University Hospital Ulm

Department of General and Visceral Surgery

Medical Director: Prof. Dr. med. Christoph Michalski

Colorectal Cancer: Impact of CEA and CA19-9

Dissertation submitted for the Doctoral Degree of Medicine at the Medical Faculty of Ulm University

Leilani Ellen Lakemeyer

born in Toronto, Canada

2021

Acting Dean: Prof. Dr. T. Wirth

First reviewer: Prof. Dr. J. Lemke

Second reviewer: Prof. Dr. P. Hermann

Day of Graduation: 18.11.2022

Parts of this dissertation were published in:

Lakemeyer L, Sander S, Wittau M, Henne-Bruns D, Kornmann M, Lemke J: Diagnostic and Prognostic Value of CEA and CA19-9 in Colorectal Cancer. *Diseases* 9: 21 (2021) doi:10.3390/diseases9010021

Table of Contents

List of abbreviations

BMI	Body mass index
CA19-9	Carbohydrate antigen 19-9
CCCU	Comprehensive Cancer Center Ulm
CEA	Carcinoembryonic antigen
CI	Confidence interval
CREDOS	Cancer Retrieval Evaluation and Documentation System
Fig.	Figure
G	Histological grading
HR	Hazard ratio
ICD-10	10th Revision of the International Statistical Classification of Diseases and Related
M	Status of metastatic spread
N	Status of infiltration of the lymph nodes
n	Absolute number of patients
N/A	No data available
Pat.	Patients
R	Residual tumor
TNM	TNM classification of malignant tumors
T	Primary tumor size
UICC	Union for International Cancer Control
WHO	World Health Organization

1 Introduction

1.1 Colorectal cancer and its tumor markers

1.1.1 Colorectal cancer: Epidemiology, early detection methods and staging

Colorectal cancer is a very important topic in our society. Cancer in general, after cardiovascular diseases, is the most frequent cause of death in Germany (Barnes *et al.*, 2016). There has been an impressive development in cancer research in the past years so that the early detection methods and further possibilities in treatment have made a significant difference for the patients.

Nevertheless, for men colorectal cancer is the third most common cancer, after lung cancer and prostate cancer, and for women it is the second most common cancer, after breast cancer (Ferlay *et al.*, 2010; Robert Koch-Institut, 2017). The median age to diagnose colorectal cancer is 72 years for men and 75 years for women (Robert Koch-Institut, 2017). Lifestyle risk factors for carcinomas of the intestinal system are a daily diet that is composed of meat, fat and is low in fiber, increased alcohol consumption, smoking and obesity. Other risk factors can be colorectal adenomas and a long-lasting inflammatory bowel disease like ulcerating colitis or Crohn's disease. Only 10% of the colorectal neoplasia derives from a genetic dysfunction. The familial adenomatous polyposis (FAP) has a mutation of the tumor suppressor gene adenomatous polyposis coli (APC) that results in multiple colorectal adenomas. The Lynch syndrome, also called hereditary non-polyposis colorectal cancer (HNPCC), on the other hand, has mutations in certain DNA repair genes (MLH1, MSH2, MSH6, PMS2 and EPCAM-deletion) and a microsatellite instability that comes with a much higher risk to develop colorectal, endometrial, ovarian, gastric and urothelium carcinoma (Herold, 2015). With further changes of eating habits and living habits in many countries, it will likely be even more of a problem in the future. In many cases colorectal cancer remains without any clinical symptoms for a long time, so that a diagnosis is often made in late stages of the cancer disease (Henne-Bruns, Dürig and Kremer, 2008; Gonzalez-Pons and Cruz-Correa, 2015). The cancer can be classified either in TNM classification, where T stands for the primary tumor size, N describes the

regional lymph node involvement, and category M stands for the possible spread to distant organs. It can also be classified according to UICC stages (Union for International Cancer Control), another staging system that is derived from the TNM classification. The tumor is located in 25% of all cases in the cecum and/or ascending colon, in 15% in the transverse colon, in 5% in the descending colon and in 55% in the rectum (Henne-Bruns, Dürig and Kremer, 2008). The overall 5-year survival after R0-resection is about 65%. Patients diagnosed with UICC stage I have a good outcome with a 5-year survival of 90%, UICC stages ranging from II to IV have a 5-year survival from 13 to 70% (American Cancer Society, 2014). In Germany patients without any risk factors can get a screening for colorectal cancer beginning at the age of 50 with a test for occult blood in feces. This test may be performed every year. Even though this test has low sensitivity and specificity, it is inexpensive and non-invasive and can help to detect colorectal cancer early. From the age of 55, patients may get a preventative colonoscopy and thereafter every 10 years if nothing conspicuous has been found in the first place (Herold, 2015; Schmiegel *et al.*, 2017).

1.1.2 Treatment and follow-up program for colorectal cancer

As far as an R0 outcome is possible, a surgical resection of the solid cancer and its metastases is pursued (Henne-Bruns, Dürig and Kremer, 2008; Yu *et al.*, 2017). Nevertheless, 10 to 15% of the patients experience a recurrence of the disease after the radical operation, mainly within the first 18 months (Henne-Bruns, Dürig and Kremer, 2008). In patients with colon neoplasia, cancer cells will spread through the hepatic portal vein to the liver and peritoneum, and further to the lungs and brain. Rectal cancer will primarily metastasize through the inferior vena cava instead, which results in metastases directly in the lung (Henne-Bruns, Dürig and Kremer, 2008). To prevent or detect a relapse early, clinics use an intense follow-up program for 5 years starting after the complete resection of the tumor, which includes anamnesis, a physical examination, blood tests including the search for tumor markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), a colonoscopy, an abdominal sonography, a rectoscopy, a computed tomography and a chest X-ray (Schmiegel *et al.*, 2017).

1.1.3 Tumor markers

For both, detecting colorectal cancer in early stages and for an effective follow-up program, it is important to find further methods to improve the search for malignancies and metastases. Since tumor markers are non-invasive and cost-effective, they would be a meaningful alternative. Tumor markers are substances that are produced by cancer cells or built by normal tissue as a reaction to the tumor. They can be detected in body fluids such as blood, urine and tissue, and can be proteins, antigens or hormones (Yang *et al.*, 2011). Depending on the tumor marker and the neoplasia, tumor markers are used to back up the diagnosis, to retrieve information for making a prognosis, and to monitor the success of treatment (Yang *et al.*, 2011; Schmiegel *et al.*, 2017). Nowadays, especially the tumor markers CEA and CA19-9 are used for colorectal cancer. Much research has been done to study these two tumor markers in correlation with colorectal cancer, but there is still uncertainty about how reliable CEA and CA19-9 really are as tumor markers.

1.1.4 Carcinoembryonic antigen

CEA was first discovered in 1965 by Gold and Freedman (Gold and Freedman, 1965). It is a glycoprotein that belongs to the group of oncofetal antigens together with alpha-fetoprotein (AFP) that is used as a tumor marker for hepatocellular carcinoma and in gynecology for prenatal diagnostics. CEA is an intracellular adhesion molecule that is produced in fetal gut tissue and by epithelial tumor cells, where it helps with angiogenesis (Hammarström, 1999). Its half-life is about 1 to 3 days (Yakabe *et al.*, 2010). An increased serum CEA is found in malignancies such as colorectal, breast, gastric, lung, ovarian and pancreatic cancer (Hammarström, 1999). Therefore, it is one of the most important tumor markers representing adenocarcinomas. Nevertheless, an elevation of CEA may also be seen in many non-malignant conditions, such as cigarette smoking, alcoholism, chronic inflammatory bowel disease, diverticulitis, pancreatitis and liver disease (George *et al.*, 1982; van der Schouw *et al.*, 1992).

1.1.5 Carbohydrate antigen 19-9

CA19-9 is also a glycoprotein, which was originally described by Koprowski et al. in 1979 (Koprowski *et al.*, 1979). CA19-9 is a monoclonal antibody that is a ligand

for E-Selectin (Berg *et al.*, 1992; Nakayama *et al.*, 1997). An increase of serum CA19-9 can be found in malignant and benign processes. The tumor marker is mostly produced by pancreatic, gastric, lung, biliary tract and colorectal cancer (Steinberg, 1990). Nevertheless, patients diagnosed with liver cirrhosis, acute cholangitis, diabetes mellitus, endometriosis, or bronchiectasis also show increased levels of CA19-9 (Kim *et al.*, 2020). Whereas studies were presenting a sensitivity level for CEA ranging from 65 to 74% in colorectal cancer patients, CA19-9 only had a sensitivity ranging from 26 to 48% (Yakabe *et al.*, 2010; Bagaria *et al.*, 2013; Zhang, Lin and Zhang, 2015). Despite the low sensitivity for CA19-9 on its own, studies detected that CA19-9 correlates with the tumor marker CEA and may, therefore, improve the sensitivity of CEA (Filella *et al.*, 1992; Ueda, Shimada and Urakawa, 1994; Zhang, Lin and Zhang, 2015; Lu *et al.*, 2016; Ozawa *et al.*, 2016; Shin *et al.*, 2019).

1.1.6 Summary

Nowadays, guidelines still only recommend the use of CEA alongside other screening methods for determining prognosis, for surveillance after a curative resection, and for monitoring treatment, such as chemotherapy or radiation. Due to low sensitivity, the use of CA19-9 on its own for detecting colorectal cancer or monitoring ongoing therapy or follow-up is not recommended (Locker *et al.*, 2006; Duffy *et al.*, 2007, 2014; Sturgeon *et al.*, 2008; Labianca *et al.*, 2010). The behavior and usefulness of the combination of CEA and CA19-9 for colorectal cancer patients have not yet been investigated sufficiently to make any guideline-oriented recommendations.

1.2 Aim of the study

The aim of this study was to collect specific information about the tumor markers CEA and CA19-9 in people diagnosed with colorectal cancer and treated by the Department of General and Visceral Surgery at the University Hospital Ulm between 2000 and 2015. Particular attention was directed at the serum level of the tumor markers CEA and CA19-9 that were measured within two weeks prior to primary surgical tumor resection. Furthermore, possibly influencing parameters were selected and assembled such as the date of birth, gender, the last date of surveillance or the date of death, the life status, the date of the cancer diagnosis and of its primary surgical resection, the age at time of diagnosis, body mass index, the TNM classification, the UICC stages, the histological grading, the localization of the tumor encoded as ICD-10, the residual tumor, the localization of metastases, the number of recurrences, the localization of the recurrence and the date of detection.

The collected information was used to answer the following questions:

(i) Is there a correlation between the level of the tumor markers CEA and CA19-9 and the clinicopathological parameters mentioned in the paragraph above? Therefore, is evaluating both tumor markers combined a reliable diagnostic screening method?

(ii) Are CEA and CA19-9, either separately or evaluated together, prognostic markers for overall survival?

(iii) Is the combination of CEA and CA19-9 an independent prognostic marker for survival and how can the information be used to improve treatment?

(iv) Are CEA and CA19-9, evaluated together, prognostic markers for recurrence-free survival?

2 Material and Methods

2.1 Patients and data collection

The University Hospital Ulm created its own program for tumor documentation, called CREDOS. CREDOS stands for Cancer Retrieval Evaluation and Documentation System that was developed by the Comprehensive Cancer Center Ulm (CCCU). It runs on the platform SAP/R3 and contains two software components called CREDOS-B basic version and CREDOS-S special version. CREDOS-B shows all the information about the diagnosis, progress and therapy of the tumor and CREDOS-S gives the opportunity to expand the basic documents with special documents.

SAP GUI is a software to gather patient data, write physician letters and manage the patients in the hospital ward. Depending on the position in the hospital, employees and students get individually regulated access to the patient's data so that medical confidentiality is warranted. A patient's file provides physician letters, pathologic results, surgical and anesthesia reports, test results and scanned letters from external physicians.

First of all, the secretary's office for tumor after-care of the Department of General and Visceral Surgery created a file that listed all the patients that were diagnosed with colorectal cancer and were therefore treated at the University Hospital Ulm from 2000 to 2015. The list contained 1487 patients approved by the Ethics Committee of the University Ulm (protocol code: 108/18; date of approval: 6th June 2018).

A database was created with Microsoft Excel for Mac 2011, where the data was collected, anonymized and encoded. A lot of information was already provided with the list from the Department of General and Visceral Surgery. Missing data was then individually collected with the help of CREDOS and SAP. CREDOS provided all the information about the date of birth, the date of the diagnosis, the TNM classification and the date and kind of the recurrence. SAP listed further needed information that was found in anamnesis forms, physician letters, anesthesia letters, pathologic results, surgical reports and blood test results.

1487 patients were either diagnosed directly in the University Hospital Ulm or in other medical facilities. Everyone was then treated for colorectal cancer at the Department of General and Visceral Surgery. A follow-up program that lasted 5 years was initiated for every patient who agreed to it. The follow-up can be done, either in the University Hospital Ulm, by external hospitals or medical specialists following the criteria of the follow-up program. External examiners passed the results on to the tumor database of the University Hospital Ulm.

For this study it was important that only adenocarcinomas were included, therefore all pathologic results for each of the 1487 patients were examined one more time. As a result, 20 patients with the pathologic diagnosis of a carcinoid were excluded from the study. From this point on the study only considered the remaining 1467 patients. Furthermore, another 509 patients of the patient collective had to be excluded as they did not have both tumor markers documented (Fig. 1). Out of the remaining 958 patients, four groups were formed dependent on normal or increased preoperative CEA and CA19-9. The distribution showed that 57% had both preoperative tumor markers below the cut-off value, 22% had only CEA increased, 5% had only CA19-9 increased, and 16% had both tumor markers increased (Fig. 2).

To evaluate the correlation of the tumor markers CEA and CA19-9 combined and recurrence-free survival, it was necessary to exclude all patients that received a R1- or R2- primary tumor resection. Whereas a R0-resection means that after the operation no tumor remains can be detected in the operating area, a R1-resection stands for remaining histological neoplasia found by the pathologist, and a R2-resection means that there are macroscopic remains of the tumor left in the patient’s body. As a result, only 798 patients were included in this part of the study.

