Improving surgery and prognostication in right-sided colon cancer

Kristin Bentung Lygre

Thesis for the degree of Philosophiae Doctor (PhD)
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Abbreviations

AC	After Christ
ACT	Adjuvant chemotherapy
AJCC	American Joint Committee on Cancer
AMC	Adenocarcinoma with mucinous component
APC	Adenomatous polyposis coli
ASA	American Society of Anaesthesiologists' classification of physical status
BC	Before Christ
BMI	Body mass index
BRAF	B-Raf proto-oncogene, serine/threonine kinase
C-D	Clavien-Dindo
CE	Contrast enhanced
CEA	Carcinoembryonic antigen
CI	Confidence interval
CIMP+	CpG island methylator phenotype
CME	Complete mesocolic excision
CMS	Consensus molecular subtypes
CpG	5'-Cytocine-phosphate-Guanine-3' (cytocine preceding guanine)
CT	Computed tomography
CTC	Circulating tumour cells
CVL	Central vascular ligation
cfDNA	Cell-free DNA
ctDNA	Circulating tumour DNA

DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
F	Female
FAP	Familiar adenomatous polyposis
GEWF	Glacial acetic acid, ethanol, water, formaldehyde
HDH	Haraldsplass Deaconess Hospital
HNPCC	Hereditary non-polyposis colon carcinoma
HRQoL	Health related quality of life
HR	Hazard ratio
HUH	Haukeland University Hospital
IV	Intra-venous
KRAS	Kirsten rat sarcoma viral oncogene homolog
LN	Lymph node
LOS	Length of stay
M	Male
MDT	Multidisciplinary teams
MMR	Mismatch repair
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
MSS	Microsatellite stabile
MUC	Mucinous adenocarcinoma

NGS	Next generation sequencing
NRAS	Neuroblastoma rat sarcoma viral oncogene homolog
OR	Odds ratio
OS	Overall survival
PCR	Polymerase chain reaction
POI	Postoperative ileus
QoL	Quality of life
RAS	Rat sarcoma virus
RAF	Rapidly accelerated fibrosarcoma
RBC	Red blood cells
RCC	Right-sided colon cancer
RCT	Randomized controlled trial
REK	Regional committee of ethics
RFS	Recurrence free survival
SMA	Superior mesenteric artery
SMV	Superior mesenteric vein
TIVA	Total intravenous anaesthesia
TNM	Tumour- Node –Metastasis
TME	Total mesorectal excision
TP53	Tumour protein 53
UICC	Union for International Cancer Control
WMA	World Medical Association
WHO	World Health Organization

Scientific environment

The project “Open D3 hemicolectomy compared to laparoscopic CME right hemicolectomy for right-sided colon cancer” from which this thesis originates have been conducted at Department of Surgery, Haraldsplass Deaconess Hospital and Department of Gastrointestinal Surgery, Haukeland University Hospital. The candidate has been admitted to the Ph. D programme at the Department of Clinical Medicine (K1), Faculty of Medicine, University of Bergen. The Liquid biopsy-project has been conducted in collaboration with Department of Medicine and Section for Cancer Genomics, Haukeland University Hospital, Bergen.

Acknowledgements

My interest in colon cancer arouse in 2009 when I started the gastrointestinal part of my surgical training at the department of gastrointestinal surgery at Haraldsplass. When I was introduced to laparoscopy as a student, I believed that the introduction of minimally invasive surgery was a disaster for the surgeons. Eventually, Kristian Storli taught me the art of laparoscopy, and I was conquered. The previous colon cancer project at Haraldsplass, initiated by late professor Karl Søndenaa, made data-collection an integral part of my clinical work. Still, it was the clinical aspects of work I prioritized until I finished my surgical training and became consultant surgeon. Having achieved that milestone, I was ready for new challenges and addition of an academic dimension to my working life. I first knocked at professor Viste's door. He sent me next door to Professor Frank Pfeffer, who had an idea of the perfect project for me. A randomized study comparing open and laparoscopic colon cancer surgery for right-sided colon cancer. To start a project that was so integral in my clinical work, was like winning a lottery. Inspired by Karl's project we also asked permission to collect biological tissue for further analyses without actually knowing what to look for. It was not until Lars Thomas Seeberg encouraged me to read about liquid biopsies and circulating tumor cells that an idea started to form. The random meeting of Professor Bjørn Tore Gjertsen at the watch shop sent me further into tumor biology and circulating tumor DNA. This was the start of the collaboration on the "Liquid biopsy"-study. The two dimensions of this project are a great strength. The collaboration of molecular biologists with their excellent and detailed knowledge of the basics and the genetic landscape and surgeons with their overview of the real world and clinical realities are priceless.

This project has been an endless series of logistics. Especially the coordination of sampling and preparation of samples from two hospitals have been a challenge. It would not have been possible without Trude Høysether. Her overview and systematics are invaluable. Biobank Haukeland have always been positive and dynamic. The staff at the laboratory at Haukeland and Haraldsplass, at the Department of Oncology and even at Voss have been cooperative and planned for good logistics. A great thanks to

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Inger Marie Løes, thank you for your positivism and for helping me keep track of patients receiving oncologic treatment. The data collection for patients receiving chemotherapy was a breeze with your contribution. Professor Olav Dahl, your immense knowledge and concern for this project have been of great value. I appreciate that you have introduced me to Nordic capacities in biomarkers and colleagues working on adjacent projects. Idun Augland and Marjolein Liedenbaum, thank you for spending time performing repeated measurements. Ingfrid Haldorsen, thank you for introducing us to inspiring colleagues and for taking interest in our passion for aspects of right-sided colon cancer. Bjørn Tore Gjertsen, thank you for your inspiring and optimistic attitude. You are an expert in connecting and facilitating collaborations. This project owes you a great thanks for seeing opportunities and connecting the right people.

Rakel Brendsdal Forthun have performed most of the genetic analysis. This project would not have been possible without your effort. Randi Hovland, thank you for teaching me so much of genetics and analysis together with Rakel. You have opened a whole new world for me. I look forward to further collaboration. Professor Geir Egil Eide, your contribution to this project has been invaluable. You have led me steady through formalities all the way from writing the initial protocol, through all applications for grants and trough analysis and writing of the three papers in this thesis. I will miss your guidance, your accuracy and attention to details in my further research. Håvard M. Forsmo, Aly Dicko, Frank Pfeffer, Kristian E. Storli, and Maria Decap; thank you for adhering to protocol and for performing safe and steady surgery for the patients included in this project. Håvard, I appreciate your continuous support and contribution to the project all the way from the initial idea through inclusion, treatment, and publishing. Kristian, my gratitude to you for teaching me the art of laparoscopy is endless. Your geeky interest in laparoscopic colon cancer surgery and your openness to change and improvement are inspiring. Frank, I love working with you. You are the best supervisor and mentor I could have wished for. I am glad we have further projects ahead.

To all the patients who have been willing to contribute to research by participating in this project; thank you. It would not have been possible without you. It is a privilege to meet you and be allowed to ask for your contribution when you are in a vulnerable position. I am impressed and grateful of your positivism and desire to contribute to new knowledge.

Last, but not least to my always supporting and listening parents, Gunvor and Henning. Thank you for encouragement, love, and support. To my children and greatest pride, Karoline, Eirik, Torstein and Ingrid; thank you for being just who you are and maintain chaos and joy. Øystein, the man of my dreams. Thank you for your patience and never-ending support, I love you.

Bergen, January 2024

Kristin Bentung Lygre

Sammendrag

Bakgrunn

Kreft i tykktarm er blant de vanligste kreftformene i Norge. Kirurgi er viktigste behandling for tykktarmskreft uten spredning, men har ikke gjennomgått samme forbedringer som behandling for endetarmskreft. En faktor som bidro til bedre overlevelse for endetarmskreft var standardisering av og mer radikal kirurgi. Det er på tide at behandling for tykktarmkreft gjennomgår tilsvarende forbedringer. Forskjeller i anatomi, biologi og prognose relatert til svulstens lokalisasjon i tykktarmen førte søkelyset til høyresidig tarmkreft. Kirurgi for proksimale svulster er teknisk vanskelig i tillegg til at de har en særegen biologi og dårlig prognose. Kjennskap til svulstens biologi er nødvendig for persontilpasset kreftbehandling, og inspirerte til en studie med flytende biopsi og analyse av sirkulerende DNA fra svulsten (ctDNA).

Formål

Målet med studien var å sammenlikne komplikasjoner etter radial kirurgi hos pasienter operert med åpen- eller kikkhullsoperasjon for høyresidig tykktarmskreft. Spesifikke komplikasjoner ble også undersøkt. Lengden på gjenværende karstump etter reseksjon ble vurdert som et mål på kvalitet. Målet med biomarkørstudien var å undersøke om ctDNA kan forutsi tilbakefall.

Metode

I studien ble 128 pasienter randomisert til åpen- eller kikkhulls-operasjon. Kirurgien ble utført standardisert og med mål om å fjerne de sentrale lymfeknutene i operasjonsområdet. De første 40 pasientene, 20 fra hver gruppe, ble undersøkt med måling av gjenværende stump fra blodkaret etter operasjon. Målingen ble utført på CT tatt 6 måneder etter operasjon, og ble utført to ganger av to uavhengige radiologer. I den prospektive observasjonsstudien for biomarkører ble 50 pasienter undersøkt med neste generasjons sekvensering og digital dråpe PCR (ddPCR) for kreftrelaterte mutasjoner i svulst og blod.

Resultat

Det var ingen forskjeller i komplikasjoner mellom de to gruppene. Det var ingen livstruende komplikasjoner og få alvorlige komplikasjoner med 8 % i den åpne gruppen og 5 % i kikkhulls-gruppen. Ingen pasienter ble reoperert for lekkasje i tarmskjøten. Blodoverføring eller infusjon av jern var den vanligste komplikasjonen (22 % åpen vs. 15 % kikkhull). Postoperativ tarmparalyse var den nest vanligste komplikasjonen, og ble registret hos 16 % operert åpent og 19 % operert med kikkhull. Det ble i gjennomsnitt fjernet like mange lymfeknuter ved åpen operasjon (n=32) som ved kikkhullsoperasjon (n=29). Gjenværende karstump var kort og lik i begge grupper (4mm). Det var godt samsvar mellom målingene til hver radiolog, mens det var forskjeller mellom de to. Forskjellene forekom i den åpne gruppen hvor det ikke var markør på karet som var delt. Kreftrelaterte mutasjoner ble funnet hos 49/50 pasienter, og 47 av disse var mulig å følge med ddPCR. ctDNA kunne påvises hos 31/47 pasienter før operasjon, og ble redusert etter operasjon hos 27/31. Risiko for tilbakefall var forhøyet hos pasienter hvor ctDNA kunne påvises etter operasjon (justert hazard ratio: 173).

Konklusjon/implikasjoner

Høyresidig kolektomi med sentral lymfeknutedisseksjon foran vena mesenterica superior er en anvendelig, trygg og reproduserbar metode som kan bli fremtidig standard ved høyresidig tykktarmskreft. Lengden av gjenværende karstump kan være kvalitetsmarkør for omfang av lymfeknutedisseksjon. ctDNA kan forutsi tidlig tilbakefall ved høyresidig tykktarmskreft.

Abstract

Background

The incidence of colon cancer is high in Norway. Surgery is the mainstay in the treatment of colon cancer but has not undergone the same improvements as rectal cancer. Standardisation of and more radical surgery was one of the measures that lead to increased survival for rectal cancer. It is time for colon cancer to undergo the same improvements. Differences in tumour biology, anatomy and prognosis related to

localisation of the tumour in colon, led to focus on right-sided colon cancer. The proximal tumours have a distinct tumour biology with poor prognosis in addition to technical demanding surgery. Knowledge of tumour biology is necessary to personalize cancer treatment and inspired to launch a project with liquid biopsy and analysis of circulating tumour DNA (ctDNA).

Aim

The aim of the study was to compare the differences in complications after radical surgery between open and laparoscopic surgery for right-sided colon cancer. Specific complications were also explored. Surgical quality was evaluated by comparing length of remaining vascular stump length after resection. The aim of the biomarker study was to investigate ctDNA as a predictor of recurrence.

Method

In the trial, 128 patients were randomised to receive either open or laparoscopic colectomy. The surgery was standardized and focused on central lymphadenectomy. The first 40 patients, 20 in each group were explored for length of remaining vascular stump length after resection by measurements in computed tomography 6 months postoperative. The measurements were conducted twice by two independent radiologists. In the prospective observational biomarker study 50 patients were explored by next generation sequencing and digital droplet PCR (ddPCR) for cancer related mutations in tumour and blood.

Results

There was no difference in complications between the two groups. There were no life-threatening complications and a low incidence of serious complications with 8 % in the open group and 5 % in the laparoscopic group. No patients were reoperated due to anastomotic leaks. Transfusion or infusion of i.v iron was the most common complication (22 % open vs 15 % laparoscopic). Postoperative ileus was the second most common complications and occurred in 16 % after open and 19 % after laparoscopic surgery. Equal number of mean lymph nodes was removed in open (n=32) and laparoscopic (n=29) group. The remaining vascular stump length after resection was short and equal in the two groups (4 mm). Interclass correlation for each

observer was good but interclass correlation between observers was poor. The discrepancy occurred in the open group where no marker was present at the vessel stump. Cancer related mutations was detected in 49/50 patients of which 47 available for surveillance with ddPCR assays. ctDNA was present in liquid biopsy prior to surgery in 31/47 patients and was reduced after surgery in 27/31 patients. Risk for recurrence was elevated in the patients with postoperative positive ctDNA (adjusted hazard ratio: 173).

Conclusion/implications

Right-sided colectomy with central lymphadenectomy along superior mesenteric vein is applicable, safe, and reproducible and can be the future standard for right-sided colon cancer. Vascular stump length can be a quality marker for central lymph node dissection. ctDNA is a strong predictor for early recurrence in right sided colon cancer and must be further explored in clinical trials to establish its role in routine diagnostics.

List of papers

Complications after open and laparoscopic right-sided colectomy with central lymphadenectomy for colon cancer: randomized controlled trial

Kristin B Lygre, Geir E Eide, Havard M Forsmo, Aly Dicko, Kristian E Storli, Frank Pfeffer

BJS Open, Volume 7, Issue 4, August 2023, zrad074, https://doi.org/10.1093/bjsopen/zrad074

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Short and equal vascular stump length after standardized laparoscopic and open surgery with central lymphadenectomy for right-sided colon cancer

Kristin B Lygre, Geir E Eide, Marjolein H Liedenbaum, Idun M B Augland, Ingfrid S Haldorsen, Frank Pfeffer

British Journal of Surgery, Volume 111, Issue 1, January 2024, znad410, https://doi.org/10.1093/bjs/znad410

Published: 08 December 2023 **Received:** 09 November 2023 **Revision received:** 17 November 2023 **Accepted:** 24 November 2023

Assessment of postoperative circulating tumour DNA to predict early recurrence in patients with stage I–III right-sided colon cancer: prospective observational study

Kristin B Lygre, Rakel B Forthun, Trude Høysæter, Sigrun M Hjelle, Geir E Eide, Bjørn T Gjertsen, Frank Pfeffer, Randi Hovland

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1. Introduction

Focus on preoperative staging, standardized surgical treatment and introduction of neoadjuvant radio-chemotherapy led to improved survival for rectal cancer. At the same time colon cancer did not experience the same focus or measures. It is time for colon cancer to undergo a corresponding quality improvement. The extent of central lymphadenectomy in right-sided colon cancer varies. There is no consensus or definition of the medial border of the right colon's mesentery. Terms are used interchangeably due to ambiguously defined terminology. There is a need for standardization of the surgical approach to oncologic right-sided colectomy with focus on the central lymphadenectomy. With increasing radicality it is crucial to perform the surgery safely and with low risk of major complications. The aim when designing this project, was to improve outcome of right-sided colon cancer by standardization of the surgical technique and central lymphadenectomy. The setup with randomization between two hospitals and different surgical approaches will harbour competitive elements, which will benefit the patients. Although the focus is on surgery, this study will also inspire colleagues at the departments of radiology and pathology and eventually lead to increased quality of the perioperative diagnostics. The prospective registration leads to increased awareness of clinical outcomes and launches opportunities for improvement. This project is also important for the cooperation between the two hospitals and their common treatment chain for colon cancer patients. Our belief that knowledge of tumour biology is essential for prognosis has led to a valuable interdisciplinary collaboration in translational medicine with analysis of circulating tumour DNA (ctDNA) in this cohort of non-metastatic colon cancer.

1.1 Epidemiology and pathogenesis of colon cancer

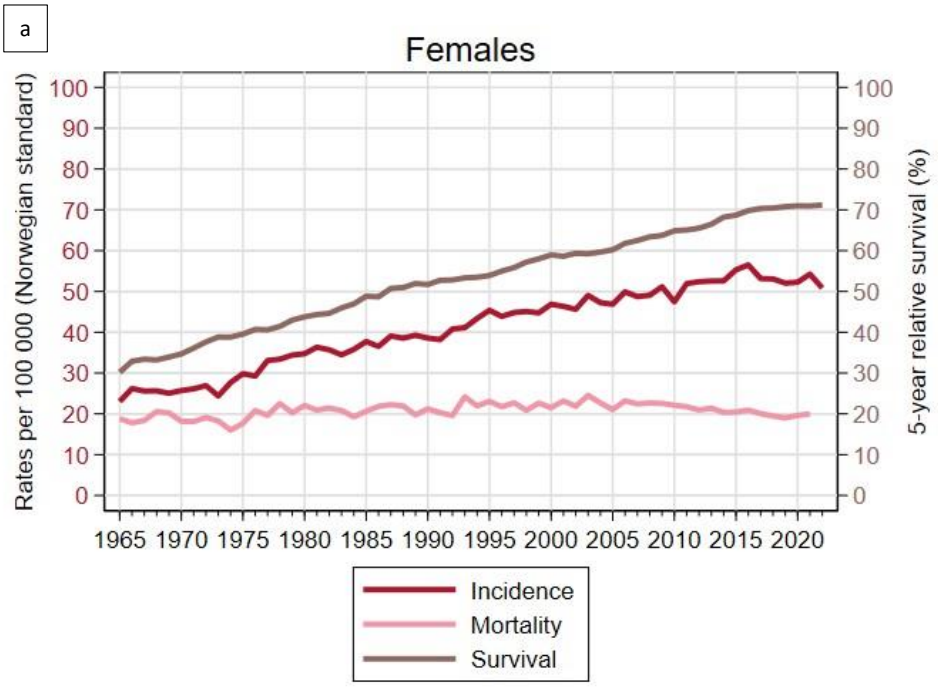
The definition of colon cancer is adenocarcinoma originating from the mucosa of colon. Colorectal cancer is the most common cancer in Europe and the third most common cancer in the world. Colon cancer causes a huge burden of disease with 1.15 mill new cases diagnosed per year and 577 000 deaths annually (2020) (1).

