Prognostic factors in colon cancer

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holder or the unit which grants the doctorate.

This thesis is dedicated to my fantastic family,

who has supported me during the entire study period;

my dear wife Janne,

my lovely daughters Ida and Eline,

and my wonderful sons Ole Jørgen and Sverre.

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Fredrikstad, May 2011

Preface

The studies presented in this Thesis are based on a research project initiated in 1993 by the late Professor Knut Nygaard. His large interest and competence in colorectal cancer treatment was a great inspiration for the younger colleges. All patients admitted to Oslo University Hospital, Aker were prospectively included, and a comprehensive set of clinical and pathological data from the primary treatment and follow-up was registered and entered into a local database. Professor Nygaard saw the potential for quality assessment and clinical research based on these registrations. Professor Arild Nesbakken attended the department in 1995, and immediately became responsible for the registry. Since Professor Nygaard resigned in 1997, he has been responsible for the whole research project.

In the early nineties, a standardised technique for rectal resection, total mesorectal excision (TME) as described by R. J. Heald, was introduced in our hospital and nationwide in Norway. TME has been one of the most important contributions to oncological surgery during the last 25 years, leading to decreased rates of local recurrence and improved survival[1]. Several studies on TME for rectal cancer, based on the research project which started in 1993, have been published, describing local recurrence rates and survival[2], early complications[3], and functional outcome – including neorectal function[4], sexual- and bladder function[5] and the long term consequences of anastomotic leakage[6].

The operative technique for treatment of colon cancer has been basically unchanged for decades. However, in recent years new attention has been given to the importance of lymph node removal for correct staging and improvement of oncological outcome, and to the importance of correct dissection in the mesocolic plane to secure free radial margins. Corresponding to the TME for rectal cancer, the concept of complete mesocolic excision (CME) for colon cancer has been introduced. However, the surgical technique still varies between centres and individual surgeons, both in Norway and internationally.

Ole H. Sjo was introduced to and engaged in the research project in 2003, and had a special focus on clinical research on colon cancer. A number of clinicopathological factors with prognostic impact were known at that time. Other factors were supposed to have prognostic impact, but reports were equivocal, and further studies were warranted. Our large, prospective and population based patient series was considered suitable for such studies, and the results are presented in this Thesis.

A translational research program in cooperation with The Institute for Cancer Research, Oslo University Hospital, Radiumhospitalet and based on the same patient cohort, was started in 2005 in order to evaluate the prognostic and predictive impact of molecular genetic factors.

Abbreviations

AJCC	American Joint Committee on Cancer
CC	Colon cancer
CEA	Carcinoembryonic antigen
CIN	Chromosomal instability
CRC	Colorectal cancer
CSS	Cancer specific survival
DFS	Disease free survival
FAP	Familial adenomatous polyposis
HE	Haematoxylin Eosin
HNPCC	Hereditary Non Polyposis Colon Cancer
HR	Hazard ratio
IHC	Immunohistochemistry
ITC	Isolated tumour cells
LN	Lymph nodes
LNR	Lymph node ratio
MM	Micrometastases
MMR	Mismatch repair
MSI	Microsatellite instability
OR	Odds ratio
OS	Overall survival
PBT	Perioperative blood transfusion
PC	Peritoneal carcinomatosis
RT-PCR	Reverse transcriptase - Polymerase chain reaction
RS	Relative survival
SN	Sentinel node(s)
TNM	Tumour - Node - Metastasis
TTR	Time to recurrence
UICC	International Union Against Cancer (Union Internationale Contre Cancer)

List of Papers

1: Sjo OH, Lunde OC, Nygaard K, Sandvik L, Nesbakken A. Tumour location is a prognostic factor for survival in colonic cancer patients. Colorectal Dis. 2008 Jan;10(1):33-40. Epub 2007 Aug 2.

2: Sjo OH, Larsen S, Lunde OC, Nesbakken A. Short term outcome after emergency and elective surgery for colon cancer. Colorectal Dis. 2009 Sep; 11(7):733-9. Epub 2008 Jul 9.

3: Faerden AE, Sjo OH, Bukholm IR, Andersen SN, Svindland A, Nesbakken A, et al. Lymph node micrometastases and isolated tumor cells influence survival in stage I and II colon cancer. Dis Colon Rectum 2011;54(2):200-6.

4: Sjo OH, Berg M, Merok MA, Kolberg M, Svindland A, Lothe RA, Nesbakken A. Peritoneal carcinomatosis of colon cancer origin: Highest incidence in women and in patients with right sided tumours. J Surg Oncol 2011 May 5. doi: 10.1002/jso.21959. [Epub ahead of print]

5: Sjo OH, Merok MA, Svindland A, Nesbakken A. Prognostic impact of lymph node harvest and lymph node ratio in colon cancer patients. (Submitted)

General introduction

Epidemiology

Incidence

Cancer is a major health problem in the world, and colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer-related death worldwide. The incidence is highest in North America, Australia/New Zealand and Western Europe and lowest in Africa and Asia. More than a million new cases are diagnosed every year and approximately half of these patients will die from the disease within five years. The lifetime risk of developing colorectal cancer is 4-5% in the Western World. In Norway, the cumulative risk of developing colon cancer by the age of 75 years was 3.0% for males and 2.7% for females[7] during 2004-2008, and CRC is the second most frequent cancer after breast cancer in women, the third most frequent after prostate and lung cancer in men, and the most common cancer for both genders together[7]. The incidence has doubled for both genders during the last 35 years. The rates are among the highest in the world, and for women highest in Europe[8]. In the Nordic countries, Norway has the highest rates for both genders (Figure 1 and 2). Overall, the incidence in Norway over the last years seems to stabilise, at least for women[7].

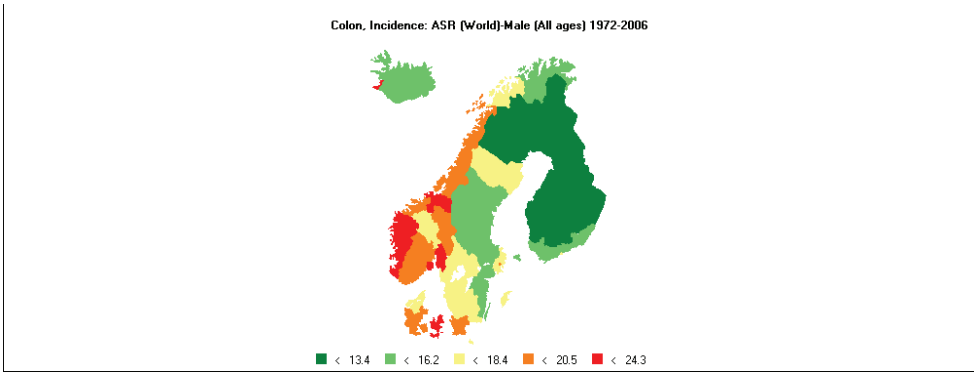

Figure 1: **Incidence for males in the Nordic countries 1972-2006, all ages**
Source: (NORDCAN; http://www-dep.iarc.fr/nordcan/English/frame.asp)

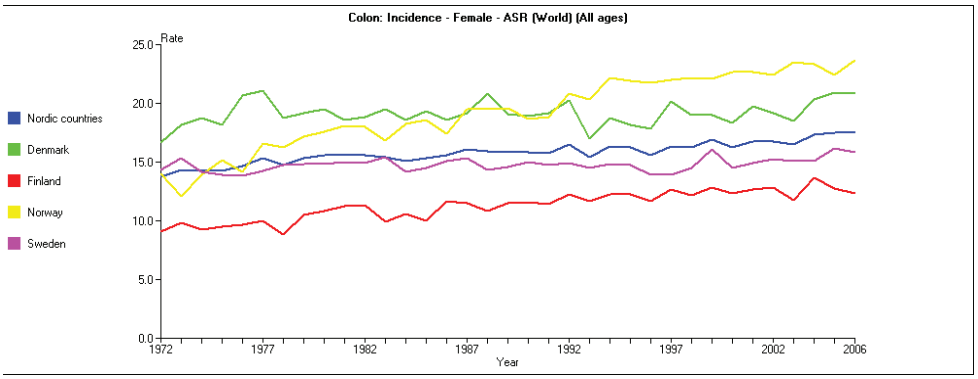

Figure 2: **Development of incidence in the Nordic countries 1972-2006, females**

Source: (NORDCAN; http://www-dep.iarc.fr/nordcan/English/frame.asp)

Survival

Long term survival in colon cancer patients has improved over the last decades; from 1965 to 2007, the 5 year relative survival rates in Norway, adjusted for survival in the general population, have increased from below 30% to approximately 60% for men and 65% for women[9], paralleling the increased rates of incidence.

Long term survival in colon cancer patients is strongly dependent on stage of disease at the time of diagnosis. For all stages, the relative risk of cancer associated mortality is highest during the first years after diagnosis and treatment, then stabilising after about five year (Figure 2).

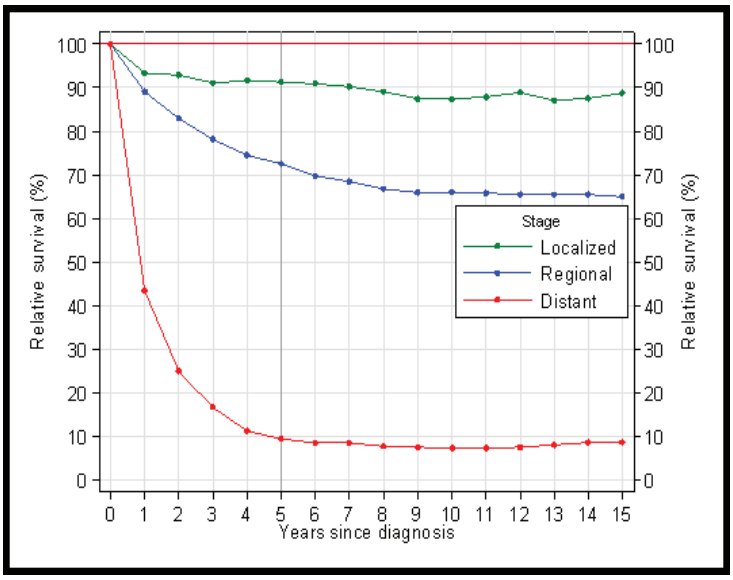

Figure 3: 15 year relative survival in colon cancer patients for both genders, related to stage of disease at the time of diagnosis [9].

Aetiology / genetics in colon cancer

The present studies are made on adenocarcinomas, the most frequent histological type of colon cancer, which accounts for some 98% of all malignancies in the large bowel[10]. The majority of adenocarcinomas occur sporadically (75-85%)[11], but 25-30% of the patients have a family history of cancer. In about 5% of the CRC patients, an inherited germline gene defect is confirmed. The study of these inherited defects has yielded insights into the genetics of sporadic colorectal carcinogenesis.

Hereditary CRC syndromes

The most common of these syndromes is the Lynch Syndrome, also known as Hereditary Non Polyposis Colon Cancer (HNPCC), which is an autosomal dominant disorder and accounts for 1-6% of all malignancies of the colon[12]. This syndrome is characterised by early onset of CRC (mean age < 45 years), with tumours predominantly located in the right colon (70%)[12], and often associated with synchronous (18%) or metachronous (24%) tumours. In Type I, the only affected organ is the large bowel; in Type II, there are additional extra colonic tumours[13]. The syndrome is caused by mutation in one of the five human mismatch repair (MMR) genes, essential in repairing the nucleotide repeats (microsatellites) that are prone to slippage during replication[14]. The second most frequent inherited syndrome, the Familial Adenomatous Polyposis syndrome (FAP), accounts for less than 1% of all

CRC[15]. From early adolescence and onwards, patients with this condition develop hundreds to thousands of polyps in the colon and rectum. There are several clinical variations of FAP, such as Gardner's syndrome[16], Turcot's syndrome[17] and the attenuated form of FAP[18]. FAP is caused by germline mutations in the tumour suppressor gene *APC* (adenomatous polyposis coli), also found to be frequently mutated in sporadic colorectal cancers. Other inherited CRC syndromes are rare and includes the Peutz-Jegher's syndrome with mucocutaneous pigmentation and gastrointestinal hamartomas, the juvenile polyposis syndrome with multiple hamartomatous polyps spread throughout the gastrointestinal tract, and Cowden's disease with multiple hamartomatous polyps, neurologic and dermatologic symptoms[12].

Colorectal polyps

The two main histological types of polyps in the colorectal mucosa are hyperplastic and adenomatous polyps. Carcinogenesis starting in hyperplastic polyps develops through serrated adenomas, and is suggested to be caused by microsatellite instability[19, 20]. This link to serrated adenomas may represent a carcinogenetic pathway largely independent of the adenoma-carcinoma sequence[19]. Risk factors are polyp size (>1 cm), multiple polyps (>20), family history of hyperplastic polyposis or CRC [21]

Most colon carcinomas develop from adenomas, which are separated into three histologially different types: tubular (75%), villous (10%) and tubulovillous (15%). Increased size, grade of dysplasia and villous structure is associated with increased risk of malignancy. Removal of adenomas in the colon and rectum decreases this risk of developing CRC[22, 23]. The transformation of adenomas to invasive cancer involves a wide spectre of genetic events including alterations of oncogenes, tumour suppressor- and mismatch repair genes[24-26].

The adenoma-carcinoma sequence / sporadic colorectal cancer

A number of genetic and epigenetic changes affecting genes controlling cell proliferation and/or cell death trigger the development of carcinoma[27] . Most CRCs arise sporadically from adenomatous polyps. The evolvement of carcinomas through different histopathological steps was suggested in 1974[28], and some years later Vogelstein et al[24] described genetic alterations in several genes accompanying this stepwise progression from a benign adenoma to a malignant carcinoma. This has been named the adenoma-carcinoma sequence and include mutations in the Adenomatous polyposis coli (*APC*), *Kirsten-ras* (*K-ras*) and *TP53* genes amongst others[29]. Mutations in the *APC* gene, which cause FAP if mutations are inherited, is one of the most frequently mutated genes in CRC and an early event in development of CRC[30]. *K-ras* mutations are also observed as early events[25, 31], whereas mutations in the tumour suppressor gene *TP53* is considered to be a relatively late event in colorectal carcinogenesis[25, 32]. However, many other genes are involved in the development of CRC, causing

changes in the chromosomal composition, instabilities in microsatellites or epigenetic changes that may induce malignant transformation with different patterns of proliferation, invasion and metastasis[33-37].

The knowledge of aberrant genes causing instability of the genome[38], suggests at least two different main genetic pathways in the development of CRC[39], the chromosomal instability (CIN) and microsatellite instability (MSI) pathways.

Chromosomal instability

The expression of a mutator phenotype in human cancers as an early step in tumour progression has been described[27, 40]. This is known as the chromosomal instable phenotype, and these cells typically display numerous chromosomal aberrations. The cause for this genetic instability still remains unknown, but changes in genes that are responsible for normal chromosomal number and integrity during cell division have been suggested as initiating events.

Microsatellite instability

Nucleotide repeats (microsatellites) numerous and distributed throughout the entire DNA sequence. They are prone to slippage during replication[14], and functional mismatch repair (MMR) genes are essential for these aberrations to be repaired.

The five MMR genes described above encode for most proteins of the MMR system, phylogenetically a highly conserved system and present across species. Mutations or methylation of *MMR* genes inactivate their function and give rise to uncorrected defects in the nucleotide repeats and subsequent MSI. Characteristically, MSI tumours are bulky and most commonly occur in the proximal colon. They have cells that are diploid, are histologically poorly differentiated, and of the mucinous type[41]. MSI tumours often present Crohn-like lymphocytic infiltrates[42]; the frequency of lymph node and distant metastases are lower than in CIN tumours[43].

