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ANAEMIA, BLOOD LOSS and COLORECTAL CANCER

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Anaemia, blood loss and colorectal cancer

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Till John och Agnes

“Sometimes you are the fly

Sometimes you are the windshield”

-Barry Weiss

ABSTRACT

Introduction: Colorectal cancer (CRC) is the fourth most common cancer worldwide and strikes both sexes. The age-adjusted 5-year survival in Sweden was 60% in the early 21st century. Approximately 50% of patients are anaemic at the time of diagnosis. Many patients need blood transfusion due to anaemia or surgical blood-loss. Blood transfusions administered to patients with CRC have been associated with an increase in the risk for cancer recurrence, independent of tumour stage. One long-term complication to abdominal surgery is small bowel obstruction (SBO) necessitating further surgery.

Hypotheses:

- Anaemia prior to surgery and perioperative red blood cell transfusion increase overall mortality and risk of recurrence in patients after curative resections for CRC.
- Blood-loss in surgery for colon cancer impairs overall survival.
- The amount of blood lost at index surgery for colon cancer increases the risk of future surgery for SBO due to adhesions.
- Major blood loss during surgery for rectal cancer increases the risk for SBO due to adhesions or tumour recurrence and reduces overall survival.

Materials and methods: All studies are retrospective cohort studies of prospectively collected data. All patients who had abdominal resection for CRC stage I-III at Karolinska University hospital from 2007 to 2010 were included in the study considering the effects of anaemia and blood transfusion. Information was retrieved from the Swedish Colorectal Cancer Registry and linked to information on transfusion and laboratory data on haemoglobin. Patient records were validated for recurrent disease. The studies on blood loss are based on all patients with CRC in the Uppsala-Örebro region 1997 to 2003. Data from the Swedish Rectal and Regional Colon Cancer Registries were linked to information on hospital admissions for SBO and abdominal pain. Patient charts for those undergoing surgery for diagnoses congruent with SBO were validated for cause of SBO. Statistics were calculated using non-parametric methods, logistic regression, and Cox Proportional Hazards regression analyses.

Results: Anaemia prior to surgery for CRC increased the risk of overall mortality (HR 2.2; 95% CI: 1.4-3.3). The analyses also revealed a trend towards an association between preoperative anaemia and recurrence (HR 1.6; 95% CI: 0.99-2.6). No association between perioperative blood transfusion and risk of recurrence or overall mortality was found. Blood loss \geq median (250 ml) impaired overall survival (HR 1.1; 95% CI: 1.0-1.2) after surgery for colon cancer. There was no association between blood loss and survival for the rectal cancer patients. A blood loss \geq median for patients with colon cancer (250 ml) increased the risk of future surgery for SBO caused by tumour recurrence (HR 2.2; 95% CI: 1.1-4.3). The same was found for patients with rectal cancers who had blood loss \geq median 800 ml (HR 10.5; 95% CI: 1.4-81.5). There was no increased risk for surgery for SBO caused by adhesions for colon or rectal cancer patients.

Conclusions: Anaemia prior to surgery for CRC is a predictive factor for mortality and a trend was seen towards an association with recurrence. Additional effort should be given to study this topic. No association was established between a perioperative blood transfusion and future risk of recurrence or mortality. Blood loss at surgery for CRC should be kept to a minimum to decrease mortality in patients with colon cancer and reduce future risk for SBO due to tumour recurrence.

LIST OF SCIENTIFIC PAPERS

The thesis is based on the following papers.

- I. Malin E. M. Mörner, MD, Ulf Gunnarsson, PhD, Pia Jestin, PhD, and Monika Svanfeldt, PhD. The importance of blood loss during colon cancer surgery for long-term survival. *Annals of Surgery*: June 2012 - Volume 255 – Issue 6 - p 1126–1128. Wolters Kluwer Health Lippincott Williams & Wilkins©
- II. Mörner, M, MD; Gunnarsson, U, PhD; Jestin, P, PhD and Egenvall, M, PhD. Volume of blood loss during surgery for colon cancer is a risk determinant for future small bowel obstruction caused by recurrence – a population based epidemiological study. Springer and *Langenbecks Archives of Surgery*, 400, 2015, 599-607, reproduced and printed with kind permission from Springer Science and Business Media.
- III. Egenvall, M, MD, PhD; Mörner, M, MD; Pahlman, L, MD, PhD; Gunnarsson U, MD, PhD. Degree of blood loss during surgery for rectal cancer: surgical complications and survival - a population-based epidemiologic study. *Colorectal Dis*, 2014: Sep;16(9):696-702.
- IV. Mörner, M, MD; Edgren, G, PhD, Martling, A, PhD; Gunnarsson, U, PhD; Egenvall, M, PhD. Preoperative anaemia and perioperative red blood cell-transfusion as prognostic factors for recurrence and mortality in colorectal cancer – A Swedish cohort study. *Manuscript*

TABLE OF CONTENTS

| | | |
|-------|---|----|
| 1 | Introduction | 1 |
| 2 | Background | 2 |
| 2.1 | Epidemiology and etiology | 2 |
| 2.2 | Symptoms | 3 |
| 2.3 | Survival..... | 3 |
| 2.4 | Colorectal cancer staging | 4 |
| 2.4.1 | Clinical staging..... | 4 |
| 2.4.2 | Histopathological staging | 4 |
| 2.5 | Treatment..... | 7 |
| 2.5.1 | Neoadjuvant oncological treatment..... | 7 |
| 2.5.2 | Rectal cancer | 7 |
| 2.5.3 | Colon cancer..... | 8 |
| 2.5.4 | Lymph node metastases | 9 |
| 2.5.5 | Distant metastases | 9 |
| 2.5.6 | Adjuvant oncological treatment..... | 10 |
| 2.5.7 | Follow up..... | 10 |
| 2.6 | Blood loss and Colon cancer surgery | 11 |
| 2.7 | Small bowel obstruction..... | 11 |
| 2.8 | Anaemia and colorectal cancer disease | 12 |
| 3 | Aims..... | 15 |
| 4 | Methods | 16 |
| 4.1 | Settings | 16 |
| 4.1.1 | Personal Registration Number | 16 |
| 4.1.2 | The Swedish Cancer Register | 16 |
| 4.1.3 | The National Patient Register | 17 |
| 4.1.4 | The local blood transfusion database | 18 |
| 4.1.5 | KarDa and Structured Patient Data | 18 |
| 4.2 | Study populations | 19 |
| 4.2.1 | Studies I-III..... | 19 |
| 4.2.2 | Study IV | 24 |
| 4.3 | Statistical analysis | 25 |
| 4.3.1 | Study I | 25 |
| 4.3.2 | Study II | 26 |
| 4.3.3 | Study III..... | 28 |
| 4.3.4 | Study IV | 29 |
| 5 | Results | 31 |
| 5.1 | Study I..... | 31 |
| 5.2 | Study II | 32 |
| 5.2.1 | Surgery for SBO according to blood loss..... | 33 |
| 5.2.2 | Admission to hospital for SBO without surgery according to blood loss | 35 |
| 5.2.3 | Admission to hospital for emergency abdominal pain according to blood loss | 35 |
| 5.3 | Study III | 35 |
| 5.3.1 | Surgery for ASBO and TSBO according to blood loss | 36 |

| | | |
|-------|--|----|
| 5.3.2 | Admission to hospital for SBO without surgery according to blood loss..... | 37 |
| 5.3.3 | Overall 5-year survival according to blood loss | 37 |
| 5.3.4 | Surgical complication according to blood loss | 37 |
| 5.4 | Study IV | 37 |
| 5.4.1 | Risk of recurrent disease | 39 |
| 5.4.2 | Overall mortality | 39 |
| 6 | Methodological discussion..... | 40 |
| 6.1 | Study design | 40 |
| 6.1.1 | Precision | 41 |
| 6.1.2 | External validity | 41 |
| 6.1.3 | Internal validity | 41 |
| 6.2 | Studies I-III..... | 43 |
| 6.2.1 | Precision | 43 |
| 6.2.2 | External validity | 45 |
| 6.2.3 | Internal validity | 45 |
| 6.3 | Study IV | 47 |
| 6.3.1 | Precision | 47 |
| 6.3.2 | External validity | 47 |
| 6.3.3 | Internal validity | 48 |
| 7 | General Discussion..... | 50 |
| 7.1 | Anaemia, Blood transfusion and recurrence..... | 50 |
| 7.2 | Anaemia, blood transfusion and survival | 51 |
| 7.3 | Surgical Blood loss and overall survival | 52 |
| 7.4 | Surgical Blood loss and small bowel obstruction | 53 |
| 7.4.1 | Surgery for SBO..... | 53 |
| 7.4.2 | Hospital admission for SBO without surgery | 54 |
| 8 | Conclusion..... | 56 |
| 9 | Sammanfattning på svenska..... | 57 |
| 9.1 | Bakgrund | 57 |
| 9.2 | Metod | 58 |
| 9.3 | Resultat | 58 |
| 9.4 | Slutsats | 59 |
| 10 | Acknowledgements..... | 60 |
| 11 | References | 63 |

LIST OF ABBREVIATIONS

| | |
|-----------|--|
| APR | Abdomino perineal resection |
| ASBO | Adhesive small bowel obstruction |
| CEA | Carcinoembryonic antigen |
| CI | Confidence interval |
| CME | Complete Mesocolic Excision |
| CRC | Colorectal Cancer |
| DFS | Disease-free survival |
| Hb | Haemoglobin |
| HR | Hazard Ratio |
| IDA | Iron deficiency anaemia |
| ICD | International Statistical Classification of Diseases and Related Health Problems |
| LAR | Low anterior resection |
| MRI | Magnetic Resonance Imaging |
| NPR | National Patient Register |
| OR | Odds Ratio |
| PRN | Personal Registration Number |
| RBC | Red blood cells |
| RCC | Regional Cancer Center |
| ROC | Regional Oncology Center |
| RT | Radiotherapy |
| SBO | Small Bowel Obstruction |
| The Board | National Board of Health and Welfare |
| TME | Total Mesorectal Excision |
| TNM | Tumour Node Metastases |
| TSBO | Small bowel obstruction due to tumour recurrence |

1 INTRODUCTION

While colorectal cancer (CRC) is one of the most common malignancies, scientific documentation of the effects of anaemia and blood loss on complications, overall survival and oncological outcome is sparse.

This thesis analyses CRC epidemiology focusing on the effects of anaemia prior to surgery and peroperative bleeding based on the Swedish Colorectal Cancer Registry. The thesis consists of four studies exploring; whether preoperative anaemia and blood transfusion influence the risk of recurrent disease and overall mortality (IV), if the volume of blood lost during surgery influences overall survival in colon (I) and rectal (III) cancer and the risk of future surgery and in hospital stay for small bowel obstruction (SBO), (II and III).

Chapters 3-6 describe the studies in the order they were conducted (I-IV). Abstract, introduction, chapter 7, and 8 describe the thesis from a more clinical/chronological perspective starting with study IV (preoperative anaemia) followed by study I-III.

2 BACKGROUND

2.1 EPIDEMIOLOGY AND ETIOLOGY

Colorectal cancer is the third most common cancer in Sweden with an annual incidence exceeding 6,000 cases¹. Worldwide, it is the fourth most common type of cancer constituting 9.4% of all incidental malignancies in men (third most common cancer after lung and bronchus cancer, and prostate cancer²) and 10.1% in women (second most common after breast cancer²)³. The highest incidence is seen in Australia and New Zealand, Europe, North America and Eastern Asia whereas the lowest incidence has been observed in Middle Africa².

Risk factors for CRC are both genetic and environmental. It has been suggested that environmental factors play a larger role than previously assumed. Now, environmental factors are believed responsible for about 70-80% of all CRC³. Risk factors for CRC include high age, personal history of inflammatory bowel disease or adenomatous polyps, family history of the two previous factors, dietary and life-style factors.^{3, 4} Women have a higher risk than men of developing colon cancer whereas men have a higher risk of developing rectal cancers than women⁵.

Most colorectal neoplasms emanate from premalignant intestinal adenomas or “polyps” transforming to adenocarcinomas⁶. Out of all CRC’s, approximately 60% are localised in the colon and 40% in the rectum⁷. Prevention of CRC disease is accomplished through screening programs with detection of faecal blood⁸ and/or colonoscopy with polypectomy⁶. In Sweden it is recommended that all citizens aged 60-74 years should be afforded screening through detection of faecal occult blood⁹. Nowadays many screening programs use an immunologic test for faecal blood since it is easy to use and has a high sensitivity⁹. Introducing screening programmes has been shown to decrease CRC incidence^{10, 11} and mortality¹¹. During the past 25 years, management of CRC has changed regarding both surgical¹²⁻¹⁴ and oncological treatment¹⁵. Examples are implementation of preoperative radiotherapy (RT) and Total Mesorectal

Excision (TME)¹⁶ for rectal cancer and the use of adjuvant chemotherapy in colon cancer¹⁷.

2.2 SYMPTOMS

In its initial stage, CRC often remains asymptomatic. The main symptom from CRC is altered mode of defecation such as altering diarrhoea and constipation, rectal blood and/ or mucus when passing stool, pain and an urgent need for defecation.

Patients with right-sided colon cancers are often anaemic¹⁸ (iron deficiency anaemia, IDA¹⁹) with poor general state of health at the time of diagnosis. Left-sided tumours and rectal cancers often present with changed mode of defecation, pain and blood/ mucus in stool¹⁹.

2.3 SURVIVAL

For patients diagnosed with CRC in the late 1980s, the age-adjusted 5-year overall survival for CRC in Sweden was 51.5%. In the early 21st century, the corresponding figure was 60.3%. The gain in age-adjusted 5-year survival was more pronounced among Swedish men (+11.0%) than in women (+6.8%). The same pattern was seen throughout Europe⁷.

Approximately 25% of the colon cancers present as emergencies²⁰. Emergency cases are associated with decreased stage-specific survival²⁰.

Stage at time of diagnosis is the strongest predictor for survival. The more advanced the stage, the poorer the prognosis^{7, 21-23}. The regimen for treatment of distant metastases has been subject to constant development^{24, 25} since the mid - 90s. Today, a larger proportion of patients with distant metastases are eligible for treatment with curative intention. In patients having a liver resection due to metastasis from CRC, the 5-year survival has increased by about 25% during a 20-year period²⁴.

Comorbidity plays an important role for overall survival. A Danish study showed that comorbidity at diagnosis increased mortality mainly during the first year

after diagnosis²⁶. In Sweden, approximately 60% of all CRC are diagnosed in persons aged 70 years or older²⁷.

2.4 COLORECTAL CANCER STAGING

2.4.1 Clinical staging

In Sweden, patients with a CRC diagnosis, or suspected CRC diagnosis are referred to a surgical clinic for investigation. Assessment is designed to stage for local growth or spread to regional lymph nodes or distant organs. Staging is performed using clinical examination, imaging and pathology¹⁹. After clinical investigation and use of appropriate imaging modalities, all patients are discussed at an MDT conference to ensure they meet prevailing guidelines.

Clinical investigation includes digital rectal examination, colonoscopy and investigation with rigid rectoscopy for rectal cancer. Confirmation of a malignant diagnosis necessitates a tumour biopsy. In all cases of colorectal tumours, a clean colon assessment preferably by colonoscopy shall be performed and preferably prior to surgery¹⁹.

The preferred imaging method for screening for metastases is computed tomography (CT) of the thorax and abdomen. In addition local staging is performed with CT in colon cancer and Magnetic Resonance Imaging (MRI) in rectal cancer²⁸. Sometimes appropriate radiological assessment necessitates several imaging modalities, including Positron Emission Tomography PET-CT²⁸.