1.1.1 Incidence in Norway

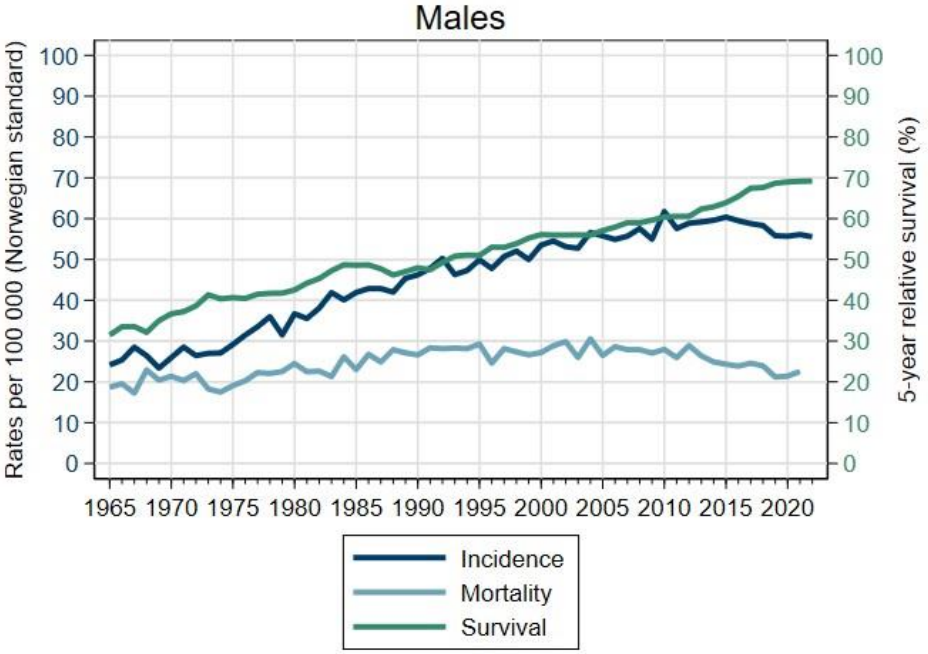
The incidence of colon cancer in Norway today is amongst the highest in the world. It remains the second most common cause of cancer-related deaths both national and global (2).

Figure 1: Trends in incidence, mortality and survival in colon cancer in Norway from 1965 to 2020 for females (a) and males (b) respectively

Reprinted with permission. Reference: *Cancer Registry of Norway. Cancer in Norway 2022 - Cancer incidence, mortality, survival, and prevalence in Norway. Oslo: Cancer Registry of Norway, 2023.*



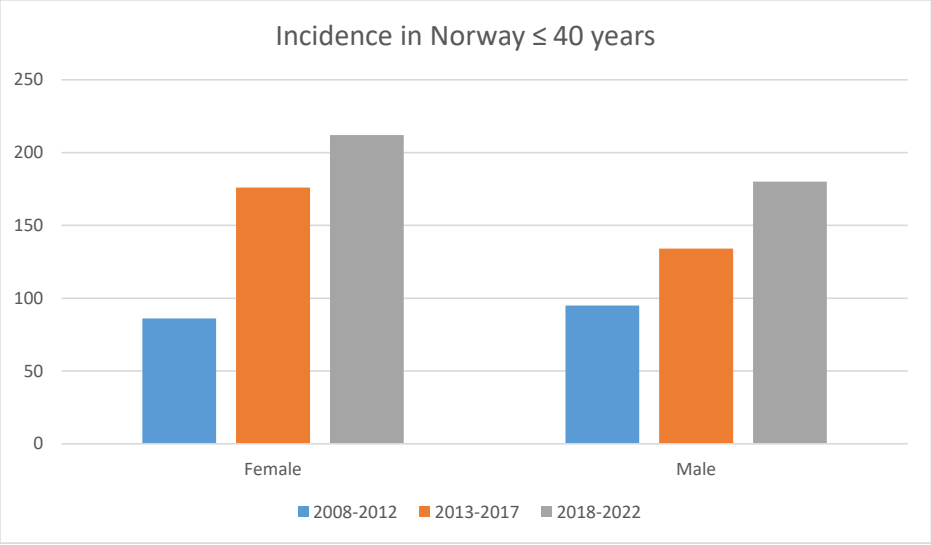
b



In 2018-2022 there was a decrease in colon cancer incidence in Norway for both men and women (-5.5 % M and -2.9 % F) after years with increasing incidence. Rectal cancer has experienced declining incidence for decades. Almost 3500 patients are diagnosed with colon cancer in Norway each year. More than 1200 patients died due to colon cancer in 2021. Five-year relative survival (RS) for colon cancer is 70 %, and the five-year relative survival rates for regional and localized cancer are 84 % and 97 %, respectively (2). Ninety % of the recurrences present within the first five years, and most of them occur during the first three years (3). In total 30-50 % of colon cancer patients will experience recurrent disease. About 25 % of these patients were initially diagnosed as colon cancer stage I or II (4). The median age at diagnosis of colon cancer in Norway is 74 years. The risk of colon cancer increases with advancing age. The incidence for patients under 40 years of age is increasing in Norway and other high-income countries such as US, UK, Denmark, Canada, Australia, and New Zealand (5-9). Colon cancer in the lower age groups has traditionally been related to

inherited predispositions, but the increase under 40 years also includes sporadic colon cancer.

Figure 2 The incidence for colon cancer in females and males ≤ 40 years/5-year period in Norway from 2008 to 2022. *Based on numbers from the Cancer Registry of Norway*



1.1.2 Risk factors

Lifestyle and dietary habits are recognized risk factors for colon cancer. Abundant alcohol intake, high intake of red and processed meat, smoke and obesity leads to increased risk. Type 2 diabetes is an independent risk factor for colorectal cancer (10, 11). Physical activity seems to protect and so does abundant intake of dietary fibre (12). The Western high-fat, low-fibre diet induces inflammation and proliferation in the colon mucosa (13). Long-standing inflammatory bowel disease (IBD) with colitis increases the risk for colon cancer due to the impact of inflammation on the colon mucosa (14). The common feature of the risk factors is that they create a genotoxic environment, which in turn increases genetic alterations and thus facilitates cancer formation.

1.1.3 Symptoms

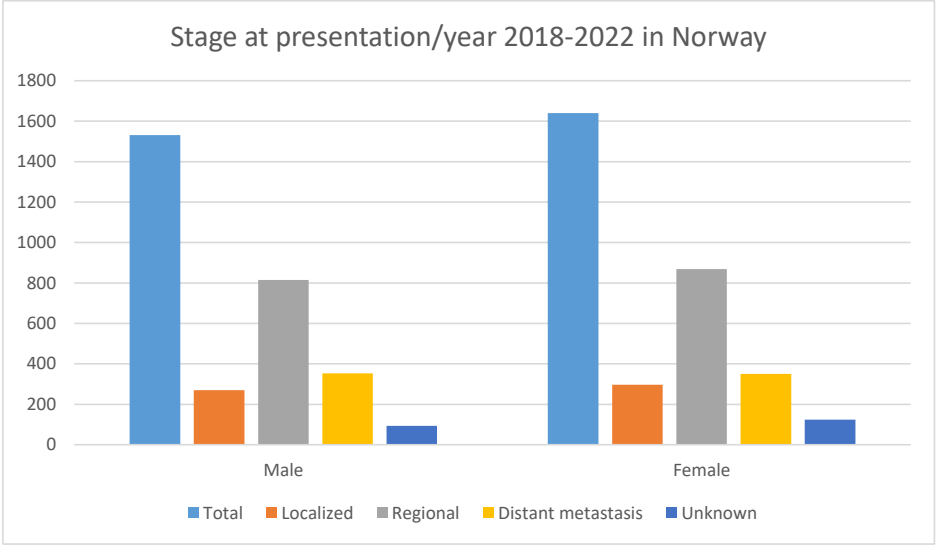
The symptoms of colon cancer vary. Tumours of the left colon are more commonly annular or encircling lesions, which will result in narrowing of the lumen and altered

bowel movements. The more distal the tumour is the more likely a presentation with rectal bleeding and altered bowel habits as diarrhoea, constipation, and narrowing of the stool. Between 25-40 % of adenocarcinoma in the colon appear in the right colon (15, 16). Proximal tumours present more diffuse, and often without abdominal specific symptoms. Anaemia is the presenting symptom of 60-75 % of patients with tumours in the coecum or ascending colon. The unspecific symptoms of tumours of the proximal colon can lead to delayed diagnosis and more advanced tumour stages (17).

1.1.4 Staging and risk assessment

Colon cancer is staged according to the Tumour Node Metastasis (TNM) -system of the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC), introduced in 1978. The staging is dependent on the pathological and clinical interpretation of the anatomical extent of the patient's disease.

Figure 3 The stage at presentation/year for colon cancer in females and males in Norway 2018-2022. *Based on numbers from the Cancer Registry of Norway*



The three components are T – the local extent of primary tumour, N – the extent of regional lymph node metastases, and M – the absence or presence of distant metastases. Prognosis is dependent on the stage at diagnosis with the best prognosis for localized disease. Surgery is the main treatment for non-metastatic colon cancer

with or without the addition of systemic chemotherapy. TNM allows prediction of prognosis on a population level, not for the individual patient. Treatment recommendations are currently based on the risk stratification by TNM staging.

Table 1 TNM classification and subcategories with explanation of criteria and corresponding stages for colon cancer

N-category	**T-category**				
	T1	T2	T3	T4a	T4b
N0	I	I	II	II	II
N1a	III	III	III	III	III
N1b	III	III	III	III	III
N1c	III	III	III	III	III
N2a	III	III	III	III	III
N2b	III	III	III	III	III

T-category	T-criteria
T1	Tumour invades the submucosa (through the muscularis mucosa but not into the muscularis propria)
T2	Tumour invades muscularis propria
T3	Tumour invades through the muscularis propria into the peri-colorectal tissues
T4	Tumour invades the visceral peritoneum or invades or adheres to adjacent organ or structures
T4a	Tumour invades through the visceral peritoneum (including gross perforation of the bowel through areas of inflammation to the surface of the visceral peritoneum
T4b	Tumour directly invades or is adherent to other organs or structures

N-category	N-criteria
N1	Metastasis to 1-3 regional lymph nodes
N1a	Metastasis to 1 regional lymph node
N1b	Metastasis to 2-3 regional lymph nodes
N1c	Tumour deposits in subserosa, mesentery of non-peritonealised, pericolic or perirectal/mesorectal tissues without regional nodal metastasis
N2	Metastasis to 4 or more lymph nodes
N2a	Metastasis in 4-6 regional lymph nodes

N2b	Metastasis in 7 or more regional lymph nodes

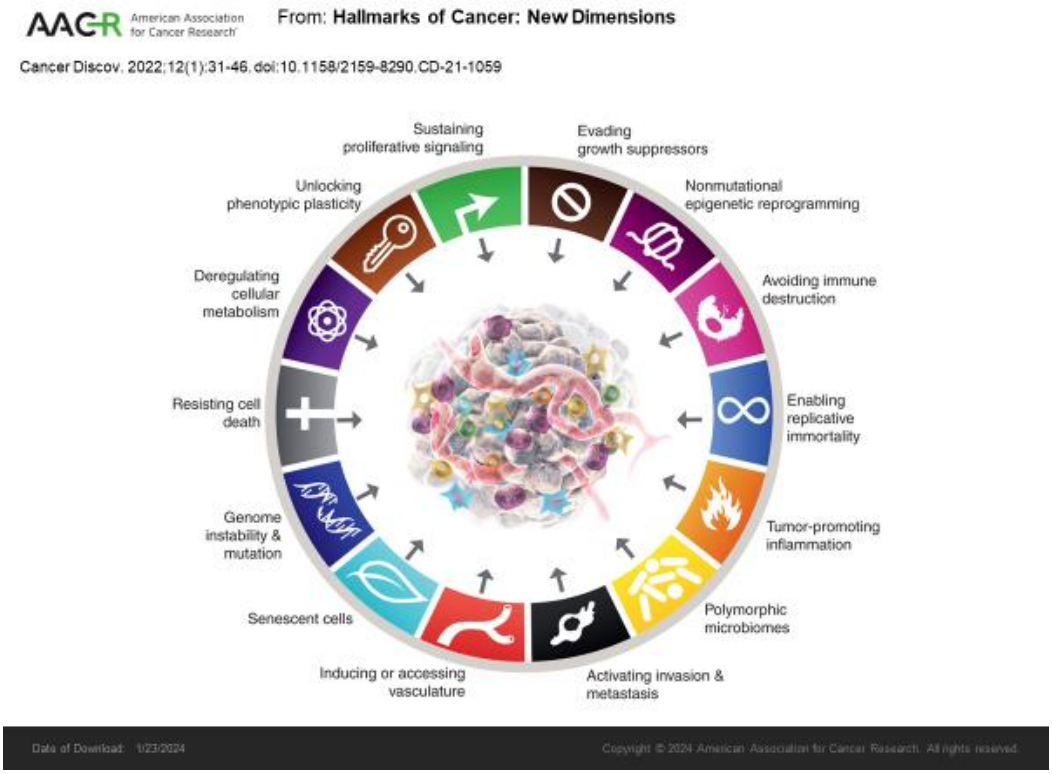
M-category	M-Criteria
M0	No distant metastasis by imaging
M1	Metastasis to one or more distant sites or organs or peritoneal metastasis identified
M1a	Metastasis to one site or organ identified without peritoneal metastases
M1b	Metastasis to two or more sites or organs identified without peritoneal metastases
M1c	Metastasis to the peritoneal surface is identified alone or with another site or organ metastases

STAGE I
LOW-RISK STAGE II
HIGH-RISK STAGE II
STAGE III

1.1.5 Pathways to colon cancer

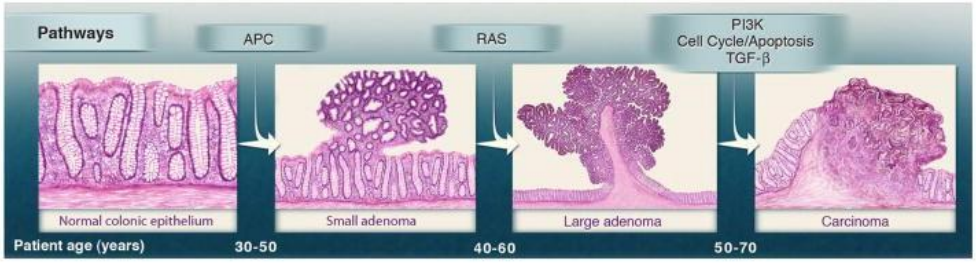
Except for congenital genetic defects (germ line mutations), predominantly represented by hereditary non-polyposis colon carcinoma (HNPCC) including Lynch syndrome and familiar adenomatous polyposis (FAP), the development of colon cancer takes decades. The mechanism is related to epi-genetic and genetic alterations and an accumulation of mutations with increasing age. Mutations occur frequently and are related to cell division. A mutation is only carcinogenic if it directly or indirectly causes a growth advantage in the cell in which it occurs. Activation of oncogenes or inactivation of tumour suppressor genes promotes net cell growth of the mutated cells. The sequential mutation of more than one gene is necessary for the development of cancer (18).

Figure 4 HALLMARKS of cancer with updated additions in 2022. Reprinted with personal permission from Hanahan D. *Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022 Jan;12(1):31-46. doi: 10.1158/2159-8290.CD-21-1059. PMID: 35022204.*



The selective growth advantage is the first step in cancer formation, often called "breakthrough phase". Additional driver-gene mutations lead to the "expansion phase" and formation of a benign tumour. The last step in cancer formation is the "invasive phase" in which additional driver-gene mutations are necessary to enable the cells to invade surrounding tissue (18). There are more than one pathway leading to the development of a malignant colon tumour. The most known is the adenoma-carcinoma pathway accounting for approximately 60 % of colon cancers. It was first described by Fearon and Vogelstein (19).

Figure 5 Adenoma-carcinoma pathway from *Bert Vogelstein et al., Cancer Genome Landscapes.Science**339**,1546-1558(2013).DOI:10.1126/science.1235122/ Reprinted with permission from AAAS*



The model proposes a sequence of mutations based on statistical analysis. However, this is a simplification. The accumulation of the mutations is more important than the order of appearance. The recognition that adenomas are not precursors of all cancers has led to the description of other pathways as for example the serrated pathway where broad-based serrated polyps are the initial lesion (20). They are often small lesions localized in the proximal colon. Due to their broad base and location in the thin-walled proximal colon, they are often difficult to eradicate endoscopically (21). They are characterized by mutations in tumour suppressor genes and lack the expression of mismatch repair genes (MMR) due to hypermethylation of promotor genes (22). The pathways to colon cancer are described with its own genetic alterations inducing the transformation from benign to malignant. Increased understanding has led to a more complex picture: these are overlapping and colon cancer cannot always be grouped into one or the other exclusively (23). There exists more than one genetic mean for carcinogenesis. The combinations of mutations are huge, but a limited number of genes play a crucial role in the carcinogenesis of colon cancers.

1.1.6 Tumour biology

Exploration and understanding of cancer biology is the premise for personalized cancer therapy. The discovery of biological markers leads to a perception of colon cancer as many sub-group diseases instead of a uniform disease. The development of colon cancer requires multiple steps and results from the progressive accumulation of

epi-genetic and genetic alterations, broadly classified in three groups: modulation of stability genes, inactivation of tumor suppressor genes and activation of oncogenes.

A simplified explanation of epi-genetic and genetic alterations in colon cancer follows:

1.1.6.1 Epi-genetic phenotypes

CpG island methylator phenotype (CIMP+) exhibits an epigenetic silencing of genes, characterized by simultaneous methylation of multiple CpG islands located in the promotor region of the genes. MMR genes can be amongst them. Hypermethylation of MMR genes lead to transcriptional silencing of MMR and thus microsatellite instability (MSI). Approximately 15 -20 % of colon cancers are CIMP+ (24, 25) and they are often located in the proximal colon, occur in elderly females, are poorly differentiated, and often discovered at a higher tumor stage. They exhibit a lower rate of p53 mutations (26) than CIMP- colon cancers. The combination of CIMP+ and MSS tumors, which is present in 20 %, exhibit a poor prognosis (27).

1.1.6.2 Mismatch repair genes

Stability genes act normally to keep genetic alterations to a minimum. They repair subtle mistakes frequently occurring during normal DNA replication. When they are inactivated, mutations occur at a higher rate. The mutations are not confined to oncogenes or tumor suppressor genes but affect all genes. Stability genes are represented by mismatch repair genes. Failure of the cells mismatch repair mechanism leads to genetic instability caused by accumulation of base-pair mismatch due to deletion or insertion of single base pair in repeated DNA sequences called microsatellites. The result is microsatellite instability. Approximately 15 % of colon cancers are MSI. MSI occurs in about 30 % of proximal cancers, but the incidence declines the more distally in the colon the tumor is presented. Less than 10 % of rectal cancers are MSI. HNPCC or Lynch syndrome is caused by germ line mutation in MMR genes, and these cancers are always MSI. For sporadic cancers with MSI, the silencing of MMR is caused by hypermethylation (12 %) (28). MSI can be labeled MSI-high (H) or MSI-low (L). MSI-H means instability in > 30 % of loci, MSI-L instability in 10-30 % of loci, although this sub-classification is rarely used in clinical practice. Microsatellite stabile (MSS) means no instability. MSI colon cancer have

improved survival compared to MSS. They do not benefit from traditional fluorouracil-based chemotherapy (29). MSI colon cancer responds to immunotherapy (30), currently only approved routinely for metastatic disease.

1.1.6.3 Tumour suppressor genes

APC- Adenomatous polyposis coli gene is a tumour suppressor gene. Wild-type APC controls how often the cell divides, how it attaches to other cells within a tissue, how the cell polarize and moves within or away from a tissue. The hereditary familiar adenomatous polyp syndrome (FAP) is caused by a germ-line mutation in the APC-gene. Somatic mutation of the APC is often the initial genetic mutation in a sporadic colon cancer and is described as the gateway-mutation in the adenoma-carcinoma pathway.