Epigenetic changes

Epigenetic changes are chemical modifications of DNA resulting in changes in gene expression. The main epigenetic modifier is methylation of cytosine located within the dinucleotide CpG[44, 45], and CpG sites are widely and unsymmetrically distributed in the genome. When CpG islands are located in the promoter region of a gene it will result in transcriptional silencing of the gene expression. The methylation of multiple CpG islands, defined as CpG island methylator phenotype (CIMP), seems to be an event in about half of all sporadic CRCs[46], and MSI tumours are often caused by epigenetic silencing of the DNA mismatch repair gene *MLH1* [47]. Aberrant methylation linked to MSI is described as a third pathway of tumour suppressor gene inactivation in carcinogenesis[48-50].

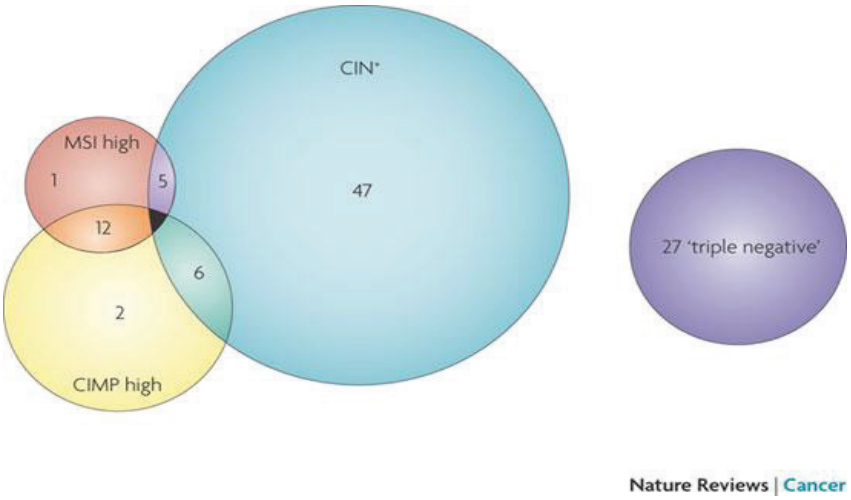

Figure 4: Distribution of chromosomal instability, microsatellite instability and CpG island methylator phenotypes in colorectal cancers[51] All numbers are percentages of the overall number of patients. The subset of CRC displaying no genomic instability might be smaller than shown here ('triple negative') as flow-cytometry used to identify CIN is relatively insensitive. A proportion of these cancers might display minor chromosomal abnormalities. It is likely that many would become overtly CIN^+, but changes in CIMP status over time cannot be ruled out at present.

***TP53* tumour suppressor gene**

The *TP53* tumour suppressor gene is located at chromosome 17p, where allelic deletions are recognised to be involved in colorectal carcinogenesis[24].

TP53 encodes a tumour suppressor with multifaceted functions in the maintenance of genomic stability, regulation of the cell cycle and apoptosis[52]. Mutations in *TP53* are typically found in CIN tumours, and are reported in approximately 50% of all colorectal carcinomas[53, 54]. Most mutations occur in the DNA binding domain, encoded by exons 5 to 8[53, 55]. The incidence of *TP53* mutations is highest in the distal colon[56-58]

Staging of colon cancer

The Tumour Node Metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC)[59, 60] and the International Union Against Cancer (UICC) is the international clinicopathological staging system for colorectal cancer. It is based on three components of the anatomic extent of the disease: T for the local extent of the primary tumour, N for the extent of regional lymph node metastases and M for the absence or presence of metastases to other regions/organs. The TNM classification system has been revised several times since the first edition in 1982, and three revisions have been published since our database was established in 1993.

Table 1: The TNM staging system of the AJCC/UICC for colorectal cancer; Comparison of the 4th, 6th and 7th editions.

Primary tumour (T)

4th edition [61, 62]	6th edition [63, 64]	7th edition [59, 65]	Definition
TX	TX	TX	Primary tumour cannot be assessed
T0	T0	T0	No evidence of primary tumour
Tis	Tis	Tis	Carcinoma in situ; intraepithelial or invasion of lamina propria
T1	T1	T1	Tumour invades submucosa
T2	T2	T2	Tumour invades muscularis propria
T3	T3	T3	Tumour invades through the muscularis propria into pericolorectal tissues
T4	T4	T4a	Tumour penetrates the visceral peritoneum
T4	T4	T4b	Tumour invades or is adherent to other organs or structures

Regional Lymph Nodes (N)

4th edition	6th edition	7th edition	Definition
NX	NX	NX	Lymph nodes cannot be assessed
N0	N0	N0	No regional lymph node metastasis
N1	N1	N1a	Metastasis in 1 regional lymph node
N1	N1	N1b	Metastases in 2 to 3 regional lymph nodes
Not defined	Not defined	N1c	Tumour deposit(s) in the subserosa, or nonperitonealised pericolic or perirectal tissues without regional lymph node metastasis
N2	N2	N2a	Metastases in 4 to 6 regional lymph nodes
N2	N2	N2b	Metastases in 7 or more regional lymph nodes
N3	Not defined	Not defined	Metastases to nodes along the ileocolic, right, middle, left or inferior mesenteric artery, and/or apical lymph node as described by the surgeon

Distant metastasis

4th edition	6th edition	7th edition	Definition
MX	MX	MX	Distant metastases cannot be assessed
M0	M0	M0	No distant metastasis
M1	M1	M1a	Metastasis to single organ or site (e.g. liver, lung, ovary, non-regional/distant lymph node)
M1	M1	M1b	Metastases to more than one organ/site or to the peritoneum

There are different prefixes used to indicate the basis of information used for classification:

m = multiple primary tumours on primary site

y = classification after neoadjuvant radio- and/or chemotherapy

r = recurrent tumour following a disease free interval

c = classification based on clinical and radiological assessment

p = classification based on pathological evaluation

a = classification at autopsy

The initial staging based on clinical information (cTNM) is essential for planning of treatment. Normally this includes endoscopy with biopsy of the primary tumour and CT scan of the chest and abdomen/pelvis.

After surgical removal of the tumour - and metastases if necessary - a pathological examination will establish whether nodal or distal metastases are present. This staging is considered the most accurate and reliable (pTNM).

The Dukes staging system, originally intended for rectal cancer only[66] but subsequently modified and made applicable to colon cancer[67], was formerly in common use. This system is now often replaced by the more differentiated TNM staging system based on combinations of the TNM stages listed in Table 1.

The main purpose of staging is to stratify patients according to prognosis, with possible implications for adjuvant treatment and follow-up. Table 2 compares the (p)TNM and Dukes classification systems.

Table 2: TNM staging system (sixth edition) compared with Dukes' staging system

TNM stage	T	N	M	Dukes
0	Tis	N0	M0	A
I	T1 – T2	N0	M0	A
IIa	T3	N0	M0	B
IIb	T4	N0	M0	B
IIIa	T1-T2	N1	M0	C
IIIb	T3-T4	N1	M0	C
IIIc	Any T	N2	M0	C
IV	Any T	Any N	M1	D

Basically, prognosis worsens with advancing stage. For clinical purposes, the subdivision of TNM stages II-IV (6th and 7th edition) is rarely practised in Norway.

Peritoneal carcinomatosis (PC)

PC refers to the complex sequence of events by which tumour cells disseminate from their primary organ of origin to establish independent metastatic deposits on the visceral and parietal peritoneal lining of the abdominal cavity[68]. Distant dissemination may occur through several mechanisms. First, malignant cells may invade bowel wall veins and spread haematogenously to distant organs; second, through lymphatic vessels to regional and distant lymph nodes; third, when the serosal surface is involved, directly from the primary tumour to adjacent and distant peritoneal surfaces.

The peritoneal metastatic cascade

A complex sequence of events, described as 'the peritoneal metastatic cascade', characterises the development of PC[69]. The first step is the dissemination of free tumour cells to the abdominal cavity. Cells may also be liberated by tumour perforation, either spontaneously or inadvertently during surgery. Furthermore, tumour cells may be seeded from transected lymphatic- and blood vessels during surgery. The second step is the adhesion of liberated tumour cells to the innermost layer of the peritoneum, the mesothelium. Several adhesions molecules have been implicated in this process[69]. Tumour cells then penetrate the mesothelial monolayer and its basement membrane with subsequent invasion of the underlying connective tissue, tumour proliferation and the establishment of discrete metastatic tumour deposits. Finally, angiogenesis is induced to sustain tumour proliferation and enable further metastatic growth.

Clinical features of PC

The natural history of CRC patients presenting with PC at diagnosis is sparsely documented and based on data from selected series. The incidence of PC at the time of diagnosis is 7-10%[70, 71]. In recurrent disease, about 25% have PC[71, 72] and 40% to 80% of those who die of the disease will have PC[73].

Imaging to detect PC is difficult as signs on CT scan can be subtle in early stages with limited tumour load and absence of ascites. In recent years, positron emission tomography (PET) has gained favour in assessing metastatic disease, but lesion less than 1 cm are difficult to detect[74]. In the majority of cases, the diagnosis is established at operation.

The extent and distribution of PC can be described according to different scoring and staging systems[69]. The Peritoneal Cancer Index is widely used and includes assessment of small bowel involvement, which is an important selection criterion for operative treatment.

Genetic changes in patients with PC have been investigated, and PC seems to be associated with CIN tumours, including mutation(s) in the *TP53* gene.

Treatment

Surgical treatment

Surgical treatment of colon cancer depends on the extent of the disease at the time of diagnosis, and staging is essential when planning the operation. Preoperative work-up includes endoscopy, CT scans of the chest and abdomen/pelvis and in some cases PET-CT if distant metastases are suspected. Following staging, two possible situations may arise with different implications for surgical strategy – emergencies excluded.

I. Colon cancer without distant metastases

In this situation, surgery is performed with curative intent. The primary tumour should be removed with free resection margins including the mesocolic lymphovascular pedicle to the tumour bearing bowel segment. Precautions should be made to avoid intra-operative dissemination of tumour cells.

Extent of bowel resection

For oncological reasons, a free resection margin along the bowel wall of at least 5 cm at both sides of the tumour is necessary; however, several authors advocate a resection margin of 10 cm[75-77].

A free radial (circumferential) resection margin around the tumour and the mesentery is essential and is secured by an accurate dissection following the anatomical retroperitoneal plane[75, 78]. This technique is described as complete mesocolic excision (CME) by Hohenberger et al.[75, 79], and improved local recurrence rates and cancer specific survival at five years have been reported. Adherence to the CME technique also resulted in reduced rates of involved resection margins and improved OS in the study by Bokey et al.[78], and increased distance between the tumor and the high vascular tie, the length of large bowel and the area of mesentery[80]

In approximately 5-10% of cases, the tumour is adherent to, or infiltrates adjacent organs/structures, either because of true tissue invasion or inflammatory adherence. Intra-operatively, tumour infiltration and inflammatory adherence cannot be reliably discerned, and the resection should be extended to include the infiltrated part of the neighbour organ/structure to secure a potentially curative operation[76, 77]. Perforation of the bowel is to be avoided, as it is associated with poor prognosis[81, 82]

It is mandatory to secure an adequate blood circulation the bowel ends planned for anastomosis (or stoma). The remaining circulation after the oncological necessary lymphovascular dissection (see below) decides how much of the colon to be removed. By right sided cancer a right hemicolectomy is required; by cancer in the descending colon a left hemicolectomy; by cancer in the sigmoid a resection of the sigmoid or left hemicolectomy is necessary, depending on the circulation. Tumours in the flexures are treated with extended hemicolectomies or a subtotal colectomy. Tumours in the transverse

colon are treated with resection of the transverse colon including both flexures or by subtotal colectomy. By synchronous cancers (i.e. two or more tumours simultaneously), a subtotal colectomy is warranted in most cases.

Extent of lymph node dissection

Regional lymph nodes may contain tumour cells as ITC, MM or ordinary metastases. Removal of all LN containing tumour cells is mandatory to obtain cure. Moreover, correct pN staging requires dissection of a certain number of lymph nodes.

The regional lymph nodes are divided into three groups according to their localisation, and the nomenclature is different in Japan / the western world: N1 / paracolic or epiploic, N2 / intermediate nodes, and N3 / central or apical nodes (Figure 5). N1 denotes the nodes close to the bowel wall; N2 nodes are situated along the main vessels (i.e. the ileocolic, the middle colic, the left colic and the superior rectal arteries and veins) and N3 are the central nodes at the origin of the main vessels (superior mesenteric vessels and inferior mesenteric artery).

This classification of node stations parallels the classification of lymph node dissection as D1- D3. Thus, D1 dissection includes the N1 nodes, D2 the N2 nodes and D3 includes dissection and removal of the central N3 nodes (Figure 6).

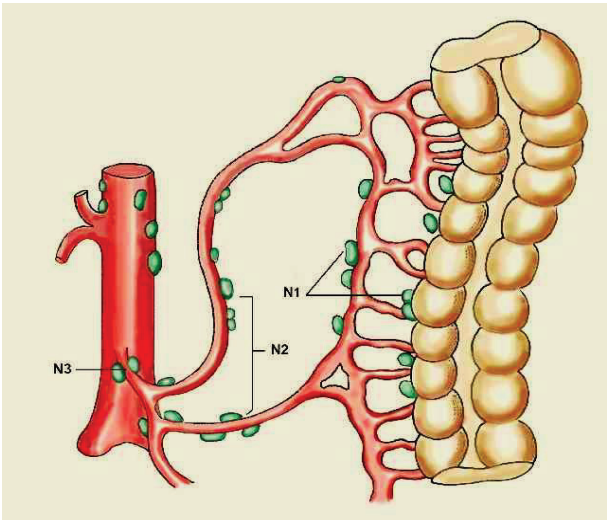

Figure 5: Regional lymph node groups (N1, N2 and N3) in relation to bowel wall

For right sided colon cancer, a D3 dissection is widely recommended; for tumours in the transverse or left colon, at least a D2 dissection should be performed. It is a matter of debate whether a D3

dissection is necessary in this situation, but metastases to central lymph nodes (N3) in absence of distant metastases have been reported in 2-4% of patients[83-85]. These are patients who could be cured by a D3 dissection; following a D2 dissection, however, they might have a locoregional recurrence. Some authors therefore recommend D3 dissection also for left sided tumours[79, 86, 87].

Hohenberger also recommends removal of nodes along the greater curvature of the stomach (including subpyloric nodes) in transverse and right flexure cancers, as some of these patients have metastases in these nodes[85].

Increasing the number of examined lymph nodes in colon cancer specimens makes staging more accurate. This is important, as advanced stages (IIIa-c) regularly receive adjuvant chemotherapy, which improves prognosis significantly. Examination of more than 12 lymph nodes is associated with improved long time survival[88, 89] indicating the advantages of the more radical D3 dissection.

A B

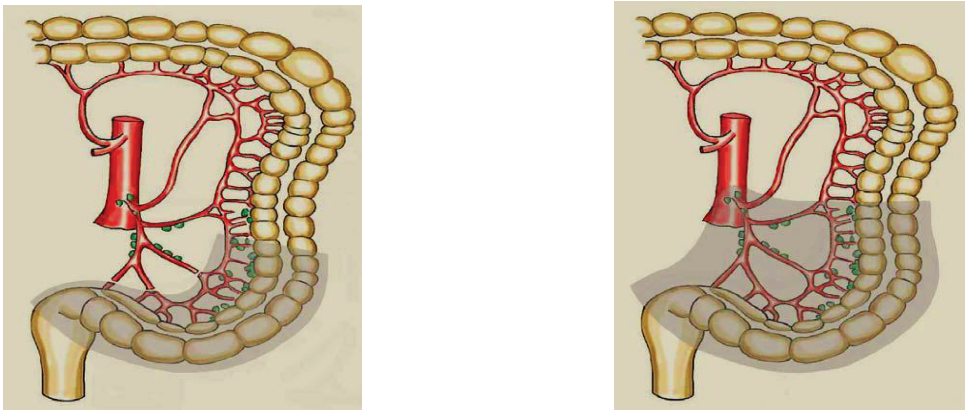

Figure 6: Lymphovascular dissection in sigmoid cancer: D3 dissection

No touch technique

The term 'no-touch technique' was introduced by R. Turnbull in 1967[90] and implies central ligation of the vessels before mobilising the bowel, which should be manipulated as little as possible to avoid the intra-abdominal spread of cells from the tumour surface. Strangulation of the bowel lumen within the area of resection is advocated to reduce the risk of intra-luminal spread of tumour cells and implantation in the anastomosis. The benefit of this technique described by Turnbull[91], Slanetz[87] and Wiggers[92] has been disputed[93]. Despite the lack of evidence, this technique is based on reasonable oncological principles and should be considered when operating colon cancer for cure.

