Diplomarbeit

Small Platelets and Prognosis in Patients with Colorectal Cancer

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Graz, am 13.12.2018 *Dominik Barth eh*

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Zusammenfassung

Hintergrund: Die Rolle des mittleren Thrombozytenvolumens (MPV) als Prädiktor des Krankheitsverlaufes und Therapieansprechens wurde bereits bei verschiedenen Tumorarten untersucht, unteranderem auch beim kolorektalen Karzinom (KRK). Allerdings fehlen noch immer Daten zum prognostischen und prädiktiven Wert der Thrombozytengröße beim KRK über mehrere systemische Therapielinien.

Material und Methoden: In diese retrospektive Single-Center-Studie wurden 690 Patientinnen und Patienten mit UICC Stadium II, III oder IV KRK, die eine adjuvante und/oder palliative Chemotherapie erhalten hatten, inkludiert. Die primären Endpunkte waren das 3-Jahres-rezidivfreie Überleben in adjuvanter beziehungsweise das 6-Monats-progressionsfreie Überleben in palliativer Behandlungssituation, sowie nach Einstellung aller kurativen Therapieversuche im Sinne einer „best supporitve care“ (BSC) das 6-Monats Gesamtüberleben. Kaplan-Meier Schätzer, log-rank Tests und uni- und multivariable Cox Modelle wurden zur Analyse von rezidiv-freiem, progressionsfreiem und Gesamtüberleben verwendet. Der Cut-off-Wert zur Definition von Patientinnen und Patienten mit niedrigem MPV wurde empirisch bei der 25-Perzentile der MPV Verteilung in der jeweiligen Behandlungssituation gewählt.

Ergebnisse: Das 3-Jahres-rezidivfreie Überleben lag bei 76%. Das mittlere progressionsfreie Überleben in Erst-, Zweit- und Drittlinientherapie betrug 59%, 37% beziehungsweise 27% und das mittlere Gesamtüberleben in BSC Setting 31%. Kleine Thrombozyten, definiert als niedriges MPV, waren kein Prädiktor für ein kürzeres rezidiv-freies Überleben. Außerdem konnte in den ersten drei palliativen Therapielinien kein konsistenter Zusammenhang zwischen niedrigem MPV und verkürztem progressionsfreiem Überleben beobachtet werden. In der „best supportive care“ hatten Patientinnen und Patienten mit niedrigem MPV zwar ein kürzeres Gesamtüberleben, dies war jedoch nicht statistisch signifikant. Erhöhtes MPV war kein konsistenter Prädiktor der Therapieansprechrate in allen drei palliativen Therapielinien.

Schlussfolgerung: Diese Studie zeigt, dass die Thrombozytengröße kein Indikator für Krankheitsverlauf und Therapieansprechen sind und daher keinen Einfluss auf klinische Entscheidungen hat.

Abstract

Background: The role of mean platelet volume (MPV) as a predictor of outcomes in various cancer entities including colorectal cancer (CRC) has already been analysed. However, data on the prognostic and predictive value of small platelets in CRC over multiple lines of systemic therapy are still missing.

Material and Methods: In this retrospective single-centre cohort study, 690 patients with UICC stage II, III or IV CRC receiving adjuvant and/or palliative chemotherapy were included. Primary endpoints in the adjuvant setting, palliative setting and best supportive care (BSC) setting were 3-year recurrence- free survival (RFS), 6-months progression- free survival (PFS), and 6-months overall survival (OS), respectively. Kaplan-Meier estimators, log-rank tests, and uni- and multivariable Cox models were used to analyse RFS, PFS and OS. A cut-off defining patients with low MPV was chosen empirically at the 25th percentile of the MPV distribution in the respective treatment setting.

Results: Three-year RFS was 76%. Median 6-month PFS estimates in 1st, 2nd, and 3rd line therapy were 59%, 37%, and 27%, respectively, and median 6-month OS in BSC was 31%. Small platelets as indicated by low MPV did not predict for shorter RFS. Also, in the first 3 palliative treatment lines we did not observe a consistent association between low MPV and decreased 6-month PFS. In the BSC setting, patients with low MPV had numerically but not significantly shorter OS. Higher MPV levels did not consistently predict for ORR or DCR across the first 3 palliative treatment lines.

Conclusion: This study shows that the platelet volume does not predict CRC outcome, and thus is hardly useful for influencing clinical decision-making.

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Abbreviations

5-FU *5-Fluorouracil*
APC ..... *adenomatous polyposis coli*
BMI ..... *body mass index*
BSC ..... *best supportive care*
CEA ..... *carcinoembryonic antigen*
CIMP ..... *CpG island methylator phenotype*
CRC ..... *colorectal cancer*
DCR ..... *disease control rate*
EGFR ..... *epidermal growth factor receptor*
FAP ..... *Familial adenomatous polyposis*
FIT ..... *fecal immunochemical test*
gFOBT ..... *guaiac-based fecal occult blood test*
HNPCC ..... *hereditary non-polyposis colorectal cancer*
HR ..... *Hazard Ratio*
IBD ..... *inflammatory bowel disease*
MMR ..... *Mismatch repair deficiency*
MPV ..... *mean platelet volume*
MSI ..... *Microsatellite instability*
MSI-H ..... *microsatellite instability - high*
MSI-L ..... *microsatellite instability - low*
ORR ..... *objective response rate*
OS ..... *overall survival*
PFS ..... *progression-free survival*
PPV ..... *positive predictive value*
RFS ..... *recurrence-free survival*
UICC ..... *Union for International Cancer Control*
VEGF ..... *vascular endothelial growth factor*

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1 Introduction

Colorectal cancer (CRC) is the third most common cancer among both men and women. However, due to changes in risk factors and the increased attendance in screening programmes in developed countries, incident rates have been declining over the last years (1). Nevertheless, 30% of patients with UICC stage II or III experience recurrence after resection in curative intention, 80% of which have stage III disease at diagnosis (2). Approximately 20% of all patients present with initially metastasized UICC stage IV disease at diagnosis (3). Therefore, it is crucial to find cost-effective and reliable prognostic biomarkers to identify patients at high risk of local or distant recurrence as well as for outcome prediction in metastasized CRC (4).

The mean platelet volume (MPV), a marker for platelet activation, is easily available in routine blood tests and has already been demonstrated to be a predictor of thrombotic events in patients with cardiovascular and cerebrovascular disease (5). Furthermore, an association of MPV and the risk of venous thromboembolism in cancer patients has been found (6). Evidence suggests that activated platelets might also play an important role in tumour progression by interacting with various cell types and participating in tumour proliferation related processes (7). In addition, platelets have been shown to promote cancer angiogenesis by releasing angiogenic growth factors such as the vascular endothelial growth factor (VEGF) (8).

Altered MPV has previously been analysed as a prognostic and predictive biomarker for various tumour entities including cancers of the lung, bladder, kidney, endometrium, stomach and pancreas (9-14). In CRC it has been shown that higher values of MPV relate to the presence of this cancer entity, shorter overall survival rates (OS), as well as detrimental effects on progression-free survival (PFS) (15-18). However, to the best of our knowledge MPV and its impact on the recurrence-free survival in the adjuvant setting as well as its prognostic and predictive value over multiple palliative treatment lines and best supportive care have not been investigated yet. The aim of this study is to fill in this gap and evaluate the predictive and prognostic potential of pre-treatment MPV in both the adjuvant and palliative setting in CRC.

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1.1 Epidemiology

Colorectal cancer is the third most commonly diagnosed cancer among both men and women and the second (males) or third (females) most common cause of cancer death (1).

There are an estimated 1.4 million cases and 693,900 colorectal cancer associated deaths in 2012 worldwide, however, a difference in incidence rates can be found between developed and developing countries. Approximately 55% of colorectal cancer patients live in developed countries, with the regions of the highest incidence rates to be Australia and New Zealand, Europe, and Northern America (19,20).

In Austria the overall incidence rate is about 55 per 100,000 people per year, with a higher incidence in males (72.3/100,000/year) than in females (42.2/100,000/year). The probability for colorectal cancer until the age of 75 years is 3.6% in men and 2.1% in women (21).

Due to changes in risk factors, the use of screening methods such as the faecal occult blood test, and the introduction of and increased attendance in colonoscopy-based screening programmes, incident rates declined by 3% per year from 2004 to 2013 (1,19,22).

The median age at diagnosis depends on the localization and is 63 years in men and 65 years in women for rectal cancer and 69 years and 73 years for colon cancer, respectively (22).

1.2 Etiology and Risk Factors

1.2.1 Genetic Factors

Only 5% of all colorectal carcinomas are hereditary with an underlying genetic syndrome characterized by high penetrant inherited mutations, such as hereditary non-polyposis colorectal cancer (HNPCC), the so-called Lynch-Syndrome, or familial adenomatous polyposis (FAP). However, approximately 30 - 35% have an underlying familial background with genetic polymorphisms and low-penetrance susceptibility loci (23,24).

These patients can be divided into two groups, the first of which consists of patients who meet the Amsterdam I or II criteria, but do not have DNA mismatch repair (MMR) deficiency. Patients with this so-called high-risk familial or non-syndromic

colon cancer are at lower risk of developing an invasive carcinoma than in Lynch-Syndrome. Furthermore, they become diagnosed 10 years later and do not develop microsatellite instability (MSI) (23).

The second group are patients with common familial-risk colon cancer who have at least one first-degree relative diagnosed with colorectal cancer. Having a first-degree relative being diagnosed at the age of 50 years or older or a first-degree relative being diagnosed at 45 years or younger is associated with a 2 – 3 or 3 – 6 times higher risk, respectively (23).

However, the individual risk does not only depend on the age at diagnosis of the relatives, but also on the age of the person at risk. Lower age is associated with an increased risk, whereas higher age is linked to a decrease in risk. The prevalence of having at least one first-degree relative diagnosed with colorectal cancer is between 3.6 and 10% (25).

A US-study (26) has shown that also an increased number of affected first-degree relatives or one first-degree relative combined with one or more affected second- or third-degree relatives is related to a further increase in risk.

1.2.1.1 Lynch-Syndrome

Lynch-Syndrome or hereditary non-polyposis colorectal cancer (HNPCC) is the most common form of hereditary colorectal cancer. It is an autosomal dominant inherited syndrome. Single allele mutations in germline cells in one of the DNA-mismatch repair genes such as MLH1, MSH2, MSH6 and PMS2 leave only one remaining functional allele of the affected tumour suppressor gene. A second event in somatic cells (two-hit hypothesis) causes the remaining second copy of the gene to completely lose its function and results in an accumulation of mutations and an increased likelihood of malignant degeneration (27).

In up to 90% of cases there is a mutation in MLH1 or MSH2, MSH6 accounts for 10%, however, PMS2 mutations are rare. Between 50 and 80% of patients with Lynch-Syndrome develop colorectal cancer through their life time (23).

Typically, the tumours develop at younger age and are located proximal to the splenic flexure. Unlike patients with FAP, in Lynch-Syndrome there are only few adenomas at the age of 50, nevertheless, the malignant degenerative timeline in the adenoma-carcinoma-sequence is abbreviated from 10 – 15 years to only 35 months.

Therefore, the average age at diagnosis (44 – 61 years) is earlier, compared to sporadic colorectal carcinoma (28).
Histologically, the carcinomas are more often poorly differentiated and additionally have signet cell histology, extracellular mucin and tumour-infiltrating lymphocytes. Microsatellite instability is also characteristic (23,24,28).
Besides the increased risk of colorectal cancer, patients are more likely to develop other extra-colonic HNPCC typical tumours, such as especially endometrial (39 - 50%) and ovarian (7 - 8%) cancer in women, within 10 years after diagnosis. Other HNPCC-associated tumour entities are gastric cancer, cancer of the renal pelvis or ureter, cancer of the bile ducts, small bowel cancer, central nervous system tumours and pancreatic cancer (24).
To identify potential patients with Lynch-Syndrome the Amsterdam II Criteria and the Revised Bethesda Guidelines have been defined. The Amsterdam II Criteria have higher specificity, yet lower sensitivity compared with the Bethesda Guidelines, which have higher sensitivity but lower specificity (27).

<u>Amsterdam II Criteria (29), all criteria must be met:</u>

- three or more relatives with HNPCC associated cancer, one affected relative being a first-degree relative of the other two
- two or more successive generations are affected
- one or more relative was diagnosed before the age of 50 years
- exclusion on FAP

<u>Revised Bethesda Guidelines (30), one or more criteria must be met:</u>

- colorectal cancer diagnosed before the age of 50
- synchronous, metachronous colorectal cancer, or other HNPCC-associated tumours, regardless of age
- colorectal cancer with the MSI-high histology before the age of 60
- colorectal cancer and at least one first-degree relative with colorectal cancer or other HNPCC-associated tumours, one of them diagnosed before the age of 50
- colorectal cancer and two or more first- or second-degree relatives with colorectal cancer or other HNPCC-associated tumours, regardless of age at diagnosis

1.2.1.2 Familial Adenomatous Polyposis (FAP)

FAP is autosomal dominant inherited and the second most frequent form of inherited colorectal cancer. A mutation of the APC (adenomatous polyposis coli) tumour suppressor gene causes the development of hundreds or up to thousands of colorectal adenomas. At the age of 35 years 95% have multiple adenomas. Without treatment the average age of developing a colorectal carcinoma is 39 years, at the age of 50 years 95% of untreated patients are affected.

Attenuated FAP is a less severe form, with the mutation often found in a different part of the APC gene. Patients develop an average of 30 adenomas, however, they have a greater risk of developing proximal neoplasms and have a 70% life time risk of colorectal carcinoma.

In addition, FAP features some extra-colonic manifestations, both benign and malignant, such as gastric fundic gland polyps, stomach polyps, adenomatous duodenal polyps as well as duodenal and gastric cancer. Moreover, patients can develop osteomas, epidermoid cysts, fibromas and desmoids (23,28).

1.2.1.3 MUTHY-associated Polyposis

MUTHY-associated polyposis is a rare, autosomal recessively inherited biallelic germline mutation of the MUTHY-gen, the product of which is a base excision repair enzyme. This leads to the development of about 10 to a few hundred adenomas, usually around the age of 40. The mean age of colorectal cancer onset is 48 years (28).

1.2.2 Non-genetic Risk Factors

1.2.2.1 Colorectal Adenomas / Polyps

Colorectal cancer mostly develops from precancerous colorectal adenomas through the adenoma-carcinoma sequence *(see chapter 1.3 Pathogenesis).* After 50 years of age the prevalence for adenomas increases excessively, leading to an overall prevalence of up to 50% in patients at average risk. Consequently, adenomas and age are two important risk factors for colorectal cancer. There are two main types of adenomas, firstly, conventional adenomas, which are most common, and secondly, sessile serrated polyps.