TP53-Tumor protein 53 is a tumour suppressor gene. It maintains genomic stability through control of cell cycle progression. It recognizes DNA-damage and induce cell cycle arrest and DNA repair or initiates apoptosis in response to genotoxic stress. It is called "the guardian of the genome". The p53 mutation type cancers occur in 40-60 % (31), and are more often left sided colon cancer (45 %) than right-sided colon cancer (RCC) (35 %). A p53 mutation is a negative prognostic factor (24). It is associated with lymphatic invasion in RCC (32). p53 mutation occurs late in the adenoma-carcinoma pathway.

1.1.6.4 Oncogenes

Two genes in the epidermal growth factor receptor (EGFR) signalling pathway are often involved in the development of colon cancer through driver gene mutations:

KRAS- Ki-ras2 Kirsten rat sarcoma viral oncogene homolog belongs to the RAS family in the EGFR/RAS/RAF signalling pathway. In wild-type KRAS, the K-ras protein functions as an on-off switch. Its role is to transduce stimuli from the cell surface through intracellular signalling cascade to induce cell proliferation and differentiation. Mutation in KRAS leads to stimulus-independent activation of intracellular signalling. This leads to cancer if the mutation occurs after APC mutation. 40 % of colon cancers have a mutated KRAS. It occurs more often in coecal than more

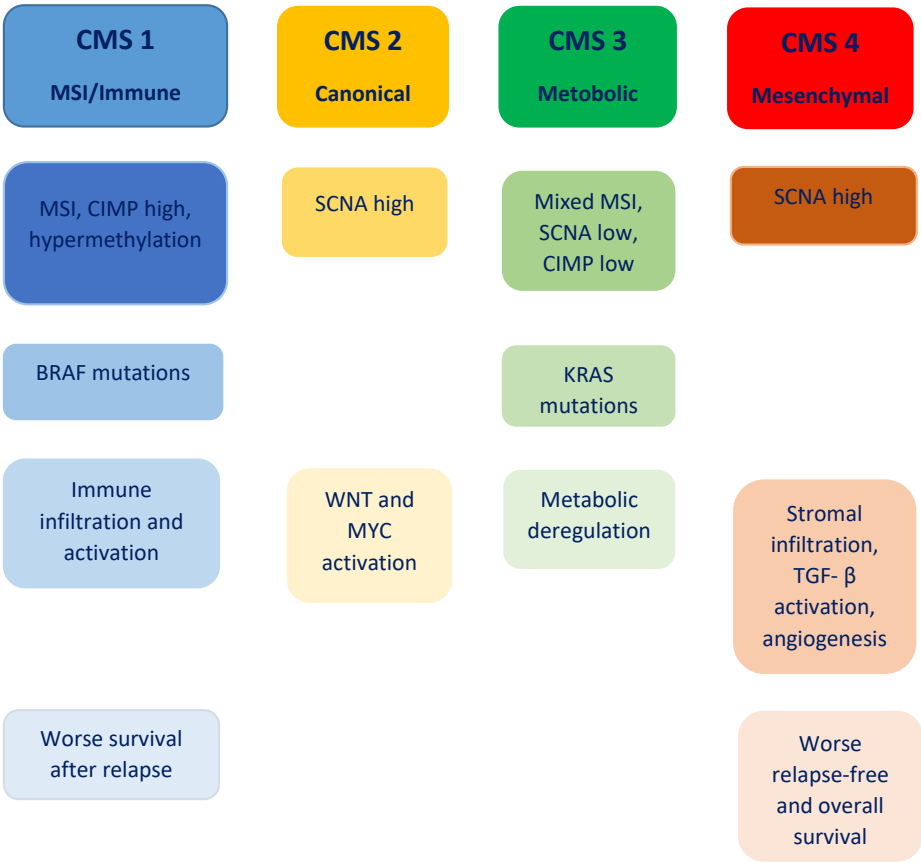
distal cancers. There are other genes coding for RAS-protein, for example NRAS, in which mutations occurs in 10 % of colon cancer. These mutations are mutually exclusively (33, 34). RAS-mutations are associated with resistance to EGFR inhibitor (cetuximab and panitumumab) therapy.

BRAF- B-Raf protooncogene belonging to the RAF kinase family. It is a downstream effector in the same signalling pathway as KRAS. Wild-type BRAF also functions as an on-off switch. It regulates the cell proliferation, differentiation, and migrations in addition to apoptosis. The overactive protein may contribute to the growth of cancers by allowing abnormal cells to grow and divide without external signals. Mutations in BRAF are mutually exclusively to mutations in RAS-genes. BRAF mutations occurs in 10 – 15 % of colon cancers. They are more frequently located in the proximal colon. Thirty-five to seventy percent of sporadic MSI cancer experience simultaneous BRAF mutation. EGFR-treatment is not efficient in BRAF-mutated tumours. The prognosis of BRAF mutated colon cancer in combination with MSS is especially poor.

1.1.6.5 Consensus molecular subtypes (CMS)

The mutational heterogeneity in colorectal cancer, varying immunogenicity, divergent response to systemic drug treatment and different outcomes lead to an international consortium agreeing on grouping colorectal cancer into four consensus molecular subtypes covering 87 % or colorectal cancers (35). CMS 1 consists predominantly of right-sided tumours (77 %) with MSI, high mutational load and high neoantigen load causing immunogenicity. However, the right-sided tumours are heterogenous and represented in all four CMS (35, 36).

Figure 6 Consensus molecular subtypes and main characteristics. Figure based on information from *Guinney, J., Dienstmann, R., Wang, X. et al. The consensus molecular subtypes of colorectal cancer. Nat Med **21**, 1350–1356 (2015). https://doi.org/10.1038/nm.3967*



Abbreviations: CMS = consensus molecular subtypes; MSI = microsatellite instability, CIMP = CpG island methylator phenotype; SCNA = somatic copy number alterations; BRAF = B-Raf proto-oncogene, serine/threonine kinase; KRAS = Kirsten rat sarcoma viral oncogene homolog; WNT = wingless-related integration site; MYC = myelocytomatosis oncogene; TGF-β = transforming growth factor β

1.2 Preoperative radiologic staging

The increased survival of rectal cancer was a result of improvement in the whole treatment chain from initial radiologic diagnostics with the introduction of magnetic resonance imaging (MRI), neo-adjuvant treatment and standardization of the surgical approach (37, 38). Focus on preoperative diagnostics and improved detection of tumor specific risk factors with MRI led to the establishment of neo-adjuvant radio- and/or chemotherapy treatment given the presence of certain radiological high-risk features like affection of circumferential resection margin, invasion of lympho-vascular vessels, presence of tumor deposits and lymph node metastasis (39). The preoperative radiological staging for colon cancer has not kept pace. Standard preoperative investigation is contrast-enhanced (CE) thoraco-abdominal computed tomography (CT). In the early phases of preoperative CT diagnostics, focus on the preoperative investigation was mainly directed at detection of distant metastasis. The interest in meticulous preoperative radiologic investigation in colon cancer has increased. Eventually, the focus has been on detection of lymph node metastasis. The accuracy of this is not satisfactory due to interpersonal variance in the interpretation and a gap between radiological and pathological staging (40, 41). Preoperative evaluation of lymph nodes is possibly of less relevance for prognosis than the presence of tumor deposits and lympho-vascular and perineural invasion (42). Neo-adjuvant treatment for colon cancer is not established as a routine treatment option but have been explored in trials given the presence of certain radiological criteria, like suspected lymph node metastases and extramural tumor growth (> 5 mm) (43, 44). So far, the results show diverging effect on 2-years disease-free survival. This can possibly be due to inaccurate radiological evaluation and risk stratification. The evolvement in surgical technique with meticulous dissection of central lymph nodes give rise to the need for detailed information of the vascular anatomy. The relation of arteries and veins, especially for proximal tumors, in addition to anatomical variants are important to depict (45-47). Preoperative CE CT in both portal venous and arterial contrast phases should therefore be conducted. The construction of tree-dimensional models is time consuming and challenging due to different enhancement of the vessels depending on contrast phases but can be an investment in safe surgery.

1.3 The development of oncologic colon surgery

1.3.1 Historic overview

The earliest known descriptions of cancer were found in Egyptian papyruses dating 3-2000 BC (48-50). Cancers of the skin, uterus, stomach, and rectum were described. The term *"carcinos"* was first introduced by Hippocrates (ca. 460 BC-370 BC) (48). It was translated from Greek to the Latin term *"cancer"* by Celsus (ca. 25 BC-50 AD). *"Carcinos"* and *"cancer"* mean crab or crayfish because the veins visual on the cut surface of a tumor resembled crab- or crayfish-legs. The term *"oncos"*, meaning swelling, from which *"oncology"* is derived, was introduced by Galen of Pergamon (Greek physician 130-200 AC). He was the first to describe arteries as *"carriers of blood"*. He is linked to the first description of vascular anatomy (51). Galen was a highly respected medical authority and stated that a patient with cancer was incurable. This dictated the perception of cancer management for centuries. The first scientific human dissection on cadavers was performed by Herophilus of Chalcedon (330-260 BC), called the *"Father of Anatomy"*, and Erasistratus of Ceos (304-250 BC). This work became stagnant as human dissections were abandoned until the Renaissance (around 1530 AC). The link between the pathologic/anatomic finding and the patient`s illness was described first in 1761 by Giovanni Morgagni of Padua. By his autopsies he established anatomy as an instrument to identify etiology and seat of diseases. The suggestion that some cancers might be cured by surgery was not proposed until the 18th century by the Scottish surgeon John Hunter (1728-1793). He said that if the tumor was movable and did not invade surrounding tissue *"there is no impropriety in removing it".* Before the development of anesthesia in 1846, surgery was not a usual treatment option for cancer.

In the book *"Chirurgie"* from 1719, it says: *"it does not matter which technique is used to repair bowel injuries since the majority of patients are not salvageable".* The first right hemicolectomy (with double-barrel ileostomy) was performed in 1732, and the first successful resection with anastomosis is reported in 1833. Bowel surgery had to overcome several obstacles before it was established as routine-treatment. Only

cancer operations due to bowel obstruction or perforation were performed initially. It was unthinkable to do elective oncologic surgery. It was not until the 19th century that bowel-anastomoses were performed routinely. In Nordiskt Medicinskt arkiv Årg 1892 Nr.8*: «Om extra-abdominal Behandling af cancer intestinalis (rectum derfra undtaget) Af Overkirurgi Oscar Bloch: Hovedresultatet er at 145 Pasienter med cancer intestinalis ere behandlede operativt; af disse 145 ere 100 döde».*

The tree surgeons, Billroth from Germany (1829- 1894), Halsted in Baltimore (1852 – 1922) and Handley in London (1872 – 1962), stand out because of their contribution to the development of surgery, especially oncologic surgery. Billroth was considered the founder of modern abdominal surgery. He was the first surgeon to successfully remove parts of abdominal organs like esophagus, rectum, and ventricle for cancer. He adhered to the antiseptic techniques and introduced a surgical training program. He advocated that results, good and bad, should be published and discussed. Halsted trained under Billroth. He was known as the surgeon who performed the first radical mastectomy for breast cancer. He was concerned about antiseptic technique and was also known for the introduction of local anesthesia and his surgical training program. Handley was a surgeon with interest in pathology. He studied the dissemination of cancer. He discovered that breast cancer mainly spread along the lymphatics and added a new dimension to our understanding of cancer and the surgical treatment of it.

Annals of Surgery published a material of colon resections for malignancies in 1949. The reported recurrence-rate after colon resection for malignancies were well over 90 % before 1907. Mortality-rates in operations before 1941, when perioperative antibiotics were introduced, were almost 50 %. Interestingly the authors state that: *"While it is obvious that no one method is best for all cases, we are convinced that adherence to certain fundamental principles will enable the surgeon to choose the method best suited for most of his patients. First and foremost is earlier diagnosis and surgical intervention; Second, is radical extirpation of the lesion and its lymphatic pathways; …; Fifth, is simplicity and adaptability of technic"*. These are principles we adhere to today. Regardless of their wisdom of fundamental principles, they were not right when they stated: *"The rise in resectability rate and lowering of operative*

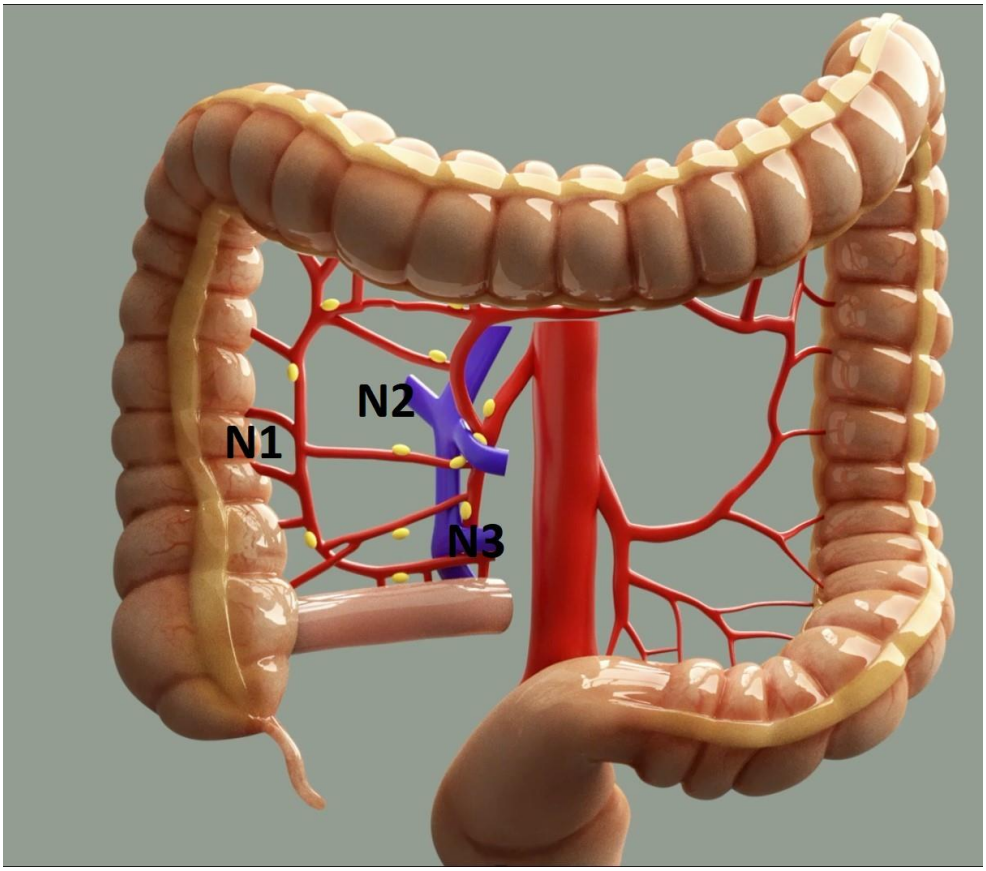
mortality (to 5 %) leave little to be gained by advances in technic or management alone".

Before World War II the treatment of oncologic patients was dependent on the individual physician's practice. It is only after mid 1900 that existing treatment methods have been standardized and globalized. The no-touch technique spread after Turnbull's documentation of increased survival if the tumor feeding arteries were dissected and divided centrally before the tumor-bearing bowel segment (52). The publication and discussion of results are cornerstones for the development of surgical technique. The era of minimally invasive colorectal surgery was initiated by a case report of the first laparoscopic-assisted right hemicolectomy in 1991 (53), followed by randomized-controlled trials to establish its role as an alternative to open surgery (54-59).

1.3.2 Terminology

There are two sets of terms used to describe radical colon cancer surgery. The lymphatic vessels and nodes draining colon is describes in detail by the Japanese (60). The division in pericolic (N1), intermediate (N2) and central lymph nodes (N3) gives rise to the expressions D1, D2 and D3, namely **d**issection of the corresponding lymph node groups. In the Western term, complete mesocolic excision (CME), the central lymphadenectomy is described by the term central vascular ligation (CVL). CME is defined as follows: the main component involves dissection between the mesenteric plane and the parietal fascia and removal of the mesentery within a complete envelope of mesenteric fascia and visceral peritoneum that contains all lymph nodes draining the tumour area (61, 62). The second component is a central vascular tie to completely remove all lymph nodes in the central direction. The third component is resection of an adequate length of bowel to remove involved pericolic lymph nodes in the longitudinal direction (63, 64).

Figure 7 Lymph nodes along the vessels in the proximal colon. Pericolic (N1), intermediate (N2) and central (N3). *Figure by Frank Pfeffer, printed with permission.*



1.3.3 The modern history

Parallel to the development of minimally invasive surgery, focus on a more radical surgical technique in colon cancer has been increasing. The realization that surgical method is crucial has led to numerous studies comparing operative technique in colon cancer. Many studies compare open to laparoscopic surgery, but the study-populations are heterogeneous and include patients operated with completely different methods due to scattered tumour-localizations (65, 66). The anatomical difference between right and left colon makes them non-comparable in terms of cancer surgery. Most of the studies are biased by the fact that the surgeons are more experienced in one of the

methods studied, and only a minority are randomized, controlled trials. The studies have only defined the access as a difference and lack a description of possible dissimilarities in the intraabdominal surgical technique. It is reason to believe that also the extent and implementation of intraabdominal dissection can differ. The Norwegian gastrointestinal cancer group recommends D3 resection/CME with CVL as the standard operative technique for colon cancer (67). There is evidence that time to recurrence and survival improves with the number of lymph nodes harvested at surgery (61, 68-72). However, current practice in Norway, while performing right colectomy for cancer varies from ligation of the feeding vessels somewhere on the right-hand side of the superior mesenteric vein (SMV) to ligation at their origin. This might be due to lack of a uniform definition of the medial border of the right colon's mesentery. Significant remaining arterial stumps have been demonstrated in patients operated for right colon cancer (73-76). This leaves reason to believe that a certain number of central lymph nodes can remain after the procedure (77). The complex anatomical relationship between the ileocolic-/right colic artery with the SMV makes central lymphadenectomy in right-sided colon cancer demanding (78, 79). European society of coloproctology launches a course in minimal invasive CME to promote and standardize minimal invasive complete mesocolic excision for colon cancer. There are ongoing efforts of European standardization of oncologic right colectomy, but consensus is not yet reached (80). The lack of a unified definition and terminology of the central lymphadenectomy is a major problem when discussing and comparing oncologic colon surgery.

Medical understanding has evolved over thousands of years. There are still black holes in our understanding of colon cancer. Surgery is the mainstay in our treatment repertoire, but elective oncologic colon surgery has only been an established treatment option for less than one hundred years. Bowel surgery have existed and evolved through a few hundred years. Although the development has undergone leaps since 1800c, further progress in surgical technique is necessary. The interesting aspect is that both standardization of the surgical procedure and personalization of treatment is necessary to further improve the prognosis of colon cancer.

