deze studie met name gekeken naar de incidentie van ernstige acute (graad ≥3) radiatie geïnduceerde toxiciteit en ernstige (graad ≥3) postoperatieve complicaties. Bij 6 van de 21 CRT-behandelde patiënten (28%) ontstond graad ≥3 acute toxiciteit. Vergeleken met incidentiecijfers die in de literatuur worden genoemd voor sporadische (niet-IBD gerelateerde) rectumcarcinoom patiënten is dit percentage aan de hoge kant. In de groep die was behandeld met korte radiotherapie (direct gevolgd door chirurgie) werd er geen ernstige toxiciteit gevonden (0/32; 0%).

Het percentage postoperatieve complicaties binnen 30 dagen van graad ≥3 was 28% en daarmee ook relatief hoog in deze populatie van IBD-patiënten in vergelijking met rectumcarcinoom patiënten zonder IBD. Er was echter geen verschil tussen de patiënten die een preoperatieve behandeling kregen met (chemo)radiatie en de patiënten die alleen een operatie ondergingen. Het hoge percentage postoperatieve complicaties is daarom waarschijnlijk het beste te verklaren doordat de IBD-patiënten vanwege hun ziekte in het verleden vaker buikoperaties hadden ondergaan en/of corticosteroïden gebruikten rond de operatie. Dit zijn allebei factoren die zijn geassocieerd met een verhoogd risico op complicaties. Op het moment van preoperatieve behandeling werd door slechts 10,5% van de patiënten systemische corticosteroïden gebruikt. Dit geeft aan dat de meeste patiënten op dat moment een relatief rustige IBD hadden. Samenvattend liet deze studie geen onacceptabel hoge percentages van (chemo) radiatie geïnduceerde toxiciteit of postoperatieve complicaties zien. De resultaten ondersteunen hiermee het gebruik van standaard preoperatieve therapiën voor rectumcarcinoom (en met name korte radiotherapie) bij IBD-patiënten met relatief weinig ziekteactiviteit. Helaas kon op basis van de retrospectief verzamelde gegevens in deze studie niet worden vastgesteld wat de exacte invloed is van actieve ontsteking van de darm ten tijde van preoperatieve therapie op het ontstaan van toxiciteit en complicaties. Er is daarom nog steeds voorzichtigheid geboden bij het toepassen van (chemo)radiatie op patiënten met actieve IBD op het moment van behandeling. Toekomstige studies moeten licht werpen op de invloed die een actieve IBD heeft op het ontstaan van acute en late toxiciteit bij patiënten die bestraald worden op het kleine bekken.
Curriculum Vitae

Publications

List of attended courses, workshops, seminars, and international conferences

Acknowledgements | Dankwoord
Curriculum Vitae

Steven Bosch was born on January 18th, 1984, in Enschede, the Netherlands, and was raised in Berkel en Rodenrijs. After graduating from the Marnix Gymnasium in Rotterdam, he started his medical education at the University of Maastricht. During his medical training he did a research project at the department of pathology’s laboratory for oncology and angiogenesis (supervisor prof. dr. Arjan Griffioen), where his interest in the field of pathology was sparked. He graduated in 2009 and later that year began his training as a general pathologist at the Radboudumc in Nijmegen (supervisors prof. dr. Piet Slootweg and dr. Willeke Blokx). During his residency he started working on his research investigating clinical and histopathological characteristics of sporadic and IBD related rectal cancer (supervisors prof. dr. Iris Nagtegaal and prof. dr. Hans de Wilt). This research, which included collaborations with the University of Leeds (dr. N. West) and the Catharina hospital in Eindhoven (prof. dr. Harm Rutten), culminated in the present doctoral thesis. In 2017 he completed his medical specialist training and began working at Stichting PAMM in Eindhoven as a general pathologist with a special interest in gastrointestinal and endocrine pathology. He lives in Nijmegen together with his wife Cathelijn and three children; Wende, Roosmarijn and Jelte.
Publications


List of attended courses, workshops, seminars, and international conferences

Conferences

Dutch Colorectal Cancer Group (DCCG) symposium; December 2010; Amersfoort, The Netherlands

Dutch Surgical Colorectal Audit (DSCA) conference; April 2011
- Speaker at the workshop “neo-adjuvant therapy”
- Title of the presentation: “Clinical significance of mucinous lakes in rectal carcinoma resection specimens after neo-adjuvant therapy”

Dutch Colorectal Cancer Group (DCCG) symposium, November 2011, Utrecht, The Netherlands

Multidisciplinary rectal cancer conference; December 2011; Piacenza, Italy
- Speaker
- Title of the presentation: “The pathologist... that is to say the judge of good quality surgery”

European Multidisciplinary Colorectal Cancer Congress (EMCCC); April 2012; Prague, Czech Republic
- Speaker at the workshop “Pathology”
- Title of the presentation: “Clinical relevance of quality of surgery evaluation – results of a meta-analysis”

European Congress of Pathology (ECP); September 2012; Prague, Czech Republic.
- Poster: “Predicting lymph node metastasis in pT1 colorectal cancer – a meta-analysis providing rationale for therapy decisions”

European Multidisciplinary Colorectal Cancer Congress (EMCCC); November 2014; Amsterdam, The Netherlands
- Poster: “Histopathological characteristics predict presence of lymph node metastases in downstaged (ypT0-2) rectal cancer after neo-adjuvant chemoradiotherapy”

Courses

Short course “Statistical Package for the Social Sciences (SPSS)”; August 2011; Radboudumc Nijmegen, The Netherlands

Short course “How to write a medical scientific paper”; November 2011; Radboudumc Nijmegen, The Netherlands
Dankwoord

Promoveren is een proces van persoonlijke groei en ontwikkeling waarbij de hulp en ondersteuning van mensen binnen en buiten de wetenschap onmisbaar is. Een aantal van deze mensen wil ik hier in het bijzonder noemen.

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