Conventional adenomas can be flat, sessile, sub-pedunculated or pedunculated and may have tubular or villous histology.

In contrast, sessile serrated polyps are flat, have heterogeneous histology with saw-toothed, luminal serrations with dilatation branching and distortion in the base of the colonic crypts. They are usually found in the right colon, which, together with their flat appearance, makes them harder to detect through colonoscopy. Therefore, sessile serrated polyps can often be the cause of interval carcinomas (carcinomas that occur between two planed colonoscopies).

A bigger size of adenomas increases the risk of advanced pathohistological features such as villous histology, high-grade dysplasia and the presence of colorectal cancer. (31)

1.2.2.2 Cigarette Smoking

Smoking increases the risk of colorectal adenomas, which are precancerous lesions, and colorectal cancer (32-35).

Studies have shown that the elevated risk for smokers might be irreversible and up to the total lifetime exposure even if they stop smoking, since there is only a slight difference between former and current smokers (33,34). Botteri et al. (33) noticed an increase of colorectal incidence after 30 years of smoking supporting the hypothesis that there is a long period between smoking and the development of cancer.

In addition, former and ongoing smokers have an increased mortality after colorectal cancer diagnosis and shorter disease-free survival after resection (32,34). This might be due to accelerated tumour growth by induction of angiogenesis, suppression of cell-mediated immunity, a suggested minor response to chemotherapy and smoking-associated comorbidities such as cardiovascular disease. Also, an unhealthier lifestyle and decreased participation in screening programmes of smokers could play a role (34). Furthermore, the 30-day mortality after tumour resection is 49 – 100% higher among ongoing smokers (32).

Smoking relates to MSI-H in colorectal cancer, which usually is associated with longer survival compared to microsatellite stable patients, however, among smokers with MSI-H this does not take effect (32).

1.2.2.3 Alcohol

Alcohol mainly is a risk factor for liver cancer and cancer of the upper gastrointestinal tract, however, it also plays a role in colorectal cancer development (24,36). Fedirko et al. (36) showed the impact of daily alcohol consumption in a dose-risk relation. Daily intake of 12.6 – 49.9 g/day, classified as moderate drinking, and heavy drinking, defined as ≥ 50 g/day, were associated with a 21% and 52% elevated risk compared to non-drinkers, respectively.

There is a slight difference in the relation between alcohol consumption and colorectal cancer risk between studies from Asia and other regions. The slow-metabolizing variant of aldehyde dehydrogenase enzyme, which has a high prevalence in the Asian population, leads to elevated blood levels of acetaldehyde after drinking alcohol, and could be a cause of the stronger association of alcohol drinking and colorectal cancer in this population (36).

1.2.2.4 Obesity

Obesity increases the risk of colorectal cancer (37-39). A systematic review of 41 prospective studies has shown that obesity, defined by body-mass-index (BMI) of 30 or more, is associated with a higher risk of colorectal cancer. Pooled relative risks for obese people were 1.334 compared to normal BMI categories (38).

Besides BMI, obesity measured by waist circumference has also proven to increase colorectal cancer risk, with pooled relative risks of 1.455 for the highest vs. the lowest category of waist circumference (38).

Karahalios et al. (39) analysed the association between change in weight and waist circumference and an increasing risk for colorectal cancer. A weight gain of 5 kg only leads to a minimal increased risk.

Reasons may be metabolic and endocrine abnormalities associated with obesity, such as alterations in sex hormone metabolism, insulin and insulin-like growth factor signalling and adipokines or inflammatory pathways (37).

1.2.2.5 Diet

Numerous observational studies have shown a link between high long-term consumption of red and processed meat and a significantly increased risk of colorectal cancer (40,41).

1.2.2.6 Inflammatory bowel disease (IBD)

A higher incidence of colorectal cancer can be observed in both Crohn's disease and ulcerative colitis, however, Crohn's disease without colitis does not lead to an increased colorectal cancer risk (42,43).

As for ulcerative colitis, age at diagnosis, extent, duration and activity of disease are strong, independent risk factors for colorectal neoplasms. For instance, pancolitis is associated with a 5- to 15-fold increase in risk, while proctitis alone does not influence colorectal cancer risk (44). After 20 years of disease duration the cumulative estimated incidence is 5% (45).

Although the pathogenesis of IBD-associated colorectal cancer in not fully understood, mucosal inflammatory mediators such as cytokines and chemokines, oxidative stress and intestinal microbiota are considered to play an important role in the development of colorectal cancer. P53 mutations occur much earlier in the carcinogenesis in IBD-associated than in sporadic colorectal cancer and can also be detected in not dysplastic mucosa (45,46).

1.2.2.7 Diabetes mellitus

Diabetes increases the risk of colon and of rectal cancer by 38% and 20%, respectively. Elevated insulin levels and insulin-like growth factor (IGF) promote cell proliferation and play a role in carcinogenesis (47).

In addition, overall and colorectal cancer specific mortality showed to be increased in patients with diabetes (48).

1.3 Pathogenesis

1.3.1 Adenoma-carcinoma sequence

Adenomas are the precursor lesions of most colorectal carcinomas. Fearon and Vogelstein (49) were the first who introduced the hypothesis of the adenoma-carcinoma sequence that describes the development of adenomatous polyps to invasive colorectal cancer through germline mutations and/or a stepwise accumulation of critical somatic mutations of oncogenes and tumour suppressor genes. It is also referred to as the chromosomal instability pathway, that applies for 85% of colorectal carcinomas (50). Macroscopically, it begins with the genesis of a

small polyp that becomes larger and eventually dysplastic, until it becomes an invasive carcinoma (49).

Important affected genes in the adenoma-carcinoma sequence are APC, K-RAS and p53 (49-51).

The adenomatous polyposis coli (APC) gene is an important tumour suppressor gene that regulates intercellular β-catenin levels by forming a complex with β-catenin and another co-factor, resulting in an accelerated degradation of this protein. β-catenin can activate gene transcription, meaning that a loss of APC function leads to its accumulation and enhanced gene transcription activity on that account. In addition, APC plays a role in the microtubule-chromosome attachment and therefore mutations can cause chromosomal instability. APC mutations are considered to happen early in the colorectal carcinogenesis, since already small polyps of 0.5 cm and 80% of adenomas show this mutation (50,51).

In familial adenomatous polyposis (FAP) (*see chapter 1.2.1 Genetic Factors*) an autosomal dominant inherited mutation of APC causes the development of hundreds or thousands of colorectal adenomas (28,50,51).

K-RAS is an oncogene of the RAS gene family, which is a part of the normal regulation of mitosis and differentiation. K-RAS transduces the signals of the epidermal growth factor receptor (EGFR) on the cell surface to the nucleus. An activating K-RAS mutation causes a constant signalling in the downstream pathway and results in constant mitogenic signalling. The K-RAS mutation, found in 95% of dysplasia and 30 - 50% of colorectal cancer, occurs early in the adenoma-carcinoma sequence and can cause the progression of dysplastic adenomas with pre-existing APC mutation to an invasive carcinoma. (50,51)

p53, referred to as “guardian of the genome”, is an important tumour suppressor gene that can shut down mitosis in the G1/S-phase in case of DNA damage to allow DNA repair systems to work. However, if the detected damage is too severe to be repaired, p53 and the caspase pathway activate apoptosis. The mutation of this gene is crucial in the carcinogenesis, for it may turn a non-invasive dysplasia into an invasive carcinoma. Therefore, p53 mutations typically occur late in the adenoma-carcinoma sequence. 75% of colorectal cancers carry this mutation. (50,51)

1.3.2 Serrated pathway

However, an alternative route of carcinogenesis has been defined in recent years, the serrated polyp pathway. Taking account for 10% of colorectal carcinomas, it describes the development of serrated adenocarcinoma from serrated polyps. These include hyperplastic polyps, sessile serrated adenomas and mixed polyps (52).

The serrated pathway can be split into two sub-pathways, the sessile serrated pathway and the traditional serrated pathway, both starting with the same genetic mechanism, the CpG island methylator phenotype (CIMP) (53). CpG islands are noncoding DNA sections consisting of repetitive cytosine-guanine dinucleotides near to promotor regions. Hypermethylation of CpG islands next to promotors of tumour suppressor genes silences these respective genes and enables mutations and consecutive malignant degeneration. These tumours are referred to as CIMP-positive (54).

The sessile serrated pathway comprises the development of hyperplastic polyps to sessile serrated adenomas that eventually have dysplasia, and finally evolve to mainly right-sided serrated adenocarcinomas. This, accounting for most cases, involves the inactivation of the mismatch repair gene MLH1 by hypermethylation of its promotor region, leading to high microsatellite instability (MSI-H). Sporadic colorectal carcinomas, which are microsatellite unstable, are associated with a high-level form of CIMP (CIMP-H) and additionally, unlike hereditary MSI-H tumours such as HNPCC, commonly have mutations of the BRAF gene (53). *(also see chapter 1.9.1 Prognostic and predictive biomarkers*)

In the traditional serrated pathway hyperplastic polyps develop to traditional serrated adenomas and over dysplasia into serrated adenocarcinomas, however, typically located in the left colon or rectum. The tumours are CIMP-H, yet they are microsatellite stable (MSS) or have low microsatellite instability (MSI-L) and have BRAF-wild type. (53)

1.4 Morphology

1.4.1 Localization

The most common location of colorectal cancer is the rectum, making up for half of the cases. Other localizations in the order of their frequency are the sigmoid colon (30%), the caecum and ascending colon (10%) and the rest of the colon (10%) (35). The differentiation between colon and rectal cancer is defined by the distance of the tumour from the anocutaneous line, measured by rigid sigmoidoscopy. Tumours higher than 15 cm are colon cancers, tumours at 15 cm or below are rectal cancers. Moreover, rectal cancer is divided in low (≤ 5 cm), middle (> 5 – 10 cm) and high (> 10 – 15 cm) rectal cancers (55).

1.4.2 Macroscopic appearance

Colorectal carcinomas can be found with a polypoid (exophytic), ulcerating (endophytic) or diffuse-infiltrating growth (56).

1.4.3 Histopathology

Most colorectal cancers are adenocarcinomas that produce mucus in varying extent, based on which the carcinomas can be divided into subcategories. The mucus can be presented at an intracellular level or is secreted in the extracellular space. Two important subcategories of adenocarcinomas are the mucinous and the signet cell carcinoma (56).

The mucinous adenocarcinoma consists of mucus to an extent of 50% or more of the tumour volume, accounts for 4 – 19% of all colorectal cancers and is associated with a poorer prognosis than non-mucinous cancers. This might be due to increased metastatic potential, since extracellular mucus is taken up by lymphatic vessels and can access the peritoneal cavity. Additionally, mucinous carcinomas show less response to chemotherapy (57).

Adenocarcinomas are defined a signet cell carcinoma if at least 50% of the tumour cells have significant amounts of intracellular mucin, filling the cytoplasm and marginalizing the nucleus. Signet cell carcinomas are very rare, aggressive tumours that are associated with a poor outcome (58).

Besides the adenocarcinoma other rare colorectal carcinomas are the adenoid-squamous, the medullary, the small-cell and the serrated carcinomas (56).

Dependent on the percentage of the cells with glands and their differentiation the carcinomas are graded from G1 to G3. Tumours with >95% of glandular structures are defined G1, with 50 – 95% or 0 – 49% they are defined as G2 and G3, respectively (56).

1.4.4 Tumour dissemination

Colorectal carcinomas disseminate by local invasion of tissue and organs in their surroundings, by invading lymphatic vessels with the consecutive development of lymph node metastasis and by infiltrating blood vessels eventually leading to distant metastasis in other organs (56).

1.4.4.1 Dissemination per continuitatem

Intraperitoneal segments of the large bowel, for instance caecum, transverse colon and the sigmoid loop, are covered by serosa and have a higher risk of peritoneal seeding if the tumour is extending through the bowel wall.
In contrast, extraperitoneal parts, such as the ascending and descending colon, and the hepatic and splenic flexure, have no serosa on their posterior walls, and more often invade the retroperitoneal soft tissue and structures and organs like ureters, kidneys or the pancreas (59).

1.4.4.2 Lymphatic dissemination

The probability and extent of lymph node metastases strongly correlates with the depth of the tumour invasion (56). Lymph node metastases mainly occur in the pericolonic and perirectal lymph nodes. Also, metastasis can be found along the first vascular arcade, along major arterial trunks (ileocolic, right colic, middle colic, left colic, inferior mesenteric and superior hemorrhoidal arteries) and along the periaortic chain (59). Furthermore, middle and low rectal caners can also spread to lymph nodes of the pelvic wall and to the inguinal lymph nodes, respectively (35,60).

1.4.4.3 Hematogenic Dissemination

Already 20% of patients show metastatic disease at the initial diagnosis (3).
The most common location of distant metastasis in colorectal cancer is the liver since most of the large bowel drains into the portal vein, the second common is the lung. Less frequent metastatic sites are the bones and the brain. Colon and rectal

cancer show different metastatic patterns, as rectal cancer tends to develop more metastases at extra-colonic sites, such as the lung, than colon cancer. Also, the left colon has a higher incident rate of metastasis, specifically of liver, lung and bone metastasis, than the right colon (35,61).

The reason why cancers of this location have a higher incidence of pulmonary metastasis is the different venous drainage of the lower rectum. The middle and inferior hemorrhoidal veins drain to the pelvic veins and further to the inferior vena cava, enabling direct hematogenic spread to the lung (59).

1.4.5 Staging

The staging is performed on the base of the TNM-system of the Union for International Cancer Control (UICC). The letter T represents the depth of the infiltration of the primary tumour, N represents the number of local lymph node metastasis and M refers to the number of distant metastasis.

Depending on how these categories were diagnosed, different prefixes are used. cTNM means that the diagnosis is only based on clinical parameters such as CT-imaging or endoscopy, whereas pTNM stands for a verification by pathologic examination and histology and has higher validity (56).

Based on the TNM-system patients can be assigned to 5 different prognostic stages. The TNM classification and staging system for colorectal cancer is listed below (62).

