Diplomarbeit

THE PLASMA AST/ALT RATIO (DE RITIS) IS AN INDEPENDENT PROGNOSTIC MARKER FOR DISEASE FREE SURVIVAL IN STAGE II AND III COLORECTAL CANCER

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Zusammenfassung

Hintergrund:
Ein hoher AST/ALT Quotient bei Tumordiagnose ist ein negativer prognostischer Marker bei diversen malignen Tumorerkrankungen. In dieser Studie untersuchten wir den prognostischen Stellenwert des Plasma – AST/ALT (De Ritis) Quotienten bei Patienten mit nicht metastasiertem Kolorektalkarzinom im Stadium II und III.

Material und Methoden:
In dieser retrospektiven Studie wurden insgesamt 536 Patienten und Patientinnen mit lokalisiertem Kolorektalkarzinom (Stadium II und III) und vorhandenen AST/ALT Quotienten bei Tumordiagnose eingeschlossen. Die primären Endpunkte der Studie waren einerseits das krankheitsfreie Überleben sowie das Gesamtüberleben.

Ergebnisse:
In der univariablen Analyse zeigte sich ein deutlich verkürztes krankheitsfreies Überleben bei Patienten mit erhöhten AST/ALT Quotienten (HR 1.568, 95% 1.10-2.23, p = 0.012).
Auch in der multivariablen Analyse unter Berücksichtigung von Stadium, Grad sowie erhaltener adjuvanter Chemotherapie konnte eine statistisch signifikante Assoziation zwischen erhöhtem AST/ALT Quotienten und einem verschlechterten krankheitsfreien Überleben gezeigt werden (HR 1.53, 95% 1.05-2.22, p = 0.026). Es zeigte sich kein Zusammenhang zwischen einem erhöhten AST/ALT Quotienten und dem Gesamtüberleben (HR 1.4, 95% CI 0.89 – 2.22, p = 0.14).

Conclusio:
In dieser Studie ging der Serum - AST/ALT Quotient bei Tumordiagnose als valider prognostischer Marker für das krankheitsfreie Überleben bei Patienten mit Kolorektalkarzinom im Stadium II und III hervor. Der AST/ALT Quotient könnte daher als neues und günstiges prognostisches Tool für die Identifikation von Patienten und Patientinnen mit hohem Rezidivrisiko dienen.

Abstract

Introduction:

A high AST/ALT quotient at cancer diagnosis has been associated with poor prognosis in multiple malignancies. In the present study we evaluated the prognostic value of the serum AST/ALT (De Ritis) ratio in a large cohort of non-metastatic CRC patients.

Material and Methods:

Five-hundred-thirty-six patients with stage II and III CRC as well as available AST/ALT ratio at cancer diagnosis were included in this single-center retrospective analysis. Laboratory data were measured within two weeks before histological tumor diagnosis. Co-Primary endpoints for this analysis were disease free survival (DFS), defined as the time from surgery to disease progression or death and overall survival (OS). Survival analysis was performed by using Kaplan-Meier estimators as well as uni- and multivariable Cox models.

Results:

In univariate cox regression disease free survival was significantly shorter in patients with an elevated AST/ALT ratio. (HR 1.568, 95% 1.10-2.23, p = 0.012) In multivariable analysis, adjusting for grade, stage and adjuvant chemotherapy, the prognostic association between an elevated AST/ALT ratio and a worse DFS prevailed statistically significant (HR 1.53, 95% 1.05-2.22, p = 0.026). No statistically significant association between the AST/ALT ratio and OS was observed. (HR 1.4, 95% CI 0.89 – 2.22, p = 0.14)

Conclusion

In this study the serum AST/ALT ratio emerged as a valid prognostic marker for disease free survival in non-metastatic colorectal cancer patients at stage II and III. These findings suggest that the serum AST/ALT ratio might represent a novel and inexpensive prognostic tool to aid in the identification of patients at high risk of recurrent disease.

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Glossar und Abkürzungen

5-FU) *5-fluorouracil*

AFAP ..... attenuated familial adenomatous polyposis

ALT ..... *alanine aminotransferase*

ASCO ..... *American Society of Clinical Oncology*

AST ..... Aspartate aminotransferase

BMI ..... *body mass index*

CEA ..... *Carcinoembryonic antigens*

CI ..... *confidence interval*

CIMP ..... *CPG island methylator phenotype*

CIN ..... *chromosomal instability phenotype*

CRC ..... colorectal cancer

CRP ..... *c- reactive protein*

DFS ..... *disease free survival*

EGFR ..... *epidermal growth receptor*

ESMO ..... *European Society for Medical Oncology*

FAP ..... *familial adenomatous polyposis*

FDA ..... *food and drug administration*

FIT ..... *fecal immunochemical test*

FOBT ..... *fecal occult blood test*

gFOBT ..... *guaiac fecal occult blood test*

HNPCC ..... *hereditary non-polyposis colorectal cancer*

HR ..... *hazard ratio*

IBD ..... *inflammatory bowel disease*

LMR ..... *Lymphocyte - to- monocyte ratio*

mCRC ..... *metastasized colorectal cancer*

MMR ..... *mismatch repair*, *mismatch repair*

mSEPT9 ..... *hypermethylated septin 9*

MSI ..... *microsatellite instability*

NCCN, ... *National Comprehensive Cancer Network*

NIH .....*national institute for health*

NLR .....*Neutrophil-to-lymphocyte ratio*

OS .....*overall survival*

PCR..... *Polymerase chain reaction*

PFS ..... progression free survival

ROS ..... *Reactive oxygen species*

RR ..... *relative risk*

UICC .....*Union for International Cancer Control*

WHO ..... *word health organisation*

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1 Introduction

Introduction:

Colorectal cancer (CRC) is the third most common form of cancer and the second leading cause of cancer related deaths worldwide.[1] Since the early 1980s the mortality rate has constantly declined, mainly due to extensive screening methods and improved treatment options.[2]

Surgical resection followed by adjuvant chemotherapy is considered the standard therapy for CRC UICC Stage III.[3–5] In stage II patients adjuvant chemotherapy is case-dependent, with an ongoing debate on whether benefits outweigh possible severe side effects. Despite these treatment options, approximately 30% of all patients with UICC stage II or III CRC undergoing treatment with curative intention develop recurrent disease.[6]

Identifying patients at high risk of recurrent disease and adapting the treatment and follow-up regime accordingly can reduce the risk of recurrence. However, to date only limited clinical, laboratory or histopathological factors exist to predict the risk of recurrent disease in CRC patients.

It is therefore crucial to explore novel biomarkers that help in the identification of patients at high risk of recurrent disease. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are commonly used biomarkers for hepatocellular damage.[10] Both enzymes are produced by malignant and non-malignant cells and are expressed in different types of cells in the body. The ratio of plasma activities of AST and ALT, the so called De Ritis ratio, has initially been described as a diagnostic marker for acute viral hepatitis. Recent studies, however, have shown that the AST/ALT ratio may be used as a prognostic tool for certain kind of malignancies.[11–14] To the best of our knowledge there has been no study to date that reported a correlation between the AST/ALT ratio and the DFS in patients with localized CRC. Therefore, the purpose of this thesis is to summarize the most important screening as well as prognostic and predictive treatment markers and elucidate the role of the AST/ALT ratio as a novel and inexpensive marker for DFS in patients with localized CRC.

1.1 Epidemiology

CRC is the third most frequently diagnosed cancer in males and the second in females according to the WHO Globocan database. In 2018 CRC was responsible for close to 8,610,000 deaths worldwide, which makes it the second leading cause of cancer related deaths only topped by lung cancer.[15]
In the western world – including the USA and Europe – both incidence and mortality are slowly decreasing.

CRC incidence underlies regional differences and varies up to 10-fold. The countries with the highest incidence rates are North America, Europe, Australia and New Zealand, while in Africa and South-east Asia the chance to develop CRC is the lowest.[16] These differences in incidence rates indicate that lifestyle habits, including diet and exercise, as well as environmental exposures and genetic background are important factors in the development of the disease. In addition to that, people with low socioeconomic status are also at an increased risk compared to people with average or high socioeconomic status. Poor diet, smoking, obesity, physical inactivity as well as lower screening rates are all associated with low socioeconomic status and are thought to be the major factors for the increased incidence rates.[17]

In general men are more likely to develop CRC than women with an incidence of 72.3/100,000 compared to 42.2/100,000 for females. The incidence of CRC also greatly increases with age. The median age at diagnosis for colon cancer is 68 years in men and 72 years in women. For rectal cancer the median age of patients is 63 years for both genders.[1]

While the total incidence rate has been slowly declining for western countries, such as the USA or Europe, recent data has shown increasing numbers of CRC patients under the age of 50.[18] As of today the reasons for this trend are unclear.

1.2 Etiology

1.2.1 Genetic factors

1.2.1.1 Familial adenomatous polyposis (FAP)

FAP is an autosomal dominant inherited disease that usually causes multiple colonic adenomas during childhood with an average onset at the age of 16.[19] The syndrome is caused by a germline mutation and accounts for approximately 1% of all CRC. If untreated, the disease will lead to the development of CRC in almost all patients with an average age of diagnosis at 39 years. The increased CRC risk comes from the high number of adenomas. Patients usually present with hundreds to thousands of colorectal polyps. Since the polyps develop at such an early age, it is almost certain that at least one of them progresses into CRC. In up to 40% of all FAP patients synchronous CRC develops.[20]

Attenuated FAP (AFAP) is a milder form of the disease which is usually characterized by the presence of less than 100 adenomatous colorectal polyps, although the disease lacks a clear definition.[21] The risk of developing CRC is approximately 80% of that compared to FAP. Colorectal polyps are diagnosed at a mean age of 44 years, much later compared to classic FAP. The average age of CRC diagnosis in attenuated FAP patients is 56.[22]

1.2.1.2 Lynch Syndrome (HNPCC)

More common than FAP is Lynch syndrome or hereditary non-polyposis colorectal cancer (HNPCC), which accounts for approximately 3% of all CRC. The disease is caused by a defect in one or more of the DNA mismatch repair genes and is inherited in an autosomal-dominant manner. The genes most commonly mutated are hMLH1, hMSH2, hMSH6 and hPMS2.[23]

Depending on the mutated gene as well as the sex of the patient the risk of developing CRC in Lynch syndrome reaches from 10 to 47 percent.[24–26] In addition to the increased risk, CRC is also diagnosed at an earlier age in Lynch syndrome

patients compared to sporadic disease. Apart from that, patients suffering from the disease are also at an increased risk for developing other malignancies such as cancer of the endometrium, the ovaries, stomach and the small bowels.[24]

1.2.2 Non-genetic factors

1.2.2.1 Inflammatory bowel disease (IBD)

Patients suffering from chronic inflammatory bowel disease, such as Crohn`s disease or ulcerative colitits, are more likely to develop CRC compared to the general population. Chronic inflammation as well as an altered immune response associated with IBD are thought to be the reason for the higher incidence.[27]
Patients suffering from pancolitis are associated with an up to 15 times increased risk for developing CRC compared to healthy individuals. The increased risk starts approximately 10 years after initial diagnosis and increases with disease duration.[28] While there is less data for the association between Crohn´s diseases and increased CRC risk, it seems that the increase in incidence is similar to ulcerative colitis.[27]

1.2.2.2 Diabetes mellitus

Patients with diabetes mellitus have an 38% increased relative risk of developing colon cancer and a 20% increased relative risk of developing rectum cancer.[29] Increased insulin levels are thought to be responsible for the increased risk, as they act as a stimulant in the growth of colon mucosal cells. Furthermore, type II diabetes mellitus patients with CRC may have worse prognosis compared to patients without diabetes. In a study by Dehal et al. type II diabetes mellitus patients with CRC had a significantly increased risk of cancer-specific mortality in comparison to the non-diabetic CRC patients.[30]

1.2.2.3 Obesity

Excess body fat measured by an increased BMI has been associated with a higher risk of CRC. A 5 kg/m^2 increase in BMI has been associated with an elevated risk ratio of 1.24 for CRC.[31] An even stronger association has been shown for abdominal obesity with a 50% increased risk of CRC in the highest category of waist circumference.[32] A meta-analysis comprised of 13 studies investigated the effect of weight gain in adults and found a significant association for weight gain and increased CRC risk, although the increase in risk is only modest.[33]

1.2.2.4 Smoking

Cigarette smoking has been associated with increased incidence and mortality rate for CRC. In a recent meta-analysis including 106 observational studies, smokers had a RR of 1.18 for developing CRC as well as an increased risk of dying from the disease compared to people who never smoked. While the increased risk for developing CRC is only modest, smokers have a much higher risk of developing colonic polyps. Botteri et al. explain this discrepancy mainly by the long latency period for smoking and the development of CRC as well as the hypothesis that smoking has a stronger effect on the development of nonprogressive polyps.[34]

1.2.2.5 Diet

An increasing number of studies show an association between the consumption of red meat and especially processed meat with an increased risk of developing CRC, even though the data are not completely consistent. In a meta-analysis consisting of 10 cohort studies, 100 g/day of red meat were associated with an 17% increase in CRC incidence. This dose-depending relationship was also shown for processed meat, with an 18% increase for every 50g/day of processed meat consumption.[35] Other studies only show weak to no association for meat consumption and increased risk of CRC.[36]

The same discrepancy in study findings can be seen in diets that have been associated with decreased CRC risk. Several studies have found a diet high in vegetable, fruit and fiber intake to be protective against the development of CRC. In those studies, the groups with the highest intake of vegetables and fruit had a relative risk of 0.5 for the development of CRC compared to those with the lowest intake.[37] Contrary to these findings, a large prospective cohort study with around 135 000 participants found no association between fruit and vegetable intake and a reduced CRC risk.[38]

1.3 Morphology

1.3.1 Gross appearance

Colorectal carcinomas can vary in their morphologic appearance, depending on whether they present with an invasive or expansive growth pattern and can show signs of bleeding. Tumors in the right colon often have a polypoid, exophytic appearance, while left side tumors more often present as annular lesions.

1.3.2 Histology

Most tumors of the colon and rectum are adenocarcinomas, although different histologic subtypes, such as neuroendocrine or mesenchymal tumors, exist. The histologic subtype can have a prognostic impact. The signet cell carcinoma for example is characterized by cells containing a large mucin filled vacuole. It is associated with a more aggressive growth behavior and a worse prognosis compared to adenocarcinomas.[39]

The histologic grade assesses the degree of cellular differentiation in the tumor. In adenocarcinomas this is done by examining the gland formation of the tumor. In well differentiated tumors, gland formation is present and well defined. Poorly differentiated tumors lack well defined glandular structures and show cellular abnormalities and a high mitotic rate. The internationally used grading system recommended by the UICC for CRC consists of four tiers:

- **Grade 1** – Well differentiated (>95 percent gland formation)
- **Grade 2** – Moderately differentiated (50 to 95 percent gland formation)
- **Grade 3** – Poorly differentiated (<50 percent gland formation)
- **Grade 4** – Undifferentiated (no gland or mucin formation; no squamous or neuroendocrine differentiation)

Contrary to the recommendations of the UICC the WHO recently recommended the use of a two-tiered system, which only distinguishes between low grade and high grade tumors. This system is thought to simplify the grading process while retaining the same prognostic value.

1.3.3 Staging

Cancer staging is the classification of the anatomic extent of the disease. It is used in order to assess the patient's prognosis as well as to determine the proper treatment course.[40] The most commonly used staging system recommended by the UICC is the tumor, node and metastasis (TNM) staging system. If the staging is based on radiographic, endoscopic or intraoperative findings the term clinical stage is used (cT, cN,cM). For pathologic staging (pT,pN,pM), which has a greater validity, histopathologic examination is necessary. Depending on the TNM classifications one of five tumor stages is assigned. The detailed staging classifications are listed below.[41]

Table 1: Primary Tumor

	Primary tumor (T)
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ: intraepithelial or intramucosal carcinoma (involvement of lamina propria with no extension through the muscularis mucosa)
T1	Tumor invades submucosa (through the muscularis mucosa but not into the muscularis propria)
T2	Tumor invades muscularis propria

T3	Tumor invades through the muscularis propria into the pericolorectal tissues
T4	Tumor invades the visceral peritoneum or invades or adheres to adjacent organ or structure
T4a	Tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
T4b	Tumor directly invades or is adherent to other organs or structures

Table 2: Regional lymph nodes

	Regional lymph nodes (n)
NX	Primary tumor cannot be assessed
N0	No evidence of primary tumor
N1	Carcinoma in situ: intraepithelial or intramucosal carcinoma (involvement of lamina propria with no extension through the muscularis mucosa)
N1a	Tumor invades submucosa (through the muscularis mucosa but not into the muscularis propria)
N1b	Tumor invades muscularis propria
N1c	Tumor invades through the muscularis propria into the pericolorectal tissues
N2	Tumor invades the visceral peritoneum or invades or adheres to adjacent organ or structure
N2a	Tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
N2b	Tumor directly invades or is adherent to other organs or structures

Table 3: Metastasis

	Distant metastasis (m)
M0	No distant metastasis by imaging or other studies, no evidence of tumor in distant sites or organs.
M1	Metastasis to one or more distant sites or organs or peritoneal metastasis is identified
M1a	Metastasis confined to 1 organ or site is identified without peritoneal metastasis
M1b	Metastasis to two or more sites or organs is identified 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	No	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-T4	N1/N1c	M0
	T2-T3	N2a	M0
	T1-T2	N2b	M0
IIIC	T4a	N2a	M0
	T3-T4a	N2b	M0
	T4b	N1-N2	M0
IVA	Any T	Any N	M1a
IVB	Any T	Any N	M1b
IVC	Any T	Any N	M1c

1.4 Treatment of localized CRC

1.4.1 Therapy for stage I

Surgical resection is the mainstay in the curative treatment of CRC for stages I-III. For a curative outcome, the complete removal of the tumor including en bloc excision of the associated lymphatic drainage system is imperative. The current guidelines suggest that at least 12 lymph nodes should be resected to guarantee proper staging and reduce the risk of recurrence. Laparoscopic surgery may be an option for non-locally advanced colon cancer with the benefit of a faster recovery time. Both techniques offer the same long term oncologic outcome.[42]

In rectal cancer, total mesorectal excision is the standard surgical procedure for tumors located in the middle or lower third of the rectum. For tumors located in the upper part of the rectum, sphincter-sparing surgery may be an option if a sufficient tumor-free safety margin can be guaranteed.