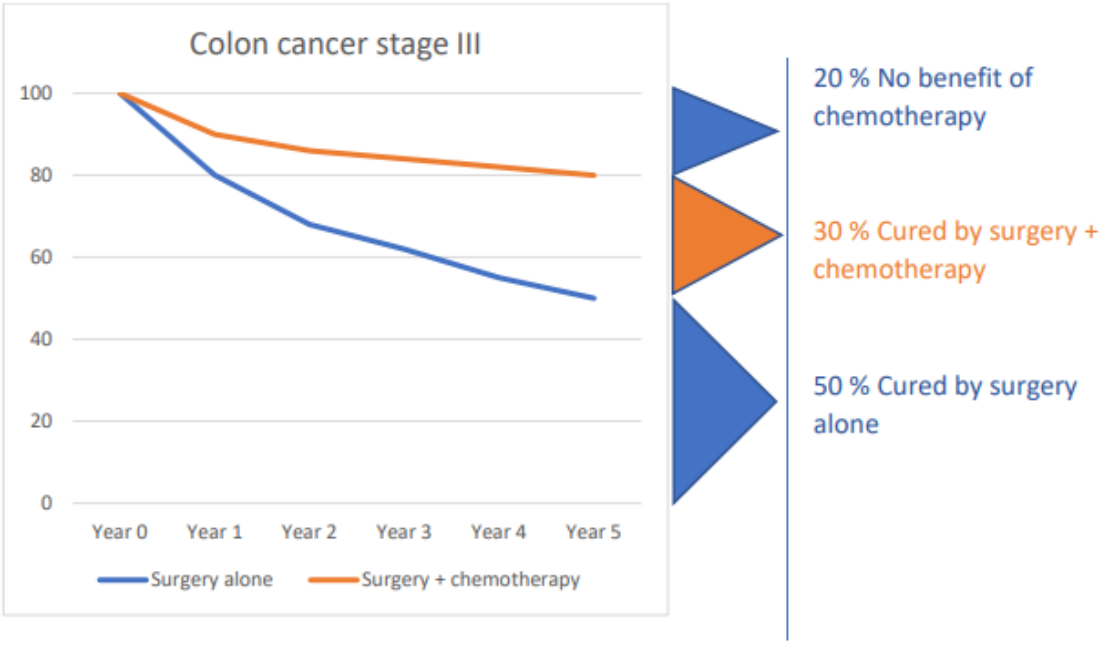
1.4 Systemic oncologic treatment

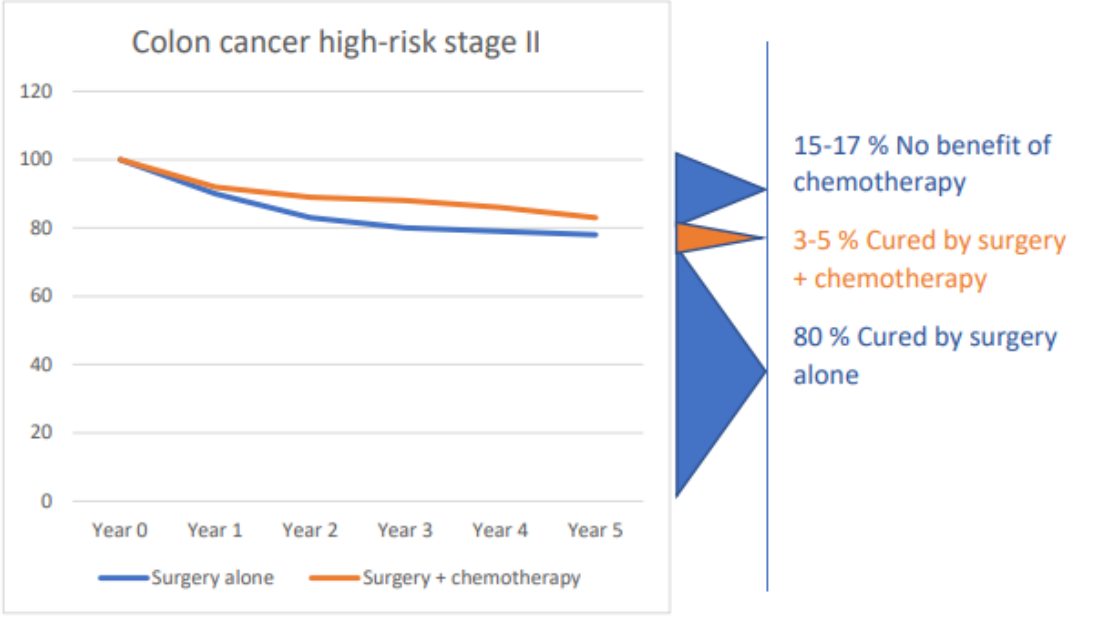
Systemic oncologic treatment in an adjuvant setting is indicated to reduce the risk of recurrence in patients classified as stage III (lymph node positive) or high-risk stage II (in Norway defined as lymph node negative pT4, perforation close to tumour or lymph node count < 12).

1.4.1 Chemotherapy

Although surgery constitute the cornerstone in colon cancer treatment, chemotherapy is the other central component in the treatment chain. The intention of systemic treatment is to eradicate micro-metastases and improve long term survival. Benefit of adjuvant chemotherapy (ACT) has been established for high-risk stage II and stage III patients (81-86). However, identification of high-risk patients needs improvement as the current criteria to select patients for ACT are based on population based risk stratification from the TNM-classification (87) and data from older patient cohorts. This has not been modernised despite the progress in preoperative diagnostics, treatment, pathological evaluation, and the fact that that lymph node negative patients also develop metastatic disease (88). The TNM-classification focuses solely on tumour, node, and metastasis. The risk stratification lacks evaluation of biological markers. To date, the Norwegian recommendations for ACT do not take morphological evaluations like lympho-vascular infiltration and grade of differentiation into account. As the treatment algorithm is based on cohort studies, it is known that some of the patients receiving ACT would have equally good prognosis without. Many patients with colon cancer are above age 75. The potential benefit from ACT must be balanced by the potential for risk attributable to increased toxicity for elderly patients. As chemotherapy has a wide range of side effects that can contribute to morbidity and a reduced health related quality of life (HRQoL), tools that allow improved selection and avoidance of overtreatment is crucial. The field of biological markers and new techniques for detection of individual tumour biology harbours the solution.

Figure 8 Effect of chemotherapy for stage III and high-risk stage II, respectively. Based on numbers from *Taieb J, Gallois C. Adjuvant Chemotherapy for Stage III Colon Cancer. Cancers (Basel). 2020 Sep 19;12(9):2679. doi: 10.3390/cancers12092679* and *Efficacy of adjuvant fluorouracil and folinic acid in B2 colon cancer. International Multicentre Pooled Analysis of B2 Colon Cancer Trials (IMPACT B2) Investigators. J Clin Oncol. 1999 May;17(5):1356-63. PMID: 10334519*





1.4.2 Targeted therapy drugs

In contrast to chemotherapy which has a non-targeted effect on cells with rapid cell division, targeted therapy drugs have specific effect on for example vascular endothelial growth factors or epidermal growth factor receptor. These drugs can be combined with chemotherapy, and the effect is mainly inhibition of the cells' growth advantage. These drugs are used in the setting of metastatic colon cancer.

1.4.3 Immunotherapy

A new systemic oncologic treatment has become available in recent years. Immunotherapy, or immune checkpoint inhibitors. These checkpoint inhibitors are effective only in colon cancer patients with deficient MMR. Currently, only patients with metastatic disease are offered this treatment routinely in Norway. Immunotherapy is investigated in a neo-adjuvant setting in the Phase 3 trial AZUR-2 (CLinicalTrial.gov ID NCT05855200). The mechanism of action is to inhibit the "breaks" of the immune system and boost an immune response against colon cancer cells. The most serious adverse effects are related to the removal of the safeguard of the immune system and thereby inducing an autoimmune reaction.

1.5 Metastatic process

About half of colon cancer patients exhibit distant metastases at some point in the course of disease. The mechanism of the metastasise is not fully understood. In the metastatic process, cancer cells from the primary tumour detach, disseminate trough blood or lymphatics to settle down and proliferate into metastases (89). The process is stepwise and depends on the cells ability to survive and adapt to distinct microenvironments. The timing of spread is unknown. Two proposed models for metastatic development exist. In the linear model, tumour cells are considered to undergo multiple successive mutation steps. The result is tumour cells most apt to settle in a new environment followed by transport to new sites where they form distant metastasis. In the parallel progression model, dissemination occur early and tumour cells continue to mutate and mature at ectopic sites (90). In the stepwise model mutations in the metastatic tumour cells are expected to be identical to the primary

tumour. In the parallel model metastatic tumour cells can have a different genomic profile than the primary tumour. Evidence supports that early seeding and micro-metastases occur before clinically detectable tumour in 80 % of metastatic colorectal cancers (91). The described two main routes for metastasise in colon cancer are the lymphatic and hematogenous dissemination. They occur after cancer cells penetrate either lymphatic vessels or blood vessels by direct growth at the primary site. The relation between the two routes is not completely explored. Up until date the presence of lymph node metastases has had the highest impact on treatment decisions because of the assumption that they represent the gateway to distant metastases. The perception that lymphatic spread always proceeds hematogenous dissemination can be debated. The recognition that lymph node negative patients do exhibit metastases (4) and that vascular invasion, perineural growth and tumour deposits also harbour metastatic potential (42), challenges this understanding, and should promote the development of an improved model for risk stratification.

Figure 9 Models of metastatic process; linear a) and parallel model b). *Klein, C. Parallel progression of primary tumours and metastases. Nat Rev Cancer **9**, 302–312 (2009). https://doi.org/10.1038/nrc2627/Reprinted with permission from Springer Nature and personal permission from Klein*

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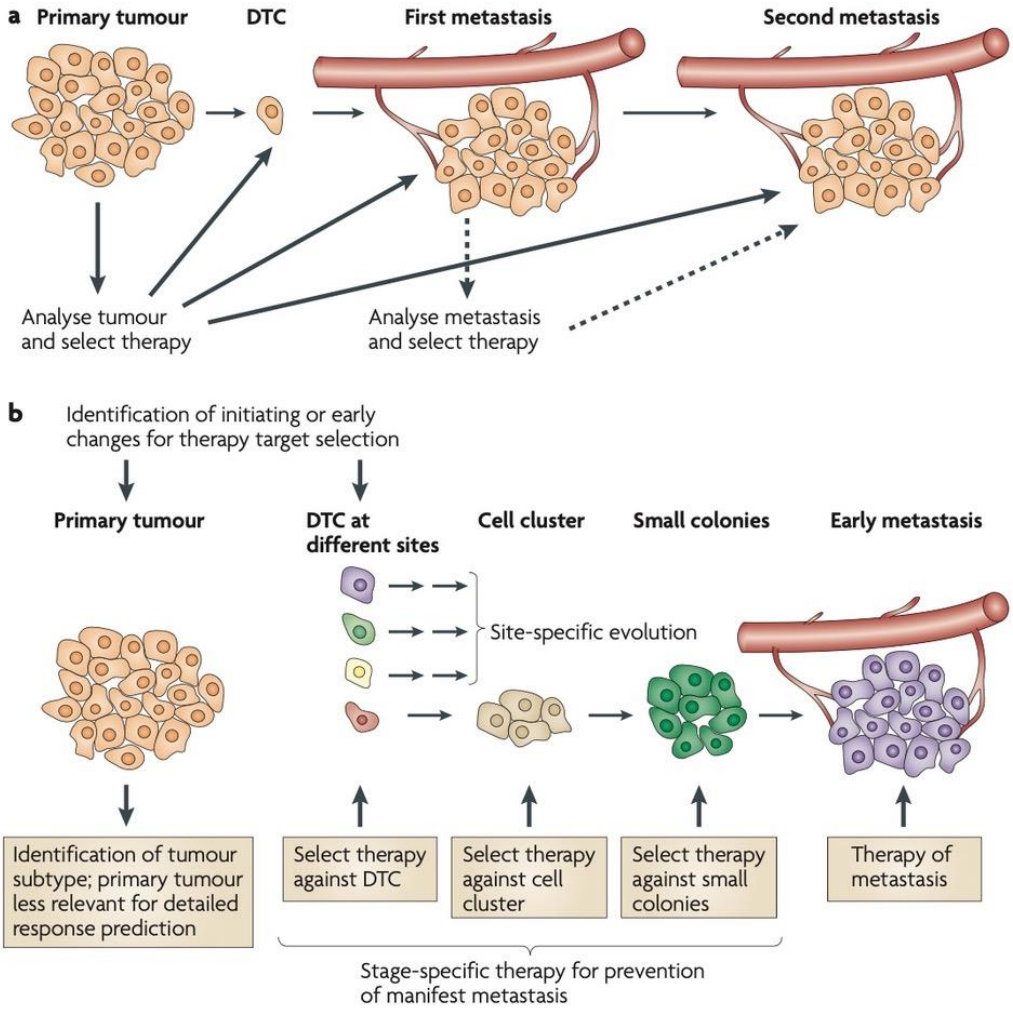


Abbreviations: DCT = disseminated tumour cells

1.5.1 Distant organ metastases

Besides loco-regional lymph node metastases, the liver and lungs are the two most frequent organs affected. Approximately 25-40 % of colon cancer patients will exhibit metachronous liver metastases at some point (92). The metastatic route to the liver is through the venous drainage of the right colon into the portal vein. Colon cancer patients often exhibit lung metastases after liver metastases as the lympho-venous drainage is through the mesenteric circulation into the liver. Few patients metastasise solely to the lungs. The peritoneal cavity is also a predilection site for metastases from colon cancer, especially proximal tumours where poorly differentiated tumours are more common. Currently, the detection of metastasise relies upon radiological presentation. The metastasis must reach a certain size to be detected by imaging (93-95). Advances in genetic profiling and analyses for liquid biopsies may enable earlier detection of systemic disease when early seeding is present, and these tumours fall below the detection limits for current imaging modalities.

1.6 Liquid biopsy

Genetic alterations are necessary for malignant tumours to evolve. The transition from a benign to a malignant lesion is caused by acquiring a series of mutations over time (19, 96). A small proportion of colon cancer (3 %) can be attributed to inherited genetic defects. The rest is caused by accumulated somatic mutations. A limited number of known mutations and epi-genetic alterations are important in the development of colon cancer, but the possibilities for combinations are large. These genetic alterations harbour prognostic value. Exploring the individual genetic landscape is the tool to personalize cancer treatment. Liquid biopsy is an evolving field, analysing cancer biomarkers isolated from nonsolid tissues. Liquid biopsy from blood is an established approach to map biological properties and measure treatment response (97, 98). When this project started, most liquid biopsy-studies involved patients with known macroscopic metastatic disease, first and foremost to monitor the effect of treatment. The TNM-classification is the basis of today`s risk stratification, as discussed earlier. The fact that lymph node negative patients develop recurrence proves that this classification does not capture all prognostic factors (4). Liquid biopsy

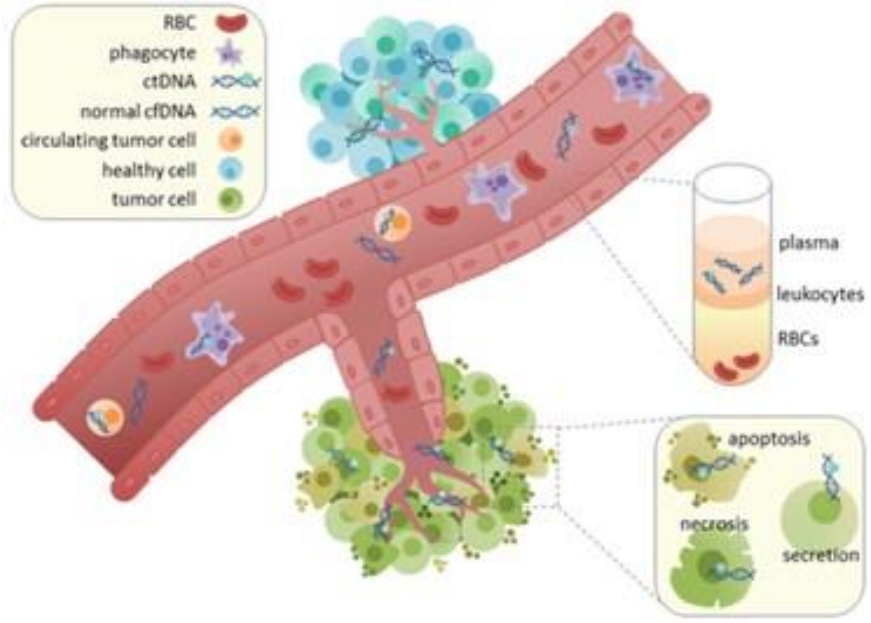
holds strong potential as a tool to estimate prognosis and predict and detect recurrence (99-104) in non-metastatic cancer. Genetic subtyping and expression profiling will enhance patient selection. Various tumour-derived products can be detected in blood and further analysed. Circulating tumour DNA (ctDNA) is one of the most investigated and promising products (97, 105-107). Tumour tissues and plasma can be the source of the initial genetic profiling. There is a heterogeneity in the mutational landscape. The mutation profile can differ between the primary tumour and metastasis. There can be differences within the primary tumour with expression of diverging mutations in different areas of the tumour. Genetic profiling of liquid biopsy can potentially capture this diversity better than profiling from a tumour biopsy.

1.6.1 Circulating tumour DNA

All cells shed DNA to the circulation. Cells that undergo apoptosis and necrosis shed large amounts of DNA to the blood stream. When blood is centrifuged, the various components can be separated, and it is possible to remove intact cells. This way you can analyse plasma with fragments of cell free DNA. Circulating tumour DNA constitutes a small portion of total cell free DNA (< 1 %) (108). The amount of ctDNA correlates with tumour load (109). Detection of postoperative ctDNA indicate the presence of remaining cancer cells (99). Since DNA in blood has a short half-life, the presence of tumour DNA after surgery is a strong indicator of so called minimal residual disease (MRD). ctDNA can give a genetic profile of the tumour, identify treatment targets, and better reflect the heterogeneity and progression than a tumour biopsy alone (98). There are also indications that ctDNA can supplement conventional surveillance and lead to earlier detection of recurrent disease (110). Early detection of recurrence might improve survival (111). Disease surveillance protocols varies to great extent, but intensive surveillance is time and cost consuming. It includes clinical examination, imaging test, colonoscopy, serum carcinoembryonic antigen (CEA) and other lab tests. The goal is to detect and treat recurrence in an early stage before symptoms occur, and before disseminated metastasise. Different methods for detection of ctDNA exist. The two main alternatives are a tumour agnostic approach where the diagnostic marker is selected in advance and independent of each individual patients' genetic landscape. Digital droplet PCR can be the method for analysis with this

approach. The other approach is a broad genetic mapping with next generation sequencing (NGS), detecting each patient's mutation profile.