2.4.2 Histopathological staging

Several systems for histopathological staging have been used. For example, the Dukes system and the Astler-Coller classification (mostly used in US literature)¹⁹. Currently, these systems have been replaced by the TNM classification system²⁹. In 1953, the International Commission on Stage-Grouping in Cancer and Presentation agreed to use the TNM system for classification of cancer³⁰.

The TNM classification is basically divided in three parameters. T describes the primary tumour, N the nodal (lymph node) status, and M, the presence of distant metastases or not.³⁰ Tumours are classified by the clinical TNM (cTNM) based on imaging and clinical examination and the histopathological TNM (pTNM) based on examination of the resection specimen.³⁰.

TNM-Classification 7th edition³⁰

T – Primary tumour

| | |
|-----|---|
| TX | Primary tumour cannot be assessed |
| T0 | No evidence of primary tumour |
| Tis | Carcinoma in situ: intraepithelial or invasion of lamina propria |
| T1 | Tumour invades submucosa |
| T2 | Tumour invades muscularis propria |
| T3 | Tumour invades subserosa or into non-peritonealised pericolic or perirectal tissues |
| T4 | Tumour directly invades other organs or structures and/ or perforates visceral peritoneum |
| T4a | Tumour perforates visceral peritoneum |
| T4b | Tumour directly invades other organs or structures |

N – Regional Lymph Nodes

| | |
|-----|--|
| NX | Regional lymph nodes cannot be assessed |
| N0 | No regional lymph node metastasis |
| N1 | Metastasis in 1-3 regional lymph nodes |
| N1a | Metastasis in 1 regional lymph node |
| N1b | Metastasis in 2-3 regional lymph nodes |
| N1c | Tumour deposit(s), i.e., satellites in the subserosa or in non-peritonealised pericolic or perirectal soft tissue without regional lymph node metastasis |
| N2 | Metastasis in 4 or more regional lymph nodes |
| N2a | Metastasis in 4-6 regional lymph nodes |
| N2b | Metastasis in 7 or more regional lymph nodes |

M – Distant metastasis

| | |
|-----|---|
| M0 | No distant metastasis |
| M1 | Distant metastasis |
| M1a | Metastasis confined to one organ (liver, lung, ovary, non-regional lymph node(s)) |
| M1b | Metastasis in more than one organ or the peritoneum |

TNM stage grouping³⁰

| Stage | T | N | M |
|--------------|----------|----------|----------|
| Stage 0 | Tis | N0 | M0 |
| Stage I | T1, T2 | N0 | M0 |
| Stage II | T3, T4 | N0 | M0 |
| Stage IIA | T3 | N0 | M0 |
| Stage IIB | T4a | N0 | M0 |
| Stage IIC | T4b | N0 | M0 |
| Stage III | Any T | N1, N2 | M0 |
| Stage IIIA | T1, T2 | N1 | M0 |
| | T1 | N2a | M0 |
| | T3, T4a | N1 | M0 |
| Stage IIIB | T2, T3 | N2a | M0 |
| | T1, T2 | N2b | M0 |
| | T4a | N2a | M0 |
| Stage IIIC | T3, T4a | N2b | M0 |
| | T4b | N1, N2 | M0 |
| Stage IVA | Any T | Any N | M1a |
| Stage IVB | Any T | Any N | M1b |

Modern pathology reports often include information on assessment of tumour deposits, extramural vascular invasion, perineural growth, mucinous characteristics and tumour growth threatening the mesorectal/ mesocolic fascia.

Residual tumour after treatment is classified as R0 if no residual tumour is found macroscopically and no tumour cells are found in the resection margins; R1 if there is tumour cells in the resection margin of the specimen; and R2 if there is macroscopically residual tumour³⁰. If presence of residual tumour cannot be assessed; Rx is used.

2.5 TREATMENT

Nowadays, the treatment regimen is a structured mix of neoadjuvant and adjuvant chemotherapy, RT (for rectal cancer) and surgery following distinct protocols²⁸. Still, the only curative treatment is surgical removal of the tumour.

2.5.1 Neoadjuvant oncological treatment

Rectal cancer treatment regimen is more complex than for colon cancer. Depending on tumour stage, some patients should receive preoperative RT or preoperative RT combined with chemotherapy. RT has been proven to significantly reduce the risk of local recurrence³¹ and improve survival³²⁻³⁵.

The oncological regimens are continuously improving and given according to distinct evidence-based protocols²⁸ or patients are included in study protocols.

2.5.2 Rectal cancer

Since the mid-1980s, the gold standard in rectal cancer surgery is TME^{13, 14}. It has proven to significantly reduce recurrence rate^{12, 13} and increase both cancer-specific and overall survival¹². Surgery for rectal cancer is challenging and demands a high degree of specialisation, ensuring improved 5-year local recurrence rate and 5-year cancer specific survival^{12, 19}. However, there are studies pointing in a different direction³⁶ that suggest surgeon specialisation or hospital case-load *per se* are not independent predictors for survival after CRC-surgery.

Rectal cancer surgery offers the surgeon a considerable challenge due to anatomical circumstances. The tumours are located in the pelvis, which constitutes narrow conditions for surgery, especially in men and in advanced cases, and neighbouring organs may have to be resected en bloc.

The TME technique aims to remove a complete bloc of tumour, blood vessels and lymph nodes wrapped in an intact visceral fascia. An intact fascia combined with an R0 resection margin is crucial for minimising the risk of local recurrence³⁷.

It is also possible to perform surgery for CRC laparoscopically³⁸ and this is considered an attractive alternative to open surgery³⁹ with comparable outcome³⁸. In Sweden, the proportion of laparoscopically performed procedures is still relatively low (with regional differences) but this is steadily increasing²².

An emerging technique in CRC is robotic surgery⁴⁰. One study reported overall 3-year survival as 93.1% and the cumulative disease free survival (DFS) to 79.2% for stage I-IV rectal cancer⁴⁰.

2.5.3 Colon cancer

The anatomical structures and embryological planes used as natural borders for surgical dissection in rectal cancer surgery can also be applied when operating on patients with colon cancer. Tissue layers surrounding most of the colon, runs behind the spleen and pancreas including the mesenteric root on the right hand side. This makes it possible to remove a complete mass of intact tumour and lymphatic drainage⁴¹. In the event of a spread to the lymph nodes, this spread will occur alongside of the supplying arteries, where the corresponding lymph drainage is situated⁴¹.

In order to achieve radical surgery, the surgeon is prompted to sharply dissect in the avascular embryological plane between the visceral and parietal planes¹⁹, Complete Mesocolic Excision (CME)⁴¹. The colic arteries are divided proximally and it has been debated whether a very central ligature is beneficial for the total outcome⁴¹. For patients having a procedure with curative intent, it is possible to achieve R0 in 97% of surgeries⁴¹.

Over the past 20 years, it has also become possible to perform colon cancer surgery laparoscopically, even with the CME technique^{42, 43}. In Sweden, the proportion of laparoscopic surgeries for colon cancer is increasing. However, from an international perspective the proportion of laparoscopy vs. open resections remains low²¹.

2.5.4 Lymph node metastases

Survival for patients with N-positive disease has shown a positive trend in recent decades (Table 1).

| | Rectal cancer Sweden 1995-2000²³ | Rectal cancer Sweden 2001-2009²³ | Colon cancer Sweden, Norway and Denmark late 20th century⁴⁴ | Colon cancer Sweden 2007-2012²¹ |
|------------------|--|--|--|---|
| Stage II | Just below 80 [‡] | Just over 80 [‡] | - | ≈90 [‡] |
| Stage III | ≈50 [‡] | ≈65 [‡] | ≈50 [*] | ≈70 [‡] |

Table 1. The trend in survival among Swedish patients with CRC in recent decades. Figures in per cent. †: 5-year survival. ‡: Relative 5-year survival. *: Overall 5-year survival.

It is still difficult to assess dissemination to the lymph nodes by imaging prior to surgery²⁸. Therefore, it is important during surgery to harvest as many lymph nodes as possible²⁸ to assure correct staging and thus appropriate treatment. Currently, the recommendation is to examine ≥ 12 lymph nodes^{19, 28, 45}. The higher the ratio between cancer-infiltrated lymph nodes and examined lymph nodes, the shorter the DFS⁴⁶.

2.5.5 Distant metastases

Approximately 20% of CRC patients have synchronous distant metastases at cancer diagnosis^{22, 21, 47}, most commonly to the liver, lungs and the peritoneum.

Liver resection for metastatic CRC can be performed with an overall median 5-year survival between 22-44 % depending on disease severity and oncological therapy⁴⁸. Some studies report a survival rate of 58%⁴⁹. A continuous increase in overall 5-year survival has occurred since the mid-90s²⁴.

In a recent study, approximately 10% of all CRC cases had peritoneal metastases⁵⁰. If no other distant metastases are present, the treatment strategy has changed from palliative to treatment with curative intent⁵⁰. The technique for treatment is cytoreductive surgery with intraperitoneal chemotherapy⁵¹

(hyperthermic intraperitoneal chemotherapy²⁸). This treatment significantly improves both disease specific survival and progression free survival⁵⁰. Five-year survival has been reported to 45% for patients with a complete cytoreduction at surgery⁵⁰.

2.5.6 Adjuvant oncological treatment

The objective of adjuvant chemotherapy is to eradicate micro metastases in order to prevent future generalisation of disease⁵².

Patients with stage III colon-cancer are recommended postoperative chemotherapy^{28, 44, 52} decreasing recurrence rate and increasing overall survival⁵². In 2013, 84% of stage III colon cancer patients aged <75 were planned for adjuvant therapy, whereas the corresponding figure for patients aged >75 years was 34%. The use of adjuvant treatment was equally distributed between acute and elective cases⁵³.

For rectal cancer, there is no clear evidence of benefit in survival or recurrence from adjuvant chemotherapy. Findings in studies regarding this question are contradictory⁵⁴⁻⁵⁶. Some speculate that the differences in outcome can be ascribed to differences in localisation of the tumours⁵⁷. Guidelines still provide no consensus^{28, 52}.

2.5.7 Follow up

Yet, there is no scientific consensus on surveillance of CRC patients after surgery, though there are ongoing international studies. One is the COLOFOL study⁵⁸, in which many Swedish centres have participated. The study randomises patients with stage II and III between low frequency follow-up, *i.e.* CT of the abdomen and chest combined with carcinoembryonic antigen (CEA) at 12 and 36 months and a high frequent follow up arm, *i.e.* the same investigations at 6, 12, 18, 24 and 36 months. Until those studies have reported complete results, patients with low risk for recurrent disease are followed according to the low frequency arm at many Swedish hospitals, while patients with high risk of recurrence are followed according to the high frequency follow-up arm.

One month after surgery, the patients come to a clinical follow up including CEA¹⁹. From 3 years after the surgery and every 5 years until the biologic age of 75, patients also undergo a colonoscopy¹⁹.

Patients with poor physical health or advanced biological age not considered candidates for oncological or surgical treatment if diagnosed with recurrence are usually not followed

2.6 BLOOD LOSS AND COLON CANCER SURGERY

Intraoperative haemorrhage is dependent on a number of factors, such as surgical technique, complexity of surgical procedure, patient coagulation status and intraoperative body temperature. Intraoperative systemic warming has been shown to decrease blood loss^{59, 60}. To minimise the amount of blood lost during surgery, preventive measures such as discontinuing medication potentially interfering with the coagulation system⁶¹ should be undertaken. Over recent years, surgical techniques have improved for both colon^{41, 62} and rectal cancer⁶³.

There are a variety of methods to measure haemorrhage during surgery. The different methods of estimation (spectrophotometric haemoglobin analysis, gravimetric analysis^{64, 65} and visual estimation⁶⁶) all risk miscalculation. In one study, the authors concluded that the gravimetric, *i.e.* weighing of surgical swabs, is preferable⁶⁵. Another study recommends visual estimation, if performed routinely⁶⁶. The method used in this thesis is visual estimation by the anaesthetic nurse immediately after surgery. This estimate is specified in the national quality register on which the studies are based.

2.7 SMALL BOWEL OBSTRUCTION

Small bowel obstruction is a common, well-known complication to abdominal surgery^{67, 68}. This thesis addresses benign and malignant SBO.

It has been shown that approximately 65% of intra-abdominal adhesions are secondary to traumatising (abdominal surgery) of the peritoneum. This fraction increases with type of surgery (minor versus major) and number of surgeries⁶⁹. Development of adhesions is likely caused by lack of equilibrium between

formation of fibrin by activation of fibrinogen and the degree of fibrinolysis⁷⁰. It is known that longer duration of surgery is associated with a decrease in fibrinolytic activity⁷¹. This supports a theory that haemorrhage possibly induces activation of fibrinogen and concurrent decreasing or disturbed fibrinolytic activity. Such a complex of reactions may result in increasing formation of adhesions.

In some materials, the incidence of surgery for SBO after colorectal surgery in long-term postoperative course is reported at 10%^{72, 73}. The risk of admission to hospital for symptoms possibly or directly related to adhesions following colorectal surgery has been reported to 19% four years after surgery⁷⁴. A Swedish population-based study reported mechanical bowel obstruction as the dominant symptom from local or regional recurrence in approximately 25% of the patients⁷⁵. In patients operated for SBO who were previously operated for colon cancer, about 80% of adhesions have been reported as benign in patients with no known recurrence, and 30% in patients with known recurrence⁷⁶.

Before starting the studies for this thesis, little was known about whether haemorrhage during surgery can contribute to future morbidity due to SBO. Only one study was available⁷². In that study of 472 patients operated for colorectal cancer (121 for palliative surgery), the authors state that blood loss of 1,000 ml or more was associated with the occurrence of late episodes of SBO. Of the patients with SBO, approximately 50% had a malignant reason for their SBO and 50% had SBO from benign reasons⁷².

2.8 ANAEMIA AND COLORECTAL CANCER DISEASE

Many patients with CRC have intestinal bleeding from their tumour before diagnosis, but symptoms before diagnosis vary according to tumour localisation¹⁹. Bleeding from a proximal tumour is more likely to pass unnoticed than bleeding from a distal tumour^{18, 77}.

Anaemia is common in cancer⁷⁸ and prevalence in CRC disease has been reported from 21% to 75%^{18, 77-79} in various materials. This thesis uses the WHO classification for anaemia (Table 2)⁸⁰. Anaemia can be classified by morphology

or aetiology. Aetiological classification is roughly subdivided into excessive blood loss or destruction of red blood cells, and insufficient regeneration of red blood cells from bone-marrow⁸¹. In CRC patients, anaemia is usually due to iron deficiency, IDA⁸² secondary to blood loss.

| Population | Non Anaemia | Anaemia (Hb g/l) | | |
|---------------------------|-------------|------------------|----------|--------|
| | | Mild | Moderate | Severe |
| Non-pregnant women | ≥120 | 110-119 | 80-109 | <80 |
| Pregnant women | ≥110 | 100-109 | 70-99 | <70 |
| Men | ≥130 | 110-129 | 80-109 | <80 |

Table 2. WHO classification of anaemia for adult population (above 15 years of age). Haemoglobin levels to diagnose anaemia at sea-level⁸⁰.

Red blood cells (RBC) contain iron. The human body is able to absorb approximately 1-2 mg of iron per day. Daily demand from haematopoiesis is approximately 20-30 mg. Most of this is provided to the body by macrophages, facilitating recycling of iron from old RBC⁸³.

In a study⁸⁴ preoperative anaemia, defined as haemoglobin (Hb) <110g/l, was an independent predictive factor for impaired survival in a multivariate model including patients with both curative and palliative surgery⁸⁴. Another study showed negative association between preoperative mild anaemia and overall survival 3-8 years after surgery in patients with CRC stage I-IV⁸⁵. Iron deficiency anaemia has been reported to be associated with impaired DFS for patients with T3N0M0 stage colorectal cancer⁸⁶. In that paper (Zhen et. al.) made no correction for ASA stage, or transfusion. The multivariate analysis in the same paper, also stated that IDA was a predictor for impaired DFS in the T3N0M0 group but not in the T4N0M0 group. The only independent predictor for DFS coherent between the two groups was lymphovascular infiltration⁸⁶.

