Advances in treatment and new insights in molecular biology of rectal cancer

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Summary and conclusive remarks

SUMMARY

The general introduction in **Chapter 1** presents clinical aspects and molecular backgrounds of (colo)rectal cancer. In this thesis, we have focused on both of these aspects since understanding of the molecular background of rectal cancer can provide useful information for the determination of clinical strategies. The results are mainly obtained from a trial performed by the Dutch ColoRectal Cancer Group "Total mesorectal excision with or without preoperative radiotherapy in the treatment of primary rectal cancer" (TME-trial).

PART I: ADVANCES IN TREATMENT

Local recurrences (LR) have been a major problem in the treatment of rectal cancer. A high incidence of local recurrence (15-45%) is associated with conventional, non-standardised procedures, which consists of blunt dissection of the rectal fascia and often results in incomplete removal of mesorectal tissue. **Chapter 2** describes a population-based study of local recurrence rates in curatively resected patients with rectal cancer, diagnosed between 1988 and 1992, in the west Netherlands. The first objective was to make an inventory of the overall local recurrence rate after non-standardised conventional surgery, inter-institutional local recurrence rate variability, and correlations between patient- and tumour-related factors and local recurrence rate. A second objective was to investigate the compliance to guidelines for postoperative radiotherapy. The overall local recurrence rate was 22.5% with a range of 9-36% between the 12 hospitals. These differences were not significant. Dukes' Astler-Coller stage, tumour location and residual tumour were significant independent prognostic factors for the risk of local recurrence. Indications for postoperative radiotherapy were Dukes' Astler-Coller B2 and C tumours, positive surgical margins and tumour spill, but compliance to these guidelines was only 50%. However, no significant difference in local recurrence rate was found between patients treated according to the guidelines and those not treated according to the guidelines. In conclusion, this study shows a high local recurrence rate with conventional surgery and variability in local recurrence rate between the participating hospitals. Furthermore, it confirms that the risk of local recurrence in primary rectal cancer is dependent on Dukes' Astler-Coller stage, tumour location and residual tumour. Lastly, this study contributes to the discussion about the feasibility of guidelines for postoperative radiotherapy.

To improve results of surgery, various additional treatments, such as radiotherapy, chemotherapy and immunotherapy, have been applied. The Swedish Rectal Cancer Trial (SRCT) was the first trial to show that better local control, achieved with preoperative radiotherapy, resulted in improved survival. A major problem of published studies on adjuvant therapy however, is that surgery has not been standardised. Furthermore, quality control of the surgical technique by standardised pathological examination of the specimen is absent in most studies. In Europe, TME has become the preferred standard of operative management for rectal cancer. Adjuvant therapies should now be re-examined based upon a platform of standardised, optimal surgery and pathology. In **Chapter 3** we studied the current European trials in which TME-surgery is intentionally performed. Most of these trials are still in progress or have too short follow-up, so definitive results, apart from interim-analyses, are not known yet. The TME-trial however, has already shown that performing a large, multicentre trial with quality control of both surgery and pathology is feasible.

Reports on improved local control after short-term 5x5 Gy preoperative radiotherapy

and TME-surgery have led to the conduction of the TME-trial, in which the effect of TME surgery with or without short-term preoperative radiotherapy was evaluated. However, any benefit regarding a reduced local recurrence rate and possible improved survival must be weighed against potential adverse effects. The study in **Chapter 4** was undertaken to assess the acute side effects of short-term, preoperative radiotherapy in rectal cancer patients undergoing TME and to study the influence of 5x5 Gy on surgical parameters, postoperative morbidity and mortality. We analysed 1530 Dutch patients entered in the TME-trial of which 1414 were evaluable. Toxicity during radiotherapy hardly occurred. Irradiated patients had 100 ml more blood loss during the operation ($P<0.001$) and showed more perineal complications ($P=0.008$) in case of an abdominoperineal resection. The total number of complications was slightly increased in the irradiated group ($P=0.008$). No difference was observed in postoperative mortality (4.0% vs. 3.3%) or in the number of reinterventions. In conclusion, preoperative hypofractionated RT is a safe procedure in patients treated with TME surgery, despite a slight increase in complications when compared to TME surgery only.