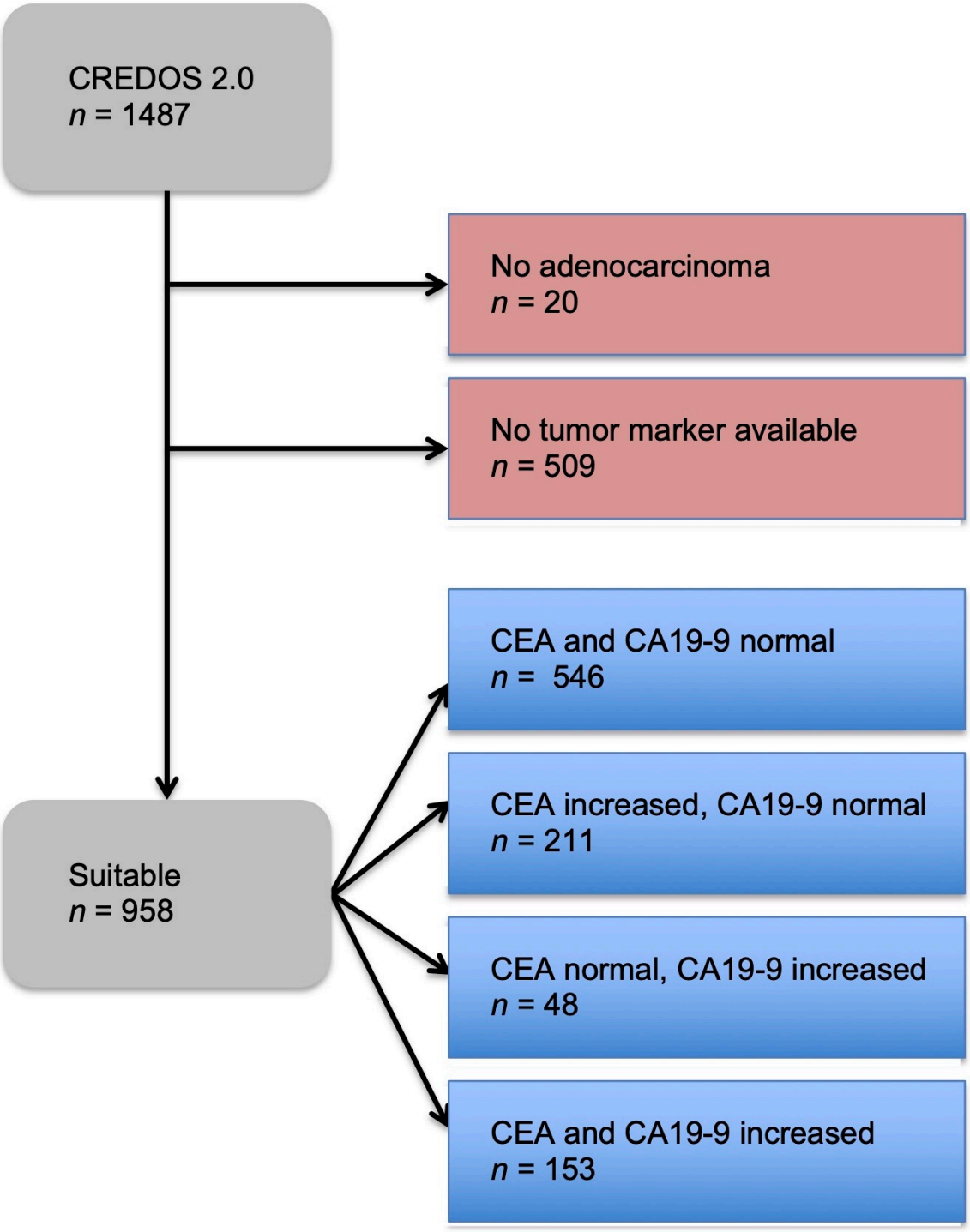

Figure 1: Study design of the patient collective in this study. The two red boxes represent the patients that were excluded from the study. The blue boxes represent the patients that were suitable for the study and their subdivision into four groups: both tumor markers below cut-off value, only carcinoembryonic antigen (CEA) increased, only carbohydrate antigen 19-9 (CA19-9) increased, and both tumor markers increased. (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

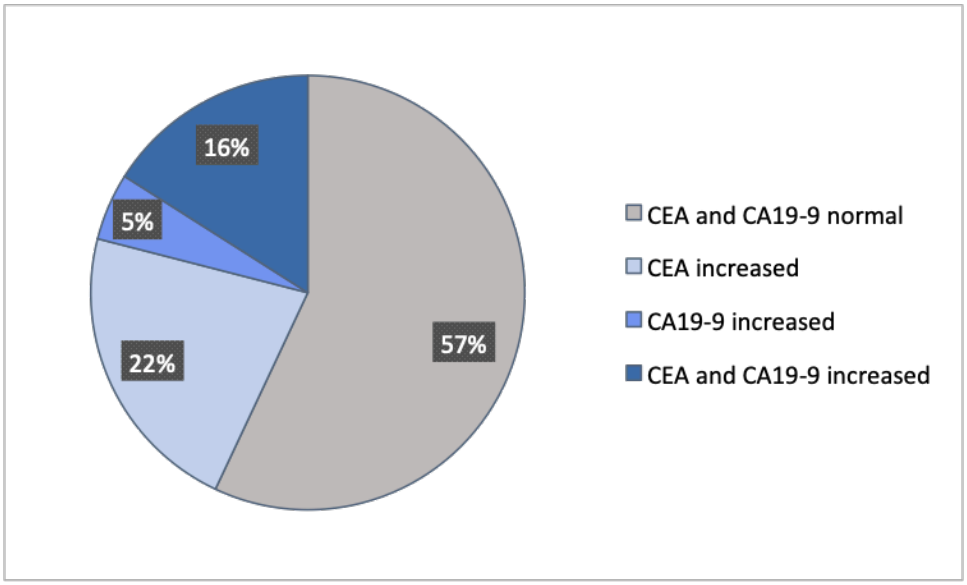

Figure 2: Distribution of the patient collective. (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/
Note. CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9

2.2 Patients' data

The patients' data mentioned in 2.1 and listed in Table 1 were all collected in a table by Microsoft Excel using the list provided by the secretary office for tumor after-care of the Department of General and Visceral Surgery and the hospital software SAP and CREDOS. The patients were anonymized using only their hospital file number, and their data was encoded into numbers.

The following table lists all the data collected for this study, categorized by general information of the patient, information about the primary tumor and information about recurrences.

Later in this study, information about the number of recurrences, their localization and the tumor marker value at the time of the recurrence were not included in further evaluations.

Table 1: This table shows the collected data filed in three different groups. The data refers to the patient's general information and diagnosis of colorectal cancer and the detection of a local recurrence or metastases in the follow-up program.

General Information	**Information about the primary tumor diagnosis**	**Information about the first detection of the first local recurrence and/or metastases**
Patient's file number	Date of diagnosis	Number of recurrences
Date of birth	Age at time of diagnosis	Localization of metastases
Gender	Date of surgical resection	Date of detection
Last date of surveillance	Body mass index	CEA, CA19-9
Date of death	TNM Classification	
Life status	UICC stage	
	Histological grading	
	ICD-10	
	Residual tumor (R-classification)	
	Localization of metastases	
	CEA, CA19-9	

Note. Life status = alive without recurrence/alive with recurrence/death caused by tumor/death cause unrelated to tumor/death cause unknown; TNM Classification: T = primary tumor site, N = regional lymph node involvement, M = possible spread to distant organs; UICC stage = Union for International Cancer Control; ICD-10 = 10th revision of the International Statistical Classification of Diseases and Related Health Problems; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9

2.2.1 General information

The category general information covers the patient's file number, the date of birth, gender, last date of surveillance, and the date of death. The last date of surveillance and the date of death are given by the registration office or the day of their last appearance at the University Hospital Ulm. The life status describes once more whether the patient is alive without recurrence, alive with recurrence, dead

caused by the tumor, dead caused non-related to the tumor, and dead with an unknown cause. The information about the patient's file number, the date of birth and the gender was documented in the list by the office of tumor after-care. The date of surveillance or the date of death was provided by the software CREDOS.

2.2.2 Age at time of diagnosis

The age was calculated from the date of birth and the date of diagnosis.

2.2.3 Body mass index (BMI)

The body mass index helps to categorize people as underweight, normal weight and obese (Table 2). It demonstrates easily the correlation of health problems that come with each weight class (WHO, 2018).

The body mass index is defined as a person's weight in kilograms (kg) divided by the square of the person's height in meters (m):

$BMI = kg/m^2$

Table 2: The body mass index (BMI) categorizes people into nutritional status (WHO, 2018)

BMI	Nutritional status
<18,5 kg/m²	Underweight
18,5 – 24,9 kg/m²	Normal weight
25,0 – 29,9 kg/m²	Pre-obesity
30,0 – 34,9 kg/m²	Obesity class I
35,0 – 39,9 kg/m²	Obesity class II
>40 kg/m²	Obesity class III

The body mass index was provided by the anesthesia reports from the primary resection or the anamnesis forms.

2.2.4 TNM classification

The TNM classification categorizes neoplasia into different stages that help to give a quick overview of the severity of the disease and to find the best treatment possible (Table 3). Therefore, three categories are evaluated. T stands for the size

and extension of the tumor, N stands for number of lymph nodes infiltrated by the tumor and M stands for possible occurrence of metastases (Comprehensive Cancer Center Ulm (CCCU), 2018).

Table 3: The TNM classification of the colorectal carcinoma (Comprehensive Cancer Center Ulm (CCCU), 2018)

T: Primary tumor size	
Tx	Main tumor cannot be assessed due to lack of information
T0	No evidence of primary tumor
TIS	Carcinoma in situ: intraepithelial or infiltration of the lamina propria
T1	Tumor infiltrates submucosa
T2	Tumor infiltrates muscularis propria
T3	Tumor infiltrates subserosa and/or pericolic/perirectal tissue that is not surrounded by peritoneum
T4	Tumor directly infiltrates other organs and/or structures and or perforates the visceral peritoneum
N: Regional lymph node involvement	
Nx	Regional lymph nodes cannot be assessed due to lack of information
N0	No regional lymph node metastases found (at least 12 lymph nodes were inspected)
N1	Metastases in 1 – 3 regional lymph nodes
N2	Metastases in 4 or more regional lymph nodes
M: Possible spread to distant organs	
Mx	Remote metastases cannot be evaluated
M0	No remote metastases found
M1	Metastases in at least one organ found (most often in liver, lung and lymph nodes; less often in the brain and skeleton)

Small letters preceding the TNM classification code give further information about the cancer status (Table 4) (Herold, 2015).

Table 4: Additional expansion of the TNM classification (Herold, 2015)

c	Clinical
p	Pathological (most often after surgery)
y	After therapy (neoadjuvant)
r	Recurrence

Note. Neoadjuvant therapy = radiation or chemotherapy before surgery

2.2.5 UICC staging system

The UICC staging system is an alternative classification system that is used to find the best treatment depending on the tumor stage. It is based on the categories of the TNM classification (Table 5).

Table 5: The UICC staging system for colorectal cancer (Comprehensive Cancer Center Ulm (CCCU), 2018).

UICC 2010	**TNM-system**		
Stage 0	Tis	N0	M0
Stage I	T1/T2	N0	M0
Stage II A	T3	N0	M0
Stage II B/C	T4	N0	M0
Stage III	All T	N1/N2	M0
Stage IV	All T	All N	M1

Note. UICC = Union for International Cancer Control, T = primary tumor size; N = status of infiltration of the lymph nodes; M = status of metastatic spread

2.2.6 Histological grading

The grading categorizes the tumor into one of four groups that shows how similar the tumor cells are in comparison to the cells of the original organ (Table 6). The classification can be made after surgery by pathologists and is another factor that decides over the kind of treatment (Comprehensive Cancer Center Ulm (CCCU), 2018). The information was gained from the histopathologic report.

Table 6: Histologic grading classification of the colorectal carcinoma (Comprehensive Cancer Center Ulm (CCCU), 2018)

	Grading
GX	Tissue histologically not assessable
G1	Cancer cells are highly differentiated
G2	Cancer cells are moderately differentiated
G3	Cancer cells are poorly differentiated
G4	Cancer cells are undifferentiated

2.2.7 International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10)

The ICD-10 is an internationally recognized classification system for diseases. It is published and updated by the World Health Organization (WHO) and assigns a code to every individual disease (Table 7).

Table 7: ICD-10 (International Statistical Classification of Diseases and Related Health Problems 10th Revision) codes for colorectal cancer (WHO, 2016)

C18	**Malignant neoplasm of colon**
C18.0	Caecum
C18.1	Appendix
C18.2	Ascending colon
C18.3	Hepatic flexure
C18.4	Transverse colon
C18.5	Splenic flexure
C18.6	Descending colon
C18.7	Sigmoid colon
C18.8	Overlapping lesion of colon
C18.9	Colon, unspecified
C19	**Malignant neoplasm of rectosigmoid junction**
C20	**Malignant neoplasm of rectum**

2.2.8 Residual tumor (R-classification)

For a curative outcome of the treatment, it is important that the tumor is surgically fully resected. The R-classification gives information if residual tumor remains in the patient's body after primary resection (Table 8). The information was taken from the histopathological report.

Table 8: The classification of the residual tumor after surgery (R-classification) (Comprehensive Cancer Center Ulm (CCCU), 2018)

	Residual tumor
RX	The presence of the residual tumor cannot be assessed
R0	No residual tumor
R1	Microscopic residual tumor
R2	Macroscopic residual tumor

2.2.9 Tumor markers: CEA and CA19-9

The levels of the tumor markers CEA and CA19-9 were obtained from the laboratory records. They were measured within two weeks prior to primary surgery and again every six months in the first two years after operation, followed by once a year for another three years in the follow-up program. In this study preoperative tumor markers and tumor markers from the time of local recurrence or metastases were collected. Later in this study, only the preoperative tumor marker values were used for evaluation.

2.3 Measurement and definition of CEA and CA19-9 levels

An electro-chemiluminescence immunoassay (ECLIA) is used for the in vitro quantitative determination of the tumor markers CEA and CA19-9. For this procedure a serum or plasma sample is used that is usually obtained from a blood sample coming from the cubital vein.