2.8.1.1 Blood transfusion

In 2006, a Cochrane report stated that perioperative blood transfusion, defined as one month prior to until one month after CRC surgery, was associated with an increased risk for tumour recurrence⁸⁷. Transfusions have also been reported to

decrease survival after surgery for CRC⁸⁸. When deciding on whether a patient with CRC should receive a blood transfusion or not, the most important parameters in the equation are the amount of blood lost during surgery and anaemia prior to surgery.

3 AIMS

- Study I: To evaluate the risk for impaired overall survival according to blood loss at index surgery for colon cancer.
- Study II: To test the hypothesis that the volume of blood lost during surgery for colon cancer increases the risk for future SBO, mainly due to adhesions.
- Study III: To investigate if major blood loss during surgery for rectal cancer increases the risk for surgical complications, SBO due to adhesions, or tumour recurrence; or reduces overall survival.
- Study IV: To test the hypothesis that anaemia prior to surgery and perioperative red blood cell transfusion increases the risk of recurrence and overall mortality in patients with stage 0-III CRC after curative abdominal resections.

4 METHODS

4.1 SETTINGS

4.1.1 Personal Registration Number

All Swedish citizens have a unique identification number (Personal Registration Number (PRN)). This number cannot be changed during the person's lifetime and is used for identification. The number includes the date of birth (8 digits) and four control digits (YYYYMMDD-CCCC). The third control digit indicates sex. Even numbers designate female and odd designates male⁸⁹. Swedish authorities use the PRN for identification and registration. It is also used in national quality registers. This provides a unique capability to trace individuals between databases or registers and link these datasets to each other.

4.1.2 The Swedish Cancer Register

The Swedish Cancer Register was founded in 1958 to create a nationwide population based register to monitor cancer statistics in Sweden. It also enabled comparison of Swedish cancer statistics with cancer incidence in other countries. Initially, all data was collected by the Swedish National Board of Health and Welfare (the Board).

In the 1970s, six Regional Oncology Centres (ROCs) were established for data collection. These institutions were renamed Regional Cancer Centres (RCC) when their responsibilities were broadened to include working with guidelines and common principles. These centres are responsible for quality control of the cancer register and provide the Board annual data updates⁹⁰.

Every Swedish physician must report all new tumours and tumour like conditions to the Cancer register. Both the clinician in charge of the patient investigation and the pathologist are urged to submit separate reports⁹¹.

4.1.2.1 The Uppsala-Örebro regional oncology centre for colorectal cancer

Since 1995, all surgeries for rectal adenocarcinomas are registered in a national quality register held by regional centres. Since 1997, all colon adenocarcinomas

in the Uppsala-Örebro region are also registered at ROC⁹². The Swedish national colon cancer registry was founded in 2007⁹³. These registers, now run by the successor RCCs, have been shown to have high validity⁹².

Register data are prospectively collected. The system includes automatic grids and logic checks to prevent registration of incongruous data⁹⁴. The register steering committee compiles an annual national report for colon and rectal cancer^{21, 22} including reports on long-term follow up. These reports are published officially to maintain national quality assurance.

4.1.2.1.1 Contents of the registers provided by the ROC

The ROC registers include for example data on PRN, pretherapeutic staging (radiology, biopsies), date of surgery, type of surgery, complication, resection margin, blood loss, histopathology staging and date of death. These parameters were retrieved from the register. Some parameters were not included in the registers from the start. ASA score (American Society of Anaesthesiologists⁹⁵) was introduced to the register in 2007⁹⁶. Recurrence was added to the rectal cancer register in 1995 whereas for colon cancer it was added in 2007. From 1995 to 2006 data on recurrence (local, distant, radiation etc.) was recorded on a separate form annually 1-5 years after surgery.

Surgical complications are classified as: none, wound infection, perineal infection, intraabdominal infection, postoperative bleeding, anastomotic complication, ostomy, indwelling catheter when leaving the hospital, and other complication. In the ROC register data is limited to surgical complications occurring during the same hospital stay as the CRC surgery.

4.1.3 The National Patient Register

The National Patient Register (NPR), is a complete nationwide register of the Board. Reporting to the register is compulsory for each in-patient care institution. NPR has almost complete data from somatic and psychiatric care since 1987⁹⁷. The register provides data on the patient (PRN), date of hospital-admission and –discharge, ICD-code, and codes for classification of surgical

procedure^{97, 98}. In 2009 it had complete data on diagnoses for 99% of the hospital admissions in Sweden⁹⁷. From 1997 and on, the register allows registration of one main-diagnosis and up to seven secondary diagnoses at discharge⁹⁷. Each registration includes data on one hospital-episode⁹⁷. The largest proportion of missing data is in psychiatric care⁹⁷.

4.1.3.1 The ICD system

The ICD-system (International Statistical Classification of Diseases and Related Health Problems) is an international system in use for about a century. The WHO has managed the ICD since 1948. It has been revised several times and the current edition (ICD 10) was published in 1990. ICD 10 has been in use in Sweden since 1997⁹⁹.

4.1.4 The local blood transfusion database

The local blood transfusion database is based on a system called ProSang. ProSang is a database system used for storage of transfusion data. Every completed transfusion must be reported to the system. The PRN and the transfusion bag ID number are scanned. The user reports completion of the transfusion. Returned unused units can also be reported¹⁰⁰.

Validation of the local blood transfusion database has proven it has a high degree of accuracy¹⁰¹.

4.1.5 KarDa and Structured Patient Data

KarDa (Karolinska Datalagring) was originally created for patient follow-up at the Karolinska University hospital. This database contains data from the computerised patient record system, TakeCare®, at Karolinska University Hospital. Data are complete from 2007 and linked to PRNs, including data on laboratory results, blood pressure, weight and other parameters registered as measurements or values in the TakeCare system¹⁰².

4.2 STUDY POPULATIONS

All studies adhere to the Helsinki declaration and were conducted after approval from the Stockholm Regional Ethics Committee.

4.2.1 Studies I-III

Studies I-III are sprung from the same cohort. Data were retrieved from ROC on all patients operated for colorectal cancer during 1997-2003 in the Uppsala-Örebro region. In 1997, this region consisted of seven counties with one to five hospitals in each county. Currently, none of the counties runs more than three hospitals. Altogether, the region contains approximately 20% of the Swedish population⁹². Data were extracted from the register on July 29th 2009. This ensured data on 5-year survival for the entire cohort. In total, the material constituted 7,047 patients having CRC surgery.

Data on recurrence was not included in the extracted dataset. ASA score was not included in the registry during the timespan studied.

4.2.1.1 Study I

The original cohort constituted 4,502 cases of colon cancer resections. Exclusions were made according to Table 3.

| Study I | | | Study II | | |
|-------------------------------------|------------|--------------|---|------------|--------------|
| | N excluded | N left | | N excluded | N left |
| N at start | | 4,502 | N at start | | 4,492* |
| Not curative surgery | 699 | 3,803 | Not open resection, not locally radical | 721 | 3,771 |
| Stage IV/missing/undeterminable | 381 | 3,422 | Undeterminable tumour stage | 19 | 3,752 |
| Death within 6 months after surgery | 204 | 3,218 | Blood loss missing or 0 ml | 195 | 3,557 |
| Blood loss missing | 45 | 3,173 | Complication missing | 1 | 3,556 |
| Unusual surgical procedures | 111 | 3,062 | Date for surgery missing | 2 | 3,554 |
| N left for analysis | | 3,062 | N left for analysis | | 3,554 |

Table 3. Exclusions for colon cancer patients in studies I and II. * When evaluating the data for study II, 10 patients out of 4,502 who had had a resection for both colon and rectal cancer were identified. Cases with colon cancer surgery were excluded.

4.2.1.2 Studies II and III

Data from ROC of the 7,047 CRC patients were sent to the Board. PRNs were matched to the NPR to identify cases with a diagnosis indicating hospital admission due to abdominal pain or small bowel obstruction according to certain ICD codes. ICD 9¹⁰³: (560B Paralytic ileus, 560D Impaction of intestine, 560W Other intestinal obstruction, 560X Other intestinal obstruction without information of hernia, 568A Peritoneal adhesions, 789A Abdominal pain) and ICD 10:¹⁰⁴ (K567 Bowel obstruction, unspecified, K566 Other and unspecified intestinal obstruction, K565 Intestinal adhesions [bands] with obstruction, K560 Paralytic ileus, K564 Other impaction of intestine R104 Abdominal pain).

Identified in-patient episodes were scanned for codes indicating surgical treatment of the diagnoses listed above⁹⁸. The resulting datasets were anonymised with unique study codes for each patient. The Board saved a key for the study codes.

Following extraction of data from the Board, it was possible to link the anonymised datasets from the ROC and the Board to each other. This matching provided the study codes of 377 patients who had a surgical procedure >30 days¹⁰⁵ after the CRC-operation until the censoring date, July 29th 2009. A new request was made to the Board for the PRN of the 377 patients. This was made possible by the key between the PRNs and the study code saved by the Board. The hospitals where these patients had their CRC surgeries were asked to send a copy of the patient record including the surgical report and a pathology specimen report for the specified hospital stay (Figure 1).

Data from the patient records were entered into an Access[®] database. The main outcome was surgery for SBO. Collected parameters were: study code, date of surgery and reason for surgery. The exclusion criteria included surgery for other reasons than SBO and surgery more than 30 days after surgery obviously due to complications in the postoperative course. In case of uncertainties about the reason for surgery, the record was reviewed by Egenvall and Gunnarsson for a consensus discussion.

After entering all the data into the Access[®] database, it was possible to merge these with the previously merged file of data from the ROC and the Board using the study code (Figure 1).

For rectal cancer, 182 patients were registered to the Access[®] database. The corresponding figure for colon cancer was 186.

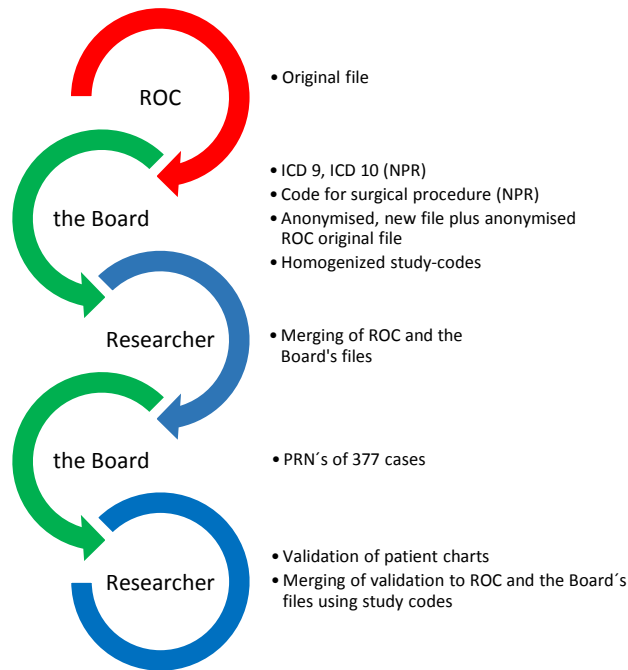


Figure 1. Flowchart of data collection for studies II and III. Text inside circles indicates where the dataset was located and who did the alterations indicated in the dotted lists.

4.2.1.2.1 Study II

This study includes the colon cancer patients described in the section above.

Exclusions were made according to Table 3, leaving 3,554 patients for analysis.

Of the 3,554 patients, 110 had the outcome “surgery for SBO”. Forty-nine of these were operated due to adhesion, 43 due to a tumour recurrence and 18 were defined as “other reason” (8 hernia, 2 benign strictures, 1 volvulation, 1 postoperative complication, 3 other malignancies, 1 Mb von Recklinghausen and 2 indeterminable) (Table 4).

Analyses were also made for the outcomes “hospital admission for SBO without surgery” (n=228) and “hospital admission for abdominal pain” (n=370) (Table 4).

4.2.1.2.2 Study III

Study III includes the rectal cancer patients in the cohort described above. The material constituted 2,555 patients. After exclusion of patients according to Figure 2, 1,843 patients remained for analysis. At this level, the exclusions took three different paths according to the dependent variable chosen for analysis (Figure 2).

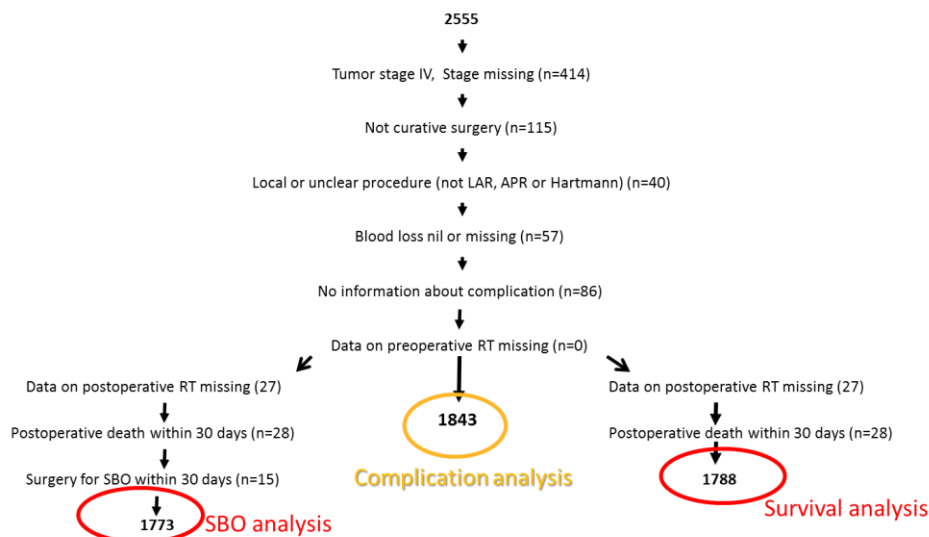


Figure 2. Study cohort for study III. Red circles indicate Cox proportional hazard Regression analysis. Yellow circle indicates Logistic Regression Analysis.

Of the remaining 1,843 patients, 82 had the outcome “surgery for SBO due to adhesions”. Twelve patients had had surgery for SBO due to cancer recurrence (Table 4).

As in study number two, an analysis was made for hospital stay due to SBO not requiring surgery (Table 4).

| | Colon | Rectum |
|------------------------------------|--------------|---------------|
| Number | 3,554 | 1,843 |
| SBO – | | |
| Adhesive | 49 | 82* |
| Tumour recurrence | 43 | 12 |
| Other | 18 | 26 |
| Hospitalized SBO | 228 | 287 |
| Hospitalized abdominal pain | 370 | - |

*Table 4. Number of cases in study II (colon) and III (rectum). * 1 patient with adhesive SBO was excluded in the exclusions for SBO-analysis.*

4.2.2 Study IV

All patients with surgically and microscopically radical resections with curative intent for CRC stage I-III at Karolinska University hospital 2007-2010 (n=546) were included. Data was collected from RCC, in the Stockholm-Gotland region at January 7th 2014. Patient charts were validated for recurrence, prior surgery for colorectal cancer, and surgery for synchronous liver metastasis. This information was entered into an Access[®] database.

Data on blood transfusion two months prior to and one month after surgery were retrieved from the local blood transfusion database. Laboratory values for Hb two months prior to surgery were collected from KarDa. The files from ROC, the local blood transfusion database, KarDa and the Access database were sent to the Board which anonymised the files and provided each observation with a study code instead of PRNs. Study codes were harmonised between the files. The datasets were then merged for statistical analysis.