II. Colon cancer with distant metastases

If the distant metastases can be radically resected, there is a curative potential and the same oncological principles as listed above apply. Up-front chemotherapy is then to be considered. Whether first to resect the primary tumour, the metastasis or both simultaneously is an unsettled issue. In most cases, a tailored multimodal treatment should be given. Therefore, these patients should be evaluated by a multidisciplinary team.

Most patients with distant metastases cannot be cured. Treatment is then palliative, and how to address the primary tumour is debatable[93]. If the tumour does not cause symptoms, the indication for resection is unclear[94, 95]. When symptoms are present, the treatment should be directed against these: by obstruction, an intraluminal stent may be considered in fragile patients when the tumour is located in the left colon; otherwise, operation with a bypass, diverting stoma or resection is required. Sometimes, bleeding may warrant a resection if the patient tolerates surgery.

III. Colon cancer with emergency presentation

Approximately 10-30% [96] of colon cancer patients present with acute symptoms, mostly because of obstruction (80-85%) or perforation (10-20%). Profuse bleeding rarely occurs.

Treatment for acute colon obstruction (according to the Norwegian guidelines) depends on location: By right sided obstruction, a right hemicolectomy is recommended, or a bypass/diverting stoma if the tumour cannot be removed. By left sided obstruction, an intraluminal stent could be inserted for relief of symptoms[97], either as a permanent palliative procedure or as a temporary 'bridge to surgery' followed by potential curative resection after one to two weeks[98-100]. Reports on stenting of colonic obstruction have been presented since the nineties, but this method was not used in our department before 2003.

Stent placement is recommended by several authors in order to reduce postoperative morbidity and mortality and to avoid stoma formation following emergency operations[97, 98, 100-102]. The overall technical and clinical success rates of colonic stenting are reported to be 89-96% and 85-92%, respectively[103, 104]. Procedure related mortality is less than 1%, and the most common complications are recurrent obstruction (7.3-12%), migration of the stent (4.4-11.8%) and perforation (2.5-4.5%)[103]. Stenting is recommended both as bridge to surgery[102, 104-109], and as permanent palliative treatment[97, 99, 102, 104-106, 110]. Fit patients can then start chemotherapy immediately and avoid the immunosuppression and delay caused by surgery. If bowel function after stent placement is poor, bowel resection can be done at a later stage. In frail patients palliative stenting is a permanent treatment.

In case of stent failure, there are different surgical options, depending on situation; with curative intent, the operation can be done as one-, two- or three stage operations. In two-stage procedure resection with end colostomy (Hartmann's operation) is performed initially, followed by reversing the stoma and restoration of bowel continuity after 3-6 months. A three- stage procedure includes proximal decompressing colostomy, colon resection after 1-3 weeks, and finally closure of the stoma after some months. In the non-curable situation and in frail patients, a diverting stoma or bypass of the tumour without resection is an option, even though associated with high mortality (>20%).

In case of perforation, peritonitis or obstruction with a blind loop, primarily operation is mandatory.

IV. Adjuvant treatment (AT)

Following potential curative resection, the possibility of microscopic residual tumour tissue/cells in the patient exists. This is the target of postoperative adjuvant chemotherapy. Treatment over 6 to 12 months with 5-fluorouracil (5-FU) combined with Leucovorin (FLV regimen) has improved five year DFS in stage II patients with 2-4%, and stage III with 10-15%[111-114]. The combination FLV with Oxaliplatin (FLOX regimen) has increased DFS in stage III patients[115, 116].

Combination of FLV and Irinotecan (FLIRI regimen) has not proved to add survival benefit in stage III patients[117, 118], but could be effective in tumours with MSI[119].

The treatment with antibodies to Vascular Endothelia Growth Factor (VEGF) or Epithelial Growth Factor Receptor (EGFR) has to date not proven to be effective in adjuvant settings.

AT to stage III patients $\leq$ 75 years is widely used. The Norwegian guidelines have changed during the research project. Since 1997 FLV were recommended to stage III patients under 75 years. From 2003 FLOX was recommended to fit patients up to 70 years of age, and since 2005 FLV was recommend to healthy patients up to 80 years.

In stage II patients, subgroups with high risk of recurrence are considered for treatment[120], and the Norwegian guidelines recommended chemotherapy to patients who have bowel perforation in the tumour area before or during the operation, and to those who have 8 or less lymph nodes examined.

V. Treatment of colon cancer with peritoneal carcinomatosis

Over the last 15 to 20 years, different treatment options in patients with PC of colorectal origin have been described. Selection of patients to operative treatment with extended cytoreduction/peritonectomy (removal of all visible tumour tissue from the peritoneum) combined

with hyperthermic intraoperative chemotherapy (HIPEC) has improved survival in selected patients. The extension of the intra-abdominal spread and the general condition of the patients are basis for selection. This treatment is resource demanding, and in Norway it is performed only in Oslo University Hospital, Radiumhospitalet.

Histopathological examination

The methods used by the pathologists to identify, retrieve and examine lymph nodes from the specimen vary. Mostly, formalin fixed specimens are examined after 3-5 days, however, with a risk of missing nodes in the mesocolic fat. Identification of nodes is easier when fat clearance techniques are utilised[121-123], and increases the number of nodes examined.

One slice is then obtained from each node, followed by Haematoxylin Eosin (HE) staining of 3-4 μm thick paraffin-embedded sections which undergo microscopic examination. Ultra-sectioning - obtaining multiple slices from each node - are not routinely used, nor is the use of immunohistochemical (IHC) examination or reverse transcriptase - polymerase chain reaction (RT-PCR) to identify small ordinary metastases, micro-metastases (MM) or isolated tumour cells (ITC). Such techniques contribute to increase the detection rate of metastases.

The sentinel node (SN) concept

The concept was primarily described in the treatment of penis cancer, and is used regularly in treatment of breast cancer and malignant melanoma It is based on the hypothesis that if lymph node metastases are present in any node, metastases will also be present in the LN next to the primary tumour, defined as 'sentinel nodes'. Extended examination of these nodes is then sufficient for accurate LN staging, including the identification of MM or ITC. Time and resource demanding examinations (Ultra sectioning, IHC, RT-PCR) is therefore necessary in only a limited number of LN.

In colon cancer this concept has been evaluated in numerous studies, but with conflicting results[124]. We participated in a prospective study on SN[121] which did not show any benefit of this method in the staging of CC.

Predictive factors

Factors which have impact on the patients' response to a certain therapy are called *predictive* factors. Some bio-molecular factors that predict the response to cytotoxic therapy have been identified, and are therefore important when allocating patients to different therapeutic regimens.

The predictive value of *K-ras* mutation status in therapy with antibodies to epidermal growth factor receptors (EGFR) is well documented[125-127], and in regular clinical use.

Human cancer cells lines with disruption of *TP53* have shown reduced therapeutic response to fluorouracil in experimental studies[128], and reduced effect of FU-based chemotherapy has been reported in *TP53*mutated tumours has been reported[129, 130]. However, the potential predictive value of *TP53* mutation must be investigated in future studies. The research in this field is expanding rapidly, and opens up the perspectives of personalized medicine.

Prognostic factors

The prognosis of CRC differs widely among patients, and depends on a number of factors. Currently, the gold standard of prognostication is the clinicopathological staging based on the TNM classification system. Stage of the disease at presentation has profound effect on the prognosis. However, prognosis also differs between patients within the same TNM stage, and many clinical, histopathological and biomolecular markers have potential impact on outcome.

Previously the research was focused on different clinical and histopathological factors and a limited number of protein markers, such as CEA. During the last two decades, an extended number of proteins, biomolecular and genetic markers have been subjects of intensive research, and both prognostic and predictive impact has been investigated. Such studies are, however, often small (underpowered), performed on selected materials and retrospective. Multivariate analyses with adjustment for known prognostic factors are necessary when investigating the effect of new factors, and very large, prospective studies are then needed. So far, despite many published recommendations to include new prognostic markers, no consensus has been reached to incorporate any of these in the daily routines.

At the beginning of this study period (2003), the clinical prognostication of patients was mainly based on the TNM stage, and less attention was paid to other factors. Knowledge about prognosis is important for two main reasons; firstly, to identify patients in stage I-II at high risk of recurrence who might benefit of AT, and to identify patients in stage III with low risk of recurrence, who should not be over-treated with AT. Secondly, to decide structure and intensity of follow up programs; this

should be based on calculated risk of recurrence in the individual patient to avoid unnecessary and costly examinations.

When our studies started, many factors had demonstrated prognostic impact in several studies and were therefore well established. Other factors with a possible prognostic impact had been identified, but the findings were equivocal, and new studies warranted. In the following factors with a definitive prognostic impact as per 2003, and those with equivocal impact, are presented separately.

Histopathological prognostic factors

Established histopathological factors at start of the studies

The depth of tumour growth (pT), the lymph node status (pN) and the presence of distant metastases are independent prognostic factors. There is also a strong correlation between these three factors.

Tumour (pT) stage: Advanced T-stage is associated with reduced long term outcome[131]. Patients with stage II tumours (pT3-4, pN0, pM0) experience recurrence in about 20-30% of the cases[132, 133].

Lymph node (LN) metastases: The presence of lymph node metastases is associated with reduced survival, and prognosis worsens with an increasing number of metastatic nodes[134]. For a correct interpretation of LN status it is necessary to know the total number of LN examined, which depends on the extent of the surgical dissection and the quality of the pathological examination, and to know if metastases are present and the number of metastatic nodes.

Distant metastases: Tumours presenting with distant metastases have the poorest prognosis, which is obvious and well documented. We excluded these patients in most studies on prognostic factors.

Histologic subtype is always reported if assessable. The prognosis is most favourable in adenocarcinomas and worst in small cell carcinomas[135, 136]. In adenocarcinomas, tumours with extracellular mucin in more than 50% of the tumour volume are classified as mucinous. These are most prevalent in men and in the right colon, and patients with mucinous tumours have reduced survival[137].

Tumour differentiation grade: The traditional assessments include high, middle, low and undifferentiated tumours. Lower differentiation grade is associated with poorer outcome[131]. Because of bias caused by inter-observer differences in assessments, a two grade system (high or low grade) has been proposed[131, 138], but this is not implemented in clinical practise in Norway.

Venous invasion: A negative prognostic impact of venous invasion is well documented, and it is recommended to include it in the pathological report[131]. Venous invasion is proposed as part of an

extended system for prognostication[139, 140], but is still not included in the generally used staging systems.

Lymphovascular invasion has been regarded as a step in the pathway of spread to the regional lymph nodes, and increases the risk of metastases in these nodes[141], and is associated with poorer outcome[131].

Perineural invasion of tumour is reported to have a negative prognostic impact in most series[142-144] and is recommended to be routinely included in pathologic reports[131, 136] .

Tumour residual classification (R-classification) system defines the extent of residual tumour tissue in the patients following resection, and is based on histopathology, intraoperative exploration and preoperative radiological examinations. R-stage is strongly associated with outcome[64, 145]. R0 refers to the situation with neither macroscopic nor microscopic residual tumour tissue. R1 refers to microscopic tumour tissue at the resection margins, but without macroscopic tumour left in the patient. R2 refers to macroscopic residual tumour after surgery, locally or distant, found at laparotomy or by radiological examinations. A curative resection is usually defined as R0 resection, but in some studies R1 resected patients are also included, (not all patients develop recurrence after a R1 resection).

Histopathology; not well established prognostic factors

Number of examined lymph nodes: The problem of potential under-staging has led to research on the prognostic impact on number of examined lymph nodes, showing an association between low number of examined lymph nodes and poor outcome in terms of recurrence and survival in stage (I-)II patients[88, 146-148], whereas results in stage III are diverging[88, 147, 149]. At the start of these works, examination of at least 12-14 lymph nodes was recommended[65], but the minimum number of nodes needed for correct staging and prognostication varied among studies[150-154].

Lymph node ratio (LNR): LNR is the ratio of positive LN to the total number of examined LN in a specimen. At the beginning of our studies, the prognostic impact of LNR was not investigated in colon cancer patients. Most studies are published after 2005[155-160], and demonstrate a significant prognostic impact in stage III patients.

Lymph node size: It is unclear if LN size is of prognostic significance: Up to 77% of metastatic nodes are less than five mm in greatest diameter[161-164]. LN volume was not of prognostic significance in the study by Wong et al.[165]. However, these reports are contradicted by two studies, one showing significantly higher proportion of metastases in lymph nodes $\geq$ 10 mm[166] and another showing size $\geq$ 10 mm as an independent prognostic factor in stage III patients[167].

Micrometastases (MM) / isolated tumour cells (ITC) in lymph nodes (LN): Metastases of colorectal origin are defined according to size; Ordinary metastases as deposits of tumour cells $\geq$ 2

mm in diameter, MM as deposits between 0.2 and 2 mm of size, and ITC as malignant cell clusters less than 0.2 mm or single isolated tumour cells[63, 65]. Ordinary metastases are normally detected in routine microscopic examination of HE stained samples. This method is cheap, easy and little resource demanding compared to more advanced methods, like IHC and RT-PCR, which are required to detect MM and ITC. The presence of MM/ITC has been reported in up to 30% of stage I/II patients when using IHC technique to examine the LN[168-173].

Despite the logical assumption that spread of viable tumour cells (as ITC or MM) to the lymphovascular draining system might lead to recurrence and thus have impact on survival after potential curative surgery, the results varies among reported series[168, 171, 174-177]. In 2007, Compton stated that the prognostic significance of minute tumour deposits in regional lymph nodes remains unclear[178]. This important clinical issue was investigated in Paper 3, which is a follow up of the patients included in our two-centre study of the SN concept in the surgical treatment of CC published in 2008[121].

Host lymphoid response to tumour (Crohn's like lymphoid reaction) is as an expression of a more powerful immune response in the host, and has been reported to predict a better survival[179-181], findings contradicted by other authors[182-184]. It has therefore been evaluated with evidence grad IIB as not sufficient studied for routine documentation like lymphovascular and venous invasion[136].

Clinical prognostic factors

Established clinical factors at start of the studies

Emergency presentation (obstruction, perforation): The incidence and causes of emergency presentation of CC is described above (page 22).

Postoperative mortality (10-25%)[185, 186] and morbidity (>50%) are increased in patients who have undergone emergency operation[187-191].

Since the early eighties, patients operated for obstruction have been reported with poor long term survival[144, 189, 191], findings confirmed in later studies[143]. In a German multi-centre study from 1994[192], overall survival was 33% and 51%, respectively in emergency versus elective patients. The corresponding figures for relative survival were 47% and 65%, respectively. Patients admitted emergently have more advanced tumours and consequently the rate of curative resection is lower[185, 193]. However, even after curative resection five year survival is lower than following elective operation[187, 188, 193, 194]. In 2006, McArdle et al. reported poor outcome in emergency patients presenting with the symptoms blood loss, obstruction or perforation [195]

Adherence to other organs: The tumour might adhere to neighbour organs / structures due to direct invasion of tumour tissue (pT4), or due to inflammatory adherence. It is not possible to distinguish between these causes intra-operatively. Therefore, 'en-bloc' resection of the tumour-bearing bowel segment and a part of (or the whole) affected organ should be carried out[196]. If radical resection with microscopic free resectional margins is achieved in a pT4 tumour, the outcome is comparable to that of a pT3 tumour[138], and 'en bloc' resection is the gold standard in these situations[76, 77]. If en bloc resection is not performed, there is a risk of involved margins and there is also an increased risk of intraoperative tumour perforation, which is associated with increased risk of recurrence and reduced survival[82].