Table 1: Primary tumour (T)

Tx	Primary tumour cannot be assessed	
T0	No evidence of primary tumour	
Tis	Carcinoma in situ: intraepithelial or intramucosal carcinoma (involvement of lamina propria with no extension through the muscularis mucosa)	
T1	Tumour invades 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 pericolorectal tissues	
T4	T4a	Tumour invades through the visceral peritoneum
	T4b	Tumour directly invades or is adherent to other organs or structures

Table 2: Regional lymph nodes (N)

Nx	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Metastasis in 1-3 lymph nodes or any number of tumour deposits are present and all identifiable nodes are negative	
	N1a	Metastasis in 1 regional lymph node
	N1b	Metastasis in 2-3 regional lymph nodes
	N1c	Tumour deposit(s) in the subserosa, mesentery, or non-peritonealised, pericolic or perirectal/mesorectal tissues without regional nodal metastasis
N2	Metastasis in 4 or more lymph nodes	
	N2a	Metastasis in 4-6 regional lymph nodes
	N2b	Metastasis in 7 or more regional lymph nodes

Table 3: Distant metastasis (M)

M0	No distant metastasis by imaging or other studies, no evidence of tumour in distant sites or organs	
M1	Metastasis to one or more distant sites or organs or peritoneal metastasis	
	M1a	Metastasis confined to 1 organ or site
	M1b	Metastasis to two or more sites or organs without peritoneal metastasis
	M1c	Metastasis to the peritoneal surface alone or with other site or organ metastases

Table 4: UICC Stages

Stage	T	N	M
0	Tis	N0	M0
I	T1	N0	M0
	T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1-T2	N1/N1c	M0

	T1	N2a	M0
IIIB	T3-T4a	N1/N1c	M0
	T2-T3	N2a	M0
	T1-T2	N2b	M0
IIIC	T4a	N2a	M0
	T3-4a	N2b	M0
	T4b	N1-N2	M0
IVA	any T	any N	M1a
IVB	any T	any N	M1b
IVC	any T	any N	M1c

1.5 Clinical presentation

Although screening programmes for colorectal cancer based on colonoscopy have been introduced, almost 90% of colorectal cancer cases are still diagnosed in a stage of symptomatic or advanced disease. Cancers detected by screening are more likely to be diagnosed in stage I and therefore are associated with a better prognosis. About 15% present as surgical emergency, in most cases because of obstruction (63).

Typical symptoms are rectal bleeding, abdominal pain and changes in bowel habits, such as diarrhoea or constipation and iron deficiency anaemia. Other symptoms associated with colorectal neoplasms are weight loss and anorexia, nausea and fatigue. Nevertheless, these symptoms are uncharacteristic and have low specificity, since they are also common in healthy individuals. For instance, up to 15% of the population have episodes of rectal bleeding of other origin in their life, yet only 3% of these patients have colorectal cancer (64). Moreover, bright red rectal bleeding and a diagnosis of haemorrhoids neither rule out cancer nor reduce the likelihood of a malignoma, since many colorectal cancer patients have coexisting perianal symptoms (65). The positive predictive values (PPV) of colorectal cancer symptoms are low, with a PPV of 2.4% for rectal bleeding, 1.1% for abdominal pain or 2.3% for anaemia with haemoglobin below 10 g/dl, respectively. However, the combination of two symptoms significantly improves the PPV. Referring to the

example above the simultaneous occurrence of abdominal pain and anaemia leads to an increase of the PPV to 6.9% (66).

The duration of the primary symptoms neither affects staging nor mortality. However, the type of the symptom influences the outcome, as rectal bleeding is associated with a better prognosis than mild anaemia (67).

The location of the tumour also plays a role in its manifestation. Left-sided constricting tumours more often become symptomatic with changes of the bowel habits and obstruction, because the stool has already been more thickened at this site (68). Left-sided and rectal carcinomas are also associated with a higher occurrence of rectal bleeding, whereas right-sided tumours more likely appear with anaemia (64).

1.6 Diagnostic tools

1.6.1 Digital rectal examination

10% of rectal tumours are palpable, therefore digital rectal examination should be performed in case of suspected rectal cancer (35). Also, the distance from the anal verge and the functional status of the sphincter can be determined. Digital rectal examination is also part of the assessment of primary tumour response following preoperative therapy (55).

1.6.2 Guaiac-based faecal occult blood test (gFOBT)

The test uses guaiac-impregnated paper slides to detect occult blood in stool samples. If there is haemoglobin in the stool, the paper slides turn blue through a peroxidase reaction. The slides should not be rehydrated as on the one hand it increases the sensitivity of the test, but on the other hand decreases the specificity. Physiologically, the blood loss in the stool is 1 mg/dl; the common Hemoccult® test detects blood losses of 20 – 40 mg/dl in 50% of cases (35).

An annually performed test improves the sensitivity and leads to a reduction in the colorectal cancer mortality by 33% as shown in the Minnesota colon cancer control study. However, in this respective study the all-cause survival did not improve due to the test compared to the control group (69). Ederer et. al (70) launched a discussion when they showed that 16 – 25% of the suggested mortality reduction of

the very same study were due to chance detection, as false positive tests lead to a higher number of colonoscopies.
Dietary restrictions, such as a reduction of red meat, raw fruits and vegetables for a certain time before and during the gFOBT, showed no decrease in the number of false positive screenings. The intake of ascorbic acid, however, should be avoided 3 days before a gFOBT, as it indeed could inhibit the test (71).

1.6.3 Faecal immunochemical tests (FIT)

Faecal immunochemical tests detect human haemoglobin in the stool, using labeled antibodies. There are two different analytical methods that represent two different approaches in defining a performed test “positive”. On the one hand, lateral flow immunochromatographic analysis gives a positive signal at a pre-set cutoff and therefore is a qualitative test. On the other hand, the results of quantitative testing with immunoturbidimetric methods are not indicated by a signal but by definite haemoglobin concentrations and the cutoff is recommended by the manufacturer. However, as a lower cutoff leads to a higher sensitivity and reversed, cutoffs of quantitative testing could be adjusted to fit best to the available colonoscopy resources, since a higher sensitivity increases the rate of colonoscopies. To define the best threshold for FIT further research is still required. FIT have a sensitivity of 79% with a specificity of 94% (72).

1.6.4 Faecal DNA test

Faecal DNA tests aim to detect mutations in oncogenes or tumour suppressor genes of colonocytes in the stool. Depending on the used marker panel, the sensitivity and specificity strongly vary. The best-achieved result had a sensitivity of 90% and a specificity of 89% for colorectal cancer and large adenomas (72). However, faecal DNA testing is expensive and still experimental and consequently is not part of the current screening guidelines (73).

1.6.5 Endoscopy

1.6.5.1 Sigmoidoscopy

Flexible sigmoidoscopy is used for colorectal cancer screening to both detect and remove premalignant lesions from the rectum up to the splenic flexure. In a UK study

(74) patients aged between 55 years and 64 years underwent once-only sigmoidoscopy. After a median follow-up time of 17 years the screening led to a 35% reduction in colorectal cancer incidence and a 41% reduction in colorectal cancer mortality in the per-protocol analysis. Nevertheless, sigmoidoscopy cannot screen for adenomas and colorectal neoplasms in the proximal colon, which account for 39% of colorectal cancer cases in men and even 52% in women up to 80 years of age. Therefore, although both men and women have the same risk reduction for rectal and distal colon cancer by once-only sigmoidoscopy screening, women do not benefit as much as men regarding the all-site colorectal cancer mortality (74). Colonoscopy showed better results in detecting proximal colon cancers or premalignancies, however, the almost doubled risk for perforation after the intervention should be considered (75).

1.6.5.2 Colonoscopy

Colonoscopy is the gold standard for the diagnosis of colorectal cancer and the detection and removal of adenomas to prevent their degeneration to invasive carcinomas.

A long-term follow-up study observed patients who underwent conlonoscopic removal of adenomas and compared the death rate in this group with the colorectal cancer mortality of the general population. After a long follow-up period of up to 23 years the mortality from colorectal cancer was reduced by 53% in the polypectomy group (76). Polyps and adenomas can be missed in a colonoscopy or a polypectomy can be ineffective, and a so-called interval cancer may develop. An interval carcinoma is defined as the occurrence of colorectal cancer within 3 years after a polypectomy or within 10 years after unsuspicious colonoscopy (35). The risk of interval cancer significantly correlates with the adenoma detection rate of the performing endoscopist. An adenoma detection rate below 20% was associated with a higher risk of interval cancer, indicating its importance in the quality assessment of colonoscopies and showing the need of careful inspection of the mucosa when performing the intervention (77).

Although colonoscopy is a safe procedure, adverse events such as gastrointestinal bleeding or even bowel perforation, occur at a cumulative rate of 1.98 per 1000 colonoscopies. The rate of complication increases with the complexity of the performed procedure, for example the removal of large adenomas (78).

1.6.5.3 Colon capsule endoscopy

Capsule endoscopy can be an alternative method for patients incompliant to existing screening programmes based on colonoscopy because of embarrassment, the requirement of bowel preparation or discomfort during the procedure. In the procedure patients swallow a small capsule with a camera that is able to image the bowel mucosa. In a meta-analysis the sensitivity and specificity for polyps with a size greater than 6mm or 3 or more polyps of any size were 69% and 86%, respectively.

However, a big disadvantage of capsule endoscopy compared with the gold standard is the impossibility of obtaining tissue samples or performing necessary therapeutic interventions if finding required it (79).

1.6.6 CT-Colonography

Another minimally invasive alternative to colonoscopy is computer tomographic (CT-) colonography.

Disadvantages of CT-colonography are radiation and dose accumulation after repeatedly performed screenings as well as the missing option of obtaining biopsies of suspicious lesions. On the other hand, no sedation is required and there is no risk of uncontrollable gastrointestinal bleeding or even bowel perforation. Bowel preparation is still required though. In a meta-analysis the pooled sensitivity and specificity were 67% and 80% and as a result inferior to regular colonoscopy, however, the sensitivity improves with the size of polyps. For instance, for polyps with a diameter of more than 10 mm the sensitivity increases to 90% (80).

CT-colonography uses software image reconstruction to make two-dimensional and three-dimensional views of the colon, but information about extra-colonic structurers are also obtained. Important extra-colonic findings are for example lung nodules and indeterminate kidney lesions, which may require further investigation. However, there is no evidence that the detection of previously asymptomatic lesions has any advantages for the patient that exceed the risks of a higher radiation dose and of further diagnostic procedures. Additionally, the costs for this evaluation must also be considered (81).

1.6.7 Carcinoembryonic antigen (CEA)

CEA does not qualify for screening purposes in the general population, since it indeed is very specific for cancer but in fact has a very poor sensitivity. However, it plays a role in the treatment evaluation as well as in the pre- and postoperative setting. CEA determination is indicated preoperatively if it can contribute to staging and surgical treatment panning. After surgery CEA should be tested every three months for at the least 3 years if the patient is a candidate for surgery or systemic therapy as elevated CEA could advert to metastatic disease and therefore demand further investigation. In metastatic colorectal cancer constantly increasing CEA during chemotherapy indicates progressive disease even without radiological evidence and could make a change in the therapy plan necessary (82).

1.6.8 Clinical staging evaluation

After the diagnosis of colorectal cancer, a clinical staging evaluation is necessary. This includes laboratory tests including liver enzymes and CEA if indicated, ultrasonography or better a CT scan of the liver and the abdomen and lung X-ray or chest CT scan. In case of suspected metastatic disease, additionally a fluorodeoxyglucose-positron emission tomography (FDG-PET) possibly combined with a CT scan is useful for the detection of metastasised sites. However, FDG-PET is not indicated at the initial diagnosis, as there is no consequence for the treatment for most of the patients (73,83).

1.7 Screening

Colorectal cancer screening methods comprise digital rectal examination, stool-based screening methods, endoscopy and CT-colonography. They have already been described in detail in the previous chapter (*see 1.6 Diagnostic tools*). This chapter focuses on the screening recommendations depending on the individual risk and pre-existing and predisposing diseases.

1.7.1 Patients at average risk

Colonoscopy is the method of choice for screening purposes in an asymptomatic population. People 50 years of age are recommended to have a screening colonoscopy to detect and remove precursor lesions of colorectal cancer. If no adenomas or polyps are found, it should be repeated after 10 years.

If patients do not wish to undergo colonoscopy, a gFOBT should be performed annually, a positive result indicates for a complete colonoscopy (84).

1.7.2 Positive family history

First-degree relatives of patients with sporadic colorectal cancer have a higher risk of developing the very same cancer, therefore a colonoscopy should be performed 10 years before the age at which their respective relative was diagnosed, however, in any case before the age of 40 – 45 years. If no polyps are detected, the interval for the next screening is 10 years (84).

1.7.3 Inflammatory bowel disease

Colitis is a major risk factor in patients with inflammatory bowel disease. Therefore, 6 – 8 years after the initial diagnosis the extent of the disease and risk factors should be assessed by a screening colonoscopy. Patients with high risk of colorectal cancer, for instance, due to persistent histological inflammation or long-standing colitis (>20 years) are recommended to undergo colonoscopy every one or two years. Annual colonoscopies are also necessary in patients with coexisting primary sclerosing cholangitis. If the risk is low the screening interval can be stretched to 3 or 4 years. Proctitis alone does not demand screening by colonoscopy. A preventive colectomy is indicated if high grade-dysplasia has been detected in the screening (85).

1.7.4 Familial adenomatous polyposis

To detect FAP as early as possible, first-degree relatives are recommended to participate in screening programmes, preferably APC gene testing at the age of 10 – 12 years. If gene testing is not possible, an endoscopic screening with sigmoidoscopy or colonoscopy should be performed every year. Patients should undergo subtotal colectomy or total proctocolectomy if more than 20 adenomas have developed, adenomas of > 1 cm have been found, or advanced histology appears. Endoscopic surveillance of any remaining rectal segment is necessary for the rest of the entire life. Chemoprevention with non-steroidal anti-inflammatory drugs or selective cyclooxygenase-2 inhibitors such as celecoxib can be considered to reduce the number and size of colorectal polyps. However, endoscopic

surveillance is still required. Esophagogastroduodenoscopy is recommended every three years for patients with FAP starting at 25 to 30 years of age (23,28).

1.7.5 Lynch syndrome (HNPCC)

Patients with Lynch syndrome and their first-degree relatives should have a colonoscopy every one or two years to remove polyps or diagnose possible carcinomas at an earlier stage with a more favourable outcome. Screening should be initiated between 20 to 25 years of age or 2 – 5 years sooner than the earliest occurrence of colorectal cancer in the family. In addition, affected patients are recommended to undergo an endoscopy of the upper gastrointestinal tract including inspection of the duodenum and a gastric biopsy of the antrum at the age of 30 – 35 years. In case of helicobacter pylori infection eradication is advised. Annual screening for endometrial cancer, the second most common cancer in Lynch syndrome, is recommended for female patients starting at the age of 30 – 35 years. Transvaginal ultrasound and endometrial sampling should be performed. In addition, hysterectomy and bilateral salpingo-oophorectomy can be offered to patients with completed family planning or 40 years old. The screening for other extra-colonic HNPCC related cancers also includes an abdominal sonography and physical examination especially of the skin. (23,27,28)

1.8 Therapy

Treatment options of colorectal cancer comprise surgery, radiotherapy, chemotherapy and targeted therapy with high specific antibodies. They are described in detail below.