Patients were excluded from the material according to the algorithm specified in Figure 3, leaving 496 patients for analysis; 282 colon cancer patients and 214 rectal cancer patients.

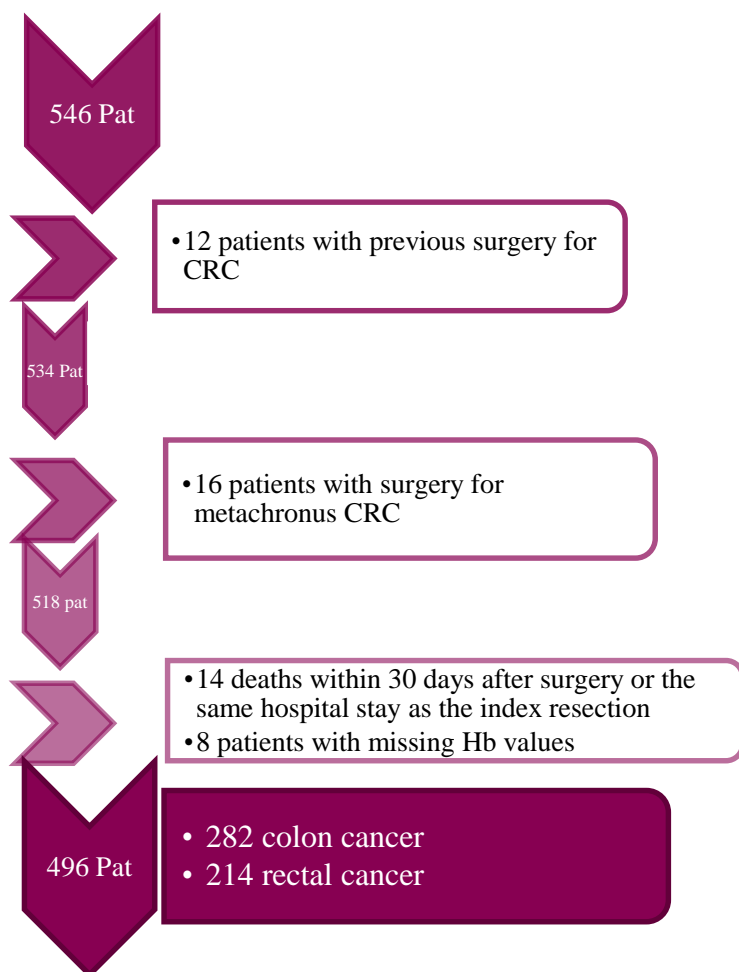


Figure 3. Algorithm for exclusions leaving 496 patients for analysis.

4.3 STATISTICAL ANALYSIS

4.3.1 Study I

Survival analysis was calculated using the Cox proportional hazard Regression analysis with a 95% confidence interval (CI). Each patient contributed risk exposure from the date of surgery until death or censoring date, July 29th 2009. A univariate analysis was performed for parameters potentially influencing

outcome. Factors significant in the univariate model were entered into the multivariate model.

Kendall Tau tests were performed between factors suspected not independent of each other. Blood transfusion and complication correlated significantly with blood loss. Thus, three separate multivariate models were performed with overall survival as dependent variable and with blood loss, transfusion and complication respectively as independent variables. Male gender, stage I-III disease and age <75 years were used as independent variables in all three multivariate models (Table 6).

Blood-loss was split into two groups according to the median blood loss for the studied cohort of 3,062 patients, <250 ml or \geq 250ml.

Each procedure was classified into one of three groups (right hemicolectomy and ileocecal resection; left hemicolectomy and sigmoid resection; colectomy and subtotal colectomy). This grouping was used for stratification of the survival analyses.

Mann-Whitney U test was performed to analyse whether there was a difference in blood-loss between the three groups. This test did not reveal any significant difference in the amount of blood lost.

The software used for statistical calculation was Statistica version 7 (Statsoft, Tulsa, OK, USA).

4.3.2 Study II

Three main outcomes were investigated; surgery for SBO (adhesive SBO, ASBO, and SBO due to tumour recurrence, TSBO); in hospital admission for SBO not requiring surgery; and hospital admission for abdominal pain. The patients contributed with exposure-time in a hierarchical way depending on analysis.

In analysis of surgery for SBO, patients contributed with risk exposure time from the surgery date until the first of censoring date (July 29th 2009), death or date of surgery for SBO.

In analysis of hospital admission for SBO, patients contributed with risk exposure time from surgery date until the first of death, censoring date (July 29th 2009), surgery date for SBO, or hospital admission date for SBO.

Analysing in hospital episode of “abdominal pain” as dependent variable, patients contributed with risk exposure-time from the surgery date until the first of death, censoring date (July 29th 2009), date of surgery for SBO, date of hospital admission for SBO, or date for hospital admission for abdominal pain.

Median blood loss was 250 ml. Three cut-offs for blood loss were used in the analyses: \geq median, \geq 800 ml (median blood loss for rectal cancer patients having surgery during the corresponding time period) and \geq half that volume, 400 ml. Patients with recorded blood-loss of 0 ml were excluded, since it was considered unlikely to have zero blood loss for open resection surgery.

Three separate Cox proportional hazard analyses of surgery for SBO were made. At first, surgery for SBO independent of pathogenesis was analysed. In a second step, SBO was split into TSBO and ASBO according to the pathogenesis and Cox proportional hazard analyses were made using ASBO and TSBO as independent variables. Analyses were made with a 95% CI. Univariate and multivariate analyses were performed in the same manner as in study I, but with no stratification for type of surgery.

Kaplan Maier curves were prepared for SBO surgery independent of pathogenesis, for TSBO, ASBO and for hospital admission for SBO not requiring surgery for each amount of blood loss. Log Rank tests were done to calculate significance between the Kaplan Maier curves. A p-value <0.05 was set as level of significance.

The Mann-Whitney U test was performed to show any significant difference in volume of blood loss between the ASBO and TSBO groups.

Statistica version 10 (StatSoft, Tulsa, OK, USA) was used for the Cox analyses, preparation of Kaplan Maier curves and Log Rank tests. STATA IC/11.0® was used for the Mann-Whitney U-test.

4.3.3 Study III

Patients contributed with risk exposure time from date of index surgery until death or censoring the date (July 29th) in all analyses. In addition to these dates, in analysis of surgery for SBO, patients were also censored at the date of surgery for SBO, whichever came first. Analogously, in analysis of hospital admission for SBO without surgery, patients were censored at the first of previously mentioned dates or date of hospital admission.

Haemorrhage during index surgery for the cohort studied was divided into four groups according to quartiles. These ‘classes’ of haemorrhage were used as independent variables in the uni- and multivariate Cox proportional hazard regression analyses and the Logistic regression analyses.

A Chi-square test was performed for comparisons between the parameters sex, age, stage, surgical complication, surgery for SBO, hospital admission for SBO, RT, death within 6 months and death within 5 years for the analysed quartiles of blood loss.

Cox proportional hazard regression analysis with 95% CI was used for calculation of hazard ratios (HR) for ASBO, TSBO, 5-year overall death, and hospital admission for SBO not requiring surgery as dependent variables (Figure 2). Uni- and multivariate analyses were performed in the same manner as in studies I and II, but without stratification for type of surgery (as in study I).

Analysis for surgical complication was calculated using logistic regression analysis providing odds ratios (OR) with 95% CI. Significant parameters in the univariate model were entered into the multivariate model analogously to the procedure used for the Cox calculations (Figure 2).

A Kaplan Maier curve for cumulative survival grouped for the occurrence of a surgical complication at index surgery was prepared. Log rank test was used to

calculate differences between the two curves. P-value <0.05 was set as level of significance.

Statistica 10 (StatSoft, Tulsa, OK, USA) was used for all statistical analyses except for the Chi-square tests of parameters requiring a larger matrix than a 2 by 2 table, for which R by C-tables in OpenEpi was used

4.3.4 Study IV

Anaemia was classified according to the WHO classification shown in (Table 2). The Hb value used for classification was the lowest observed value two months before the date of surgery. In the analyses, material was grouped as no anaemia and anaemia (mild moderate and severe).

A patient was considered transfused if he or she had received an allogeneic red cell blood transfusion within one day before or after surgery.

Time to recurrence was calculated from date of surgery until the first of date of recurrence, death, or censoring date. A recurrence was considered as an occurrence in the statistical analysis.

Overall mortality was calculated as time from surgery until death or censoring date, whichever came first. Death was considered as an occurrence in the statistical analyses.

Patients with pT reported as 0 (n=19) and pTNM=0 (n=16) were classified as pT=1 and pTNM=1. Patients were classified according to type of surgery into one of three groups: colonic resections, low anterior resections (LAR), and Hartmann's procedure for rectal cancers and APR.

Kaplan-Meier curves were prepared for anaemia and transfusion for the outcomes risk of recurrence and overall mortality for which p-value <0.05 was considered significant.

Parameters (sex, ASA grade, pTNM, pT, type of surgery and neo-adjuvant treatment) suspected to influence the outcomes for risk of recurrence and overall

mortality were entered as independent variables in the multivariate Cox proportional Hazards regression analysis. Analyses were also adjusted for age and haemorrhage as restricted cubic splines with three degrees of freedom.

A Chi-square test was performed to test correlation between anaemia and transfusion and to analyse statistical differences between anaemia and sex, colon or rectal cancer, ASA class, pTNM and pT. Level of significance was set to $p < 0.05$.

STATA IC/11.0[®] was used for statistical calculations and preparation of figures.

5 RESULTS

5.1 STUDY I

After exclusions, 3,062 patients were eligible for analysis. Patient characteristics are given in Table 5. The median follow-up time for the included patients was 6.25 years and the total years at risk for the studied cohort was 18,504.

| Patient characteristics | |
|--|--------------------|
| Sex Man : Woman (n) | 1,481 : 1,581 |
| Blood transfusion Y : N : missing (n) | 660 : 2,347 : 55 |
| Surgical complication Y : N : missing (n) | 650 : 2,411 : 1 |
| Stage I : II : III (n) | 452 : 1,539 : 1071 |
| Age (years) <75 : ≥75 (n) | 1,665 : 1,397 |
| Age (years) median (mean) | 73.8 (72) |
| Blood loss (ml) <250 : ≥250 (n) | 1,474 : 1,588 |
| Surgical procedure (n) | |
| Right hemicolectomy + ileocecal resection | 1,548 |
| Left hemicolectomy + sigmoid resection | 1,361 |
| Colectomy + subtotal colectomy | 153 |

Table 5. Patient characteristics.

Volume of blood lost above the median, male gender, stage III disease and age ≥75years were identified as risk factors for impaired survival in the multivariate model (Table 6).

| | Cox Proportional Hazard regression HR (95% CI) | |
|----------------------------|---|------------------|
| | Univariate | Multivariate |
| Haemorrhage ≥250 ml | 1.18 (1.07-1.31) | 1.12 (1.01-1.24) |
| Men | 1.19 (1.08-1.31) | 1.23 (1.11-1.35) |
| Stage I | 0.66 (0.57-0.77) | 0.87 (0.73-1.02) |
| Stage II | 0.65 (0.59-0.72) | Ref |
| Stage III | 1.97 (1.78-2.18) | 1.93 (1.74-2.14) |
| Age <75 years | 0.44 (0.39-0.48) | 0.42 (0.38-0.47) |

Table 6. Uni- and multivariate Cox proportional hazards regression analysis of risk factors for death.

When entering blood loss and age as continuous variables in the same Cox regression analysis, the risk for overall mortality was still significantly elevated, (Hazard Ratio (HR) 1.0002; 95% CI 1.00004-1.0003) and (HR 1.05 95% CI 1.05-1.06) respectively¹⁰⁶.

The Mann-Whitney U test did not reveal a significant difference in haemorrhage between the three groups of surgical procedures.

Analyses using Cox proportional hazard regression was also done for complication and blood transfusion. The occurrence of a complication was associated with significantly increased mortality (HR 1.30; 95% CI 1.16-1.45) with approximately the same HR for the other parameters as in Table 6. Transfusion did not increase the risk for overall mortality in a multivariate model.

5.2 STUDY II

After exclusions, 3,554 patients were eligible for analysis. Of these, 110 had surgery for SBO >30 days after their index surgery (Table 4). Fifty % of the studied cohort was female, the median age at index surgery was 74 years and 13.6% had suffered a surgical complication. Stage distribution was as follows: I+II 58.0%, III 32.5%, IV 9.5%. Patient characteristics for each volume of blood loss are given in Table 7.

| | Blood-loss <250 ml (n=1619) | Blood-loss ≥250 ml (n=1935) | Blood-loss ≥400 ml (n=1245) | Blood-loss ≥800 ml (n=425) |
|-------------------------------------|---|--|--|---|
| Age median (range) (y) | 75 (12-98) | 74 (22-96) | 74 (24-96) | 72 (28-92) |
| Female (%) | 58.2 | 43.3 | 40.2 | 39.3 |
| Stage I+II : III : IV (%) | 62.3 : 29.3 : 8.4 | 54.7 : 35 : 10.3 | 51.9 : 37 : 11.1 | 50.8 : 37.9 : 11.3 |
| Complication (%) | 11.6 | 15.3 | 16.9 | 18.1 |
| ASBO (n) | 27 | 22 | 16 | 5 |
| TSBO (n) | 12 | 31 | 22 | 11 |

Table 7. Patient characteristics for each amount of blood loss

5.2.1 Surgery for SBO according to blood loss

There was no evidence that greater blood loss increased the risk for ASBO in the univariate or the multivariate model (Figure 4¹⁰⁷).

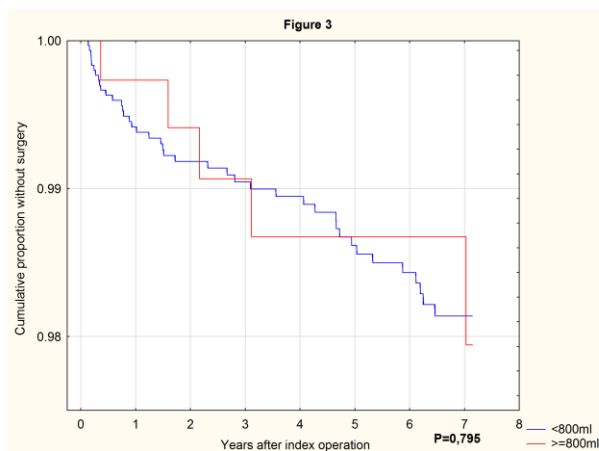


Figure 4. Kaplan-Maier curve for the cumulative proportion of patients without surgery for small bowel obstruction caused by adhesions according to blood loss of ≥ 800 ml or less. P value represent log-rank test¹⁰⁷.

There was a significant increase in risk of surgery for SBO for a blood-loss ≥ 400 ml. The risk for future surgery for TSBO was significantly elevated for a blood loss ≥ 250 and remained elevated with further increase of blood loss (Table 8).

Median time from index surgery until surgery for SBO was 632 days (range 97 to 2,640 days).

There was no increased risk for future surgery for SBO or TSBO in men compared to women in the uni- or multivariate Cox proportional hazard analyses. Suffering from a surgical complication increased the risk for future surgery for SBO but not for TSBO in the multivariate Cox proportional hazard regression analysis. Stage III and IV disease were risk factors for future surgery for both SBO and TSBO (Table 8).