Clinical factors; prognostic impact uncertain

Tumour site: A Norwegian study from 1987 demonstrated increased mortality for patients with tumour in the distal colon and rectum[197]. Aldridge et al.[198] reported increased rates of obstruction and recurrence, and reduced age adjusted 5-year-survival in tumours located in the splenic flexure. In contrast, single centre studies reported no influence of tumour location on prognosis[138, 199, 200]. Gupta reported in 2005 slightly higher survival in left sided cancers[201], but in the Norwegian national study from 2004[202], there was reduced relative survival in rectal cancer patients, but no difference between right and left colon.

Age: The incidence of CRC increases with age, as well as the morbidity and mortality from other causes than cancer. Overall survival is therefore decreased in older patients with CRC. The impact of age on cancer specific survival or relative survival varies in reported studies. An independent impact of age has been reported by some[144, 202], whereas others describe no impact on survival or local recurrence[138, 199].

Gender: The association between gender and outcome is unclear, as studies are reporting conflicting results. An assumption of difference seems logical, as the general patterns of diseases are different between the genders. This could also imply differences in tumour biology and in host related immunological response to the disease, as well as different response to adjuvant therapy.

Blood loss / Perioperative blood transfusion (PBT): some patients presents with severe anemia, and some have perioperative blood loss, and need PBT. The effect of PBT on oncological outcome in CC patients has been investigated for decades. Several retrospective reports from the 1980ies were inconclusive[203-208]. A review of 31 retrospective studies in 1991[209] was inconclusive. Several later reports showed no prognostic impact of PBT[210-213]. In contrast, Edna and co-workers from Norway found that PBT had a negative impact on survival[214], and increased the rate of postoperative infections[215]. The latter finding was supported in a later review including 20 articles published in 1986 – 2000[216].

Symptom duration: In 1981, McDermott et al. reported that patients with symptom duration less than 3 months had lower CSS than the other patients[217]. Several other series during the eighties showed significantly shorter duration of symptoms in more advanced stages[218], and better prognosis with long duration of symptoms[138, 144, 219]. In the last two decades, few studies have included this variable, most likely because of the difficulties in defining and registration symptom duration. This parameter is not included in our local database.

Surgeon related factors: Surgery for CRC is demanding, and requires surgical skill and experience. The formal levels of skill can be graded in three groups; the trainee / resident, the general surgeon, and the specialized colorectal surgeon. In all three groups the volume of colon cancer operations may vary widely.

Studies have shown a more favourable outcome in patients operated by specialised colorectal surgeons[200, 220], but there are also differences among surgeons with the same level of formal specialisation[221, 222]. In a more recent report, there was no significant difference in 30-day mortality between the different surgeons[223]. However, most series report significantly higher postoperative morality when operated by low volume surgeons[224-226].

Hospital related factors: Several authors have reported improved outcome in large volume hospitals compared with low volume hospitals[192, 227-229], and Blomqvist et al. found better survival in regional/University hospitals versus small local hospitals[230]. These results are contradicted by other reports showing no survival difference between high and low volume hospitals[231, 232]. Most studies[224-227], but not all[231, 233], report better results in high volume hospitals. There are however no consensus on definition of low and high volume hospitals.

Biomolecular factors

There has been intensive research on the prognostic effect of a diversity of bio-molecular markers the last 15-20 years. Various terms for such markers are used in the literature; biomarkers, molecular markers, biomolecular markers and genetic markers. An immense development in laboratory methods, including large scale analyses of protein expression using tissue micro arrays, new methods for sequencing DNA and RNA, and many other methods, have made such studies feasible. Studies on the genome, transcriptome and protein level are performed, and tissue from the primary tumour and metastases, blood, faeces and urine can be analysed. A detailed description of all these factors is beyond the limits of this thesis.

Carcinoembryonic antigen (CEA) is a glycoprotein involved in cell adhesion and found in the blood of some 50% of CRC patients. It is an established prognostic factor and used in clinical practice, and will therefore be discussed.

CEA: Preoperative elevation of CEA, and the degree of elevation, is associated with increased risk of recurrence[234] and decreased long term survival[235, 236] with the highest level of evidence[131]. Also when analysing subsets of patients (stage I/II), preoperative CEA is reported to be a significant prognostic factor[236].
Following potentially curative resection, CEA may rise if recurrence occur, and the reported sensitivity and specificity are 64% and 91%, respectively[237]. Even when normal preoperative, it will rise in at least 50% of patients with recurrent disease[238], making it useful in routine follow up programmes.

Follow-up

The main objectives of systematic follow-up after potential curative treatment for CRC are to detect recurrence as early as possible, thereby securing optimal treatment, whether curative or palliative. Moreover, surveillance should detect synchronous or metachronous disease; and finally, follow-up is required for registration of treatment outcome.

Secondary objectives are management of late complications after surgery, follow-up of adjuvant chemotherapy, counselling regarding risk factors and information about signs of recurrent disease as approximately half of the recurrences occur between follow-up consultations. Inclusion in a surveillance programme should also convey a feeling of security.

According to the Norwegian guidelines, patients in TNM stage II - III who are candidates for curative resection or chemotherapy in case of recurrence, should be included in a follow-up programme. General health and comorbidity have to be considered, particularly in elderly patients. Normally, patients older than 75 years of age are not included, but individual assessments are made in patients up to 80 years.

Stage I patients are not included in structured follow-up programmes, as the risk of recurrent disease is very low. A second colonoscopy should be made after five to ten years for the detection of metachronous disease. The cumulative risk for developing a second primary tumour is approximately 2% after five years, and 3-4% after ten years[239, 240].

The benefit of structured follow-up after potential curative operation for CRC has been debated[241, 242]. A meta-analysis from 2007 [243] detected an overall survival benefit for patients included in structured follow-up programmes. However, specific recommendations were not given, mainly because of heterogeneity in the different studies included[244-251]. At present, three international randomised trials are recruiting patients: the GILDA trial[252], the COLOFOL Study[253] and the FACS Trial 2009 (web-document available at http://www.facs.soton.ac.uk).

Some 85% of recurrences occur during the first three years after surgery; follow-up should therefore be most intensive in this period.

In the recently published Norwegian guidelines, follow-up, includes measurement of CEA, imaging of the liver, abdomen and chest, and examination of the colon / rectum according to the schedule presented in Table 3[254]. The first postoperative examination is made by the surgeon; otherwise, follow-up could be made by the general practitioner.

The benefit of CEA is unsettled, sensitivity and specificity varying with different cut-off values[237, 255]. In cases with elevated CEA preoperatively, a control should be made four weeks after surgery. If CEA is not normalised, residual disease should be suspected.

Contrast enhanced ultra sound (CEUS) is without carcinogenic side effects and has higher sensitivity (80-84%) and specificity (84-98%) than CT/MRI and is therefore recommended[256, 257].

Table 3: Recommended follow-up programme for patients operated with curative intent in whom resection of local recurrence or metastases could be indicated.

Months after operation	1[1]	6	12	18	24	30	36	48	60
CEA	●	●	●	●	●	●	●	●	●
CT scan of liver / abdomen		●[2]							●
CEUS [2]			●	●	●	●	●	●	
Low dose chest CT			●		●		●	●	
Colonoscopy or CT colonography									●

[1] At the first consultation, the surgeon should assess the clinical outcome and document histopathology. It should take place within four weeks for adjuvant treatment to be scheduled in due time if indicated.

[2] CEUS six months postoperatively is base for subsequent examinations. By considerable steatosis or cirrhosis, CT scan should replace CEUS. In departments of radiology where CEUS is not offered, CT scans should be done.

Aims of the study/thesis

Colorectal cancer surgery constitutes one of the principal fields of activity of our department. Continuous, prospective registration of all patients is mandatory for evaluation of treatment quality and outcome. Our project, in which we wanted to assess the quality of surgery and of the histopathological evaluation of resected specimens as well as the oncological outcome after surgery, is based on the Aker colorectal cancer registry. Close collaboration with the pathologists is important for postoperative staging and subsequent selection of patients for adjuvant chemotherapy. We suspected that histopathological factors of potential prognostic significance were inconsistently reported. Our principal aim was to investigate the prognostic impact of possible clinical and histopathological factors registered in our database. In addition, clinical characteristics, genetic mutations and long term outcome were investigated in the subgroup of patients presenting with PC at diagnosis.

Our specific aims were:

To evaluate survival in an unselected population based series of colon cancer patients (Paper 1)

To identify clinical prognostic factors for long term outcome after surgical treatment of colon cancer (Paper 1)

To evaluate short term outcome after surgery for colon cancer (Paper 2)

To identify possible risk factors for postoperative mortality and complications in patients treated as emergency cases (Paper 2)

To evaluate the prognostic impact on long term recurrence and survival of MM and ITC in regional lymph nodes from stage I/II colon cancer specimens (Paper 3)

To examine clinical characteristics of colon cancer patients who presented with PC (Paper 4)

To evaluate survival in patients with PC of colon cancer origin (Paper 4)

To examine the *TP53* mutation status in primary tumours from PC patients as compared with tumours in patients without PC (Paper 4)

To evaluate longitudinally the quality of histopathological reports on colon cancer specimens (Paper 5)

To investigate the prognostic impact of the number of examined regional lymph nodes following R0 resection in stage I-III patients (Paper 5)

To evaluate the prognostic impact of LNR in stage III patients (Paper 5)

Patients and methods

Patients and database

Our research project was initiated in January 1993. All patients with cancer of the colon and rectum admitted to our department were registered in a prospective database. Patients with colon cancer, i.e. with tumours located > 15 cm from the anal verge, were included in the present studies.

During the period 1993 – 2005, our catchment area included approximately 210,000 inhabitants, increasing in 2006 to 270,000. Data supplied from the Norwegian Cancer Registry who receive copy of all pathology reports on colorectal cancer from all departments of pathology in Norway, secured the inclusion of all patients diagnosed with colon cancer during the study period.

Preoperative diagnostic data, intra-operative findings and complications, histopathological data and postoperative morbidity were recorded.

During the study period, patients younger than 75 years of age were included in a standardised follow-up programme; patients > 75 years were included at the discretion of the surgeon. Data on recurrence, treatment for recurrence and death, including cause of death if known, were registered. The follow-up programme was in accordance with national guidelines and consisted of CEA control every third months for two years, then at three and five years postoperatively. Clinical examinations supplemented with X-ray of the chest an ultrasound (US) of the liver was undertaken every six months for two years, then at three and five years. X-ray and US were replaced by CT scan in 2007. Colonoscopy was part of the diagnostic work-up; if missing or incomplete, it was performed postoperatively and repeated at one and five years.

Study design / data quality

The studies on survival and time to recurrence were observational cohort studies which included all patients admitted from 1993. The end of inclusion varies from 2000 (Paper 1) to 2009 (Paper 5), ensuring the necessary observation periods for survival analyses of minimum twelve months (Paper 5) (Figure 7). All patients from the catchment area were consecutively registered and we therefore consider the studies to be prospective and population based.

Paper 3 comprises patients from two centres: Akershus University Hospital (AUH) and Oslo University Hospital, Aker (OUHA). The study was conducted from AUH, which included 140 patients from December 2000 to September 2005. OUHA included 53 patients (Figure 1).

Comparison of *TP53* tumour suppressor gene mutations in PC and non-PC patients presented in paper four was designed as a matched case-control study. A historic cohort with known mutation status from the same (Aker) population was chosen for comparison instead of patients without PC as a full mutation analysis of the Aker patients without PC would have been resource demanding and time consuming and not possible to perform within the time limits of the studies. The historic cohort was taken from the same region and matched the Aker non PC patients with regard to gender, tumour location and stage, but a selection bias cannot be ruled out.

The quality of preoperative examination, intra- and postoperative data was high, and patients were registered at discharge from the hospital. Some patients were not registered prospectively because they were transferred to another department (internal medicine) or died in hospital leading to autopsy not reported to the study secretary. These patients were registered retrospectively when identified by the hospital registry and the Norwegian Cancer Registry. In Paper 2, additional data on emergency patients were registered retrospectively.

Follow-up data were registered on patients included in the follow-up programme; however, for patients who moved from the catchment area during the study period, some data were missed. All patients were followed at the department. Time and causes of death were collected from hospital records for those who died in hospital. For others, data were obtained from the Norwegian Cause of Death Registry, which has a delay in registration extending to three years. In addition, the causes of death reported on the public death certificate may in some cases be erroneous due to low autopsy rates.

Survival analyses

In the first paper, we performed overall (OS) and relative survival (RS) analyses on prognostic factors mainly to avoid possible false causes of death as autopsy rates were low during the study period. OS does not distinguish between causes of death; RS is adjusted for the general mortality in the population of interest with regard to gender, age and time period. We therefore consider these analyses to be more reliable than cancer specific survival (CSS) in which the exact cause of death has to be known in all cases. In Paper 4 however, the causes of death were known in all stage IV patients. We therefore used CSS analyses with definitions of event, censors and ignored cases as described by Punt et al.[258] (Table 5).

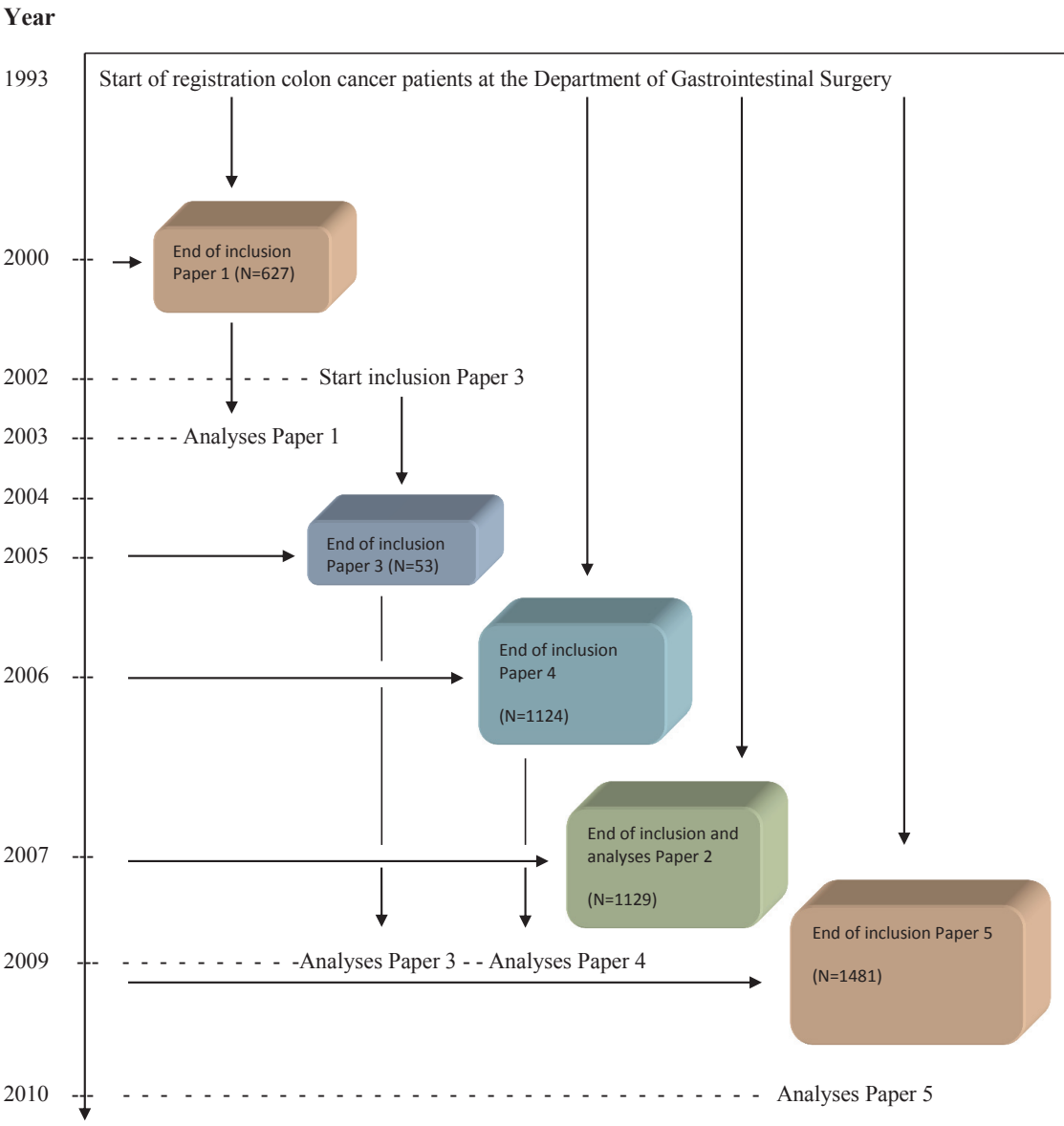

Figure 7: Inclusion periods of the different studies / papers:

Paper 1: Tumour location is a prognostic factor for survival in colonic cancer patients
Paper 2: Short term outcome after emergency and elective surgery for colon cancer
Paper 3: Lymph node micrometastases and isolated tumor cells influence survival in stage I and II colon cancer
Paper 4: Peritoneal carcinomatosis of colon cancer origin: Highest incidence in women and in patients with right sided tumours
Paper 5: Prognostic impact of lymph node harvest and lymph node ratio in colon cancer patients

Disease free survival (DFS) would have been another option; however, the standard follow-up programme only includes patients younger than 75 years of age, and recurrences in elderly patients were missed. In Paper 3, we applied DFS analyses as all patients were included in the follow-up programme and the recurrence status was known in all patients.