For stage I CRC, adjuvant chemotherapy does not improve the prognosis and is therefore not recommended.

1.4.2 Therapy for stage II

Primary goal for Stage II CRC is a curative treatment, therefore complete surgical resection is the central element of the treatment. In this stage, local recurrence after radical tumor resection is low. Adjuvant therapy only leads to a small increase in DFS and a minimal overall survival benefit and is therefore optional. For patients without any risk factors, therapy should be carefully considered since the associated impact on quality of life may outweigh the benefits. Risk factors in this stage include: T4 TNM Stage, tumor perforation, surgery in an emergency setting, histologically confirmed lymph- or vascular infiltration. [43]

1.4.3 Therapy for stage III

In stage III CRC, complete surgical resection is also the most important factor for a curative outcome. In this stage the benefits of adjuvant chemotherapy have been clearly shown in multiple studies and it is therefore the standard approach. A combination therapy of 5-FU, folic acid and oxaliplatin or Capecitabin/Oxaliplatin (CAPOX, XELOX) are considered as standard therapy. For patients with a low risk of recurrence (T1-3 and N1) a 3-month therapy of CAPOX is preferred to a 6-month 5-FU/ folic acid/ oxaliplatin therapy as the shortened therapy duration has been shown to reduce long term neurotoxicity, whereas for patients in a higher risk setting (T4 or N2) a 6-month course of either 5-FU/ folic acid/ oxaliplatin or capecitabine/oxaliplatin therapy is associated with the highest DFS and considered standard of care.[44]

In addition to the above, radiotherapy plays an important role in the treatment of localized rectal cancer. Neoadjuvant or adjuvant radiotherapy in combination with either a fluoropyrimidine or capecitabine is the standard choice of therapy.[45] Preoperative chemoradiotherapy is equal to postoperative chemoradiotherapy in regard to overall survival, but is associated with reduced toxicity and reduced local recurrence rate.[46] According to the ESMO guidelines, adjuvant chemotherapy should only be considered for stage III as well as "high-risk" stage II rectal cancer patients, as the evidence today suggests that there is only a benefit to DFS and not OS.[47] The length of preoperative chemoradiotherapy and adjuvant chemotherapy should add up to 6 month total.[46]

1.5 Definition Biomarker

The term biomarker was first used in a medical publication in 1977 on the influence of renal insufficiency on serum RNase in patients with multiple myeloma by Karpetsky et al.[48] With the increased scientific interest over the last decades came a variety of different definitions and understandings regarding the question what a biomarker is.

In 2001 the American National Institute of Health (NIH) formed the Biomarker Definition Working Group that proposed the following definition: A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response to a therapeutic intervention.[49]
This definition for the term biomarker includes simple medical signs such as heart rate and blood pressure as well as laboratory measurements and complex molecular genetic changes.

1.6 Current state of research

With the ongoing shift from standardized medicine to more personalized approaches in both diagnosis and treatment of disease, the importance of new and properly validated biomarkers continues to grow.[50,51] Over the last two decades the search for new biomarkers has increased year to year. A literature search on PubMed showed a total of 812,570 results for publications with the keyword biomarker. While for the year 2000 the total number of published articles was 17,295, in 2017 the number grew to over 55,000.

Constant advances in sequencing techniques as well as an increasing knowledge of the underlying tumor biology help in the search for novel biomarkers. However, proper biomarker validation represents a major obstacle in their translation to clinical use. Many promising prognostic and predictive biomarkers emerge from small retrospective studies. The validation process for these biomarkers requires

large, randomized clinical trials and meta-analysis, which are associated with time and high costs.[52]

1.7 Screening markers

Colorectal carcinomas have a significantly better prognosis when diagnosed and treated in an early stage. The five-year relative survival rate for CRC in a localized stage is 90,3%, for local progression and involvement of adjacent organs or lymph nodes 70,4% and just 12,5% for a metastatic spread.[53] Given that colorectal cancer usually develops in a slow manner from precancerous lesions that are very often removable, it is an obvious candidate for regular screenings.

The benefits of various screening methods have been shown in multiple studies and are far reaching, especially with the implementation of an organized screening program. An example for a successful screening program was shown by a Californian health care provider. Patients between 51-75 years where screened by either colonoscopy, gFOBT or FIT over a course of 15 years. Initiation of this program greatly increased the screening rate from 38,9% in 2000 up to 82,7% in 2015 and led to a reduction in CRC mortality of 52.4% and a reduction of annual CRC incidence of 25.5%.[54]

In addition to the above stated benefits, studies show that CRC screening is also cost-effective. A meta-analysis by Ran et al. in 2019 comprised 33 studies from Europe, North America, Asia and Australia regarding the cost effectiveness of CRC screening strategies. All of the most common screening procedures, including annual and biennial fecal occult blood tests, immunochemical tests as well as colonoscopy every 10 years and flexible sigmoidoscopy every 5 years were cost effective compared to no screening.[55]

Today organized screening programs have been implemented in many countries. In 2013, 19 out of 28 countries in the European Union have established a nationwide organized screening program.[56] At this time Austria follows an opportunistic approach to CRC screening; however, recent organized screening

projects in Vorarlberg and Burgenland showed promising results that could be implemented in other parts of Austria as well.[57]

1.7.1 Stool

1.7.1.1 FOBT

The fecal occult blood test is the most commonly used stool-based screening method for CRC. The earliest representative of this method is the gFOBT (guaiac fecal occult blood test), widely known as Hemocult®. A fecal sample is placed on a guaiac paper (i.e.a paper impregnated with alpha-guaiaconic acid), that turns blue in the presence of heme, a hemoglobin component, when mixed with hydrogen peroxide.

The sensitivity for a single gFOBT is relatively low, with reported numbers reaching from 10% to 30% and is therefore not sufficient for screening. This is due to the fact that not all colorectal tumors bleed, and if they bleed it is often in an intermittent fashion. [58] However, the sensitivity rises to 92% when the test is performed on 3 consecutive stool samples.[59] If a test shows a positive result, further stool testing is not appropriate, and a colonoscopy should be performed.

There have been multiple large randomized trials that support the effectiveness of gFBOT. These studies showed a reduction in CRC mortality ranging from 13% to 18% over a follow up time of 13-14 years.[60,61] Despite the proven effectiveness and relatively cheap cost, there are several shortcomings that became apparent over the years of clinical use. These include the requirement of 3 samples, dietary restrictions such as the avoidance of red meat, false negatives with ascorbic acid and positive tests from other non-malignant disease that present with bleeding.

A newer approach, the so called immunochemical fecal occult blood test (iFOBT) or fecal immunochemical test (FIT) was developed to address these issues. This method utilizes specific antibodies that bind to human globin. Since stomach acid alters the structure of globin, FIT does not react to upper gastrointestinal bleedings and is therefore more specific than gFOBT. Additionally, several trials report a

higher sensitivity as well as an increased participation rate, since only one stool sample is needed.[62,63]

1.7.1.2 Fecal DNA

Fecal DNA can be used as a disease-specific biomarker because malignant colonocytes continuously exfoliate into the colonic lumen. These new tests usually evaluate KRAS mutations, aberrantly methylated BMP3 and NDRG4 promoter regions, β-actin and a hemoglobin assay. As of today only one fecal DNA test was approved by the Food and Drug Administration (FDA) and is commercially available under the name Cologuard™.

In a large trial by Imperiale et al. a multitarget stool DNA test was compared to the more established fecal immunochemical test (FIT) in a large patient population who underwent screening colonoscopy (n= 9989). Fecal DNA testing showed a sensitivity for CRC of 92,3% compared to 73,8% when tested with FIT. Precancerous lesions were detected with a sensitivity of 46,2% by DNA testing and 23,8% by FIT. The specificity of DNA testing was inferior to FIT, with 86,6% compared to 94,9%. One reason for the higher sensitivity of fecal DNA and a major advantage when compared to FIT is that a proximal location of the tumor does not affect the test.[64]

In contrast to the trial above, a recent German publication found fecal DNA testing to be just equivalent or even worse in diagnostic performance despite its almost 20-fold cost.[65] One limiting factor in the performance of fecal DNA test is the fact that only 0,01% of the fecal DNA is human, and only a small part of that DNA derives from the tumor cells.[66]

1.7.2 Blood

1.7.2.1 Septin – 9

While Colonoscopy remains the gold standard as the screening method for CRC, non-invasive stool-based methods such as FIT or the fecal DNA test have been

proven to be effective alternatives. However, the inconvenience of stool collection and the required need for shipping can have a negative impact on screening participation. A simple yet effective blood test could encourage those, who rejected other forms of screening.

There are 13 septin genes in humans that translate into various proteins with widespread functions across many cellular processes. These processes include crucial functions in cell division[67], cytokinesis[68], microtubule regulations[69], vesicle targeting[70], exocytosis[70], cell motility and bacterial-cellular interactions[71].

An overexpression of Septin-9 has been found in various tumors, such as hepatic, breast and ovarian carcinomas.[72] In CRC the gamma promoter of the SEPT9 gene V2 transcript is hypermethylated (mSEPT9). Necrotic or apoptotic cells as well as cell-free DNA shed from the tumor and circulate in the peripheral blood stream. Specialized assays can identify mSEPT9 in the circulating plasma with the use of PCR.

The accuracy of mSEPT9 in CRC detection has been verified in many studies. However, the results of the different studies vary widely, with rates for sensitivity ranging from 48,2% to > 90%.[73,74] The Interpretation of these studies is additionally complicated by the lack of a standardized testing algorithm. Algorithms used to investigate the diagnostic properties of mSEPT9 were 1/1, 1/2, 1/3, 2/3.
A 1/3 algorithm means that the test is positive, if one of the three PCR measurements exceeds the predefined limit of plasma mSEPT9.

In 2017 a meta-analysis was done by Song et al, including 24 studies. The sensitivity for the 1/3 algorithm was 78% and the specificity 84% compared to the 2/3 algorithm with a sensitivity of 73% and a specificity of 96%. The results of the meta-analysis confirmed that mSEPT9 is a reliable biomarker, especially when used in the 2/3 algorithm.[75]

1.8 Prognostic and Predictive Markers

1.8.1 Clinicopathologic prognostic markers in localized CRC

Certain clinicopathological features have been associated with higher risk of recurrence and a worse prognosis for stage II CRC. In a study by Quah et al. T4 tumor stage (HR 2.7; 95% CI 1.1–6.2; P=0.02), a preoperative carcinoembryonic antigen greater than 5 ng/ml (HR 2.1; 95% CI, 1.1–4.1; P=0.02) and the presence of lympho-vascular or perineural invasion (HR, 2.1; 95 % CI, 1–4.4; P=0.04) were associated with a shorter DFS. The 5-year disease specific survival for patients lacking any of these features was 95%. If the tumor carried one of the above features the 5-year disease specific survival dropped to 85% and with 2 or more features to 57%.[76]

Additional clinicopathological high risk features for stage II CRC identified by other studies include:

- High grade histology [77]
- Bowel obstruction or perforation [78]
- Close or positive surgical margins [79]
- Less then 13 sampled lymph nodes [80]
- Occult nodal micometastases [79]

Despite being associated with a worse prognosis, only limited evidence exists that patients carrying these features benefit from adjuvant chemotherapy. Nevertheless, it is advised by both the ASCO and the NCCN, to take these factors into account when deciding on whether or not an adjuvant chemotherapy is appropriate.[79]

Since chemotherapy is recommended to stage III CRC patients regardless of specific tumor features, clinicopathological markers are of less importance in this regard. However, they can be used to predict the prognosis and outcome. Most of

the above stated risk factors are also associated with a worse prognosis for stage III CRC although less data exists.
With the intention of identifying possible clinicopathological biomarkers aiding in the accurate prognostic prediction for stage III CRC Li et al. conducted a study in which tumor grade, positive lymph nodes, intravascular emboli, high preoperative serum CEA levels, albumin to globulin ratio, T stage as well as N stage emerged as prognostic factors for OS. Furthermore, Tumor grade, lymph node metastasis ratio, intravascular emboli , albumin to globulin ratio and N stage were found to be prognostic factors for DFS in stage III CRC. [81]

1.8.2 Molecular Pathways behind CRC

Before looking into specific prognostic and predictive markers, it is important to understand the underlying molecular pathways behind CRC. The carcinogenesis of CRC has been extensively studied. At least three molecular pathways underlying the incurrence of CRC have been identified. 65-70% of all sporadic CRC develop on basis of the chromosomal instability phenotype (CIN).[82] This group is characterized by chromosomal alterations with frequent chromosomal amplifications and loss of heterozygosity resulting in a high number of APC, TP53 and KRAS mutations.[83] Tumors of this phenotype develop more frequently in the distal colon.[84]

The second pathway underlies a mismatch repair deficiency leading to genetic hypermutability and the occurrence of high microsatellite instability (MSI-H). This pathway accounts for approximately 15% of all CRC tumors and occurs more often in stage II (22%) than in stage III (12%) or IV (4%) CRC. [85,86] The specifics of this pathway will be discussed in the following chapter “MSI”.

The third common pathway, in contrast to the above, does not rely on changes in the tumor’s DNA sequence but on epigenetic molecular alterations. One example of these epigenetic alterations is the aberrant promoter hypermethylation that occurs at CpG dinucleotide dense regions, the so called CpG islands. CRCs of the CPG island methylator phenotype (CIMP) are associated with proximal locations

of the colon, older individuals, females, a family history of CRC, a mucinous cell differentiation and serrated adenomas as precursor lesions. [87–93] Around 35% of all CRC develop via this pathway.

1.8.3 Molecular Markers

1.8.3.1 MSI

The DNA mismatch repair proteins are responsible for recognizing and repairing nucleotide mispairings as well as erroneous insertions or deletions that occur during the process of DNA replication and recombination. [94] There are several genes that encode for these proteins, including hMSH2, hMSH6, hPMS2 and hMLH1. Mutations in these genes can occur in a sporadic manner in CRC or be the result of a hereditary germline mutation as it is the case with Lynch syndrome. Patients with Lynch syndrome have a greater risk to develop CRC in their early adolescence, most commonly between 20 and 30 year and in contrast to sporadically acquired MSI seldom present with a BRAF-V600E mutation.[95]

Microsatellites are simple repeated sequences of DNA, that are particularly exposed to damage of the DNA mismatch repair system. Cells with an impaired mismatch repair system are not able to correct errors that occur during DNA replications. This condition leads to either a shortening or an extension of these microsatellite regions and is called Microsatellite instability (MSI). According to the Bethesda Guidelines, MSI can further be classified into MSI-high (MSI-H) or MSI-low (MSI-L), depending on the extent of unstable MSI loci. If none of the microsatellite sequences are mutated, the tumor is termed microsatellite stable (MSS). [96,97]

MSI testing can either be performed by using a PCR based assay or by an analysis of MMR protein expression via Immunohistochemistry (IHC). In sporadic CRC only the loss of hMLH1 protein expression has been described. If a CRC tumor shows a loss of hMLH1 expression in IHC, additional testing for BRAF mutations are a cost-effective approach to exclude Lynch Syndrome, since BRAF V600E mutations are strongly associated with sporadic disease. [98] Furthermore,

BRAF V600E mutations are associated with a poorer prognosis in metastasized CRC and therefore testing has additional prognostic value. [99,100] An example of an MSI testing procedure is shown in the figure below.