The Mann-Whitney U test revealed that TSBO cases bled more than ASBO cases ($p=0.0072$) at the index operation (Figure 5). Stage distribution among TSBO cases is shown in Figure 6.

| Cox Proportional Hazard regression HR (95% CI) | | | | |
|---|---------------------------------|----------------------------------|---|---|
| | Surgery for SBO [†] | Surgery for TSBO [†] | Hospital admission SBO [†] | Hospital admission abdominal pain [†] |
| ≥250 ml | 1.26(0.85-1.85) [†] | 2.20(1.12-4.31) [†] | 1.78(1.34-2.36) [†] | ns |
| ≥400 ml | 1.58(1.08-2.33) [†] | 1.97(1.07-3.63) [†] | 1.87(1.43-2.43) [†] | ns |
| ≥800 ml | 2.11(1.33-3.36) [†] | 2.68(1.34-5.37) [†] | 1.78(1.28-2.49) [†] | ns |
| Complication* | 1.81(1.12-2.93) | 1.95(0.93-4.08) | 2.32(1.71-3.16) | |
| Stage III* | 1.74(1.16-2.62) | 2.36(1.21-4.58) | 1.85(1.39-2.47) | |
| Stage IV* | 2.28(1.11-4.70) | 3.78(1.36-10.49) | 3.23(2.04-5.11) | |
| Male* | 0.70(0.48-1.02) | 0.63(0.34-1.17) | 0.98(0.75-1.27) | |

Table 8. Results from multivariate cox proportional hazard regression. *HRs are given for surgical haemorrhage ≥250 ml. [†]Adjusted for complication, stage and gender.

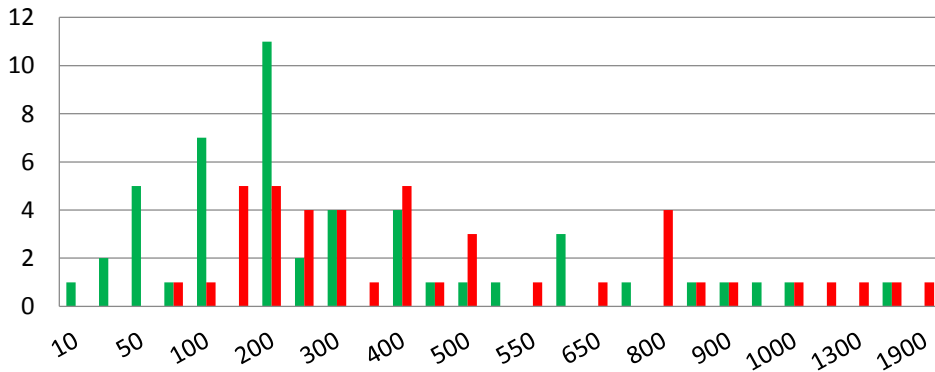


Figure 5. Distribution of blood-loss (ml) for ASBO (green) and TSBO (red). Y-axis indicates number of cases within each amount of blood lost.

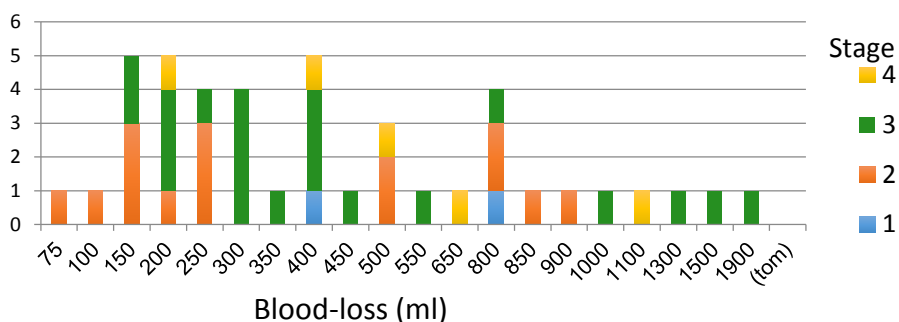


Figure 6. Distribution of tumour stage for TSBO according to blood-loss (ml). Y-axis indicates number of cases.

5.2.2 Admission to hospital for SBO without surgery according to blood loss

Blood loss equal to or above median (250 ml) increased the hazard for future hospital admission for SBO not necessitating surgery in both the uni- and multivariate model (Table 8). This hazard also remained elevated for blood loss of ≥ 400 ml and ≥ 800 ml. Also, surgical complication, stage III and IV disease increased the hazard. There was, however, no elevated hazard for future hospital admission for men compared to women.

5.2.3 Admission to hospital for emergency abdominal pain according to blood loss

When calculated with a Cox proportional hazard regression analysis, there was no elevated hazard risk for hospital admission for emergency abdominal pain regardless of blood loss at index surgery (blood loss ≥ 400 ml (HR 1.16; 95% CI 0.94-1.44)) (Table 8). Nor did the log rank test show significant increase in risk (p-value 0.075).

5.3 STUDY III

After basic exclusions, 1,843 patients remained for statistical analyses, (Figure 2).

Men bled more than women and so did patients receiving preoperative RT. There was an increased risk for surgical complication with greater haemorrhaging at the index surgery.

There was no significant difference in stage distribution or deaths within six months, or five years after index surgery between the blood loss quartiles analysed. Median blood-loss was 800 ml.

5.3.1 Surgery for ASBO and TSBO according to blood loss

There was no evidence that blood loss during index surgery increased the hazard for future surgery for ASBO. Blood loss ≥ 800 ml increased the hazard for future surgery for TSBO in both the uni- and multivariate Cox analysis. After adjustment for stage in a multivariate model, the increase in hazard for TSBO was (HR 10.52; 95% CI 1.36-81.51). The number of cases for each quartile are given in Table 9. The distribution of blood loss for ASBO and TSBO is shown in Figure 7.

| | <450 ml | 450- <800 ml | 800- <1400 ml | ≥ 1400 ml |
|---------------------|---------|--------------|---------------|----------------|
| Patients (n) | 424 | 432 | 455 | 462 |
| ASBO (n) | 24 | 20 | 20 | 17 |
| TSBO (n) | 0 | 1 | 5 | 6 |

Table 9. Number of patients in each quartile of blood loss in the Cox proportional hazard analysis of surgery for SBO.

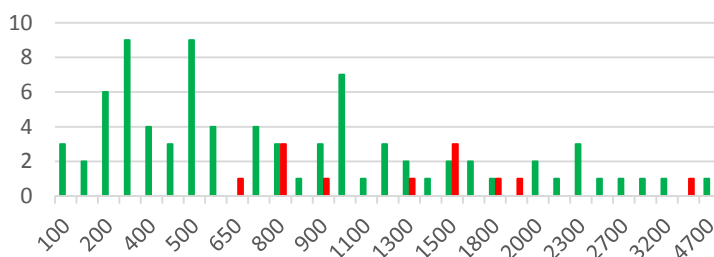


Figure 7. Distribution of blood-loss (ml) for ASBO (green) and TSBO (red) in the Cox proportional hazard regression analysis of SBO. Y-axis indicates number of cases for each amount of blood loss (ml).

5.3.2 Admission to hospital for SBO without surgery according to blood loss

There was no association between blood loss at index surgery and the risk for future hospital admission for SBO not necessitating surgery. There was an increased hazard of future hospital admission for SBO not requiring surgery for those who had received RT (HR 1.34 95% CI 1.01-1.78).

5.3.3 Overall 5-year survival according to blood loss

There was no association between the volume of blood loss and overall 5-year survival in the univariate Cox proportional hazard regression analysis. Age, stage III disease, and those who had suffered from a surgical complication had an impaired 5-year survival in the multivariate model. Radiotherapy protected from death during the initial five years after index surgery but this protective effect disappeared when the variable age was introduced to the multivariate model (Table 10).

| | Cox proportional hazard regression HR (95% CI) | |
|------------------------------|---|-----------------|
| | Univariate | Multivariate |
| Stage III | 2.31(1.97-2.69) | 2.46(2.10-2.87) |
| RT | 0.73(0.62-0.86) | 0.91(0.76-1.08) |
| Surgical complication | 1.23(1.04-1.46) | 1.28(1.08-1.52) |
| Age (continuous) | 1.04(1.04-1.05) | 1.05(1.04-1.06) |

Table 10. Results from multivariate Cox proportional hazard regression for overall 5-year survival.

5.3.4 Surgical complication according to blood loss

Blood loss ≥ 800 ml (median) (OR 1.46; 95% CI: 1.18–1.81), male gender and preoperative RT increased the risk for a surgical complication in a multivariate logistic regression analysis.

5.4 STUDY IV

Approximately 50% of the patients were anaemic before surgery. Fifty-eight % of anaemic patients received a blood transfusion in association with their

abdominal resection. The corresponding proportion for the non-anaemic group was 21%. Patient characteristics are shown in Table 11.

| | Patient characteristics | |
|------------------------------------|-------------------------|-------------|
| | No anaemia | Anaemia |
| No subjects, N (% of total) | 239 (48) | 257 (52) |
| Female | 115 (48.1) | 119 (46.3) |
| Male | 124 (51.9) | 138 (53.7) |
| Age at dx Median (IQR)* | 67 (61-74) | 70 (62-79) |
| CRC, N (%) | | |
| Colon | 115 (48.1) | 167 (65) |
| Rectum | 124 (51.9) | 90 (35) |
| Blood transfusion, N (%) | | |
| Yes | 49 (20.5) | 150 (58.4) |
| ASA, N (%) | | |
| 1 | 50 (20.9) | 30 (11.7) |
| 2 | 132 (55.2) | 113 (44.0) |
| 3 | 48 (20) | 100 (38.9) |
| 4 | 9 (3.8) | 14 (5.5) |
| pTNM, N (%) | | |
| 1 | 91 (38) | 34 (13.2) |
| 2 | 82 (34.3) | 144 (56.0) |
| 3 | 66 (27.6) | 79 (30.7) |
| pT, N (%) | | |
| 1 | 43 (18) | 14 (5.5) |
| 2 | 66 (27.6) | 27 (10.5) |
| 3 | 109 (45.6) | 154 (60.0) |
| 4 | 21 (8.8) | 62 (24.1) |
| Type of resection, N (%) | | |
| Colonic | 115 (48.1) | 166 (64.6) |
| LAR + Hartmann | 83 (34.7) | 50 (19.5) |
| APR | 41 (17.1) | 41** (16.0) |
| Neoadjuvant treatment N (%) | | |
| (Chemo)radiotherapy [†] | 99 (79.9) | 73 (81.1) |
| Chemotherapy only | 1 (0.8) | 6 (2.4) |

Table 11. Patient characteristics for the 496 abdominal resections of stage I-III CRC. Percentages are given for number of patients within group of anaemia/ no anaemia unless stated otherwise. *Median age for colon cancer 71 years, median age rectal cancer 65 years. **Including one colon cancer. [†]Only patients with rectal cancer.

5.4.1 Risk of recurrent disease

The Log-Rank test revealed a significantly increased risk of recurrence in anaemic patients (Log Rank $p=0.002$) but not those given a transfusion (Log Rank $p=0.97$).

Anaemia was close to significantly associated to greater risk for recurrent disease in the multivariate analyses for anaemia. When analysed as an independent variable in the combined analysis of anaemia and transfusion anaemia was a significant predictor for recurrent disease. No association was detected between risk of recurrent disease and transfusion (Table 12).

| Multivariate Cox proportional hazard regression HR (95% CI)* | | | |
|---|----------------|-------------------|-----------------------------|
| | Anaemia | Blood transfusion | Anaemia + Blood transfusion |
| Risk of recurrence | | | |
| Anaemia | 1.6 (0.99-2.6) | - | 1.7 (1.1-2.8) |
| Blood transfusion | - | 0.8 (0.4-1.3) | 0.6 (0.4-1.1) |
| Overall mortality | | | |
| Anaemia | 2.2 (1.4-3.3) | - | 2.4 (1.5-3.7) |
| Blood transfusion | - | 1.0 (0.6-1.5) | 0.7 (0.5-1.1) |

Table 12. Multivariate analyses of anaemia, transfusion or both as independent variables. *All analyses are adjusted for sex, ASA-grade, pTNM, pT, type of resection and neoadjuvant treatment. Analyses are also adjusted for age and haemorrhage as restricted cubic splines, 3df.

5.4.2 Overall mortality

The Kaplan Maier curves and Log-Rank tests revealed a significant relationship between anaemia (Log Rank $p<0.001$) and transfusion (Log Rank $p=0.003$), and decreased overall survival. Also in the multivariate Cox analyses, anaemia was a significant predictor for increased overall mortality, both when analysed separately and in the same model as transfusion. Transfusion was not associated to an increase in overall mortality (Table 12).

6 METHODOLOGICAL DISCUSSION

Chapter 7 includes general discussion of the studies included.

This section will survey methodological difficulties in epidemiological research. A methodological discussion of the included studies (I-III) and IV follows an introduction to the subject.

6.1 STUDY DESIGN

There are, naturally, different angles to approach the difficult task of designing and reviewing a study. The structure below was presented by Professor Albert Hofman¹⁰⁸. This chapter addresses the yellow, blue and green boxes in the tree structure (Figure 8). When designing a study, there are three main topics to address: Does this study add new knowledge? What is the relevant prior knowledge in the area? How do we secure the accuracy of the study? Accuracy is the measurement tool to establish the goodness of the study and can be divided into two sections – precision and validity – which can be divided into subgroups. (Figure 8).

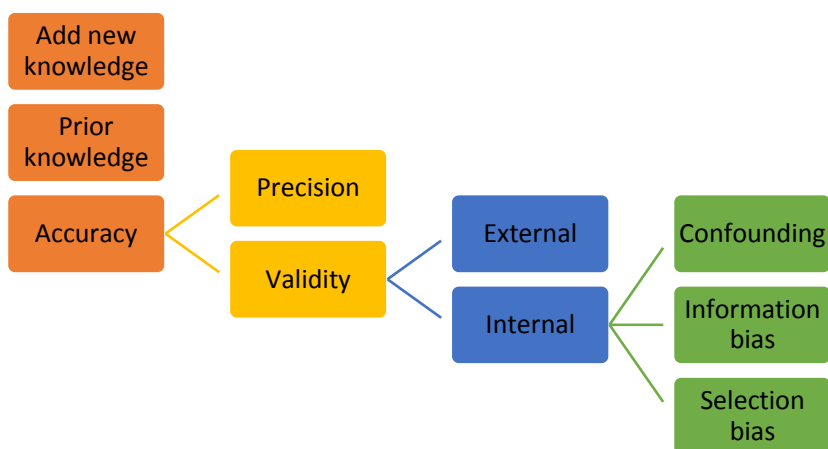


Figure 8. Study design

6.1.1 Precision

The precision of a study depends on sample size. A larger sample size will result in lower standard error in calculations. One way to express precision is through confidence intervals. In this thesis, 95% CI are used. The inference here is that “we are 95% confident that the population value of X is between the ‘lower limit’ and the ‘upper limit’”. The wider the CI, the poorer the precision.

Precision is also an important topic in order to establish reproducibility of the study. After adjusting for confounders and minimising systematic errors (bias) in the study, this might still include random errors.

6.1.2 External validity

External validity is crucial in order to enable generalisation of the results. Are the results of the study true outside the cohort studied?

6.1.3 Internal validity

Are the results ‘within’ the study really true? Does the study measure what it was intended to? In order to maximise internal validity, it is important to minimise systematic errors.

Internal validity can be divided into three main topics: confounding, information bias, and selection bias. Statistical analyses can be done to examine confounders but there are no analyses to detect or adjust for bias. Bias must be reflected upon in the study design. In the validity-section, there is a risk for systemic errors. A correct measurement of exposure and outcome makes it possible to draw inferences from the results.

6.1.3.1 Confounding

A confounder is a systematic error that can be adjusted for. The definition of a confounder is that it; 1) is associated with the exposure, 2) is associated with the outcome, and 3) is not an intermediate link between exposure and outcome (Figure 9)¹⁰⁹.