Local control and survival of rectal cancer have been improved by the introduction of the TME-technique. In addition to the surgical technique, hospital volume and specialisation can be important prognostic factors. In **Chapter 5** the effect of training in TME-surgery was assessed on short- and long-term outcomes in rectal cancer in the TME-trial and outcomes were compared with results from a former randomised trial (Cancer Recurrence And Blood transfusion (CRAB) trial), in which conventional surgery was performed without quality control. We analysed the eligible, preoperatively non-irradiated, curatively operated patients. The influence of hospital volume was investigated in both trials, while the role of hospital specialisation was analysed only in the TME-trial. We corrected for differences in clinicopathological characteristics by means of multivariate analyses and to ensure valid comparisons, only events occurring within 2 years of surgery were analysed for long-term outcomes. Hospital volume was analysed as a continuous factor. Local recurrence rate decreased from 16.3% in the CRAB-trial to 8.6% in the TME-trial, and type of surgery (conventional (CRAB-trial) vs. TME (TME-trial)) was an independent predictor for local recurrence ($P=0.002$). Type of surgery was also an independent predictor for overall survival ($P=0.019$) with a higher survival rate in the TME-trial. Higher hospital volume was significantly associated with lower distant recurrence ($P=0.006$) and higher overall survival ($P=0.011$) in the CRAB-trial. However, in the TME-trial hospital volume and specialisation were not of significant value for short- and long-term outcomes. In conclusion, training of surgeons with TME-surgery, leads to improved long-term outcome of rectal cancer patients without volume- or specialisation-related differences.

In **Chapter 6**, the outcome of the main objective of the TME-trial is reported: is short-term preoperative radiotherapy still beneficial in rectal cancer patients undergoing TME? The combination of these treatment modalities was never investigated. Between January 1996 and December 2000, 1861 Dutch and foreign patients with resectable rectal cancer were randomly assigned to preoperative radiotherapy of 5x5 Gy followed by TME or to TME alone. Of the 1861 randomised patients, 1805 were eligible. The 2-year overall survival rate for the 1805 eligible patients was 82.0% in the RT+TME group and 81.8% in the TME group ($P=0.84$). For the 1748 patients who underwent a macroscopically local complete resection, 2-year local recurrence rate was 5.3%. The 2-year local recurrence

rates were 2.4% in the RT+TME group and 8.2% in the TME group (P<0.0001). In conclusion, in a setting of standardised TME-surgery, short-term preoperative radiotherapy still has a beneficial effect on local recurrence risk.

PART II: NEW INSIGHTS IN MOLECULAR BIOLOGY

Observations from other studies support the theory that development of left- and right-sided colorectal cancers may involve different mechanisms. In this study, different genes involved in tumourigenesis of colon vs. rectal cancers, were investigated, and their prognostic value was analysed. **Chapter 7** compares a series of colon cancers with standardised treated rectal cancers obtained from the pilot-study of the TME-trial, with regard to different genes involved in tumourigenesis of colorectal cancer. Mutation and expression profiles were investigated and related to tumour site and prognosis. *APC* mutation analysis of the mutation cluster region showed truncating mutations in 18 of 22 rectal tumours (82%), but presence of an *APC* mutation was not related to nuclear β-catenin expression (P=0.75). Rectal cancers showed significant more nuclear β-catenin than colon cancers (65% vs. 40%, P=0.04). *p53* mutation analysis corresponded well with p53 immunohistochemistry (P<0.001) and with this, rectal cancers showed significant more p53 expression than colon cancers (64% vs. 29%, P=0.003). In rectal cancers a significant correlation was found between positive p53 expression and worse disease-free survival (P=0.008), but not in colon cancers. Cox regression showed that p53-expression (P=0.03) was an independent predictor for disease-free survival in rectal cancers. This study shows that rectal cancers may involve more nuclear β-catenin in the APC/β-catenin pathway than colon cancer and/or nuclear β-catenin may have another role in rectal cancer independent of APC. The p53-pathway seems to be more important in rectal cancer, in which p53 expression also has independent prognostic value. When prognostic markers are investigated in larger series, differences in biological behaviour between colon and rectal cancer should be considered.