These two tumor markers are measured at the time of diagnosis and within two weeks prior to surgical resection. Due to the follow-up program the tumor markers are then measured every six months for the first two years after the operation, followed by once a year for another three years. For this study, the measured

tumor markers before the primary surgical resection and at the time of the first recurrence were collected, while the preoperative levels were of particular interest.

In the years 2000 to 2015 the recommended cut-off value for both tumor markers were changed by the laboratory of the University Hospital Ulm. To compare the results with other studies, the currently recommended and most frequently used reference values were chosen. CEA levels below 5 ng/ml were defined as normal and everything equal to 5 ng/ml and above was defined as increased levels. For CA19-9 a level below 37 U/ml was defined as normal and a level equal and above 37 U/ml was defined as an increased value.

The patients were then divided into four subgroups:

- CEA and CA19-9 normal
- CEA increased; CA19-9 normal
- CEA normal; CA19-9 increased
- CEA and CA19-9 increased

2.4 Literature search

The literature search started with the help of the database PubMed. PubMed is a free archive developed by the National Center for Biotechnology Information (NCBI) at the National Library of Medicine that gives access to literature about biomedicine and life sciences. In addition, a literature search was performed with Medline via Ovid. It is a licensed database that provides access to journals and literature in the medical field.

The following search terms were used to find relevant publications that focused on similar topics compared to this study: “colorectal cancer”, “colorectal neoplasia”, “CEA”, Carcinoembryonic antigen”, “CA19-9”, “Carbohydrate antigen 19-9”, “biomarkers” and “tumor markers”. All search items were linked with a logical connective (either “and” or “or”) so that the outcome showed a wide, but specific range of journals and articles discussing topics similar to this study.

At last, further information was provided by the World Wide Web and books from the library at the University Ulm.

2.5 Statistical analysis

The patient data was collected and listed in tabular form with the help of Microsoft Excel (Microsoft Excel for Mac 2011, Version: 14.2.5).

The first part of this study analyzes a possible correlation of characteristics such as gender, age, BMI, tumor localization, UICC classification, grading, and the TNM classification to the level of the tumor markers CEA and CA19-9 (Table 9 to 11).

The tables reflect the number of patients assigned to every group, as well as the percentage, the Median, the Minimum and the Maximum of the age and the level of the preoperative tumor markers CEA and CA19-9.

Bar charts were then used to visualize the results (Fig. 3 to 12).

The overall survival depending on different levels of CEA and CA19-9, evaluated separately and combined, was presented with the help of the Kaplan-Meier Method (Fig. 13 to 17; Table 12 to 16). The survival time was calculated from the time of the diagnosis of the primary tumor until the date of death or the date of the last surveillance. In this process the date of death presents an incident, and the date of the last surveillance is marked as censored.

Further, to examine which parameters could be a higher risk for a shorter life expectancy when diagnosed with colorectal cancer, a univariate and a multivariate analysis was performed as the Cox proportional hazards model (Table 17 to 18).

Then again, the Kaplan-Meier Method was used to present the recurrence-free survival of the patients in correlation to preoperative CEA and CA19-9 combined (Fig. 18; Table 19). Therefore, the date of diagnosing the first recurrence and the date of death of patients who did not have a recurrence documented but had the tumor documented as the cause of death present an incident and the date of the last surveillance is censored.

The statistical analysis and the presentation of the Kaplan-Meier curves were made with the help of Mrs. Sander of the Institute of Epidemiology and Medical Biometry of the University of Ulm.

3 Results

3.1 Frequency distribution of all patients at time of diagnosis

The first step to analyze the collected data was to create a table that included the frequency distribution. Table 9 to 11 present the patients' data at time of the diagnosis and the primary operation and are divided as follows:

1. General information
2. Data of the primary tumor
3. Life status

In the following tables, the left column shows the parameters that were analyzed. Further to the parameters, the patients are divided into five groups shown in the upper line of the table. The groups contain patients with both tumor markers below cut-off value, patients with an increased CEA value and CA19-9 below cut-off value, patients with a normal CEA value and an increased CA19-9 value, then patients with both tumor markers increased and, at last, patients with no tumor markers filed. The last column gives information about the total number of patients belonging to the parameter.

Every group is divided into two further columns that present the absolute number of patients and its percentage.

Table 9: General information about the patients who were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015. Modified from (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

General Information											
Parameter	CEA: < 5ng/ml; CA19-9: < 37U/ml		CEA: ≥ 5ng/ml; CA19-9: < 37U/ml		CEA: < 5ng/ml; CA19-9: ≥ 37U/ml		CEA: ≥ 5ng/ml; CA19-9: ≥ 37U/ml		No tumor marker available		Total
	n=546	%	*n*=211	%	*n*= 48	%	*n*=153	%	*n*=509	%	*n*=1467
Gender											
Male	354	40	130	15	22	2	95	11	291	33	892
Female	192	33	81	14	26	5	58	10	218	38	575
Age at time of diagnosis											
< 65 years	229	38	106	17	13	2	56	10	197	33	606
≥ 65 years	317	37	105	12	35	4	97	11	312	36	861
Median (Min;Max)		67 (20;93)		64 (29;89)		68 (34;89)		67 (36;95)		69 (26;96)	67 (20;96)
BMI											
< 18,5	13	37	7	20	1	3	4	11	10	29	35
≥ 18,5 - <25	176	35	88	17	24	5	51	10	164	33	503
≥ 25	351	41	114	13	21	2	92	11	268	32	681
N/A	6	7	2	2	2	2	6	7	67	81	83

Note. CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; n = absolute number of patients; Min. = minimum; Max. = maximum; BMI = body mass index, N/A = no data available

Table 10: Information about the primary tumor of the patients who were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015. Modified from (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

Information about the primary tumor											
Parameter	CEA: < 5ng/ml; CA19-9: < 37U/ml		CEA: ≥ 5ng/ml; CA19-9: < 37U/ml		CEA: < 5ng/ml; CA19-9: ≥ 37U/ml		CEA: ≥ 5ng/ml; CA19-9: ≥ 37U/ml		No tumor marker available		Total
	n=546	%	*n*=211	%	*n*= 48	%	*n*=153	%	*n*=509	%	*n*=1467
Tumor localization											
Cecum	42	35	17	14	6	5	21	18	33	28	119
Vermiform appendix	3	19	1	6	0	0	4	25	8	50	16
Ascending colon	49	29	25	15	10	6	19	11	68	40	171
Hepatic flexure	15	39	4	11	3	8	6	16	10	26	38
Transverse Colon	20	27	14	19	2	3	7	10	30	41	73
Splenic flexure	4	29	2	14	0	0	1	7	7	50	14
Descending colon	13	23	11	20	0	0	0	0	32	57	56
Sigmoid colon	89	34	35	13	7	3	34	13	95	37	260
C 18.8	2	29	2	29	0	0	1	14	2	29	7
C 18.9	27	31	13	15	2	2	14	16	32	36	88
Right colon	126	31	60	15	21	5	53	13	141	35	401
Left colon	106	32	48	15	7	2	35	11	134	41	330
In total: Colon	264	31	124	15	30	4	107	13	317	38	842
Rectosigmo-idal colon	35	40	14	16	1	1	6	7	31	36	87
Rectum	247	46	73	14	17	3	40	7	161	30	538
In total: Rectum	282	45	87	14	18	3	46	7	192	31	625
UICC classification											
Stage I	200	58	21	6	8	2	4	1	113	33	346
Stage II	128	36	61	17	8	2	24	7	137	38	358
Stage III	159	41	48	12	23	6	26	7	134	34	390
Stage IV	52	15	77	22	9	3	99	29	106	31	343
N/A	7	23	4	13	0	0	0	0	19	63	30

Note. CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; n = absolute number of patients; Left colon = including cecum, ascending colon, hepatic flexure, transverse colon; Right colon = including splenic flexure, descending colon, sigmoid colon; C18.8 = malignant neoplasm of overlapping sites of colon; C19.9 = malignant neoplasm of colon, unspecified; UICC = Union for International Cancer Control; N/A = no data available

The table continues on page 22.

Continuation of Table 10.

Parameter	CEA: < 5ng/ml; CA19-9: < 37U/ml		CEA: ≥ 5ng/ml; CA19-9: < 37U/ml		CEA: < 5ng/ml; CA19-9: ≥ 37U/ml		CEA: ≥ 5ng/ml; CA19-9: ≥ 37U/ml		No tumor marker available		Total
	n=546	%	*n*=211	%	*n*= 48	%	*n*=153	%	*n*=509	%	*n*=1467
Grading											
G1	45	48	8	9	2	2	4	4	34	37	93
G2	374	39	146	15	26	3	100	11	304	32	950
G3	93	30	42	13	17	5	43	14	117	38	312
G4	3	15	1	5	3	15	3	15	10	50	20
GX	31	34	14	15	0	0	3	3	44	48	92
Stage T											
Tis	2	100	0	0	0	0	0	0	0	0	2
ypT0	5	71	1	14	0	0	0	0	1	14	7
T1	85	56	6	4	4	3	2	1	54	36	151
T2	166	56	27	9	8	3	11	4	87	29	299
T3	232	32	130	18	26	4	79	11	257	35	724
T4	48	19	44	17	10	4	61	24	93	36	256
TX	8	29	3	11	0	0	0	0	17	61	28
Stage N											
N0	338	45	100	13	17	2	39	5	261	35	755
N1	125	33	60	16	20	5	46	12	123	33	374
N2	70	25	44	16	10	4	65	23	94	3	283
NX	13	24	7	13	1	2	3	5	31	56	55
Stage M											
M0	475	45	125	12	39	4	52	5	370	35	1061
M1	50	15	77	23	9	3	99	29	106	31	341
Number of located metastases											
1	45	17	57	22	8	3	69	26	84	32	263
≥ 2	5	6	20	26	1	1	30	38	22	28	78
MX	21	32	9	14	0	0	2	3	33	51	65

Note. CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; n = absolute number of patients; G = histological grading; T = primary tumor; N = status of infiltration of the lymph nodes; M = status of metastatic spread

Table 11: Life status of the patients diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015.

	Life status										
Parameter	CEA: < 5ng/ml; CA19-9: < 37U/ml		CEA: ≥ 5ng/ml; CA19-9: < 37U/ml		CEA: < 5ng/ml; CA19-9: ≥ 37U/ml		CEA: ≥ 5ng/ml; CA19-9: ≥ 37U/ml		No tumor marker available		Total
	n=546	%	*n*=211	%	*n*= 48	%	*n*=153	%	*n*=509	%	*n*=1467
Alive											
Remission	281	49	78	14	20	3	22	4	173	30	574
Recurrence	28	45	17	27	2	3	4	6	11	18	62
In total: Alive	309	49	95	15	22	3	26	4	184	29	636
Dead											
Caused by tumor	83	21	70	18	16	4	72	18	156	39	397
Independent of the tumor	63	39	15	9	4	3	11	7	67	42	160
Unknown	91	33	31	11	6	2	44	16	102	37	274
In total: Dead	237	29	116	14	26	3	127	15	325	39	831

Note. CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; n = absolute number of patients

3.2 Graphic representation of the frequency distribution

To visualize the patients' data that was collected and presented in Table 9 to 11, the following step in this study was to create bar charts. The bar charts show the distribution of patients in percentage of each parameter belonging to the groups "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value", "CEA below cut-off value, CA19-9 increased" and "both tumor markers increased".

The following charts are organized according to the parameters mentioned in Table 9 to 11. Since the group "tumor marker not available" has no informative value in this study, it was only included in the tables but was ignored in the bar charts. The same applies to the parameters GX, TX, NX and MX.

3.2.1 Gender

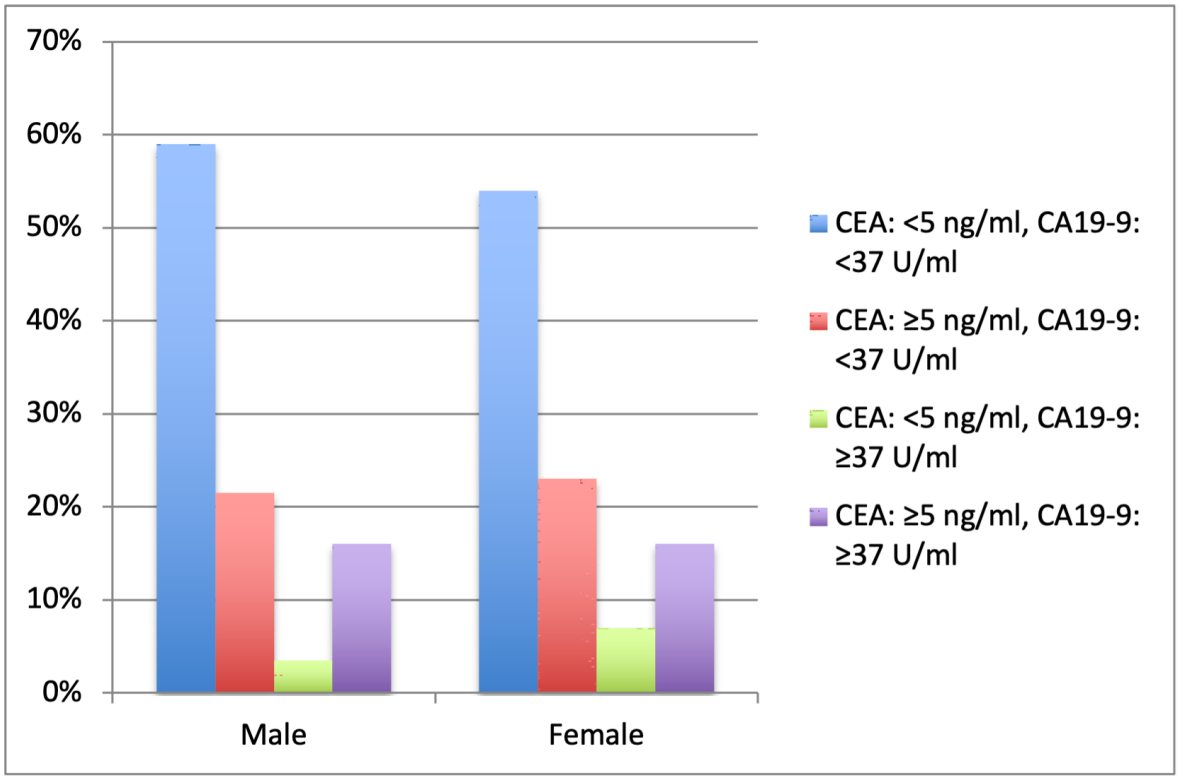

Figure 3: The distribution of male and female patients who were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015. Both bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".