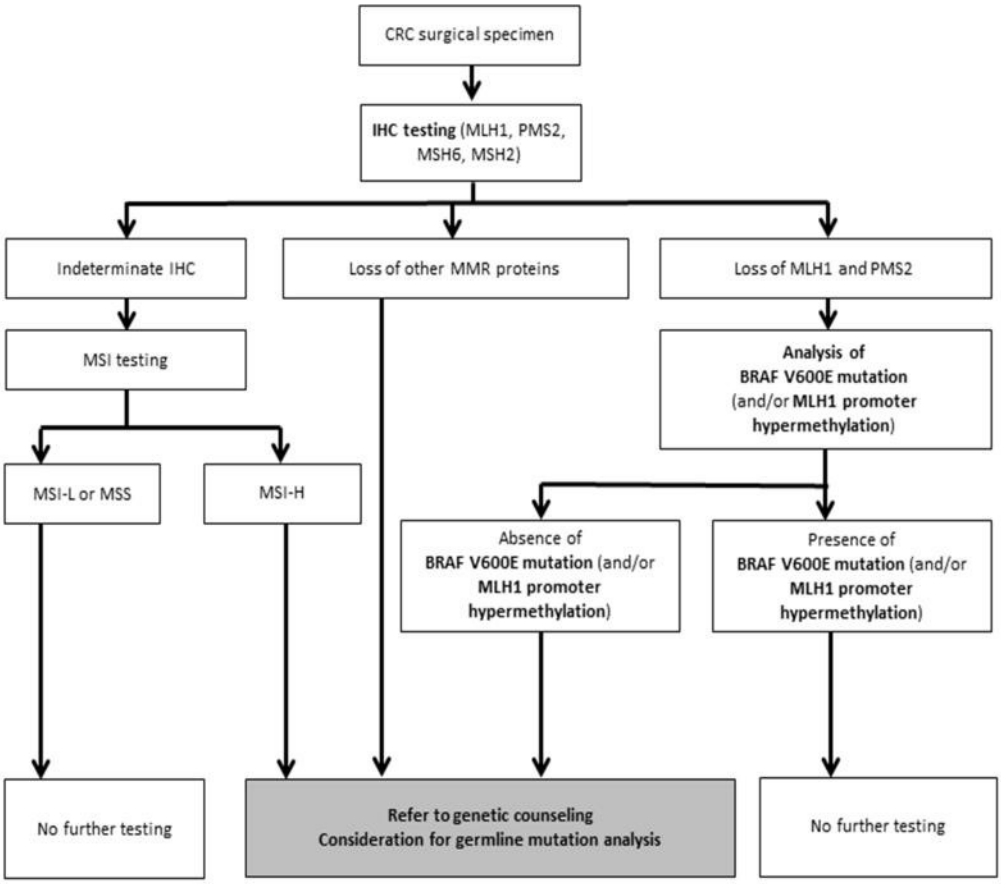

Figure 1: MSI testing

CRC with MSI-H is associated with several characteristic clinicopathological features. Cancers arising as a result of MSI are more often localized proximal to the splenic flexure and have a higher prevalence in women. Histologically they are associated with a mucinous cell type, poorer differentiation, a greater depth of invasion with an increased number of tumor-infiltrating lymphocytes and a lower stage. [101]

Taking all the histopathological features into account, MSI-H tumors show a better overall surviving compared to colorectal carcinomas that are microsatellite stable

(MSS). A meta-analysis by Popat et al including 32 studies with a study population of 7,642 patients confirms MSI as an independent prognostic marker. In this study tumors with MSI-H were associated with a significantly better prognosis regardless of tumor stage (combined hazard ratio (HR) 0.65, 95% CI 0.59–0.71).[102]

In addition to the associated clinicopathological features, the MSI status also plays an import role when assessing a patient's further treatment. A large study investigated whether the MSI status affects the outcome of stage II and III CRC patients undergoing surgery alone and surgery followed by a 5- fluorouracil- (5-FU) based adjuvant chemotherapy. The results showed that stage II patients with MSI-H CRC do not benefit from an adjuvant 5-FU-based chemotherapy, in contrast to patients with proficient MMR tumors. [103]

In 2015 a phase II study by Dung et al published in the New England Journal of Medicine showed a correlation between MSI status and the susceptibility of pembrolizumab. Pembrolizumab is a humanized antibody that targets the programmed cell death (PD-1) receptor of lymphocytes. In this study the clinical activity of pembrolizumab was evaluated in 41 patients with progressive metastatic CRC with or without a mismatch repair deficit. The response rate and progression-free survival rate (PFS rate) were 40% and 78% for mismatch repair- deficient CRC and 0% and 11% for mismatch repair-proficient CRC. The team suggests that the greatly increased number of mutation associated neoantigens that result from a defective mismatch repair system is the basis for the enhanced anti-PD1 responsiveness; however, the exact mechanisms are not fully understood hitherho. [104] Further studies have shown that the susceptibility of pembrolizumab in relation to the MSI status is not restricted to CRC but rather independent on the type of tumor.[105]

In the ongoing phase II Checkmate- 142 trial ,Nivolumab, a PD-1 antibody was combined with low dose Ipilimumab as first-line therapy for MSI-H metastatic CRC patients. The latest data, presented at the ESMO 2018, showed an ORR of 60% at a median follow-up of 13.8 months. At 12 months the progression free survival rate was 77% and the OS was 83%. Nivolumab and ipilimumab synergistically promote T-cell antitumor activity with a better manageable safety profile and more

preferable results than Nivolumab monotherapy. While the final results of the study are still pending, the combination of low dose Ipilimumab and Nivolumab represent a promising new treatment option that may become the new standard first line therapy for metastatic MSI-H CRC patients.[106]

1.8.3.2 RAS

The discovery of the epidermal growth factor receptor (EGFR) was a tremendous milestone in the way of targeted therapy. Ever since the validation of EGFR as a therapeutic target there have been numerous trials that proved the beneficial effect of anti-EGFR antibody therapy in patients with metastatic CRC.[107–109] However, there was a certain percentage of patients who did not respond to this therapy. In further studies a correlation between KRAS mutations and poor response to anti-EGFR antibody therapy was discovered. [110]

There are 3 RAS genes in humans: HRAS, NRAS and KRAS. KRAS encodes for a GTPase protein that plays an important role in signal transduction, cellular growth, differentiation, proliferation and survival.[111] It increases cell proliferation and induces tumorigenesis.[112] Approximately 40% of all CRC show activating mutations in the KRAS genes, most commonly in exon 2.[113] These mutations are thought to impair GTPase activity, with the effect of increased and unregulated cellular proliferation and the transformation to a malignant tumor. [114] KRAS mutations, involving either codon 12 or 13, have been associated with a poorer prognosis compared to wild-type KRAS tumors. [115]

Cetuximab and panitumumab are two commonly used anti-EGFR-antibodies in CRC therapy. Their effectiveness relies on a KRAS exon 2 wild type, as multiple studies have shown. [116,117] Karapetis et al showed that for patients with mutated KRAS CRC the median overall survival was 4,5 months in the group receiving cetuximab, compared to 4,6 months in the supportive care group. For patients with wild-type KRAS tumors, a median overall survival of 9,5 months could be achieved, while for the supportive care group the median OS was 4,8 months. The median progression-free survival for the wild-type KRAS patients receiving

cetuximab was 3,7 months, compared to 1,8 months for the group of patients with mutated KRAS CRC receiving cetuximab.[117] Similar results where shown for panitumumab.[116] However, there have also been conflicting studies that did report a benefit of cetuximab therapy for certain kind of KRAS mutations.[118]

These studies confirm that exon 2 KRAS mutations are a negative predictive biomarker for the therapy with anti-EGFR antibody therapy in metastasized CRC. More recent studies have also concluded that other RAS mutations as well as BRAF, NRAS and PIK3CA exon 20 mutations are also associated with a resistance to anti-EGFR antibody therapy. [119,120]

As a result of these studies, it is now recommended that all patients who are potential candidates for anti-EGFR antibody therapy should be tested for mutations in KRAS and NRAS in exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146) by the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN). [121,122] While sanger sequencing of tumor tissue seems to be the standard method of RAS testing, there are several new methods that are expected to become increasingly popular in the next few years. Next generation sequencing platforms are able to detect single nucleotide changes, insertions, deletions and translocations in several genes or loci in a single complex PCR. Furthermore, this technology requires only around 1-5% of RAS mutated cells compared to 10-25% for sanger sequence.[123,124]

The high sensitivity of next generation sequencing devices allows the detection of genetic alterations in circulating tumor DNA (ctDNA) from a simple blood draw. This method is called ‘liquid biopsy’. Targeted agents, such as cetuximab and panitumumab, often require associated molecular testing. However, as the patients are mostly in an advanced stage, the molecular testing is often performed on tumor tissue that has been collected years before and the results might not be consistent to the current genomic state of the disease. In cases where a new tissue biopsy cannot be obtained, ctDNA could offer more accurate molecular information about the current tumor than tumor tissue from long preceding biopsies.[125]

Recent studies have demonstrated that wild-type KRAS patients treated with anti-EGFR- antibodies develop mutated KRAS clones, that lead to therapy resistance. However, upon antibody withdrawal, these KRAS clones decay and the patients regain drug sensibility, which explains the efficiency of rechallenge therapies. Liquid biopsies can be used to monitor the molecular dynamics of CRC and guide the use of specialized treatments. [126]

In addition, several studies investigated the prognostic and predictive correlations of RAS in localized CRC. In a study by Deng et al. the association between the KRAS status and the 3-year DFS for stage II and III CRC patients receiving FOLFOX was analyzed. In this study KRAS status was significantly associated with a worse 3-year DFS (HR, 1.572; 95% CI, 1.058–2.335; P = 0.025). Patients with wild type KRAS status did not benefit from adjuvant chemotherapy (3-year DFS 84.3% vs 82.0%, P = 0.66). However, a difference in 3-year DFS was observed for patients with stage III KRAS mutant tumors. Here, the 3-year DFS was 74.4% for patients receiving FOLFOX compared to 50.2% for patients without any chemotherapy (P = 0.020). [127]

1.8.3.3 BRAF

BRAF is the direct downstream target of KRAS in the RAS/RAF/MEK/ERK pathway (MAPK/ERK pathway). RAF activation leads to the phosphorylation of MEK1/2 proteins, which further activates ERKs. ERKs are responsible for the phosphorylation of multiple transcription factors and thereby regulate important cellular activities. [128]

Between 8 and 12 % of all metastatic CRC harbor a BRAF mutation with the distinct BRAF V600E mutation accounting for >90% of these numbers. This point mutation has been associated with a poor prognosis. While the data for the prognostic value of BRAF mutations in the adjuvant setting is limited, it has been reported as an independent negative prognostic factor for stage II and III colon cancer patients. [129] In the metastatic setting, BRAF V600E mutation has been

shown to be a powerful prognostic marker. In a study by Yokata et al the median overall survival for BRAF mutated mCRC was 11 months compared to 40 months of patients with KRAS and BRAF wild-type mCRC.[130] The FOCUS trial also reported a worse OS for BRAF mutated mCRC [HR1.82; 95% confidence interval (CI), 1.36–2.43] but showed no difference in the progression free survival (PFS) (HR, 1.14; 95% CI, 0.86–1.52). [131]

In addition to being a prognostic marker, BRAF V600E mutations have also been associated with distinct clinicopathological characteristics. The mutation appears to be more common in female patients as well as patients over the age of 60. There is a significant association between BRAF V600E and advanced TNM stage at diagnosis, mucinous histology and poor differentiation, proximal location, MSI and KRAS wild-type.[132] Because of the association with these characteristics and a poor prognosis, early detection of BRAV V600E mutation can have an impact on the therapeutic options for a patient. It is therefore recommended by the NCCN guidelines to include BRAF testing at the time of diagnosis of mCRC.[133]

BRAF V600E mutations are a potential therapeutic target. In malignant melanoma, the mutation is present in around 50% of all cases and associated with very high response rates to therapy with BRAF-inhibitors such as vemurafenib, dabrafenib and encorafenib.[134] Unfortunately, CRC appears to be resistant to monotherapy with BRAF- inhibitors. The lack of response has been hypothesized to be due to feedback activation of the EGFR/PI3K/AKT pathway. [135] However, recent studies suggest that combinations of BRAF, MEK and other pathway inhibitors improves response rates by targeting the adaptive feedback pathways responsible for the primary resistance to BRAF inhibitors.[136] The BEACON CRC trial is the first phase III trial evaluating the efficacy and safety of the triplet combination encorafenib , a BRAF inhibitor, binimetinib, a MEK inhibitor and cetuximab, an anti-EGFR antibody, in patients with BRAF V600E mutated mCRC.

The first results of the safety lead-in were presented in June 2018. Among the 30 patients, a confirmed overall response rate (ORR) of 48% was reported. The one-year overall survival rate was 62%.[137] These results show substantial improvements to the currently approved therapy options for patients with BRAV V600E mutated mCRC.

The currently recommended first line therapy for fit patients with BRAF V600E mutated mCRC is FOLFOXIRI plus bevacizumab.[138]
Furthermore, several studies evaluated the predictive value of BRAF V600E mutation on the effect of anti-EGFR-antibody therapy. A meta-analysis including 7 studies and a total of 1,352 patients associated BRAF V600E mutation in wild-type KRAS mCRC patients treated with anti-EGFR-antibody therapy with a deterioration in PFS and OS (hazard ratio=2.78, 95% CI=1.62-4.76; hazard ratio=2.54, 95% CI=1.93-3.32).[139] Therefore the use of anti-EGFR therapy in patients with known BRAV V600E mutations is currently not recommended. [138]

Regarding the localized setting, BRAF has been shown to be a prognostic marker for both stage II and III CRC. A meta-analysis by Zhu et al. consisting of seven phase III randomized clinical trials with a total of 1,035 BRAF mutation stage II/III CRC was conducted to assess the negative prognostic role of BRAF in localized CRC in a quantitively way. In this study BRAF mutation was associated with a worse OS (HR = 1.42, 95% CI: 1.25–1.60; P < 0.00001) as well as a shorter DFS (HR = 1.26, 95% CI: 1.07–1.48, P = 0.006) compared to BRAF wild type tumors.[140]

1.8.4 Tumor location as a prognostic and predictive marker

The proximal colon is derived from the embryonic midgut and includes the appendix, the colon ascendes and the proximal two-thirds of the colon transversum. The distal colon originates from the hindgut and includes the distal part of the colon transversum, the descending colon, the sigmoid colon and the rectum. While the proximal colon is perfused by the superior mesenteric artery, the distal colon is served by the inferior mesenteric artery. Apart from the different embryonic origin and vascular supply there are also various distinct histological, immunological, and molecular differences between the proximal and the distal colon. [141–143]

Considering these profound differences, CRC can be separated into left-sided and right-sided CRC. These two subgroups of CRC show differences in the pathogenetic development, prognosis and clinical responses to chemotherapy.[144–

[146] While there is no consistent definition, the splenic flexure is the most common dividing point between left- and right sided CRC. [147] Since the phenotype of a tumor is determined by its molecular characteristics, the site of tumor origin remains a useful approximation until a clearly defined molecular marker exists to distinguish between those two subgroups.[148]

In a meta-analysis by Petrelli et al, including 66 studies and a total of 1,427,856 patients, left sided primary tumor location was associated with a significantly reduced risk of death (HR, 0.82; 95% CI, 0.79-0.84; P < .001). The association was independent of stage, ethnicity, adjuvant chemotherapy, year of study, number of participants, and quality of included studies.[144]

Assessing survival and recurrence patterns between left and right sided stage I and II colon cancer, Lee et al. found no difference in the 5-year OS as well as the 5-year recurrence free survival (RFS) However, in their study, stage III right sided colon cancer was associated with both worse 5-year OS (HR, 1.53; 95% CI, 1.02–2.30; P = 0.037) and an increased risk for recurrence (HR, 1.56; 95% CI, 1.13–2.14; P = 0.006).[149]

Patients with right-sided colon cancer appear to be significantly older. While Patients with a tumor located in the caecum were the oldest, patients with a tumor located in the sigmoid colon were the youngest.[150] A likely explanation for these findings is the delayed diagnosis of CRC in the proximal colon. Left-sided CRC is associated with pain and more frequent bleeding, while tumors of the right side of the colon tend to be more subtle in the expression of symptoms.[151,152] Additionally the likelihood of a prior polypectomy, and thus the prevention of a transition to a malignant disease, is also greater on the left side of the colon. [153]

There is also a difference between the average tumor size at time of resection between left- and right-sided CRC. Tumors of the right side of the colon tend to grow more frequently in a flat manner. This in addition to the generally wider lumen of the right colon, allow it to achieve larger sizes while still remaining clinically asymptomatic. [151]

Recent studies suggest that laterality might also be a predictive marker for response to targeted therapy in patients with RAS wild-type metastatic CRC. In a large US intergroup trial 1,137 patients with KRAS exon 2 wild-type mCRC were randomly assigned to cetuximab or bevacizumab with a backbone chemotherapy of either FOLFOX or FOLFIRI (individual choice of the oncologist). The study concluded that there was no significant difference in overall survival between the addition of cetuximab vs bevacizumab to the given chemotherapy.[154] However, in a retrospective analysis of the data from this study an association between the tumor location and the benefit of cetuximab vs bevacizumab was found. Cetuximab provided superior median OS (37,5 vs 32,1 month) for patients with left-sided CRC, while bevacizumab showed better results for right-sided CRC (median OS 24,2 vs 16,7 month). Similar findings were reported in other studies.[155]

1.8.5 Blood based markers

1.8.5.1 CEA

Carcinoembryonic antigens (CEA) are glycoproteins, that are produced during fetal development by cells of the gastrointestinal tract. As production stops shortly before birth, adult serum levels are usually low. However, certain kinds of cancer – including CRC – can lead to an elevation of CEA levels. CEA was discovered in 1965 and has since then been a prominent biomarker for CRC.

CEA is most commonly used as a marker for disease recurrence during the follow-up period after surgical resection. After a successful tumor resection the elevated CEA serum levels should normalize within 4 to 6 weeks.[156,157] A failure of the serum CEA levels to normalize can imply an incomplete resection or a systemic disease and thus needs further investigation.[156] The sensitivity and specificity of CEA to detect recurrence in patients who underwent surgical resection depends on the used threshold values. A Cochrane review reported a pooled sensitivity of 82% and a specificity of 80% for a cutoff value of 2.5 mcg/L. When using a higher threshold of 10 mcg/L the sensitivity dropped to 68% but the specificity was higher (97%). The authors of this study concluded that CEA is insufficiently sensitive to

be used alone in the detection of recurring CRC, even with a low threshold. Therefore they recommend the addition of another diagnostic modality, but using the high cut-off value for CEA of 10 mcg/L to avoid high numbers of false postives.[158] The sensitivity of CEA is also dependent on the site of recurrence. The rate of detection is high for hepatic and retroperitoneal metastasis, with a sensitivity of over 70%, but below 50% for local, peritoneal or pulmonal metastasis. For solitary lung metastasis the sensitivity is just 15%. [159]

Currently most CRC guidelines including ASCO, NCCN, ESMO and the ACS, suggest the monitoring of CEA every 3 to 6 months for the first 2 to 3 years after surgery. However recent publications question the need for such frequent testing. The COLOFOL trial compared two alternative follow-up schedules for patients with stage II or III colorectal cancer who underwent surgery with curative intent. Patients were randomized either to follow-up testing with computed tomography of the thorax and abdomen and serum CEA at 6, 12, 18, 24, and 36 months after surgery (high-frequency group) or at 12 and 36 months after surgery (low-frequency group). There was no significant difference in the 5-year overall mortality or in the incidence of recurrence detection for the two groups. [160]

CEA concentrations are significantly higher in patients with CRC than those with benign colorectal disease. [161] Nonetheless CEA has little use in the diagnosis of colorectal cancer, as diagnostic sensitivity and specificity are rather low. Furthermore, the fact that elevated CEA levels are usually only detected in advanced stages of CRC make it an ineffective method for screening.