1.8.1 Surgery

Curative surgery can be performed open or laparoscopically with the laparoscopic approach having some advantages over the conventional open procedure. For instance, laparoscopic surgery is associated with less intraoperative blood loss, decreased postoperative pain and a reduction in hospitalization time. However, the operating time is longer for this procedure. The long-term oncologic outcome is the same for both the laparoscopic and open surgery, as there are no differences in cancer related death, local recurrence or the number of resected lymph nodes (86).

Despite the advantages of laparoscopic surgery, depending on complicating circumstances such as previous abdominal surgeries or complicated locally advanced colorectal cancers, previous proctectomy or Crohn's disease, the open procedure still has its place (87).

Emergency surgeries are necessary if the tumour causes an ileus or tumour or bowel perforation, respectively. In this case, the same radical resection as in elective surgeries, depending on the tumour site, should be performed (84).

The extent of the bowel resections depends on the tumour location and is determined by the vascularization of the affected bowel segment. Tumours of the coecum or the ascending colon require right hemicolectomy due to their vascularization by the ileocolic and right colic artery, which must be ligatured in the procedure. Carcinomas of the right colic flexure and the proximal transverse colon additionally need a ligature of the middle colic artery, resulting in an extended right hemicolectomy with a resection of the right parts of the omentum. This is due to a possible tumour dissemination in the omentum. A transverse colectomy with a resection of both colic flexures is to be performed if the tumour is situated in the middle of the transverse colon. In this case a ligature only of the middle colic artery is necessary. Cancers of the distal transverse colon and the left colic flexure or the descending colon demand a left hemicolectomy. The blood supplying arteries of these sites are the middle colic artery and the left colic artery or the inferior mesenteric artery, respectively. The latter also supplies the sigmoid colon. Generally, the respective bowel segment and the adherent mesentery are resected en-bloc and in no-touch technique to prevent tumour cell dislocation. Surgeons should also harvest a number of at least 12 lymph nodes for staging purposes and to improve the outcome of the patient (35,84).

In rectal cancer the choice of the surgical procedure depends on the localization, size and local infiltration of the tumour. Patients with high or middle rectal carcinomas may have sphincter-sparing surgery if a sufficient tumour-free safety margin can be obtained. It is 5 cm for high and 2 cm for middle rectal tumours, respectively. In addition, a total mesorectal excision (TME) is necessary for middle and low rectal cancers, as it significantly decreases the risk of local recurrence.

If a sphincter-sparing surgery is not possible, an abdominoperineal resection with a permanent colostomy is indicated (35).

The resection of isolated liver metastasis is a potentially curative surgical approach. However, certain criteria for the possibility of a resection of the metastasis are to be met:

- the primary tumour has been or can be controlled
- the liver metastasis can be resected completely
- no or controllable distant metastasis apart from the liver
- sufficient liver function after the resection.

The remaining liver volume should be at least 20% or 30% of the volume before surgery in patients with normal or cirrhotic liver, respectively. Up to 20% of patients with liver metastasis may have surgery with a respective 5-year survival of 50% after resection (88).

Radiofrequency ablation is an alternative strategy for patients inoperable due to comorbidities, lesion size or location; however, the results are inferior to an open surgical resection of the metastasis (88).

Patients with isolated peritoneal carcinosis that meet the requirements (peritoneal cancer index under 20, absence of extra-abdominal metastasis, complete resection of all visible tumour manifestation) can undergo cytoreductive surgery with hyperthermic intraperitoneal chemotherapy following (84).

1.8.2 Radiotherapy

Radiotherapy is important in the treatment of stage II and III rectal cancer but usually not used in the curative treatment of colon cancer (*see chapter 1.8.5 Adjuvant therapy*). Both, direct and indirect effects of radiation are responsible for the effectiveness of radiotherapy. On the one hand, ionizing radiation directly interacts with intracellular molecules through energy absorption causing single- and double-strand breaks, DNA cross-links and single losses of bases in the DNA. On the other hand, it enhances the genesis of free radicals and indirectly results in the same molecular effects (89).

Early side effects due to radiation toxicity in the treatment of rectal cancer are diarrhoea, cystitis and perineal dermatitis. Late toxicity effects are bowel dysfunction, faecal incontinence, bleeding, perforation, genitourinary dysfunction and pelvic fractures (90).

1.8.3 Chemotherapy

Chemotherapy is a major part in the treatment of colorectal cancer patients. Chemotherapeutic drugs used are 5-flurouracile, capecitabine, oxaliplatin and irinotecan. Common treatment regimens are 5-FU/Leucovorin monotherapy, FOLFOX (5-FU, leucovorin, oxaliplatin), FOLFIRI (5-FU, leucovorin, irinotecan) XELOX (capecitabine, oxaliplatin) and FOLFOXIRI (5-FU, leucovorin, oxaliplatin, irinotecan). The individual chemotherapeutic agents are explained in detail below.

1.8.3.1 5-Fluorouracil (5-FU)

5-Fluorouracil is an antimetabolite and pyrimidine analogue and one of the most important chemotherapeutic drugs in colorectal cancer treatment, as it is used in many treatment regimens (e.g. FOLFOX or FOLFIRI). The effect of the drug is based on the inhibition of the thymidylate synthase by FdUMP, which is the active metabolite of 5-Fluorouracil. As a result, tumour cells are not able to synthesize enough thymidine nucleotides, thus DNA replication and cell proliferation are inhibited (91). The modulation of fluorouracil-based chemotherapy by leucovorin (folinic acid) improved both the response rate and the overall survival in patients with advanced colorectal cancer as it enhances the effect of fluorouracil. The response rate of 5-Fluorouracil alone is only 10 – 15%, in combination with leucovorin, however, it increases almost two-fold (92).

Common adverse effects of fluorouracil are (91):

- myelosuppression
- stomatitis, mucositis
- diarrhoea
- alopecia
- neurotoxicity
- hand-foot syndrome
- coronary spasm (rare)

1.8.3.2 Capecitabine

Capecitabine is an orally administered prodrug of 5-fluorouracil with a bioavailability of 80%. The transformation of capecitabine is a three-step process, with the first and second steps to be carried out in the liver by carboxylesterase and cytidine

deaminase, respectively. The third step of the capecitabine activation, the final transformation to 5-fluorouracil through thymidine phosphorylase, already takes place in the tumour cell itself, as this enzyme is more active in malignant cells. Capecitabine is equally effective as 5-fluorouracil plus leucovorin. Side effects of capecitabine are similar to fluorouracil/leucovorin, however, the onset of grade 3 or 4 toxic effects is delayed (91,93) They are:

- diarrhoea
- nausea or vomiting
- stomatitis
- hand-foot syndrome (more frequent and severe than 5-FU/Leucovorin)
- abdominal pain
- fatigue or asthenia
- neutropenia
- alopecia
- hyperbilirubinemia

1.8.3.3 Oxaliplatin

Oxaliplatin is an intravenously administered alkylating antineoplastic agent in the treatment of colorectal cancer that reacts with guanine and adenosine. It induces both intra-strand cross-links in the DNA and less frequent cross-links between two DNA strands that are next to each other. Consequently, DNA replication is prevented, and cell-death is induced. Oxaliplatin is the only platin-based chemotherapeutic drug that is effective in colorectal cancer (91).

The combination of oxaliplatin and 5-FU/Leucovorin improves the outcome in both the adjuvant and palliative setting and therefore is more effective than fluorouracil plus leucovorin alone (94,95). Adverse effects associated with oxaliplatin-based chemotherapy are (96):

- neurotoxicity/peripheral neuropathy (dose limiting)
- nausea or vomiting
- diarrhoea
- neutropenia
- thrombocythemia

1.8.3.4 Irinotecan

Irinotecan is an inhibitor of topoisomerase-I that stabilizes the cleavable complex of this enzyme causing reversible single- and irreversible double-strand breaks if the unrepaired site of the single-strand break is being replicated. The formation of the replication fork then causes the irreversible strand break.

Irinotecan is a prodrug that becomes activated to its active metabolite (SN-38) in the liver. Glucanosyltransferase UGT1A1 is important for the biliary elimination of the drug. Consequently, polymorphisms of this enzyme and therefore the irinotecan clearance play a major role in the development of severe adverse effects, such as severe diarrhoea (91).

A combination of irinotecan and fluorouracil (FOLFIRI) is effective as both first- and second-line therapy in patients with metastatic colorectal cancer and improves the outcome compared to 5-FU/leucovorin alone. Also, in patients who experienced no benefit of fluorouracil-based therapy, irinotecan monotherapy increased the one-year survival rate compared to best supportive care (36% vs 14%) (97,98).

Common side effects are:

- delayed diarrhoea (dose limiting)
- neutropenia (dose limiting)
- early cholinergic syndrome
- nausea or vomiting
- alopecia
- asthenia

1.8.4 Targeted therapy

1.8.4.1 Bevacizumab

Bevacizumab is a monoclonal humanized antibody targeted against the vascular endothelial growth factor (VEGF). Tumours often express VEGF, which plays an important role in angiogenesis and endothelium proliferation. The antibody binds VEGF and consequently inhibits the activation of its associated receptors (VEGF-R1 and VEGF-R2), and therefore prevents angiogenesis in the growing tumour. Another effect is the normalization of the cancer's unorganized vascular system which may enable a better transport of chemotherapeutics to the tumour (91).

The addition of bevacizumab to chemotherapy regimens in metastatic colorectal cancer significantly improves the outcome (99), however, there is no benefit in the adjuvant treatment (100). Observed side effects are (91):

- hypertension
- nausea
- abdominal pain
- diarrhoea
- proteinuria
- bowel perforation (rare)

1.8.4.2 Cetuximab

Cetuximab is a chimeric monoclonal antibody that specifically binds to the epidermal growth factor receptor (EGFR) and blocks it for the natural ligands, and consequently inhibits the signalling cascade. Furthermore, the antibody induces the endocytosis and degradation of the receptor. Cetuximab is indicated for metastatic patients with wild-type RAS only, as mutated K-RAS or N-RAS activate the signalling pathway below the receptor, and the receptor's inhibition would have no effect (91,101). An adjuvant outcome benefit of standard chemotherapy combined with cetuximab could not be observed (102). Important side effects of cetuximab are (91):

- acneiform rash (more than 80%)
- hypomagnesaemia
- infusion reactions (fever, vertigo, dyspnoea)
- mucositis
- elevation of liver enzymes

1.8.4.3 Panitumumab

Another EGFR inhibitor available in the first- and second-line treatment of stage IV colorectal cancer is panitumumab. Just as cetuximab it is a monoclonal antibody, however fully humanized, and binds to the EGFR, inhibiting the proliferation and angiogenesis of the tumour and inducing apoptosis. Panitumumab, too, requires RAS wild-type. Adverse effects are (103):

- acneiform rash

- pruritus
- hypomagnesaemia
- diarrhoea
- infusion reactions
- pulmonary fibrosis

1.8.4.4 Aflibercept

Aflibercept is a recombinant fusion protein and a soluble receptor for VEGF A and B, as distinguished from bevacizumab, which only targets VEGF A. It prevents VEGF as well as the placental growth factor from binding to their associated receptors and consequently inhibits the tumour angiogenesis. Studies showed an outcome benefit of FOLFIRI with aflibercept in metastatic colorectal cancer in first- and second-line therapy compared to FOLFIRI alone. Adverse effects of aflibercept are (104):

- neutropenia
- thrombocytopenia
- diarrhoea
- stomatitis
- abdominal pain
- proteinuria
- hypertension

1.8.4.5 Regorafenib

Regorafenib is an orally administered tyrosine kinase inhibitor that targets multiple kinases associated with tumour progression including amongst others VEGF, BRAF and platelet derived growth factor. Regorafenib monotherapy is a treatment option for patients with metastatic colorectal cancer who have already received all standard therapies and VEGF- and EGFR-inhibitors. Best supportive care and regorafenib showed an improvement of overall survival compared to best supportive care alone. Most of this effect is due to disease stabilization.
The most common side effects of regorafenib are (105):

- fatigue
- hand-foot skin reaction

- diarrhoea and nausea
- anorexia and weight loss
- hypertension
- oral mucositis
- rash

1.8.5 Adjuvant therapy

1.8.5.1 Stage I

Both colon and rectal cancer in UICC stage do not require any adjuvant therapy after a complete surgical removal of the tumour (R0 resection) (84).

1.8.5.2 Stage II

1.8.5.2.1 Stage II colon cancer

Adjuvant chemotherapy for patients with UICC stage II colon cancer is not generally indicated (84). A British study showed that the benefit of an adjuvant chemotherapy with fluorouracil only means a 4% reduction of the relative risk of death for patients at low risk of recurrence (106).
However, adjuvant therapy should be considered in the presence of certain unfavourable prognostic factors that come along with a higher recurrence rate. These risk factors are: advanced tumour invasion (TNM T4), perforation of the tumour, surgery emergency setting and few harvested and examined lymph nodes. A determination of the microsatellite status is mandatory before the start of adjuvant chemotherapy in stage II colon cancer, as MSI-H tumours do not benefit from it (84). A monotherapy with fluorouracil is the only option if the decision for an adjuvant treatment has been made, since the combination with oxaliplatin showed no significant benefit (107).

1.8.5.2.2 Stage II rectal cancer

Patients with UICC stage II and III low and middle rectal cancer should undergo neoadjuvant chemoradiotherapy with a total radiation dose of 45 – 50 Gy or alternatively short-term radiotherapy consisting of 5 x 5 Gy and immediate consecutive surgical resection within 10 days or after 4 – 8 weeks.

Chemoradiotherapy contains infusional fluorouracil or its orally administered prodrug capecitabine, the interval until the surgical removal of the remaining tumour is 6 – 8 weeks. The aim of the neoadjuvant treatment is the preoperative downsizing of the tumour and the improvement of the local recurrence rate.

