

Aspects on Local Recurrence after Rectal Cancer Surgery

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UNIVERSITY OF GOTHENBURG

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"Perfection of rectal cancer surgery is in the ability of adhering to detail"
-Anonymous

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ABSTRACT

Background: Local recurrence after rectal cancer surgery has a profound impact on affected patients' lives. The overall aim of this thesis was to acquire a deeper understanding of local recurrence after rectal cancer surgery, with a long-term hope of minimising the incidence and mitigating the effects.

Methods: Analysis of quality assurance data from the Swedish Rectal Cancer Registry, review of medical records and analysis of tumour DNA with array-comparative genomic hybridisation and quantitative polymerase chain reaction.

Results: The majority of studied patients were symptomatic when diagnosed with local recurrence, deemed incurable, not well palliated and had a poor outcome. Aspects on the surgical management could partly explain a difference in local recurrence rate between regions. The local recurrence rate was significantly lower in patients that had perioperative rectal washout than in those who had not. The favourable outcome also remained after adjustment for known confounding factors. DNA in primary rectal carcinomas in tumours that subsequently recurred locally differed from DNA in tumours that did not recur.

Conclusions: Local recurrence after rectal cancer surgery is still a reality in modern day medicine and is associated with intractable symptoms and premature death. Genetic differences might be contributory to the pathogenesis but the quality of surgery is of fundamental importance. Rectal washout is an integral part of good medical practice and should be performed routinely.

Keywords: Rectal neoplasms. Neoplasm recurrence, local. Methodology. Rectal washout. Rectal irrigation. Anterior resection. Quality assurance registry. Follow-up. Array-CGH. Tumour DNA.

LIST OF PAPERS

This thesis is based on the following publications and manuscript, which are referred to in the text by their Roman numerals (I-IV):

- I. Kodeda K, Holmberg E, Steineck G, Nordgren S
Regional differences in local recurrence rates after rectal cancer surgery.
Colorectal Dis. Epub 2009 Nov 14.
Colorectal Dis. 2010 Oct;12(10 Online):e206-15. doi: 10.1111/j.1463-1318.2009.02137.x.
- II. Kodeda K, Derwinger K, Gustavsson B, Nordgren S.
Local recurrence of rectal cancer - A cohort study with focus on diagnosis, treatment and outcome.
Colorectal Dis. 2011 Nov 22. doi: 10.1111/j.1463-1318.2011.02895.x.
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- III. Kodeda K, Holmberg E, Jörgren F, Nordgren S, Lindmark G
Rectal washout and local recurrence after rectal cancer surgery.
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Br J Surgery website -your views: Posted Oct 31, Author reply Nov 30 2010.
<http://www.bjs.co.uk/details/yourviews/892833/Rectal-washout-and-local-recurrence-of-cancer-after-anterior-resection-.html> [accessed 30 October 2011].
Br J Surg. 2011 Apr;98(4):601-2; author reply Br J Surg. 2011 Apr;98(4):602
- IV. Kodeda K, Gustafsson Asting A, Lönnroth C, Derwinger K, Wettergren Y, Nordgren S, Gustavsson B, Lundholm K.
Genomic CGH assessed structural DNA alterations in rectal carcinoma as related to local recurrence following primary operation for cure.
Manuscript.

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THESIS AT A GLANCE

Paper	Study design	Specific aim	Main methods used	Patients	Main Results	Conclusions in brief
I	Quality Assurance Study	To analyse a substantial regional difference in local recurrence rate after rectal cancer surgery focusing on management.	Analysis of registry data.	651 regional patients compared with 3132 national patients diagnosed 1998-2000. Identified in the Swedish Rectal Cancer Registry.	6.6% difference - 7.1 vs 13.7%. Partial explanations: systematic errors of underreporting ($\leq 1.4\%$), differences in patient populations and indications for surgery difference in the use of neo-adjuvant radiotherapy ($\leq 1.0\%$) and some aspects of surgical strategy.	Registry data valuable - knowledge of specific limitations required. Surgical aspects – fundamental in reducing local recurrence rate. Educational effort initiated.
II	Medical Record Review	To evaluate the consequences of local recurrence.	Analysis of medical records.	57 patients with local recurrence treated at the Sahlgrenska University Hospital/Östra 1995-2003.	70% diagnosed in the interim between planned visits. 86% symptomatic. Most deemed incurable. Attempted resection in 18%. Poor outcome. Not well palliated. Resource demanding.	Local recurrence reality – severe symptoms and premature death. Yearly clinical follow-up post surgery – inadequate if cure desired.
III	Cohort study	To test the hypothesis that the risk of local recurrence is reduced by rectal washout.	Analysis of registry data.	4677 patients operated with anterior resection in Sweden 1995-2002. 3749 had rectal washout, 851 no washout and 77 with missing information. Identified in the Swedish Rectal Cancer Registry.	Local recurrence rate 6.0 vs 10.2% in washout vs no washout groups ($p < 0.001$). OR in favour of washout 0.56 in univariate analysis 0.61 in multivariate ($p < 0.001$). RR in all analysed subgroups < 1 in favour of washout (range 0.49-0.91).	Rectal washout – of importance for prevention of local recurrence. Rectal washout- should routinely be used in anterior resection for rectal cancer.
IV	Experimental Study	To test the hypothesis that there is a genetic difference in primary tumours that recur locally compared with non-recurrent.	DNA-extraction array-CGH quantitative PCR	From a total of 2576 patients in a local database 77 eligible patients. 6 patients with isolated early local recurrence and 12 matched controls with long term recurrence free survival.	Copy number variations in several known regions of relevance for colorectal cancer in both groups. Less pronounced in locally recurrent. The 4q31.1-31.22 region- significant copy number gain in recurrent group in array-CGH. Finding confirmed on group level in qPCR.	Genetic differences between recurrent and non-recurrent primary tumours - possibly contributory in the pathogenesis of local recurrence. The 4q31.1-31.22 region - of interest for further investigations.

ABBREVIATIONS

array-CGH	Array comparative genomic hybridisation, aCGH.
CEA	Carcinoembryonic antigen.
CGH	Comparative genomic hybridisation.
CIMP	CpG island methylator phenotype.
CIN	Chromosomal instability.
CpG	A dinucleotide consisting of the two DNA-bases cytosine-guanine linked with a phosphodiester bond and CpG islands are regions with a high frequency of this dinucleotide.
CRM	Circumferential resection margin.
DNA	Deoxyribonucleic acid.
FAP	Familial adenomatous polyposis.
GWAS	Genome-wide association study. Also referred to as WGAS. whole genome association study.
HNPCC	Hereditary non-polyposis colorectal cancer.
MDT	Multi-disciplinary team (conference).
MSI	Microsatellite instability.
MRF	Mesorectal fascia.
NGS	Next generation sequencing. Also referred to as third generation sequencing.
PCR	Polymerase chain reaction.
PME	Partial mesorectal excision.
qPCR	Quantitative polymerase chain reaction. Also referred to as real-time PCR.
TEM	Transanal endoscopic microsurgery.
TME	Total mesorectal excision.
TNM	Classification of malignant disease based on primary tumour, regional nodes and distant metastases. Published by the International Union Against Cancer (UICC) and also used by the American Joint Committee on Cancer (AJCC).

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INTRODUCTION

Background

“All these cases are condemned to die either from the immediate effects of intestinal obstruction or... ..they are abandoned without hope to linger on for a few months until death relieves them from their loathsome condition”. This was written in the Lancet by HW Maunsell in 1892, who was referring to patients with unresected rectal tumours, but has often been quoted in reference to the situation for patients with local recurrence after rectal cancer surgery¹. Left untreated, local recurrence is associated with severe, often intractable, symptoms and premature death. The predominant symptom is reported to be pain²⁻⁴. Patients can also suffer from bleeding, discharge, odour and affected bowel function with, e.g., bowel obstruction or faecal incontinence.