In **Chapter 8** we investigated molecular profiles of sporadic rectal cancers using 12 microsatellite markers and DNA ploidy analysis in order to classify tumours in terms of genetic instability. Screening of 81 rectal cancers revealed one tumour with high frequency of microsatellite instability (MSI). The majority of tumours (74%) showed loss of heterozygosity (LOH) for at least one marker. Most of the LOH-positive tumours (81%) and 47% of the LOH-negative tumours were aneuploid. The data indicate that chromosomal instability (CIN) rather than microsatellite instability (MSI) plays a role in rectal cancers. We found a subset of rectal tumours without hallmarks of gross genetic instability (n=13). Five of these diploid, MSI-stable tumours, of which 4 did not show LOH, were further characterised for *p53* mutation status and expression of 1700 cancer-related genes, and compared to two aneuploid tumours. Clustering of gene expression profiles revealed that the *p53* mutant diploid tumours seemed more similar to the *p53* wild type aneuploid tumours than to the *p53* wild type diploid tumours. Within the diploid tumour subset, differential gene expression patterns related to *p53* mutation status were found. The expression analysis also revealed a lack of mRNA expression of *hMSH2* and *hMSH3* in a diploid tumour originating from a 29-year-old patient. In addition, hMSH2 and hMSH6 were lost at the protein level. No mutation was detected in *hMSH2* and *hMSH6*. Since this tumour was MSI-stable, the loss of expression of these mismatch repair genes may be a late event. In conclusion, as others we identified a group of rectal tumours without evidence of gross genetic instability

by molecular analysis with microsatellite markers and flow cytometry. We show that tumour heterogeneity in this class of tumours can be defined by molecular characteristics, such as *p53* mutation status and differential expression profiles.

In **Chapter 9**, the influence of radiotherapy on the expression of p53 and $p21^{waf1}$ was investigated in normal mucosa and rectal carcinomas in patients from the TME-trial. In vitro, ionising radiation of epithelial cells leads to upregulation of wild type *p53* and subsequent induction of $p21^{waf1}$. The effect of radiotherapy on the expression of these proteins in patients is unknown. p53 and $p21^{waf1}$ expression was determined in 51 irradiated and 52 non-irradiated patients using immunohistochemistry. In normal mucosa, both p53 and $p21^{waf1}$ were strongly upregulated after radiotherapy, compared with the expression in unirradiated normal tissue ($P<0.001$). In tumour cells, no significant difference in the expression of p53 or $p21^{waf1}$ was found in the irradiated vs. the non-irradiated group. In the few rectal tumours with wt *p53*, induction of p53 after radiotherapy did not necessarily lead to upregulation of $p21^{waf1}$. These findings demonstrate that in normal mucosa a functional p53-$p21^{waf1}$ pathway is present, whereas in tumour cells it is defective in almost all cases due to either *p53* mutation or down- or upstream disruption in tumours with wild type *p53*. Therefore, we believe that the role of p53 expression as a single prognostic marker in rectal cancer needs reconsideration.

In the process of invasion and metastasis, cell adhesion and angiogenesis are important. In **Chapter 10** we investigated 97 rectal tumours from the TME-trial to analyse the influence of irradiation on the expression of cell adhesion molecules and microvessel count, and to examine the prognostic value of these factors. Immunohistochemical expression of E-cadherin, α-, β-, γ-catenin, EpCAM and CD31 were investigated in patients who had undergone surgery with or without preoperative radiotherapy. Irradiated tumours showed more nuclear β-catenin expression ($P=0.004$) and a lower microvessel count ($P=0.03$). No other differences were found between irradiated and non-irradiated tumours. Loss of EpCAM expression was significantly associated with local recurrence ($P=0.015$) for the total group of tumours. Low microvessel count was associated with an increased distant recurrence risk ($P=0.04$) and lower overall survival ($P=0.02$). The overall results of this study show that loss of EpCAM expression is associated with increased local recurrence risk and low microvessel count with increased distant recurrence risk in rectal cancer. Furthermore, irradiation has an influence on nuclear β-catenin expression and microvessel count.

CONCLUSIVE REMARKS

In the last decades, major advances have been made in the treatment of rectal cancer by the introduction of new surgical techniques and additional technical improvements (e.g. staplers). During the last years, quality assurance of surgery has become an important topic in rectal cancer treatment. Quality assurance is of major importance for standardisation of treatment in (neo)adjuvant therapy studies and for improvement of outcomes.