OS and time to recurrence (TTR) were used in Paper 5, adopting the definitions of endpoints listed in Table 5[258]. We accepted the application of TTR analyses as we considered the quality of data on causes of death to be sufficiently high after corroboration with the Norwegian Cause of Death Registry. However, the possibility of false cause of death in some cases cannot be ruled out.

Histopathological examinations

All specimens were examined at the Department of Pathology, Oslo University Hospital Aker. Eight consultant pathologists were involved during the study period. After formalin fixation, paraffin embedded blocks was prepared, followed by standard microscopic examination of 3-4 µm haematoxylin-eosin (HE) stained sections. Tissue-sections from primary tumour(s), resection margins of both bowel ends, of circumferential mesocolic resection margins and all identified lymph nodes were examined.

In Paper 3, all identified lymph nodes in stage I and II patients were examined immunohistochemically using a monoclonal antibody to cytokeratin CAM 5.2 (Becton-Dickenson, mountain View, CA, USA) as described in the paper. All immunostained sections were examined by two pathologists at each of the two participating hospitals.

TNM stage classification

The prospective registration of patients began on 1 January 1993 and inclusion periods varied as shown in Figure 1. Until 2003, when results from our first study were analysed, we applied the TNM classification system according to the UICC/AJCC fourth edition. In the subsequent studies, the sixth edition was applied with conversion of data for patients registered earlier than 2003.

DNA isolation and *TP53* mutation analyses (Paper 4)

DNA isolation was carried out on formalin-fixed, paraffin-embedded tissue samples from 55 PC patients. For each tissue sample the tumour area and differentiation grade in a 5µm HE-stained section were evaluated by a pathologist. If necessary, tumour tissue was manually dissected prior to sectioning

for DNA isolation. DNA was extracted by standard procedure from four consecutive 25µm sections of each sample. Tumour DNA from two additional patients was isolated by standard phenol – chloroform extraction from fresh frozen tissue.

Each of the exons 5, 6, 7 and 8 was amplified in independent polymerase chain reactions (PCR) using flanking intronic primers, which generated products of 289, 267, 218 and 281 bp, respectively (Table 4). Each PCR was performed according to the procedure shown in Table 4a. The direct sequencing reaction was performed using several different inputs of PCR product. The same primers were used as for the initial PCR. The resulting sequence product was further purified using Millipore multiscreen plates and subjected to further sequencing by a 3730 DNA Analyzer (Applied Biosystems).

Owing to the possibility of false positives resulting from formalin fixation[32, 53, 259, 260], all DNA sequences of interest for each sample were submitted to two independent PCR and sequencing analyses.

Tumours with mutations and those in which the mutations could not be scored were submitted to a second new PCR procedure (Table 4b). The high-fidelity polymerase lowers the probability of errors introduced by the polymerase itself. The results were also compared with IARC *TP53* mutation database[1], version R12, November 2007.

Table 4: Polymerase chain reactions performed with a) HotStar polymerase and b) HotStar HiFi polymerase (QIAGEN, GmbH, Hilden, Germany) for *TP53* mutation detection.

a) HotStar polymerase

	HotStar Buffer without MgCl2	$MgCl_2$ (mM)	dNTP (mM)	Polymerase (U)	Q - solution	5' primer (pmol/µl)	3' primer (pmol/µl)	DNA (ng)	Anneal temp (°C)	cycles	Product size (bp)	5' Primer sequence	3' Primer sequence
ex5	1x	1.5	0.17	1.0U	0	0.2	0.2	50	54	40-45	289	TTC AAC TCT GTC TCC TTC CT	GCA ATC AGT GAG GAA TCA GA
ex6	1x	2.5	0.17	1.0U	0	0.2	0.2	50	54	40-45	267	GCT GCT CAG ATA GCG AT	CCA CTG ACA ACC ACC CTT
ex7	1x	1.5	0.17	1.0U	0	0.2	0.2	50	54	40-45	218	AGG CGC ACT GGC CTC ATC TT	AGG GGT CAG CGG CAA GCA GA
ex8	1x	2.5	0.17	1.0U	0	0.2	0.2	50	58	40-45	281	TTG GGA GTA GAT GGA GCC T	AGG CAT AAC TGC ACC CTT GG

[1] www-p53.iarc.fr/index.html

b) HotStar HiFi polymerase

	HiFi Buffer with dNTP	$MgSO_4$ (mM)	dNTP (mM)	Polymerase (U)	Q-solution	5' primer (pmol/µl)	3' primer (pmol/µl)	DNA (ng)	anneal temp (°C)	cycles	Product size (bp)	5' Primer sequence	3' Primer sequence
ex5	1x	2.5	1.0	1.25	1x	1.0	1.0	50	56	40	289	TTC AAC TCT GTC TCC TTC CT	GCA ATC AGT GAG GAA TCA GA
ex7	1x	2.5	1.0	1.25	1x	1.0	1.0	50	56	40	267	GCT GCT CAG ATA GCG AT	CCA CTG ACA ACC ACC CTT
Ex8	1x	2.5	1.0	1.25	1x	1.0	1.0	50	56	40	281	TTG GGA GTA GAT GGA GCC T	AGG CAT AAC TGC ACC CTT GG

Statistical methods

For comparison of proportions, Pearson chi squared or Fisher Exact test were used, as appropriate. When comparing means in two independent samples, student t-test was used if data were of normal distribution. If data were skewed, a non-parametric test was used; means in two independent samples were compared by Mann-Whitney U test and in analyses of three or more independent samples, Kruskal-Wallis H test was used. For multivariate analysis on prognostic factors for mortality and complications (Paper 1) and risk factors for PC (Paper 4), binary logistic regression was used, as step-forward model including all factors with significant differences ($P \leq 0.05$) in univariate analysis.

Overall survival, disease free survival, cancer specific survival and time to recurrence were calculated with Kaplan Meier Method, and Log-rank test was used for comparison of curves. The endpoints were defined according to Punt et al.[258] (Table 6) as described above. For multivariate evaluation of independent prognostic factors, cox regression analyses were performed, as step-forward model including all factors with significant impact in univariate analysis.

Relative survival was calculated with adjustment for general mortality in the population according to gender, age and time period, using Statistics Norway (www.ssb.no) as source of required data.

In all tests, a P value of ≤ 0.05 was considered statistically significant.

Summary of papers

Paper 1:

Tumour location is a prognostic factor for survival in colonic cancer patients.

Prognostic factors for survival were evaluated in a consecutive, unselected series of 627 colon cancer patients admitted to Department of Gastrointestinal Surgery, Aker University Hospital, between 1993 and 2000. Median follow-up was 44 months.

A total of 501 (81%) patients were operated on with major tumour resection. The rate of major resection was lowest for tumours located in the splenic flexure (73%) and the descending colon (67%). The intention was curative in 410 (65%) patients, of whom 378 (60%) had a R0 resection and 32 (5%) a R1 resection.

Five year OS was calculated with the Kaplan-Meier method. Five year RS was estimated with adjustment for age, gender and time period related mortality of the general population in the area.

For all patients admitted, five year OS rate was 41%. Five year RS was 50% in females and 52% in males. Following resection with curative intent, the five year OS was 59% with no difference between genders; five year RS was 74% in females and 79% in males.

Following R0 resection, five year OS was 62% and five year RS was 78% in females and 82 % males, a difference which was not statistically significant.

Cox regression analyses were performed to identify independent prognostic factors. Patients with tumours located in the transverse colon, splenic flexure and descending colon (these locations were stratified into one group) had reduced survival (hazard ratio [HR] 1.8, P=0.048) compared to patients with tumours located in the right colon.

Blood transfusion of more than 2 units during hospital stay (HR 1.8, P=0.008), emergency operation (HR 1.7, P=0.005), advanced TNM stage (Stage II: HR 1.8, stage III: HR 2.9) and advanced age (P<0.001) were independent prognostic factors for reduced survival.

Patients with tumours in the splenic flexure and descending colon have reduced survival due to a low resection rate. However, additional factors also contribute; even following major resection, tumour location in the transverse colon, splenic flexure and descending colon were independent negative prognostic factors in multivariate analyses.

Paper 2:

Short term outcome after emergency and elective surgery for colon cancer

Postoperative mortality and complications were evaluated in a consecutive, population based series of 1129 patients registered in the period 1993 – 2007, and results following elective and emergency surgery were compared.

A total of 850 (75%) elective and 279 (25%) emergency patients were admitted; 999 (89%) underwent surgical treatment and 924 (82%) had major resections. The rate of major resection was 58 % in emergency patients and 90% in elective patients (P<0.001). After resection 82% of emergency patients and 98% of elective patients had a primary anastomosis (P<0.001).

The main causes of emergency admittance were obstruction (80%), obstruction with proximal perforation 5% and perforation in the tumour area (8%).

After major resection, the mortality rate was 3.5% in elective and 10 % in emergency patients (P<0.01), and the overall complication rates were 24% and 38%, respectively (P<0.01). The odds ratio for mortality was 2.5 (1.4-4.5) and for complications 2.0 (1.4-2.8)

In patients operated on for left sided obstruction (without perforation), the mortality rate following Hartmann's procedure was 19%, whereas it was 3% following resection with primary anastomosis (P<0.01).

High mortality rates were noted in patients who underwent surgery without resection, 17% in the elective and 24% in the emergency setting (P=0.45). Overall complication rates were 25% and 45 %, respectively (P=0.07)

For all patients who underwent surgery, logistic regression analyses demonstrated that emergency operation (P=0.001), male gender (P<0.01), advanced age and advanced TNM stage were associated with increased complication rates. Emergency operation (P<0.001), advanced age and ASA (American Society of Anaesthesiologists) score IV (P<0.001) were associated with an increased risk of postoperative death.

In conclusion, the study shows that a large proportion of patients with colon cancer presents with acute symptoms. Emergency operation is associated with high mortality and complication rates. Hartmann's procedure and surgery without resection was associated with a particularly high mortality rate, probably due to patient selection. Alternatives to emergency surgery should be considered but need further evaluation in future studies.

Paper 3:

Lymph node micrometastases and isolated tumour cells influence survival in stage I and II colon cancer

The influence of MM and ITC on recurrence rates and the survival – using time to recurrence (in the article incorrectly called disease free survival) as endpoint, was investigated in this prospective study of 193 patients with colon cancer operated on at two centres between 2000 and 2005. Median follow-up was five years.

All retrieved lymph nodes were examined by routine microscopy in haematoxylin and eosin-stained sections. If no metastases were identified in any node, all nodes were examined immunohistochemically with the monoclonal antibody CAM 5.2.

Ordinary metastases were defined as malignant cell cluster larger than 2 mm in diameter; MM as malignant cell clusters with diameter between 0.2 and 2 mm; and ITC as malignant cell clusters less than 0.2 mm in diameter or single tumour cells.

Ordinary metastases were found in 67 (35%) patients, leaving 126 patients in stage I/II. In these, immunohistochemistry detected 6 (5%) patients with MM and 33 (26%) with ITC.

There were 31% recurrences in stage III and 12% in stage I/II patients (P=0.002). In stage I/II patients with MM/ITC, recurrences were seen in 23%, whereas 7% in the MM/ITC negative group had recurrent disease (P=0.01).

Five year TTR was 91%, 86% and 66% in stage I, II and III, respectively (P=0.002). In stage I/II, TTR was 75% for patients with MM/ITC and 93% for patients without MM/ITC (P=0.012). When analysing stage I (n=39) and II (n=87) separately, there was a trend towards improved survival in patients without MM/ITC (P=0.06 in stage I and P=0.07 in stage II, respectively). There were no significant differences in recurrence rates or TTR between stage I/II and stage III patients with MM/ITC.

Cox regression showed that the presence of ordinary lymph node metastases (HR 4.8, P=0.001) in stage I-III patients, as well as MM/ITC (HR 3.5, P=0.02) in stage I/II patients, were independently associated with lower TTR.

In conclusion, the presence of MM/ITC in the mesocolic lymph nodes of stage I/II patients has a statistically significant negative prognostic impact. The prognosis in patients with ITC is probably similar to that of patients with MM and not much different from the prognosis in stage III patients. Larger studies are needed to confirm these findings.

Paper 4:

Peritoneal carcinomatosis of colon cancer origin: Highest incidence in women and in patients with right sided tumours

The incidence of PC was evaluated in a prospectively recorded series of all colon cancer patients admitted in the period 1993-2006. Clinical and pathological characteristics, survival and *TP53* tumour suppressor gene mutation status in primary tumours were compared in patients with and without PC.

A total of 1124 patients were included in the study. At the time of diagnosis, 94 (8.3%) patients had PC; 10% in women and 7% in men (P=0.05). PC was diagnosed at laparotomy (n=89) or by CT scan (n=5).

PC patients were younger than patients without PC, median (range) age 71 (33-91) and 75 (30-96) years, respectively (P=0.002). The incidence of PC was 10.3% in right sided tumours and 6.2% in left sided tumours (P=0.025). Liver metastases were present in 33% of PC patients and in 17% of patients without PC (P< 0.001). Major resections were performed in 66 % of PC patients and in 84% of non-PC patients (P< 0.001); emergency operations were more frequent in PC patients (34% vs. 18% in non-PC patients).

PC patients were compared with patients with distant metastases at other sites (n=201): in PC patients, tumours were more often right sided (69% in PC patients vs. 51% in non-PC patients, P=0.006); in those who underwent major resection, PC patients had more advanced pT-stage (pT4 in 65% of PC patients vs. 17% in non-PC patients, P<0.001).

In multivariate analyses of all patients following major resection (n=928), pT4 stage (OR 10.4, pT1-2 used as reference, P=0.003) and stage pN+ (OR 3.2, P=0.002) were independently associated with PC.

CSS was median 9 (range 0-60) months in PC patients. Patients with a single metastatic peritoneal deposit (n=13) had better survival, median 31 months (P=0.03).

TP53 mutation status could be fully scored in 49 patients with PC. Mutation status being unknown in patients without PC, comparisons were made with a group of 148 non-PC colon cancer patients from a historical multicentre series treated in the period 1987-1989[41, 53, 261]. Patients in the historical series were younger than non-PC patients in the unselected series, median age 71 and 75 years, respectively (P=0.003), but were well matched with regard to gender, TNM stage and tumour location.