A meticulous surgical technique with a specimen-oriented dissection has been demonstrated to have a profound impact on the local recurrence rate⁵⁻⁹. The concept of TME (total mesorectal excision) is widely accepted. It is also established that radiotherapy, and especially preoperative radiotherapy, further improves the results¹⁰⁻¹³. Today, chemoradiation is used as oncological “conversion therapy” and can facilitate a resection with clear resection margins in patients in whom this was not possible with acceptable morbidity. However, no universally agreed-upon criteria yet exist for selection of patients for different treatment modalities. Despite continuous advances in medical oncology and reports of selected patients cured without surgical intervention¹⁴⁻¹⁸, surgery remains a prerequisite for cure in the overwhelming majority of patients.

The serious implications of a local recurrence make it a commonly used endpoint in clinical trials. However, the consequences for affected patients and the management thereof are less well described in modern day practice.

Reporting on local recurrence rate

The local recurrence rate can be calculated in several ways, which affects the rate reported. Most commonly, it is given as a crude rate in percent (No of patients with local recurrence / No of patients at risk)¹⁹. This should make it easy enough to compare results. However, several pitfalls need to be considered.

First of all, the time of surveillance should be comparable. Currently, the five-year recurrence rate is considered the gold standard. Possibly, the rates after shorter intervals are correlated to the rate after five years. The actual time span can be considered less relevant as long as it is consistent. Obviously, the proportions of patients followed for a shorter period of time or lost to follow up are important. Previously, the rate at two years was said to reflect more than half of the final local recurrence rate and that 95 percent of all recurrences were diagnosed at five years. However, some reports show that the proportion of late recurrences is higher after radiotherapy^{11, 13, 20, 21}. The possible further deferring effect after more aggressive chemoradiotherapy is not clear. An argument used by proponents of even longer follow-up periods is to avoid deflating the final local recurrence rate.

Secondly, if the denominator is restricted to “curative” resections, the rate will likely be different from when all resections are included. Subjectivity is a risk when defining a “curative resection”. If only patients with “excellent prognosis” are included, the results are likely to be “excellent”. On the other hand, the other extreme poses the competing risk of death (vide infra). A pragmatic definition of the denominator, i.e., patients at risk, could be *all patients who have undergone an excision of the tumour or resection of tumour-bearing segment of the bowel*.

The numerator should also be clearly defined. For instance, if only patients with isolated local recurrence are reported, approximately 40-50% will be omitted due to simultaneous distant metastases²²⁻²⁶. This figure might be further affected if the diagnosis is based on radiology, clinical examination or histological confirmation. In PubMed, the mesh-term “Neoplasm Recurrence, local” is defined as *“The local recurrence of a neoplasm following treatment. It arises from microscopic cells of the original neoplasm that have escaped therapeutic intervention and later become clinically visible at the original site”*²⁷. Applying this definition strictly, tumour growth after surgery with, e.g., grossly involved margins should be termed “persistent” rather than “recurrent” disease. The consequences for afflicted patients are likely to be similar, regardless of semantics, and one can argue that all patients that develop a tumour growth in the pelvis post surgery (according to the defined denominator) should be accounted for. A wider definition, more suitable for reporting purposes, would be *any recurrent tumour growth below the level of promontory, regardless of distant metastases, histological confirmation and means of diagnoses*.

Finally, whether the crude local recurrence rate represents the optimal reporting variable is debatable. Theoretically, a low local recurrence rate can be due to bias caused by a competing risk. For instance, an extremely high mortality in the first post-operative year or study of an extremely aged patient population will reduce

the number of local recurrences. This can be accounted for by competing risk methodology. Some argue for the routine use of the Kaplan-Meier method for reporting a local recurrence rate¹⁹. The argument is that this also adjusts the number at risk through censoring; for instance, those lost to follow-up and death. Finally, a graph with cumulative incidence related to time elapsed is also more informative than just a numerical value, regardless of method used.

Rectal washout

Adenocarcinomas in the rectum are known to shed viable cells into the rectal lumen²⁸⁻³⁰. Strong evidence indicates that these cells have the ability to implant³⁰⁻³⁶. Raw surfaces, such as the areas of pelvic dissection or the suture line at the bowel anastomosis, are potential sites of implantation.

Intra-operative rectal washout, with a clamp distal to the tumour, diminishes the number of exfoliated tumour cells in proportion to the amount of solution used^{37, 38}. A number of solutions with cytocidal properties have been used with somewhat conflicting results^{39, 40}. In Basingstoke, the centre where the TME-concept was coined and excellent figures on local recurrence have been produced, rectal lavage has always been “*a part of the routine*”⁷. However, the evidence for an effect of rectal washout on local recurrence after rectal cancer surgery is not conclusive. No randomised controlled studies have been published and only a few comparative studies exist. A meta-analysis from 2008 of five non-randomised studies demonstrated a local recurrence rate of 10.2 percent in the ‘no washout’ group and 4.8 in the ‘washout’ group⁴¹, albeit not statistically significant (OR 0.64, 95% CI 0.20-2.04, $p=0.45$) in the limited patient cohort (246 and 166 patients respectively). In 2011, another meta-analysis was published⁴². However, more than 90% of the patients in this meta-analysis were derived from *paper III* included in this thesis and the conclusions were thus based mainly on one publication.

Follow-up after rectal cancer surgery

The overall aim of follow-up is to improve chances of long-term survival by treating new or recurrent disease. Non-oncological aspects of follow-up are also relevant^{43, 48}. In 2002, the general understanding that no survival benefit was associated with follow-up of patients treated for colorectal cancer was challenged by two systematic reviews^{44, 45}. To date, at least 8 randomised

controlled trials and 6 meta-analyses have been published on the topic⁴⁶⁻⁴⁸. These can be criticised on several accounts, but a simplistic and positive interpretation is that “something is better than nothing” and “more is better than less” with a survival benefit for intense follow-up.

Despite the number of studies with mostly high-quality design, insufficient support exists for determining the optimal surveillance modalities and intervals. This is reflected in the variation in current recommendations^{43, 49-54}. Commonly, the same follow-up is recommended for patients with colon- and rectal cancer and focus is on diagnosing distant metastases. Additional data are awaited from three large randomised controlled trials. GILDA stopped recruiting in 2006 and the results were planned to be published in 2010⁵⁵. The final results from FACS will be analysed in 2013⁵⁶, and the results from COLOFOL a few years later⁵⁷. However, certain issues will likely remain unresolved even after the results from these studies are reported. Among these: a decision on the optimal follow-up protocol for early detection of local recurrence after rectal cancer surgery in order to improve survival.

Molecular basis of rectal cancer

Colorectal cancer has a relatively high proportion of familial cases when compared with other common malignancies⁵⁸. The study of these has also provided insight and understanding of the genetic changes associated with the disease in sporadic cases. Some 15 (-30%) of colorectal carcinomas are estimated to have a major hereditary component^{58, 59}. However, <5% are attributed to highly penetrant mutations described in a clinical entity. Approximately 10 different groups of hereditary syndromes are described, with the most prevalent and well known being HNPCC (Hereditary non polyposis colorectal cancer) and FAP (Familial adenomatous polyposis) ^{58, 59}.

Several alterations in the genome of a cell are required for cancer to develop. This is facilitated by genetic instability. Three major pathways of genetic instability are described in the context of colorectal carcinogenesis⁵⁹⁻⁶¹.

- 1) Chromosomal instability (CIN) is present in approximately 85% of sporadic colorectal cancers. The mechanism is unclear but genes that regulate the alignment and segregation of chromosomes at mitosis may be involved.
- 2) Microsatellite instability (MSI) is characteristic in approximately 15% of cases and involves defects in mismatch-repair genes. These genes are crucial for repair

of defects in the many repetitive sequences (microsatellites) that are common and scattered in the genome.

3) CpG island methylator phenotype (CIMP) is present in approximately 15% of colorectal cancers and is caused by epigenetic silencing of genes by methylation of the DNA-base cytosine. (CpG is an abbreviation for a dinucleotide consisting of the two DNA-bases cytosine-guanine linked with a phosphodiester bond and CpG islands are regions with a high frequency of this dinucleotide).