Even though many patients do not present with elevated CEA levels at the time of diagnosis, multiple studies found preoperative CEA status to be a valuable prognostic marker. In a study including 17,910 patients diagnosed with CRC of all stages elevated preoperative CEA levels (p-CEA) were independently associated with a 60% increased risk of overall mortality (hazard ratio of death = 1.60, 95% confidence interval = 1.46 to 1.76, P<.001). It was also observed that N0 patients with elevated p-CEA had a worse prognosis (HR of death = 1.75, 95% CI = 1.48 to 2.09) than N1 patients with normal p-CEA (HR of death = 1.58, 95% CI = 1.30 to 1.91).[162]

1.8.5.2 MicroRNA (miRNA)

MicroRNA are small, non-coding RNA molecules that play an important role in post transcriptional regulation of gene expression. They regulate diverse cellular processes including cell cycle progression, cell differentiation, apoptosis, developmental transitions and organ morphology [163].

A connection between miRNA and cancer has first been described in a study by Calin et al, in which they described frequent deletions and down-regulations of miR15 and miR16 in chronic lymphocytic leukemia.[164] In the complex process of carcinogenesis the expression of miRNA is dysregulated. This can be explained by the fact that a large number of miRNA genes that are known today are frequently located at regions of loss of heterozygosity, regions of amplification, common breaking points or other cancer-related genomic regions.[165] A dysregulation of miRNA expression can either result in an over or under expression. Overexpressed, miRNA can act as an oncogene, underexpressed its function as a tumor suppressor can be impaired.[166] A dysregulated expression of miRNA has subsequently been shown in numerous types of cancer including brain, breast, pancreas, thyroid, leukemia and CRC. [163]

Their properties of being expressed in a tissue-specific manner and their protection from endogenous RNase activity makes them a promising potential non-invasive biomarker for cancer and other disease.[167,168] Numerous miRNAs and their correlation to CRC have been studied; however, miR21 stands out as the most extensively explored representative. The results of a two cohort study by Schetter et al showed an overexpression of miR21is associated with both a poorer prognosis and a worse therapeutic outcome in CRC.[169] A high expression of miR21 in colorectal tumor cells results in a reduced expression of hMSH2, a protein that – together with hMSH6 – is part of the core mismatch repair protein complex. In addition, tumor cells with increased expression on miR21 exhibit significantly reduced 5-fluorouracil (5-FU)-induced G2/M damage arrest and apoptosis compared to normal cells.[170]

miR21 has also been studied in the hope for a new non-invasive diagnostic biomarker for CRC. A meta-analysis by Shan et al involving 6 studies found miR21 to have a pooled sensitivity of 77,4% and a specificity of 84,6% in the detection of CRC.[171] Even better results could be achieved in a recent study, where a serum panel combining 5 mRNAs was used. This 5-serum miRNA panel reported a sensitivity of 91,6% and a specificity of 91,7%.[172]

Given these promising results it is not surprising that ever since their discovery in the early 2000s, miRNAs have been a very active area of study. In the last years there has been an enormous effort in the development of plasma miRNA biomarkers for both diagnostic and prognostic use in cancer. [173,174] However, widespread inconsistencies in the results among those studies are hindering their translation into clinical use. To this day, no miRNA biomarkers are routinely used neither for screening nor as a predictive marker in CRC.

1.8.6 Inflammatory biomarkers

In recent years the connection between inflammation and cancer development has been well-established and became a noticeable focus in research. [175,176] The presence of inflammatory bowel diseases such as ulcerative colitis and Crohn´s disease is a significant risk factor for the development of CRC. Colitis-associated cancer is a subtype of CRC that is preceded by inflammatory bowel disease and associated with a poor prognosis. [177,178]

In sporadic CRC, inflammation is most likely not the primary cause. However, in the progress of tumor development immune cells are recruited and lead to a localized inflammatory microenvironment. Reactive oxygen species (ROS) produced by the intratumoral immune cells can induce DNA damage and mutation. Additionally, several proinflammatory cytokines are released by the tumor cells, attracting further immune cells such as neutrophils, dendritic cells, macrophages, eosinophils, mast cells, and lymphocytes. These cells are capable of producing a number of different angiogenic and growth stimulating cytokines and chemokines, that induce cancer cell proliferation and promote tumor spread.[175] Since the

beginning of the 2000s numerous studies have investigated the prognostic values of widely available inflammatory biomarkers in cancer.[179,180]

1.8.6.1 Neutrophil-to-lymphocyte ratio (NLR)

An elevated neutrophil-to-lymphocyte ration has been associated with a poor prognosis in patients with advanced CRC receiving chemotherapy. In a study by Riedl et al. a 1 standard deviation (SD) increase in NLR correlated with a 7% absolute lower ORR in first line (95%CI: 6-9, p<0.0001), 4% lower ORR in second line (3-5, p< 0.0001), and 2% lower ORR in third line (-1-11, p=0.68). Riedl et al. additionally showed that the NLR was slightly higher in patients entering first line, than in those before second and third line and highly elevated in patients entering BSC. The NLR is an indicator of systemic inflammation response. Therefore the results support the hypothesis that inflammation plays an important role in the progression and survival outcome in patients with CRC.[181]

The NLR is also a useful prognostic marker for localized CRC. In a study by Dimitriou et al. a elevated NLR was significantly associated with a worse DFS, 5-year survival as well and OS in stage II CRC. [182] A different study by Absenger et al. showed that an elevated preoperative NLR is statistically significant associated with a decreased time-to-recurrence both in stage II and III CRC. [183]

In a study by Pine et al. a high NLR was associated with a higher pT- and pN-stage and a greater incidence of extramural venous invasion but no correlation between NLR and MMR status was reported.[184] This is in contrast to a recently published study by Wen-Zhuo He et al. where systemic inflammatory factors, including NLR and CRP, were associated with MMR status. In dMMR CRC patients a higher neutrophil count was observed than in proficient MMR (pMMR) CRC patients, though only in non-metastatic settings. The authors hypothesize that one reason for this finding might be that dMMR CRC patients are more susceptive to somatic mutations. Hence, dMMR CRC is recognized earlier by the immune system, which leads to a systemic inflammatory response at an early stage.[185]

1.8.6.2 Lymphocyte - to- monocyte ratio (LMR)

Another readily available inflammatory biomarker is the lymphocyte -to- monocyte ratio (LMR). Lymphocytes play a crucial role in the containment of tumor growth and spread via cytotoxic cell death. On the contrary, monocytes have been shown to be able to promote tumor progression by the secretion of several proinflammatory cytokines.[186] Therefore a decreased LMR may be favorable for tumor growth and spread.

Several studies have shown that the LMR has valuable prognostic properties in solid tumors.[187] In a meta-analysis by Qingbin et al. a low LMR was associated with both a worse OS and DSF for patients with CRC. Regarding OS in stage I-III the pooled HR for patients with low LMR was 1.7. The cut-off values used to distinguish between low and high LMR in the included studies ranges from 2.14 to 3.78. For stage IV CRC the HR was 1.45.[188]

1.8.6.3 C – reactive Protein

C-reactive protein (CRP) is one of the most commonly used markers for inflammation. Additionally, CRP has been reported to be a prognostic factor for several malignancies including CRC.[189]In a study by Shiu et al. CRC patients with elevated preoperative CRP had a lower cancer-specific survival than those with normal CRP values. However, stratified by stage, the statistical significance only remained for stage III and not for stage II.[190]

1.8.7 The AST/ALT ratio

The serum AST/ALT ratio was first described in the year 1957 as a marker for acute viral hepatitis by Italian pathologist Ferdinand De Ritis. While both AST and ALT are usually elevated in the course of acute viral hepatitis, De Ritis found that ALT is usually higher than AST at the time of diagnosis, resulting in a low AST/ALT ratio. This is mainly due to the fact that at the time of the study, when

laboratory testing was performed the infection was already declining. Since ALT has a longer half-life (36h) compared to AST (18h) the ratio was usually well below 1.[191,192]

Over the following decades the AST/ALT ratio has subsequently been shown to be a valuable tool in several other liver related disease and some of these findings are still relevant today. A high AST/ALT ratio of greater than 2 is a good indicator for alcoholic hepatitis.[193] Alcoholic hepatitis causes mitochondrial damage resulting in an increase of serum AST.[194] Additionally, chronic alcohol abuse leads to the depletion of Vitamin B6 which has been shown to decrease serum levels of ALT.[195]

Although the hepatic proportions of AST and ALT are approximately 2.5:1, the shorter half-life of AST results in close to similar serum levels for healthy individuals. AST and ALT are both part of the laboratory liver function test and therefore very commonly assessed. In comparison to more complex molecular markers or invasive tissue biomarkers, the AST/ALT ratio is a low cost, convenient and readily available parameter.[192]

1.8.7.1 Aspartate amino transferase (AST)

AST catalyzes the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate with the use of Vitamin B6 as a cofactor. Two different isoenzyme forms of AST are found in our body, the mitochondrial and the cytoplasmic form. The enzyme is expressed in multiple tissues and organs including heart, liver, muscle and kidneys.
In the liver, the mitochondrial isoenzyme form is responsible for around 80% of the total enzyme activity.[196] The normal serum level of a healthy individual is between 0 to 35U/L.[197,198] An elevation of serum AST levels does not necessary indicate liver inflammation since it is expressed in multiple organs and tissues.

1.8.7.2 Alanine amino transferase (ALT)

ALT is found in different tissues throughout the body but is most common in the cytoplasm of hepatic tissue. The enzyme is responsible for the conversion of L-Alanine and α-ketoglutarate to pyruvate and L-glutamate. ALT is routinely measured as part of the liver function test. The normal serum level ranges from 0 to 40 U/L.[198] Elevated serum levels of ALT can be suggestive for any kind of hepatocellular damage. However, mildly elevated liver transaminase levels can be normal as their values fluctuate during the day and can increase to a certain extend after physical activity.[199] Serum ALT levels greater than 500 U/L are in most cases the result of liver cell damaging disease such as viral hepatitis or toxin-induced liver damage, although the peak of the ALT elevation does not correlate with the actual cellular damage.[200]

1.8.7.3 AST/ALT ratio in cancer

In recent years the AST/ALT ratio has been given new attention as several studies showed that the ratio can be used as a prognostic and predictive biomarker in cancer. A correlation between an increased AST/ALT ratio and a worse prognosis has been described for the following malignancies: Renal cell carcinoma[11], hepatocellular carcinoma[201], urinary tract urothelial carcinoma[12], prostate cancer[14], cancer of the bladder[202], head and neck cancer[203] and pancreatic cancer[204]

Although a lot of these findings are promising, a lack of consensus, mainly due to different study designs and sample size, complicate the interpretation. In 2019 a meta-analysis by Wu et al. consisting of 18 studies and a total of 9400 patients was conducted to assess the prognostic properties of the pretreatment serum AST/ALT ratio in solid tumors and to get an overview of the current state of research. The study showed the following results: renal cell carcinoma (pooled HR=1.64) liver cancer (pooled HR=1.16), urinary tract urothelial carcinoma (pooled HR=1.96), bladder cancer (pooled HR =2.66) other cancers (pooled HR=1.44)[205]

These studies have shown that the AST/ALT ratio can be a useful biomarker for risk stratification in several malignancies. However, since the quality of some of these studies suffer from short follow-up periods or low sample sizes further clinical validation is needed before the marker can be implemented into clinical use.

2 Materials and Methods

2.1 Study Design, patient cohort and clinical outcome

This retrospective single-center observational cohort study included data on patients with histologically confirmed stage II and III (UICC) CRC. The initial cohort consisted of a total of 1018 CRC patients (UICC Stage I – VI) who were treated in our department (Division of Oncology, Department of Internal Medicine, Medical University of Graz, Austria) between March 2010 and January 2016. According to predefined criteria (**Fig. 2**), we excluded 66 Stage I and 245 Stage IV patients. From a total of 695 stage II and III patients, AST and ALT values obtained within two weeks prior to the histological diagnosis, were available from 536 patients and thus were ultimately included in this study. Baseline data was extracted from the electronic health record database of our hospital trust (includes all public hospitals in the province of Styria, Austria), our departments internal documentation system as well as paper-chart archives of our hospital. Plasma AST and ALT were measured in each patient by standard clinical testing methods in lithium heparin plasma (upper reference level AST and ALT 35 and 45 U/L, respectively).

The co-primary endpoints for this analysis were DFS, defined as the time from curative surgery of the primary tumor to recurrence or death from any cause, whatever occurred first, and OS.

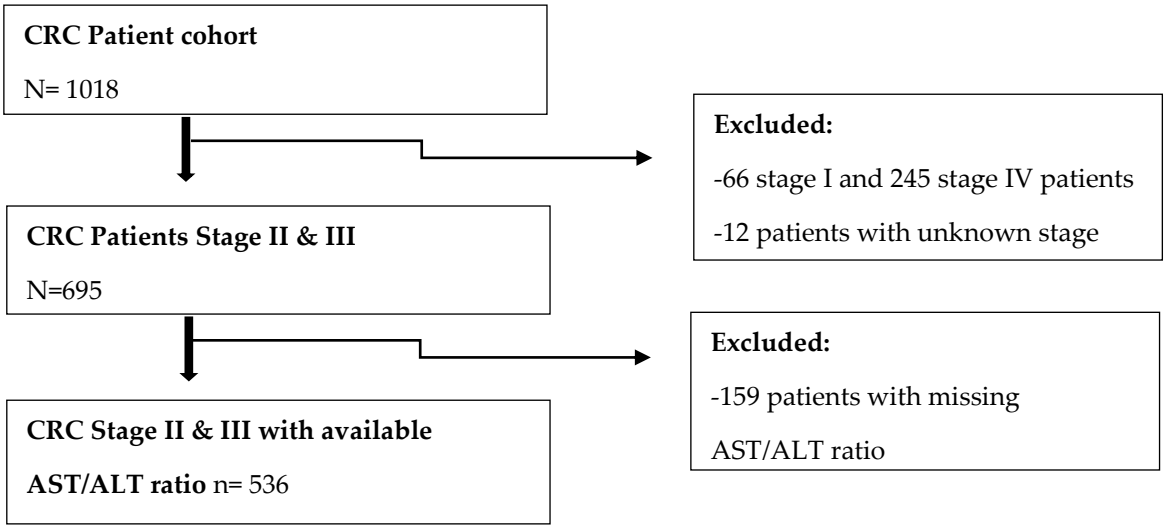

Figure 2: Flowchart of the study population.

Abbreviations: CRC- Colorectal cancer, AST - Aspartate Amino-Transferase ALT - Alanine Amino-Transferase.

2.2 Ethics statement

The study was approved by the local ethics committee (Ethikkommission der Medizinischen Universität Graz, IRB00002556) prior to any patient-related activities (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.

2.3 Statistical analysis:

Medians (with interquartile ranges [IQR]) and means (with standard deviations [SD]) were reported for non-normally and normally-distributed variables, respectively. Derits ratio was calculated by dividing ALT through AST. Youden index was used to estimate the optimal cut-off value for the Deritis ratio with regards to DFS. Uni- and multivariate Cox-regression analyses were used to assess risk factors for DFS and OS, respectively. Statistical analyses were carried out using Stata Version 15.1 (*StataCorp, TX, USA*). A p-value of <0.05 was considered statistically significant.

3 Results:

3.1 Analysis at baseline:

A total of 536 patients were included in this study, of which 334 were male (62%) and 202 were female (38%). The median age at diagnosis was 65 years (IQR 64-66). 196 patients (37%) were assessed as UICC stage II and 340 (63%) as stage III. Regarding tumor location, 292 tumors (56%) were located in the colon, while 236 (44%) were tumors of the rectum. 260 patients (49%) received adjuvant chemotherapy. The median AST/ALT ratio was 1.16 (IQR 0.92-1.53). Further baseline characteristics of our cohort are summarized in **Table 5**.

In the comparison of the low and high AST/ALT ratio groups, defined by an empirical cut-off of 0.96 suggested by the Youden Index, there were statistically significant differences in sex, age, BMI, hemoglobin and the proportion of patients who received adjuvant chemotherapy. Apart from that, the distribution of all other investigated baseline parameters was highly similar between patients with and without an elevated AST/ALT ratio (**Table 5**).

3.2 Cohort outcome:

During a median follow up of 31 months the median DFS and OS was not reached. Out of 536 patients a total of 102 (19%) developed tumor recurrence of which 86 patients developed distant metastases, 9 had a local recurrence and 7 patients had a local recurrence and distant metastases. One-, two- and 5-year DFS rates were 89.5%, 82.3% and 76.7%, respectively. Sixty-two patients (12%) died within the follow up period, resulting in one-, two- and 5-year OS rates of 98.5%, 95.9% and 81.6%.

Table 5: Baseline characteristics of the study population (n=536).