However, patients with cT3a/b rectal cancer, no suspected lymph node involvement, no mesorectal fascia (MRF) infiltration and no extramural vascular invasion (EMVI) can have a total mesorectal excision without preoperative treatment (84). In this case, an adjuvant chemoradiotherapy or a fluorouracil-based chemotherapy is recommended. Besides, patients with histological verified risk factors for local recurrence need an adjuvant chemoradiotherapy. These risk factors are (84):

- R1-resection
- intraoperative tumour perforation
- tumour resection margin positive
- insufficient quality of total mesorectal excision
- pT4
- extra-nodal mesorectal tumour invasion
- T3 low rectal cancer

1.8.5.3 Stage III

1.8.5.3.1 Stage III colon cancer

Generally, patients with UICC stage III colon cancer should have adjuvant chemotherapy within 8 weeks after a complete (R0) surgical resection of the tumour given that there are no contraindications and the patient is fit for treatment (84).

The MOSAIC trial was the first to show a significant benefit in disease- free survival as well as in overall survival from adding oxaliplatin to a fluorouracil/leucovorin based chemotherapy (108). In the 10-years follow-up of this study the overall survival for the FOLFOX group was 4.6% higher compared to the patients receiving 5-FU/leucovorin alone (109).

Consequently, oxaliplatin containing chemotherapy regimens (FOLFOX or XELOX) are the adjuvant treatment of choice in stage III colon cancer. However, patients older than 70 years should receive fluoropyrimidine monotherapy as there is a higher rate of adverse effects in this group and there is no clear improvement of the outcome. Besides, patients not fit enough or with contraindications for oxaliplatin should also receive 5-FU monotherapy. VEGF- and EGFR-inhibitors such as

bevacizumab and cetuximab showed no outcome benefit and should not be used in the adjuvant setting (84).

1.8.5.3.2 Stage III rectal cancer

There is no difference in neoadjuvant and adjuvant treatment between UICC stage II and III rectal cancer (84).

1.8.5.4 Stage IV

Patients with metastasized colorectal cancer may still be treated with curative intent if an R0 resection of all metastases can be achieved. The criteria for technically primary resectable metastatic disease of the liver amongst others include the number, size and localization of the metastasis as a sufficient liver function must be obtained. Furthermore, the individual tumour biology and its aggressiveness are important to consider deciding whether a resection brings a reasonable chance of success. Indicators therefore are synchronous versus metachronous metastases, short time to progression or short disease-free interval (83,84). Depending on these factors the guidelines of the European Society of Medical Oncology (ESMO) on metastatic colorectal cancer recommend either an adjuvant chemotherapy for 6 months or a neoadjuvant chemotherapy for 3 months followed by surgery and postoperative adjuvant chemotherapy for another 3 months. Both strategies should use the FOLFOX regimen, monoclonal antibodies are not to be used (83). As opposed to this, the German S3-guidelines for colorectal cancer do not recommend adjuvant chemotherapy after primary resection of liver metastasis (84).
Besides primary resectable metastasis, patients may be potentially operable after downsizing of the metastasis by cytoreductive induction chemotherapy. For this purpose, the FOLFOX, FOLFIRI or FOLFOXIRI regimen with or without biologicals can be used in suitable patients. The general principle is to use the most effective treatment as possible (83).

1.8.6 Palliative therapy

Patients with non-resectable metastatic disease receive palliative treatment with the aim to prolong their survival whilst maintaining a good quality of life and to reduce the cancer symptoms. Therefore, patients should be offered chemotherapy, depending on the tumour biology and the general condition of the patient various

treatment options are recommended. Generally, more intensive first-line chemotherapy regimens such as FOLFOX, XELOX, FOLFIRI or even FOLFOXIRI are recommended for fit patients in good general condition. Elderly and unfit patients should receive 5-FU or capecitabin monotherapy combined with the VEGF inhibitor bevacizumab.

Oxaliplatin-based first-line therapy should be applied for 4 – 6 months until deescalating the treatment and continuing with a fluoropyrimidine maintenance therapy or deciding for a therapy pause. After this time oxaliplatin does not bring a further significant survival benefit, it does however come along with a higher toxicity.

In case of RAS wildtype an anti-EGFR therapy, for instance with cetuximab, is recommended for left-sided tumours, whereas right-sided and mutated RAS metastasized colorectal cancer should be treated with bevacizumab.

After progression, in the second line oxaliplatin-based regimens should be switched to irinotecan-based chemotherapy and reverse. Bevacizumab also shows a benefit beyond the first-line setting and should be continued after progression.

For patients who have already received all available treatment options of chemotherapy and the options for biologicals are exhausted, the multikinase inhibitor regorafenib is recommended (84).

1.8.7 Follow-up

Up to 50% of colorectal cancer patients will experience recurrence after a resection with curative intent and adjuvant chemotherapy. Therefore, the right follow-up and surveillance strategy is crucial to identify relapses in an early yet curatively resectable extent to improve survival. Possible tests are clinical examination, blood CEA testing, CT-scans of the abdomen, the pelvis and lung, liver imaging with ultrasound and endoscopic follow-up (73). A recent meta-analysis (110) including eleven studies with altogether 4055 patients showed that patients undergoing an intensive follow-up protocol compared to a less intensive or no follow-up have a significantly higher detection rate of asymptomatic recurrence and a shorter recurrence detection time with a higher likelihood of curative surgery. Consequently, in the intensive follow-up group there was a significant survival benefit. However, in most of the included studies the definition of intensive and less intensive follow-up was different (110).

In Austria the recommended follow-up for colorectal cancer includes an anamnesis, a clinical examination and blood CEA testing every 3 months for 3 consecutive years and then every 6 months for another 2 years. Moreover, a CT-scan of the abdomen and thorax every 12 months for 3 years is advised. Colonoscopy should be performed at year one and four after curative treatment (111,112).

1.9 Prognosis

Prognosis strongly depends on UICC stages and TNM classification. The stage dependent 5-year survival rates for colon and rectal cancer are listed below (35).

Table 5: 5-year survival rate

UICC stages	Colon cancer	Rectal cancer
I	95%	95%
II	85%	85%
III	55%	65%
IV	5%	5%

Further prognostic and predictive markers are the Eastern Cooperative Oncology Group (ECGO) performance score, blood-based markers such as the white blood cell count, albumin, lactate dehydrogenase and CEA, tumour location (left- vs right-sided) and molecular markers like RAS and BRAF mutations and MSI status (113,114). Histologically, tumour grading is an independent risk factor for poor outcome and variants like the mucinous and signet-cell carcinoma are associated with poor prognosis (113). Some important biomarkers are elaborated in more detail below.

1.9.1 Prognostic and predictive biomarkers

1.9.1.1 CEA

The carcinoembryonic antigen and its role in colorectal cancer diagnosis and surveillance has already been described previously (*see chapter 1.6 Diagnostic tools*).

As for prognostic use, increased preoperative CEA levels (C stage) were shown to be associated with poor prognosis. Thirunavukarasu at al. (115) found a 60% increase in overall mortality risk in colorectal cancer patients with elevated CEA (C1

stage) compared to patients with no CEA alteration (C0 stage). This difference in prognosis even persisted when C1 cancers of lower stages were compared with higher stages without CEA elevation.

The 2015 follow-up of the study population hardened the evidence that the inclusion of C stage into the TNM-based staging system may be important to select patients who benefit from adjuvant chemotherapy. To date, the decision for adjuvant treatment is mainly based on lymph node status, however, C1 cancers without lymph node invasion (N0) have a similar or worse prognosis than N1 but C0 cancers. Therefore, preoperative CEA levels might become an indicator for treatment decisions (116).

1.9.1.2 RAS

The role of mutated RAS in carcinogenesis has already been described in previous chapters (*see chapter 1.3 Pathogenesis*).

Both, mutations of K-RAS in exon 2 or 3 and mutated N-RAS (exon 2, 3 or 4), predict the resistance of tumours to anti-EGFR targeted therapy with biologicals such as cetuximab and panitumumab. Wild-type RAS is a requirement for the use of these therapies as mutated RAS causes a persistent activation of the downstream signalling pathway. Consequently, an inhibition of EGFR as the top of the pathway has no effect (91,101,114). Therefore, the RAS mutation status has to be evaluated in all patients with metastatic colorectal cancer considered for anti-EGFR therapy.(114)

1.9.1.3 BRAF

BRAF is also part of the RAS pathway, activated by EGFR, and the direct downstream target of K-RAS. Eight percent of colorectal cancers have the characteristic V600E mutation of the BRAF oncogene, most of which in MSI-H colorectal cancers, resulting in a constitutive downstream signalling and enhancement of proliferation and tumour growth (114). BRAF mutation is a predictor of poor prognosis in MSS tumours with a 5-year survival rate of 40.5%, however, this does not account for MSI colorectal cancer. Survival rates of MSI tumours with or without BRAF V600E are similar, with corresponding 5-year survival rates of 84.6% and 80.7%, respectively (117).

Besides RAS mutations, mutated BRAF may also exclude patients from treatment with cetuximab and panitumumab. A meta-analysis showed that EGFR targeted therapy did not improve the outcome compared to standard chemotherapy in patients with BRAF mutation, concluding that patients should be tested for BRAF mutations before treatment with anti-EGRF biologicals (118). However, another meta-analysis of the same year using different statistical methods and inclusion criteria found only insufficient evidence for mutated BRAF to predict poor benefit from anti-EGFR therapy. Therefore, BRAF should currently not be used to limit the access to cetuximab or panitumumab treatment and further research is required (119).

Finding and establishing new cost-effective prognostic and predictive biomarkers is crucial to select the best available treatment options for colorectal cancer patients. The impact of platelets on tumour progression has been described previously (7,8). To the best of our knowledge there is no existing data on pre-treatment MPV and a possible influence on treatment outcomes across multiple treatment settings as well as on clinical response rates. The intention of our study was to analyse a potential biomarker applicable for many CRC patients in different settings rather than for a selected cohort.

Since a recent study (11), by our department, that investigated platelet size as a prognostic biomarker in nonmetastatic renal cell carcinoma showed a significant prognostic value of MPV. We expected to find similar results in colorectal cancer, given that platelet size has already successfully analysed in this cancer entity in terms of diagnosis and prognosis (16-18,120).

2 Material and Methods

Study Design & Patients

In this single-centre observational cohort study, we retrospectively included patients with histologically-confirmed non-metastatic (UICC stage II and III) and metastatic (UICC stage IV) carcinomas of the colon or rectum who were referred to our department (Division of Oncology, Department of Internal Medicine, Medical University of Graz, Austria) between January 1st, 2010 and March 1st, 2016. From these 1,054 patients we excluded 364 patients according to pre-defined criteria (**Figure 1**). Data at baseline were extracted from the electronic health record system of our hospital trust (which includes all public hospitals in the Austrian state of Styria), the internal documentation system of our department, and from paper-chart archives of our hospital. MPV results were derived from routine laboratory analyses of whole blood samples drawn into EDTA-coated collection tubes (Vacuette®, Greiner Bio-One, Kremsmünster, Austria). MPV was computed as the ratio of plateletcrit (PCT) to the number of platelets (PLT). In detail, PLT is determined via impedance after hydrodynamic cell focusing, while PCT results from the summation of the single impulses during PLT measurement. All measurements were performed on analysers by Sysmex®, during that six-year span various models (XN-1000™, XE-5000™) were used in the local clinical laboratory. The models do not differ in method of detection. For patients with metastatic disease, we extracted MPV values from the day of treatment initiation (for each one of the first three systemic treatment lines and at the timepoint of Best Supportive Care (BSC) initiation). For patients with non-metastatic disease, we extracted MPV values that were closest to the time of histological tumour diagnosis (within one week before and at a maximum of two weeks after histologic diagnosis), but always before definitive surgery.

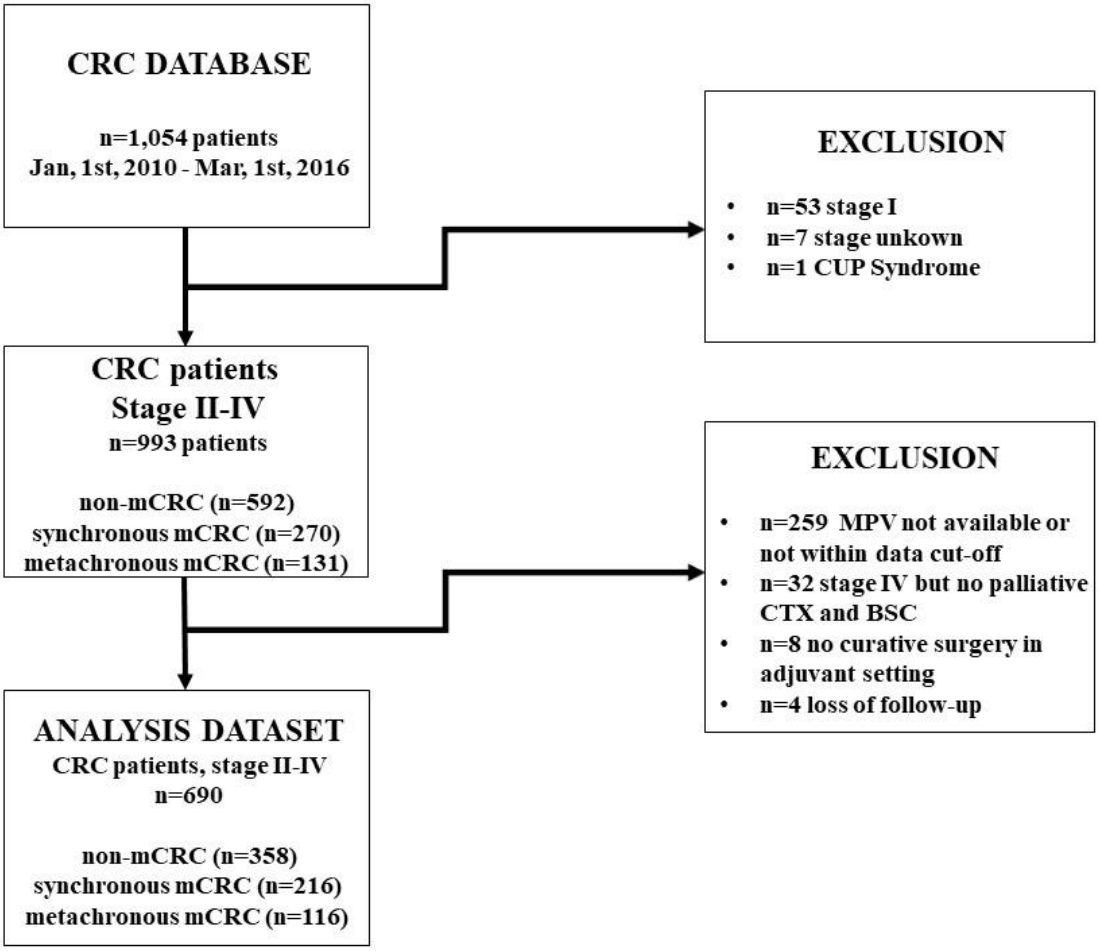

Figure 1: Flowchart of the study population selection including exclusion criteria.