Chromosomal instability (CIN) can cause chromosomal aberrations that are either balanced or unbalanced. The result of unbalanced aberrations is that the genome will contain more (copy number gain) or less (copy number loss) of a whole or parts of a chromosome. In colorectal cancer, copy number gains and losses are mainly described on chromosome 5, 8, 13, 17, 18 and 20^{59, 62-64}. The relevance of chromosomal aberrations specific for rectal carcinoma is less well characterised.

Genetic instability increases the genetic variability between cells in a tumour, leading to increasing heterogeneity. However, clonal selection causes some cells to dominate. Not surprisingly, then, tumours of the same anatomical and histological origin can vary in several aspects of aggressiveness, such as ability to set distant metastases⁶¹. Genetic differences between rectal tumours also possibly affect their ability to recur locally. This issue has not been explored.

AIM

The overall objective of this thesis was to acquire a deeper understanding of local recurrence after rectal cancer surgery including pathogenesis, pathophysiology, symptoms, diagnosis, treatment and prevention.

The specific aims of the included studies were:

- Paper I: To analyse a substantial regional difference in local recurrence rate after rectal cancer surgery focusing on management.
- Paper II: To evaluate the consequences of local recurrence.
- Paper III: To test the hypothesis that the risk of local recurrence is reduced by rectal washout.
- Paper IV: To test the hypothesis that a genetic difference exists in primary tumours that recur locally compared with non-recurrent tumours.

PATIENTS

Paper I

This study included national data from the Swedish Rectal Cancer Registry with patients diagnosed from 1998 through 2000. The study group comprised patients from the Western Sweden Health Care Region (775 patients). The reference group consisted of patients from Sweden excluding the regional cases (3764 patients). Specific focus was put on patients that had the tumour resected/excised and had a valid 5-year follow-up (651 and 3132 respectively).

Paper II

At the time of the study, data from valid 5-year follow-up was available in the Swedish Rectal Cancer Registry 1995-2003, defining the study period. Of all patients registered, a total of 671 were managed at the Sahlgrenska University Hospital. The final studied cohort consisted of 57 patients where the registered local recurrence could be confirmed by evaluation of the medical records.

Paper III

Included in this study this study were patients who had undergone anterior resection, Dukes' A-C (\approx TNM Stage I-III) and a valid 5-year follow-up in the Swedish Rectal Cancer Registry at the time of data extraction (1995-2002). A total of 4677 patients were analysed (3749 who had rectal washout, 851 no washout and 77 with information missing).

Paper IV

The patients for this study were identified in the clinical database linked to the bio-bank at the Surgical-Oncology Laboratory, Sahlgrenska University Hospital/Campus Östra. From a total of 2576 patients with colorectal cancer, two groups of patients operated for rectal cancer with negative resection margins (R0) between 2002 and 2006 were selected. Six study patients were diagnosed with early local recurrence, whereas 12 matched control patients had no sign of local or systemic recurrence at long term follow-up. These patients were selected among the 77 finally eligible patients in TNM stage I-III (excluding T4) without preoperative radiotherapy.

METHODOLOGICAL CONSIDERATIONS

Overview of methods used

	Study design	Main methods used	Main statistical methods used	Main methodological pros and cons with chosen study design
I	Quality Assurance Study	Analysis of registry data	Descriptive statistics Kaplan-Meier estimate Competing risk method Relative risk estimates Simulated effect estimates Observed and relative survival analysis.	+ Commonly large study base, + Availability of data. + External validity often good if population-based with acceptable coverage and loss to follow-up. - Knowledge of specific limitations required. - Validity of data crucial. - Internal validity questionable if confounders not accounted for.
II	Medical Record Review	Analysis of medical records	Descriptive statistics only	+ Can address issues of exposures impossible to randomise. (e.g. harmful) + Analysis of rare events hard to record prospectively. + Studies of disease patterns, quality assurance and pilot studies. - Risk of selection bias. - Risk of systematic under-reporting and missing data. - Risk of bias in data abstraction due to subjectivity of unblinded abstractor, ambiguous variable definitions and inter-abstractor variability –observer bias.
III	Cohort study	Analysis of registry data	Descriptive statistics Chi-square test Two-sample <i>t</i> -test Univariate logistic regression Multivariate logistic regression Relative risk estimates	As Paper I
IV	Experimental Study	DNA-extraction aCGH, qPCR. Analysis of pooled array-data and individual PCR-data	Descriptive statistics One sample <i>t</i> -test ANOVA Comparative Cq-method	Array-CGH: + Powerful tool for whole genome analysis of gDNA or cDNA. + Possible to compare several combinations of samples simultaneously. + Can be used on both individual and pooled material. - Cost and availability. - Lack of accepted criteria of evaluation and analysis. - Explorative and may need confirmation by other method.

Table 1. Overview of methods used in Paper I-IV.

The Swedish Rectal Cancer Registry

From a practical point of view, the Swedish Rectal Cancer Registry covers all patients with rectal cancer in Sweden since it was launched in 1995. However, when analysing data from the registry, note that not all patients with a malignant tumour in the rectum are registered. Included patients, are those with a primary rectal adenocarcinoma with the lower border 15 cm or less from the anal verge. Other tumours (e.g., squamous cell carcinoma, sarcoma, malignant melanoma, carcinoid tumours, teratomas and overgrowth from adenocarcinoma in another organ) are not included. Patients with carcinoma in situ and patients diagnosed at autopsy are also excluded. These restrictions are of minor importance in most analyses of registry data, but could be of relevance in a comparison with data from other national registries. The national coverage varies from 96 to 100 percent among years. Random selection of the missing and ineligible patients does not affect analysis of registry data, but if a systematic reason exists why some are missing, this increases the risk of selection bias in certain analyses, especially certain sub-group analyses. The reasons for missing patients have not been fully investigated, but the most common reason in later years is that individual hospitals fail to report a proportion of their patients in time for analyses.

Patients are followed for 5 years in the registry and less than 2 percent are lost during follow-up. The definition of a valid follow-up is either death within the follow-up period or a form sent to the registry at least 54 months post surgery. Date of death is retrieved from official demographic registries and is unlikely to be faulty. In this case, the slight discrepancy between the studied and actual cohorts is also likely to be insignificant in most analyses. However, the risk of bias in certain analyses is present if loss to follow-up is systematic. Information about why patients are lost is limited in most cases. The assumption is that some patients are underrepresented among the lost since they are less likely to be still living five years post surgery (e.g., patients, patients with advanced tumours, disseminated disease, unclear resection margins, serious co-morbidities and elderly patients).

The abovementioned potential causes of bias are irrelevant for the analyses in this thesis, except in *paper I*, where special consideration has been taken. For most purposes, the Swedish Rectal Cancer Registry can be viewed as a population-based registry with near complete national coverage and minimal loss to follow-up.

An important issue in all research based on registries is the accuracy and validity of the data. The Swedish Rectal Cancer Registry is currently being validated. All variables are compared to the medical records of 500 randomly selected patients treated in recent years. The results are not available at the time of writing this thesis. A previous validation of six variables in the 1996 cohort showed 94-97% accuracy. Several variables have also been validated in limited cohorts within different research projects. According to a study by Jörgren et al,⁶⁵ a registered “local recurrence” could not be confirmed in 35 of 326 cases (11%) when the medical records were reviewed. A similar finding was noted in *paper II*, where 11 of 68 registered local recurrences were registered erroneously. As discussed in *paper I*, in all likelihood, the local recurrence is underreported in some specific subgroups. A local recurrence is defined in the registry as a recurrent tumour growth below the promontory. Clinical diagnoses or imaging are accepted. Histological confirmation is not required. A variable that gains some attention in *paper III* is “rectal washout”. This variable is difficult to validate from the medical records since the absence of its mentioning does not mean it has not been performed, while the opposite is less likely. Rectal washout is not clearly defined in the registry and a potential risk of misconception exists. The intended understanding is transanal washout of the rectal lumen, distally to an occluding clamp, before the bowel is transected.