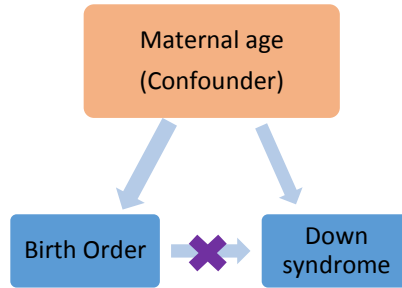


Figure 9. Confounding. Maternal age is associated with both birth order and Down syndrome but it is not an intermediate link between exposure (birth order) and outcome (Down syndrome).

There are various ways to handle confounding in a study, as with: restriction, stratification, randomisation, adjustment through regression analysis, and matching (primarily case-control studies). To be able to use data on confounders, it is important to collect data on possible confounding factors and to do so in an appropriate manner – otherwise, the analyses will have poor quality.

6.1.3.2 Information bias

Information in a study gathered in a systematically incorrect manner referred to as *information bias*. Information of a categorical nature (for example, blood pressure of all patients in a study on diet and blood pressure is measured with a broken device) is referred to as misclassification. Misclassification can be categorised as *differential* or *non-differential*¹⁰⁹. It is not possible to adjust for information bias in statistical analyses.

6.1.3.2.1 Differential misclassification

This means that misclassification (the systematic error) is only present in one of the studied groups (exposed or unexposed), leading to under or overestimation of the risk. A common example is recall bias in retrospective studies. A person diagnosed with CRC might be more likely to recall the amount of red meat they ate five years ago compared to a person without that outcome (CRC). This might overestimate (or underestimate) the calculated risk of consuming red meat in the development of CRC¹⁰⁹.

6.1.3.2.2 Non-differential misclassification

This misclassification is randomly distributed between the two groups (exposed and unexposed). This type of misclassification will lead to a dilution of a possible true increase in risk, the estimate will show a regression against zero¹⁰⁹ (bias towards the null). This is true if the parameter is dichotomous. If the parameter has dependent or ordinal nature, the mathematical relationship is more complex¹¹⁰.

6.1.3.3 *Selection bias*

Which are the selection criteria for inclusion in the study? If the cases are selected in a systematically incorrect manner according to both exposure and outcome, the study suffers from a systematic error due to selection bias.

The result of selection bias will be a risk estimate calculated as higher or lower than the true level. Selection criteria must be contemplated when designing a study. Selection bias can occur at recruitment or when conducting the study. Who signed up for the study? Why are some subjects lost to follow up?

6.2 STUDIES I-III

6.2.1 Precision

The studies comprise a large number of cases with CRC which is a rare outcome in the Swedish population, though it is one of the most common cancers¹. Study I includes a large number of cases (Table 5) and this large sample size provides good precision to the analyses. In studies II and III, there are small numbers of occurrences (surgery for SBO) (Table 4) collected using a well-defined algorithm (Figure 1). The data used from the NPR is collected annually from the Board. It seems easy to argue that the number of cases in papers II and III is too small. Even so, we were able to obtain the original patient records in 97% of the cases in study II and 98% in study III. The CI of TSBO in paper III is wide, most probably due to a low number of occurrences (Table 9). The low number of

occurrences thus (Table 4) contributes to less robust results, particularly in paper III. However, the results from study II are confirmed in study III, strengthening the argument that greater blood loss at index surgery increases the hazard of future surgery for SBO due to tumour recurrence. The low number of occurrences, rather, increases the risk of missing a true relationship (type II error) than falsely proving one (type I error).

Blood loss at index surgery was extracted from the ROC register. This provided us with the unique capability to investigate whether there is a relationship between surgical blood loss at index surgery for CRC and SBO, without tracing and assessing all patient records from the index surgery. Instead, focus could be kept upon identifying, assessing, and classifying the surgeries for SBO.

In both studies II and III, multivariate Cox regression analysis is performed with multiple adjustments. Due to the low number of occurrences, especially in paper III (Table 4, Table 9) these adjustments can pose a problem.

In paper II and III, random error is possible in the hospital diagnoses (although this ought to be very small). In order not to miss any of the surgeries for SBO, all surgeries for a wide range of ICD codes was requested for each hospital admission. This measure was taken to reduce possible errors due to misdiagnosis. It is highly unlikely that individuals who have had surgery during a hospital stay would not have the reason for this as one of his or her diagnoses. It is also very unlikely that individuals admitted for abdominal pain or small bowel obstruction of any kind would not have this registered as first or any secondary diagnoses. Since 1997, the NPR contains data on 1-8 diagnoses. In 2009 the NPR reached a completeness of 99% of all Swedish hospital admissions.

All register data used in the present studies are prospectively recorded to the registers. The registers held by ROC are continuously used for research projects. Should the researchers using the registers detect any errors, they are obligated to report this to the holder of the register for correction⁹⁴. This system further decreases the incidence of random errors in the register.

6.2.2 External validity

The studies include all persons with a CRC diagnosis, both men and women, from a large catchment area containing approximately 20% of the Swedish population. This region constitutes both larger cities (Uppsala being the fourth largest county in Sweden) and rural areas, assuring a representative cross section of the Swedish population.

6.2.3 Internal validity

6.2.3.1 Confounding

One drawback is that it was not possible to adjust for comorbidity (ASA grade) in the calculations. Comorbidity is a known confounder in the survival analyses since it can affect both the exposure (blood loss), and the outcome (survival).

In study I, the material was restricted to stage I-III disease. Statistical analyses were performed using Cox proportional hazard regression analyses with stratification for surgical procedure.

The cohorts in studies II and III were restricted using specified exclusion criteria (Table 3, Figure 2). The statistical analyses were adjusted using regression models (Cox, Logistic).

6.2.3.2 Information bias

Estimating perioperative haemorrhage is challenging and may convey uncertainty⁶⁴⁻⁶⁶.

Some patients were claimed to have a blood loss of 0 ml. Such a low volume of bleeding is considered improbable but can potentially have been registered when patients bleed very little or might be an erroneous registration. In paper I (colon cancer), patients indicated with blood loss of 0 ml were included (n=118), but in papers II and III, they were not. Fortunately, the studies include many patients and the proportion of patients claimed to have bled nil is low.

Stage is another parameter, which could possibly interfere with the results. Currently, pathologists are urged to examine ≥ 12 lymph nodes^{19, 28, 45} if possible. Earlier, the pathologist sometimes gave a diagnosis regarding regional disease based on the number of lymph nodes found without extra effort to extract more nodes. This might lead to underestimation of the proportion of lymph node positive patients. In study III, neoadjuvant RT might lead to down staging. The registry held only data on pTNM and therefore a proportion of patients may be included in the analyses with a lower stage than their true preoperative stage.

The autopsy rate in Sweden is generally low. For cancer diagnoses, it is difficult to get an appropriate registration of cause of death. Physicians tend to classify cause of death as 'cancer-death' if the patient has suffered from a malignant disease. This will lead to a differential misclassification. Because of this, survival analyses are performed for overall survival and not for cancer-specific survival.

In studies II and III, the surgeries were classified according to categories. With the slightest doubt when classifying a case, the record was later assessed in consensus between three of the authors.

6.2.3.3 *Selection bias*

All studies are population based and data were requested and gathered in a systematic manner. The ROC registers cover both colon and rectal cancers since 1997. Data are limited to that reported in the registers. The sources used (ROC, NPR) have a proven high validity^{92, 97}.

Initially, the material provided from ROC contained more than 7,047 surgeries for CRC. Some patients had had surgery more than once and some for both colon and rectal cancer. In those cases, we selected the episode with the most advanced cancer according to stage. Analogously, for patients having surgery for both rectal and colon cancer, the treatment episode for rectal cancer was selected.

Papers I and II emanate from the same cohort. Different exclusions were made, however, because of the different outcomes studied.

Exclusions and restrictions were made at the initial phase in all the studies. This strategy brings the possibility of skewed exclusions

When making exclusions, one will lose power but on the other hand, the material will probably become more homogenous – resulting in narrower confidence intervals. One problem with exclusions is that it is impossible to adjust for qualities within the excluded material in the analysis. If an exclusion is made for a group of cases that withhold certain statistical qualities (not randomly distributed in the entire material), this can introduce uncertainty into the final study results. In papers I and III, stage IV disease is excluded, but not in paper II. In study II, the multivariate analyses are instead made with adjustment for stage IV. Stage IV cases differ considerably from stage III compared to the difference between stages I and II, for example in respect to 3-year survival⁹⁴. In study II, no analysis of survival is performed. The survival analysis for the colon cancer patients is made in paper I, where stage IV is excluded.

6.3 STUDY IV

6.3.1 Precision

Information on all consecutive abdominal resections stage 0-III during 2007-2010 at Karolinska University hospital was extracted from the Stockholm-Gotland ROC register. Exclusion criteria are specified in Figure 3. Since all patients were treated at the same hospital, it was possible to link the ROC data to the hospital's laboratory results and to validate 100% of the patient records for recurrent disease.

6.3.2 External validity

The study comprises patients, both men and women, from one of the largest clinics in Sweden where modern techniques are used. The results are based on prospectively registered data where one of the outcomes (recurrence) has been validated in 100% of the cases. Calculations are performed after linkage of these different registers. Comprehensive attempts were made to keep the studied cohort of 496 patients as homogenous as possible.

6.3.3 Internal validity

6.3.3.1 *Confounding*

Analyses were restricted to a homogenous group of patients (Figure 3) and adjusted for possible confounders (sex, ASA-grade, pTNM, pT, type of surgery, neoadjuvant treatment, age and blood-loss) in a multivariate regression model. The reasoning behind adjusting for ASA grade, pTNM and pT as ordinal and not dichotomised variables was to minimise residual confounding.

Age and blood loss were adjusted for as restricted cubic splines with three degrees of freedom. These variables were calculated as continuous predictors and therefore, in this setting it did not seem appropriate to dichotomise any of the independent variables as above/below median. Furthermore, colon and rectal cancer are known to differ in volume of blood loss during surgical resection and thus this design seems more appropriate.

6.3.3.2 *Information bias*

Both the ROC register⁹² and the local blood transfusion database¹⁰¹ are validated with a well-renowned accuracy. Patient records were validated for recurrent disease in 100 % of the cases. Laboratory test results were extracted via a computerised system at the hospital (KarDa).

Patients were classified as anaemic or not based on Hb-values two months prior surgical resection. This classification is not based on other co-morbidities, intake of medicine, or other conditions, which may influence the Hb value. Therefore, it might be that some patients suffer from impaired renal function (for example) resulting in an elevated Hb value. Though this proportion of patients ought to be low. The value chosen for classification as anaemic or not was the lowest value observed during two months prior surgery. If a patient presents at a health care provider and receives treatment for anaemia and then another value is sampled, the lowest value would be chosen.

As discussed earlier, blood loss is difficult to measure. The value used is the amount prospectively reported to the register by the anaesthetic nurse immediately after surgery.

6.3.3.3 Selection bias

The study included prospectively registered data on all consecutive abdominal resections for stage I-III CRC at Karolinska University Hospital during a four-year period. There are other clinics in Stockholm performing CRC abdominal resections and patients living in the catchment area are free to choose the clinic of their preference. However, this proportion of patients is considered to be low.

7 GENERAL DISCUSSION

In this thesis, preoperative anaemia and blood loss during surgery for colorectal cancer are explored. The most important findings are:

- Preoperative anaemia is associated with impaired survival. The analyses also indicate a trend for increased risk of recurrence.
- Blood loss above median during surgery for colon cancer impairs overall survival. This effect was not seen after surgery for rectal cancer, probably due to a more complex and multifactorial situation where irradiation delivered preoperatively increased blood loss.
- Blood loss over the median at surgery for colon and rectal cancer increases the risk for later surgery for SBO due to cancer recurrence.

It can be admitted that it is difficult to differentiate the influences of anaemia, iron medication, blood transfusion, and major blood loss during surgery on the outcome of CRC-surgery. All four factors can potentially impair outcome. The first three studies focus on the importance of blood loss and the last on preoperative anaemia. Data on blood transfusion was available in studies I and IV, while data on preoperative anaemia were available only in study IV.

7.1 ANAEMIA, BLOOD TRANSFUSION AND RECURRENCE

Preoperative anaemia was associated with increased risk of recurrence (Table 12) in the Log-Rank test. No statistically significant association could be established in the multivariate model but the analyses revealed a trend towards an increased risk for recurrence for the patients with anaemia. Other factors increasing the risk of recurrence were advanced TNM and T stage. Also, type of surgery (low anterior resection and Hartmann and rectal surgery with APR) increased the risk of recurrence, APR carrying the highest hazard.

The association between preoperative anaemia in CRC and increased risk of recurrence has, to our knowledge, only been investigated in a study by Zhen on stage II colon cancers⁸⁶. It was concluded that disease free survival was impaired

for anaemic patients with T3N0M0 cancers but not in patients with T4N0M0 cancers⁸⁶. In study IV, the multivariate analyses are adjusted for patient and tumour (pTNM and pT) characteristics, while in Zhen's study, adjustments were made only for tumour characteristics⁸⁶.

In the present study, blood transfusion was not a risk factor for cancer recurrence. Previous studies, summarised in a Cochrane report, have shown that patients receiving blood transfusion within one month before or after surgery for CRC have an increased risk of recurrence, independent of tumour stage⁸⁷. There, it was not possible to adjust for type of surgery or haemorrhage during surgery⁸⁷. Those adjustments were possible in the present study, and interestingly, no association was seen between transfusion and recurrence (Table 12). Blood transfusion might impair the immune response and defence against circulating tumour cells in close association with the resection resulting in an increased risk of cancer recurrence. Therefore, the time-span of one day before or after surgery was chosen. Analyses were also performed for transfusion during one month before and after the abdominal resection (data not shown) with virtually the same results.

7.2 ANAEMIA, BLOOD TRANSFUSION AND SURVIVAL

Anaemia was significantly associated with decreased overall survival in the multivariate model (Table 12). Stage, previously described as the strongest risk factor for impaired survival was significantly associated with survival in the univariate analysis but when adjusted for preoperative anaemia this association was no longer significant.

The relationship between preoperative anaemia and impaired survival has been described in two previous studies^{84, 85}. Mild anaemia was shown to be associated with a more advanced stage and impaired overall survival. In spite of this, stage was not adjusted for in the subsequent multivariate analysis⁸⁵. Qui, et. al. investigated the relationship between preoperative hematologic abnormalities and overall survival⁸⁴. Their study included patients with both curative and

palliative surgery and the authors found that preoperative anaemia was a risk factor for impaired overall survival in a multivariate analysis⁸⁴.

Blood transfusion did not influence overall survival in the present study. A negative effect of blood transfusion on survival for CRC patients has, however, been indicated by point estimates in a study by Khanbhai et. al⁸⁸.

7.3 SURGICAL BLOOD LOSS AND OVERALL SURVIVAL

Study I shows that blood loss greater than median (≥ 250 ml) in colon cancer surgery, impairs overall survival. Due to collinearity with the volume of blood lost, the effect of transfusion was calculated in a separate multivariate model and found not to be significant. Thus, it is concluded that greater volume of blood loss is the primary determinant for the increased hazard of overall mortality for patients with a blood loss above median (Table 6). 250 ml is a fairly small amount of blood loss and in most cases, it is possible to perform resection of a colon cancer with a lower blood loss. Trying to keep the volume of blood loss low might thus improve the chance for long-term survival.

The impaired survival was also confirmed when analysing blood loss as a continuous variable, indicating that the hazard increases continuously with greater blood loss¹⁰⁶. Patients who died within 6 months of surgery were excluded in order to analyse only the long-term effect on survival.