The introduction of TME-surgery has led to a major reduction in local recurrence rates and improved survival. We showed that short-term preoperative radiotherapy gives a further reduction in local recurrence rate when standardised TME-surgery is used. TME-based operations are now established as the standard of care for rectal cancer, and should form the basis for trials concerning the role of (neo)adjuvant therapy.

In general, it is thought that high volume and specialist care produces superior results to

low volume and non-specialist care, especially for those less frequent forms of cancer and in technically difficult operations, like those for rectal cancer. However, limiting the performance of rectal cancer surgery to surgeons who work in specialised centres or to only those general surgeons who perform more than a certain volume is impractical in view of the prevalence of rectal cancer. The concentration process can also take place within one hospital surgical unit with 1-3 surgeons performing rectal cancer surgery. This has been demonstrated in the TME-trial, in which training in TME-surgery to surgeons who are dedicated to oncology, has led to improved outcome without volume- or specialisation-related differences.

Quality assurance of the surgical technique requires besides training, adequate knowledge of the anatomy of the organs and nerves in the pelvis and other related structures. Furthermore, standardisation in the description of operations and reporting of pathology specimens should be implemented as important features of quality control. In addition, a multidisciplinary approach provides the best care for patients, since the access and use of standardised and up-to-date therapy is better organised. Similarly, patients participating in clinical trials generally experience a survival advantage over non-participating patients, which is probably due to standardised treatment.

Within the TME-trial structuralisation and audit of rectal cancer treatment has led to improvement of treatment results and this infrastructure provides optimal conditions for conducting future rectal cancer trials. The successor trial of the TME-trial, the Preoperative Radiotherapy and/Or adjuvant Chemotherapy combined with Tme surgery in Operable Rectal cancer (PROCTOR)-trial, is currently investigating the role of postoperative chemotherapy in TME-treated patients. However, it is of utmost importance that outside the setting of trials, standardisation of treatment is also applied and sustained. Population-based cancer registries, covering an increasing proportion of the world's population, are an invaluable source of data for this goal.

In addition to clinical improvements, the molecular biology of colorectal cancer will be unravelled even more in the coming years. New techniques in cancer research comprise genome-wide analysis techniques such as chromosome painting, comparative genomic hybridisation, high-throughput analysis of LOH, serial analysis of gene expression (SAGE) and expression microarray analysis. These techniques are now accelerating the high resolution of aberrations in human tumours. By these new techniques identification of affected genes, elucidation of their functions and associations of these genes with tumour progression will be disentangled by which the tumourigenesis of colorectal cancer will be more fully understood. Furthermore, these techniques can help to predict sensitivity or resistance of individual patients to adjuvant therapy. Hereby, individual patients can be offered their own most "suitable" therapy. This "tailor-made" therapy will emerge most likely in the next decade for several diseases.

In the TME-trial, the criteria for analysis of individual risk factors as stated by R.A.E.M. Tollenaar in his thesis were completely met.[1] The criteria of uniform collection of clinical findings according to strictly defined criteria, detailed documentation and standardisation of therapeutic procedures, uniform collection of macroscopic and histological tumour characteristics, standardised documentation of the course of the disease and lastly, evaluation of the data using multivariate statistical methods, were all fulfilled. The TME-trial with its unique and thorough setup, still offers a challenge to future investigators and will certainly

provide more answers to questions concerning the molecular biology, prognostic factors and the mechanisms of radiation-induced damage in tumour cells. In addition, more clinical outcomes of the TME-trial will be known in the coming years, such as the long-term side-effects of preoperative radiotherapy and the influence of irradiation on overall survival. However, the most important objective of this trial has already been achieved; improvement of the treatment for rectal cancer patients with much lower local recurrence rates as compared to a decade ago when conventional surgical techniques were applied. This thesis has dealt with clinical and molecular aspects of rectal cancer and shows that by investigating the combination of these aspects in a large randomised multicentre trial, advances in treatment and new insights in molecular biology have been obtained.

REFERENCES

1. RAEM Tollenaar. Aspects of tumour progression in colorectal carcinoma. University of Leiden. Thesis/Dissertation, 1997

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Samenvatting en afsluitende opmerkingen
Lijst van deelnemers TME-trial
Publicaties
Curriculum Vitae
Bijlage