Another pertinent issue is the delay in updating data and thus, the question at which point data are reliable. Data regarding the initial treatment are readily available but 5-year data on outcome have not been reliable until 6-7 years post the initial treatment. Initially, reports regarding each patient were requested for the registry on a yearly basis and at the time of an event such as recurrence. Unfortunately, adherence to this practice was low and data were often only reported at the end of the follow-up period of five years. The compilation, analyses and final release of data on outcome has extended the delay by another year. Thus, data on outcome have been criticised for reflecting “historical” conditions. The process has since been revised and 3-year data will be published a few months into each new year while waiting for the final 5-year follow-up data release.

In summary, the Swedish Rectal Cancer Registry is a unique source of information, but data should be analysed thoroughly while bearing in mind the known and potential limitations related to selection, validity and study design.

The local database and biobank

The local database and biobank at Sahlgrenska University Hospital/Campus Östra are maintained by the Surgical Oncology laboratory. In the *biobank*, samples from, e.g., blood, tumour and mucosa are stored at -80°C. Consecutive tissue samples have been collected since 2002 from virtually all elective resections for colorectal cancer. Occasional tissue samples may be lacking in cases where the procedure is not completed in office hours or when laboratory personnel were not at hand at the time of specimen extraction for other reasons. Identification or analyses of cases with missing tissue samples has not been performed. Theoretically, this should be taken into account in specific study designs, but was not considered relevant in the study described in this thesis (*Paper IV*).

The biobank is linked to a database with clinopathological variables of all patients with colorectal cancer treated at this institution since 1999. The *database* is cross-checked against records of hospital admissions and operative planning systems to verify that no patients are missing. The patient cohort is derived from a catchment area of approximately 500.000 and can, in that respect, be considered population-based. However, a few patients are referred from hospitals outside the catchment area for reasons such as locally advanced tumours or personal preference. A few patients from the catchment area have also undergone operations in other hospitals due to emergently presenting symptoms. More importantly, a non-negligible cohort of patients, mainly with less-advanced colon cancer, underwent operations in other hospitals prior to 2006 and some also during specific periods of time thereafter. Reasons for this included educational purposes and, at times, inability to deal with the caseload of the catchment area. These limitations raise questions whether the cohort in the local database is really population-based. Nevertheless, this does not affect the design or methodological aspects of *Paper IV*, where the database was utilised. The data in the local database have not been systematically validated. This is in part compensated for by the fact that the database is updated at regular intervals.

The delay in updating data and the question at what point data are reliable have hampered the use of outcome measures from the Swedish Rectal Cancer Registry. However, these are not issues regarding the local database, which is updated twice yearly to assure reliable and current data. Furthermore, patient files are screened for new events without a defined time limit, thereby enabling long-term follow-up.

At the time of writing, no data transfer occurs between the local database and the Swedish Rectal Cancer Registry.

In conclusion, the cohorts in both the database and the biobank should be viewed as “institution derived” rather than “population based”. Keeping this in mind in study design and interpretation, the combination of a large biobank and a linked clinopathological database of this extent should be acknowledged as possibly the only one of its kind in the colorectal field worldwide.

Array-CGH, Comparative genomic hybridisation

Comparative genomic hybridisation (CGH) was developed to detect large chromosomal differences between two DNA-samples, where the chromosomes are studied in metaphase. Array-CGH allows for a detailed analysis of the entire genome, by comparing two different samples at tens of thousands of sites of the genome. In the specific array-CGH used in *Paper IV*, more than 55 000 sites are compared. The median spacing is 33.3 kb in coding sequences and 78.9 kb in non-coding sequences.

In a simplified description of the method, DNA from a sample and DNA from a control are “chopped” up into minute pieces (oligonucleotides) and tagged with two different fluorescent colours. DNA from sample and control are mixed and distributed on an array-plate with thousands of DNA-probes (other oligonucleotides). For probes where the pieces from the sample have adhered (hybridised), the sample’s fluorescent colour will predominate. This is interpreted as the sample having more DNA with that particular sequence than the control (copy number gain). Advanced software can thereafter present data for the entire genome, a specific chromosome or part of a chromosome (Fig 1). Even affected genes can be represented.

Array-CGH can only detect copy number changes (i.e., gains and losses) caused by unbalanced chromosomal aberrations. Consequently, balanced changes, where the actual copy number remains the same, will not be detected.

As is found with many methods in molecular biology, potential causes of bias can arise in several steps of an array-CGH analysis, ranging from the extraction of DNA, via labelling and hybridisation to detection of fluorescence. A separate challenge arises when analysing the data, as conventional statistical methods are

often inadequate⁶⁴. The field of bioinformatics is evolving, but no consensus yet exists for presentation and analysis of array-data in biomedical journals.

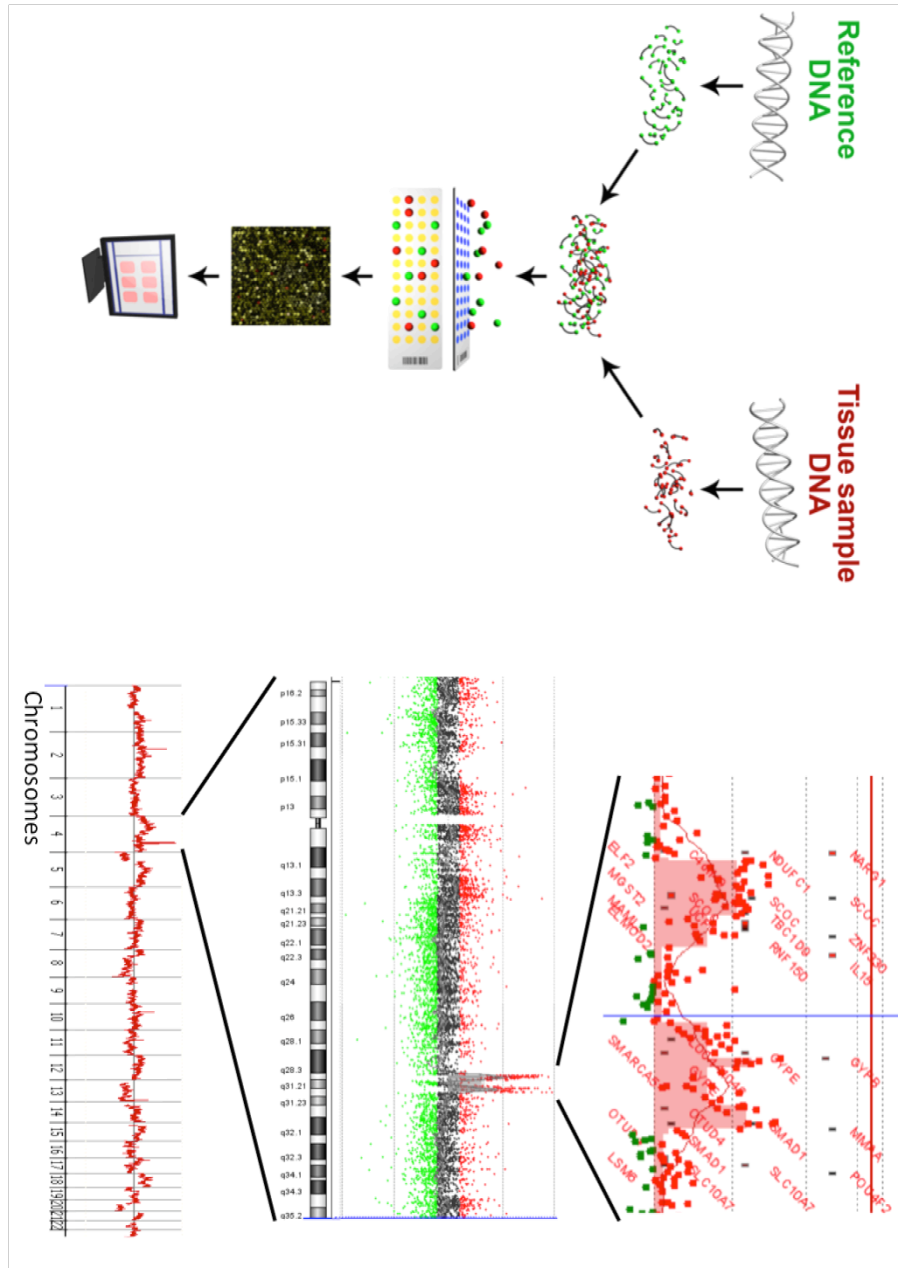


Fig 1. Schematic presentation of array-CGH analysis. Illustration by Jacob Kodeda.