For this bar chart (Fig. 3) the study group was divided into male (*n* = 601) and female patients (*n* = 357).

The bar chart shows a very similar distribution for both, male and female patients. 59% of the male patients and 54% of the female patients had both tumor markers below the cut-off value. Then, 21.5% of the male and 23% of the female patients had only the tumor marker CEA increased. Only 16% of male and female patients had both tumor markers increased and even fewer patients, 3.5% of male patients and 7% of female patients had only the tumor marker CA19-9 increased.

3.2.2 Age at time of diagnosis

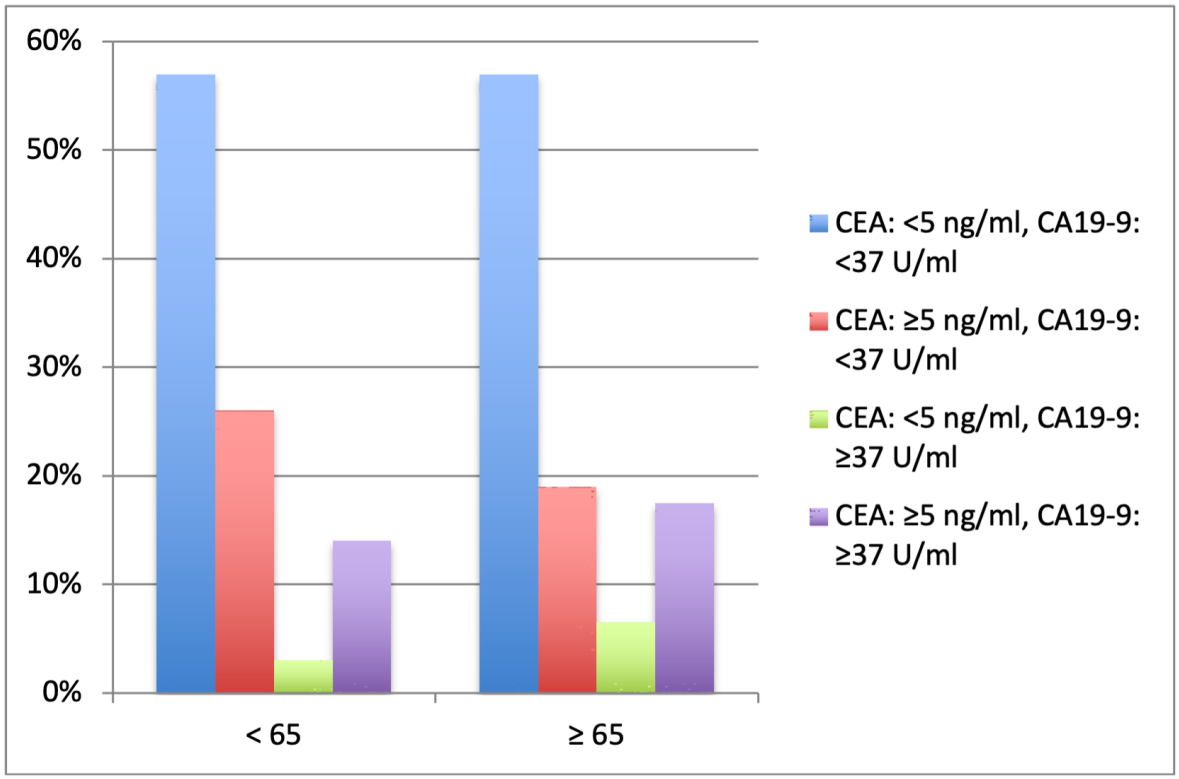

Figure 4: The distribution of patients who were diagnosed with colorectal cancer either at age 64 and younger or 65 and older and treated at the University Hospital Ulm from 2000 to 2015. Both bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".

For this bar chart (Fig. 4) the study group was divided into patients that were diagnosed with colorectal cancer at age 64 and younger (*n* = 408) or at age 65 and older (*n* = 550).

Here again, the bar chart shows a similar distribution of both groups. 57% of patients in both age groups had both tumor markers below the cut-off value. Only 26% of patients below 65 years and 19% of patients 65 years and older had the tumor marker CEA increased. 14% of patients with both tumor markers elevated were below 65 years and 17.5% of patients 65 years and older. 3% of patients below 65 years and 6.5% of patients 65 years and older had only the tumor marker CA19-9 increased.

3.2.3 Body mass index

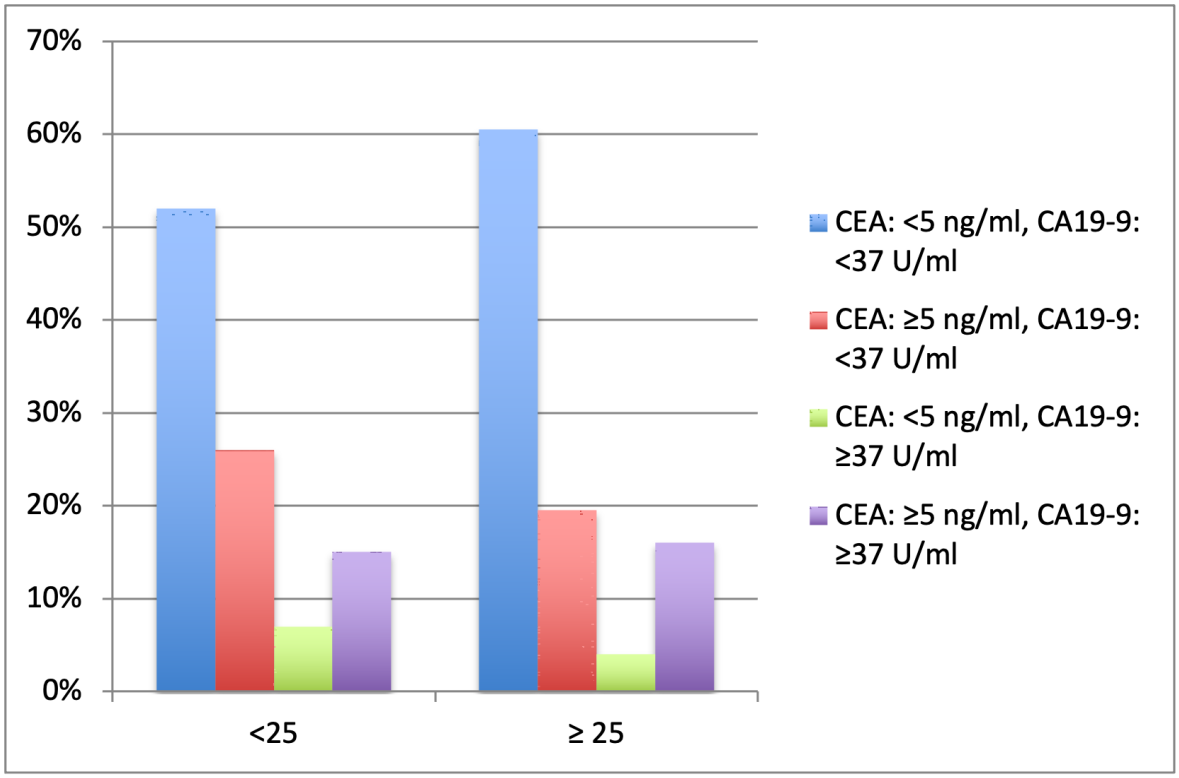

Figure 5: The distribution of patients with a body mass index below 25 kg/m^2 and equal or above 25 kg/m^2 who were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015. Both bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".

For this bar chart (Fig. 5) the study group was divided into patients with a body mass index below 25 kg/m^2 (*n* = 364) and patients with a body mass index of 25 kg/m^2 and above (*n* = 578).

Patients with a body mass index below 25 kg/m^2 had slightly less often both tumor markers below the cut-off value (52%), and instead slightly more often the tumor marker CEA increased (26%). Therefore, 60.5% of patients with a body mass index of 25 kg/m^2 and above had both tumor markers below the cut-off value and 19.5% had the tumor marker CEA increased. Only 15% of the patients with a body mass index below 25 kg/m^2 had both tumor markers increased and 7% had only the tumor marker CA19-9 increased. Similar to that, only 16% of the patients with a body mass index equal or above 25 kg/m^2 had both tumor markers increased and 4% had only the tumor marker CA19-9 increased.

3.2.4 Tumor localization

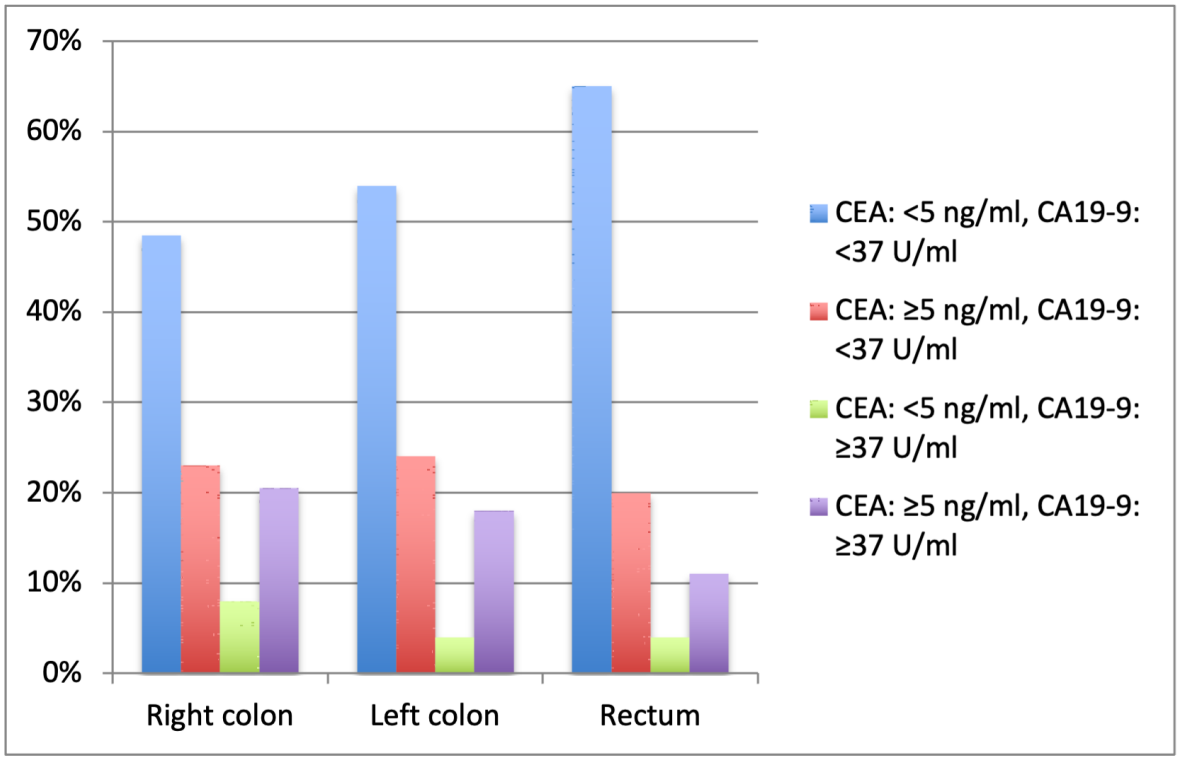

Figure 6: The distribution of patients who were diagnosed with colorectal cancer either located in the left colon, the right colon or the rectum and treated at the University Hospital Ulm from 2000 to 2015. All three bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".
Note. Right colon = cecum, ascending colon, hepatic flexure, transverse colon; Left colon = splenic flexure, descending colon, sigmoid colon; Rectum = rectosigmoidal colon, rectum

The localization of the colorectal cancer was assigned to twelve different locations within the intestinal tract. To obtain a better overview, the locations were merged into three groups. The right colon includes the cecum, the ascending colon, the hepatic flexure and the transverse colon. The left colon includes the splenic flexure, the descending colon and the sigmoid colon. The rectum includes the rectosigmoidal colon and the rectum. The vermiform appendix and the patients with an ICD code C18.8 (malignant neoplasm of overlapping sites of colon) and C18.9 (malignant neoplasm of colon, unspecified) could not be assigned to any of the groups mentioned above and were therefore excluded from the graphic illustration.

In this bar chart (Fig. 6) 260 patients had a tumor located in the right colon, 196 patients in the left colon, and 433 patients in the rectum.

While the groups presenting the patients diagnosed with an adenocarcinoma in the right or the left colon show a similar distribution, the group including rectosigmoidal and rectal cancer patients shows a slightly different division.

Patients with a tumor located in the rectum had more often both tumor markers below the cut-off value at the time of diagnosis (65%) compared to the patients with a tumor located in the right (48.5%) or left colon (54%). Instead, more patients with a carcinoma located in the colon (Right: 20.5%; Left: 18%) had both tumor markers elevated compared to the patients with a rectosigmoidal or rectal carcinoma (11%). In all three locations a similar number of patients had only the tumor marker CEA elevated, in the right colon 23%, in the left colon 24% and in the rectal area 20% of the patients. Only 8% of the patients with colon cancer on the right side and 4% on the left side or in the rectum had only the tumor marker CA19-9 elevated.