Mutations in the *TP53* gene were present in 57% of the PC patients and in 40% of non-PC patients (P= 0.04), odds ratio 2.4 (CI 1.2-4.8) in multivariate analyses. These analyses also confirmed that female gender was independently associated with PC (OR 2.5, CI 1.2-5.2)

In conclusion, this study demonstrates for the first time that PC of colon cancer origin is more frequent in women and in tumours of the right colon. PC patients were slightly younger than non-PC patients. PC was independently associated with mutation in the *TP53* tumour suppressor gene, but selection bias cannot be ruled out when comparing mutation status with a historical series.

Paper 5:

Prognostic impact of lymph node harvest and lymph node ratio in colon cancer patients

The prognostic impact of the total number of examined lymph nodes in stages I-III and of lymph node ratio in stage III patients were investigated. Development over time was evaluated by stratifying the patients into three groups according to dates of operation (1993-98 [first period], 1999-2004, 2005-2009 [last period]). A total of 1481 patient were registered, of whom 950 stage I-III patients with R0 resection were included in the analyses.

Median number of lymph nodes increased from 7 during the first to 15 during the last period ($P<0.001$). The proportion of patient with $\geq$12 nodes increased from 18% to 85% ($P<0.001$). During the last two periods, 33% had stage III disease compared to 25% during the first period ($P=0.02$).

LNR in stage III patients was median 0.33 during the first period decreasing to 0.14 during the last period ($P<0.001$).

OS in all patients improved from 39% to 46% during the study period ($P=0.002$). TTR improved from 81% to 95% ($P=0.02$) in stage I patients and from 66% to 85% ($P=0.003$) in stage II patients.

Following R0 resection, increased number of examined lymph nodes was associated with improved OS and TTR in both univariate and multivariate analysis.

Stage III patients were separated into four categories according to quartiles of LNR. The proportion of patients in the lowest quartile (0-0.10) increased from nine % during the first period to 33 % during the last period ($P=0.002$).

High lymph node ratio was significantly associated with poor OS and TTR, 40% vs. 70% and 46% vs. 83% in the groups with highest and lowest LNR, respectively. Multivariate analyses confirmed the prognostic impact of LNR.

Selection bias is unlikely in this prospective, single centre population based series. Surgical technique has probably not changed much during the study period, but the quality of the pathological reports

improved. Therefore, improved pathological examination most likely explains the increase in the number of investigated lymph nodes, although other factors cannot be ruled out.

In conclusion, OS in all registered patients improved during the study period, in accordance with national data. There are probably several reasons for this improvement. In patients who underwent curative resection, improved pathological examination with evaluation of more regional lymph nodes was associated with stage migration, which probably contributed to the improved stage specific survival. In stage III patients, LNR was a stronger prognostic factor than the total number of examined lymph nodes.

General discussion

Curative treatment of colon cancer is based on surgical resection of the tumour bearing bowel segment with the regional mesocolic lymphovascular pedicle, containing the lymph nodes draining the tumour area. There are three main pathways of spread of the disease; haematogenous, lymphatic or direct spread of tumour cells to the abdominal cavity, all representing diagnostic and treatment challenges. The premise for cure is usually removal of all viable tumour tissue, although microscopic remaining tumour can be cured by adjuvant chemotherapy, and/or be destroyed by the immune-system of the diseased. Evaluation of outcomes is mandatory for quality assurance in a department, and research is necessary to further optimize the treatment, which must be individualised according to many factors with influence on patient tolerance for different treatment modalities, and impact on the long term results. Our research project with a local registry was founded to make this possible.

The database

These studies are based on a cohort containing all patients with colon cancer treated at a single institution from 1993 to 2009. Consecutive registration of patients required participation of all surgeons involved in the treatment and follow-up of patients. The registration was performed on case record forms (CRF), and the data were then entered into a database by researchers involved in the study project, or by a secretary trained in such registration. The CRF with clinical information on initial treatment was supposed to be finalized at discharge after the first hospital admission (or out-patient visit in those not treated as in-patients).

The follow-up of the patients was performed at the department during the entire study period, and all blood tests and radiological investigations were taken at the hospital. Follow-up data were also noted on special CRF's by the surgeons.

The compliance of the surgeons varied and some information was registered with some delay, either by the surgeon who had missed the primary registration, or by researchers more directly involved in the research project. To avoid missing cases, the database was checked against the patient administrative system in the hospital and against the Norwegian Cancer Registry. Such control procedures were not made, but in relation to publication of results. Updating the database at the end of the study period revealed that a maximum of 1% of patients were missing in previous publications, and it is unlikely that missing data had significant impact on the results and conclusions.

Study endpoints

The studies were mainly focused on prognostic factors for survival in patients who underwent a (potentially) curative resection (Paper 1, 3 and 5). The primary endpoints were Relative survival (RS) and Overall survival (OS) in Paper 1 and 5, Cancer specific survival (CSS) in Paper 4, and Time to recurrence (TTR) and Disease free survival (DFS) in Paper 3 and 5.

Table 5: Definitions of endpoints in survival analyses according to a consensus conference in 2007 described by Punt et al.[258].

Event	Disease Free Survival	Time To Recurrence	Cancer Specific Survival	Overall Survival
Locoregional recurrence	E	E	I	I
Distant metastases	E	E	I	I
Second primary, same cancer	E	I	I	I
Second primary, other cancer	E	I	I	I
Death from same cancer	E	E	E	E
Death from other cancer	E	C	C	E
Non-cancer related death	E	C	C	E
Treatment-related death	E	C	C	E
Loss to follow-up	C	C	C	C

E = event, C = censor, I = ignore

OS and RS analyses are based on death of any causes, which is the most robust endpoint. These data has been conceived from the Norwegian Population Registry on a regular basis, and are correct. Relative survival is a useful surrogate marker for cancer specific survival when data on cause of death is unknown or uncertain. It describes survival in the study cohort related to the expected survival in the whole population, adjusted for gender, age and time period. Large, population based studies have used this method[96, 192, 202, 230, 262], making comparison of results meaningful.

OS is often used in outcome studies. All deaths, regardless of causes, are then events (Table 6), and accordingly, the results are influenced by variations in non-cancer mortality in the population. Obviously, elderly patients will be at higher risk of death than younger patients, regardless of other factors included in the analyses.

DFS is used with different definition across studies. In Paper 3, DFS was defined with death of the same cancer and local / distant recurrence as event. Death of other causes was censored, as done by others[171]. The correct definition, as described in the consensus report published in 2007 by Punt and co-workers[258] (Table 5), includes recurrence of CC and death of any cause as events. DFS with this definition will give lower survival curves than TTR.

In the last study on prognostic impact of lymph nodes (Paper 5), we used OS and TTR as endpoints. TTR is based on death of the same cancer and recurrence as events, and loss to follow-up and death of other causes as censors. This corresponds to our definition of DFS in Paper 3, and TTR would have been the proper term to use in that paper. TTR requires knowledge of all recurrences and the cause of death in all patients in the study cohort. We believe these data to be of good quality, but false death causes or missed recurrences in some cases cannot be ruled out.

CSS was used in Paper 4 because the patients of interest had disseminated cancer with spread to the abdominal cavity or liver/lung. Event is death of colon cancer, other causes of death is censored, and recurrent disease and metastases are ignored (Table 5). As cause of death is known in all these patients, it gives a meaningful comparison of survival between patients with PC and patients with metastases in other locations.

All survival analyses were performed using the Kaplan Meier method, with cut off after five years, corresponding 5-year-OS, RS, CSS and TTR. Most recurrences occur within three years after curative surgery, and recurrence is rare after five years. Observation shorter than five years would imply the risk of missing recurrences and death of cancer. After five years most deaths are not related to colon cancer. OS and DFS will therefore be highly influenced by non-cancer related events. The risk of having a false death cause (cancer related) increases, and might confound analyses of CSS and TTR. We therefore think that five-year survival is best for evaluation of long term outcome of colon cancer.

Cox regression analyses as a step-forward model were used for identification of independent prognostic factors for survival. All significant factors with *P*-value of 0.05 or less in univariate analysis were included.

Comparing results from different studies and centres is often difficult, and results from meta-analyses should be interpreted with care. There are more reasons for this;

1: The methods used in the survival analyses are not defined, or only referred to as '5-year survival' or similar. If the method is specified, the definitions of endpoints, i.e. events and censors, are often not clearly stated. Thus, comparison and interpretation of survival curves are difficult, and the results not reliable.

2: Selection criteria for inclusion in studies may vary between different centres. Analyses are often made on subgroups, for example patients referred to specialized centres[192]. Inclusion criteria may be curative resection (R0 resection)[199], operation with curative intent[186, 188, 193, 234], or is not clearly stated[138, 194, 263]. These studies often lack information about the complete population from which the selection has been made, with a probable risk of selection bias. This problem is illustrated by the great variation in resection rates from 99%[199], 96%[264] to 71%[96], R0 resection rates of 85%[199], 77%[192] and 55%[264], and the distribution of colon and rectum tumours[264].

In larger regional or national series, selection bias should be minimal, and relative survival[96, 202, 230, 262], observed survival[265], or both[192] is often used, as detailed information on recurrences and causes of death often lacks.

3: The definition of the criteria used for selection is often not clearly described, like the definition of resection with curative intent and curative resection[87, 263].

4: In some studies colon and rectum cancer are mixed[87]. It is generally accepted, that colon and rectum cancers have different properties, have different natural histories, and are treated differently. They should therefore be investigated separately.

In paper 2 mortality and postoperative complications were the endpoints. Data on per and postoperative deaths are complete, making the result on mortality reliable. Patients with major postoperative complications are probably nearly completely registered, as these cases are highly focused on in the clinical practice with registration of both complication(s) and treatment in the patients' records. Minor complications are probably missing in some cases.

Prognostic factors

A number of characteristics and properties of the tumour, the patient and the therapeutic team including the surgeon, pathologist, oncologist and the department/hospital have effect on the outcome of colon cancer, as presented in the introduction. Only a fraction of known prognostic variables are used regularly in clinical practice.

In the present project we have studied a number of clinical and histopathological factors, in addition to analyses of *TP53* mutations in patients with PC, and the results will be discussed in the following.

Tumour stage (Dukes' - UICC/AJCC): The patients were classified according to Dukes' / TNM stage using the TNM classification system described in the 'Patients and methods' section. Tumour stage had a strong impact on prognosis and also influenced postoperative complication and mortality rates. Consequently, adjustment for stage was always performed in multivariate analyses.

Residual tumour (R-stage): R2-stage has major prognostic impact, as described previously and shown in our studies. R1-stage also has some impact on prognosis. Adjustment for R-stage (or inclusion of only R0 / R0+R1 patients, as appropriate) was therefore performed in survival analyses when evaluating other prognostic factors.

Clinical prognostic factors

Tumour location: At the beginning of our study period, a possible prognostic impact of tumour site was not stated. Reported results were conflicting; Single centre studies showed no influence of tumour location on survival[138, 199-201, 212, 234], as did the large Norwegian study from 2003[202]. Others reported adverse impact on postoperative mortality and long term survival in left sided tumours[197, 198].

Right versus left colon

Differences in clinical and biologic characteristics[266, 267], incidence[201] and different genetic expressions[268, 269] of tumours in the right and left colon has been described, and the heterogeneity of colon cancers has become obvious[39]. These differences might have impact on the prognosis. MSI tumours with good prognosis is more prevalent on the right colon[41, 270], whereas CIN tumours with less favourable prognosis is more prevalent on the left side[271]. *TP53* mutated tumours occur more often in the left colon and these patients have reduced survival in a large, multinational study[56].

However, conflicting results are reported when comparing right- and left-sided tumours. The definitions of right versus left colon are not consistent; some define right colon as including 2/3 of the transverse colon[198], but most commonly the entire transverse colon is included in the right colon, while the splenic flexure, descending and sigmoid (with or without the rectosigmoid junction) is included in the left colon[201, 272]. This definition was used in our study. Indeed, the exact location and classification of tumours in - and close to - the left flexure may be difficult, which might influence the reported results. In the present study no difference in outcome was found, whereas other studies have shown better prognosis in right sided tumours excluding the hepatic flexure[197], the whole transverse colon[273] or part of transverse colon[198].

Right sided tumours were reported by Gupta et al. to present at a more advanced stage[201]. In this smaller study, no significant impact of tumour location on survival was found. In a large population

based prospective cohort from USA with almost 78.000 patients operated for CC[272], the postoperative mortality was higher in patients with right sided tumours than in those with left sided tumours (4.0% vs. 5.3%), median age was higher (73 and 69 years respectively), the proportion of poorly differentiated tumours was higher (25% vs. 14%) and median survival was lower; 78 vs. 89 months (P<0.001). In adjusted multivariate analysis, HR for death within five years was 1.042 (P=0.001) for right sided tumours.

Prognosis according to segmental location

We also analysed prognosis according to tumour location in each segment of the colon, but the numbers were too small to reach conclusive results. Interestingly, a trend towards less favourable prognosis was found for tumours in the transverse, left flexure, and descending colon, respectively. It was also our clinical impression that tumours in these sites – especially in the left flexure – are surgically more demanding than tumours in other sites. We therefore combined these three segments in new analyses, and found a significantly reduced survival when comparing tumour location in the transverse-left flexure-descending colon with the other colonic segments.

There are several possible explanations for poorer outcome at these locations:

- The surgical procedure may be more difficult, as already mentioned. The risk of missing the correct mesocolic plane during the dissection is greater than during operations for tumours in other locations

- Both the transverse and the left flexure cancer might have metastatic lymph nodes at the origin of the middle colic artery, and central lymphovascular dissection should be performed. Possibly, the surgery was not radical enough in some cases.

- Tumours in the descending colon and splenic flexure had a lower rate of major resection (Table 1, Paper 1), leading to reduced rates of curative operations.

- In addition, tumours in the descending and sigmoid colon were more often operated as emergencies (Table 1, Paper 1), with increased postoperative mortality and morbidity, as shown in Paper 2.

However, in multivariate analyses of patients operated with curative intent, tumour location remained as independent prognostic factor after adjustment for gender, age, emergency operation, TNM stage and perioperative blood transfusions.

Our findings have not been verified in other studies, and the results should be interpreted with caution. Stratification according to location might be confounded by misinterpretation of the exact location. Tumours in these locations are rare, accounting for 15% of all CC, and our sample size is relatively

small. In addition, the level of significance was not very high (*P*=0.048), which imply a possibility of Type I error.

Tumours of the splenic flexure have shown to be at high risk of obstruction, local recurrence and in-hospital mortality[190, 198], and a recent large study by Benedix showed that these tumours have the highest proportion of stage III-IV tumours and lymphatic invasion, which are factors associated with decreased survival[274].

We think that tumours in the area of the left flexure need special attention. It is advisable to perform a preoperative CT scan to detect possible advanced tumour growth, and the operation should be performed by highly competent colorectal surgeons.

Perioperative blood transfusion (PBT): We found (Paper 1) an increased risk of death (HR 1.8, *P*=0.008) in patients who received three or more units of blood transfusion during the hospital stay, in accordance with the report from Edna et al.[214], and in a study of younger patients operated for CC[275], but contradicted in a large population based study from Spain[212].

Allogeneic blood transfusion is suggested to have negative immunologic effects on the host through increasing the number of suppressor T-lymphocytes and stimulating the production of a variety of antibodies. The activity of natural killer cells is depressed, and transfusion promotes the release of Prostaglandin E2, which inhibits interleukin-2 production and thus suppresses the immune response[276]. In animal models, PBT showed to enhance the growth of established metastases, but not of the primary tumour[135].

Patients who need PBT are anaemic for different reasons; more advanced cancer disease, increased comorbidity or greater loss of blood during surgery. It is reasonable to assume these factors to increase the risk of complications and poorer outcome. However, PBT was an independent risk factor in our study when adjusting for gender, age, emergency operation, stage and tumour location in multivariate analyses.

In the review from Amato in 2006 (updated 2010), results from a meta-analysis of more than 12.000 patients from 36 included studies, support the hypothesis that PBT have a negative effect on recurrence rate and survival[277]. This effect was observed regardless of timing or type of transfusion. The prognostic effect seemed to increase with the dose given, and was independent of preoperative anemia or preoperative blood loss.