qPCR, Quantitative Polymerase Chain Reaction

The polymerase chain reaction (PCR) was developed to detect specific, limited regions in the DNA by serial amplifications (cycles). In quantitative polymerase chain reaction (qPCR or real-time PCR) the exponential pattern of amplification is utilised to measure the relative amount of a specific region or sequence in a sample. In brief the number of cycles required to reach a preset level is recorded. In the commonly used comparative C_q-method the number of cycles needed is compared to a reference and adjusted for the efficiency of the respective assays.

Statistical considerations

Distinction should be made between findings that are clinically significant and statistically significant. This is especially important when working with large data sets and small groups respectively. The absolute and relative risks should also be clearly differentiated. Statistical significance was in the majority of cases in this thesis defined as a two-sided p-value of <0.05. In analyses of OR (odds-ratio) and RR (relative risk) 95% confidence intervals were considered more informative than p-values and were also stated where applicable. The 95% confidence intervals not including 1 were considered statistically significant. For the comparison of non-randomly selected groups matching or multivariate analysis were utilised.

Ethical considerations

The investigations and studies included in this thesis were concluded within the frames of an approval by the regional ethical review board on May 19th 2008 (Dnr 261-08).

RESULTS AND COMMENTS

Paper I

Regional differences in local recurrence rate after rectal cancer surgery.

For patients who underwent operations in 1998-2000, the crude local recurrence rate after rectal cancer surgery in the Western Sweden Health Care Region was 13.7%. In Sweden, excluding patients from the Western Sweden Health Care Region the crude rate was 7.1%. For patients operated with curative intent, the corresponding figures were 11.9% and 6.5%, respectively. Local recurrence rates produced with competing risk estimates were 13.4 vs. 7.0%, respectively. The five-year cumulative probability using Kaplan-Meier estimates were 16.8 vs. 9.0%, respectively.

The absolute difference in crude local recurrence rate between the cohorts was 6.6% (RR 1.9, 95% CI 1.5-2.4) and could partly be explained by several factors. An effort was made to quantify the theoretical maximal effect of possible contributing factors available in registry data. The effects were not viewed as additive since the subgroups were partly overlapping.

Slight differences were noted in the patient population characteristics and in the proportion of patients that received surgical treatment, which most likely reflected differences in time of detection, selection and preoperative work-up. The—likely small—theoretical effect of these differences was not considered quantifiable. Indications of national underreporting in two specific subgroups could account for no more than 1.4 and 1.3 of the 6.6 percent difference. Identified inadequate registration, which led to over-reporting in the regional cohort, accounted for 0.9 percent. Differences in the use of preoperative radiotherapy accounted for 1.0 percent or less of the difference. A substantial part of the difference between the cohorts could not be explained by registry data. However, some indications pointed to the importance of differences in several aspects of surgical management .

Comments

Please consult the discussion on reporting practices and variations in calculation of local recurrence rate included in the introduction to this thesis (page 1).

Regardless of the methodology used for calculation, the difference between the cohorts persisted, which necessitated a more detailed analysis. This can be criticised for not accounting for all possible contributing factors, but the prerequisite of the study was analysis of data from a quality assurance registry, since this is where the difference in local recurrence rate was first detected. Certain variables would have been of interest to study, had they been registered. The analysis can be further criticised for implying that the unexplained portion should be attributed to substandard surgical care. However, this was the overall design of the study and to “leave no stone unturned”. An issue of relevance not discussed in the publication (*paper I*), is that some of the surgical departments in the region were reorganised shortly before and during the relevant period of time. As shown in *Fig. 2*, in the years preceding and following the study period, the local recurrence rate in the region more closely paralleled that of other regions. Therefore, reorganisations might also have had an effect outside the reorganised units. For patients operated in 2005, once again the regional figures underline the importance of continuous monitoring, analysis and action.

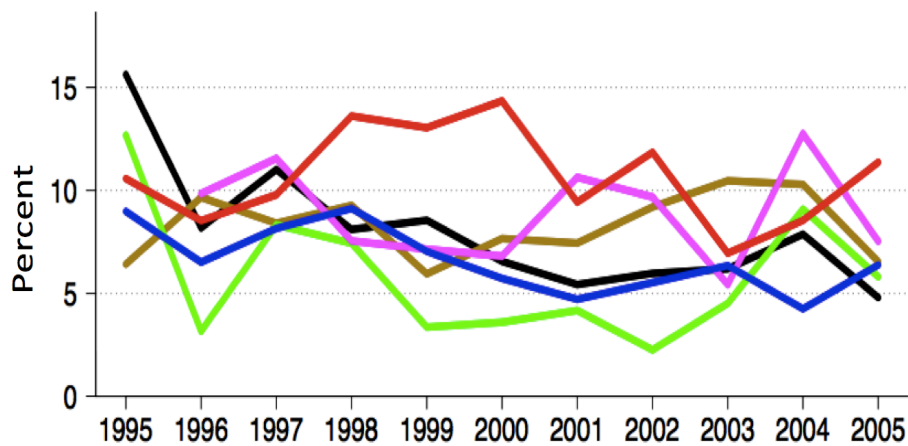


Fig 2. Five-year local recurrence rate per year of initial surgery, grouped on the basis of health care region. The red curve shows the data for the Western Sweden Health Care Region. Republication of data from official reports, with kind permission from the national working group for the Swedish Rectal Cancer Registry.

Paper II

Local recurrence of rectal cancer – A cohort study with focus on diagnosis, treatment and outcome.

The majority of patients with local recurrence were symptomatic at the time of diagnosis (86%; 49/57). Most were diagnosed in the interim between planned, annual follow-up visits (70%; 40/57). The most prevalent presenting symptom was pain, followed by bleeding (or anaemia), urogenital symptoms and changed bowel habits. The majority of local recurrences were diagnosed on clinical examination, where endoscopy played an important role. The most common finding was visible or palpable tumour growth. The predominant location was in the lower third of the pelvis and the most common site was the anastomosis or upper part of the rectal stump.

The mean and median times to recurrence after the primary resection were 22 and 18 months, respectively. The mean and median survival durations from the time of diagnosis of local recurrence were 12 and 9 months, respectively.

Resection or excision of the recurrence was attempted in only 18% (10/57), despite the fact that 40% (23/57) had no evidence of systemic disease. In total, 8 out of 10 operated patients had an R0-resection (clear resection margins) but 3 had a local re-recurrence. A total of 55 of 57 patients were dead within five years after the initial surgical procedure for rectal cancer and only one patient had a documented cause of death not related to the recurrence. The majority needed prescription of opiate-based analgesia and had symptoms that were difficult to palliate adequately. Radio- and chemotherapy was attempted in 16 and 14 patients, respectively, with mainly positive effects. During the last year in life, the patients required, on average, a full month of hospitalisation, two operative procedures and a visit to the outpatients department every second week.