Baseline Characteristics	N (%miss.)	Overall N=536	AST/ALT < 0.96 Youden Index N = 157	AST/ALT > 0.96 Youden Index N= 379	p-value
Demographic variables					
Female gender	536(0%)	202 (38%)	44(28%)	158(42%)	**0.003**
Age (years)	536(0%)	65(IQR 64-66)	62	66	**0.0007**
BMI (kg/m²)	461(14%)	26(IQR 25-26)	27,6	25,8	**0.0006**
ECOG	366 (32%)	/	/	/	0.373
--0		287(54%)	96(78%)	191(78%)	/
--1		69(13%)	26(21%)	43(17%)	/
--2		8(2%)	1(1%)	7(4%)	/
--3		2(<1%)	0(0%)	2(1%)	/
Smoker or ex-smoker	322 (43%)	122(23%)	36(49%)	86(38%)	0.639
Tumor variables					
UICC Stage	536 (0%)	-	-	-	0.610
---II	-	196 (37%)	60(38%)	136(36%)	/
---III	-	340 (63%)	97(62%)	243(64%)	/
Location of primary tumor	534 (<1%)	-	-	-	0.736
---Caecum	-	58(11%)	13(8%)	45(12%)	/
---Right ascending	-	51(9%)	19(12%)	32(8%)	/
---Right flexure	-	24(5%)	5(3%)	19(5%)	/
---Transverse colon	-	22(4%)	6(4%)	16(4%)	/
---Left flexure	-	19(4%)	5(3%)	14(4%)	/
---Left descending	-	10(2%)	3(2%)	7(2%)	/
---Sigma	-	108(20%)	35(23%)	73(19%)	/
---Rectum	-	236(44%)	68(44%)	168(44%)	/
---Appendix	-	4(1%)	2(1%)	2(1%)	/
---Multilocular	-	2(<1%)	0(0%)	2(1%)	/
Grading	531(<1%)	-	-	-	0.239
---I	-	42(8%)	10(6%)	32	/
---II	-	401(75%)	126(81%)	275	/
---III	-	85(16%)	18(12%)	67	/
---IV	-	3(1%)	1(1%)	2	/
Treatment variables					
Adjuvant Chemotherapy	507(5%)	260(49%)	88(58%)	172(48%)	**0.04**
Laboratory variables					
AST	536 (0%)	21(IQR 17-28)	21 (IQR 17-29)	21(IQR 17-27)	0.883
ALT	536 (0%)	18(IQR 14-26.5)	29 (IQR 22-42)	16(IQR 12-20)	**0.000**
AST/ALT	536 (0%)	1.16 (IQR 0.92-1.53).	0.76 (IQR 0.63-0.87)	1.33 (IQR 1.33 – 1.64)	**0.000**

Hemoglobin (g/dl)	533(<1%)	13(IQR 10.9-14.4)	13.7(IQR 11.9-14.9)	12.7(IQR 10.4-14.3)	**0.0001**
Leucocyte count (G/L)	530(1%)	7.7(IQR 6.2-9.8)	8(IQR 6.5- 10)	7.6(IQR 6.2-9.6)	0.170
CRP (mg/dl)	511(5%)	3.4(IQR 1-10.8)	3.9(IQR1.1-10.6)	3.25(IQR 0.9-11)	0.436
CEA	373(30%)	2.9(IQR 1.6-6.8)	2.62(IQR 1.5-6.1)	3.1(IQR 1.6-6.8)	0.355

Distribution overall as well as by AST/ALT-ratio ≤ and > 0.96 as suggested by the Youden Index. Data are reported as medians [25th-75th percentile] or absolute counts (%). *p-values were from rank-sum tests, χ2-tests, and Fisher's exact tests. Abbreviations: AST – Aspartate Amino-Transferase, ALT – Alanine Amino-Transferase, BMI – Body Mass Index, ECOG – Eastern Cooperative Oncology Group performance status, UICC- Union for International Cancer Control, TNM – Tumor Node Metastasis Classification, CRP – C-reactive protein, CEA- carcinoembryonic antigen.

3.3 Uni- and multivariate analysis of clinical outcomes regarding AST/ALT ratio

DFS was significantly shorter in patients with an elevated AST/ALT ratio. In univariate cox regression, one unit increase of the AST/ALT ratio was associated with a 1.57-fold relative increase in the risk of recurrence or death (HR 1.568, 95% 1.10-2.23, p = 0.012). Other univariate predictors of DFS were tumor stage (worse DFS in stage 3 compared to stage 2) and tumor grading (worse DFs in patients with grade 3 disease). In univariate analysis, adjuvant chemotherapy did not emerge to be a predictive marker for DFS. However, since the application of adjuvant chemotherapy is an established prognostic marker in clinical practice, we included it in our multivariate analysis. In the multivariate model including grade, stage and adjuvant chemotherapy, the prognostic association between an elevated AST/ALT ratio and a poor survival prevailed statistically significant (HR 1.53, 95% 1.05-2.22, p = 0.026, **Table 6**).

Treating the AST/ALT ratio as binary variable with an empirical cut-off at 0.96 suggested by the Youden index, similar results were observed. In detail, the median 12, 24 and 36-month DFS rates were 92%, 88%, 84% in the low and 88%,

79%, 76 % in the high AST/ALT group, respectively (HR 1.63, 95% CI 0.99 – 2.68, p = 0.052; **Figure 3**).

In OS-analysis, there was a trend towards worse OS for elevated AST/ALT ratio; however, no statistically significant association was observed in univariate (HR 1.4, 95% CI 0.89 – 2.22, p = 0.14, **Figure 4**) and multivariate analysis (HR 1.24, 95% CI 0.78-1.96, p= 0.35).

Table 6: Uni- and multivariable Cox proportional hazards regression models of DFS (n=536).

Uni- and multivariable Cox regression	Univariate Analysis		Multivariate Analysis	
Variable	Hazard ratio (95%CI)	p-value	Hazard ratio (95%CI)	p-value
AST/ALT per unit increase	1.57 (1.10-2.23)	0.012	1.53(1.05-2.22)	0.026
AST/ALT Youden cut-off	1.63 (0.99-2.68)	0.052	/	/
AST	0.99 (0.98-1.01)	0.814	/	/
ALT	0.99 (0.98-1.01)	0.713	/	/
Age	1.09 (0.93-1.25)	0.265	/	/
BMI	1.00 (0.95-1.03)	0.889	/	/
Grading				
--II	1.72 (0.75-3.92)	0.196	3.22(0.78-13.17)	0.104
--III	2.61 (1.08-6.27)	0.032	4.25(0.98-18.34)	0.052
Stage	1.74 (1.18-2.56)	0.005	1.83(1.12-3.01)	0.016
Adjuvant Chemotherapy	0.86 (0.62-1.20)	0.387	0.78(0.50-1.22)	0.286

All variables who were statistically significant predictors of response in univariable analysis were included in multivariable analysis. Abbreviations: AST – Aspartate Amino-Transferase, ALT – Alanine Amino-Transferase, BMI- body mass index

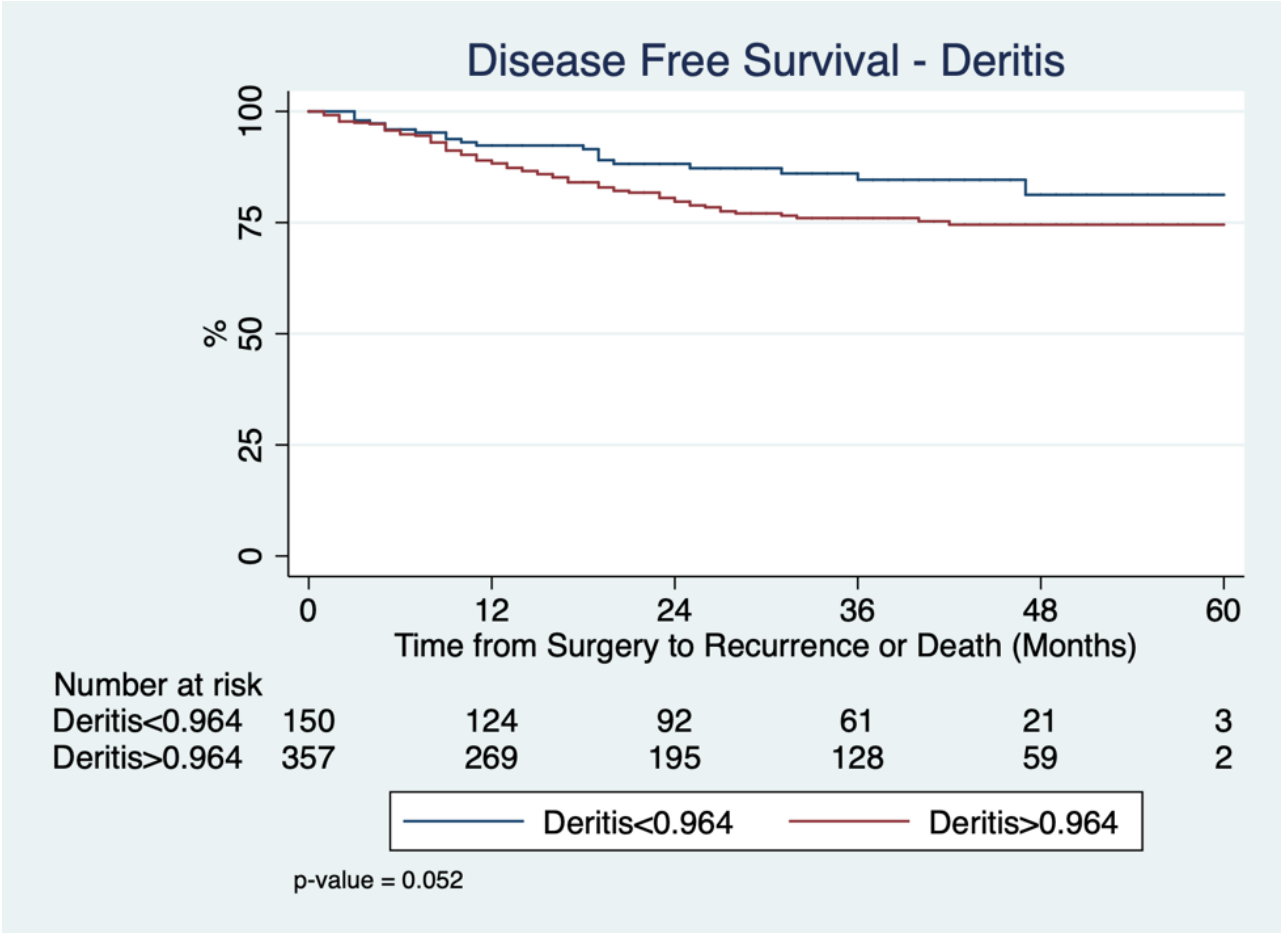

Figure 3:Disease-free survival according to AST/ALT-ratio (n=507).

DFS estimates were computed with Kaplan-Meier estimators, and the numbers below the x-axis represent a risk table.

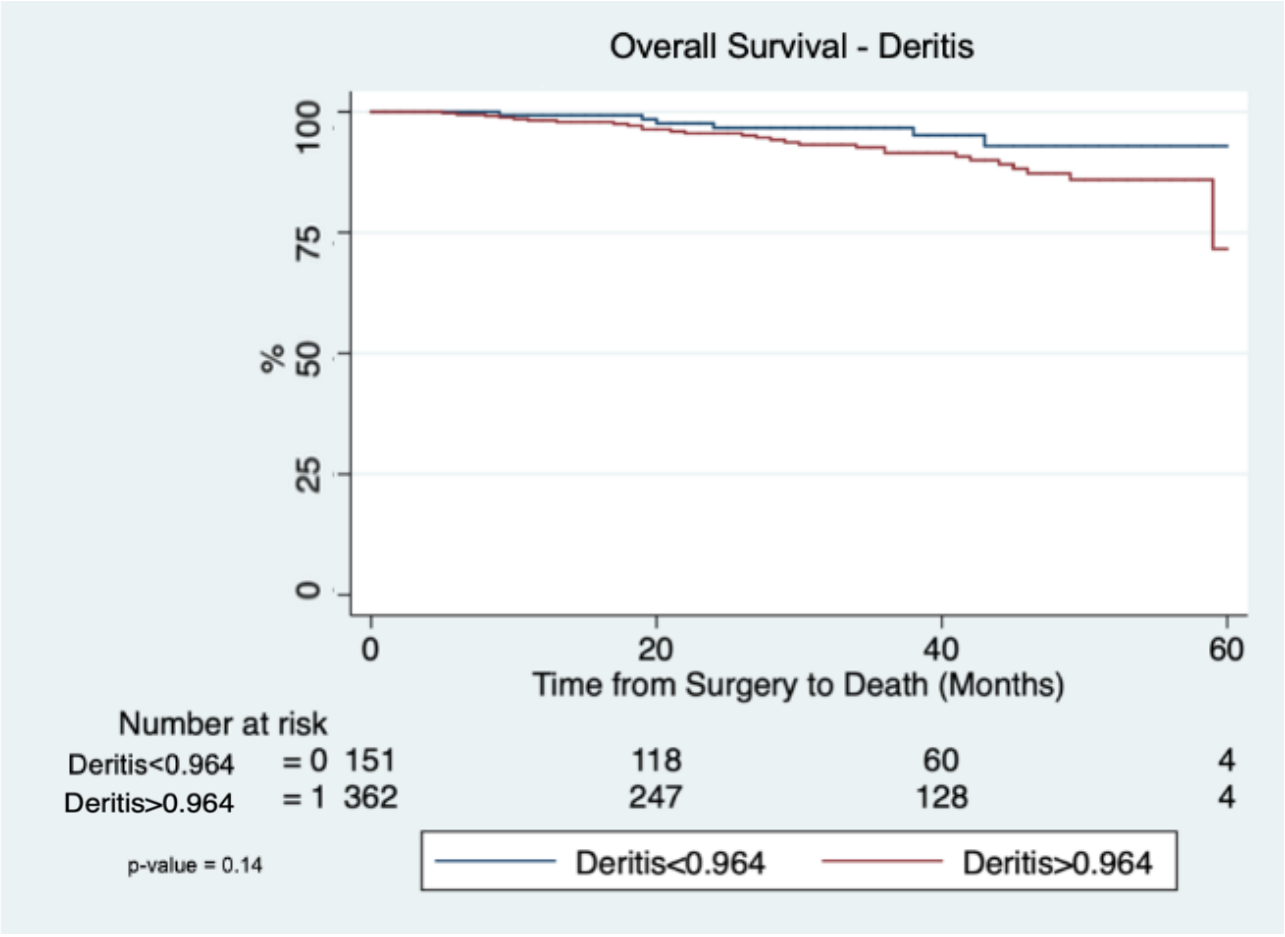

Figure 4: Overall Survival according to AST/ALT-ratio (n=513).

OS estimates were computed with Kaplan-Meier estimators, and the numbers below the x-axis represent a risk table.

3.4 Sensitivity analysis

We further performed a sensitivity analysis investigating the association of an elevated AST/ALT ratio with DFS in our cohort stratified by adjuvant chemotherapy. We observed that in patients receiving adjuvant chemotherapy, an elevated AST/ALT ratio was associated with worse DFS (HR 1.78 per unit increase, 95% CI 1.10-2.86, p= 0.017), whereas in patients not receiving adjuvant chemotherapy no statistically significant association between the AST/ALT ratio and DFS was found. (HR 1.48 per unit increase, 95% CI 0.83-2.64, p= 0.177)

In a second sensitivity analysis we investigated whether stage II and III colorectal cancer patients with missing AST/ALT ratio (n=159) differed from patients included

in our primary analysis. Here, we observed that baseline characteristics between the in- and excluded patients were all grossly similar. **(Table 7)**

Table 7: Distribution of baseline characteristics depending on whether the AST/ALT ratio was observed or missing (n=695).

Baseline characteristics	AST/ALT ratio observed n = 536	AST/ALT ratio missing n = 159	p-value
Demographic variables			
Female gender	202 (38%)	71(44%)	0.114
Age (years)	65(IQR 64-66)	65(63-67)	0.984
BMI (kg/m²)	26(IQR 25-26)	26(IQR 25-27)	0.924
ECOG	/	/	0.206
--0	287(54%)	76(77%)	/
--1	69(13%)	17(17%)	/
--2	8(2%)	6(6%)	/
--3	2(<1%)	0(0%)	/
Smoker or ex-smoker	122(23%)	27(35%)	0.732
Tumor variables			
UICC Stage	-	-	0.054
---II	196 (37%)	45(28%)	/
---III	340 (63%)	114(72%)	/
Location of primary tumor	-	-	0.540
---Caecum	58(11%)	15(9%)	/
---Right ascending	51(9%)	13(8%)	/
---Right flexure	24(5%)	8(5%)	/
---Transverse colon	22(4%)	8(5%)	/
---Left flexure	19(4%)	8(5%)	/
---Left descending	10(2%)	4(3%)	/
---Sigma	108(20%)	37(23%)	/
---Rectum	236(44%)	63(40%)	/
---Appendix	4(1%)	0(0%)	/
---Multilocular	2(<1%)	3(2%)	/
Grading	-	-	0.419
---I	42(8%)	14(9%)	/
---II	401(75%)	117(75%)	/
---III	85(16%)	23(15%)	/
---IV	3(1%)	3(1%)	/
Recurrence	102(19%)	45(28%)	0.012
Treatment variables			
Adjuvant Chemotherapy	260(49%)	89(59%)	0.098

Laboratory variables			
Hemoglobin (g/dl)	13(IQR 10.9-14.4)	13.1(IQR 11.3-13.7)	0.885
Leucocyte count (G/L)	7.7(IQR 6.2-9.8)	7.2IQR 6.3- 8.8)	0.545
CRP (mg/dl)	3.4(IQR 1-10.8)	1.35(IQR 0.51-5.45)	0.114
CEA	2.9(IQR 1.6-6.8)	2.5(IQR 1.2-9.5)	0.760

Data are reported as medians [25th-75th percentile] or absolute counts (%). *p-values were from rank-sum tests, χ2-tests, and Fisher's exact tests. Abbreviations: AST – Aspartate Amino-Transferase, ALT – Alanine Amino-Transferase, BMI – Body Mass Index, ECOG – Eastern Cooperative Oncology Group performance status, UICC- Union for International Cancer Control, TNM – Tumor Node Metastasis Classification, CRP – C-reactive protein, CEA- carcinoembryonic antigen.