Endpoints

We defined the date of definitive surgery as the baseline date for patients with non-metastatic tumours. In the metastatic setting, we defined start date of the respective chemotherapy line (1st, 2nd and 3rd) or date of BSC initiation as the baseline date, respectively. Co-primary endpoints were Recurrence-Free Survival (RFS) in the non-metastatic setting, Progression-Free Survival (PFS) in the first three treatment lines in the metastatic setting, and Overall Survival (OS) in Best Supportive Care (BSC). Follow-up was truncated at 3 years for RFS analyses and at 6 months for PFS and OS analyses, respectively.

Ethics statement

The local ethics committee (Ethikkommission der Medizinischen Universität Graz, IRB00002556) approved the study prior any patient-related activities were performed (No.25-458 ex 12/13). Written informed consent was not obtained from individual patients, because the local ethics committee specifically granted a "waiver of consent" for this retrospective database study. All investigations have been in accordance with the principles embodied in the declaration of Helsinki.

Statistical analysis

All statistical analyses were performed using Stata (Windows version 15.0, Stata Corp., Houston, TX, USA). Continuous variables were summarized as medians [25^{th}-75^{th} percentile], whereas categorical variables were reported as absolute counts (%). The association between response rates and MPV under study were analysed with uni- and multivariable generalized linear models from the Bernoulli family with an identity link. Median follow-up was estimated with a reverse Kaplan-Meier (KM) estimator according to Schemper & Smith. PFS and OS was estimated with KM estimators, compared between two groups using log-rank tests and modelled with uni- and multivariable Cox proportional hazards models. For dichotomization of MPV (necessary for all figures) an empirical cut-off at the 25^{th} percentile of the MPV distribution in the respective treatment setting was used.

3 Results

Analysis at baseline

In total, six-hundred-ninety patients were included in the analysis of which four-hundred-twenty-five, two-hundred-thirty-one, one-hundred-seventeen, fifty-five, and two-hundred-twelve patients accounted for the adjuvant, 1st-line metastatic, 2nd-line metastatic, 3rd-line metastatic, and BSC setting, respectively (**Table 6**). The average MPV levels were highly similar across all treatment settings (**Table 6**).

Table 6: Baseline characteristics of the study population.

Distribution overall and by therapy line. The column "n (% miss.)" shows the number of patients whose values of the respective variable could be collected (% missing). Continuous variables are reported as medians [25th percentile (Q1) – 75th percentile (Q3)], whereas absolute frequencies and percentages are used for categorical variables. Abbreviations: BMI – Body mass index, MPV – mean platelet volume, CRP – C reactive protein

		Adjuvant (n=425)			**1st line (n=231)**			**2nd line (n=117)**			**3rd line (n=55)**			**BSC (n=212)**	
Variable		n (%miss.)	Summary measure		n (%miss.)	Summary measure		n (%miss.)	Summary measure		n (%miss.)	Summary measure		n (% miss.)	Summary measure
Demographic variables															
Female gender		425 (0%)	156 (37%)		231 (0%)	83 (36%)		117 (0%)	46 (39%)		55 (0%)	22 (40%)		212 (0%)	75 (35%)
Age (years)		425 (0%)	66 [56-73]		231 (0%)	64 [57-72]		117 (0%)	64 [57-71]		55 (0%)	63 [59-70]		212 (0%)	68 [60-74]
BMI (kg/m²)		375 (12%)	25 [23-29]		204 (12%)	24 [22-27]		104 (11%)	25 [22-27]		49 (11%)	24 [21-27]		0 (100%)	/
Karnofsky Index		299 (30%)	90 [90-100]		152 (34%)	90 [80-100]		82 (30%)	90 [80-90]		31 (44%)	90 [80-90]		0 (100%)	/
No comorbidity		425 (0%)	335 (79%)		231 (0%)	188 (82%)		117 (0%)	96 (82%)		55 (0%)	46 (84%)		212 (0%)	163 (77%)
Smoker or ex-smoker		270 (26%)	99 (23%)		126 (45%)	54 (43%)		62 (47%)	28 (24%)		31 (44%)	12 (39%)		93 (56%)	48 (52%)
Tumor variables															
Synchronous metastases		N/A	N/A		231 (0%)	153 (66%)		117 (0%)	76 (65%)		55 (0%)	35 (64%)		211 (0%)	139 (66%)

Location of primary tumor		423 (0%)	/		231 (0%)	/		115 (1%)	/		54 (2%)	/		211 (0%)	/
---Right ascending		/	77 (18%)		/	40 (17%)		/	16 (14%)		/	9 (17%)		/	45 (21%)
---Right flexure		/	20 (5%)		/	14 (6%)		/	9 (8%)		/	3 (6%)		/	14 (7%)
---Transverse colon		/	16 (4%)		/	10 (4%)		/	5 (4%)		/	1 (2%)		/	8 (4%)
---Left flexure		/	13 (3%)		/	12 (5%)		/	6 (5%)		/	2 (4%)		/	11 (5%)
---Left descending		/	6 (1%)		/	6 (3%)		/	3 (3%)		/	1 (2%)		/	5 (2%)
---Sigma		/	80 (19%)		/	67 (29%)		/	31 (27%)		/	15 (28%)		/	52 (25%)
---Rectum		/	208 (49%)		/	79 (37%)		/	42 (37%)		/	23 (43%)		/	74 (35%)
---Multilocular		/	3 (1%)		/	3 (1%)		/	3 (3%)		/	0 (0%)		/	2 (1%)
Kras wildtype		74 (83%)	39 (53%)		214 (7%)	116 (54%)		110 (6%)	63 (57%)		54 (2%)	31 (57%)		168 (21%)	94 (56%)
Nras wildtype		34 (92%)	31 (91%)		76 (67%)	67 (76%)		36 (69%)	32 (89%)		19 (65%)	18 (95%)		52 (75%)	45 (87%)
Treatment variables															
Adjuvant chemotherapy		420 (1%)	228 (54%)		N/A	N/A		N/A	N/A		N/A	N/A		N/A	N/A
Number of adjuvant chemotherapy cycles		211 (7%)	8 [5-8]		N/A	N/A		N/A	N/A		N/A	N/A		N/A	N/A
Number of palliative chemotherapy cycles		N/A	N/A		218 (6%)	8 [4-10]		113 (3%)	5 [4-9]		52 (5%)	5 [4-8]		N/A	N/A
Polychemotherapy		220 (4%)	109 (50%)		231 (0%)	168 (73%)		117 (0%)	78 (67%)		55 (0%)	33 (60%)		N/A	N/A

Laboratory variables														
MPV (fl)		425 (0%)	9.8 [9.0-10.4]		231 (0%)	9.9 [9.3-10.7]		117 (0%)	9.9 [9.4-10.5]		55 (0%)	9.7 [9.2-10.3]		212 (0%)	9.8 [9.1-10.6]
Haemoglobin (g/dl)		403 (5%)	13.1 [11.1-14.5]		222 (4%)	12.3 [11.3-13.4]		114 (3%)	12.7 [11.7-13.9]		52 (5%)	13.2 [11.3-13.9]		208 (2%)	11.3 [10.2-12.7]
Leucocyte count (G/L)		401 (6%)	7.6 [6.2-9.8]		222 (4%)	7.7 [6.0-9.9]		114 (3%)	7.0 [5.5-9.2]		52 (5%)	7.6 [5.6-8.9]		208 (2%)	8.7 [6.3-12.0]
Platelet count (G/L)		424 (0%)	281 [231-344]		229 (1%)	301 [241-386]		115 (2%)	225 [187-301]		54 (2%)	252 [195-308]		212 (0%)	278 [210-384]
CRP (mg/dl)		385 (9%)	3.6 [1.1-11.2]		219 (5%)	13.2 [3.8-37.0]		113 (3%)	8.2 [3.7-24.1]		51 (7%)	12.3 [4.2-39.8]		197 (7%)	46 [12-97]
CEA		290 (32%)	3.0 [1.7-7.0]		211 (9%)	32 [6-226]		100 (15%)	51 [13-188]		44 (20%)	49 [11-295]		133 (37%)	69 [14-309]
CA19 9		265 (38%)	8 [3-16]		211 (9%)	79 [11-1145]		100 (15%)	84 [15-11697]		44 (20%)	87 [20-1140]		130 (39%)	217 [23-3814]

Analysis of response patterns in the 1st to 3rd-line metastatic setting and their association with MPV

Objective response rates (ORR) were 33% (95%CI: 27-40) in 1st-line therapy, 24% (16-34) in 2nd-line therapy and 19% (8-33) in 3rd-line therapy, respectively (**Figure 2**). Corresponding disease control rates (DCR) were 65% (58-72), 57% (47-67) and 42% (27-58), respectively. Higher MPV levels did not consistently predict for ORR or DCR across treatment lines, and this prevailed in multivariable analysis adjusting for polychemotherapy (**Table 7).**

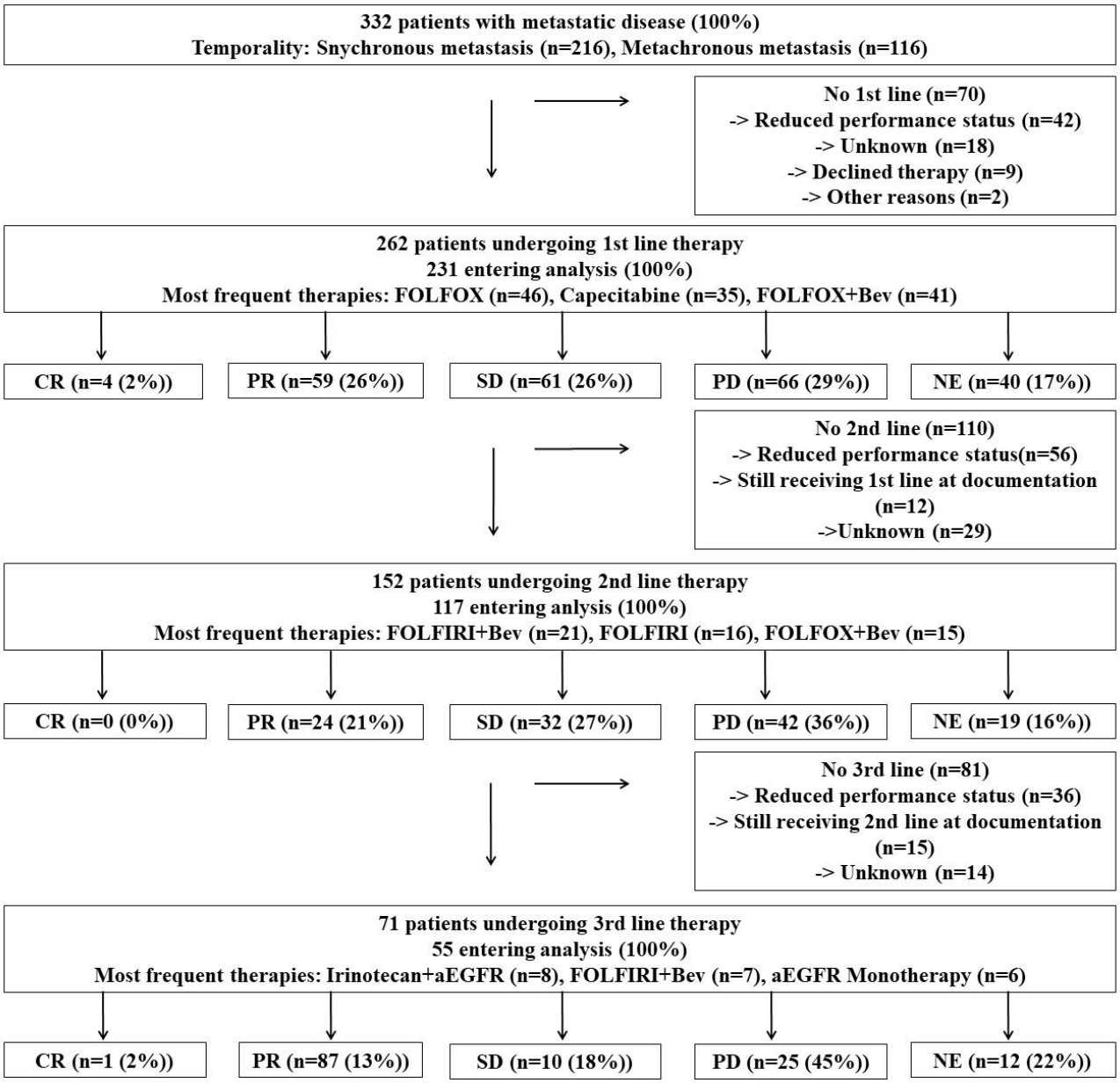

Figure 2: Flowchart of the study population with metastatic disease.