Comments

Surveillance following rectal cancer surgery, in the form of annual visits with clinical examination including rigid rectosigmoidoscopy, was not sufficient to detect local recurrences at an early, asymptomatic stage. One could argue that early recurrences might be more aggressive in nature and therefore early detection may select the poorest candidates for potential curative therapy. However, symptomatic recurrences are associated with poor prognosis^{3, 26, 66, 67}. Most recurrences in this cohort were deemed incurable at the time of diagnosis, which probably in part explains the poor outcomes. This also possibly explains

why only 60% (34/57) were confirmed on histology and why staging with imaging was not commonly used.

Patients with local recurrence after rectal cancer surgery require frequent interventions from the health care provider. Even in modern day practice, recurrence is associated with severe, intractable symptoms and premature death. Palliation has obvious room for improvement, from the patients' perspective.

Apart from the general criticism that can be applied to retrospective medical record reports from a single centre, this particular study can be questioned from the point of view that many patients were treated approximately 10 years ago. It would be interesting to repeat the analysis on patients treated after the study period, i.e., since 2004. To date, only preliminary and nonvalidated data are available (*Table 2*). Evaluation of a follow-up protocol aimed at detecting local recurrences at an early stage would also be interesting.

	Number of patients
	n/n
Operated for local recurrence	19/25
R0-resection	15/19
No new recurrence	5/15
Deceased	15/25
Alive with malignant disease	6/25
Alive without known malignant disease	4/25

Table 2. *Unpublished, nonvalidated data from the clinical database at Sahlgrenska University Hospital/ Östra regarding patients with local recurrence treated after the study period. Patients underwent operation for primary rectal tumour 2004-2010. Of patients with "curative" resection or excision 25 developed local recurrence without synchronous distant metastases. Different dates of latest follow-up, at least one year before data extraction 11 Nov. 2011. Data should be interpreted cautiously due to these limitations.*

Paper III

Rectal washout and local recurrence after rectal cancer surgery.

The local recurrence rate in the 3749 patients who underwent rectal washout was 6.0%, compared to 10.2% in the 851 patients that had no washout ($p < 0.001$).

Univariate analysis with logistic regression favoured washout with an odds-ratio of 0.56 (95% CI 0.43-0.72, $p < 0.001$). The patients differed between the groups in proportion of preoperative radiotherapy, curative resections/radical surgery, peritumoural perforations and age. These differences were taken into account by use of a multivariate analysis with logistic regression, which favoured washout with an odds-ratio of 0.61 (95% CI 0.46-0.80, $p < 0.001$).

The multivariate analysis was repeated after restricting the study base to patients without peritumoural perforations or anastomotic leakage and in whom the resection was deemed locally radical and curative (2521 patients out of 4510). The odds-ratio was consistent with the previous findings, 0.58 (95% CI 0.39-0.87, $p = 0.009$).

In total, 22 subgroups were also studied. The relative risk of recurrence was < 1 ; i.e., favouring washout in all studied subgroups (RR range 0.49-0.91). The relative risk was similar in radiated and non-irradiated patients (0.65 vs. 0.64, $p < 0.05$ for both)

Comments

The main limitation of this non-randomised study is the risk of selection bias and confounding. Some potential confounders have been taken into account, but other factors in the care of rectal cancer patients that could not be evaluated from registry data may remain. The central issue is causality. The findings in this study are supported by applying the criteria proposed by Hill for evaluating causality in observational research⁶⁸⁻⁷⁰:

1. Strength of association: Is there a strong effect, measured as relative risk or odds ratio?
2. Consistency of association: Have others seen the effect?
3. Specificity of association: Does exposure lead only to outcome?
4. Temporal sequence: Does exposure precede outcome?
5. Biological gradient: Is there a dose-response relation?

6. Biological plausibility: Does the association make sense?
7. Coherence: Is the association consistent with existing, available evidence on natural history and biology of the disease?
8. Experimental evidence: Has a randomised controlled trial been done?
9. Analogy: Is the association similar to other exposures?

In view of the lack of randomised trials on the topic and the availability of only a few underpowered comparative published trials, the authors believe that the findings are clinically relevant. Performing a study where patients would be randomised to no washout would also be considered unethical. The procedure is simple, cheap, not very time consuming and has a very favourable ratio of needed to treat/needed to harm. Therefore, the authors recommend rectal washout distally to the tumour prior to transection and anastomosis in the anterior resection of adenocarcinoma of the rectum in order to reduce the risk of local recurrence.

The above statement has initiated some discussion; please see letters to the journal and the author replies in BJS, attached in the appendix.

Patients with local recurrence/all operated	
	n/n (%)
Operated 2003	
Rectal washout	20/532 (4)
No washout	7/118 (6)
Operated 2004	
Rectal washout	25/556 (4)
No washout	6/75 (8)
Operated 2005	
Rectal washout	21/551 (4)
No washout	7/86 (8)

Table 3. Local recurrence during five-year follow-up in relation to rectal washout in patients operated for anterior resection in Sweden after the study period, national data. Republication of data from official reports, with kind permission from the national working group for the Swedish Rectal Cancer Registry.

Paper IV

Genomic CGH assessed structural DNA alterations in rectal carcinoma as related to local recurrence following primary operation for cure.

Previously described aberrations, known to be involved in colorectal carcinogenesis; e.g., on chromosomes 5, 8, 13, 17, 18 and 20^{59, 62-64}, were identified in primary rectal tumours. The magnitude of aberrations was more pronounced in non-recurrent tumours in areas with both copy number gain (chromosomes 5, 8, 13, 20) and copy number loss (1, 4, 5, 17, 18, 22).

However, an area on chromosome 4 (4q31.1-31.22) displayed a significant copy number gain specific for the tumours that recurred locally, when compared with reference DNA, $p < 0.0001$. It was also specific for tumour-DNA compared with mucosa from the same patients, $p < 0.0001$. The aberration was not detected in the non-recurrent group when compared with reference DNA.

Analysis of the specific region identified 22 affected genes, some of which code for products of interest in tumour biology, such as cellular growth, p53-regulation, progression through the cell cycle and regulation of apoptosis.

The finding on array-CGH regarding the 4q31.1-31.22 region was analytically confirmed on patient group level with qPCR.

Comments

This study would not have been possible without the availability of a large clinical database with linked clinicopathological data. The availability of tissue and data allowed for careful matching of groups. The samples in the biobank are also unique in that many patients have not been irradiated preoperatively. This eliminates some potentially important causes of bias in genetic analysis.

The role of adequate surgery and adjuvant therapy in the prevention of local recurrence is undisputable. Our null hypothesis was that these factors are of such importance that possible genetic differences between locally recurrent and non-recurrent tumours would not be detectable.

The fact that the previously described aberrations were generally more pronounced in the non-recurrent group can be interpreted as indicating that they are not of key importance in the context of local recurrence. The identified

region on chromosome 4 is of great interest for further studies and indicates that the null hypothesis might be false. However, it should be stressed that this finding needs to be confirmed in other, and preferably larger, patient series.

If these results can be reproduced and confirmed, they indicate that previously undescribed inherent differences exist between rectal tumours that recur locally and tumours that do not. If this is the case, the prudent question is how *clinically relevant* this may be. If a prognostic factor based on genetic testing can be identified, this could have implications for both oncological and surgical treatment of the disease—under the condition it is also predictive of the treatment effect.

Several limitations are recognised in this work

1. The matching of groups was not as accurate as initially designed in the selection of patients. Two patients in the non-recurrent group were excluded due to tumour-DNA degradation and one patient in the recurrent group contributed two separate tissue samples. Despite this, groups were well matched on predefined matching criteria.
2. Tissue material for this study was not micro-dissected and colorectal tumours are well known to consist of other cells than cancer cells, with approximately 50% being stroma and macrophages. However, even if the detected aberrations were confirmed to derive from non-cancerous cells, the differences between the study groups remain.
3. Pooling of samples is always a debatable practice, but in this exploratory study design, it is a prerequisite for comparison at the group level with reasonable cost-efficiency.
4. The qPCR analytically confirmed the data obtained from array-CGH at the group level, but failed to do so at an individual level. Numerical differences exist between patients, and one patient displayed a greater copy number gain compared to the others on the relevant area on chromosome 4, but the small number of patients in the study precluded a more detailed analysis.
5. Analysis of genomic DNA raises questions regarding what is transcribed to RNA, spliced, translated to amino acids and eventually folded into functional products. Detection of amplified regions does not imply expression of relevant genes, due, for instance, to possible methylation. On the other hand, an identified region might have been relevant during earlier carcinogenesis.
6. The massive amount of information in array data requires powerful software and a structured method of evaluation. The significance level was set at $\pm 0.2 \log(2)$ ratio, as determined in an earlier study⁶⁴. The significance level can be discussed, but even with a substantially higher significance level, the noted aberrations would still be detected.