4 Discussion:

In the present study, we evaluated the prognostic value of the AST/ALT (De Ritis) ratio in a large cohort of stage II and III non-metastatic CRC patients. We discovered that an elevated AST/ALT ratio is significantly associated with a worse DFS. These findings indicate that the AST/ALT ratio might represent a novel, inexpensive biomarker for risk stratification in patients with localized CRC who underwent surgical resection.

AST and ALT are the most commonly used serum biomarkers for hepatocellular damage in clinical practice. While ALT is almost exclusively found in the liver, AST is additionally expressed in various other organs including the heart, skeletal muscle, kidneys, brain and erythrocytes. Thus, many pathological processes and diseases can lead to an elevated AST activity, while elevated ALT levels are mostly associated with liver diseases. [192]

While the exact underlying pathophysiological processes linking an elevated AST/ALT ratio to a worse prognosis are yet to be understood, there are several possible explanations. Warburg discovered that cancer cells predominantly rely on glycolysis followed by lactic acid fermentation as their source of energy, even in the presence of abundant oxygen. [206] The malate-aspartate-shuttle is a biochemical system that plays an important role in the aerobic glycolysis. It consists of four proteins, with AST being one of them. Tumor metabolism can therefore lead to an elevation of AST and affect the De Ritis ratio.[207]

Furthermore, tumor progression is associated with high proliferation, cell turnover, tissue damage and necrosis. All of these pathological processes can lead to an elevation of AST while ALT levels remain mostly unaffected. [208]

Recent publications have shed new light on the well-known marker by linking an elevated AST/ALT ratio to worse prognosis in multiple malignancies. While the majority of these studies included metastatic disease in their analysis, some specifically investigated the AST/ALT ratio in the localized setting.

Wang et al. found a correlation between an elevated AST/ALT ratio and a worse prognosis for localized prostate cancer patients in their retrospective cohort study including 438 patients. In their study, a high AST/ALT ratio was associated with a higher rate of biochemical recurrence and a predictor for a higher Gleason score. The 2-year biochemical recurrence-free survival (BCRFS) rates were 97.2% for the low ratio group (AST/ALT<1.32) and 93.7 % for the high ratio group (AST/ALT>1.32), while the 5-year BCRFS were 86.1% and 69.3%, respectively. As in our study, the maximum value of the Youden index was used to evaluate the optimum cut-off point between low and high AST/ALT ratio group.[209]

In a study by Bezan et al. a high AST/ALT quotient was associated with poor survival in non-metastatic RCC. A preoperative increase in AST/ALT ratio above 1.26 was an an independent prognostic factor for both metastasis-free survival (HR 1.61) and overall survival (HR 1.76).[11] Yun-Sok Ha et al. retrospectively investigated 118 non-metastatic bladder cancer patients. In this study, patients with an elevated AST/ALT ratio had inferior metastasis-free survival (HR 2.38), cancer-specific survival (HR 2.77) and OS (HR 2.76) outcomes.[202] Nishikawa et al. investigated the AST/ALT ratio in localized upper urinary tract urothelial carcinoma patients following nephroureterectomy. In their retrospective study, an elevated AST/ALT ratio was shown to be significantly correlated with a worse extravesical recurrence-free survival in both uni- and multivariate analysis. In addition, the relationship between the AST/ALT ratio and the OS as well as the DSS of the included patients were analyzed, although no correlation was found. [210]

Following a comparable approach to these studies, we investigated the AST/ALT ratio in stage II and III disease, where risk stratification and choice of treatment are especially crucial. Although we are first to investigate the association between the

AST/ALT ratio and the prognosis of CRC patients, Lindmark et al. has already reported an association of elevated AST levels to a worse disease-specific survival in CRC patients in the year 1994. [211] Interestingly, we found no association between elevated AST levels and worse survival outcome indicated by DFS and OS in our cohort. In our analysis the De Ritis ratio emerged to be an independent prognostic indicator for DFS. Furthermore, the association was stronger in patients receiving adjuvant chemotherapy, which might indicate a potential predictive value of this biomarker.

Finally, this study contains several limitations that should be discussed. First, the retrospective design of this study may have introduced a selection bias to our cohort. We tried to address this issue by excluding patients of UICC stage I, since the majority of this patient cohort are not routinely referred to our cancer center.
Secondly, in our study an elevated AST/ALT ratio was only associated with a worse DFS but not with worse OS. However, the hazard ratio of 1.4 indicates a similar trend towards worse OS in patients with elevated AST/ALT ratio. The lack of statistical significance might be due to the low event rate caused by the relatively short follow up period of 31 months.

Furthermore, the absence of an independent validation cohort for our biomarker analysis proposes an additional limitation. We therefore encourage other study groups to validate our findings in comparable patient cohorts as a further step to clinical significance

5 Conclusion:

In this study the plasma AST/ALT ratio emerged as a valid prognostic marker for DFS in non-metastatic colorectal cancer patients at stage II and III. Within the limitations of a retrospective study we conclude that the serum AST/ALT ratio might represent a novel and inexpensive prognostic tool to aid in the identification of patients at high risk of recurrent disease.

6 Literaturverzeichnis

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7-34. doi:10.3322/caac.21551
2. La Vecchia C, Bosetti C, Lucchini F, et al. Cancer mortality in Europe, 2000-2004, and an overview of trends since 1975. *Ann Oncol*. 2009;21(6):1323-1360. doi:10.1093/annonc/mdp530
3. Andre T, Boni C, Mounedji-Boudiaf L, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med*. 2004;350(23):2343-2351. doi:10.1056/NEJMoa032709
4. Twelves C, Wong A, Nowacki MP, et al. Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med*. 2005;352(26):2696-2704. doi:10.1056/NEJMoa043116
5. Kuebler JP, Wieand HS, O'Connell MJ, et al. Oxaliplatin combined with weekly bolus fluorouracil and leucovorin as surgical adjuvant chemotherapy for stage II and III colon cancer: results from NSABP C-07. *J Clin Oncol*. 2007;25(16):2198-2204. doi:10.1200/JCO.2006.08.2974
6. O'Connell MJ, Campbell ME, Goldberg RM, et al. Survival following recurrence in stage II and III colon cancer: Findings from the ACCENT data set. *J Clin Oncol*. 2008;26(14):2336-2341. doi:10.1200/JCO.2007.15.8261
7. Heitzer E, Auer M, Gasch C, et al. Complex tumor genomes inferred from single circulating tumor cells by array-CGH and next-generation sequencing. *Cancer Res*. 2013;73(10):2965-2975. doi:10.1158/0008-5472.CAN-12-4140
8. Smolle MA, Pichler M, Haybaeck J, Gerger A. Genetic markers of recurrence in colorectal cancer. *Pharmacogenomics*. 2015;16(11):1315-1328. doi:10.2217/pgs.15.83
9. Goblirsch M, Richtig G, Slaby O, Berindan-Neagoe I, Gerger A, Pichler M. MicroRNAs as a tool to aid stratification of colorectal cancer patients and to guide therapy. *Pharmacogenomics*. 2017;18(10):1027-1038. doi:10.2217/pgs-2017-0004
10. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. *Toxicology*. 2008;245(3):194-205. doi:10.1016/j.tox.2007.11.021
11. Bezan A, Mrsic E, Krieger D, et al. The preoperative AST/ALT (De Ritis) ratio represents a poor prognostic factor in a cohort of patients with

nonmetastatic renal cell carcinoma. *J Urol*. 2015;194(1):30-35. doi:10.1016/j.juro.2015.01.083

12. Lee H, Choi YH, Sung HH, et al. De Ritis Ratio (AST/ALT) as a Significant Prognostic Factor in Patients With Upper Tract Urothelial Cancer Treated With Surgery. *Clin Genitourin Cancer*. 2017;15(3):e379-e385. doi:10.1016/j.clgc.2016.08.023
13. Cho YH, Hwang JE, Chung HS, et al. The De Ritis (aspartate transaminase/alanine transaminase) ratio as a predictor of oncological outcomes in patients after surgery for upper urinary tract urothelial carcinoma. *Int Urol Nephrol*. 2017;49(8):1383-1390. doi:10.1007/s11255-017-1613-z
14. Miyake H, Matsushita Y, Watanabe H, et al. Significance of De Ritis (Aspartate Transaminase/Alanine Transaminase) Ratio as a Significant Prognostic But Not Predictive Biomarker in Japanese Patients with Metastatic Castration-resistant Prostate Cancer Treated with Cabazitaxel. *Anticancer Res*. 2018;38(7):4179-4185. doi:10.21873/anticanres.12711
15. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424. doi:10.3322/caac.21492
16. Fitzmaurice C, Allen C, Barber RM, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study Global Burden . *JAMA Oncol*. 2017;3(4):524-548. doi:10.1001/jamaoncol.2016.5688
17. Ward EM, Sherman RL, Henley SJ, et al. Annual Report to the Nation on the Status of Cancer, Featuring Cancer in Men and Women Age 20-49 Years. *J Natl Cancer Inst*. 2019;111(12):1279-1297. doi:10.1093/jnci/djz106
18. Meester RGS, Mannalithara A, Lansdorp-Vogelaar I, Ladabaum U. Trends in Incidence and Stage at Diagnosis of Colorectal Cancer in Adults Aged 40 Through 49 Years, 1975-2015. *JAMA*. 2019;321(19):1933-1934. doi:10.1001/jama.2019.3076
19. Petersen GM, Slack J, Nakamura Y. Screening guidelines and premorbid diagnosis of familial adenomatous polyposis using linkage.

Gastroenterology. 1991;100(6):1658-1664. doi:10.1016/0016-5085(91)90666-9

20. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW. ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol*. 2015;110(2):223-262; quiz 263. doi:10.1038/ajg.2014.435
21. Lynch HT, Smyrk T, McGinn T, et al. Attenuated familial adenomatous polyposis (AFAP). A phenotypically and genotypically distinctive variant of FAP. *Cancer*. 1995;76(12):2427-2433. doi:10.1002/1097-0142(19951215)76:12<2427::aid-cncr2820761205>3.0.co;2-b
22. Hernegger GS, Moore HG, Guillem JG. Attenuated familial adenomatous polyposis: an evolving and poorly understood entity. *Dis Colon Rectum*. 2002;45(1):126-127. doi:10.1007/s10350-004-6127-y
23. Moreira L, Balaguer F, Lindor N, et al. Identification of Lynch syndrome among patients with colorectal cancer. *JAMA*. 2012;308(15):1555-1565. doi:10.1001/jama.2012.13088
24. Bonadona V, Bonaiti B, Olschwang S, et al. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA*. 2011;305(22):2304-2310. doi:10.1001/jama.2011.743
25. Dowty JG, Win AK, Buchanan DD, et al. Cancer risks for MLH1 and MSH2 mutation carriers. *Hum Mutat*. 2013;34(3):490-497. doi:10.1002/humu.22262
26. Ten Broeke SW, van der Klift HM, Tops CMJ, et al. Cancer Risks for PMS2-Associated Lynch Syndrome. *J Clin Oncol*. 2018;36(29):2961-2968. doi:10.1200/JCO.2018.78.4777
27. Stidham RW, Higgins PDR. Colorectal Cancer in Inflammatory Bowel Disease. *Clin Colon Rectal Surg*. 2018;31(3):168-178. doi:10.1055/s-0037-1602237
28. Ekbom A, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. *N Engl J Med*. 1990;323(18):1228-1233. doi:10.1056/NEJM199011013231802
29. Yuhara H, Steinmaus C, Cohen SE, Corley DA, Tei Y, Buffler PA. Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? *Am J Gastroenterol*. 2011;106(11):1911-1921; quiz 1922. doi:10.1038/ajg.2011.301

30. Dehal AN, Newton CC, Jacobs EJ, Patel A V, Gapstur SM, Campbell PT. Impact of diabetes mellitus and insulin use on survival after colorectal cancer diagnosis: the Cancer Prevention Study-II Nutrition Cohort. *J Clin Oncol*. 2012;30(1):53-59. doi:10.1200/JCO.2011.38.0303
31. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet*. 2008;371(9612):569-578. doi:10.1016/S0140-6736(08)60269-X
32. Dai Z, Xu YC, Niu L. Obesity and colorectal cancer risk: A meta-analysis of cohort studies. *World J Gastroenterol*. 2007;13(31):4199-4206. doi:10.3748/wjg.v13.i31.4199
33. Karahalios A, English DR, Simpson JA. Weight change and risk of colorectal cancer: a systematic review and meta-analysis. *Am J Epidemiol*. 2015;181(11):832-845. doi:10.1093/aje/kwu357
34. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and Colorectal Cancer. *Jama*. 2008;300(23):2765. doi:10.1001/jama.2008.839
35. Bouvard V, Loomis D, Guyton KZ, et al. Carcinogenicity of consumption of red and processed meat. *Lancet Oncol*. 2015;16(16):1599-1600. doi:10.1016/S1470-2045(15)00444-1
36. Saliba W, Rennert HS, Gronich N, Gruber SB, Rennert G. Red meat and processed meat intake and risk of colorectal cancer: a population-based case-control study. *Eur J Cancer Prev*. 2019;28(4):287-293. doi:10.1097/CEJ.0000000000000451
37. Slattery ML, Boucher KM, Caan BJ, Potter JD, Ma KN. Eating patterns and risk of colon cancer. *Am J Epidemiol*. 1998;148(1):4-16. doi:10.1093/aje/148.1.4-a
38. Michels KB, Giovannucci E, Joshipura KJ, et al. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. *J Natl Cancer Inst*. 2000;92(21):1740-1752. doi:10.1093/jnci/92.21.1740
39. Yun SO, Cho YB, Lee WY, et al. Clinical significance of signet-ring-cell colorectal cancer as a prognostic factor. *Ann Coloproctol*. 2017;33(6):232-238. doi:10.3393/ac.2017.33.6.232
40. Brierley J, Gospodarowicz M, O'Sulivan B. The principles of cancer staging.

Ecancermedicalscience. 2016;10:3-6. doi:10.3332/ecancer.2016.ed61

41. Rose LJ. Colon Cancer Staging. 2019:2-7. https://web.archive.org/web/20151007104701/http://emedicine.medscape.com/article/2006674-overview.
42. Rentsch M, Schiergens T, Khandoga A, Werner J. Surgery for Colorectal Cancer - Trends, Developments, and Future Perspectives. *Visc Med*. 2016;32(3):184-191. doi:10.1159/000446490
43. Varghese A. Chemotherapy for Stage II Colon Cancer. *Clin Colon Rectal Surg*. 2015;28(4):256-261. doi:10.1055/s-0035-1564430
44. Labianca R, Nordlinger B, Beretta GD, et al. Early colon cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2013;24(SUPPL.6):vi64-vi72. doi:10.1093/annonc/mdt354
45. Willett CG, Ryan DP. Neoadjuvant chemoradiotherapy and radiotherapy for rectal adenocarcinoma. *UpToDate*. 2016;(table 1):1-25. http://www.uptodate.com/contents/neoadjuvant-chemoradiotherapy-and-radiotherapy-for-rectal-adenocarcinoma?source=machineLearning&search=rectal+cancer+treatment&selectedTitle=1~150&sectionRank=1&anchor=H1388273260#H15.
46. Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*. 2004;351(17):1731-1740. doi:10.1056/NEJMoa040694
47. Glynne-Jones R, Wyrwicz L, Tiret E, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol Off J Eur Soc Med Oncol*. 2017;28(suppl_4):iv22-iv40. doi:10.1093/annonc/mdx224
48. Lassere MN. The biomarker-surrogacy evaluation schema: A review of the biomarker-surrogate literature and a proposal for a criterion-based, quantitative, multidimensional hierarchical levels of evidence schema for evaluating the status of biomarkers as surrogate endp. *Stat Methods Med Res*. 2008;17(3):303-340. doi:10.1177/0962280207082719
49. Atkinson AJ, Colburn WA, DeGruttola VG, et al. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89-95. doi:10.1067/mcp.2001.113989
50. Chan IS, Ginsburg GS. Personalized Medicine: Progress and Promise. *Annu*