Table 7: Uni- and multivariable predictors of clinical response rates in first, second and third line

Absolute change of ORR (objective response rate) and DCR (disease control rate) per 1fL increases of MPV (mean platelet volume). Abbreviations: ORR – objective response rate, DCR – disease control rate, CI – confidence interval, P – P value, MPV – mean platelet volume, N/E – not evaluable

Variable	Δ_{abs} in 1st-line (95%CI, p)	Δ_{abs} in 2nd-line (95%CI, p)	Δ_{abs} in 3rd-line (95%CI, p)
OBJECTIVE RESPONSE RATE (%)			
Univariable analysis			
MPV (per 1fL increase)	+0.8% (-6.8-6.9, p=0.982)	+7.3% (-2.1-16.6), p=0.129)	-7.4% (-15.0-0.2, p=0.058)
Other predictors – Univariable analysis			
Age (per 10 years increase)	-7.9% (-14.3-(-1.5), p=0.015)	-1.3% (-9.6-7.0, p=0.759)	-13.6% (-21.9-(-5.2), p=0.001)
Right side	-6.8% (-21.6-8.0, p=0.370)	13.4% (-8.1-35.0, p=0.221)	-12.8% (-35.0-9.4, p=0.259)
Right side in KRAS-wt	-15.4% (-35.2-4.5, p=0.129)	7.8% (-21.9-37.4, p=0.609)	0.0% (-39.2-39.2), p=0.999)
Polychemotherapy	+22.7% (10.5-34.8, p<0.0001)	16.9% (0.8-33.1, p=0.039)	+19.7% (-0.7-40.0, p=0.058)
Multivariable analysis			
MPV (per 1fL increase)	+1.0% (-5.6-7.7, p=0.764)	+8.2% (2.4-14.1), p=0.006)	N/E
Polychemotherapy	+19.4% (5.6-33.2, p=0.006)	+18.4% (4.8-32.0, p=0.008)	N/E

DISEASE CONTROL RATE (%)						
Univariable analysis						
MPV (per 1fL increase)		+4.9% (-1.8-11.6, p=0.154)		+10.2% (0.6-19.7, p=0.036)		-0.2% (-14.9-14.5, p=0.980)
Other predictors – Univariable analysis						
Age (per 10 years increase)		-7.1% (-13.0-(-1.2), p=0.019)		-1.2% (-10.6-8.1, p=0.796)		-2.8% (-20.1-14.6, p=0.754)
Right side		+10.8% (-5.2-26.8, p=0.187)		+21.9% (0.7-43.1, p=0.043)		+4.8% (-29.2-38.8, p=0.781)
Right side in KRAS-wt		+1.8% (-22.1-25.7, p=0.881)		+3.9% (-26.5-34.2, p=0.802)		+15.0% (-33.1-63.2, p=0.542)
Polychemotherapy		+31.5% (15.3-47.8, p<0.0001)		+31.7% (11.4-51.9, p=0.002)		+16.9% (-12.6-46.4, p=0.262)
Multivariable analysis						
MPV (per 1fL increase)		+4.5% (-1.8-10.9, p=0.162)		+12.9% (4.3-21.4, p=0.003)		-0.7% (-15.2-13.8, p=0.924)
Polychemotherapy		+31.3% (15.2-47.5, p<0.0001)		+34.9% (15.8-54.0, p<0.0001)		+17.0% (-12.6-46.6, p=0.261)

Uni- and multivariable analysis of clinical outcomes across treatment settings

Median PFS was not reached in 1st-line, 4.8 months in 2nd-line, and 4.1 months in 3rd-line therapy, respectively. Six-month PFS was 59% (52-65), 37% (28-46) and 27% (15-41) in 1st-line, 2nd-line, and 3rd-line therapy, respectively. Median OS was 2.6 months in BSC, and 6-month OS in BSC was 31% (23-40, **Figure 3**). Median RFS was not reached in the adjuvant setting, while 3-year RFS was 76% (72-81, **Figure 4**).

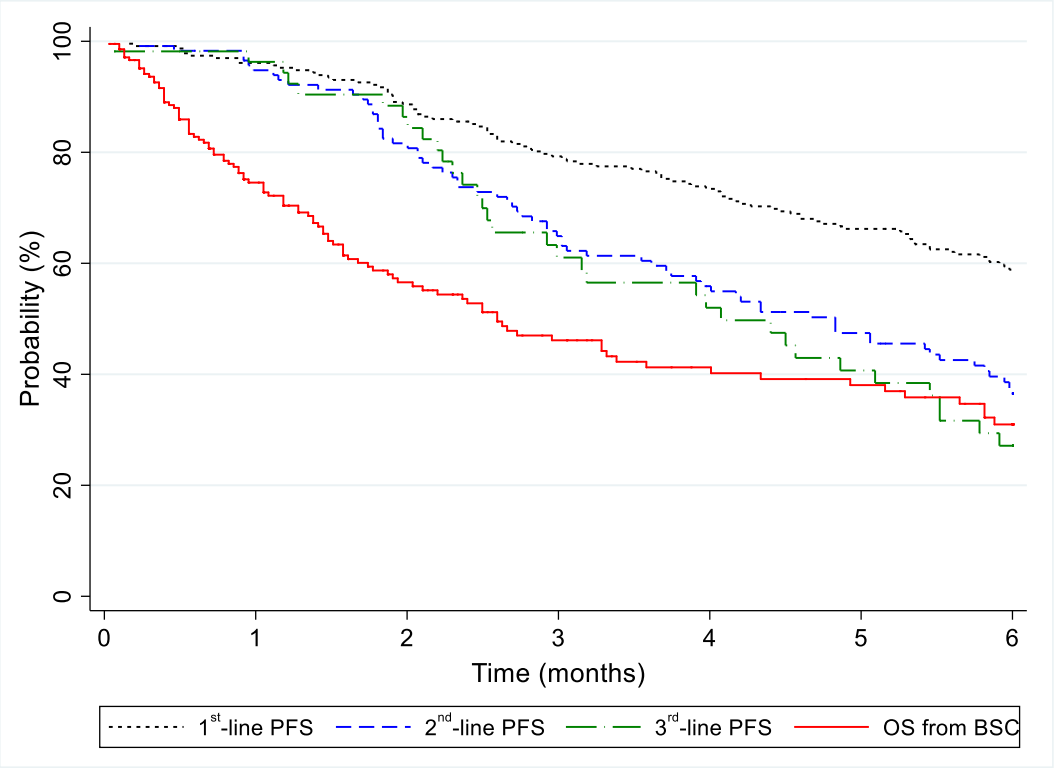

Figure 3: Cumulative probability of 6-months progression- free survival in 1st, 2nd and 3rd palliative treatment line and 6-months overall survival in best supportive care.

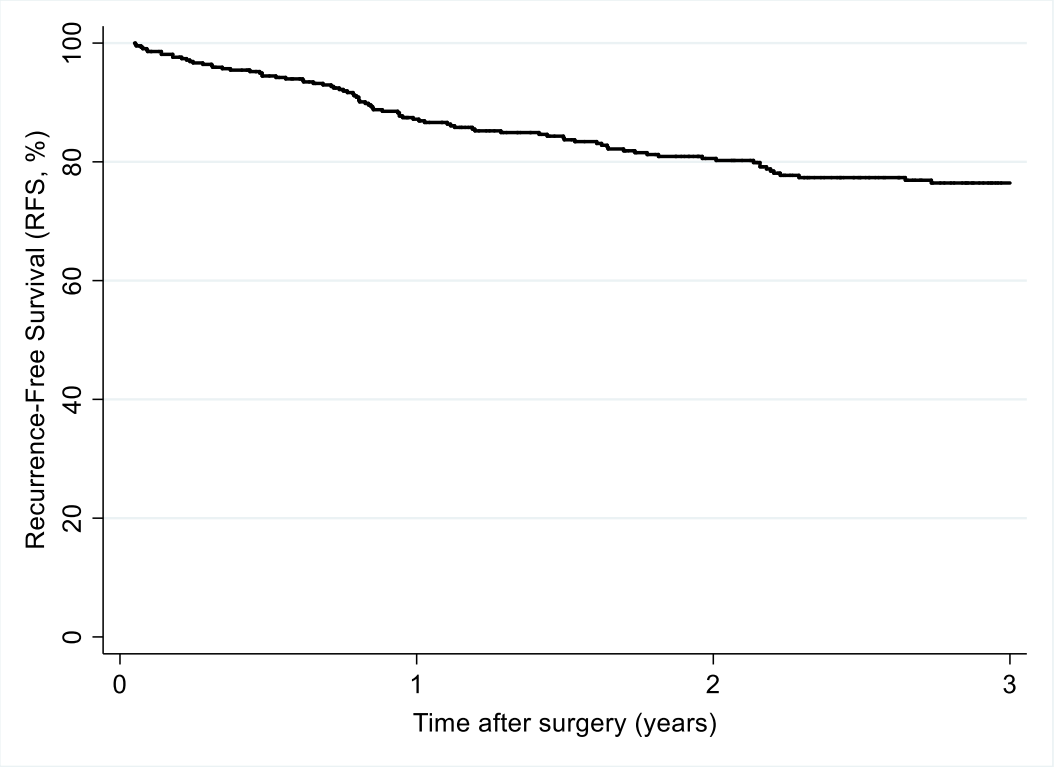

Figure 4: Cumulative probability of 3-year recurrence- free survival in the adjuvant setting.

Three-year RFS was highly similar in patients with low MPV (as defined by an empirical cut-off < the 25th percentile (<Q1) of its distribution) and patients with MPV above this cut-off, respectively (log-rank p=0.566, **Figure 5**). In univariable Cox regression, MPV levels were not associated with the rate of recurrence (Hazard Ratio (HR) per 1fL increase in MPV=0.96, 95%CI: 0.82-1.12, p=0.600, **Table 8**). This result prevailed after multivariable adjustment for stage III disease (Adjusted HR=0.95, 0.81-1.12, p=0.556, **Table 8**).

Table 8: Uni- and multivariable predictors of clinical outcomes in the adjuvant setting, first, second, third line metastatic setting, and best supportive care

Hazard ratio of 3-year RFS (recurrence- free survival), 6-months PFS (progression- free survival) and 6-months OS (overall survival) per 1fL increase of MPV. Abbreviations: BSC – best supportive care, RFS – recurrence- free survival, PFS – progression- free survival, OS – overall survival, HR – hazard ratio, CI – confidence interval, P – P value, MPV – mean platelet volume, N/A – not applicable

Variable	3-year RFS in the adjuvant setting (HR (95%CI, p))	6-months PFS in 1st line (HR (95%CI, p))	6-months PFS in 2nd line (HR (95%CI, p))	6-months PFS in 3rd line (HR (95%CI, p))	6-months OS in BSC (HR (95%CI, p))
Univariable analysis					
MPV (per 1 fL increase)	0.96 (0.82-1.12, p=0.600)	0.82 (0.67-1.00, p= 0.053)	0.82 (0.63-1.06, p=0.136)	0.87 (0.62-1.22, p=0.424)	0.81 (0.67-0.98, p=0.031)
Other predictors – Univariable analysis					
Age (per 10 years increase)	1.14 (0.95-1.36, p=0.164)	1.12 (0.91-1.37, p= 0.284)	1.01 (0.80-1.27, p=0.920)	1.34 (0.88-2.04, p= 0.179)	0.69 (0.59-0.81, p<0.0001)
Right side	N/A	1.23 (0.78-1.95, p= 0.370)	0.78 (0.44-1.37, p=0.383)	1.23 (0.57-2.63, p= 0.597)	1.56 (1.07-2.29, p=0.022)
Right side in KRAS-wt	N/A	1.31 (0.70-2.45, p= 0.398)	1.34 (0.67-2.68, p=0.412)	1.58 (0.52-4.79, p=0.423)	1.62 (0.98-2.70, p=0.062)

Stage III (vs. Stage II)		2.14 (1.29-3.53, p=0.003)		N/A		N/A		N/A		N/A
Adjuvant chemotherapy		0.69 (0.45-1.07, p=0.099)		N/A		N/A		N/A		N/A
Polychemotherapy		N/A		0.51 (0.33-0.77, p=0.002)		0.67 (0.411.09, p=0.104)		0.94 (0.47-1.86, p=0.857)		N/A
Metachronous metastases		N/A		0.98 (0.63-1.52, p= 0.916)		1.13 (0.70-1.83, p=0.616)		1.22 (0.61-2.48, p=0.573)		0.71 (0.47-1.07, p=0.105)
Multivariable analysis		Adjusted for Stage III		Adjusted for polychemotherapy		Adjusted for polychemotherapy		Adjusted for polychemotherapy		Adjusted for age and right side
MPV (per 1fL increase)		0.95 (0.81-1.12, p=0.556)		0.83 (0.68-1.02, p= 0.074)		0.81 (0.62-1.05, p=0.113)		0.87 (0.62-1.22, p=0.426)		1.11 (0.93 – 1.33, p= 0.248)
Stage III (vs. Stage II)		2.14 (1.30-3.54, p=0.003)		N/A		N/A		N/A		N/A
Polychemotherapy		N/A		0.52 (0.34-0.80, p=0.003)		0.65 (0.40-1.06, p=0.085)		0.94 (0.48-1.87, p=0.867)		0.80 (0.60-0.97, p=0.021)
Age (per 10 years increase)		N/A		N/A		N/A		N/A		0.75 (0.63-0.89, p=0.001)
Metachronous metastasis		N/A		N/A		N/A		N/A		1.65 (1.10-2.47, p=0.016)

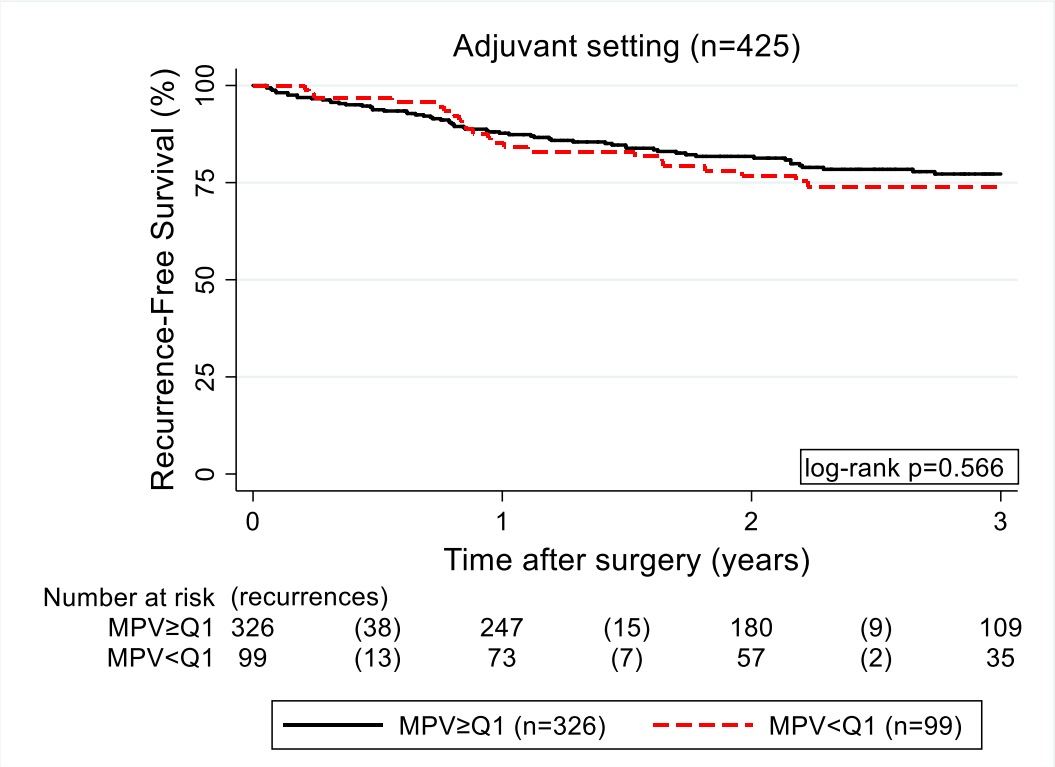

Figure 5: Kaplan Meier curve according to MPV ≥ Q1 vs. MPV < Q1 for recurrence- free survival in the adjuvant setting

Rates of progression in 1st- to 3rd-line therapy were higher in patients with low MPV (as defined by an empirical cut-off < the 25th percentile (<Q1) of its distribution in the respective treatment setting) than in patients above this cut-off (Panels A-C of **Figure 6**), although this did only reach statistical significance in the 2nd-line setting. This pattern was confirmed in uni- and multivariable Cox regression, where higher MPV levels were numerically but not statistically significantly associated with a lower rate of progression in 1st- to 3rd-line metastatic settings, as well as BSC, respectively (**Table 8**). **Figure 7** indicates the forest plot for this analysis.