GENERAL DISCUSSION

Despite recent advances in rectal cancer treatment, local recurrence remains a treatment failure for modern medicine, with disastrous consequences for affected patients. Even in modern day practice, local recurrence is associated with intractable symptoms and premature death in the majority of patients.

Detection and management

Support is sparse for different modalities of follow-up and the appropriate intervals, despite the abundance of trials and published studies. Focus has mainly been on the detection of liver metastases in patients treated for colorectal cancer, in part because this represents a large patient category and in part due to improved results. Resectability for liver metastases is no longer limited to a few deposits in one lobe, but rather by the amount of functional liver parenchyma remaining. The limit is further pushed by conversion chemotherapy (which allows possible resection of previously inoperable disease), induced hypertrophy by selective portal embolisation and staged resections. A similar development can be foreseen for the treatment of local recurrence after rectal cancer. Today, we can see multimodal therapy emerging from a previously defeatist approach. Oncological adjuncts such as chemoradiation, brachytherapy and IORT (intraoperative radiotherapy) can be combined with extensive surgical procedures, such as pelvic exenteration, partial/total sacrectomy and even hemipelvectomy. However, it should be stressed that the value of these measures needs clarification. Dedicated centres report long-term survival of selected patients with local recurrence between 23 and 57 percent^{3, 4, 23, 71-76}. (The reader is reminded of the above discussion on reporting clinical frequencies.) With improved methods of detection for recurrent disease at an early stage and increased availability of treatment options, surveillance programs will possibly aim at detecting recurrences at several levels: distant (e.g., liver, lung), regional (e.g., mesenteric, intraperitoneal) and local (e.g., pelvic). Note also that when discussing detection and treatment of local recurrence, future local recurrences might differ from the ones encountered in the past. With improved local control, the remaining local recurrences might develop later, with a higher proportion of synchronous distant metastases and more commonly lateral and presacral^{20, 21}. With this increasing complexity, risk-adjusted or personalised strategies of follow-up after surgery also should be contemplated.

Despite the encouraging prospects for treatment of recurrent disease, prevention is — by logical reasoning — the most efficacious, effective and efficient approach⁷⁷.

Prevention

In evaluating different preventive measures, it can prove beneficial to discuss the pathogenesis of local recurrence after rectal cancer surgery from a theoretical perspective.

Remaining solid tumour tissue, due to macroscopically involved margins (R2-resection), is unlikely to provide cure and the high risk of recurrent tumour growth is hardly debatable. Microscopically involved margins (R1-resection) also carry a very high risk of recurrence. Preoperative oncological treatment, such as conversion therapy, is important, but the crucial role of adequate surgery for preventing involved margins is undisputable.

Remaining solid tumour tissue in patients where the margins are clear (R0-resection) is a definite possibility. Firstly, the R0-classification depends on dedicated pathological analysis of the specimen and even so, there will be areas not assessed. Secondly, there are several possibilities of deposits with tumour tissue not in contact with the main tumour; e.g., pathological lymph nodes, areas of a previous tumour-associated abscess and lymphovascular deposits. Oncological treatment can sterilise some sites, but the role of surgery is also fundamental in eradicating these potential local recurrences. Including all tissue within the mesorectal fascia and achieving adequate distal margins are central. Other surgical aspects with relevance in this context include: level of vascular (thus lymphatic) ligation, extra-levator or ischio-anal excision in abdominoperineal resection and extended lymphadenectomy including “lateral” lymph nodes in the pelvic sidewall.

Remaining free tumour cells represent a third possible cause of local recurrence. The obvious case where a tumour is removed in pieces, rather than en-bloc with adjacent healthy tissue, often has a predictable negative clinical course. However, even the most perfect oncological resection can be futile, if viable free tumour cells are left in the operative field. This can occur after spilling of intraluminal tumour cells at the time of bowel transection or by preoperative perforation of the rectum. Free intraluminal tumour cells can also possibly be incorporated in the anastomosis or be rubbed off from the transected bowel ends during extraction of the specimen. Avoiding these situations is the rationale behind perioperative rectal washout. Interestingly, the long-term follow-up of the Dutch TME-trial, indicated that the highest risk of involved

resection margin was anterior, while the predominant location of local recurrence was posterior^{21, 78}. Theoretically, rinsing the pelvic cavity on completion of the surgical procedure could also be advantageous for eradicating remaining free tumour cells. Proponents of the latter procedure will argue that the lymphatic fluid or even blood, shed perioperatively, also can contain tumour cells²¹. However, the relative concentration of tumour cells is likely to be low compared to that of the bowel content next to the tumour.

The three theoretical pathogenic explanations of local recurrence after rectal cancer surgery should also acknowledge that rectal carcinomas are heterogeneous. Some tumours are possibly more prone to recur locally than are others and this can be linked to the tumour phenotype or genotype. However, if identified, this should not be viewed in a fatalistic manner. Specific oncological adjuncts may conceivably be developed, but we should strive for even more perfection of surgical procedures for those patients at greatest risk.

Lastly, local recurrence also can be viewed from the perspective that cancer is a systemic, chronic disease. The balance between recurrent growth and host defence will vary in different locations, over time and in relation to immune response. Relapse in different locations will, in this view, be the result of inadequate host defences where the “soil” fits the “seed”. Purely hypothetically, a local recurrence can thus be the result of haematogenous spread to the newly operated field. Even more speculative is the possible explanation of a new malignancy in the area of chronic inflammation, as described for, e.g., squamous cell carcinoma in chronic wounds and burn scars (Marjolin’s ulcer)⁷⁹ and adenocarcinoma in ulcerative colitis⁸⁰. This generalised view is arguably more relevant in the context of distant metastases and this is also the theoretical pathogenic explanation where other factors than details of the surgical management are important.

Summary

This thesis focuses mainly on aspects of local recurrence related to the surgical management of rectal cancer. This focus can be considered the main limitation, but also the main strength, of the presentation. Optimal treatment of local recurrence after rectal cancer surgery can be concluded to require detection when the recurrence is still amenable for cure, but preferably local recurrence should be avoided in the first place.

At present, meticulous surgery, where rectal washout is one component, is still the single most important determinant of patient outcome in terms of local recurrence after rectal cancer surgery.

FUTURE PERSPECTIVES

The development of new techniques in genetic research is rapid. The contemporary array-CGH used in this thesis compares two samples at over 55 000 sites (*paper IV*). Genome-wide association studies (GWAS) are already comparing multiple samples over 500 000 sites and linking findings to a specific disease or trait. Even more spectacular is the third generation, or next generation sequencing (NGS), where the entire genome can be sequenced in less than 15 minutes. The amount of data will pose new challenges in interpretation and the field of bioinformatics will be central. Broad sharing of data, made accessible online from sequencing, genome-wide association studies as well as transcriptomic and epigenomic data, will likely expand knowledge even further.

As previously discussed, few conclusions can be drawn from the literature on follow-up after colorectal cancer surgery, despite the number of studies of apparently high-quality design such as randomised controlled trials and meta-analyses that have been performed. The results from the three large trials that remain to be published will have to be evaluated in the light of ongoing improvements in imaging modalities and treatment of recurrences at different sites. The ongoing controversies will hopefully be settled regarding the only molecular marker widely used today: Carcinoembryonic Antigen (CEA). New, more specific and sensitive molecular markers are also desirable and the evolution in genetic research will possibly facilitate their development. If known genetic profiles can be linked to recurrences at different sites, small quantities of tumour-DNA in peripheral blood could be analysed. However, in this era of molecular biology and advanced imaging, the clinical examination with rectoscopy should not be forgotten for patients followed for a potential local recurrence after rectal cancer surgery (*paper II*).