Rev Genomics Hum Genet. 2011;12(1):217-244. doi:10.1038/nrclinonc.2011.14

51. Foroutan B. Personalized Medicine: A Review with Regard to Biomarkers. *J Bioequiv Availab*. 2015;07(06):244-256. doi:10.4172/jbb.1000248
52. Schirripa M, Lenz HJ. Biomarker in colorectal cancer. *Cancer J (United States)*. 2016;22(3):156-164. doi:10.1097/PPO.0000000000000190
53. Desantis CE, Lin CC, Mariotto AB, et al. Cancer Treatment and Survivorship Statistics , 2014. *CA A Cancer J Clin*. 2014;64(4):252-271. doi:10.3322/caac.21235.
54. Levin TR, Corley DA, Jensen CD, et al. Effects of Organized Colorectal Cancer Screening on Cancer Incidence and Mortality in a Large, Community-based Population. *Gastroenterology*. 2018;(September):1-9. doi:10.1053/j.gastro.2018.07.017
55. Ran T, Cheng C-Y, Misselwitz B, Brenner H, Ubels J, Schlander M. Cost-Effectiveness of Colorectal Cancer Screening Strategies-A Systematic Review. *Clin Gastroenterol Hepatol*. 2019;17(10):1969-1981.e15. doi:10.1016/j.cgh.2019.01.014
56. Safety F. Cancer Screening in the European Union (2017). 2017.
57. Ergebnisbericht W. Übersicht nationaler Kolonkrebs-Screening-Programme.
58. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus Fecal Occult Blood for Colorectal-Cancer Screening in an Average-Risk Population. *N Engl J Med*. 2004;351(26):2704-2714. doi:10.1056/NEJMoa033403
59. Church TR, Ederer F, Mandel JS. 1440 Reports. *October*. 1997;89(19).
60. Bussel JB, Berkowitz RL, McFarland JG, Lynch L, Chitkara U. The New England Journal of Medicine Downloaded from nejm.org at PENN STATE UNIVERSITY on November 25, 2015. For personal use only. No other uses without permission. From the NEJM Archive. Copyright © 2010 Massachusetts Medical Society. All rights reserved. *N Engl J Med*. 2010;319(21):1374-1378. doi:10.1056/NEJM198603063141003
61. Hardcastle JD, Chamberlain JO, Robinson MHE, et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet*. 1996;348(9040):1472-1477. doi:10.1016/S0140-6736(96)03386-7
62. van Rossum LG, van Rijn AF, Laheij RJ, et al. Random Comparison of

Guaiac and Immunochemical Fecal Occult Blood Tests for Colorectal Cancer in a Screening Population. *Gastroenterology*. 2008;135(1):82-90. doi:10.1053/j.gastro.2008.03.040

63. Hol L, Van Leerdam ME, Van Ballegooijen M, et al. Screening for colorectal cancer: Randomised trial comparing guaiac-based and immunochemical faecal occult blood testing and flexible sigmoidoscopy. *Gut*. 2010;59(1):62-68. doi:10.1136/gut.2009.177089
64. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget Stool DNA Testing for Colorectal-Cancer Screening. *N Engl J Med*. 2014;370(14):1287-1297. doi:10.1056/NEJMoa1311194
65. Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet*. 2014;383(9927):1490-1502. doi:10.1016/S0140-6736(13)61649-9
66. Clayton R, Borges-neto AA, Almeida GID, Márcia P, Riley LW, Moreira BM. NIH Public Access. 2010;60(1):79-87. doi:10.1016/j.diagmicrobio.2007.07.018.Prevalence
67. Estey MP, Di Ciano-Oliveira C, Froese CD, et al. Mitotic regulation of SEPT9 protein by cyclin-dependent kinase 1 (Cdk1) and pin1 protein is important for the completion of cytokinesis. *J Biol Chem*. 2013;288(42):30075-30086. doi:10.1074/jbc.M113.474932
68. Sellin ME, Holmfeldt P, Stenmark S, Gullberg M. Microtubules support a disk-like septin arrangement at the plasma membrane of mammalian cells. *Mol Biol Cell*. 2011;22(23):4588-4601. doi:10.1091/mbc.E11-09-0754
69. Field, C.m., & Kellog D. Septins: cytoskeletal polymers or signallin GTPases? *Trends Cell Biol*. 1999. doi:10.3864/j.issn.0578-1752.2015.15.008
70. Hall PA, Russell SEH. The pathobiology of the septin gene family. *J Pathol*. 2004;204(4):489-505. doi:10.1002/path.1654
71. Mostowy S, Janel S, Forestier C, et al. A role for septins in the interaction between the Listeria monocytogenes invasion protein InlB and the Met receptor. *Biophys J*. 2011;100(8):1949-1959. doi:10.1016/j.bpj.2011.02.040
72. Scott M, Hyland PL, McGregor G, Hillan KJ, Russell SEH, Hall PA. Multimodality expression profiling shows SEPT9 to be overexpressed in a wide range of human tumours. *Oncogene*. 2005;24(29):4688-4700. doi:10.1038/sj.onc.1208574

73. Church TR, Wandell M, Lofton-Day C, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. *Gut*. 2014;63(2):317-325. doi:10.1136/gutjnl-2012-304149
74. Tóth K, Sipos F, Kalmár A, et al. Detection of Methylated SEPT9 in Plasma Is a Reliable Screening Method for Both Left- and Right-Sided Colon Cancers. *PLoS One*. 2012;7(9):1-7. doi:10.1371/journal.pone.0046000
75. Song L, Jia J, Peng X, Xiao W, Li Y. The performance of the SEPT9 gene methylation assay and a comparison with other CRC screening tests: A meta-analysis. *Sci Rep*. 2017;7(1):1-12. doi:10.1038/s41598-017-03321-8
76. Quah HM, Chou JF, Gonen M, et al. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. *Dis Colon Rectum*. 2008;51(5):503-507. doi:10.1007/s10350-008-9246-z
77. Amri R, Bordeianou LG, Berger DL. Effect of High-Grade Disease on Outcomes of Surgically Treated Colon Cancer. *Ann Surg Oncol*. 2016;23(4):1157-1163. doi:10.1245/s10434-015-4983-4
78. Chen HS, Sheen-Chen SM. Obstruction and perforation in colorectal adenocarcinoma: an analysis of prognosis and current trends. *Surgery*. 2000;127(4):370-376. doi:10.1067/msy.2000.104674
79. Zeng Y, Lan P. Adjuvant chemotherapy for stage II colon cancer. *Zhonghua Wei Chang Wai Ke Za Zhi*. 2012;15(10):1092-1094.
80. Chang GJ, Rodriguez-Bigas MA, Skibber JM, Moyer VA. Lymph node evaluation and survival after curative resection of colon cancer: systematic review. *J Natl Cancer Inst*. 2007;99(6):433-441. doi:10.1093/jnci/djk092
81. Li C, Pei Q, Zhu H, et al. Survival nomograms for stage III colorectal cancer. *Med (United States)*. 2018;97(49):1-7. doi:10.1097/MD.0000000000013239
82. Al-Sohaily S, Biankin A, Leong R, Kohonen-Corish M, Warusavitarne J. Molecular pathways in colorectal cancer. *J Gastroenterol Hepatol*. 2012;27(9):1423-1431. doi:10.1111/j.1440-1746.2012.07200.x
83. Grady WM, Markowitz SD. The Molecular Pathogenesis of Colorectal Cancer and Its Potential Application to Colorectal Cancer Screening. *Dig Dis Sci*. 2015;60(3):762-772. doi:10.1007/s10620-014-3444-4
84. Lothe RA, Peltomaki P, Meling GI, et al. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res*. 1993;53(24):5849-5852.

85. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic Role of KRAS and BRAF in Stage II and III Resected Colon Cancer: Results of the Translational Study on the PETACC-3, EORTC 40993, SAKK 60-00 Trial. *J Clin Oncol*. 2009;28(3):466-474. doi:10.1200/JCO.2009.23.3452
86. Koopman M, Kortman GAM, Mekenkamp L, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. *Br J Cancer*. 2009;100(2):266-273. doi:10.1038/sj.bjc.6604867
87. Frazier ML, Xi L, Zong J, et al. Association of the CpG island methylator phenotype with family history of cancer in patients with colorectal cancer. *Cancer Res*. 2003;63(16):4805-4808.
88. Samowitz WS, Albertsen H, Herrick J, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroenterology*. 2005;129(3):837-845. doi:10.1053/j.gastro.2005.06.020
89. Ogino S, Kawasaki T, Kirkner GJ, Kraft P, Loda M, Fuchs CS. Evaluation of markers for CpG island methylator phenotype (CIMP) in colorectal cancer by a large population-based sample. *J Mol Diagn*. 2007;9(3):305-314. doi:10.2353/jmoldx.2007.060170
90. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet*. 2006;38(7):787-793. doi:10.1038/ng1834
91. Goel A, Nagasaka T, Arnold CN, et al. The CpG island methylator phenotype and chromosomal instability are inversely correlated in sporadic colorectal cancer. *Gastroenterology*. 2007;132(1):127-138. doi:10.1053/j.gastro.2006.09.018
92. Nagasaka T, Koi M, Kloor M, et al. Mutations in both KRAS and BRAF may contribute to the methylator phenotype in colon cancer. *Gastroenterology*. 2008;134(7):1950-1960, 1960.e1. doi:10.1053/j.gastro.2008.02.094
93. Nosho K, Irahara N, Shima K, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. *PLoS One*. 2008;3(11):e3698. doi:10.1371/journal.pone.0003698
94. Marra G, Jiricny J. DNA Mismatch Repair and Colon Cancer. *Genome*

Instab Cancer Dev. 1995:85-123. doi:10.1007/1-4020-3764-3_4

95. Domingo E, Niessen RC, Oliveira C, et al. BRAF-V600E is not involved in the colorectal tumorigenesis of HNPCC in patients with functional MLH1 and MSH2 genes. *Oncogene*. 2005;24(24):3995-3998. doi:10.1038/sj.onc.1208569
96. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer and Microsatellite Instability. *J Natl Cancer Inst*. 2004;96(4):261-268. doi:10.1093/jnci/djh034
97. Boland CR, Boland CR, Thibodeau SN, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*. 1998;58(22):5248-5257. doi:10.1158/0008-5472.can-06-1114
98. Ladabaum U, Wang G, Terdiman J, et al. Strategies to identify the Lynch syndrome among patients with colorectal cancer: a cost-effectiveness analysis. *Ann Intern Med*. 2011;155(2):69-79. doi:10.7326/0003-4819-155-2-201107190-00002
99. Sinicrope FA. The role of microsatellite instability testing in management of colorectal cancer. *Clin Adv Hematol Oncol*. 2016;14(7):476-479. doi:10.1007/s11864-015-0348-2
100. Zarkavelis G, Boussios S, Papadaki A, Katsanos KH, Christodoulou DK, Pentheroudakis G. Current and future biomarkers in colorectal cancer. *Ann Gastroenterol*. 2017;30(6):613-621. doi:10.20524/aog.2017.0191
101. Raut CP, Pawlik TM, Rodriguez-Bigas MA. Clinicopathologic features in colorectal cancer patients with microsatellite instability. *Mutat Res - Fundam Mol Mech Mutagen*. 2004;568(2):275-282. doi:10.1016/j.mrfmmm.2004.05.025
102. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol*. 2005;23(3):609-618. doi:10.1200/JCO.2005.01.086
103. Sargent DJ, Shi Q, Yothers G, et al. Prognostic impact of deficient mismatch repair (dMMR) in 7,803 stage II/III colon cancer (CC) patients (pts): A pooled individual pt data analysis of 17 adjuvant trials in the ACCENT database. *J Clin Oncol*. 2014;32(15_suppl):3507.

doi:10.1200/jco.2014.32.15_suppl.3507

104. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med*. 2015;372(26):2509-2520. doi:10.1056/NEJMoa1500596
105. Dung T. Le, Jennifer N. Durham, Kellie N. Smith, Hao Wang, Bjarne R. Bartlett L, K. Aulakh, Steve Lu, Holly Kemberling, Cara Wilt, Brandon S. Luber, Fay Wong NS, Azad, Agnieszka A. Rucki, Dan Laheru, Ross Donehower, Atif Zaheer, George A. Fisher T, et al. Mismatch-repair deficiency predicts response of solid tumors to PD-1 blockade. *Science (80-)*. 2017;6733(June):409-413. doi:10.1126/science.aan6733
106. H-J.J. Lenz, E. Van Cutsem, M.L. Limon, K.Y. Wong, A. Hendlisz MA, P. Garcia-Alfonso, B. Neyns, G. Luppi, D. Cardin, T. Dragovich US, A. Atasoy, R. Postema, Z. Boyd, J-M. Ledeine, M. Overman SL. Durable clinical benefit with nivolumab (NIVO) plus low-dose ipilimumab (IPI) as first-line therapy in microsatellite instability- high/mismatch repair deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC). 2018;29(October). doi:https://doi.org/10.1093/annonc/mdy424.019
107. Cunningham D, Atkin W, Lenz HJ, et al. Colorectal cancer. *Lancet*. 2010;375(9719):1030-1047. doi:10.1016/S0140-6736(10)60353-4
108. Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol*. 2007;25(13):1658-1664. doi:10.1200/JCO.2006.08.1620
109. Tabernero J, Van Cutsem E, Diaz-Rubio E, et al. Phase II trial of cetuximab in combination with fluorouracil, leucovorin, and oxaliplatin in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol*. 2007;25(33):5225-5232. doi:10.1200/JCO.2007.13.2183
110. Lievre A, Bachet J-B, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res*. 2006;66(8):3992-3995. doi:10.1158/0008-5472.CAN-06-0191
111. J.L. B. ras Oncogenes in human cancer: A review. *Cancer Res*. 1989;49(17):4682-4689. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed2&NEWS=N&AN=1989213649.

112. Chang YY, Lin PC, Lin HH, et al. Mutation spectra of RAS gene family in colorectal cancer. *Am J Surg*. 2016;212(3):537-544.e3. doi:10.1016/j.amjsurg.2016.02.013
113. Peeters M, Kafatos G, Taylor A, et al. Prevalence of RAS mutations and individual variation patterns among patients with metastatic colorectal cancer: A pooled analysis of randomised controlled trials. *Eur J Cancer*. 2015;51(13):1704-1713. doi:10.1016/j.ejca.2015.05.017
114. Conlin A, Smith G, Carey FA, Wolf CR, Steele RJC. The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. *Gut*. 2005;54(9):1283-1286. doi:10.1136/gut.2005.066514
115. Chua CWL, Chong DQQ, Kanesvaran R, et al. The prognostic impact of KRAS mutation in colorectal cancer patients: A meta-analysis of phase III clinical trials. *J Clin Oncol*. 2014;32(15_suppl):e14515-e14515. doi:10.1200/jco.2014.32.15_suppl.e14515
116. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26(10):1626-1634. doi:10.1200/JCO.2007.14.7116
117. Provenzano R, Wiecek A, Ph D, et al. K-ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer Christos. 2013:307-319. doi:10.1056/NEJMoa1203165
118. Tejpar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. *J Clin Oncol*. 2012;30(29):3570-3577. doi:10.1200/JCO.2012.42.2592
119. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol*. 2010;11(8):753-762. doi:10.1016/S1470-2045(10)70130-3
120. Sorich MJ, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. *Ann Oncol Off J Eur Soc Med Oncol*.

2015;26(1):13-21. doi:10.1093/annonc/mdu378

121. Morgan RJ, Armstrong DK, Alvarez RD, et al. Ovarian Cancer , Clinical Practice Guidelines in Oncology. 2016;14(9):1134-1163.
122. Allegra CJ, Rumble RB, Hamilton SR, et al. Extended RAS Gene Mutation Testing in Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. *J Clin Oncol*. 2016;34(2):179-185. doi:10.1200/JCO.2015.63.9674
123. Van Krieken JHJM, Rouleau E, Ligtenberg MJL, Normanno N, Patterson SD, Jung A. RAS testing in metastatic colorectal cancer: advances in Europe. *Virchows Arch*. 2016;468(4):383-396. doi:10.1007/s00428-015-1876-7
124. Carotenuto P, Roma C, Rachiglio AM, et al. Detection of KRAS mutations in colorectal carcinoma patients with an integrated PCR/sequencing and real-time PCR approach. *Pharmacogenomics*. 2010;11(8):1169-1179. doi:10.2217/pgs.10.86
125. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: Monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol*. 2013;10(8):472-484. doi:10.1038/nrclinonc.2013.110
126. Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. 2015;(June). doi:10.1038/nm.3870
127. Deng Y, Wang L, Tan S, et al. KRAS as a predictor of poor prognosis and benefit from postoperative FOLFOX chemotherapy in patients with stage II and III colorectal cancer. *Mol Oncol*. 2015;9(7):1341-1347. doi:10.1016/j.molonc.2015.03.006
128. Sanz-Garcia E, Argiles G, Elez E, Tabernero J. BRAF mutant colorectal cancer: Prognosis, treatment, and new perspectives. *Ann Oncol*. 2017;28(11):2648-2657. doi:10.1093/annonc/mdx401
129. Farina-Sarasqueta A, van Lijnschoten G, Moerland E, et al. The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. *Ann Oncol Off J Eur Soc Med Oncol*. 2010;21(12):2396-2402. doi:10.1093/annonc/mdq258
130. Yokota T, Ura T, Shibata N, et al. BRAF mutation is a powerful prognostic

factor in advanced and recurrent colorectal cancer. *Br J Cancer*. 2011;104(5):856-862. doi:10.1038/bjc.2011.19

131. Richman SD, Seymour MT, Chambers P, et al. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol*. 2009;27(35):5931-5937. doi:10.1200/JCO.2009.22.4295
132. Chen D, Huang JF, Liu K, et al. BRAFV600E mutation and its association with clinicopathological features of colorectal cancer: A systematic review and meta-analysis. *PLoS One*. 2014;9(3):1-9. doi:10.1371/journal.pone.0090607
133. Benson AB, Venook AP, Al-Hawary MM et al. Colon Cancer, Version 1.2018. NCCN Clinical Practice Guidelines in Oncology. https://www.nccn.org/professionals/physician_gls/pdf/colon. pdf.
134. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949-954. doi:10.1038/nature00766
135. Lito P, Pratilas CA, Joseph EW, et al. Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. *Cancer Cell*. 2012;22(5):668-682. doi:10.1016/j.ccr.2012.10.009
136. Corcoran RB, Andre T, Atreya CE, et al. Research article combined BRAF, EGFR, and MEK inhibition in patients with BRAFV600E-mutant colorectal cancer. *Cancer Discov*. 2018;8(4):428-443. doi:10.1158/2159-8290.CD-17-1226
137. ESMO GI / BEACON CRC SAFETY LEAD-IN. 2018.
138. Ursem C, Atreya CE, Van Loon K. Emerging treatment options for BRAF-mutant colorectal cancer. *Gastrointest Cancer Targets Ther*. 2018;Volume 8:13-23. doi:10.2147/GICTT.S125940
139. Wang Q, Hu WG, Song Q Bin, Wei J. BRAF V600E mutation as a predictive factor of Anti-EGFR monoclonal antibodies therapeutic effects in metastatic colorectal cancer: A meta-analysis. *Chinese Med Sci J*. 2014;29(4):197-203. doi:10.1016/S1001-9294(14)60070-5
140. Zhu L, Dong C, Cao Y, et al. Prognostic Role of BRAF Mutation in Stage II/III Colorectal Cancer Receiving Curative Resection and Adjuvant Chemotherapy: A Meta-Analysis Based on Randomized Clinical Trials.