3.2.5 UICC staging

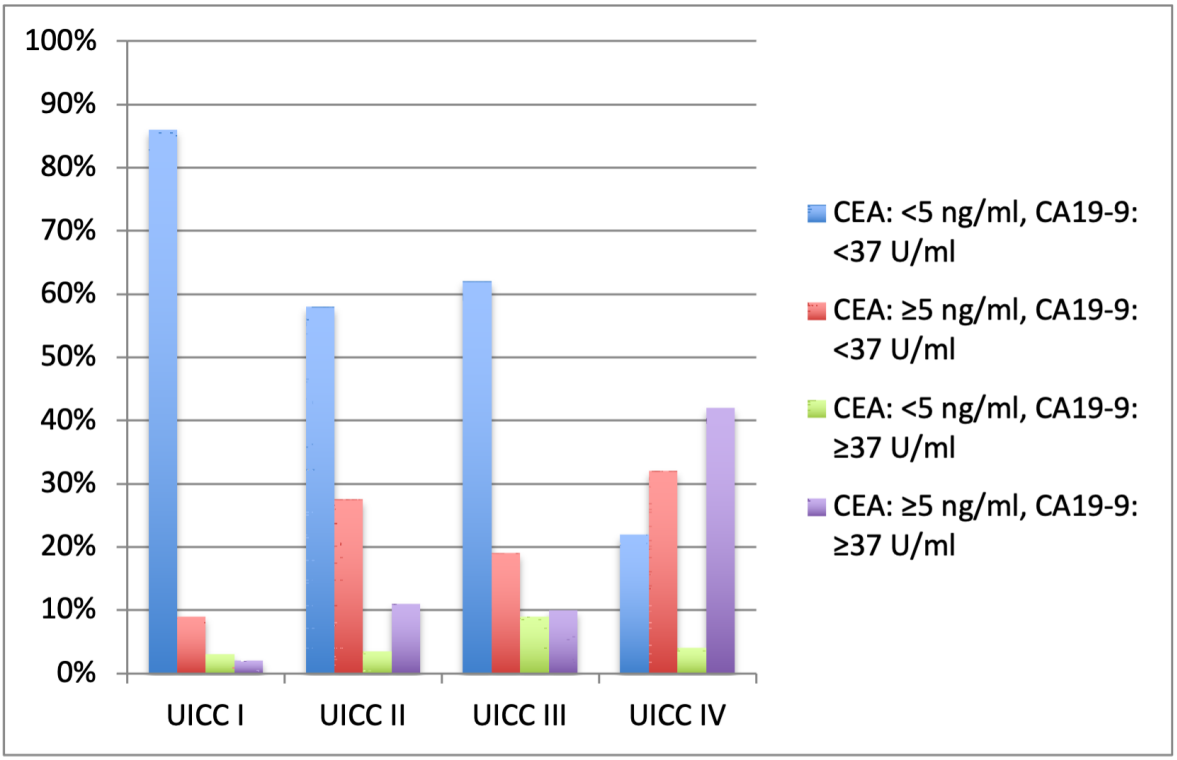

Figure 7: The distribution of patients classified with the Union for International Cancer Control (UICC) staging system who were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015. All four bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".

For this bar chart (Fig. 7) the study group was divided into the four UICC stages. All four groups include a similar number of patients. The group UICC stage I includes 233 patients, UICC stage II 221 patients, UICC stage III 256 patients and UICC stage IV 237 patients.

The bar chart reveals significant differences between the UICC stages.

In UICC stage I the majority of the patients (86%) had both tumor markers below the cut-off value. Only 9% of the patients had the tumor marker CEA elevated and CA19-9 below cut-off value and even fewer patients had only the tumor marker CA19-9 (3%) or both tumor markers (2%) elevated.

In comparison to the UICC stage I, fewer patients diagnosed with colorectal cancer in UICC stage II or III have both tumor markers below the cut-off value

(UICC II: 58%; UICC III: 62%). In return the UICC stages II and III have more patients with only the tumor marker CEA increased (UICC II: 27.5%; UICC III: 19%). While UICC stage III has 9% of patients with only CA19-9 increased, only 3.5% of patients diagnosed with UICC stage II colorectal cancer had CA19-9 elevated and CEA below cut-off value. At last, in both groups only a small cluster had both tumor markers elevated (UICC II: 11%; UICC III: 10%).

The distribution of the patients belonging to group UICC stage IV is different to the other stages. Only 22% of the patients had both tumor markers below the cut-off value, whereas most patients either had only the tumor marker CEA (32%) or both tumor markers (42%) elevated. Here again, only a few patients had only the tumor marker CA19-9 increased (4%).

3.2.6 Histological grading

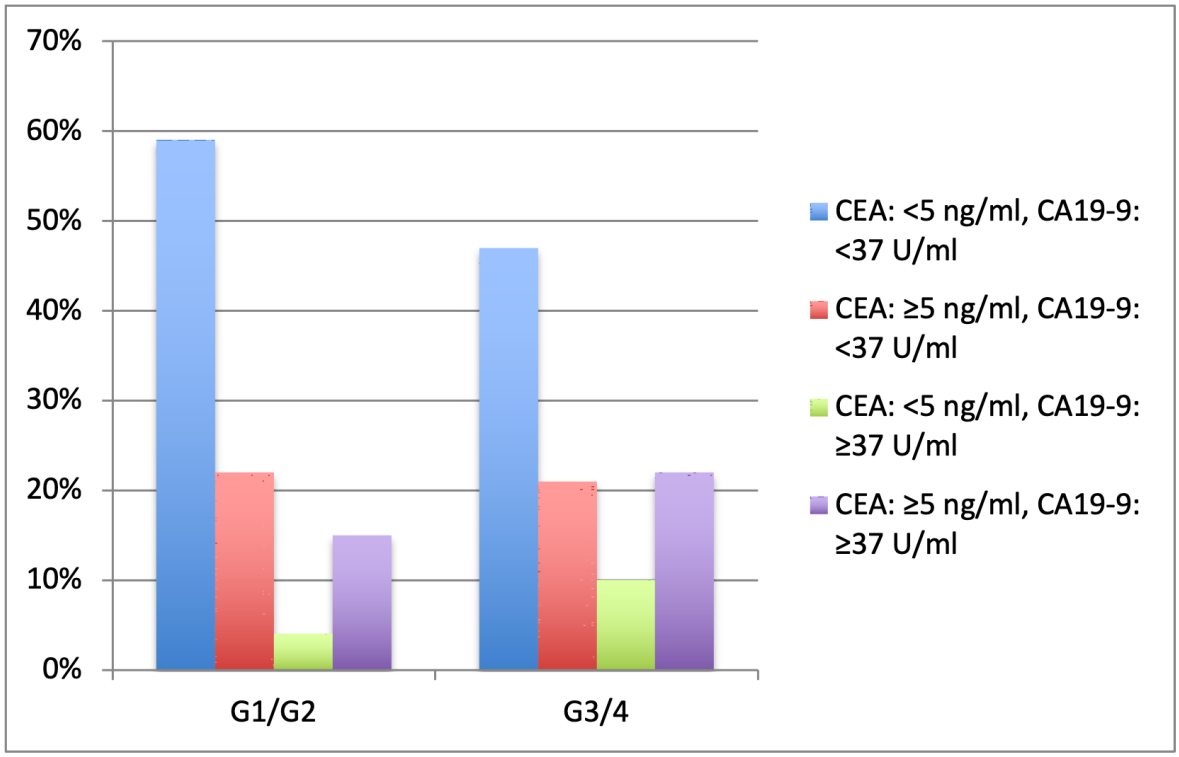

Figure 8: The distribution of patients who were diagnosed with colorectal cancer classified into histological grading (G) and treated at the University Hospital Ulm from 2000 to 2015. Both bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".

In this bar chart (Fig. 8), two groups were formed including 705 patients with well-differentiated tumors (G1/G2), and 205 patients with poorly differentiated tumors (G3/G4).

59% of the patients with well-differentiated tumors and 47% with poorly differentiated tumors had both tumor markers below cut-off value. 21% of the patients with a tumor classified as G3/G4 had only the tumor marker CEA increased whereas 22% had both tumor markers elevated. 10% of the patients had only CA19-9 increased. Compared to this group, 22% of the patients with a tumor categorized as G1/G2 had only the tumor marker CEA elevated and 15% of the patients had both tumor markers increased. Even fewer patients (4%) had only the tumor marker CA19-9 elevated.

3.2.7 Size of primary tumor (T)

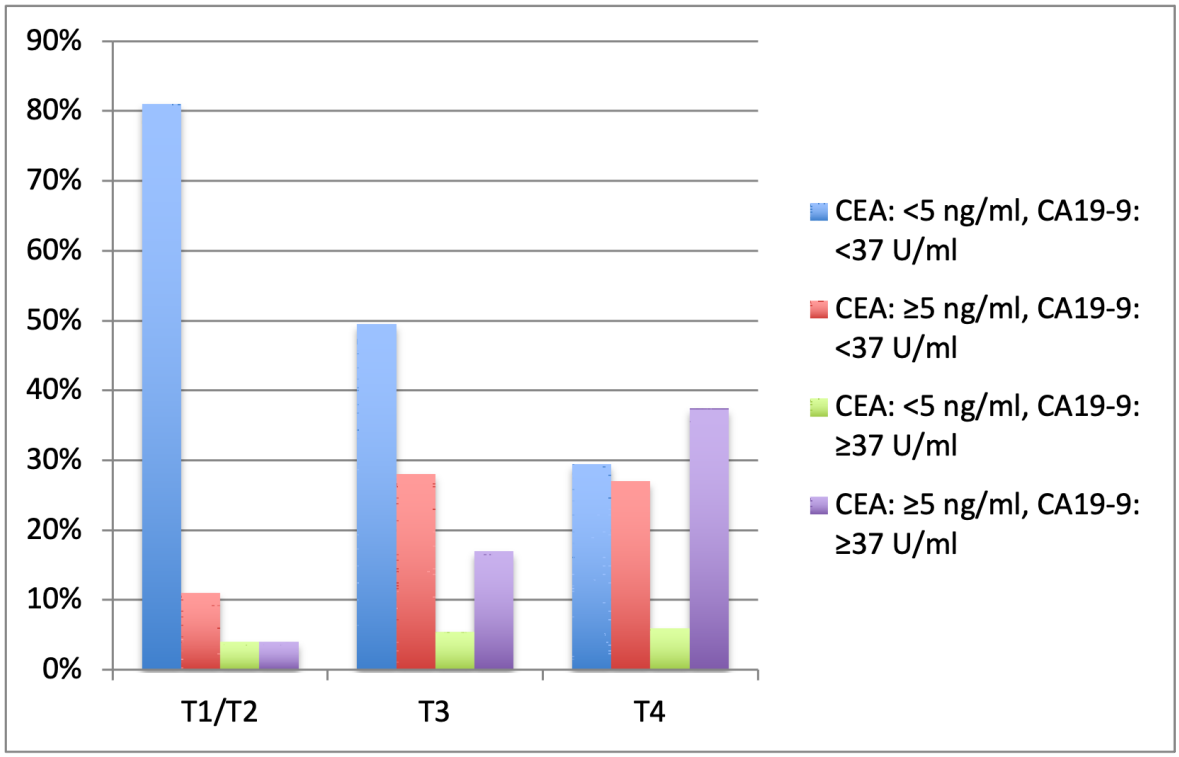

Figure 9: The distribution of patients who were diagnosed with colorectal cancer, classified in the category T (size of primary tumor) of the TNM classification and treated at the University Hospital Ulm from 2000 to 2015. All three bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".

For this bar chart (Fig. 9) the groups were divided after the TNM classification, focusing only on the tumor size. As the majority of the patients were diagnosed with a colorectal tumor in stage T3, patients with a tumor in stage T1 and T2 were merged into one group to approach comparable group sizes. To simplify the chart, patients classified with Tis or ypT0 were not included in the graphic illustrations.

Therefore, 309 patients were diagnosed with a colorectal tumor in stage T1 or T2, 467 patients in stage T3 and 163 patients in stage T4.

The bar chart presenting the tumor size (Fig. 9) shows a similar distribution compared to the individual UICC stages (Fig. 7). 81% of the patients diagnosed with colorectal cancer categorized as “T1/T2” had both tumor markers below cut-off value. Only 11% of the patients had only the tumor marker CEA elevated, 4% had only the tumor marker CA19-9 elevated and 4% had both tumor markers increased. Almost half of the patients (49.5%) with a colorectal tumor classified as T3 had both tumor markers below cut-off value. 28% had only the tumor marker CEA elevated, 5.5% of the patients had only the tumor marker CA19-9 elevated, and 17% had both tumor markers increased. At last, patients diagnosed with a colorectal tumor in stage T4 show the most significant distribution in all four tumor marker combinations. Whereas 29.5% of the patients had both tumor markers below cut-off value, 27% had only an elevated CEA value, and 37.5% had both tumor markers elevated. 6% of the patients had only the tumor marker CA19-9 elevated.

3.2.8 Lymph node status (N)

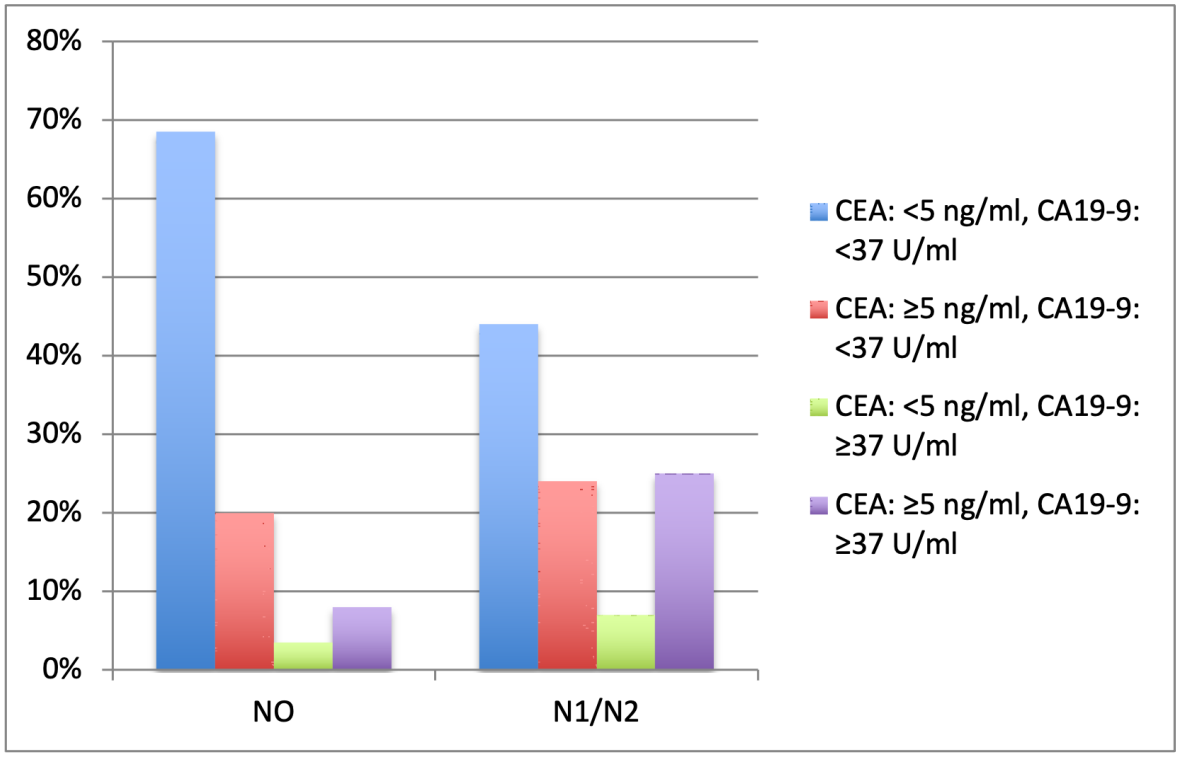

Figure 10: The distribution of patients who were diagnosed with colorectal cancer, classified in the category N (lymph node status) of the TNM classification and treated at the University Hospital Ulm from 2000 to 2015. Both bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".