Figure 10 Cell free DNA and circulating tumour DNA. Blood is centrifuged to remove cells, resulting in plasma containing cell free DNA, which is examined to find tumour specific DNA *https://en.wikipedia.org/wiki/Circulating_tumor_DNA* CC BY-SA 4.0 DEED

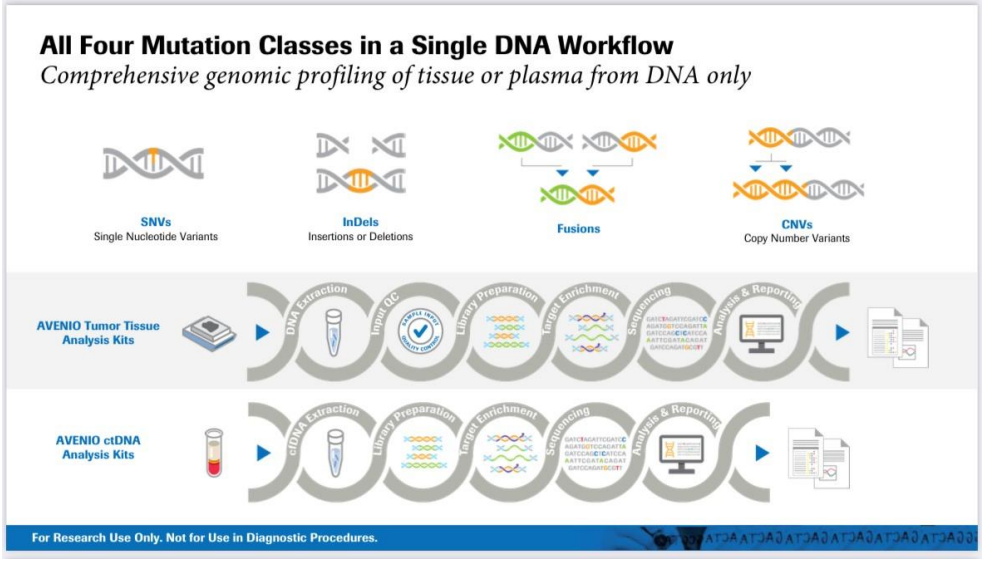


1.6.2 Next generation sequencing (NGS)

DNA sequencing originated in the late 1970s with Sanger technique and reading base by base. This technique had low throughput and high cost. The innovation with amplification and possibilities of massively parallel sequencing was a revolution. Broad genetic mapping is now becoming an established part of routine diagnostics for several cancer types. Next generation sequencing can sequence millions of DNA fragments in parallel and enables large scale genomic sequencing. It is still labour intensive and expensive, but with increased cost effectiveness and more efficient workflow with faster turnaround time compared to first generation sequencing. Sensitivity and coverage have improved. NGS can be used for whole genome

sequencing or targeted sequencing with the use of tumour specific panels as performed in this trial. NGS involves three main steps: Sample preparation with DNA extraction from samples like blood and tissue where DNA is fragmented onto shorter sequences followed by ligation of adapters, amplification, and enrichment of targeted regions. Second step is the sequencing where large amounts of genomic DNA from multiple patients can be sequenced at the same time. The method can detect a wide range of genetic alterations including single nucleotide polymorphism, small insertions, and deletions (indels) and structural variants depending on the set-up. The last step is the data analysis with the use of bioinformatic tools of data analysis applications used for quality control, alignment to reference sequence, identification of variants and interpretation to identify pathogenetic variants.

Figure 11 AVENIO tumour and ctDNA analysis kit. *Reprinted with permission from Roche [NGS Oncology Assays (roche.com)]*



1.6.3 Digital droplet PCR

Digital droplet PCR is a method with high accuracy, sensitivity, and precision in addition to being reproducible and at low-cost. ddPCR is a targeted analysis for

detection of selected mutations using assays for specific genetic alterations. The polymerase chain reaction (PCR) is literally to amplify the target nucleic acid to a detectable level. By using a probe where a fluorescent signal is generated each time an amplicon is generated, the targets can be quantified. ddPCR is a microfluidic-based digital PCR method in which a sample of DNA molecules is partitioned into thousands of water-in-oil droplets. In digital droplet PCR the amplification occurs in the oil-droplet containing the PCR reaction with ideally one target sequence. The strength of the fluorescent signal in each droplet is then read and interpreted as either positive or negative according to the selected assay analysed against.

1.7 Right sided colon cancer (RCC)

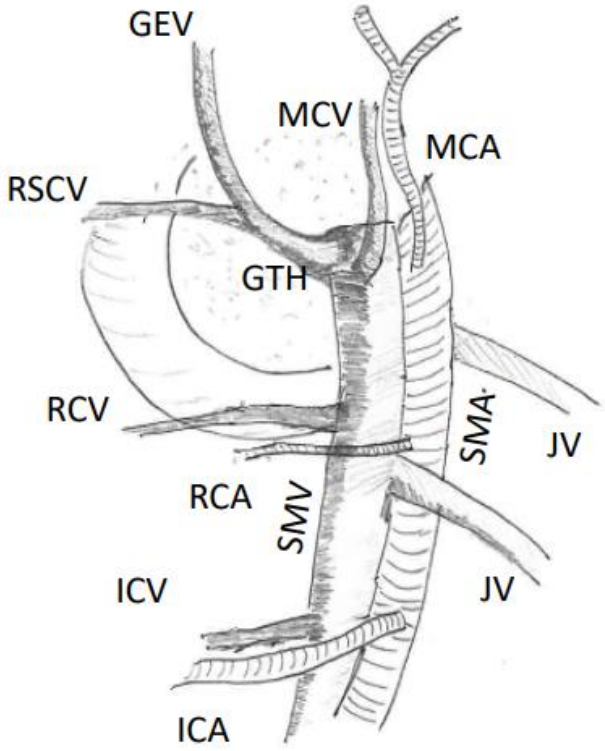
Colorectal cancer is often described as one disease, but our knowledge has increased. Rectal cancer and colon cancer have separated and are now considered two different entities. Preoperative investigation, staging, neo-adjuvant and adjuvant treatment and surgical strategy differ. It is also time for proximal and distal colon cancer to split up and be considered two different subgroups of the disease with corresponding assessment and treatment. Tumours of the right colon evolve from a different embryological origin than left colon and differ in morphological characteristics, mutation profiles and complexity of vascular anatomy. RCC is interesting both from a surgical- and a molecular biological point of view. The recognition that RCC and left sided colon cancer differ has led to a distinction between them. They are increasingly considered two different entities. Prior to this trial few studies had focused on this location in particular. Lately there is an extended number of publications where the diseases are studied separately.

1.7.1 Embryology and vascular supply

The development of the gastrointestinal tract starts with the folding of cellular layers in the 4th and 5th gestational week. The tube formed consists of foregut, midgut, and hindgut, which in turn has its own blood supply. Foregut nourishes from the coeliac trunk, midgut has its supply from the superior mesenteric artery, and the inferior mesenteric artery supplies the hindgut. Midgut gives rise to the small intestine from the ligament of Treitz and the colon until and including the proximal 2/3 of transverse

colon. The vascular supply of the midgut branches from the superior mesenteric artery with tree main vessels to the colon, namely ileocolic artery, right colic artery (60 % (112)), and medial colic artery, in addition to multiple branches supplying the small intestine. Due to the rotation and de-herniation of the midgut intrauterine, the vascular relations belonging to the right colon are complex and involves multiple branches from both vein and artery (78, 79). This makes the surgery and central lymphadenectomy for RCC more demanding than more distal colon cancer surgery, where the main tumour feeding artery branches directly from aorta.

Figure 12 Schematic overview of arteries and veins feeding the right colon. *Drawing by Frank Pfeffer. Printed with permission*

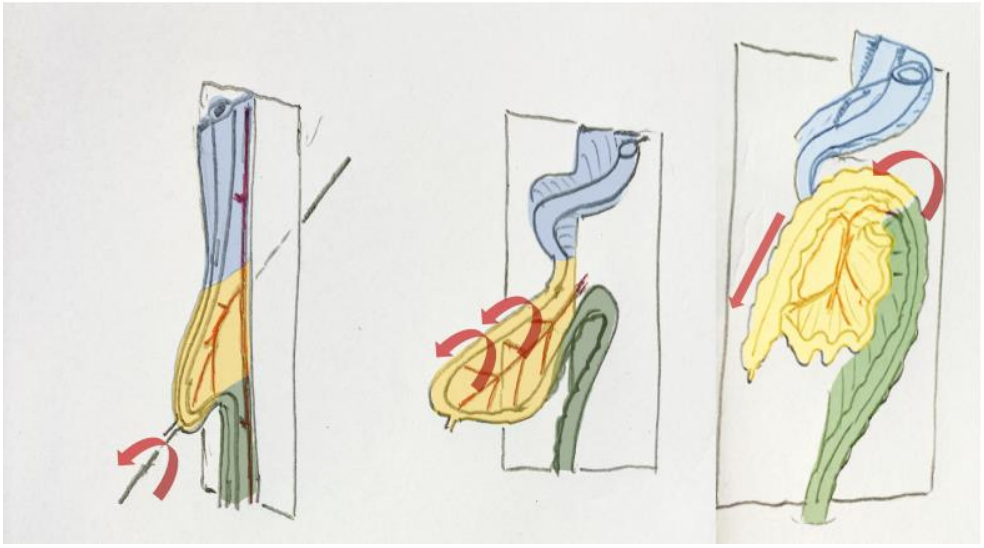


Abbreviations: ICA = ileocolic artery; ICV = ileocolic vein; SMV = superior mesenteric vein; JV = jejunal vein; SMA = superior mesenteric artery; RCA = right colic artery; RCV = right colic vein, GTH =

gastrocolic trunk of Henle; RSCV = right superior colic vein; MCA = middle colic artery; MCV = middle colic vein; GEV = gastroepiploic vein

The gut tube with its blood supply and lymphatics are lined with peritoneum. When it rotates 270° and folds back into the abdominal cavity around ten weeks of gestation, it sticks to the already peritoneal-covered anterior pararenal compartment of the retroperitoneum. The result is the posterior leaf of visceral peritoneum fusing with the parietal peritoneum forming the fusion fascia of Fredet (113) and the plane of Toldt. They are important surgical landmarks, and the plane of dissection in oncologic colon surgery. The tumour grows within the peritoneal linings unless discovered at a late stage (T4b). The rationale is that dissection along the embryological planes and the reversal of the embryology will lead to removal of all tumour cells (≤ T4a) as it is confined in the "envelope" formed by the peritoneum.

Figure 13 Rotation of midgut with corresponding feeding vessels. *Illustration by Frank Pfeffer. Printed with permission*



1.7.2 Lymphadenectomy and terminology

Colon cancer metastasise both through hematogenous and lymphatic spread. Due to the complex vascular supply to the right colon, the central lymph node dissection is demanding. The main difference when comparing CME to the total mesorectal excision in rectal surgery, where the dissection area is clearly defined (37), is the fact that the medial border of the right mesocolon still remains undefined. The medial

border of the right colon's mesentery is an arbitrary line. No international consensus exists. Complete excision is challenging to achieve when the borders are undefined. The so called D3 area is defined in slightly different ways depending on whom you ask. The terms CME, CME with CVL, CVL, modified CME, D2, extended D2, complete D2, D3 and beyond D3 exists, and the definitions are not congruent (80). Some describes denudation of the superior mesenteric vein as D2, whereas the Japanese themselves describes D3 as "removal of draining lymph nodes (station 203, 213, 223) along the superior mesenteric vein" (114). As CVL is a main component in CME, the term CME with CVL is confusing. It is assumed that lymph nodes follow the main tumour feeding arteries. On the left side, the clear anatomy makes lymph node dissection much easier compared to the right, and dissection of the central lymph nodes (N3) is accomplished when the inferior colic artery is dissected and divided close by aorta. On the right side the central nodes (N3) follow the superior mesenteric artery, which cannot be divided. Dissection along the vessel is necessary to accomplish central lymphadenectomy. Controversies of the necessary extent of central lymph node dissection exists. The disputation is whether it is mandatory to denudate both superior mesenteric vein and artery and whether dissection both anterior and posterior is essential. Unless the method is described in detail, clarification of and uniform use of the terms is a prerequisite for comparing the extent of lymphadenectomy and define a standard for the future.

1.7.3 Morphological and tumour biological characteristics

Adenocarcinoma was previously described as well, moderately and poorly differentiated. Well-differentiated tumours resemble normal tissues, both functionally and morphologically. They are characterized by high maturity, low-grade malignancy, slow progression, and late metastasise. At the other end were the poorly differentiated tumours, characterized by low maturity, high-grade malignancy, rapid progression, and early metastasise (115). Most colon cancers are moderately differentiated. Poorly differentiated tumours are more frequent at the right side (116). Poorly differentiated tumours are more likely to metastasise to the peritoneum than moderately- and well-differentiated tumours, who mainly metastasise to the liver and lungs. A Japanese study investigated risk factors for peritoneal recurrence in stage II and III colon cancer.

Approximately one fourth (26,3 %) of the patients analysed presented with cancer in coecum and ascending colon whereas two thirds (67,6 %) of the peritoneal recurrences in the study population occurred in RCC. However, tumour location was not analysed as an independent variable in their cox regression analysis (117). Updated nomenclature is low-grade (former moderately and well differentiated) and high-grade (former poorly or undifferentiated) adenocarcinoma.

Adenocarcinomas can occur with or without a mucinous component. Mucinous adenocarcinoma (MUC) is defined by WHO as adenocarcinoma with more than 50 % extracellular mucin. It is uncommon and appears in 5-15 % of colon cancers (118). Adenocarcinoma with mucinous component (AMC) are defined as less than 50 % of extracellular mucin within the tumour. Several clinical and post-mortem studies have suggested that colorectal mucinous adenocarcinoma seems to metastasise more frequently to the peritoneum compared with other types of adenocarcinoma (119).The last variant to be mentioned is the signet-ring cell carcinoma. In this variant the mucin is intracellular. The cells have their nucleus pushed to the periphery of the cell by intracytoplasmic mucin. The result resembles a signet-ring, thus the name. By definition signet-ring cell carcinoma affects > 50 % of the cells, but the poor prognosis and predominance of peritoneal metastasis also occur for the tumours with < 50 % signet-ring cells. Both signet-ring cell carcinoma, component of signet-ring cell (< 50 % of cells), MUC and ACM have a right-sided predominance (16, 120, 121). They are all considered negative prognostic factors although the results are diverging in different studies (119, 122-124). The role of different histologic subtypes in colon cancer is controversial, but several studies report unfavourable outcome for tumours with some variant of mucinous component (125-128).

Genetic and epigenetic alterations leading to changes in mismatch repair genes, tumour suppressor genes and oncogenes differs between RCC and left-sided colon cancer (129). There is a high incidence of BRAF mutations in RCC (116). BRAF hotspot mutations are negative prognostic factors. Although MSI is more common at the right side about 70 % of patients are MSS. The combination of BRAF-mutation and MSS is a predictor of especially poor prognosis.

1.7.4 Prognosis

Colon cancer is a heterogenous disease with scattered tumour location, diverging tumour and node status and different molecular subtypes. The presentation of proximal and distal disease differs in symptomatology with a more diffuse presentation of RCC. This results in an advanced stage at diagnosis. Particularly the incidence of stage I colon cancer is lower on the right side compared to the left (17). However, the stage at diagnosis cannot alone explain the outcome favouring a more distal cancer (130). Despite curative resection, RCC stage III exhibit higher recurrence rates and more frequently multiple metastatic sites in the first recurrence (131, 132). Metastatic RCC experience shorter survival than metastatic left sided colon cancer when receiving palliative chemotherapy (116, 131-133). The combination of chemotherapy and epidermal growth factor receptor targeted antibodies in RCC is also inferior to left sided colon cancer (133). The morphologic characteristics of tumours and their impact on prognosis is difficult to establish as many confounding factors exists and studies take this into account to varying degrees. The essence, however, is that RCC more often occurs in older females, is detected at an advanced stage, is high-grade adenocarcinomas with variants of mucinous differentiation and exhibits a poorer prognosis than left-sided colon cancer independent of stage at presentation (134). The tumour location can be a surrogate for different and poor biology independent of stage. This indicates that survival is not only stage specific but also that anatomic site of the primary tumour can appear prognostic.

1.8 Quality assessment

Clear and consistent terminology is a prerequisite for assessment of quality. Endpoints that are standardized, clinically relevant and universally applied are the next precondition necessary to conduct quality assessment. In oncologic surgery cancer specific outcome is the most widespread accepted quality parameter, although complications after surgery constitute a large global burden of public health issues and have a direct impact of HRQoL for the patients. Recommendations of how to assess the quality of surgical interventions was published in Nature medicine in 2023 (135). The recommendations include timepoint of assessment, postoperative complications

by C-D, patient-centered outcomes, benchmarking, and risk assessment. In addition to these general recommendations, the assessment of quality for specific procedures is also necessary. Procedure specific benchmarks for oncologic right-sided colectomy such as lymph node count, evaluation of specimen and surgical site can be defined.

2. Aim of the trial *"Open D3 right hemicolectomy compared to laparoscopic CME right hemicolectomy for right sided colon cancer"*

This dissertation consists of tree papers outgoing from the randomized-controlled trial comparing open and laparoscopic right-sided colectomy with central lymph node dissection. The clinical trial was started to improve prognostication and quality of life in patients with right-sided colon cancer by comparing two different surgical approaches performed by six selected surgeons at two neighbouring and collaborative institutions. The intent was to improve and standardize the surgical technique for RCC and clarify the terms and corresponding surgical procedures. We also launched a biomarker study to better identify patients with minimal residual disease and potentially early relapses. The aims and endpoints of the overall project in which this thesis originates were broad. A summary of the aim for this thesis is presented in the following.

2.1 The clinical trial

The surgical aim of this study was to compare short-term outcomes between open and laparoscopic colectomy for RCC. Our primary hypothesis was that laparoscopic surgery reduces postoperative complications. Secondary aim was to evaluate surgical quality by lymph node count and measuring the remaining vessel stump of the tumour feeding artery after oncologic resection for RCC.

2.2 The biomarker study

The aim of the biomarker sub-study was to explore the role of liquid biopsy with analysis of ctDNA in patients with stage I-III RCC and to test the clinical validity of liquid biopsies in identifying high-risk patients with non-metastatic RCC.

3. Methods

3.1 Design

This is an open, prospective, randomised, multi-centre clinical trial conducted at two Norwegian Institutions from September 2016 until December 2021.

Haraldsplass Deaconess Hospital (HDH) and Haukeland University Hospital (HUH) are neighbouring hospitals who cooperate in colon cancer treatment with common multidisciplinary team meetings, pathology, and oncology services. The hospitals have a close professional relationship. Patients are distributed between them based on capacity. Historically there are no differences in total 100 days survival and relative 5-year survival (136). HDH has since 2007 had focus on laparoscopic CME (137). HUH has since 2011 participated in a project focusing on open right-sided colectomy with central lymph node dissection (45, 138). Both hospitals are skilled in oncologic right-sided colectomy with central lymphadenectomy, have experienced ward-staff and routine implementation of enhanced recovery principles. Enhanced recovery after surgery (ERAS) principles includes preoperative feeding, carbohydrate loading, antimicrobial prophylactics, peroperative fluid restriction, total intravenous or gas anesthesia, epidural anesthesia (open group only), prevention of hypothermia, postoperative no routine use of nasogastric tubes or drains, enforced postoperative mobilization and feeding, and early removal of urine catheters. The perioperative care is equivalent at the two institutions. Both hospitals have performed the resection as described in this protocol since 2012. In each hospital, three high-volume oncologic colorectal surgeons were main or assistant surgeon during surgery. At the two hospitals, 110 (HUH) patients and 52 (HDH) patients were operated with the lymphadenectomy described in this protocol prior to project start. HDH operated patients allocated to the laparoscopic group and HUH operated patients allocated to the open group.