PLoS One. 2016;11(5):e0154795. doi:10.1371/journal.pone.0154795

141. Kirby JA, Bone M, Robertson H, Hudson M, Jones DEJ. The number of intraepithelial T cells decreases from ascending colon to rectum. *J Clin Pathol*. 2003;56(2):158.
142. Expression G, Glebov OK, Rodriguez LM, et al. Distinguishing Right from Left Colon by the Pattern of. *Am Assoc Cancer Res J*. 2003;12(August):755-762.
143. Araki K, Furuya Y, Kobayashi M, Matsuura K, Ogata T, Isozaki H. Comparison of mucosal microvasculature between the proximal and distal human colon. *J Electron Microsc (Tokyo)*. 1996;45(3):202-206.
144. Petrelli F, Tomasello G, Borgonovo K, et al. Prognostic Survival Associated With Left-Sided vs Right-Sided Colon Cancer: A Systematic Review and Meta-analysis. *JAMA Oncol*. October 2016. doi:10.1001/jamaoncol.2016.4227
145. Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. *Ann Intern Med*. 1990;113(10):779-788.
146. Wang F, Bai L, Liu TS, et al. Right- and left-sided colorectal cancers respond differently to cetuximab. *Chin J Cancer*. 2015;34(9):1-10. doi:10.1186/s40880-015-0022-x
147. Weiss JM, Pfau PR, O'Connor ES, et al. Mortality by stage for right- versus left-sided colon cancer: Analysis of surveillance, epidemiology, and end results-medicare data. *J Clin Oncol*. 2011;29(33):4401-4409. doi:10.1200/JCO.2011.36.4414
148. Iacopetta B. Are there two sides to colorectal cancer? *Int J Cancer*. 2002;101(5):403-408. doi:10.1002/ijc.10635
149. Lee JM, Han YD, Cho MS, et al. Impact of tumor sidedness on survival and recurrence patterns in colon cancer patients. *Ann Surg Treat Res*. 2019;96(6):296-304. doi:10.4174/astr.2019.96.6.296
150. Jess P, Hansen IO, Gamborg M, Jess T. A nationwide Danish cohort study challenging the categorisation into right-sided and left-sided colon cancer. *BMJ Open*. 2013;3(5). doi:10.1136/bmjopen-2013-002608
151. Mik M, Berut M, Dziki L, Trzcinski R, Dziki A. Right-and left-sided colon cancer-clinical and pathological differences of the disease entity in one organ. *Arch Med Sci*. 2017;13(1):157-162. doi:10.5114/aoms.2016.58596

152. Nawa T, Kato J, Kawamoto H, et al. Differences between right- and left-sided colon cancer in patient characteristics, cancer morphology and histology. *J Gastroenterol Hepatol*. 2008;23(3):418-423. doi:10.1111/j.1440-1746.2007.04923.x
153. Saltzstein SL, Behling CA. Age and time as factors in the left-to-right shift of the subsite of colorectal adenocarcinoma: A study of 213,383 cases from the California Cancer Registry. *J Clin Gastroenterol*. 2007;41(2):173-177. doi:10.1097/01.mcg.0000225550.26751.6a
154. Venook AP, Niedzwiecki D, Lenz HJ, et al. Effect of first-line chemotherapy combined with cetuximab or bevacizumab on overall survival in patients with KRAS wild-type advanced or metastatic colorectal cancer a randomized clinical trial. *JAMA - J Am Med Assoc*. 2017;317(23):2392-2401. doi:10.1001/jama.2017.7105
155. Moretto R, Cremolini C, Rossini D, et al. Location of Primary Tumor and Benefit From Anti-Epidermal Growth Factor Receptor Monoclonal Antibodies in Patients With RAS and BRAF Wild-Type Metastatic Colorectal Cancer. *Oncologist*. 2016;21(8):988-994. doi:10.1634/theoncologist.2016-0084
156. Arnaud JP, Koehl C, Adloff M. Carcinoembryonic antigen (CEA) in diagosis and prognosis of colorectal carcinoma. *Dis Colon Rectum*. 1980;23(3):141-144. doi:10.1007/BF02587615
157. Fletcher RH. Carcinoembryonic antigen. *Ann Intern Med*. 1986;104(8):66-73. doi:10.7326/0003-4819-104-1-66
158. Brian DN, Bethany S, Indika P, et al. Blood CEA levels for detecting recurrent colorectal cancer. *Cochrane Database Syst Rev*. 2015;(12). doi:10.1002/14651858.CD011134.pub2
159. Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Tangen C. An Evaluation of the Carcinoembryonic Antigen (CEA) Test for Monitoring Patients With Resected Colon Cancer. *JAMA J Am Med Assoc*. 1993;270(8):943-947. doi:10.1001/jama.1993.03510080047030
160. Wille-Jorgensen P, Syk I, Smedh K, et al. Effect of More vs Less Frequent Follow-up Testing on Overall and Colorectal Cancer-Specific Mortality in Patients With Stage II or III Colorectal Cancer: The COLOFOL Randomized Clinical Trial. *JAMA*. 2018;319(20):2095-2103. doi:10.1001/jama.2018.5623

161. Zhang SY, Lin M, Zhang HB. Diagnostic value of carcinoembryonic antigen and carcinoma antigen 19-9 for colorectal carcinoma. *Int J Clin Exp Pathol*. 2015;8(8):9404-9409.
162. Thirunavukarasu P, Sukumar S, Sathaiah M, et al. C-stage in colon cancer: implications of carcinoembryonic antigen biomarker in staging, prognosis, and management. *J Natl Cancer Inst*. 2011;103(8):689-697. doi:10.1093/jnci/djr078
163. Corté H, Manceau G, Blons H. MicroRNA and colorectal cancer. *Dig Liver*. 2012;44(3):195-200. doi:10.1016/j.dld.2011.10.010
164. Calin GA, Dumitru CD, Shimizu M, et al. CalinGA02PNAS.pdf. 2002;99(24):13-18. doi:10.1073/pnas.242606799
165. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A*. 2004;101(9):2999-3004. doi:10.1073/pnas.0307323101
166. Mishra PJ. MicroRNAs as promising biomarkers in cancer diagnostics. *Biomark Res*. 2014;2(1):4-7. doi:10.1186/2050-7771-2-19
167. Mishra PJ, Merlino G. MicroRNA reexpression as differentiation therapy in cancer. *J Clin Invest*. 2009;119(8):2119-2123. doi:10.1172/JCI40107
168. Mitchell PS, Parkin RK, Kroh EM, et al. Mitchell et al. - 2008 - Circulating microRNAs as stable blood-based markers for cancer detection.pdf. 2008. doi:10.1073/pnas.0804549105
169. A.J. S, S.Y. L, J.J. S, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA - J Am Med Assoc*. 2008;299(4):425-436. doi:http://dx.doi.org/10.1001/jama.299.4.425
170. Valeri N, Gasparini P, Braconi C, et al. MicroRNA-21 induces resistance to 5-fluorouracil by down-regulating human DNA MutS homolog 2 (hMSH2). *Proc Natl Acad Sci*. 2010;107(49):21098-21103. doi:10.1073/pnas.1015541107
171. Shan L, Ji Q, Cheng G, et al. Diagnostic value of circulating miR-21 for colorectal cancer: A meta-analysis. *Cancer Biomarkers*. 2015;15(1):47-56. doi:10.3233/CBM-140437
172. Guo S, Zhang J, Wang B, et al. A 5-serum miRNA panel for the early

detection of colorectal cancer. *Onco Targets Ther*. 2018;11:2603-2614. doi:10.2147/OTT.S153535

173. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer*. 2010;127(1):118-126. doi:10.1093/tropej/fmq111
174. Ng EKO, Chong WWS, Jin H, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: A potential marker for colorectal cancer screening. *Gut*. 2009;58(10):1375-1381. doi:10.1136/gut.2008.167817
175. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420(6917):860-867. doi:10.1038/nature01322
176. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;454(7203):436-444. doi:10.1038/nature07205
177. Lakatos P-L, Lakatos L. Risk for colorectal cancer in ulcerative colitis: changes, causes and management strategies. *World J Gastroenterol*. 2008;14(25):3937-3947.
178. Feagins LA, Souza RF, Spechler SJ. Carcinogenesis in IBD: potential targets for the prevention of colorectal cancer. *Nat Rev Gastroenterol Hepatol*. 2009;6(5):297-305. doi:10.1038/nrgastro.2009.44
179. Dalpiaz O, Luef T, Seles M, et al. Critical evaluation of the potential prognostic value of the pretreatment-derived neutrophil-lymphocyte ratio under consideration of C-reactive protein levels in clear cell renal cell carcinoma. *Br J Cancer*. 2017;116(1):85-90. doi:10.1038/bjc.2016.393
180. Richtig G, Pichler M. EBioMedicine Prediction of Response in Melanoma Therapy by Systemic In fl ammatory Response – One Size Fits Not All. *EBioMedicine*. 2017;18:13-14. doi:10.1016/j.ebiom.2017.03.032
181. Riedl JM, Posch F, Moik F, et al. Inflammatory biomarkers in metastatic colorectal cancer: prognostic and predictive role beyond the first line setting. *Oncotarget*. 2017;8(56):96048-96061. doi:10.18632/oncotarget.21647
182. Dimitriou N, Felekouras E, Karavokyros I, Alexandrou A, Pikoulis E, Griniatsos J. Neutrophils to lymphocytes ratio as a useful prognosticator for stage II colorectal cancer patients. *BMC Cancer*. 2018;18(1):1202. doi:10.1186/s12885-018-5042-x
183. Absenger G, Szkandera J, Stotz M, et al. Preoperative neutrophil-to-

lymphocyte ratio predicts clinical outcome in patients with stage II and III colon cancer. *Anticancer Res*. 2013;33(10):4591-4594.

184. Pine JK, Morris E, Hutchins GG, et al. Systemic neutrophil-to-lymphocyte ratio in colorectal cancer: the relationship to patient survival, tumour biology and local lymphocytic response to tumour. *Br J Cancer*. 2015;113:204. https://doi.org/10.1038/bjc.2015.87.
185. He W, Hu W, Kong P, Yang L, Yang Y, Xie Q. Systemic neutrophil lymphocyte ratio and mismatch repair status in colorectal cancer patients : correlation and prognostic value. 2018;9. doi:10.7150/jca.26669
186. Evani SJ, Prabhu RG, Gnanaruban V, Finol EA, Ramasubramanian AK. Monocytes mediate metastatic breast tumor cell adhesion to endothelium under flow. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2013;27(8):3017-3029. doi:10.1096/fj.12-224824
187. Teng J-J, Zhang J, Zhang T-Y, Zhang S, Li B-S. Prognostic value of peripheral blood lymphocyte-to-monocyte ratio in patients with solid tumors: a meta-analysis. *Onco Targets Ther*. 2016;9:37-47. doi:10.2147/OTT.S94458
188. Wu Q, Hu T, Zheng E, Deng X, Wang Z. Prognostic role of the lymphocyte-to-monocyte ratio in colorectal cancer: An up-to-date meta-analysis. *Medicine (Baltimore)*. 2017;96(22):e7051. doi:10.1097/MD.0000000000007051
189. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. *Crit Rev Clin Lab Sci*. 2011;48(4):155-170. doi:10.3109/10408363.2011.599831
190. Shiu Y-C, Lin J-K, Huang C-J, et al. Is C-reactive protein a prognostic factor of colorectal cancer? *Dis Colon Rectum*. 2008;51(4):443-449. doi:10.1007/s10350-007-9133-z
191. DERITIS F, GIUSTI G, PICCININO F, CACCIATORE L. BIOCHEMICAL LABORATORY TESTS IN VIRAL HEPATITIS AND OTHER HEPATIC DISEASES. EVALUATION AND FOLLOW-UP. *Bull World Health Organ*. 1965;32:59-72.
192. Botros M, Sikaris KA. The de ritis ratio: the test of time. *Clin Biochem Rev*. 2013;34(3):117-130. http://www.ncbi.nlm.nih.gov/pubmed/24353357%5Cnhttp://www.pubmedcen

tral.nih.gov/articlerender.fcgi?artid=PMC3866949.

193. Cohen JA, Kaplan MM. The SGOT/SGPT ratio--an indicator of alcoholic liver disease. *Dig Dis Sci*. 1979;24(11):835-838. doi:10.1007/bf01324898
194. Nalpas B, Vassault A, Le Guillou A, et al. Serum activity of mitochondrial aspartate aminotransferase: a sensitive marker of alcoholism with or without alcoholic hepatitis. *Hepatology*. 1984;4(5):893-896. doi:10.1002/hep.1840040517
195. Diehl AM, Potter J, Boitnott J, Van Duyn MA, Herlong HF, Mezey E. Relationship between pyridoxal 5'-phosphate deficiency and aminotransferase levels in alcoholic hepatitis. *Gastroenterology*. 1984;86(4):632-636.
196. Thapa BR, Walia A. Liver function tests and their interpretation. *Indian J Pediatr*. 2007;74(7):663-671. doi:10.1007/s12098-007-0118-7
197. Gowda S, Desai PB, Hull V V, Math AAK, Vernekar SN, Kulkarni SS. A review on laboratory liver function tests. *Pan Afr Med J*. 2009;3:17.
198. Lala V, Minter DA. Liver Function Tests. In: Treasure Island (FL); 2020.
199. Giboney PT. Mildly elevated liver transaminase levels in the asymptomatic patient. *Am Fam Physician*. 2005;71(6):1105-1110.
200. KALLAI L, HAHN A, ROEDER V, ZUPANIC V. CORRELATION BETWEEN HISTOLOGICAL FINDINGS AND SERUM TRANSAMINASE VALUES IN CHRONIC DISEASES OF THE LIVER. *Acta Med Scand*. 1964;175:49-56. doi:10.1111/j.0954-6820.1964.tb00549.x
201. Wang Z-X, Jiang C-P, Cao Y, Zhang G, Chen W-B, Ding Y-T. Preoperative serum liver enzyme markers for predicting early recurrence after curative resection of hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int*. 2015;14(2):178-185. doi:10.1016/s1499-3872(15)60353-8
202. Ha Y, Kim SW, Chun SY, et al. Association between De Ritis ratio (aspartate aminotransferase / alanine aminotransferase) and oncological outcomes in bladder cancer patients after radical cystectomy. 2019:1-8.
203. Takenaka Y, Takemoto N, Yasui T, et al. Transaminase Activity Predicts Survival in Patients with Head and Neck Cancer. *PLoS One*. 2016;11(10):e0164057. doi:10.1371/journal.pone.0164057
204. Riedl J, Posch F, Prager G, et al. The AST/ALT (De Ritis) ratio predicts clinical outcome in pancreatic cancer patients treated with first-line nab-

paclitaxel and gemcitabine: post-hoc analysis of an Austrian multicenter, non-interventional study. *Ann Oncol Off J Eur Soc Med Oncol*. 2019;30 Suppl 4:iv78. doi:10.1093/annonc/mdz155.285

205. Wu J, Chen L, Wang Y, Tan W, Huang Z. Prognostic value of aspartate transaminase to alanine transaminase (De Ritis) ratio in solid tumors: a pooled analysis of 9,400 patients. *Onco Targets Ther*. 2019;12:5201-5213. doi:10.2147/OTT.S204403
206. Warburg O, Wind F, Negelein E. THE METABOLISM OF TUMORS IN THE BODY. *J Gen Physiol*. 1927;8(6):519-530. doi:10.1085/jgp.8.6.519
207. Greenhouse W V, Lehninger AL. Occurrence of the malate-aspartate shuttle in various tumor types. *Cancer Res*. 1976;36(4):1392-1396.
208. Conde VR, Oliveira PF, Nunes AR, et al. The progression from a lower to a higher invasive stage of bladder cancer is associated with severe alterations in glucose and pyruvate metabolism. *Exp Cell Res*. 2015;335(1):91-98. doi:10.1016/j.yexcr.2015.04.007
209. Wang H, Fang K, Zhang J, et al. The significance of De Ritis (aspartate transaminase/alanine transaminase) ratio in predicting pathological outcomes and prognosis in localized prostate cancer patients. *Int Urol Nephrol*. 2017;49(8):1391-1398. doi:10.1007/s11255-017-1618-7
210. Nishikawa M, Miyake H, Fujisawa M. De Ritis (aspartate transaminase/alanine transaminase) ratio as a significant predictor of recurrence-free survival in patients with upper urinary tract urothelial carcinoma following nephroureterectomy. *Urol Oncol Semin Orig Investig*. 2016;34(9):417.e9-417.e15. doi:10.1016/j.urolonc.2016.04.001
211. Lindmark G, Gerdin B, Påhlman L, Bergström R, Glimelius B. Prognostic predictors in colorectal cancer. *Dis Colon Rectum*. 1994;37(12):1219-1227. doi:10.1007/BF02257785

Anhang -Projektplan

Anhang - Fragebogen