For this bar chart (Fig. 10) the study group was divided after the TNM classification, focusing only on the possible tumor spread to local lymph nodes. To obtain similar group sizes, patients diagnosed with a colorectal tumor in stage N1 or N2 were merged into one group. Therefore, 494 patients did not show tumor spreading to local lymph nodes. In 440 patients the tumor had already spread to local lymph nodes at time of the primary surgical resection and was classified as N1 or N2.

As this bar chart illustrates, most patients classified with N0 had no tumor marker elevated (68.5%), whereas less than half of the patients with lymph node metastases had both tumor markers below cut-off value (44%). Both groups have a similar number of patients with an elevated CEA (N0: 20%, N1/N2: 24%). More patients included in the group "N1/N2" (25%) had both tumor markers elevated

compared to “N0” (8%). At last, only a few patients with and without lymph node metastases had the tumor marker CA19-9 increased (N0: 3.5%; N1/2: 7%).

3.2.9 Status of distant metastases (M)

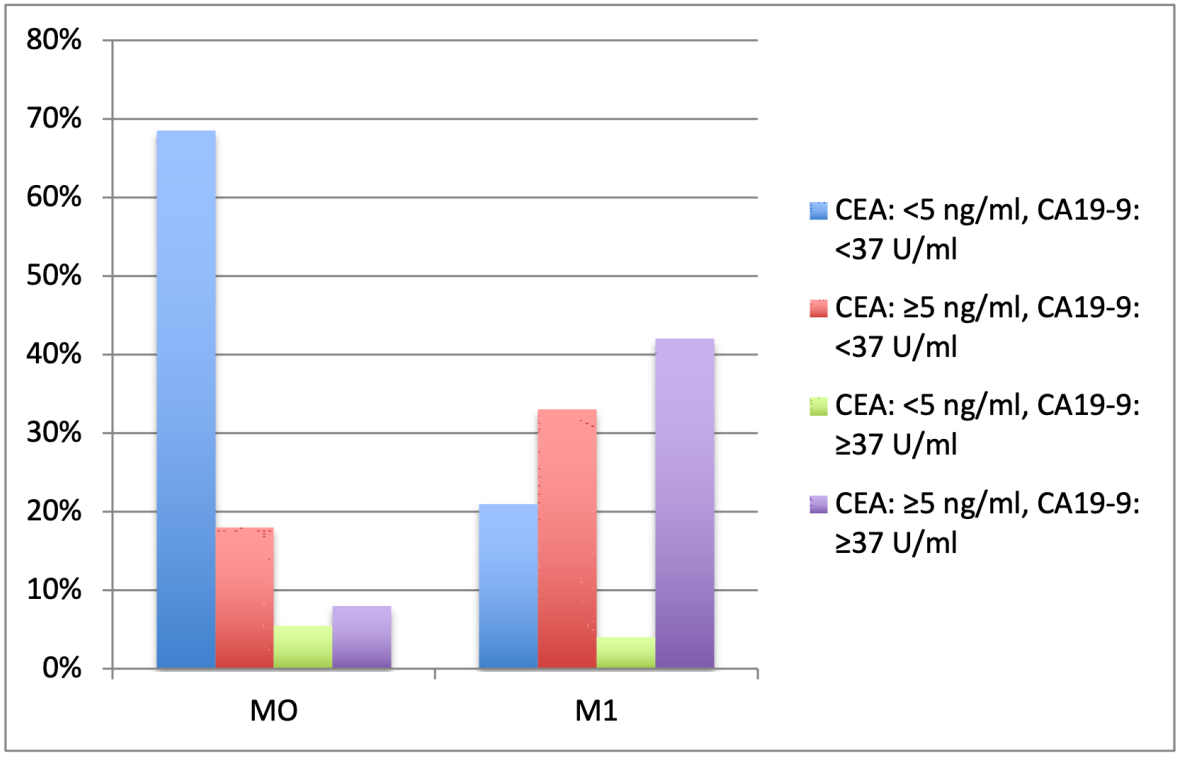

Figure 11: The distribution of patients who were diagnosed with colorectal cancer, classified in the category M (status of distant metastases) of the TNM classification and treated at the University Hospital Ulm from 2000 to 2015. Both bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: “both tumor markers below cut-off value”, “CEA increased, CA19-9 below cut-off value”; “CEA below cut-off value, CA19-9 increased” or “both tumor markers increased”.

For this bar chart (Fig. 11) the study group was divided after the TNM classification, focusing only on the distant metastases at time of the primary surgical resection. This study included 691 patients without distant metastases and 235 patients with metastases.

Whereas most patients without distant metastases had both tumor markers below cut-off value (68.5%), most patients with metastases had either both tumor markers (42%) or only the tumor marker CEA (33%) elevated. Instead, 18% of the patients classified as M0 had only the tumor marker CEA elevated and only 8%

had both tumor markers increased. 21% of the patients with a colorectal cancer classified as M1 had both tumor markers below cut-off value. In both groups, only a few patients had only the tumor marker CA19-9 elevated (M0: 5.5%; M1: 4%).

3.2.10 Life status

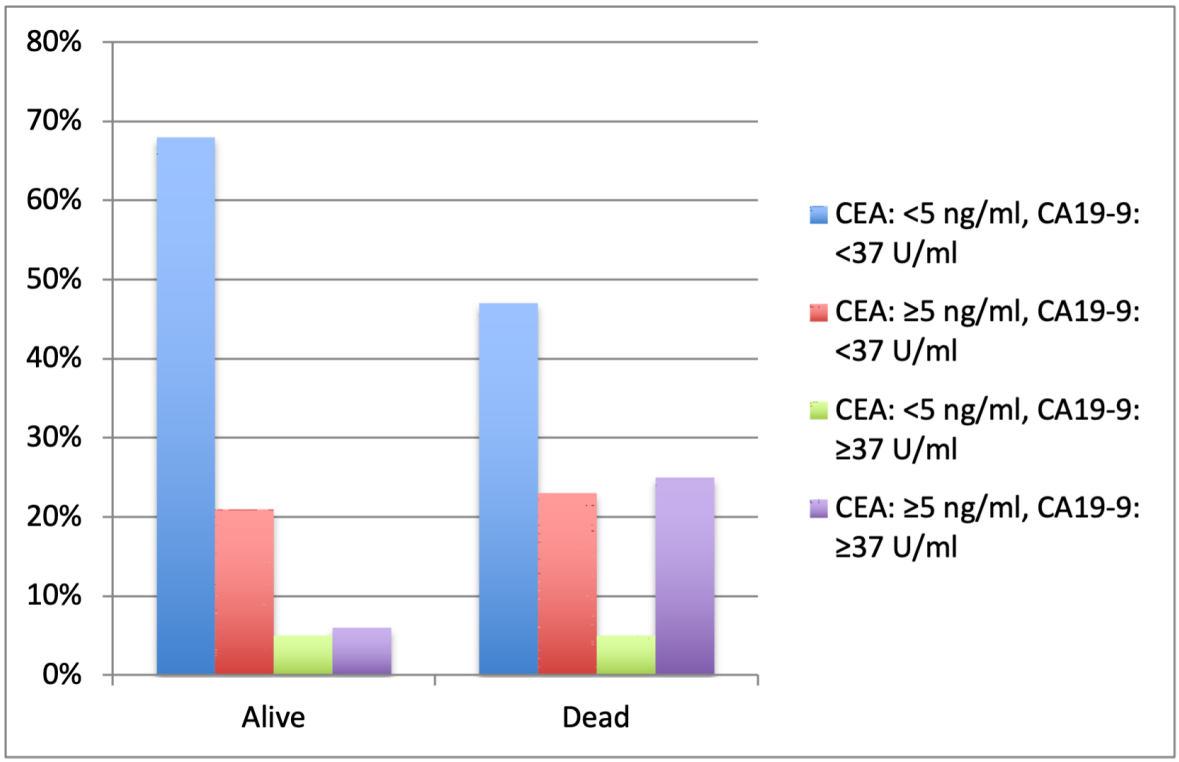

Figure 12: The distribution of patients who were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 and are documented in the follow-up as still alive or dead. Both bars are subdivided and show the ratio of patients with preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), in the following combinations: "both tumor markers below cut-off value", "CEA increased, CA19-9 below cut-off value"; "CEA below cut-off value, CA19-9 increased" or "both tumor markers increased".

For this bar chart (Fig. 12) the patients were divided into the groups "Alive" and "Dead". 452 patients in this study were still alive at the time of the last surveillance, either in remission or with a diagnosed recurrence, and 506 patients have died, either caused by the tumor, independent of the tumor or of unknown cause.

The patients still alive usually had both preoperative tumor markers below cut-off value (68%) and less often only the tumor marker CEA elevated (21%). Only 6% had both tumor markers elevated. Instead, 47% of the patients who have died had both tumor markers below cut-off value, whereas 23% had only the tumor marker

CEA and 25% both tumor markers elevated. In both groups only 5% of the patients had only the tumor marker CA19-9 increased.

3.3 The prognostic value of overall survival in correlation with the tumor markers CEA and CA19-9

The second step in this study was to create Kaplan-Meier curves to show the prognostic value of the tumor markers CEA and CA19-9 for overall survival. The date of diagnosis and the date of death or rather the date of the last surveillance was used to illustrate the overall survival. Therefore, in this method the patients who died in the follow-up were marked as an incident and the patients who are still alive were censored.

At first, the overall survival is compared for the tumor markers CEA and CA19-9 separately. First, in Figure 13 patients with a normal CEA (<5 ng/ml) are compared with patients with an increased CEA (≥5 ng/ml). Then, in Figure 14 the tumor marker CEA is divided into further parts such as below 5 ng/ml, equal or above 5 ng/ml and below 200 ng/ml, and equal or above 200 ng/ml.

A similar approach was done with the tumor marker CA19-9. At first, in Figure 15 patients with a normal CA19-9 (<37 U/ml) are compared with patients with an increased CA19-9 (≥37 U/ml). Then, in Figure 16 the tumor marker is divided into further groups such as below 37 U/ml, equal or above 37 and below 200 U/ml, and equal or above 200 U/ml.

At last, a Kaplan-Meier curve was created to show the overall survival in correlation with both tumor markers CEA and CA19-9 (Fig. 17). Here the groups are divided as follows: CEA and CA19-9 normal, CEA increased and CA19-9 normal, CEA normal and CA19-9 increased, and CEA and CA19-9 increased.

3.3.1 Overall survival of patients with a normal or an increased CEA value

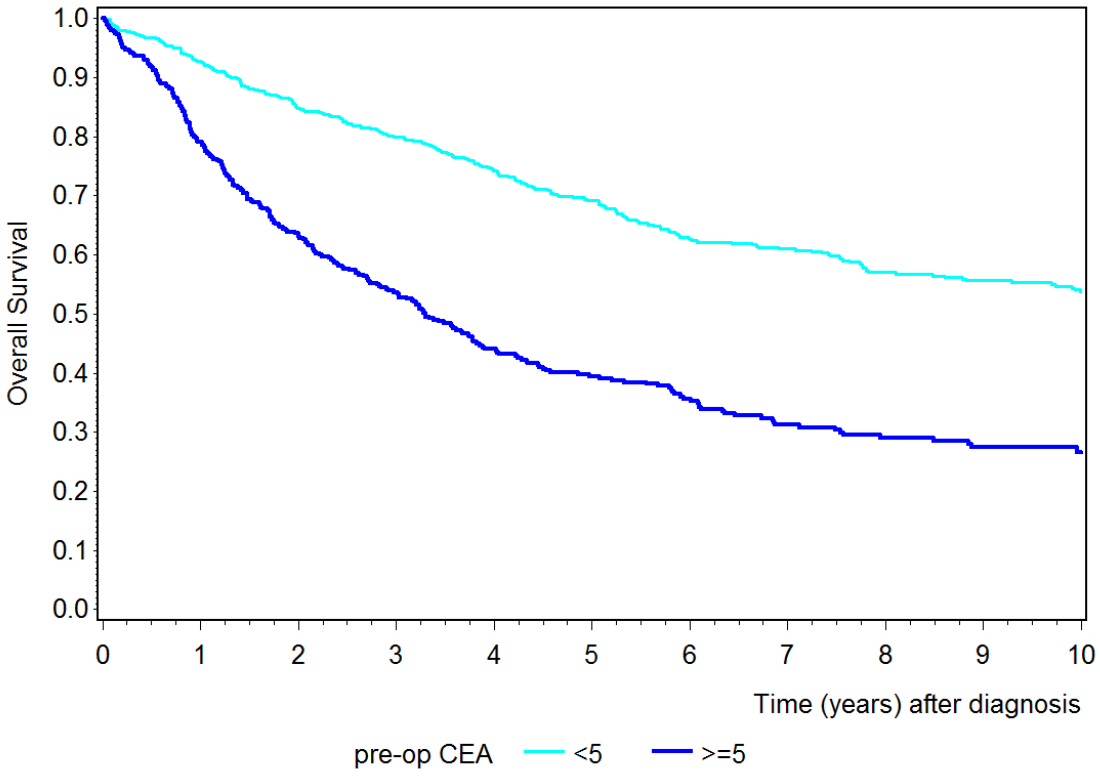

Figure 13: The overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with the preoperative tumor marker carcinoembryonic antigen (CEA). The patient collective was divided into patients with a normal CEA value (<5 ng/ml) and an increased CEA value (≥5 ng/ml). Kaplan-Meier estimator.