3.2 Patient selection

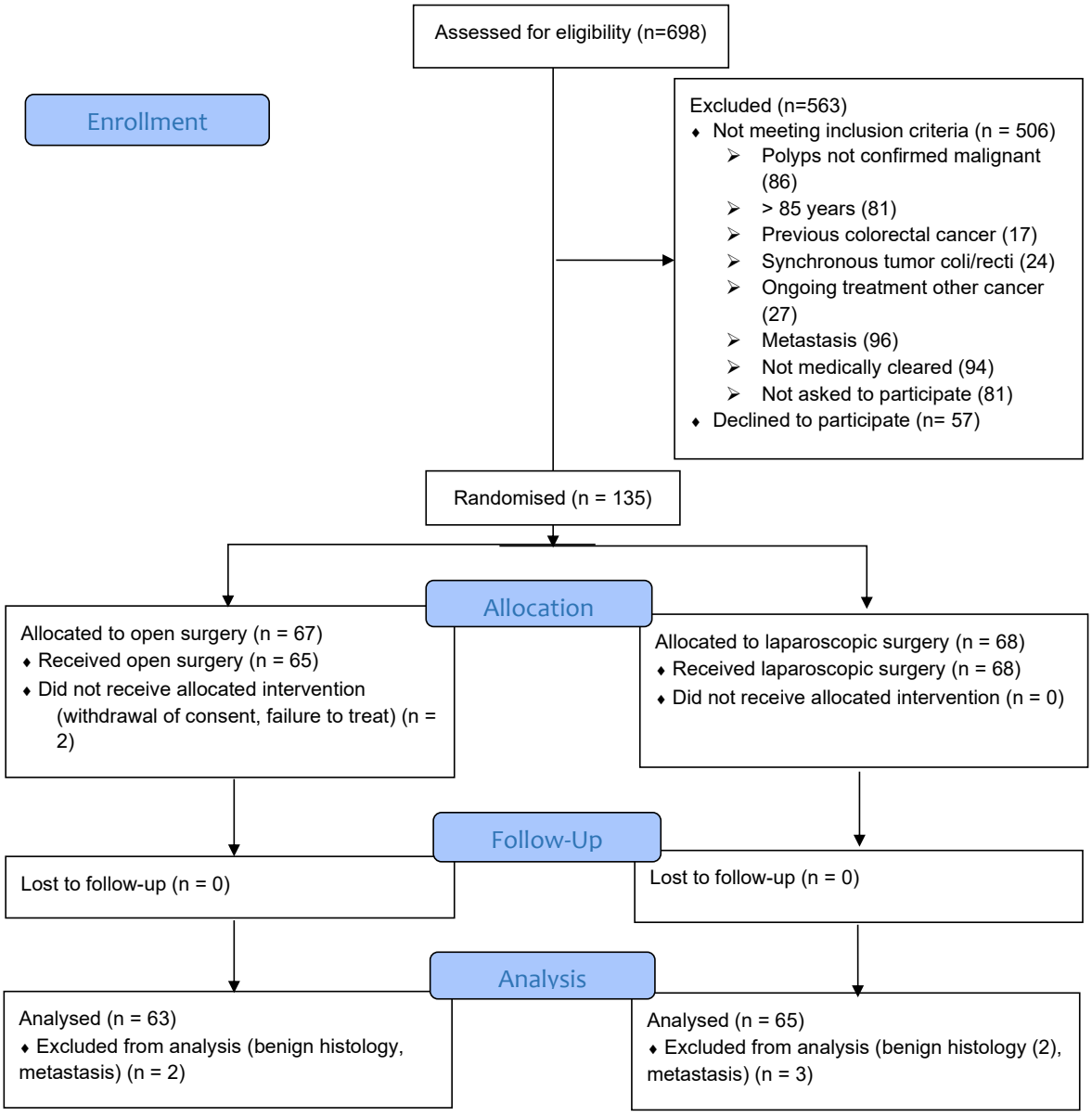
Patients between 18 and 85 years at the two recruiting hospitals with positive computed tomography, colonoscopy, or histopathological verified adenocarcinoma of the right colon without metastases were relevant for inclusion (clinical stages I-III) and considered eligible for the study. Patients were medically cleared for general anaesthesia

and oncological radical resection with central lymphadenectomy. Exclusion criteria included recurrent colon cancer, ongoing treatment for other cancer and metastases outside resection area. All patients gave written, informed consent to inclusion in the study, and a separate consent to collect and analyse biological tissue. Four patients were included in the Neo-Col protocol (Clinicaltrials.gov identifier NCT01108107) and three of them randomized to receive neo-ACT. Patients with previous colorectal cancer or ongoing treatment for other cancer were excluded. Definition of right-sided colon cancer was tumours evolving from the embryological mid-gut (caecum, ascending colon and the proximal 2/3 of transverse colon). For analysis of vessel stump, the consecutive first 40 patients were included. The prospective observational biomarker trial included the first 50 patients analysed with liquid biopsies.

3.3 Patients who did not meet inclusion criteria

Patients evaluated as not-medically cleared to receive general anesthesia and radical oncologic surgery, open or minimally invasive, were excluded. This group included patients with dementia and other psychiatric diseases who were unable to give a true informed consent due to their disease, in addition to patients with high-risk medical conditions. The decision to exclude patients was based on discussion at the multidisciplinary team meeting and physical evaluation in the outpatient clinique. Patients with negative biopsies combined with no clear assessment by preoperative computed tomography (CT; Tx or $\leq$ T2) were considered benign/premalignant and excluded.

Figure 14 Inclusion and randomisation of patients according to CONSORT 2010 flow diagram



3.4 Endpoints

3.4.1 Study I

The primary endpoint was surgical complications by Clavien-Dindo (C-D) classification ≥ II (139-141). Primary alternative hypothesis was that laparoscopic

surgery reduce postoperative complications. Postoperative complications within 30 days were registered. Secondary endpoints were perioperative blood loss, length of stay and number of lymph nodes removed. Operating time, complications C-D < II, blood transfusion/infusion of intravenous iron, postoperative ileus, reoperations, anastomotic leak, readmission and 90-days mortality were also explored. Specific complications as postoperative ileus (POI) was registered in patients with postoperative administered nasogastric tube, pronounced postoperative nausea or vomiting and/or need for administration of parenteral nutrition due to anorexia. Blood transfusion/i.v. iron was registered in all patients were this was administered after the start of surgery.

Table 2 Clavien-Dindo Classification of Surgical Complications

Grade	Definition
Grade I	Any deviation from the normal course without the need for pharmacological treatment or surgical, endoscopic and radiologic interventions Allowed therapeutic regimens are drugs as antiemetics, antipyretics, analgesics, diuretics, electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications Blood transfusions and total parenteral nutrition are also included
Grade III	Requiring surgical, endoscopic or radiological intervention
Grade III a	Intervention not under general anaesthesia
Grade III b	Intervention under general anaesthesia
Grade IV	Life-threatening complication (including CNS complications) * requiring IC/ICU management
Grade IV a	Single organ dysfunction (including dialysis)
Grade IV b	Multiorgan dysfunction
Grade V	Death of a patient

*Brain hemorrhage, ischemic stroke, subarrachnoidal bleeding, but excluding transient ischemic attacks. CNS, central nervous system; IC, intermediate care; ICU, intensive care unit.

3.4.2 Study II

The aim of this study was to compare the remaining vascular stump length of the tumour feeding artery after right-sided colectomy in the two treatment groups.

3.4.3 Study III

To establish whether ctDNA provide additional information about prognosis beyond established risk stratification in a study-population of non-metastatic RCCs, we sampled liquid biopsies and analysed ctDNA pre- and postoperatively. The main endpoint was recurrence free survival. Secondary endpoints were to investigate whether ctDNA was detectable preoperatively in non-metastatic patients, test whether

plasma or tumour was the best medium for identification of biomarkers by NGS and explore the best timing of postoperative sampling.

3.5 Surgical technique

Both groups aimed at central lymphadenectomy with ligation of tumour feeding arteries at their origin. The approach was medal to lateral in both groups. The central dissection was performed from distal to the caudal ileal vein and cranially along the superior mesenteric axis. Open resection was performed with dissection along the lateral left border of the superior mesenteric artery, and laparoscopic resection with denudation anterior to the SMV. Both groups operated following the CME-principles with dissection between the mesenteric plane and the parietal fascia and resection of adequate length of bowel. Gastrocolic trunk of Henle was not routinely divided but was exposed. Branches from the colon were divided selectively when necessary. The right gastroepiploic vein was not routinely divided. In patients with tumour location from the hepatic flexure and distally the aim was extended right colectomy where the middle colic artery was divided centrally. In the laparoscopic group, vessels were sealed with radiopaque clips. In the open group, vessels were sealed with ligatures. In the laparoscopic group, extended right-sided colectomy included resection of mesogastrium (gastrocolic ligament divided close to the greater curvature of the stomach) and prepyloric lymph nodes including division of the right gastroepiploic vein. Anastomoses in the open group were open hand sewn end-to-end, and in the laparoscopic group isoperistaltic, stapled side-to-side. All anastomoses in the laparoscopic group except one were intracorporeal.

Figure 15 Resection area in a) open surgery, b) laparoscopic surgery and corresponding photos of the resection area after c) open resection and d) laparoscopic resection. Drawing by Frank Pfeffer. Reprinted from *Kristin B Lygre et al, Complications after open and laparoscopic right-sided colectomy with central lymphadenectomy for colon cancer: randomized controlled trial,* BJS Open*, Volume 7, Issue 4, August 2023, zrad074, https://doi.org/10.1093/bjsopen/zrad074*

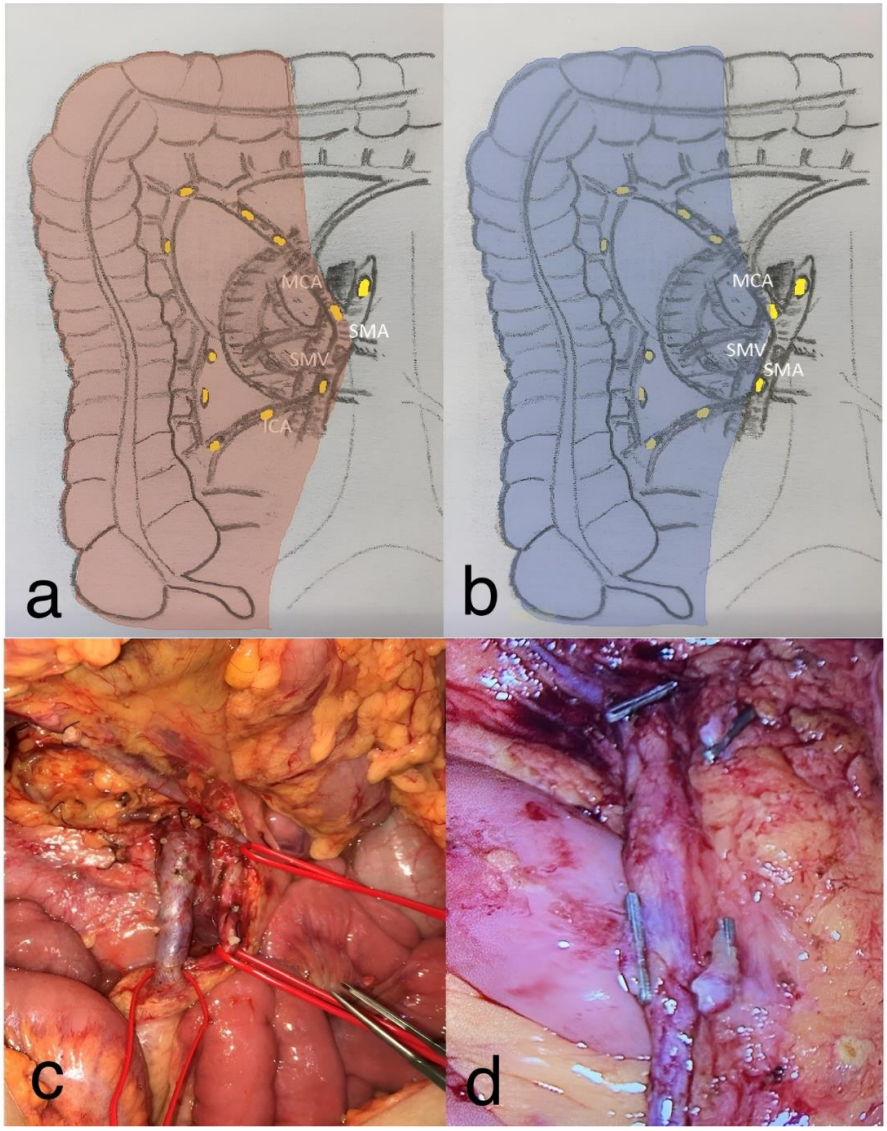
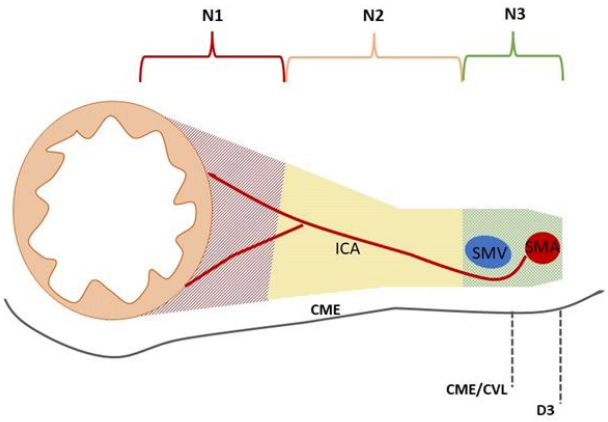


Figure 16 Illustration of level of dissection and vascular ligation in the two groups. Variants of this figure is printed in BJS Open and BJS in paper I https://doi.org/10.1093/bjsopen/zrad146 and II https://doi.org/10.1093/bjs/znad410.



3.6 Specimen

Specimens were fixated in Tarlym® (GEWF: glacial acetic acid, ethanol, distilled water and formaldehyde) (142, 143) and evaluated due to current TNM-grading system in the study period (Editions 7 and 8) (87, 144). Evaluation did not differ from standard practice in Norway. All specimens were analysed at the same pathology service. The pathologists were not blinded to operative methods.

3.7 Sample collection

Blood samples were collected between September 2017 and January 2021, prior to and after surgery (2-7 days or 1 month, 3 months (not mandatory), 6 months, and then successively every 6 months). Plasma was separated from K_2-EDTA blood within one hour of blood draw by centrifuging the blood, before a second centrifugation for the supernatant. Purified plasma was stored at -80°C in six aliquots until further processing performed with 2 years. Biopsies from the primary tumour were collected intraoperatively by the surgeon immediately after removal of the specimen and were snap frozen as four aliquots in liquid nitrogen. Samples were stored at -150°C until time of analysis. Analysis was performed on pre-surgical plasma samples (n = 29), tumour biopsies (n =45), and/or postoperative plasma samples (n = 34). ctDNA and tumour analysis were performed retrospective, blinded to patient outcome.

3.8 CT protocol

All patients were diagnosed with colon cancer and therefore scanned with CE thoraco-abdominal CT prior to surgery for staging purpose. All patients in the open group had

a preoperative CT-derived three-dimensional vascular reconstruction to guide the surgeons. Patients in the laparoscopic group had a standard two-dimensional CT preoperatively. All patients had CE thoraco-abdominal CT 6 months postoperatively as a part of the standard surveillance program. The postoperative CT, conducted in portal venous contrast phase, was used to measure the remaining vascular stump length. CT scans were performed on multi-slice CT scanners (Siemens AS+, Siemens Flash and Toshiba Prime at HUS and GE Revolution CT (GE Healthcare, Milwaukee, WI, USA) at HDS. Intravenous contrast was applied in all cases when not contraindicated. Datasets were reconstructed with a slice thickness of 1-3 mm. The remaining vascular stump length for the ileocolic artery (ICA) was measured for 40 patients included in study II.

3.9 Next generation sequencing

Next generation sequencing was performed by AVENIO® from Roche. AVENIO® is developed for analysis of ctDNA with molecular barcodes and digital error suppression which allows a sensitivity down to a variant allele frequency (VAF) of 0,1% with 20-40 ng DNA (145). NGS in general exhibit technical artifacts leading to false positive results especially at low VAF. This is reduced by the filter provided by the supplier. Results were analysed using AVENIO® ctDNA Analysis Software version 2.0.0 as recommended by the suppliers.

3.9.1 NGS tumour biopsies

Fresh frozen tumour tissue was sliced and stained before verifying tumour content in the biopsy. Necessary preparations were conducted before analyses with AVENIO® Tumour Tissue Analysis Kit paired with AVENIO® Tumour Surveillance Kit as recommended by manufacturer. AVENIO® tumour tissue and surveillance kit together increase the sensitivity. Detection threshold was 5 % for single nucleotide variants.

3.9.2 NGS ctDNA

Purified plasma (n = 62) was thawed prior to enrichment of cell free DNA by AVENIO® ctDNA Analysis Kit according to the protocol provided by the producers. Sequencing libraries were prepared from cell free DNA using AVENIO® ctDNA Analysis Kit paired with AVENIO® ctDNA Surveillance Kit as described by manufacturers. Detection threshold was 0.1 % for single nucleotide variants.

3.10 Digital droplet PCR of ctDNA

Cell free DNA was harvested from purified plasma (n = 311) using QIAamp Circulating Nucleic Acid Kit (Qiagen®) according to the protocol provided by the producer. ddPCR assays for mutations detected by AVENIO® Surveillance gene panel were purchased from Bio-Rad®. Mutations chosen for monitoring were early hits in the clonal evolution of cancer, and in cases with multiple relevant mutations, the variant with highest VAF was chosen. ddPCR was performed as previously described (146), with minor alterations. Briefly, all samples were run as triplicates, and results are presented as an

average between replicates calculating number of mutant DNA copies per mL plasma and fractional abundance (FA) as mutant DNA copies/total DNA copies. All runs included positive controls (biopsy DNA), negative controls (cfDNA from healthy blood donors) and non-template controls for each assay. ddPCR analysis was performed on 3 neoadjuvant, 46 preoperative, and 262 postoperative samples (total: 311 samples, median: 7 samples per patient, range: 2-9). Twenty-five patients were assessed by two assays (53 %), and 22 by one assay (47 %). Results were analysed using the Quantasoft version 1.7.4 software (Bio-Rad®), manually gating each assay based on positive controls, negative controls, and non-template controls. Results were presented as % FA. Samples with <12,000 droplets generated per parallel were excluded from further analysis. Based on validation of detection thresholds for each individual assay using positive controls, normal controls and non-template controls, samples generating a total of <3 mutation-positive droplets or having a FA <0.1 % were defined as having no detectable tumour DNA.

3.11 Microsatellite instability (MSI) analysis

Department of Pathology performed the MSI analysis routinely (by immunohistochemistry) for 16 patients, whereas the remaining 32 patients included in the liquid biopsy trial were analysed by the research laboratory using the MSI Analysis System, version 1.2 (Promega®) using the ABI PRISM 3100 Genetic Analyzer (Thermo Fisher®) as described by the producers.

3.12 Data collection

3.12.1 Study I

Data was collected by the project leader (Ph.D candidate) from objective information in the electronic patient chart. The clinical data with patient characteristics and postoperative complications by Clavien-Dindo classification were registered prospectively and additional clinical data obtained by reviewing electronic healthcare records. Peroperative blood loss was determined by visual estimation by the staff at the operating theater. The pathological evaluation of all specimens was performed at the same department of pathology (Gade's Institute at Haukeland University Hospital) according to the International Union Against Cancer Tumor Node Metastasis (TNM) system (seventh and eighth edition) and pathology data extracted from routine histopathology reports.