Despite advances in treatment of local recurrence after rectal cancer surgery, focus in the past, present and foreseeable future is on prevention. Some adjustments of the surgical technique and method are evolving (*Paper I and III*). However, for the majority of patients with rectal cancer, the way that surgery should be performed has been known for decades.

The contribution of one man to make this knowledge accepted and widely known cannot be underestimated. Few have put it more eloquently than this man himself, Professor R.J. Heald: *"The best cancer surgeon is the one with the capacity for taking infinite pains. The objective for the rectal cancer surgeon is the painstaking dissection in the avascular "holy plane" which surrounds the embryological hindgut with its encompassing integral lymphovascular mesorectum"*.

The challenge is to have this implemented and to ensure that every patient with rectal cancer is operated with the objective that:

-Perfection of rectal cancer surgery is in the ability of adhering to detail (Anonymous).

CONCLUSIONS

- Aspects of the surgical management are fundamentally important in reducing the local recurrence rate after rectal cancer surgery.
- Rectal washout is important for prevention of local recurrence and should routinely be used in anterior resection for rectal cancer.
- Even in modern day practice, local recurrence is often associated with intractable symptoms and premature death.
- Yearly follow-up post surgery with clinical examination is not sufficient for detection of the majority of local recurrences at a time when they are amenable for curative therapy.
- Analysis of registry data can provide insights into possible reasons for tumour recurrence, but requires knowledge of limitations of the specific registry.
- Genetic differences between recurrent and non-recurrent primary tumours are possibly contributory to the pathogenesis of local recurrence after rectal cancer surgery. The 4q31.1-31.22 region is considered of interest for further investigations. However, the relative importance, from a clinical point of view today, is likely to be small compared to the importance of surgical quality.

BRIEF SUMMARY IN SWEDISH - SAMMANFATTNING PÅ SVENSKA

Bakgrund

Tarmcancer är den näst vanligaste cancerformen i Sverige och drabbar c:a 6000 invånare/år. Cirka 1/3 av dessa cancerfall drabbar ändtarmen (rektum) och ytterst få tunntarmen. Resultaten av behandlingen av ändtarmscancer (rektalcancer) har kraftigt förbättrats under de senaste decennierna, främst beroende på förbättrad operationsteknik, men också på ökad kunskap om och användande av annan onkologisk behandling såsom strålning. En viktig kvalitetsmarkör för rektalcancerkirurgin är frekvensen av lokala recidiv, dvs återfall av tumörsjukdomen i bäckenet. Ett sådant recidiv innebär ett stort lidande för den drabbade och få kan botas. Bäckencidiv förekom i upp till 40% av opererade patienter fram till 70- och 80-talen då den moderna kirurgin implementerades. För närvarande rapporteras incidensen av bäckencidiv vara i storleksordningen 4-10% hos opererade patienter.

Inom ramen för det kvalitetsregister för rektalcancer som utarbetats i Sverige i samarbete mellan Socialstyrelsen och Onkologiskt centrum registreras samtliga patienter med diagnosen rektalcancer. Registret omfattar även kvalitetsmarkörer såsom bäckencidiv. Registerdata pekade i mitten av 00-talet på att utvecklingen av förekomst av bäckencidiv inom Västra Sjukvårdregionen var ogynnsam, och överskred det nationella genomsnittet för patienter opererade 1998-2000.

Frågeställning

Projektets målsättning var att klarlägga förekomst av bäckencidiv vid kirurgisk behandling av rektalcancer i allmänhet och förekomst inom västra regionen i synnerhet, samt att klarlägga mekanismer som predisponerar för sådant recidiv. Specifikt har delar av det kirurgiska omhändertagandet och förändringar på gennivå detaljstuderats. Ett delmål har också varit att studera det kliniska förloppet och effekten av behandling hos patienter med bäckencidiv.

Metod

Analysen utgick från ett epidemiologiskt studium av patienter med rektalcancer inom västra regionen och övriga Sverige (delarbete I). En fördjupad analys har genomförts i formen av en journalstudie (delarbete II). Kvalitetsregisterdata har analyserats avseende en specifik åtgärd i det kirurgiska omhändertagandet (delarbete III). Genetisk analys av primärtumörer har även genomförts, där en grupp patienter med tidiga, isolerade bäckenrecidiv jämförts med en jämförbar (matchad) grupp recidivfria patienter med array-CGH (comparative genomic hybridisation) och qPCR (quantitative polymerase chain reaction) (delarbete IV).

Resultat och slutsatser

Analys av skillnaden i bäckenrecidivfrekvens mellan övriga landet och västra regionen (7,1% resp 13,7%) påvisade att denna berodde delvis på skillnader i rapportering och validitet av registerdata, men att den även kan förklaras av skillnader i kirurgiskt omhändertagande och annan onkologisk behandling. Detta har resulterat i att en utbildningsinsats har initierats i regionen och ett intensifierat arbete med att ta fram klara riktlinjer rörande behandlingen (delarbete I).

Under detta arbete noterades stora skillnader i landet rörande en del av det kirurgiska omhändertagandet, nämligen sköljning av ändtarmen under operationen i syfte att minska andelen levande tumörceller inne i tarmen innan denna delas eller sammankopplas. Kunskapen rörande nyttan av denna åtgärd är mycket bristfällig i den vetenskapliga litteraturen. Data från kvalitetsregistret har analyserats och huvudsakliga fyndet är att den sköljda gruppen patienter har en nästan halverad andel bäckenrecidiv jämfört med den icke-sköljda gruppen. Skillnaderna kvarstår även då vi tar hänsyn till andra skillnader mellan grupperna (delarbete III).

Huvudfyndet i arbetet med journalgranskning var att årliga återbesök med rektoskopi inte är tillräckligt för att diagnosticera flertalet lokalrecidiv när de är asymptomatiska och behandlingsbara. Dessutom noterades att patienter drabbade av lokalrecidiv har uttalade och svårbehandlade symptom inklusive en svår smärtproblematik. Även idag kräver de frekventa och flertaliga interventioner från sjukvården, men endast ett fåtal kan botas (delarbete II).

Analys av DNA från rektal tumörvävnad påvisar tidigare kända skillnader (aberrationer) vid cancer i tjock- och ändtarm (kolorektal cancer), men mindre

uttalat i tumörer som recidiverat lokalt i ett tidigt skede efter operation. Detta tyder på att de tidigare kända förändringarna inte är av central betydelse för utvecklandet av lokalrecidiv. En tidigare i kolorektala sammanhang icke-beskriven aberration påvisas skilja mellan grupperna på kromosom 4 (4q31.1-31.22). Detta potentiellt viktiga fynd kvarstår att konfirmeras i större patientmaterial.

Bäckenrecidiv efter operation för rektalcancer utgör ett misslyckande med betydande påverkan på den drabbades livskvalitet. Kronisk smärta, sekretion och blödning kan hos dessa patienter avgörande försämra livskvaliteten under den begränsade, återstående livstiden. Det är angeläget att söka ytterligare minska incidensen av lokalt recidiv av rektalcancer och bättre palliera drabbade.

Trots framsteg avseende behandlingen av lokalrecidiv bör fokus ligga på att förebygga denna fruktade komplikation. Kvaliteten av utförd kirurgi är den enskilt viktigaste faktorn för att undvika lokalrecidiv efter rektalcancer. Vissa justeringar har gjorts, men i stora drag är det känt sedan ett par årtionden hur operationen bör genomföras. Utmaningen är att tillse att varje patient med rektalcancer erbjuds ett optimalt kirurgisk omhändertagande.

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APPENDIX (PAPER I-IV & LETTERS)