Figure 13 and Table 12 illustrate the overall survival of the 1035 patients who were diagnosed with colorectal cancer and treated at the University Hospital Ulm and had a CEA value measured prior to surgical resection. Due to missing data about the preoperative CEA values 432 of 1467 patients could not be included in this illustration. 635 patients had a CEA value below 5 ng/ml and 400 patients had a CEA value equal or above 5 ng/ml.

Figure 13 shows that patients with a normal preoperative CEA value have a longer life expectancy compared to patients with an increased CEA value. The 5-year survival rate, as shown in Table 12, is 69% for patients with a normal CEA value and 39% for patients with an increased CEA value. Further differentiated

information about the 1- and 3-year survival rate in relation to the tumor marker CEA can be taken from Table 12.

Table 12: Descriptive statistics of the overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with the preoperative tumor marker carcinoembryonic antigen (CEA). The patient collective was divided into patients with a normal preoperative CEA value (<5 ng/ml) and an increased CEA value (≥5 ng/ml).

CEA in ng/ml	Status			Overall Survival (95% CI)		
	Pat.	Dead	Alive	1 year	3 years	5 years
< 5	635	283 (45%)	352 (55%)	93% (90%;95%)	80% (77%;83%)	69% (65%;73%)
≥ 5	400	268 (67%)	132 (33%)	79% (75%;83%)	54% (49%;58%)	39% (34%;44%)
In total	1035	551 (53%)	484 (47%)			

Note. CI = confidence interval; Pat. = patients

3.3.2 Overall survival of patients with a CEA value below 5 ng/ml, equal or above 5 ng/ml and below 200 ng/ml, or equal or above 200 ng/ml

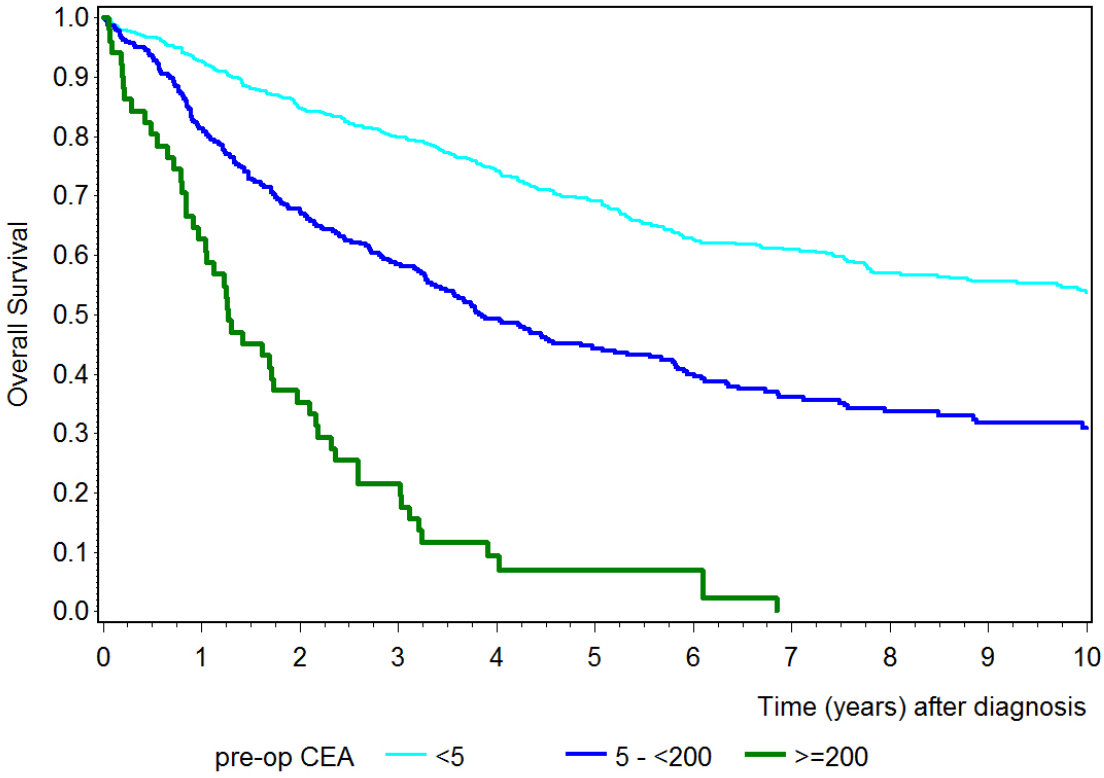

Figure 14: The overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with the preoperative tumor marker carcinoembryonic antigen (CEA). The patient collective was divided into patients with a CEA value below 5 ng/ml, equal or above 5 and below 200 ng/ml, and equal or above 200 ng/ml. Kaplan-Meier estimator. (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

Figure 14 and Table 13 illustrate the same patient collective used for Figure 13 and Table 12. Again 432 of 1467 patients could not be included in this illustration due to missing data about the preoperative CEA value. 635 patients had a CEA value below 5 ng/ml, 349 patients had a CEA value equal or above 5 ng/ml and below 200 ng/ml, and only 51 patients had a CEA value equal or above 200 ng/ml.

The division of the CEA value in three groups shows that the higher the CEA value, the lower the life expectancy. Especially patients with a CEA value equal and above 200 ng/ml had a lower life expectancy, so that no patient in this group survived longer than 7 years. Whereas the 5-year survival rate for patients with a CEA value below 5 ng/ml is 69%, and for patients with a CEA value equal or

above 5 ng/ml and below 200 ng/ml is 44%, the overall survival for 5 years is 7% for patients with a CEA value equal or above 200 ng/ml (Table 13). More differentiated information about the 1- and 3-year survival rate in relation to the tumor marker CEA can be taken from Table 13.

Table 13: Descriptive statistics of the overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with the preoperative tumor marker carcinoembryonic antigen (CEA). The patient collective was divided into patients with a CEA value below 5 ng/ml, equal or above 5 and below 200 ng/ml, and equal or above 200 ng/ml. Modified from (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

CEA in ng/ml	Status			Overall Survival (95% CI)		
	Pat.	Dead	Alive	1 year	3 years	5 years
<5	635	283(45%)	352(55%)	93%(90%;95%)	80%(77%;83%)	69%(65%;73%)
5-<200	349	218(62%)	131(38%)	81%(77%;85%)	58%(53%;64%)	44%(38%;50%)
≥200	51	50(98%)	1(2%)	63%(48%;74%)	22%(12%;34%)	7%(2%;17%)
In total	1035	551(53%)	484(47%)			

Note. CI = confidence interval; Pat. = patients

3.3.3 Overall survival of patients with a normal or an increased CA19-9 value

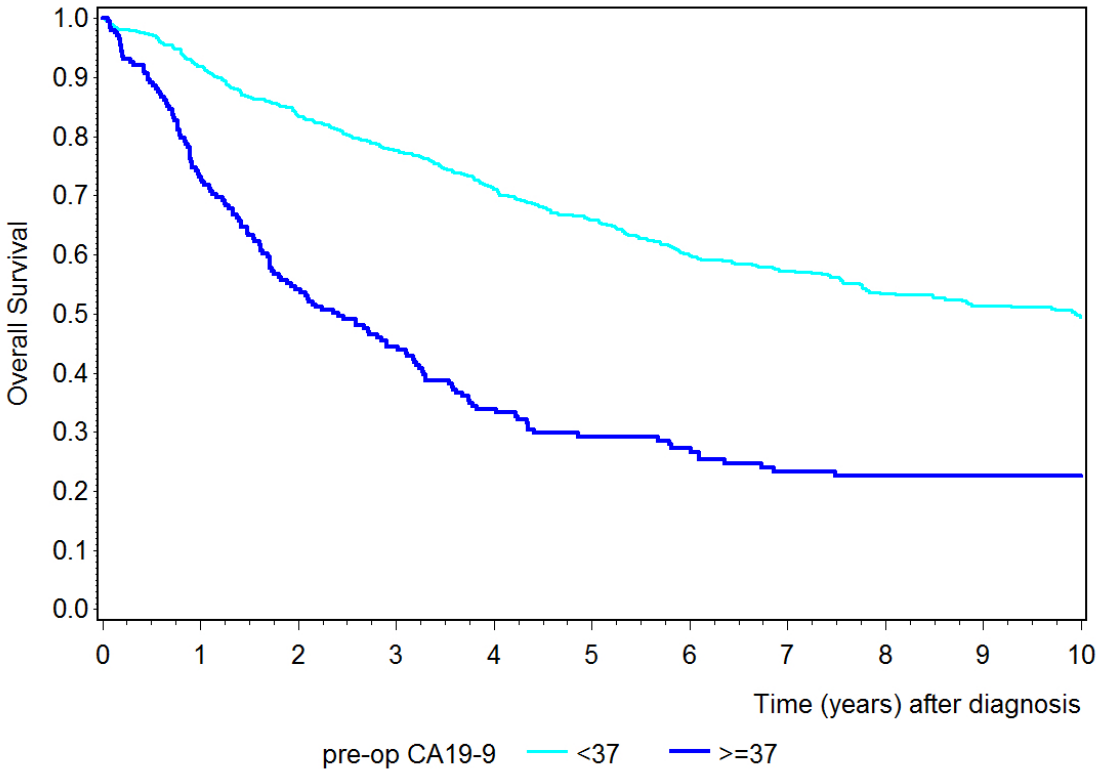

Figure 15: The overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with the preoperative tumor marker carbohydrate antigen 19-9 (CA19-9). The patient collective was divided into patients with a normal CA19-9 value (< 37 U/ml) and an increased CA19-9 value (≥ 37 U/ml). Kaplan-Meier estimator.

Figure 15 and Table 14 illustrate the overall survival of the 960 patients who were diagnosed with colorectal cancer and treated at the University Hospital Ulm and had a CA19-9 value measured before surgical resection. Due to missing data about the CA19-9 value 507 of 1467 patients could not be included in this illustration. 758 patients had a normal CA19-9 value and 202 patients had an increased CA19-9 value.

Figure 15 shows that patients with a normal CA19-9 value have a higher life expectancy compared to patients with an increased CA19-9 value. In addition, the 5-year survival rate, as shown in Table 14, is at 66% for patients with a CA19-9 value below 37 U/ml and only at 29% for patients with a CA19-9 value equal or

above 37 U/ml. Further information about the 1- and 3-year survival rate can be taken from Table 14.

Table 14: Descriptive statistics of the overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with the preoperative tumor marker carbohydrate antigen 19-9 (CA19-9). The patient collective was divided into patients with a normal CA19-9 value (< 37 U/ml) and an increased CA19-9 value (≥ 37 U/ml).

CA19-9 in U/ml	Status			Overall Survival (95% CI)		
	Pat	Dead	Alive	1 year	3 years	5 years
< 37	758	354(47%)	404(53%)	92%(90%;94%)	78%(75%;81%)	66%(62%;69%)
≥ 37	202	154(76%)	48(24%)	73%(67%;79%)	44%(37%;51%)	29%(23%;36%)
In total	960	508(53%)	452(47%)			

Note. CI = confidence interval; Pat = patients

3.3.4 Overall survival of patients with a CA19-9 value below 37 U/ml, equal or above 37 U/ml and below 200 U/ml, or equal or above 200 U/ml

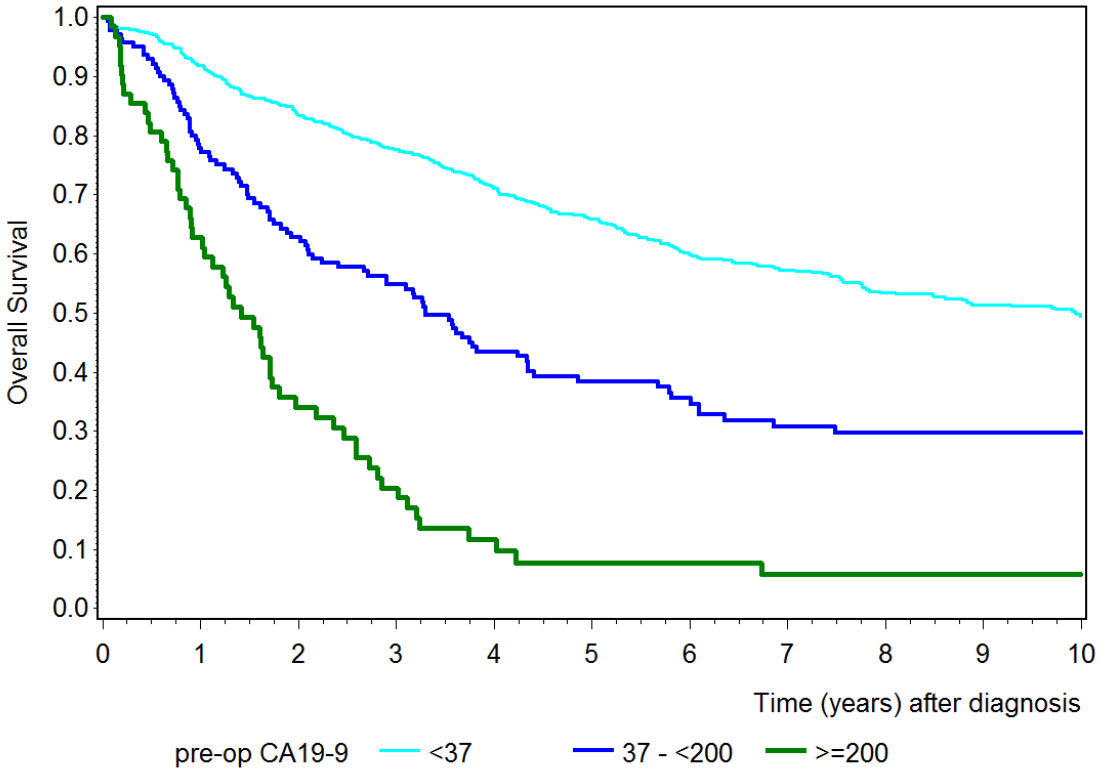

Figure 16: The overall survival of the patient collective that were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with the preoperative tumor marker carbohydrate antigen 19-9 (CA19-9). The patient collective was divided into patients with a CA19-9 value below 37 U/ml, equal or above 37 U/ml and below 200 U/ml, and equal or above 200 U/ml. Kaplan-Meier estimator. (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

Figure 16 and Table 15 illustrate the same patient collective used for Figure 15 and Table 14. Again 507 of 1467 patients could not be included in this illustration due to missing data about the preoperative CA19-9 values. 758 patients had a normal CA19-9 value, 140 patients had a CA19-9 value equal or above 37 U/ml and below 200 U/ml, and 62 patients had a CA19-9 value equal or above 200 U/ml.