3.12.2 Study II

The last postoperative CT was completed in April 2020. The analysis of the remaining vessel stumps was conducted in retrospect during 2020 and 2021. Two independent specialists in radiology, one from each institution, performed the measurements. Initially (observer 2, observation 1) from deidentified CT scans via CDs, and later (observer 1, observation 1 & 2 and observer 2, observation 2) from deidentified CT scans stored in a research picture archiving and communication system (PACS)

(SECTRA® UniView Version 22.1.10.4793). The preoperative CT scans were available in the research PACS, but not for the first observations (observer 2, observation 1). Scans were available in coronal, axial and sagittal reformats. The radiologists selected the most appropriate angles with the best display of the post resection arterial stump. All measurements were registered, and mean value was computed. Measurements were conducted from the most centrally orientated clip or the end of visible vessel along the inferior border of the resected vessel and to the right lateral border of the superior mesenteric artery. Each observer performed the measurements twice with a minimum of 6 weeks between. An additional 3D reconstruction of the vessels in SECTRA® was performed in the patients where the vessel stump visualization was challenging.

3.12.3 Study III

Patients included in the biomarker study were operated from September 2017 to July 2019 with clinical follow up until April 2023. Liquid biopsies and genetic profiling of tumour biopsies were performed by molecular biologists. The interpretation of the genetic information in a clinical context was established in collaboration with the molecular biologists, project leader and statistician.

3.13 Statistics

This trial was preregistered (ClinicalTrials.gov ID: NCT03776591) before all data analysis, but after inclusion started. Improvement from 40 % complications of Clavien-Dindo grade II-V to 20 % in the laparoscopic group was considered clinically significant. Primary sample size was revised February 2020 due to slow recruitment with reduction from originally n = 218 with 109 patients in each treatment arm (90 % power, two-sided chi-square test with 5 % significance) to n = 126 with 63 patients in each treatment arm (80 % power, one-sided chi-square test with 5 % significance).

After oral and written information, informed written consent was obtained, and patients were randomised. Included patients were assigned a sequential participant number and then referred to open resection at HUH or laparoscopic resection at HDH. Computer generated block randomisation (block size 6) was used as described in a confidential protocol addendum.

Although the study was open, the randomisation list was concealed to the hospital representative at the first consultation. Further treatment and control were at the institution the patient was randomised to. Patients who declined to participate in the study were assigned to standard treatment such as described in the Norwegian National Guidelines from the health authorities (147).

Baseline and tumour characteristics were summarized using descriptive statistics. The primary clinical endpoint examined was complications grade II-V by C-D. As planned, a one-sided exact chi-square test was used to compare this between the two randomised groups. Secondary endpoints were evaluated using the two-sided exact chi-square test. Gosset's unpaired t-test (148) was used to compare operating time and lymph nodes,

whereas the Wilcoxon-Mann-Whitney test (149, 150) was performed for length of stay and intraoperative bleeding. Risk factors for complications were explored using logistic regression (151). Results were reported as odds ratios (ORs) and adjusted ORs (aORs) with 95 % confidence intervals (CIs) and likelihood ratio p-values (LR-p). OR were adjusted based on potential predictor variables and confounding factors for complications such as age, sex, BMI, smoking status, comorbidity, ASA, operating time, and operating method.
The mean postoperative remaining arterial stump length was compared using independent samples t-test (Gosset's unpaired t-test) and reported as means and standard deviations (SD). The median postoperative remaining arterial stump length was compared using Wilcoxon-Mann-Whitney test. Inter- and intraobserver variability were calculated by interclass correlation and Bland-Altman plot.

Primary clinical endpoint examined in the liquid biopsy trial was recurrence free survival (RFS). Unadjusted RFS was explored using Kaplan-Meier-plots (152), and Cox regression (153) was used for unadjusted and adjusted analyses. Results were reported as unadjusted hazard ratios (HR) or adjusted HR (aHR) with 95 % confidence intervals (CIs) and likelihood ratio p-values (p). RFS was measured from the date of the fist postoperative sampling to the verified first radiologic recurrence (distant or local) or death from colon cancer recurrence and was censored at last follow-up or non-colon cancer-related death. Potential predictors of recurrence were ctDNA positive preoperative, ctDNA positive postoperative, tumour stage (pT1-3 versus pT4), node stage (pN0 versus pN1-3), tumour differentiation (well/moderately versus poorly), morphology of tumour (adenocarcinoma versus signet ring cell carcinoma), mucinous differentiation, tumour deposit, venous invasion, and MSI-status. Statistical analyses were performed using SPSS version 26.0.0.1.

3.14 Service user involvement

To improve relevance and quality, patients, represented by member of the service user committee (HDH), were involved. The patient information and the quality-of-life scoring were discussed before recruitment started.

3.15 Ethical considerations

The study was approved by the regional committee of ethics (REK 2015/2396) and is in accordance with the *"WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects" (154)".* Patients were informed about the study orally and written before they were asked to participate. There was a separate consent for biobanking.

4. Summary of results

4.1 Paper I

Complications after open and laparoscopic right-sided colectomy with central lymphadenectomy for colon cancer: randomized controlled study

One hundred twenty-eight patients in the randomised project were included. The perioperative results were analysed and compared between the two intervention groups. The main outcome, Clavien-Dindo complications grade II-V, were compared by one-sided exact chi squared test, and was found to be equal in the two study-groups (open 42.8 % vs laparoscopic 38.4 %, p-value: 0.372). Postoperative paralytic ileus (open 15.9 % vs laparoscopic 18.5 %, p-value: 0.698) and transfusion or administration of iv iron (open 22.2 % vs laparoscopic 15.4 %, p-value: 0.322) were the most common complications registered. There were few major complications in both groups (C-D IIIb open 7.9 % vs laparoscopic 4.6 %, p-value: 0.341) and no reoperations due to anastomotic leakages. Main risk factors for complications were anaemia and operating time. Preoperative anaemia led to higher risk of transfusion or intravenous administration of iron, but not to higher risk of complications in general. The perioperative bleeding was low in both groups with 93 % of the study population experiencing intraoperative bleeding < 200 ml. Lymph node yield was equal in the two intervention groups (mean ± SEM: open 31.9 ± 1.8 vs laparoscopic 29.3 ± 1.3, p-value: 0.235).

4.2 Paper II

Short and equal vascular stump length after standardized laparoscopic and open surgery with central lymphadenectomy for right-sided colon cancer

This paper concerned surgical quality. The selected quality indicator was remaining vascular stump length after standardized oncologic colon surgery with focus on central lymphadenectomy. The measurements were conducted in the first 40 patients operated in the main project. The two groups compared were similar in age, gender, and BMI. There were no differences in lymph node count, complications, or blood-loss between the groups. Length of remaining vessel stump was measured from the 6 months postoperative CT, conducted in the portal venous phase. Two independent radiologists conducted the measurements, and both did the measures twice with an interval of at least 6 weeks. The length of the remaining stump of the tumor feeding artery (ileocolic artery) was short (4 mm) in both intervention groups. One patient in each group were not operated as intended and they had both mean length > 15 mm. Inter- and intra-observer variance were calculated. Interclass correlation was good for each observer. The interclass correlation was poor when the two observers were compared. Sub-group analysis revealed that the discrepancy occurred in the open group where no marker was present on the vessel stump.

4.3 Paper III

Assessment of postoperative circulating tumour DNA to predict early recurrence in patients with stage I-III right-sided colon cancer: Prospective observational study

50 patients with non-metastatic RCC were investigated for ctDNA using liquid biopsies. Median follow-up was 4.4 years (1613 days), range 1 to 5.6 years (351 to 2026 days). The primary analysis was by NGS Avenio panel and ddPCR for control and follow-up. We detected cancer related mutations in 49/50 patients prior to surgery. 47/50 patients had mutations eligible for monitoring with commercially available ddPCR assays. Both tumour and plasma could be source of primary gene mapping. Mutations detected by NGS were confirmed and could be monitored by ddPCR. There was no detectable ctDNA after surgery in 42/47 patients. ctDNA positive patients at first postoperative sample had high recurrence-risk compared to patients without measurable ctDNA (adjusted hazard ratio: 172.91; 95 % confidence interval: 8.70-3437.24; p-value: 0.001). Patients with positive postoperative ctDNA experienced recurrence within mean < 6 months. An additional five patients recurred during surveillance. Three of them turned positive during monitoring (34, 22 and 9 months after surgery), of which two of them prior to radiologic verified recurrence, and one after. The ctDNA negative patients had their recurrence discovered after mean 2.3 years.

5. Discussion

5.1 How can we measure surgical quality?

What is the definition of quality? According to Cambridge Dictionary, it is *"how good or bad something is"*. According to Oxford Languages, *"the standard of something as measured against other things of similar kind; the degree of excellence of something"*. When searching for the term, there is no uniform definition. A modern definition, though, is *"fitness for intended use"*. As for surgery, intended use for whom? Patient, surgeon, society? Undeniable, quality must be assessed from different angles.

The development of oncologic surgery has undergone leaps since the early 20th century when perioperative mortality rates were 50 % and almost all patients experienced recurrence. The expectations for surgery today are completely different than they were hundred years ago. With increasing perioperative survival rates, patients' anticipations of postoperative functional performance rise. The lack of definitions of surgical procedures and diverging terminology is a major problem when comparing different surgical approaches. The lack of a definition of *quality* makes it further challenging to measure and compare surgical quality. We do also need to address the case-mix and adjust for the patients' risks if reported surgical quality measures are to be meaningful (155). We need tools to perform quality assessment. It is important that the quality indicators are relevant and represent an opportunity for improvement (135, 156).

5.1.1.1 The patients' point of view

When undergoing surgery for malignancies, most patients' priority is to be cured of their disease. This can be measured by long-term survival. Every single day after surgery, as long as the patient stays alive, it is the quality of life that matters. Complications after major intraabdominal surgery have impact on patients' quality of life (157). For older patients in particular, quality of life and functional performance can take priority above length of life.

5.1.1.2 The surgeon's point of view

Surgeons are convinced that a well performed surgery will be beneficial for the patient both in short- and long term, with high survival outcomes as the ultimate goal. The surgery is performed with the intention of achieving certain general or procedure specific benchmarks. General benchmarks can be avoidance of complications, short length of stay, low 30- or 90 days mortality. Procedure specific benchmarks for oncologic colon surgery can be lymph node count, integrity of specimen and cancer specific long-term survival. The goal is to perform the best possible surgery at the lowest possible cost for the patient.

5.1.1.3 The society's point of view

Complications after intraabdominal surgery affects up to 40 % of patients. Postoperative complications have a huge impact on the health care system due to increased length of stay and the need for a higher level of care after discharge. Treatment of complications represent an increased use of resources in the specialist health care system by for example inducing the need for intensive care. Disability due to loss of function can be the long-term consequence of postoperative complications.

5.1.2 Complications

The lack of a common classification system is a problem when interpreting and comparing complications. The Clavien-Dindo classification, initiated in 1992 and later revised (139, 140), has provided a common language for evaluation. The classification is based on the therapy needed to correct the complication(s). It has proven to be valid and applicable (141). It is widely used, although other classifications exist. The authors themselves did a five-year survey in 2009 where they discussed and specified how the classification is to be utilized. They presented different cases for colleagues from institutions worldwide and discovered divergence. The first concerning how the classification is referred to in literature. Different terms as classification, Clavien, revised Clavien, Dindo and so on. It is confusing, especially as the first classification was proposed by Clavien alone and was less detailed. The correct term is Clavien-Dindo classification (C-D). Next controversy concerned the interpretation when more than one complication occurs. The consensus in the group was to interpret only the most severe if the complications were clearly related and possible natural progression from each other. Unrelated complications are to be reported separately. All negative events occurring after surgery should be recorded, regardless of whether there was a clear correlation to the surgery performed or not. The last controversy concerned the

interpretation of negative explorative laparoscopy/laparotomy caused by suspicion of an abdominal complication. This is not to be reported if the patient recovers uneventful (141).

The tool to report and grade complications exists, but what about confounding factors? Do complications only reflect the quality of surgery? The relation of some patient characteristics and complications are established. Male gender, smoking, alcohol abuse, high BMI, steroid use, diabetes, cardiovascular disease, lung disease, age ≥ 75 years, and ASA ≥ 3 are patient characteristics associated with elevated risk for complications in colon surgery (158-161). Comparing complication rates in different surgical approaches requires equal patient selection in the two groups compared. A pitfall is too strict inclusion criteria. The actual patient population with colon cancer is often elderly and co-morbid. The operation studied must be feasible for the actual disease-population. It is crucial to explore whether a new operative technique gives rise to more complications than the established method, especially when more radical than previous technique. It is important to monitor whether a new range of complications occurs as for example chylous ascites after central lymphadenectomy (162). In this trial we did not find an increased incidence of complications. There was no C-D grade IV or V complications, no anastomotic leaks, and a low incidence of C-D grade IIIb complications. This indicates that surgery with dedicated, skilled surgeons results in good outcomes. The result was in accordance with previous studies evaluating volume and quality (163), and can possibly influence long term survival. Complications were mainly C-D grade II with transfusion and postoperative paralytic ileus. Risk factors for complications were anaemia and operating time. Like other trials, we found significantly longer operating time in the laparoscopic group (114) and confirmed increased risk for complications with increasing operating time in minutes (164, 165). The patient cohort was representative for the disease population with mean age 70 years.

5.1.2.1 Transfusion

According to the Clavien-Dindo classification for surgical complications, transfusion with red blood cells or infusion of iron postoperatively are grade II complications. The threshold for transfusion varies among surgeons and with patient comorbidity. Anaemia is defined according to the World Health Organization (WHO) as a value of Hb < 13 g/dL in men and Hb < 12 g/dL in women. As up to 75 % of patients with RCC are anaemic prior to surgery, the rate of postoperative red blood cells transfusions or infusions of iron says more about the disease and routines for transfusion than about the surgical treatment itself. This is confirmed in our material where preoperative anaemia was the risk factor for transfusion. Anaemia was associated with transfusion as a postoperative complication, but not with complications when transfusion only was excluded. Transfusion rate was not related to the amount of intraoperative bleeding. The way intraoperative blood loss is reported is

also ambiguous. Rather than measuring blood loss, the anaesthetic nurse estimates blood loss in ml based on the amount of bloodstained surgical swabs. Nevertheless, cancer associated anaemia may affect clinical outcomes. The association of transfusion and 30-days postoperative complications are established through several studies (166), but it does not appear if transfusions itself are excluded from the complication registration. Reduced recurrence free survival (RFS) and reduced five-year overall survival (OS) in patients with preoperative anaemia and postoperative transfusion have been reported (167), although no causal relationship was established.

5.1.2.2 Postoperative paralytic ileus (POI)

POI after intraabdominal surgery is a major cause of prolonged length of stay and readmission (165, 168, 169). Impairment of bowel function after colon surgery is a normal process but should usually resolve in 2-3 days. The incidence varies with varying definitions (168, 170). The incidence after right-sided colectomy is higher than after distal colon resection of unknown reasons (171, 172). In this trial POI was the second most frequent complication after transfusion. Equal incidence in the open and laparoscopic group could be attributable to the long operating time in the laparoscopic group (227 min).

5.1.2.3 Reoperation

Blank re-laparoscopy/laparotomy is not to be labelled complication according to the Clavien-Dindo classification if the patient recovers uneventfully. This is important, as the threshold for explorative laparoscopy should be low if complications are suspected. Reoperation for intra-abdominal infection and wound complications are the most common reoperations after colorectal surgery (173), and leads to prolonged length of stay. The incidence of anastomotic leaks varies for colorectal surgery with increasing incidence for distal anastomoses. The incidence of anastomotic leaks after right hemicolectomy varies in the literature from 3-7.5 % (174-176). Reoperation for intra-abdominal infection confers increased risk of mortality in addition to association with reduced cancer related outcome for anastomotic leak (177). As anastomotic leaks can be associated with poorer cancer-related outcome, it is crucial to minimize the incidence. Reoperation for superficial wound infection/wound dehisce is not associated with increased mortality (178). No reoperation in this trial was due to anastomotic leaks. C-D grade IIIb were low with 8 % in the open group, all due to wound dehiscence, and 5 % in the laparoscopic group due to mechanical bowel obstruction (2 patients) and bleeding from the abdominal wall (1 patient). One patient in each group was reoperated with blank laparotomy/laparoscopy.

5.1.3 Length of stay (LOS)

Length of stay is a commonly reported quality indicator. There are diverging ways to report LOS. Some report LOS including readmissions during the first 30-postoperative days, others report only primary LOS without readmission. LOS is related to complication rate (179), but other factors as age, comorbidity, living alone, discharge to another institution and the patient's mental health and expectations are also factors

that can contribute to longer LOS without a clear reflection of the surgical quality. Reporting LOS including readmission is better than only primary LOS, but LOS is not a parameter that displays the quality of treatment in itself. An evaluation of *"fit to depart"* related to certain objective parameters as lack of nausea, regain of nutrition and adequate pain relief might reflect quality in a better manner, but this neither considers readmission. The length of stay in this trial was short. Total length of stay was 5 days in the open group and 4 days in the laparoscopic group. Median length of stay in patient without complications were 4 and 3 days, respectively.

5.1.4 30- and 90- days mortality

A commonly referred quality indicator is 30-days mortality rate. There are studies comparing 30-days and 90-days mortality rates, which claims 90-days mortality (180) gives a more truthful impression, especially for elderly patients. According to a German consensus article, a complete 90-days mortality registration was registered for only 1/3 of the patients (181). The problem is that 30 days mortality rate is most common in use and comparison is impossible if we do not report the same parameters. 30 days is also the period in which complications traditionally are registered. 30 days mortality rates are commonly used, but 90 days mortality is a more comprehensive and better parameter. In this trial 90-days mortality was 0 %.

5.1.5 Patient reported outcome measures (PROM)

Consensus recommendations on quality assessment includes patent reported outcome measures (182). This can be Health-related quality of life (HRQoL). There exist numerous tools for HRQoL scoring. Some questionnaires are general like 15 D (183) exploring different dimensions in daily life. Other questionnaires are disease specific. As multiple tools are available, comparison is difficult. Another difficulty is the timing of the score. When is the optimal timing for assessing postoperative quality of life? Up to 40 % of colon cancer patients have histopathologic indication for adjuvant chemotherapy. HRQoL 6 months postoperative are dependent on the (adverse) effects of chemotherapy as well as the consequences after surgery. Patients included in this trial were assessed preoperatively, 6 months postoperative and after 5 years surveillance by the 15 D questionnaire. Results are not yet analysed.

5.1.6 Long-term effects

Long-term effects after oncologic colon surgery are under-investigated. The incidence of additional surgical procedures due to incisional hernia or bowel obstruction is not clear. For this trial the incidence of additional procedures will be published after five years surveillance. The functional impact of bowel resection is not fully investigated (184). The length of surveillance and systematic registration will influence the incidence of long-term effects.

5.1.7 Survival

To conduct RCTs comparing survival between different surgical approaches is difficult. These trials need a huge number of participants to have statistical power to

detect survival differences. The trials that confirmed the position of laparoscopy in colorectal surgery did not show decline in survival in the laparoscopic group compared to the open group, but most of them were not dimensioned to prove differences in survival outcome (56, 59, 185, 186). A related problem is the timespan. Development of surgical technique is a continuous process, and for long lasting studies the primary techniques often differ to some extent to the final techniques and the comparison thus becomes uncertain. For this trial the primary endpoint was complications by C-D from which the power calculation was performed. Long term results will be published when 5-year surveillance is completed.