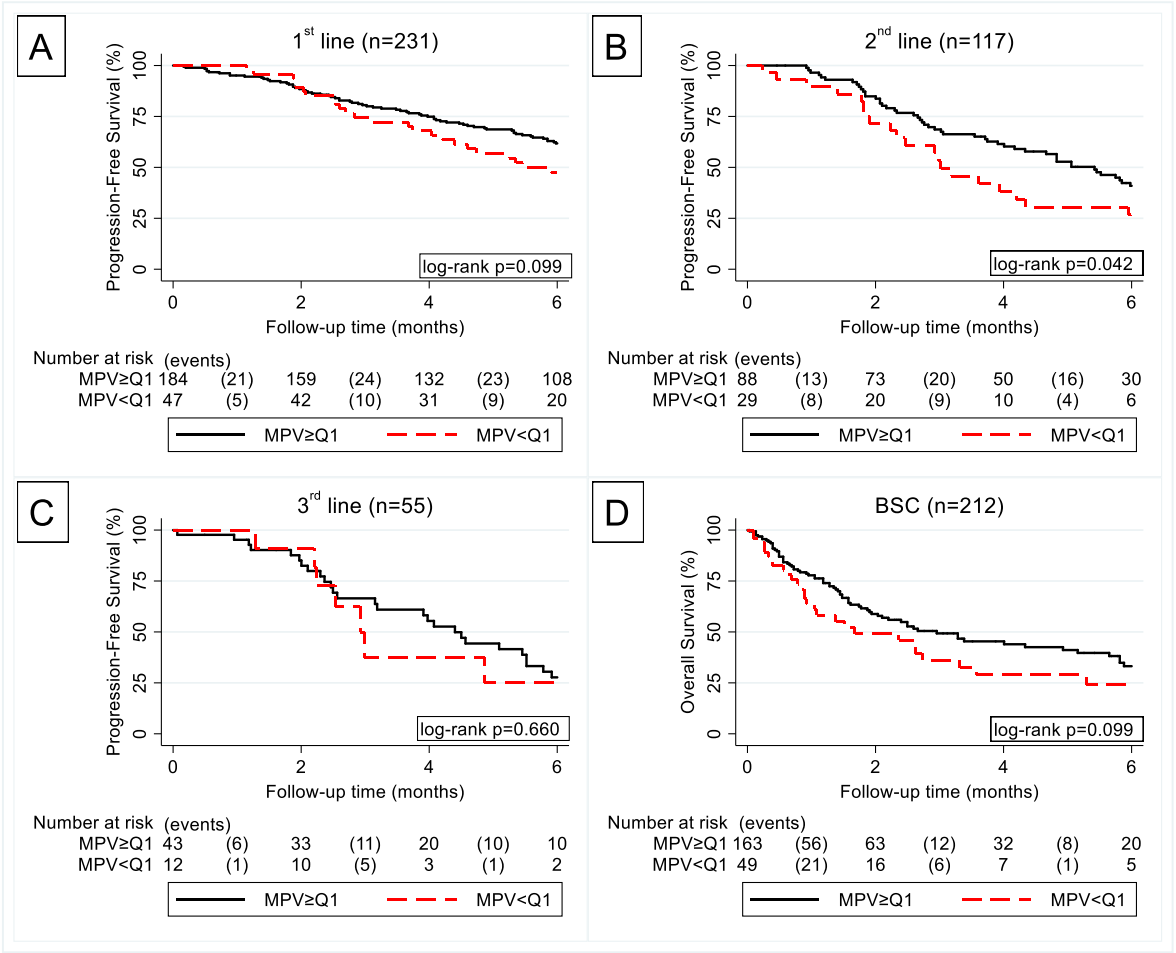

Figure 6: Kaplan Meier curve according to MPV ≥ Q1 vs. MPV < Q1 for progression- free survival in the 1st (A), 2nd (B) and 3rd (C) palliative treatment line and overall survival in best supportive care (D).

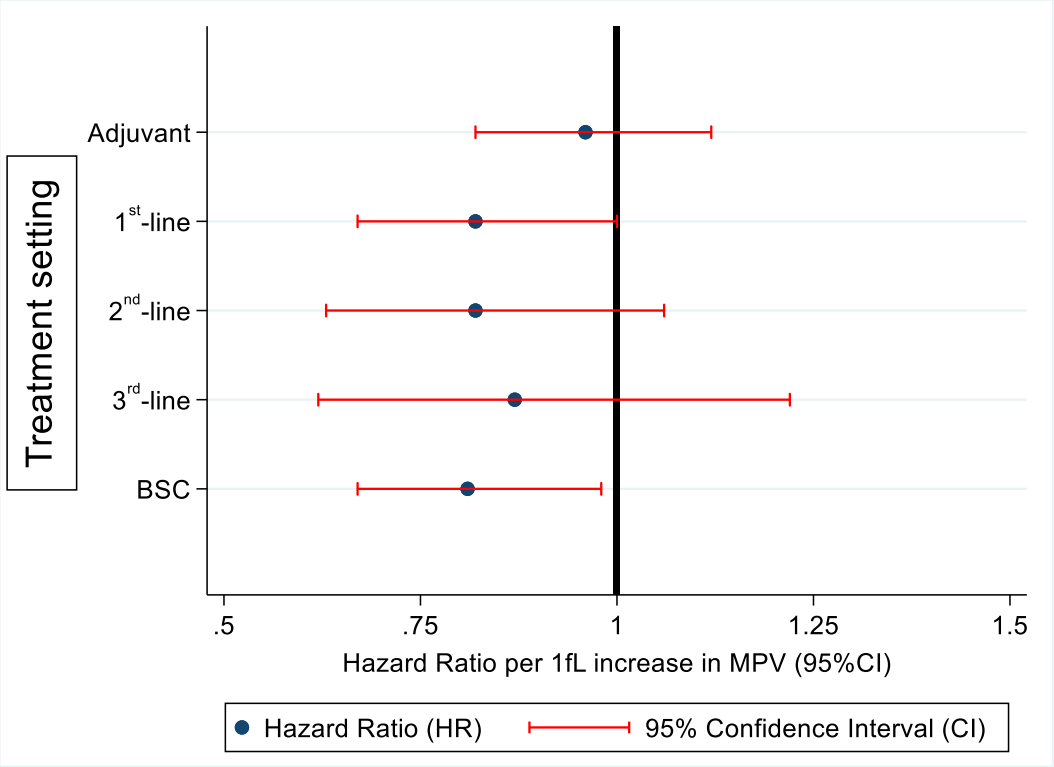

Figure 7: Forest plot indicating the association between MPV and the respective hazard ratio for 3-year recurrence- free survival in the adjuvant setting, 6-months progression- free survival in first, second and third palliative treatment line and 6-months overall survival in best supportive care.

4 Discussion

Previous studies have already analysed MPV and its potential role as a diagnostic and prognostic biomarker in different disease and treatment settings in CRC (16-18,120). However, there is a lack of information on MPV and its impact on recurrence in the adjuvant setting as well as its association with outcome in metastatic CRC over multiple systemic treatment lines. In our study, pre-treatment MPV was neither a predictor for RFS in patients undergoing potentially curative resection nor significantly associated with shorter PFS in metastasized CRC, except for 2nd line treatment. Patients in BSC with MPV below the 25th percentile had numerically but not significantly shorter OS. There was no consistent influence on clinical response rates (ORR and DCR) in patients receiving 1st, 2nd and 3rd line palliative treatment, respectively.

Platelets enhance tumour progression as they carry multiple granules containing growth factors, chemokines and proteases and form a shield around tumour cells preventing them from the immune response of natural killer cells (8). In addition, they carry prothrombotic and proinflammatory mediators and are involved in inflammatory processes and diseases (5). MPV is a marker of platelet size and rises due to enhanced platelet activation and inflammation, the last of which plays a major role in cancer progression (5,121). Markers of inflammation were already shown to relate to cancer prognosis and clinical response in metastatic CRC (122), but although an increase of MPV in some cancer entities is assumed to be the result of cancer related inflammation (13,120,123,124), the observation of decreased platelet size in cancer could also be explained by cancer-associated platelet activation and exhaustion (125). Small platelets and consequently low MPV might be the result of further enhanced inflammation and a high consumption of large activated platelets at the tumour site, causing the release and production of smaller exhausted platelets (5,10). This mechanism was shown in other diseases with systemic inflammation (5).

A meta-analysis (15), including 18 studies that investigated the diagnostic and prognostic potential of MPV in various cancer entities, found increased MPV levels to be linked to the presence of cancer. Pooled MPV levels in cancer patients were higher before than after they received curative treatment and were 8.831 fL and

8.521 fL, respectively. MPV was also lower in healthy individuals than in cancer patients. However, an analysis of two included studies that provided data on a correlation between survival outcome and platelet size could not find a significant association of altered MPV levels and shorter disease-free survival (15).

Interestingly, opposing MPV levels could be found in different cancer entities. On the one hand, increased MPV was present in gastric, endometrial, ovarian and liver cancer whereas on the other hand, it was decreased in non-small cell lung cancer (NSCLC) and renal cell carcinoma (9,12,13,15,123,124,126).

In CRC the role of MPV is still not clear. Higher preoperative MPV values could be found in patients with colon cancer compared to controls, implicating that MPV might indicate the presence of colon cancer. In addition, MPV values were associated with disease progression and rose with higher stages. However, the relatively small sample size of only 128 histologically verified cancer cases and 128 healthy controls, that where enrolled in this respective study, have to be considered as a limitation (120). Kilincalp et al (127) could further show that increased MPV at the time of diagnosis in CRC patients decreased after successful surgical removal of the tumour. Conversely, the analysis of MPV in rectal cancer alone revealed lower values at diagnosis but an increase of MPV after curative resection (18). This might indicate a difference in the role of platelets in colon and rectal cancer or left and right sided CRC but may also indicate that the cancer cells themselves influence the tumour macro-environment (i.e. platelet function and size) (128).

In contrast to our results, prior studies found increased MPV at diagnosis to be a predictor of poor prognosis in CRC, associated with shorter pooled OS and shorter PFS in metastatic patients receiving bevacizumab-combined chemotherapy. This was explained by a linkage between increased platelet size and increased platelet activation and consecutive greater inflammation (16,17). As opposed to our current study that chose the cutoff empirically at the 25^{th} percentile of the distribution, Li et al (16) and Tuncel et al (17) both used receiving operator characteristic (ROC) analysis to determine the most suitable cutoff value of MPV in their respective study population. However, in the case of Li et al (16) and their analysis of MPV and its association with OS, the area under the curve (AUC) of the ROC-curve was close to 0.5 (ROC-AUC = 0.551) and therefore, not indicating for a strong association.

Most studies, which are investigating platelet size and its link to CRC, used ROC curve analysis in their statistical evaluation. Consequently, a broad variety of different cut-off values for the determination of increased or decreased MPV were used, varying from 7.89 fL to 11.3 fL in the respective studies (13,17,18,127).

Nonetheless, although not statistically significant, regression coefficients across all treatment settings were in the direction that small platelets are associated with worse outcomes. This at least partly corroborates prior research in CRC and other tumour types, which implicate low MPV in adverse prognosis. Moreover, our clinical data are at least hypothesis-generating for further basic research studies on platelet activation and cancer progression in CRC.

Riedl et al (6) found a decreased MPV to be significantly associated with shorter OS when they analysed the impact of altered MPV in cancer patients on the risk of venous thromboembolism and mortality in a prospective cohort study including 1,544 cancer patients. However, the study summarizes both patients with solid as well as haematological malignancies and amongst others the subgroup analysis for 159 CRC cases showed no significant result. In addition, a recent study of our department found a highly significant association of decreased MPV and RFS as well as cancer-specific death in patients with non-metastatic renal cell carcinoma. (11). Low MPV values were also shown to be associated with poor prognosis in cancers of the bladder and the lung (9,10,129).

Besides the connection of decreased platelet size, as indicated by decreased MPV values, and NSCLC, Inagaki et al (9) also investigated the MPV/platelet count (PC) ratio when they enrolled 268 patients with advanced NSCLC in their retrospective study. Both MPV and MPV/PC ratio were decreased in cancer patients compared to controls. Additionally, the cohort within the NSCLC-group, which showed a low MPV/PC ratio, had a significantly reduced OS by about 4 months compared to cancer patients with a higher MPV/PC ratio.

Another study conducted by Kumagai et al (129) revealed that preoperatively low MPV beyond that is also connected to shorter disease-free survival as well as OS in patients with NSCLC after intentionally curative resection.

Yet the prognostic and predictive value of MPV might be varyingly strong in different cancer entities. Despite a relatively large sample size in many settings, the results of our study failed to reach significance in most cases, indicating that MPV is only a

weak predictor of disease outcome in CRC. Since to date only positive, significant results regarding the association of MPV and CRC prognosis have been published, a possible publication bias must be considered. Therefore, taking the results of the present study into account, further research is needed to clarify the impact of MPV on CRC prognosis.

Some limitations of this study are worth mentioning. First, selection bias cannot be excluded entirely due to the retrospective single centre study design. Second, we did not exclude patients with conditions that might influence laboratory MPV values as did other studies before, but we think that therefore our results are closer and more relevant to clinical practice. All patients included in this study who underwent surgery or received chemotherapy were fit enough for treatment, and therefore altered MPV levels due to other severe diseases seem unlikely. Third, the relatively small sample size in later treatment lines must be noticed.

In conclusion, the prognostic and predictive role of MPV in CRC patients remains unclear. On the one hand some studies have shown a significant association of platelet size and the presence of CRC as well as on progression and prognosis. On the other hand, we could neither report a consistent significant impact of platelet size on RFS, PFS or OS in the adjuvant, palliative or the BSC setting, respectively, nor could we find a consistent association between MPV and clinical response rates in 1st, 2nd and 3rd line palliative treatment.

Based on our study results, MPV is a weak biomarker in CRC and therefore hardly viable for clinical practice.

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