The volume of blood loss during surgery for rectal cancer is usually larger than that for colon cancer. Hence, rectal cancer was analysed in a separate study (paper III) where no relationship was revealed between blood loss during surgery and 5-year overall survival. In study III, blood loss was divided in quartiles and classified as a volume above or below each quartile.

As described previously, treatment of rectal cancers is more complex and multimodal than for colonic cancer²⁸. In the present study, RT was delivered to 69% of the patients and those given RT bled more. On the other hand, RT is given to reduce the risk for local recurrence³¹. In the univariate analysis, there was a significantly better five-year overall survival among irradiated patients.

However, this was not confirmed in the multivariate analysis adjusted for stage, age, and the presence of a surgical complication. Since RT down-stages the tumour¹¹¹, increases the volume of blood loss and reduces the risk for local recurrence³¹, the effect of *blood loss per se* on survival is difficult to analyse.

7.4 SURGICAL BLOOD LOSS AND SMALL BOWEL OBSTRUCTION

7.4.1 Surgery for SBO

The original hypothesis in study II – that a larger volume of blood loss at index surgery for CRC will increase the risk for future ASBO could not be confirmed. Analyses showed an increase in the risk for SBO at a volume of blood lost ≥ 400 ml (Table 8). When separating the cause of SBO into ASBO and TSBO, it was obvious that increased blood loss increased the risk for TSBO, but not for ASBO, and this was evident at a blood loss of ≥ 250 ml, which was the median for the entire cohort.

The multivariate analyses are adjusted for TNM stage. Data on T stage were not available. During surgery for locally advanced cancers, haemorrhage might increase since the bloodless embryonic planes are more difficult to respect since dissection has to be done outside the bloodless embryonic planes to ensure radicality. However, it might also be that a more advanced tumour burden will influence the immune response¹¹². Blood-loss will also contribute to an inflammatory response. A local recurrence is always caused by tumour cells left in the abdominal cavity during surgery. We do not, however, know if this is always caused by omission of lymph node metastases, tumour deposits, micro-metastases, or per continuum growth of the tumour or if it could be that tumour cells or micro-metastases are disseminated by the blood.

In paper II, the proportion of TSBO patients with stage III+IV disease is 35% higher than that of stage I+II disease. More advanced stage is a known risk factor for recurrence and was also a risk factor for TSBO. However, after adjustment for stage, blood loss was still an independent risk factor for TSBO. It is not known if the patients had a known recurrent disease from colon cancer at the

onset of symptoms. However, the original material constituted patients with a locally radical resection only (Table 3).

In study II, it was confirmed that the volume of blood lost during surgery correlated to the duration of surgery. However, the duration of surgery was not a significant determinant for SBO in the univariate analysis and did not affect the HRs when introduced in the multivariate model (also when dichotomised for duration over the median). It can thus be concluded that the haemorrhage rather than the duration of surgery is responsible for the increase in hazard for TSBO.

In paper III, the statistical results are less robust. However, in the light of the results in paper II, they point in the same direction. A blood loss above the median will lead to greater risk for future TSBO, but there is no association to an increased risk for ASBO. The fact that there is no increased risk for future TSBO for the patients in the highest quartile is probably due to too few occurrences (Table 9).

7.4.2 Hospital admission for SBO without surgery

It was possible to retrieve patient records from the surgeries for almost all cases with an operation for SBO. For the outcome hospital admission for SBO without surgery, the ICD code consistent with SBO for the hospital episode was used in the calculation. No patient records have been reviewed for validation of this outcome, leaving some uncertainty.

For the colon cancers in study II, an association between the risk for hospital admission for SBO without surgery and blood loss at index surgery was seen in a multivariate model at a blood loss of 250 ml or more at index surgery for CRC (Table 8). This association was not evident for the rectal cancer patients in study III.

In the study on colon cancer, 3% underwent surgery for SBO and 6% were admitted for SBO without surgery, while for rectal cancer, the corresponding numbers were 5% and 16%, respectively. Although admission for SBO was more common after rectal cancer, an association with blood loss was not evident

in the present study. The only potential risk factor that manifested as significant was irradiation. Radiotherapy has earlier been shown to increase the risk for in hospital episodes after surgery for rectal cancer¹¹³ and it might be that RT is one of the main reasons for the higher risk of hospital admission without surgery after resection of a rectal cancer.

8 CONCLUSION

This thesis on anaemia, blood loss and colorectal cancer shows clearly that preoperative anaemia but not perioperative transfusion is associated with impaired overall survival. The analyses also revealed a trend towards an increased risk for recurrence for patients with preoperative anaemia. Blood loss at index surgery of colon cancer above median (≥ 250 ml) was also shown to impair overall survival. Furthermore, a blood loss above median at the index surgery for colon and rectal cancer (≥ 250 ml for colon cancer and 800 ml for rectal cancer) is associated with increased risk for future surgery for SBO caused by cancer recurrence.

There are no clear guidelines for treatment of preoperative anaemia. In further research, the reason why anaemia constitutes such a strong risk for impaired outcome must be explored as well as the options for treatment. For many reasons, blood loss should be kept low during surgery for CRC. The results of this thesis show that a relatively small increase in blood loss can make a difference in cancer outcome.

9 SAMMANFATTNING PÅ SVENSKA

9.1 BAKGRUND

Cancer i tjock- och ändtarm (kolorektal cancer) är den fjärde vanligaste cancersjukdomen i världen. Sjukdomen drabbar både kvinnor och män. I Sverige insjuknar årligen cirka 6000 patienter. Den botande behandlingen är operation kombinerat med strålbehandling och/ eller cellgifter beroende på var cancern sitter och svårighetsgrad.

Ungefär hälften av patienterna har lågt blodvärde, s.k. ”anemi” när de får sin diagnos. Många patienter behöver blodtransfusion i samband med sin canceroperation. Antingen pga. lågt blodvärde eller stor blodförlust vid operationen. Tidigare forskningsresultat har visat att blodtransfusion ökar risken för canceråterfall hos patienter med kolorektal cancer oberoende av sjukdomens svårighetsgrad. Innan arbetet med denna avhandling påbörjades var det oklart om blödningsmängden under operation påverkar risken för återfall och död.

En sen komplikation till operation för kolorektal cancer är tarmvred (i denna sammanfattning avses mekaniskt stopp i tunntarmen). Tidigare forskningsrapporter har visat att upp emot 10 % av patienterna behöver en operation för tarmvred efter kolorektal kirurgi. Detta kan bero på t.ex. sammanväxningar eller återfall av cancer.

Syftet med avhandlingen var att undersöka huruvida

- Anemi före operation eller blodtransfusion vid operation för kolorektal cancer ökade risken för sjukdomsåterfall och död. Studien omfattade patienter som opererats i botande syfte.
- Blodförlusten vid operation hos patienter med tjocktarmscancer förkortar framtida överlevnad.
- Blodförlusten vid operation på patienter med tjocktarmscancer ökar risken för framtida tarmvred orsakade av sammanväxningar i buken.

- Blodförlusten vid operation på patienter med ändtarmscancer påverkar risken för framtida operationer för tarmvred orsakat av sammanväxningar, tumöråterfall och livslängd.

9.2 METOD

Alla studier baseras på befintliga svenska register över kolorektal cancer. Registren innehåller information om personnummer, diagnos, operation och eftervård. Alla studier hade etiskt godkännande. Data oidentifierades och ingen patient kan urskiljas ur presenterade data. Varken av läsare eller forskare. Resultaten gäller på gruppnivå.

I studien som utreder anemi och blodtransfusion analyserades data på patienter som opererats i botande syfte på Karolinska universitetssjukhuset 2007-2010. Data länkades till uppgifter om blodvärden och blodtransfusion. Uppgift om sjukdomsåterfall inhämtades från patientjournaler.

I de tre studierna som utreder risker med blodförlust vid operation hämtades data på alla patienter som opererats för kolorektal cancer i Uppsala-Örebro sjukvårdsområde åren 1997-2003. Uppgift om operation för tarmvred hämtades från slutenvårdsregistret hos socialstyrelsen. Orsaken till tarmvredet inhämtades ur patientjournaler.

9.3 RESULTAT

Blodbrist före operation ökade risk för död. Analyserna visade även på en trend mellan blodbrist och ökad risk för canceråterfall. Det fanns inget samband mellan blodtransfusion canceråterfall och död.

Blodförlust över 250 ml (medianvärde för patientgruppen) påverkade överlevnaden negativt hos patienter med tjocktarmscancer. Blodförlust över medianvärdet ökade också risken för framtida operation för tarmvred hos patienter opererade för tjocktarmscancer. Dessa tarmvred berodde på tumöråterfall.

Blodförlust över 800 ml (medianvärde för patientgruppen) ökade risken för tarmvred orsakat av tumöråterfall hos patienter med ändtarmscancer. Det fanns inget samband mellan blodförlust och överlevnad hos patienterna med ändtarmscancer.

9.4 SLUTSATS

Blodbrist före operation för kolorektal cancer är en riskfaktor för död. Det verkar även som att blodbrist före operation kan vara en riskfaktor för tumöråterfall. Idag finns inga riktlinjer för hur blodbrist skall behandlas. Det är ett viktigt område för mer forskning.

Operationsblödningen påverkar risk för att få återfall efter operation för tjocktarmscancer och risken att få tumörorsakat tarmvred efter operation för cancer i tjock- och ändtarm. Blödningen bör därför hållas till ett minimum för att förbättra utfallet efter kirurgi för kolorektal cancer.

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11 REFERENCES

1. Socialstyrelsen. Cancer statistics.
<http://www.socialstyrelsen.se/statistik/statistikdatabas/cancer>.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA: a cancer journal for clinicians* 2011; **61**(2): 69-90.
3. Boyle P, Langman JS. ABC of colorectal cancer: Epidemiology. *Bmj* 2000; **321**(7264): 805-8.
4. Rasool S, Kadla SA, Rasool V, Ganai BA. A comparative overview of general risk factors associated with the incidence of colorectal cancer. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2013; **34**(5): 2469-76.
5. Socialstyrelsen. Statistikdatabas för cancer. 2014.
<http://www.socialstyrelsen.se/statistik/statistikdatabas/cancer> (accessed June 24 2014).
6. Winawer SJ, Zauber AG, Ho MN, et al. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. *The New England journal of medicine* 1993; **329**(27): 1977-81.
7. Brenner H, Bouvier AM, Foschi R, et al. Progress in colorectal cancer survival in Europe from the late 1980s to the early 21st century: the EURO CARE study. *International journal of cancer Journal international du cancer* 2012; **131**(7): 1649-58.
8. Lindholm E, Brevinge H, Haglund E. Survival benefit in a randomized clinical trial of faecal occult blood screening for colorectal cancer. *The British journal of surgery* 2008; **95**(8): 1029-36.
9. Socialstyrelsen. Tjock- och ändtarmscancer, screening med test av blod i avföringen.
<http://www.socialstyrelsen.se/riktlinjer/nationellascreeningprogram/tjock-ochandtarmscancer-screen> (accessed October 29 2014).
10. Jemal A, Clegg LX, Ward E, et al. Annual report to the nation on the status of cancer, 1975-2001, with a special feature regarding survival. *Cancer* 2004; **101**(1): 3-27.
11. Schoen RE, Pinsky PF, Weissfeld JL, et al. Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy. *The New England journal of medicine* 2012; **366**(25): 2345-57.
12. Martling A, Holm T, Rutqvist LE, et al. Impact of a surgical training programme on rectal cancer outcomes in Stockholm. *The British journal of surgery* 2005; **92**(2): 225-9.
13. Heald RJ, Ryall RD. Recurrence and survival after total mesorectal excision for rectal cancer. *Lancet* 1986; **1**(8496): 1479-82.

14. Heald RJ. The 'Holy Plane' of rectal surgery. *Journal of the Royal Society of Medicine* 1988; **81**(9): 503-8.
15. Glimelius B, Cavalli-Bjorkman N. Metastatic colorectal cancer: current treatment and future options for improved survival. Medical approach--present status. *Scandinavian journal of gastroenterology* 2012; **47**(3): 296-314.
16. Birgisson H, Talback M, Gunnarsson U, Pahlman L, Glimelius B. Improved survival in cancer of the colon and rectum in Sweden. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology* 2005; **31**(8): 845-53.
17. Ragnhammar P, Hafstrom L, Nygren P, Glimelius B, Care SB-gSCoTAiH. A systematic overview of chemotherapy effects in colorectal cancer. *Acta oncologica* 2001; **40**(2-3): 282-308.
18. Edna TH, Karlsen V, Jullumstro E, Lydersen S. Prevalence of anaemia at diagnosis of colorectal cancer: assessment of associated risk factors. *Hepato-gastroenterology* 2012; **59**(115): 713-6.
19. Pählman L CB, Bohe M, Dahlberg M, Öjerskog B, Hallböök O. Kolorektal cancer. Nationellt vårdprogram 2008.
http://www.cancercentrum.se/Global/RCCUppsalaOrebro/V%c3%a5rdprocesser/kolorektal/v%c3%a5rdprogram/nat_vardprogram_08_omvvp_110412.pdf:
Onkologiskt centrum, Umeå; 2008.
20. Jestin P, Nilsson J, Heurgren M, Pahlman L, Glimelius B, Gunnarsson U. Emergency surgery for colonic cancer in a defined population. *The British journal of surgery* 2005; **92**(1): 94-100.
21. Nationella styrgruppen för koloncancer. Registret för koloncancer, nationell rapport, 2012; 2013, ISBN 91-89048-50-4.
22. Nationella styrgruppen för rektalcancer. Registret för rektalcancer, nationell rapport, 2012; 2013, ISBN: 91-89048-51-2.
23. Nationellt kvalitetsregister. Fem års uppföljning av cancer recti 2004. Sjukhusstatistik 2001-2009; 2009, ISBN 91-89048-37-7.
24. Vigano L, Russolillo N, Ferrero A, Langella S, Sperti E, Capussotti L. Evolution of long-term outcome of liver resection for colorectal metastases: analysis of actual 5-year survival rates over two decades. *Annals of surgical oncology* 2012; **19**(6): 2035-44.
25. Nordlinger B, Sorbye H, Glimelius B, et al. Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. *Lancet* 2008; **371**(9617): 1007-16.
26. Erichsen R, Horvath-Puho E, Iversen LH, Lash TL, Sorensen HT. Does comorbidity interact with colorectal cancer to increase mortality? A nationwide population-based cohort study. *British journal of cancer* 2013; **109**(7): 2005-13.

27. Arbetsgruppen för Cancergenetiska Mottagningar. Ärftlig kolorektalcancer - utredning, uppföljning och omhändertagande. http://sfmg.se/download/riktlinjer/Cancergenetik/Arftlig%20kolorektalcancer_Arbetsgruppen_2012.pdf; 2012, ISBN 978-91-85999-73-6.
28. van de Velde CJ, Boelens PG, Borras JM, et al. EURECCA colorectal: multidisciplinary management: European consensus conference colon & rectum. *European journal of cancer* 2014; **50**(1): 1 e- e34.
29. NIH consensus conference. Adjuvant therapy for patients with colon and rectum cancer. In: NIH, editor.; 1990. p. 1.
30. Sobin L. TNM Classification of Malignant Tumours. 7th ed; 2009.
31. van Gijn W, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. *The lancet oncology* 2011; **12**(6): 575-82.
32. Cedermark B, Johansson H, Rutqvist LE, Wilking N. The Stockholm I trial of preoperative short term radiotherapy in operable rectal carcinoma. A prospective randomized trial. Stockholm Colorectal Cancer Study Group. *Cancer* 1995; **75**(9): 2269-75.
33. Improved survival with preoperative radiotherapy in resectable rectal cancer. Swedish Rectal Cancer Trial. *The New England journal of medicine* 1997; **336**(14): 980-7.
34. Martling A, Holm T, Johansson H, Rutqvist LE, Cedermark B, Stockholm Colorectal Cancer Study G. The Stockholm II trial on preoperative radiotherapy in rectal carcinoma: long-term follow-up of a population-based study. *Cancer* 2001; **92**(4): 896-902.
35. Colorectal Cancer Collaborative G. Adjuvant radiotherapy for rectal cancer: a systematic overview of 8,507 patients from 22 randomised trials. *Lancet* 2001; **358**(9290): 1291-304.
36. Brannstrom F, Jestin P, Matthiessen P, Gunnarsson U. Surgeon and hospital-related risk factors in colorectal cancer surgery. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2011; **13**(12): 1370-6.
37. Phang PT. Total mesorectal excision: technical aspects. *Canadian journal of surgery Journal canadien de chirurgie* 2004; **47**(2): 130-7.
38. Penninckx F, Kartheuser A, Van de Stadt J, et al. Outcome following laparoscopic and open total mesorectal excision for rectal cancer. *The British journal of surgery* 2013; **100**(10): 1368-75.
39. Ohtani H, Tamamori Y, Azuma T, et al. A meta-analysis of the short- and long-term results of randomized controlled trials that compared laparoscopy-assisted and conventional open surgery for rectal cancer. *Journal of*

gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract 2011; **15**(8): 1375-85.