5.1.8 Lymph node count

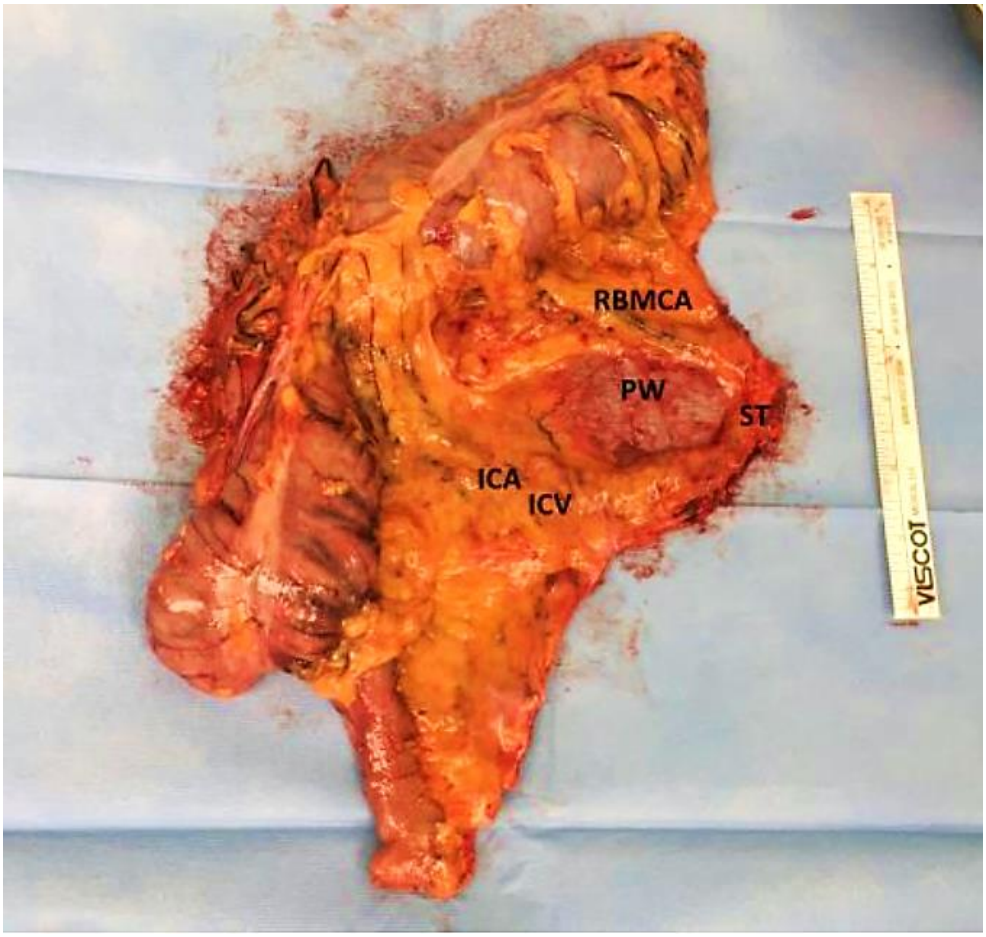
Lymph node count is a widely used quality indicator, but its role as an actual indicator of surgical quality can be questioned (187). Factors such as patient age, tumour localization and stage, evaluation by multidisciplinary team, method used for lymph node detection, dedication of the pathologist and the surgery itself have shown to influence the lymph node yield (188-190). Anatomical studies show that the number of lymph nodes varies individually in rectal cancer (191, 192). Results can be transferable to colon cancer. Advanced tumour stage and individual immune response might affect lymph node count. High lymph node harvest can be an indicator of the patient's immune response to the tumour well as much as the surgical radicality (193). CME-surgery adheres to three essential components, namely integrity of the mesentery, central vascular tie to accomplish central lymph node dissection and removal of adequate length of bowel. Increased lymph node yield may be due to extensive removal of bowel and the lymph nodes removed are not necessarily relevant for the tumour. The Japanese approach to oncologic colon surgery differs from the Western approach with resection of shorter length of bowel around the tumour-bearing segment without deterioration in prognosis (194-197). A commonly accepted benchmark for colon cancer surgery is the detection of 12 lymph nodes in the specimen. This consensus was reached early 2000s after various studies indicating this as a minimum threshold for correct staging (198) and increased likelihood to identify lymph node metastasis when analysing many nodes (199, 200). Reaching the threshold of 12 lymph nodes have different implications in patients with a high total number of mesenteric lymph nodes compared to someone with a low total lymph node count. The first scenario has the potential for many remaining unexamined nodes. Improved lymph node harvest did not lead to upstaging in a Norwegian material analysed before and after implementation of the 12-node benchmark (201). There is an increase in survival associated with higher lymph node count that cannot be accounted for by upstaging. The causal relationship has not been proved, and these studies are observational, not RCTs. Performing a standardised and central lymphadenectomy and meticulous evaluation of lymph nodes is essential to stage the patient correct. This in turn is necessary to perform risk stratification based on the TNM-staging system. In this trial dissection along the left arterial border instead of superior mesenteric vein did not lead to higher lymph node yield. The number of lymph nodes did not differ

significantly between the open and laparoscopic group. The use of lymph node count as a quality marker for radical surgical technique relies on proper assessment of the specimen, and it is important to acknowledge that many confounding factors exists.

5.1.9 Evaluation of the specimen

Evaluation of the specimen facilitates comparison of quality. BMI and the volume of the mesentery are associated (194). Calculation of the volume of the mesentery is an observation of rather scarce value in quality assessment. Both the distances from colon to the vascular pedicle and the distance from the tumor to the vascular pedicle have been measured to evaluate the quality of the specimen. These distances vary individually as well as the volume of the adipose tissue. Evaluation of these parameters are therefore inaccurate (202, 203) for measuring surgical quality. The quality of mesocolon is evaluated by inspection of the integrity of the embryological fascial envelope of the specimen. Current grading system, introduced by West (204), is valuable, and has contributed to increased quality of pathological evaluation, although results from these evaluations reportedly vary across pathologists (74). The main shortcoming is the unilateral focus on the evaluation of tears in the mesenteric fascia. Furthermore, the shape of the removed mesentery or potential central remnants indicating residual disease, are not evaluated. The proposed classification by Benz and colleagues (202) takes both the extent of lymphadenectomy and the completeness of mesocolon into consideration by the use of anatomical landmarks in the specimen.

Figure 17 Example of Type 0 (high quality) specimen according to *Benz S, Tannapfel A, Tam Y, Grunenwald A, Vollmer S, Stricker I. Proposal of a new classification system for complete mesocolic excision in right-sided colon cancer. Techniques in coloproctology. 2019. Photo by Kristin B. Lygre*



The evaluation is performed on photography of the specimen after resection but could also be implemented by the pathologist in the macroscopic evaluation of the specimen. The evaluation and classification of the specimen is valuable in assessing quality. The new classification system should be established as standard as it also takes the central lymphadenectomy in account and could be a new basis for comparing surgical quality. Although evaluation of the specimen is feasible, it is a surrogate for the actual target. Namely, are there any remnants at the surgical site that is of importance for the patient's prognosis?

5.1.10 Evaluation of the surgical site

Many colon cancer patients are included in a postoperative surveillance protocol including CT scans. These scans can be used to assess anatomical structures postoperative and evaluated potential remnant tissue. With the development of and integration of digitalized photo equipment in the operating theatre, the possibilities for documentation are unlimited. This facilitates an objective evaluation of the surgical site. A classification system based on anatomical landmarks at the surgical site can be a major contributor to standardization of surgery and allow objective evaluation and comparison. The evaluation of the surgical site is no surrogate, but the target for evaluation of potential remaining mesenterial tissue.

5.1.10.1 Length of remaining vessel stump

As lymph node distribution follows the main feeding arteries of organs, knowledge of vascular anatomy is crucial in oncologic colon surgery. The complex vascular anatomy supplying the right colon involves multiple branches from both the vein and artery (78, 79) and makes surgery for RCC more demanding than distal colon cancer surgery (205). It is especially important to depict the vascular anatomy to achieve adequate lymph node dissection and avoid complications. Measurement of the remaining vascular stump length is accessible retrospectively and is a more objective assessment than unilateral evaluation of the specimen (76, 206-208). The remaining vascular stump length is a surrogate for central lymphadenectomy in oncologic colon resection (61, 71, 209). Previous studies have shown that the remaining vascular stump after colectomy for RCC has mean length varying between 28 to 50 mm (75, 207, 208, 210). Although evaluation of the remaining vascular stump length has advantages over unilateral evaluation of the specimen, a central vascular ligation is no guarantee that an adequate central lymphadenectomy has been performed. The measurements can vary between observers, and the vessel stump can be over or underestimated due to misinterpretation, presence of "stump granuloma" (overestimation due to visible mass) or thrombotic clots (underestimation due to short contrast filled lumen). The accuracy of measurements increases with the use of a marker like metallic radiopaque clips on the vessel stump. To increase the quality of assessment of remaining vascular stump length, a photo of the vessel tie can be added to consider the extent of remaining tissue around the vessel branch. In this trial the length of the remaining vascular stump was short (4 mm) and equal in the two study-groups. The results differed between the observers for measurements in the open group where no marker was present.

5.1.11 The impact of the surgeon

The association of volume and good outcome is contentious. Several observational studies have been performed where either hospital- or surgeon- volume have been analysed as a risk factor for outcome measures such as operative mortality, 5-year mortality rates, complications, or anastomotic leaks. Results are diverging, and the quality of data is poor. The definition of high- and low- volume differs and contributes to contentious results. However, there are indications that specialisation and high case load for the individual surgeon is associated with favourable patient outcome, even

more than hospital volume (163, 211). In a Dutch survey amongst surgeons about which factors are important for achieving good outcomes in colon cancer surgery, the statement *"Elective surgery is performed by surgeons with a specialisation in gastrointestinal oncology"* was assigned the highest score (212). Six of the ten perceived most important factors for good hospital performance on colorectal cancer surgery involved *"surgeons with specialisation in gastrointestinal oncology"*. High case load and experience lead to good intraoperative decision-making. This can be due to dedicated training and practice and in-dept knowledge of surgical anatomy and pathology. The level of technical skills among surgeons differs. There is an association of the surgeons' level of technical skills and patient outcomes. The level of complications was found to be significant lower in surgeons with high level of technical skills compared with low level of technical skills (213). The complexity of right-sided colectomy leads to a greater gap in performance between experienced and unexperienced oncologic surgeons when compared to left sided colectomy (205). The surgeons in this trial were selected based on technical skills and experience with the operative approach they performed. The age range between the most and least experienced surgeon was 20 years.

5.1.12 Summary of quality assessment

A prerequisite for quality assessment is accurate terminology or unequivocally description of surgical procedure. For RCC surgery the terms (CME, D2, D3) are disturbed by different use and definitions and should therefore be avoided. A description of the procedure based on exposure of anatomical structures is recommended and should be photo documented. Quality should be assessed for the actual disease-population, and too strict inclusion criteria should be avoided. New procedures should be introduced for low-risk patients initially, but documentation of applicability for a broader patient population should be available. Complications by Clavien-Dindo, total length of stay including readmissions, 90-days mortality-rates, HRQoL and long-term survival are applicable general benchmarks. Procedure specific benchmarks should include evaluation of the specimen included lymph node evaluations, evaluation of the surgical site with photos and interpretation according to exposure of anatomical structures and long-term cancer related survival. Achieving quality is a continuous process which demands a systematic approach, registration, and comparison of results.

5.2 Prognostication and the use of biomarkers

During the years since this trials' beginning, the number of publications regarding ctDNA in non-metastatic colon cancer have exploded. When planning the trial, the existing publications were concentrated on metastatic cancer and monitoring of treatment response. The role of ctDNA in not-metastatic colon cancer was unexplored. The last few years an increasing number of such publication have appeared (100, 101,

103). The aim of the biomarker sub-study was to investigate whether ctDNA provide additional information about prognosis beyond established risk stratification, and to explore best timing and source of primary gene mapping. The biomarker trial was launched in the already existing RCC-trial where RCC was chosen based on adverse prognosis, as well as proposed distinct biological entity among colorectal cancers. The study confirmed that both tumour and plasma could be source of primary gene mapping. ctDNA was detectable preoperatively in 66 % of stage I-III RCC (214). Furthermore, mutations detected by NGS could be confirmed and monitored by ddPCR. The major finding was that presence of postoperative ctDNA is a strong predictor for early recurrence.

5.2.1 Mutational landscape

Mutated key oncogenes and tumour suppression genes were comparable with rates from The Cancer Genome Atlas Dataset. There was a higher proportion of TP53 (58 vs. 34.8 %) and BRAF (38 vs. 24.2 %) mutations than other materials with RCC (transverse excluded). The presence of APC (58 vs 63.6 %), KRAS (50 vs. 45.5 %), PIK3CA (24 vs 27.3 %) and NRAS (12 vs. 7.6 %) were comparable (35).

5.2.2 Source of primary gene mapping

Both plasma and tumour tissue can be the source for initial genomic profiling. Plasma is promising for cancer patients when tumour biopsies are not available and has been shown to be a good option for patients receiving neo-adjuvant treatment before surgery (215). Plasma will theoretically reflect the intra-tumoral heterogeneity better than a tumour biopsy (216-219). There was little discrepancy between variants selected for monitoring by NGS in tumour and plasma. There was no significant gain in capturing tumour heterogeneity by performing NGS on plasma rather than tumour. Both plasma and tumour could be reference material for detecting markers for monitoring, even in cases where only one tumour biopsy was analysed. In addition to intra-tumoral heterogeneity, there is a risk of altered mutation profile due to clonal selection during treatment and surveillance (220, 221). To increase the likelihood to capture relevant changes, two variants were followed when possible.

5.2.3 Timing of postoperative sampling

Half-life of cell free DNA is short (minutes to hours). Due to the surgical trauma, the levels of cell free DNA were high (median 38.95 ng/ml) early postoperative (2-7 days). This causes dilution of the ctDNA concentration and makes it difficult to detect cancer specific mutations. Dilution effect is less relevant for patients with tumours that shed high levels of ctDNA. For patients with low tumour burden and less ctDNA, the dilution effect can result in undetectable ctDNA early postoperative. Negative postoperative ctDNA can be related to detection threshold and should be interpreted with caution. Levels of cell free DNA dropped one month postoperative (median 7.9 ng/mL). Four

weeks after operation is a good time point for postoperative sampling (222) and allows interpretation of the results before the recommended startup for ACT.

5.2.4 Methods for monitoring of ctDNA

NGS and ddPCR are complementary methods for monitoring ctDNA. NGS is highly sensitive with unique molecular identifier and digital error suppression and give a broad mapping of the genetic profile. It is labour intensive and expensive (223) with significant turnaround time. ddPCR is sensitive, robust, and cost effective for detection of selected mutations. To identify MRD, represented by ctDNA, with targeted analyses like ddPCR, selected mutation must be present in all cancer cells. ddPCR holds potential for missing relevant mutations by selection of a suboptimal surveillance marker. It is known that intra-tumour mutational heterogeneity can be present (224, 225). Selection of surveillance mutations without knowledge of the actual mutation profile is the strategy in many ctDNA trials (108). With this tumour agnostic approach, a negative result by ddPCR is not synonymous with negative ctDNA or no MRD present. It only confirms that the selected mutation is not present. In this trial, a broad-coverage NGS assay for initial mutation profiling for plasma, tumour or both was chosen. The probability of detection of relevant mutations increased and allowed monitoring of eight patients (17 % of the patient cohort) lacking classical codon 600 BRAF and codon 12/13/61 KRAS mutations. They would not be included without a priori knowledge of tumour genotype. Monitoring of ctDNA was performed by ddPCR, and criteria for selection of surveillance markers were that mutations were detected by NGS and confirmed by ddPCR with commercially available assays. There was a high concordance between NGS and ddPCR with 100 % confirmation of NGS with ddPCR for tumour and 79 % for plasma. Selection bias was reduced with broad NGS-based approach. The high concordance and cost effectiveness of ddPCR makes it possible to implement in routine diagnostics, especially for surveillance.

5.2.5 Predictive value of positive postoperative ctDNA

In accordance with previous studies, this trial confirms that ctDNA is a marker for MRD (100-102, 226). Surgery is the curative treatment for localised colon cancer and successful surgery should theoretically lead to undetectable postoperative ctDNA. Exploration of MRD after surgery is not included in traditional risk assessment. Early postoperative ctDNA positivity was associated with risk of recurrence, whereas traditional risk stratification variables were non-significant. Five of ten patients with recurrence were positive for ctDNA in their first postoperative sample. The positive predictive value of postoperative positive ctDNA was 100 %. Recurrence was low in the negative group (11 %) but is not negligible. Traditional tools for surveillance are limited to coarse diagnostics as CEA measurement and CT imaging. CEA does not detect recurrence at an early stage (215, 227, 228), whereas CT scanning has a threshold of 5-10 mm for detection of lesions, and often yields unspecific findings (93-95). Due

to limited number of patients and sampling period in this trial, evaluation of the role of ctDNA as a diagnostic tool for early detection of recurrence was restricted and we cannot evaluate the predictive precision of ctDNA during surveillance. Analysis of the remaining study population, with complete surveillance of five years, may clarify this.

5.2.6 Future perspectives

Today's selection criteria for ACT in colon cancer are ready for revision. ctDNA holds potential to guide a tailored adjuvant treatment decision. The clinical breakthrough would be if ctDNA-status could select the high-risk stage II patients who benefit from ACT and identify the stage III patients with no advantage of adjuvant treatment. The potential for downscaling of ACT or possibly skip adjuvant treatment for selected patients is currently being further explored in clinical intervention trials with ctDNA-guided management (229-235). Additional information from ctDNA-status compared to established risk stratification is difficult to interpret, especially for ctDNA negative patients. Postoperative ctDNA status alone cannot yet guide treatment decisions but can supplement traditional risk stratification. ctDNA is a reliable predictor for early recurrence by detecting MRD. To implement this in routine diagnostics the result of the test must have implications for treatment decisions, and the endpoint must be modulable with existing treatment options. Real-time analysis of ctDNA is cost- and labour intensive, especially in Norway with scattered populations. The turnaround time is currently too long. The logistic necessary to sample, prepare samples and analyse is also cost- and labour intensive. Although ctDNA holds potential to guide treatment decisions, the knowledge is not established yet. The information of a high risk of recurrence is of no value if there are no possibilities of changing the course of the disease. The gain for the patient with todays' knowledge is currently not established and it is currently not ready for implementation in routine diagnostics.

5.3 Proposed level of central lymphadenectomy

With a low level of intra- and postoperative complications when performing central lymphadenectomy, the procedure was considered safe. There was little extra gain in performing dissection along the left side of the superior mesenteric artery with no significant difference in lymph node yield. Therefore, our suggested level of central lymphadenectomy in right-sided colon cancer is denudation of the superior mesenteric vein. The surgery should be performed by experienced surgeons with good technical skills for good postoperative patient outcomes.

6. Conclusion

Standardised oncologic right-sided colectomy performed by experts resulted in good short-term outcome and did not differ between open and laparoscopic approach. No patients were reoperated on due to anastomotic leaks. There was a low incidence of major complications (C-D $\geq$ IIIb in both groups. Assessment of the surgical site by measurement of the remaining vascular stump length of the tumour feeding artery (ICA) confirmed short vessel stump in both groups (4 mm). ctDNA is a precise predictor for

early recurrence by detecting MRD. Postoperative positive ctDNA predicts recurrence with 100 % accuracy.

7. References

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