Our results support the evidence for a negative prognostic effect of PBT. Great effort should be undertaken to reduce perioperative bleeding and blood transfusion in CC patients.

Erythropoietin given pre- or perioperative in order to reduce the need for transfusions has been evaluated, but so far, no evidence for the use of this medicament has been reported[278].

Emergency operation: The present studies show an adverse impact of emergency operation on overall survival (Paper 1), postoperative mortality and complication rate (Paper 2) and on the risk for PC (Paper 4).

Incidence

Altogether, 25% of all admitted patients presented as emergencies (Paper 2), as compared to figures from 12%[279] to 30%[280] in other studies, possibly reflecting selection bias and differences in the definition of emergency presentation/operation. Such differences can also influence on reported outcomes.

A total of 68% of the patients admitted as emergencies underwent surgical treatment. Among these, obstruction was present in 80%, and emergency operation was associated with more advanced stage and higher age, which has been shown in other studies[280-282]. The overall incidence of obstruction due to colon cancer varies from 7% to 34%, and has been reported to be more common in the left colon[107, 189, 194, 279], a finding not verified in our study.

Perforation was present in 13% of emergency operated patients, and 5% were coecal perforations in patients with obstruction, in accordance with the study from McArdle et al. [193], but somewhat lower than in other reports [195, 281, 283], where only patients who undergo surgical treatment are included.

The proportion of patients in need of emergency operation varied according to tumour location:

- Coekum / ascending colon 15 %
- Right flexure, transverse colon 24 %
- Left flexure 30 %
- Descending colon / sigmoid colon 21 %
- Rectosigmoid flexure 16 %

Surgical strategies in cases of left sided obstruction have been debated. In the present study, Hartmann's procedure was associated with very high postoperative mortality and complication rates compared to one stage resection with primary anastomosis, in accordance with other studies[284-288]. This was probably caused by patient selection. Hartmann's procedure is often recommended in high risk patients[289], and this was the routine in our hospital. One randomized study concludes that there is no difference in morbidity or mortality between the two methods[290]. A review from 2007 of 29 studies including two RCT's concluded that one-stage surgery appeared to be superior to two- or three stage procedures[291]. The item seems not to be settled.

Short term outcome

Emergency surgery was associated with an increased risk of in-hospital mortality (OR 2.5, $P= 0.001$) and postoperative complications (OR 2.0, $P< 0.001$) after adjustment for tumour stage, age and gender. Similar findings have been presented from many other studies.

Overall complication and mortality rates were not different in patients with obstruction and perforation, but the numbers were too small to draw firm conclusions. Other studies demonstrate higher postoperative mortality[281, 282, 292] and morbidity[281, 293] in patients with perforation.

Long term outcome

Emergency patients are older and probably frailer, and they present with disease at a more advanced stage, and the resection rate is lower than in elective patients (Paper 2). In addition, the postoperative mortality is increased, as discussed above. All these factors contribute to reduced survival in emergency patients. Even in patients who underwent R0 resection and survived the primary operation, emergency operated patients had a 1.7 time increased risk of death within five years (Paper 1)

Tumour perforation, spontaneously or inadvertently during surgery is associated with increased rates of local recurrence and reduced survival[82, 294]. Analyses of stage II patients from our cohort (not published) showed no difference in five year CSS, but reduced five year TTR in patients operated for perforation versus obstruction (Figure 8). However, no difference in recurrence rates, OS and CSS[293] or DFS[263] was found between patients with perforation and obstruction in two smaller series. In a series of 83 patients with tumour associated perforation, patients with perforation proximal to the obstruction had higher operative mortality, but better cancer specific survival than patient with perforation at the tumour itself[295]

Due to the poor prognosis, emergency operation should be avoided if possible. Reducing patient and doctor delay in symptomatic patients is important. In a recent Norwegian population based study the proportion of patients admitted with obstruction decreased over time[296]. Presentation at an earlier stage over time has been reported from Minnesota, USA[201] and from Norway[296]. Screening programs may contribute to earlier diagnosis[297, 298].

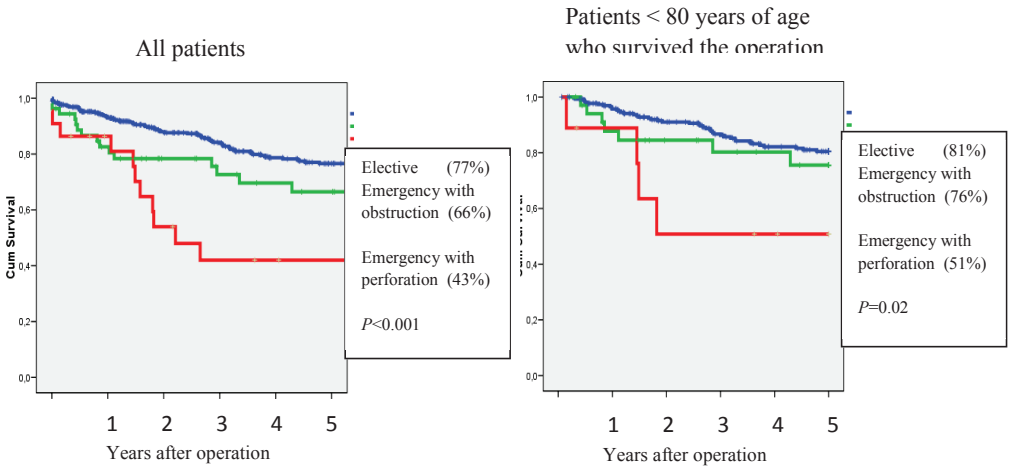

Figure 8: Time to recurrence following R0 resection, stage II (n=460)

In patients with left sided colon obstruction the emergent problem can often be solved by intraluminal stenting as a 'bridge to surgery'. Recent reports suggest that preoperative stenting of malignant obstruction followed by resection[299, 300] or palliative chemotherapy[101] is favourable. Limitations of preoperative stenting are failure to place the stent, and the complication rates, especially perforation in the tumour area (see 'General introduction'). Two recent multicentre RCT's showed no decreased rate of stoma formation in patients treated with stent as bridge to surgery compared with emergency operation[301]. In one of the studies this was caused by a high rate of procedure related perforation, and of anastomotic leakage after resection in the bridge to surgery group[302]. The long term oncological outcome is also unclear. Consequently, more studies are needed to clarify the optimal treatment of obstructing colon cancer[103, 303]. At the moment, many authors recommend stenting in cases of left sided obstruction, especially in high-risk surgical patients[304].

Age: In the present studies, OS survival following resection with curative intent decreased with advancing age (Paper 1). However, RS related to age was not calculated, like in the Norwegian national report showing lower RS in older patients[202]. These findings are also supported by a large regional study from Sweden that showed a decrease in CSS with increasing age[305], and the results were similar when analysing stage II and III separately. Another large cohort study from USA[272] reported an increase in relative risk of 3.6% for every one-year increase in patient age. However, many studies find no independent impact of age on recurrence or survival in multivariate analyses[138, 199, 234, 263, 306]

In our study, high age was associated with increased risk of postoperative complications (Paper 2), in accordance with other studies[305, 307]. Poorer short and long term outcome in elderly patients may partly be explained by a higher frequency of comorbidity, advanced tumour stage and emergency operation[305]. The latter was however not statistically significant in our study. In addition, older patients are less likely to receive adjuvant chemotherapy[305].

Gender: The age-adjusted incidence of colon cancer is higher in men than in women in Norway, but since women live longer than men, the actual number of cases is higher in women. The present study (Paper 1) demonstrates no significant survival difference between genders following resection with curative intent, neither in overall nor in relative survival, in accordance with other reports[138, 263].

The exception was stage I patients; our study showed significantly better relative survival in females than in males, 101% (95% CI: 100-103) versus 80% (95% CI: 66-94).

Chapuis reported better overall survival in females[144], and Angell-Andersen[202] reported lower cancer specific mortality in females with colorectal cancer. In a larger, multicentre series from Glasgow, comprising 2300 patients, five-year-OS and CSS following curative resection was better in women, when adjusted for age, site, presentation and stage[308]. Another study found better overall and disease free survival in females[309]. In contrast, a larger regional population based series from Sweden[305] shows no difference in cancer specific survival among genders.

In our study, postoperative mortality following major resection was not different between genders, but postoperative complication rate was higher among men than women (HR 1.6, $P<0.01$) (Paper 2), corresponding to findings from Sweden[305].

In the studies that show differences in outcome, it seems to be in favour of females. If there are any difference, a possible explanation may be that females have a stronger immune response following major abdominal surgery[310]. Some experimental observations show better posttraumatic immune competence in women than in men[311-315], possibly due to genetic differences. Such differences might contribute to different outcome in CRC.

Histopathological prognostic factors

Number of lymph nodes: The number of harvested lymph nodes increased two-fold during the study, from a median of seven in the first to 15 in the last period (paper 5). The proportion of specimens in which at least 12 nodes were examined increased, and the proportion with examination of less than eight decreased.

Many factors determine how many lymph nodes are examined. Surgical factors are essential and both hospital caseload[316], surgeon caseload[88, 317] and surgeon specialisation[318] influence on number of nodes. The importance of the surgical dissection is obvious and well documented[79, 80, 134, 152]. The standard surgical technique in our department has been central lymphovascular dissection with removal of all regional nodes including the apical nodes, corresponding to D3 dissection (see introduction), and the technique has probably not changed much during the study period.

We found a variation from six nodes in specimens from the transverse colon to nine nodes in sigmoid specimens and 13 nodes in right hemicolectomy specimens (Table 6). This probably reflects the normal anatomical distribution of LN in the mesocolon, and not differences in surgical dissection. However, the low number found after transverse colon resection might also imply less radical surgery. Similar variations in the number of nodes according to type of resection have also been reported by others[317, 319-321].

Table 6: Lymph node harvest in relation to type of colonic resection (R0 resection)

Resection type	N	No of lymph nodes Median (range)	
Right hemicolectomy	423	13	(0–44)
Resection of the transverse colon	18	6	(0–22)
Left hemicolectomy	95	11	(0–36)
Resection of the sigmoid	204	9	(0–43)

Obviously, the quality of the pathological examination also has great influence on the number of lymph nodes detected and analysed. We observed improvement in the reporting of several other histopathological factors, like Crohn like lymphoid reaction and perineural invasion (seldom described in the first study period, reported in > 50 % in the later periods), and venous invasion (increased from 7% to 88% during the study period). This indicates an increased focus on high quality examination of CC specimens, including retrieval and examination of lymph nodes.

There are several methods of specimen fixation[122, 164], and the use of GEWF solution could improve detection of lymph nodes[123] as suggested in our previously published study on the diagnostic value of SN concept[121]. The use of fat clearing techniques are often used in Japanese laboratories, probably improve the identification of LN. The level of pathologist specialisation might have significant influence on LN yield[318, 322, 323], and the use of report templates has proven to increase the quality[324]. At our hospital, the Department of Pathology don't use templates, but still have improved considerably

from 1993 to 2009. We think that improvement of the pathological examinations is the main reason for improved LN harvest.

We found no influence of age on LN harvest, in contrast to other studies reporting lower numbers of LN in older patients[147, 317]. Some authors have suggested that a weaker immune response in older patients might explain a reduced lymph node harvest. A stronger immune response causes lymph node enlargement which makes them easier to detect.

An adequate lymph node examination is necessary for correct staging of the disease. This was demonstrated in the present study; the increased LN harvest was followed by stage migration with reduced proportion of stage II and increased proportion of stage III patients. It is not likely that the true stage distribution changed during the study period, and this stage migration was therefore caused by better lymph node evaluation.

How many nodes must be analysed in order to obtain correct staging? We observed stage migration when more than eight nodes were examined, but no migration when the group with 8–11 nodes was compared with the group with $\geq$ 12 nodes. Stage migration was demonstrated when up to 13 LN were examined in a large national series from USA including more than 57.000 cases of T3N0-3 tumours[151]. A Canadian national series on more than 11.000 cases with pT3 tumours[321] showed improved staging with increased number of examined nodes up to 5-7. Above this level, marginal effect on staging was seen with increasing number of nodes. The threshold value of minimal number of nodes to secure correct staging has been debated, varying from 6 to 20[150-154]. However, it seems logical that the minimal number should be related to tumour location[319], due to the anatomic variations of LN in different colonic segments.

The present study also demonstrated that increased number of examined lymph nodes was associated with improved overall survival and time to recurrence in stage II cancers, which is in accord with other series[89, 146]. In stage III patients the results are conflicting[88, 147, 149, 322, 325], and the number of examined lymph nodes had no significant prognostic impact on OS or TTR in the present series.

Stage migration is often stated as the explanation for better outcome when more LN have been examined. Analysing more nodes decreases the risk of missing a positive node, and when false negative stage I/II patients are correctly diagnosed as stage III, the prognosis improves in both groups. This is referred to as Will Rogers phenomenon[326] after the famous US comedian Will Rogers, who stated “when the okies left Oklahoma and moved to California, they raised the average intelligence level in both states.”

Generally speaking, the Will Rogers phenomenon is obtained when moving an element from one set to another set raises the average values of both sets. The effect will occur when both of these conditions are met:

1) The prognosis of patients being moved is below average for the group (stage I/II).
2) The prognosis of patients being moved is above the current average of the set it is entering (stage III).

This phenomenon is thought to explain many observations of apparent improved outcome in epidemiological studies from a variety of biomedical fields, and is a type of information bias associated with better diagnostics. Changes in the criteria for assigning patients to the various stages of a disease can produce spurious improvements in stage-specific prognosis, even though the outcome of individual patients has not changed. In oncology, both improvements in the diagnosis of LN metastases and new imaging tools, which detects more distant metastases before they become evident clinically, can cause stage migration. Most likely it explains some of the improvement shown in stage I-III patients in the present study, which is in accordance with other reports[148, 154, 327]. In the large study from USA[151], improved overall survival was observed when up to 25 LN were examined.

In patients with a diminished immune response - an established negative prognostic factor[136] - LN are more difficult to detect[146]. This might partly explain why patients with few nodes have an inferior prognosis.

Other factors also have contributed to improved survival throughout the study periods. We found improved overall survival for all admitted patients, in accord with large national studies[202, 230, 262]. Several non-surgical and non-stage-migration factors may explain this, for instance better perioperative patient care. The use of adjuvant chemotherapy in stage III patients from 1997 might also have contributed.

Lymph node ratio (LNR): The present study demonstrates a significant impact of LNR on OS and TTR in stage III patients (Paper 5) both in uni- and multivariate analyses, in accordance with other publications[155, 158, 159, 328, 329]. LNR remained a significant prognostic factor when adjusted for the total number of LN examined.

LNR decreases with increased number of negative nodes, and in our study, the proportion of patients with low LNR increased parallel to increased total number of LN due to equal median number of metastatic nodes was equal during the study period. One could assume that the number of metastatic nodes would increase when more nodes were examined. However, a low number of metastatic LN in upstaged patients would counteract this.

Stage III patients represent a heterogeneous group according to TNM classification (Table 2)[64, 65]. A low number of examined LN implies the risk of incorrect classification within stage III (N1a – N2b)[157]. In the present study, we found median two positive LN in stage III patients, and this number was independent of the total number of examined LN (1-7, 8-11 and >11). Regarding LNR, over-staging happen when negative nodes are not discovered and under-staging if positive nodes are undiscovered. In the large series on stage III patients by Wang et al. (n > 24.000)[157], a minimum of 14 nodes are recommended to obtain correct staging, and thus a reliable LNR classification of the patients. This study concludes that LNR is a more accurate prognostic factor than the total number of examined nodes, in accordance with other reports[158, 330] including a review comprising 12 studies on colon cancer[160]. The total number of examined LN seems to have less prognostic impact than LNR in stage III patients. However, LNR should not be regarded as a substitute for adequate LN dissection[155].

The prognostic impact of the total number of positive LN in stage III patients has been investigated, and the results are diverging; some report significant impact[331, 332] whereas others does not[156, 329, 333]. Our study does not include corresponding analysis.