40. Baik SH, Kim NK, Lim DR, Hur H, Min BS, Lee KY. Oncologic outcomes and perioperative clinicopathologic results after robot-assisted tumor-specific mesorectal excision for rectal cancer. *Annals of surgical oncology* 2013; **20**(8): 2625-32.

41. Hohenberger W, Weber K, Matzel K, Papadopoulos T, Merkel S. Standardized surgery for colonic cancer: complete mesocolic excision and central ligation--technical notes and outcome. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2009; **11**(4): 354-64; discussion 64-5.

42. Adamina M, Manwaring ML, Park KJ, Delaney CP. Laparoscopic complete mesocolic excision for right colon cancer. *Surgical endoscopy* 2012; **26**(10): 2976-80.

43. Gouvas N, Pechlivanides G, Zervakis N, Kafousi M, Xynos E. Complete mesocolic excision in colon cancer surgery: a comparison between open and laparoscopic approach. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2012; **14**(11): 1357-64.

44. Glimelius B, Dahl O, Cedermark B, et al. Adjuvant chemotherapy in colorectal cancer: a joint analysis of randomised trials by the Nordic Gastrointestinal Tumour Adjuvant Therapy Group. *Acta oncologica* 2005; **44**(8): 904-12.

45. Onitilo AA, Stankowski RV, Engel JM, Doi SA. Adequate lymph node recovery improves survival in colorectal cancer patients. *Journal of surgical oncology* 2013; **107**(8): 828-34.

46. Lee HY, Choi HJ, Park KJ, et al. Prognostic significance of metastatic lymph node ratio in node-positive colon carcinoma. *Annals of surgical oncology* 2007; **14**(5): 1712-7.

47. Thomassen I, van Gestel YR, Lemmens VE, de Hingh IH. Incidence, prognosis, and treatment options for patients with synchronous peritoneal carcinomatosis and liver metastases from colorectal origin. *Diseases of the colon and rectum* 2013; **56**(12): 1373-80.

48. Kanas GP, Taylor A, Primrose JN, et al. Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors. *Clinical epidemiology* 2012; **4**: 283-301.

49. Neeff H, Horth W, Makowiec F, et al. Outcome after resection of hepatic and pulmonary metastases of colorectal cancer. *Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract* 2009; **13**(10): 1813-20.

50. Verwaal VJ, Bruin S, Boot H, van Slooten G, van Tinteren H. 8-year follow-up of randomized trial: cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy in patients with peritoneal carcinomatosis of colorectal cancer. *Annals of surgical oncology* 2008; **15**(9): 2426-32.
51. Regionala cancercentrum i samverkan. Kolorektal cancer - Nationellt vårdprogram. http://www.sfam.se/wp-content/uploads/2014/11/K-C-V%C3%A5rdprogram_20141013.pdf (accessed October 14 2015).
52. Beets GL, Glimelius BL. Adjuvant chemotherapy for rectal cancer still controversial. *The lancet oncology* 2014; **15**(2): 130-1.
53. Nationella styrgruppen för koloncancer. Koloncancer - Nationell kvalitetsrapport för år 2013 från Svenska Kolorektalcancerregistret. 2014, ISBN 91-89048-53-9.
54. Tiselius C, Gunnarsson U, Smedh K, Glimelius B, Pahlman L. Patients with rectal cancer receiving adjuvant chemotherapy have an increased survival: a population-based longitudinal study. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2013; **24**(1): 160-5.
55. Bosset JF, Calais G, Mineur L, et al. Fluorouracil-based adjuvant chemotherapy after preoperative chemoradiotherapy in rectal cancer: long-term results of the EORTC 22921 randomised study. *The lancet oncology* 2014; **15**(2): 184-90.
56. Bosset JF, Collette L. Adjuvant chemotherapy for rectal cancer--authors' reply. *The lancet oncology* 2014; **15**(6): e197-8.
57. Bujko K, Glimelius B. Adjuvant chemotherapy for rectal cancer. *The lancet oncology* 2014; **15**(6): e194-5.
58. Wille-Jørgensen P, Laurberg S, Pahlman L, et al. An interim analysis of recruitment to the COLOFOL trial. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2009; **11**(7): 756-8.
59. Wong PF, Kumar S, Bohra A, Whetter D, Leaper DJ. Randomized clinical trial of perioperative systemic warming in major elective abdominal surgery. *The British journal of surgery* 2007; **94**(4): 421-6.
60. Widman J, Hammarqvist F, Sellden E. Amino acid infusion induces thermogenesis and reduces blood loss during hip arthroplasty under spinal anesthesia. *Anesthesia and analgesia* 2002; **95**(6): 1757-62, table of contents.
61. Shander A, Javidroozi M, Perelman S, Puzio T, Lobel G. From bloodless surgery to patient blood management. *The Mount Sinai journal of medicine, New York* 2012; **79**(1): 56-65.

62. Bokey EL, Chapuis PH, Dent OF, Mander BJ, Bissett IP, Newland RC. Surgical technique and survival in patients having a curative resection for colon cancer. *Diseases of the colon and rectum* 2003; **46**(7): 860-6.
63. Anderin C, Martling A, Hellborg H, Holm T. A population-based study on outcome in relation to the type of resection in low rectal cancer. *Diseases of the colon and rectum* 2010; **53**(5): 753-60.
64. Johar RS, Smith RP. Assessing gravimetric estimation of intraoperative blood loss. *Journal of gynecologic surgery* 1993; **9**(3): 151-4.
65. Lee MH, Ingvertsen BT, Kirpensteijn J, Jensen AL, Kristensen AT. Quantification of surgical blood loss. *Veterinary surgery : VS* 2006; **35**(4): 388-93.
66. Budny PG, Regan PJ, Roberts AH. The estimation of blood loss during burns surgery. *Burns : journal of the International Society for Burn Injuries* 1993; **19**(2): 134-7.
67. Ellis H, Moran B, Thompson J, et al. Adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study. *The Lancet* 1999; **353**(9163): 1476-80.
68. Parker MC, Wilson MS, van Goor H, et al. Adhesions and colorectal surgery - call for action. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2007; **9 Suppl 2**: 66-72.
69. Weibel MA, Majno G. Peritoneal adhesions and their relation to abdominal surgery. A postmortem study. *American journal of surgery* 1973; **126**(3): 345-53.
70. Holmdahl LE, Al-Jabreen M, Risberg B. Role of fibrinolysis in the formation of postoperative adhesions. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society* 1994; **2**(3): 171-6.
71. Holmdahl L, Eriksson E, al-Jabreen M, Risberg B. Fibrinolysis in human peritoneum during operation. *Surgery* 1996; **119**(6): 701-5.
72. Edna TH, Bjerkeset T. Small bowel obstruction in patients previously operated on for colorectal cancer. *The European journal of surgery = Acta chirurgica* 1998; **164**(8): 587-92.
73. Gunnarsson U, Karlbom U, Docker M, Raab Y, Pahlman L. Proctocolectomy and pelvic pouch--is a diverting stoma dangerous for the patient? *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2004; **6**(1): 23-7.
74. Parker MC, Wilson MS, Menzies D, et al. Colorectal surgery: the risk and burden of adhesion-related complications. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2004; **6**(6): 506-11.

75. Sjøvall A, Granath F, Cedermark B, Glimelius B, Holm T. Loco-regional recurrence from colon cancer: a population-based study. *Annals of surgical oncology* 2007; **14**(2): 432-40.
76. Ellis CN, Boggs HW, Jr., Slagle GW, Cole PA. Small bowel obstruction after colon resection for benign and malignant diseases. *Diseases of the colon and rectum* 1991; **34**(5): 367-71.
77. Sadahiro S, Suzuki T, Tokunaga N, et al. Anemia in patients with colorectal cancer. *Journal of gastroenterology* 1998; **33**(4): 488-94.
78. Ludwig H, Muldur E, Endler G, Hubl W. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2013; **24**(7): 1886-92.
79. Ludwig H, Van Belle S, Barrett-Lee P, et al. The European Cancer Anaemia Survey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients. *European journal of cancer* 2004; **40**(15): 2293-306.
80. WHO. WHO - Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. *Vitamin and Mineral Nutrition Information System*, 2011. <http://www.who.int/vmnis/indicators/haemoglobin.pdf> (accessed 2014 oct 27).
81. Health E. Anaemia - Introduction and classification. <http://www.us.elsevierhealth.com/media/us/samplechapters/9780443103629/9780443103629.pdf> : Elsevier Health; 2007: 22-37.
82. Acher PL, Al-Mishlab T, Rahman M, Bates T. Iron-deficiency anaemia and delay in the diagnosis of colorectal cancer. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2003; **5**(2): 145-8.
83. Munoz M, Gomez-Ramirez S, Martin-Montanez E, Auerbach M. Perioperative anemia management in colorectal cancer patients: a pragmatic approach. *World journal of gastroenterology : WJG* 2014; **20**(8): 1972-85.
84. Qiu MZ, Yuan ZY, Luo HY, et al. Impact of pretreatment hematologic profile on survival of colorectal cancer patients. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2010; **31**(4): 255-60.
85. Stapley S, Peters TJ, Sharp D, Hamilton W. The mortality of colorectal cancer in relation to the initial symptom at presentation to primary care and to the duration of symptoms: a cohort study using medical records. *British journal of cancer* 2006; **95**(10): 1321-5.
86. Zhen L, Zhe S, Zhenning W, et al. Iron-deficiency anemia: a predictor of diminished disease-free survival of T3N0M0 stage colon cancer. *Journal of surgical oncology* 2012; **105**(4): 371-5.

87. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane database of systematic reviews* 2006; (1): CD005033.
88. Khanbhai M, Shah M, Cantanhede G, Ilyas S, Richards T. The problem of anaemia in patients with colorectal cancer. *ISRN hematology* 2014; **2014**: 547914.
89. Skatteverket. Personnumrets uppbyggnad. 2014, http://www.skatteverket.se/privat/folkbokforing/omfolkbokforing/personnumrets_oppbyggnad.4.18e1b10334ebe8bc80001502.htm (accessed May 5 2014).
90. Socialstyrelsen. Hälsodataregister /Cancerregistret /Historik 2014, <http://www.socialstyrelsen.se/register/halsodataregister/cancerregistret/historik> (accessed May 5 2014).
91. Socialstyrelsen. Cancerregistrert - För uppgiftslämnare. <http://www.socialstyrelsen.se/register/halsodataregister/cancerregistret/foruppgiftslamnare> (accessed April 30 2014).
92. Gunnarsson U, Seligsohn E, Jestin P, Pahlman L. Registration and validity of surgical complications in colorectal cancer surgery. *The British journal of surgery* 2003; **90**(4): 454-9.
93. Sjøvall A, Blomqvist L, Martling A. Pretreatment staging of colon cancer in the Swedish population. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland* 2013; **15**(11): 1361-6.
94. Kodeda K, Nathanaelsson L, Jung B, et al. Population-based data from the Swedish Colon Cancer Registry. *The British journal of surgery* 2013; **100**(8): 1100-7.
95. Owens WD, Felts JA, Spitznagel EL, Jr. ASA physical status classifications: a study of consistency of ratings. *Anesthesiology* 1978; **49**(4): 239-43.
96. Pahlman L. Register inom kirurgin - Svenska rektalcancerregistret. *Svensk kirurgi* 2010; **68**(5): 240-2.
97. Socialstyrelsen. Kvalitet och innehåll i patientregistret. June 2009 2009, Artikelnummer 2009-125-15. www.socialstyrelsen.se/Statistik/statistik_amne/sluten_vard/Patientregistret.htm.
98. Socialstyrelsen. Klassificering och koder /Åtgärds-koder (KVÅ) 2014, <http://www.socialstyrelsen.se/klassificeringochkoder/atgardskoderkva> (accessed May 5 2014).
99. Socialstyrelsen. Diagnoskoder (ICD 10). 2014, <http://www.socialstyrelsen.se/klassificeringochkoder/diagnoskoder#3> (accessed June 6 2014).
100. ProSang – the leading blood management system in Scandinavia. 2013. <https://www.prosang.com/prosang.pdf> (accessed october 29 2014).

101. Halmin M, Bostrom F, Brattstrom O, et al. Effect of plasma-to-RBC ratios in trauma patients: a cohort study with time-dependent data*. *Critical care medicine* 2013; **41**(8): 1905-14.
102. SLL IT V-f. Användarinstruktion - Strukturerade Patientdata. 2011.
103. Socialstyrelsen. Klassifikation av sjukdomar 1987, systematisk förteckning, Svensk version av International Classification of Diseases, Ninth Revision (ICD-9). 1987.
104. ICD 10. WHO.
<http://apps.who.int/classifications/icd10/browse/2010/en#/K55-K63>.
105. Nakajima J, Sasaki A, Otsuka K, Obuchi T, Nishizuka S, Wakabayashi G. Risk Factors for Early Postoperative Small Bowel Obstruction After Colectomy for Colorectal Cancer. *World J Surg* 2010; **34**(5): 1086-90.
106. Morner ME, Gunnarsson U, Egenvall M. Reply to Letter: "Analyzing the Influence of Blood Loss on Outcomes of Cancer Surgery". *Annals of surgery* 2014.
107. Morner M, Gunnarsson U, Jestin P, Egenvall M. Volume of blood loss during surgery for colon cancer is a risk determinant for future small bowel obstruction caused by recurrence-a population-based epidemiological study. *Langenbeck's archives of surgery / Deutsche Gesellschaft fur Chirurgie* 2015; **400**(5): 599-607.
108. Hofman A. <http://www.hsph.harvard.edu/albert-hofman/>.
109. Rothman K. Epidemiology - an introduction. 2 nd ed: Oxford; 2012.
110. Rothman K. Modern Epidemiology. 3 rd ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
111. Pettersson D, Holm T, Iversen H, Blomqvist L, Glimelius B, Martling A. Preoperative short-course radiotherapy with delayed surgery in primary rectal cancer. *The British journal of surgery* 2012; **99**(4): 577-83.
112. Kersten C, Louhimo J, Algars A, et al. Increased C-reactive protein implies a poorer stage-specific prognosis in colon cancer. *Acta oncologica* 2013; **52**(8): 1691-8.
113. Birgisson H, Pahlman L, Gunnarsson U, Glimelius B, Swedish Rectal Cancer Trial G. Adverse effects of preoperative radiation therapy for rectal cancer: long-term follow-up of the Swedish Rectal Cancer Trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005; **23**(34): 8697-705.