LNR has been analysed as a continuous variable in a few studies showing significant impact on OS, CSS[155] and DFS[155, 159]. Most studies analyse the prognostic impact of LNR by stratification into groups; we used quartiles of LNR, like other authors[155, 160, 334]. Additionally, some reclassify these categories based on maximal separation of the survival curves in univariate log rank analysis[156, 332, 333]. Others use categories based on literature (anticipated minimum number of LN required for accurate staging)[157, 330], mean of LNR[328] or classification and regression trees to determine high discriminating cut off points[158]. The threshold values for such LNR categories will differ due to differences in the total number of examined LN and the number of positive LN among study populations. Our data based on quartiles suggests that the optimal cut off value for prognostication was between the lowest (LNR < 0.11) and the three highest quartiles, similar to the results in the study by Lee et al.[333].

The interpretation of studies on prognostic impact of LNR is also difficult due to varying routines with regard to adjuvant chemotherapy. Some authors describe the use of chemotherapy clearly[156], others do not[157], and the chemotherapy regimens also vary[155]. These differences might have impact on the results, confounding the prognostic value of LNR. In the present study we adjusted for adjuvant chemotherapy in the multivariate analyses, but not for the two alternative regimens used, as the data were missing.

To sum up, LNR is a promising indicator for prognostication of stage III patients. However, high quality surgery and pathology is mandatory for an adequate harvest and examination of lymph nodes, and consensus with regard to the method used to stratify patients into prognostic groups is necessary.

Micrometastases / Isolated tumour cells: Occult metastases (MM/ICT) was discovered in 31% of stage I-II colon cancer (paper 3), and was associated with reduces survival; in patients with and without MM/ITC, TTR was 75% and 93% (P=0.012), respectively. This population based, prospective study was made on the patient cohort investigated for staging accuracy of the sentinel node concept[121]. All lymph nodes in the 126 patients without ordinary lymph node metastases were examined with IHC, not only the sentinel nodes, and none of these patients received adjuvant chemotherapy, which is strength of the study. When comparing MM/ITC positive patients with stage III patients, no difference in survival was found. However, adjuvant chemotherapy was administrated to most stage III patients, which probably influenced on the results.

The possible impact on prognosis of occult nodal metastases was postulated in the early nineties[172, 173], and has since then been debated. Some studies using immunohistochemistry to detect occult metastases have demonstrated significant prognostic value of MM/ITC in stage I-II patients[171, 177, 335], including a Norwegian retrospective study (reduced relative and cancer specific survival)[169], whereas others have not[133, 175, 184].

There are several possible reasons for different results across studies:

- Many studies are analysing both colon and rectal cancer, despite the differences in anatomy, surgical technique, complications, (neo) adjuvant treatment and subsequent outcome between these two cancers.

- The definition of MM/ITC varies[133, 175, 176], or is not clearly stated[171, 177, 336]. In some studies ITC is not included as occult metastases[184],

- The different use of adjuvant chemotherapy have impact on results, as MM/ITC positive patients who receive such treatment are excluded in some studies[175, 177, 184], and included in others[335-337].

- Examination has been made on different tissue samples, some on formalin fixed and paraffin embedded tissue[133, 176], and some on fresh (frozen) tissue[177, 336]

- Studies use different endpoints of survival; Overall survival[133, 177], relative survival[169], cancer specific survival[338] or disease free survival[171, 335]. The definitions of endpoints (event / censor) is often not clearly defined[176], which make comparisons difficult, as discussed earlier in this chapter.

  We used the endpoint defined as TTR[258], which includes only colon cancer related events.

Methods for identification of MM/ITC

At present, two methods are used for identification of MM/ITC. Detection of MM/ITC with reverse transcriptase polymerase chain reaction (RT-PCR) was described as superior to IHC in a meta-analysis from 2006[339], where IHC upstaged 21-39% of the patients versus 31-54% by RT-PCR. Another meta-analysis reached the same conclusions[340], but most series included are retrospective and small, which gives low statistical power[341]. In the multicentre trial of Bilchik (n=152)[337], four IHC negative patients were upstaged by TR-PCR. The high sensitivity of RT-PCR is however associated with low specificity, giving risk of false positive results[341-343]. Still, most series using this technique shows significant prognostic impact of MM/ITC[336, 338]. In the present study, 31% of stage I-II patients proved to have occult nodal disease in accordance with other studies using IHC. This number might still be too low, and false negative cases might influence on our results.

Median 12 nodes were examined in the present study, which implicate that about half of the patients had less than the minimum number of nodes recommended for accurate staging. The fraction of MM/ITC positive patients was equal when analysing patients with less than 12 and 12 or more nodes, indicating a correct staging of patients with lower number of examined LNs. Nevertheless, the possibility of under-staging cannot be ruled out. Seven per cent of the MM/ITC negative patients experienced recurrence, which may be a consequence of possible under-staging.

Prediction of occult nodal disease has been investigated by Wasif et al.[344], showing association with low differentiation, advanced T-stage (pT3-4) and lymphovascular invasion in univariate analysis. These factors have adverse impact on prognosis. In the future, supplementary analyses to detect occult nodal disease could be an option in node negative patients with unfavourable characteristics of the primary tumour.

Peritoneal carcinomatosis: The present study (Paper 4) demonstrates that 8.3% of all admitted patients had PC at time of diagnosis. Probably this number is underestimated, as some patients who were not operated or autopsied, could have undetected PC. CT of the abdomen was not routinely done until the last few years of the study.

There are few reports on the true incidence of synchronous PC in unselected, prospective series. Some investigate both patients with synchronous and metachronous PC[345, 346]. The retrospective series from Jayne et al.[347] report 7% PC at time of diagnosis, in according with our findings.

Our study revealed some new and interesting findings. Firstly, there was a higher incidence of PC in tumours located in the right colon. One possible explanation for this could be the differences in tumour genetics[39, 266, 268, 269] between right- and left-sided cancers, which could contribute do different patterns of spread. Other studies on the incidence of PC in relation to location of the primary tumour have not been presented until recently; one author reported higher incidence of PC among right sided

tumours (univariate analysis)[348], and another showed higher rate of patients with multiple peritoneal metastases in right sided tumours[72]. The study by Meguid[272] showed poorer prognosis in right sided tumours, but association with PC was not analysed.

Secondly, females had higher risk of PC than men, as demonstrated in both univariate and multivariate analyses. Gender related incidence is scarcely described in the literature. In a recent retrospective series from Japan of more than 2000 patients who all underwent surgery for colon or rectal cancer, there was no difference between genders[72]. The association between PC and female gender might be confounded by other, unknown factors, and has to be confirmed in future studies.

Furthermore, our study showed that PC patients were younger than the others (median age 71 versus 75 years, respectively). The corresponding figures for *mean* age was 69 and 73 years (P=0.002). Mean age was 59 years[346] and 62 years[345] in two selected, small series of patients with mix of synchronous and metachronous PC. A recent study of similar size as the present (Non-PC: n=975, PC: n=75), report that PC patients were significant younger than non-PC patients in univariate analysis[348]. Strength of our study is the inclusion of all patients with a diagnosis of colon cancer from a defined population, irrespective of the treatment given. The reason why PC is more frequent in younger patients might be that younger patients have tumours with more aggressive biology.

Survival

Median survival in PC patients was 9 months in the present study, slightly higher than other reports of median five to seven months[345-347]. Improved survival has been reported in patients with limited spread, treated with complete removal of all visible tumour tissue combined with intra-abdominal chemotherapy[349]. The study by Pestieau[350] showed median survival 24 months and five year survival (actuarial) of 30% in patients with complete cytoreduction. In the present study, none of the patients received HIPEC treatment, but several of them had palliative chemotherapy. 13 patients (14% of all patients with PC) had limited/single spread to the peritoneum, which could be removed radically, and survival in these patients was median 31 months. These figures correspond to the findings of Sadahiro[348], who report median survival of 21 months in patients with limited PC and no LN involvement. The variations in survival illustrates a need of clear definitions of PC to make reliable comparisons of outcome analyses, and a focus should be held on patients with limited PC, who could be cured by surgery.

***TP53* mutations:** Mutation in *TP53* gene was significantly more frequent in patients with peritoneal carcinomatosis than in those without signs of PC. This finding is in accordance with another report[271], and supported by association between *TP53* gene products and PC[351]. However, when comparing *TP53* mutation status in PC patients and non-PC patients with distant metastases in other locations, no difference was found. The mutation rate was approximately 50%, which correspond with figures from

other authors[54, 56]. This might indicate that *TP53* mutations are associated with advanced disease rather than PC itself.

The distribution of *TP53* mutation types within exons five to eight compared with the literature is shown in Figure 9; both frequencies and types of mutation in PC patients are in accordance with multicentre non-PC patients[261], and colorectal cancers in the IARC database[352], supporting our findings.

TP53 gene mutations are common in CRC, and known to be associated with both CIN tumours and distal location of the tumour (see 'Introduction'). Association with tumour location was not found in our study, but in left sided cancers, mutation was more prevalent in men than in women. This is to our knowledge not reported earlier.

A negative prognostic impact of *TP53* mutations is reported in two meta-analyses comprising 68 studies[129, 353]. Data from a large multicentre study indicate that the prognostic significance is dependent on both tumour location (proximal versus distal colon), and location of the mutations[56].

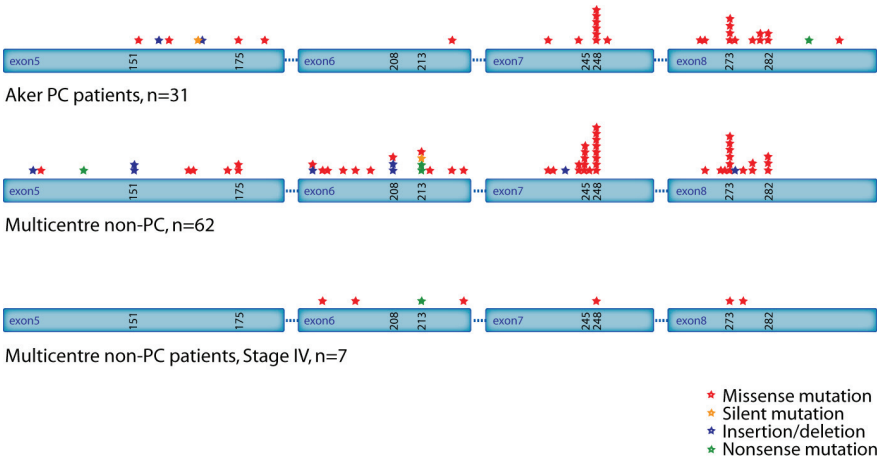

Figure 9: **Schematic overview of codon distribution and type of *TP53* mutations in exons 5 to 8.** The upper panel shows the distribution of mutations in Aker patients with peritoneal carcinomatosis (PC), the panel in the middle is the multicentre non-PC patients and the lower panel is stage IV tumours from the multicentre non-PC patients (Paper 4). Hot-spot codons known from the literature to be mutated are indicated with codon number.

Conclusions

The present studies have revealed an independent prognostic impact of several clinical and histopathological factors that formerly have been debated:

- Tumour location in the splenic flexure and descending colon was associated with reduced survival due to low resection rates. Following major resection with curative intent, tumour location in the transverse colon, splenic flexure and descending colon was an independent negative prognostic factor.

- In stage I/II patients, the presence of micrometastases or isolated tumour cells in the regional lymph nodes has a significant negative prognostic impact. The prognosis in patients with ITC is probably similar to patients with MM, and not much different from the prognosis in stage III patients.

- In patients who underwent curative resection, the lymph node harvest increased during the study period. The most likely explanation is improvement of the pathological examination of the specimens.

- Evaluation of more regional lymph nodes was associated with stage migration, with upstaging of stage I-II cancers to stage III

- Evaluation of more lymph nodes was associated with improved survival in stage I-III patients. This is probably partly explained by stage migration and Will-Rogers phenomenon, an example of classification bias due to better diagnostics

- Overall survival in all admitted patients improved during the study period, showing that other factors than stage migration contributed, and indicate a true improvement during the period

- In stage III patients, LNR was a stronger prognostic factor than the total number of examined lymph nodes. Stage III patients with LNR < 0.11 have a favourable prognosis

- Perioperative blood transfusion of three or more units had a negative prognostic impact

The studies also revealed the novel findings that peritoneal carcinomatosis of colon cancer origin is most frequent in women, and in patients with tumour in the right colon. The PC patients are slightly younger than non-PC patients.

Peritoneal carcinomatosis is independently associated with mutations in the *TP53* tumour suppressor gene.

Other prognostic factors that were well established when our studies started, have been confirmed:

Advancing TNM stage and R-stage, and emergency operation is associated with high postoperative mortality and complication rates and reduced long time survival. Hartmann's procedure and surgical treatment without resection was associated with very high mortality, probably due to patient selection. Emergency operation should be avoided if possible.

Future studies / perspectives

The list of well-established prognostic factors and of factors which probably have prognostic impact is long, including a variety of clinical, histopathological and molecular / genetic factors. Future studies are therefore demanding, because large studies are needed to define the effect of new factors while adjusting for other, known factors.

Two new developments in surgical technique need further evaluation; the technique of complete mesocolic excision and the use of laparoscopic surgery. To evaluate outcome of these techniques, adjustment for prognostic variables is necessary. Great focus on improvement of the surgical technique is important, and specialised colorectal surgeons taking part in all operations, including emergency operations, should contribute to improve results.

Future studies should ideally be population based to avoid selection bias; if not, comprehensive information on patient selection is obligatory. Analyses should be carried out based on consensus of endpoint definitions, which is mandatory for meaningful comparisons of results. Several hypotheses should be tested in randomized trials.

Many of the findings in the present study may have impact on the clinical management of colon cancer patients, but need confirmation in future studies:

- Most stage II patients do not receive adjuvant therapy, but some 20% - 30% will have a recurrence. Investigations with regard to MM/ITC seems to be a promising tool for identification of patients at high risk of recurrence, and who might gain benefit of adjuvant chemotherapy. A randomised controlled trial (RCT) would be necessary to test this.

- About half of stage III patients are cured by surgery alone. A low lymph node ratio indicates a favourable prognosis. Patients with relative contra-indications to the use of AT who have a low LNR, should probably not be treated by AT. Fit patients with low LNR could enter a RCT to investigate a potential survival benefit of AT. The optimal cut-off value for a “low” LNR needs to be established. The potential prognostic value of MM/ITC in stage III patients with low LNR should be studied to identify patients with less favourable prognosis in this group.

- Future studies of the prognostic impact of the total number of examined lymph nodes should be stratified according to subsites of the tumours, as recent data suggests that tumour properties and number of nodes varies with different locations.

High quality surgery and high quality pathological examination are preconditions for correct staging and correct interpretation of results from the studies proposed above.

- Standardisation of methods used to identify MM/ITC would be an advantage.
- There is still not agreement on how many lymph nodes must be examined for correct staging and optimal prognostication
- Our finding of the prognostic impact of tumour location needs confirmation. The surgery for CC should be standardised; however, we think that tumours at certain 'difficult' locations (like the splenic flexure) need special attention.
- Emergency operation is still a challenge, and should be avoided if possible. Earlier diagnosis through better public information and screening programs, and avoidance of patient and doctor delay, might contribute to reduce the number of emergency operations. The use of endoluminal stents for immediate decompression in obstructing cancer needs further evaluation.

Extensive research is going on worldwide in order to investigate the prognostic and predictive value of biomolecular / genetic markers. We hope to contribute through our translational research program, including investigations of ploidy, microsatellite instability, protein markers, gene signatures and transcriptional profiling.

In the future, staging of the patients should be based on more extensive systems based on clinical, histopathological and biomolecular markers with prognostic impact. A more exact prognostication is necessary for optimal treatment and follow up of the individual patient.

Improvements in preoperative staging methods, the possible use of neo-adjuvant therapy to some patients, the use of more sophisticated prognostic tools and patient tailored treatment implies that the treatment of colon cancer patients will be a multidisciplinary task in the future.

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