Patients with a CA19-9 value equal or above 200 U/ml have the shortest overall survival, followed by patients with a CA19-9 level equal or above 37 U/ml and below 200 U/ml. Patients with a normal preoperative CA19-9 value have the best outcome. The 5-year survival rate, as shown in Table 15, is 66% for patients with a

normal CA19-9 value, 38% for patients with a CA19-9 value equal or above 37 U/ml and below 200 U/ml and only 8% for patients with a CA19-9 value equal and above 200 U/ml.

More differentiated information about the 1- and 3-year survival rate in relation to the tumor marker CA19-9 can be taken from Table 15.

Table 15: Descriptive statistics of the overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with the preoperative tumor marker carbohydrate antigen 19-9 (CA19-9). The patient collective was divided into patients with a CA19-9 value below 37 U/ml, equal or above 37 U/ml and below 200 U/ml, and equal or above 200 U/ml. Modified from (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

CA19-9 in U/ml	Status			Overall Survival (95% CI)		
	Pat	Dead	Alive	1 year	3 years	5 years
< 37	758	354(47%)	404(53%)	92%(90%;94%)	78%(75%;81%)	66%(62%;69%)
37-<200	140	98(70%)	42(30%)	78%(70%;84%)	55%(46%;63%)	38%(30%;47%)
≥200	62	56(90%)	6(10%)	63%(50%;74%)	20%(11%;31%)	8%(3%;17%)
In total	960	551(53%)	484(47%)			

Note. CI = confidence interval; Pat = patients

3.3.5 Overall survival of patients with CEA and CA19-9 normal, CEA increased and CA19-9 normal, CEA normal and CA19-9 increased, and CEA and CA19-9 increased

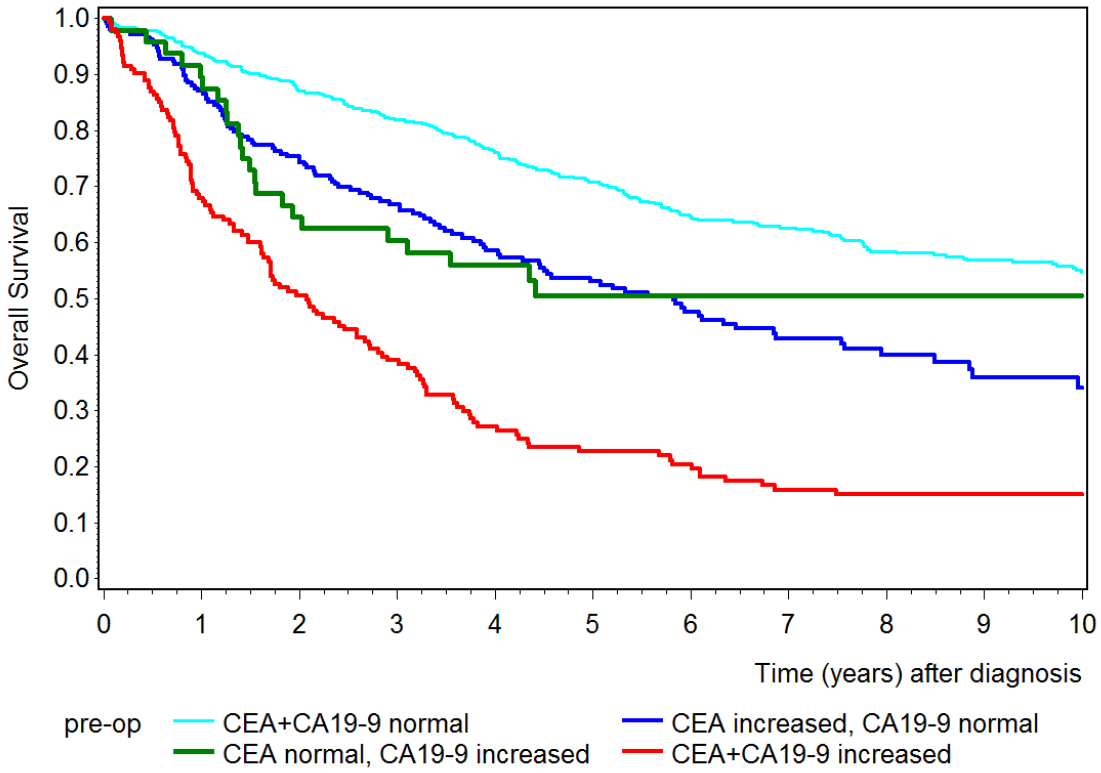

Figure 17: The overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with both preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9). The patient collective was divided into patients with CEA and CA19-9 normal (CEA < 5 ng/ml; CA19-9 < 37 U/ml), CEA increased and CA19-9 normal (CEA ≥ 5 ng/ml; CA19-9 < 37 U/ml), CEA normal and CA19-9 increased (CEA < 5 ng/ml; CA19-9 ≥ 37 U/ml), and CEA and CA19-9 both increased (CEA ≥ 5 ng/ml; CA19-9 ≥ 37 U/ml). Kaplan-Meier estimator. (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

Figure 17 and Table 16 illustrate the overall survival of the 957 patients who were diagnosed with colorectal cancer and treated at the University Hospital Ulm and had a CEA and CA19-9 value measured prior to primary surgical resection. Due to missing data about either the tumor marker CEA or CA19-9 510 of 1467 patients could not be included in this illustration. Out of the remaining 957 patients, 544 patients had both tumor markers below cut-off value, 212 patients had only the

tumor marker CEA elevated, 48 patients had only the tumor marker CA19-9 elevated, and 153 patients had both tumor markers increased.

Figure 17 emphasizes that patients with both tumor markers, CEA and CA19-9, below the cut-off value have the best prognosis of survival. The curves of patients with either CEA or CA19-9 elevated have similar survival rates and have poorer overall survival than patients with both tumor markers below cut-off value. Patients with both tumor markers increased had the lowest life expectancy.

The 5-year survival rate for patients with both tumor markers below the cut-off value is 71%, for patients with only the tumor marker CEA elevated 53%, for patients with only the tumor marker CA19-9 elevated 51%, and for patients with both tumor markers increased 23%.

More differentiated information about the 1- and 3-year survival rate in relation to the tumor markers CEA and CA19-9 can be taken from Table 16.

Table 16: Descriptive statistics of the overall survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with both preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9). The patient collective was divided into patients with CEA and CA19-9 normal (CEA < 5 ng/ml; CA19-9 < 37 U/ml), CEA increased and CA19-9 normal (CEA ≥ 5 ng/ml; CA19-9 <37 U/ml), CEA normal and CA19-9 increased (CEA < 5 ng/ml; CA19-9 ≥37 U/ml), and CEA and CA19-9 both increased (CEA ≥ 5 ng/ml; CA19-9 ≥ 37 U/ml). Modified from (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

		Status		Overall Survival (95% CI)		
Group	**Pat.**	**Dead**	**Alive**	**1 year**	**3 years**	**5 years**
CEA - CA19-9 -	544	237(44%)	307(56%)	94%(92%;96%)	82%(78%;85%)	71%(67%;75%)
CEA + CA19-9 -	212	116(55%)	96(45%)	87%(82%;91%)	67%(60%;73%)	53%(46%;60%)
CEA - CA19-9 +	48	26 (54%)	22 (46%)	90%(77%;96%)	60%(45%;73%)	51%(35%;64%)
CEA + CA19-9 +	153	127 (83%)	26 (17%)	68%(60%;75%)	39%(31%;47%)	23%(16%;30%)
In total	957	506(53%)	451(47%)			

Note. CI = confidence interval; Pat. = patients; - = below cut-off value; + = increased

3.4 Multivariate analysis

Further, the collective of patients was used to apply the Cox proportional hazards model to determine the prognostic value of clinicopathological parameters in overall survival. All 1467 patients were included in this calculation. The following parameters were used:

- Gender (Female vs. Male)
- Age at diagnosis (Unit=1)
- Body mass index (≥25 kg/m^2 vs. <25 kg/m^2)
- Location (Rectum vs. Colon)
- UICC classification (UICC I, II vs. UICC III, IV)
- Grading (G1,2 vs. G3,4)
- Tumor markers (CEA and CA19-9 normal vs. CEA increased and CA19-9 normal vs. CEA normal and CA19-9 increased vs. CEA and CA19-9 increased)
- Status of resection (R0 vs. R1/R2)

The primary tumor size, the regional lymph node involvement and the remote metastases had to be excluded in this representation, as they are already included in the UICC classification.

First, for all parameters mentioned above, the individual hazard ratio was calculated in a univariate analysis, which is presented in Table 17. The univariate analysis shows the age at the time of diagnosis ($p < 0.0001$), body mass index ($p = 0.0004$), location of the tumor ($p = 0.0037$), UICC classification ($p < 0.0001$), histological grading ($p < 0.0001$), preoperative tumor markers CEA and CA19-9 (CEA+, CA19-9-: $p < 0.0001$; CEA-, CA19-9+: $p = 0.015$; Both +: $p < 0.0001$), and the status of resection ($p < 0.0001$) as significant parameters for survival. The associated p-value, if significant ($p < 0.05$), is highlighted in red in Table 17.

Table 17: Univariate analysis of overall survival in patients that were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015. Significant associations (p < 0,05) are highlighted in red.

Parameter	Classification	Pat.	HR	95% CI	*p*-value
Gender	Female	575	1.00		0.953
	Male	892	0.996	0.9 – 1.1	
Age at diagnosis	Unit=1	1467	1.032	1.03 – 1.04	<0.0001
Body mass index	≥ 25	846	1.00		0.0004
	<25	538	1.3	1.3 – 1.5	
Location	Rectum	625	1.00		0.0037
	Colon	842	1.2	1.1 – 1.4	
UICC	UICC I, II	704	1.00		<0.0001
	UICC III, IV	733	2.5	2.2 – 2.9	
Grading	G1, G2	1043	1.00		<0.0001
	G3, G4	332	1.7	1.4 – 1.9	
preoperative tumor markers	Both -	544	1.00		
	CEA +, CA19-9 -	212	1.7	1.4 – 2.2	<0.0001
	CEA -, CA19-9 +	48	1.7	1.1 – 2.5	0.015
	Both +	153	3.7	2.9 – 4.6	<0.0001
R-Status	R0	1224	1.00		<0.0001
	R1/R2	243	5.1	4.4 – 6.1	

Note. Pat. = patients; CI = confidence interval; HR = hazard ratio; UICC = Union for International Cancer Control; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; + = increased; - = below cut-off value; R-Status = status of resection

The multivariate analysis, emphasized in Table 18, identifies both tumor markers, CEA and CA19-9 combined, as independent predictors for overall survival ($p < 0.0001$). Further independent parameters for a poorer life expectancy are male gender ($p = 0.043$), age at time of diagnosis ($p < 0.0001$), body mass index below 25 ($p = 0.018$), UICC III/IV ($p < 0.0001$), G3/4 ($p = 0.002$), and R1/R2-resection (p

< 0.0001). The associated significant *p*-values ($p < 0.05$) are highlighted in red in Table 18.

Table 18: Multivariate analysis as the Cox proportional hazards model of overall survival in patients that were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015. Significant associations (p < 0,05) are highlighted in red. Modified from (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

Parameter	**Classification**	**Pat.**	**HR**	**95% CI**	***p*-value**
Gender	Female	575	1.00		0.043
	Male	892	1.228	1.0 – 1.5	
Age at diagnosis	Unit=1	1467	1.034	1.0 – 1.05	<0.0001
Body mass index	≥ 25	846	1.00		0.018
	<25	538	1.254	1.0 – 1.5	
UICC	UICC I, II	704	1.00		<0.0001
	UICC III, IV	733	1.737	1.4 – 2.2	
Grading	G1, G2	1043	1.00		0.002
	G3, G4	332	1.408	1.1 – 1.7	
preoperative tumor markers	Both -	544	1.00		<0.0001
	CEA +, CA19-9 -	212	1.343	1.1 – 1.7	
	CEA -, CA19-9 +	48	1.263	0.8 – 1.9	
	Both +	153	1.961	1.5 – 2.5	
R-Status	R0	1224	1.00		<0.0001
	R1/R2	243	3.596	2.8 – 4.6	

Note. Pat. = patients; CI = confidence interval; HR = hazard ratio; UICC = Union for International Cancer Control; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; + = increased; - = below cut-off value; R-Status = status of resection

3.5 The prognostic value of recurrence-free survival in correlation with the tumor markers CEA and CA19-9

Once more, a further step in this study was to create a Kaplan-Meier curve to show the prognostic value of the tumor markers CEA and CA19-9 regarding the recurrence-free survival. Again, the tumor markers were investigated in the following groups: CEA and CA19-9 normal, CEA increased and CA19-9 normal, CEA normal and CA19-9 increased, and CEA and CA19-9 both increased.

Here, patients who developed metastases or a local recurrence or who died caused by the tumor according to the hospital documentation were marked as an incident and patients without any recurrences documented were marked as censored.

3.5.1 Recurrence-free survival of patients with CEA and CA19-9 normal, CEA increased and CA19-9 normal, CEA normal and CA19-9 increased, and CEA and CA19-9 increased

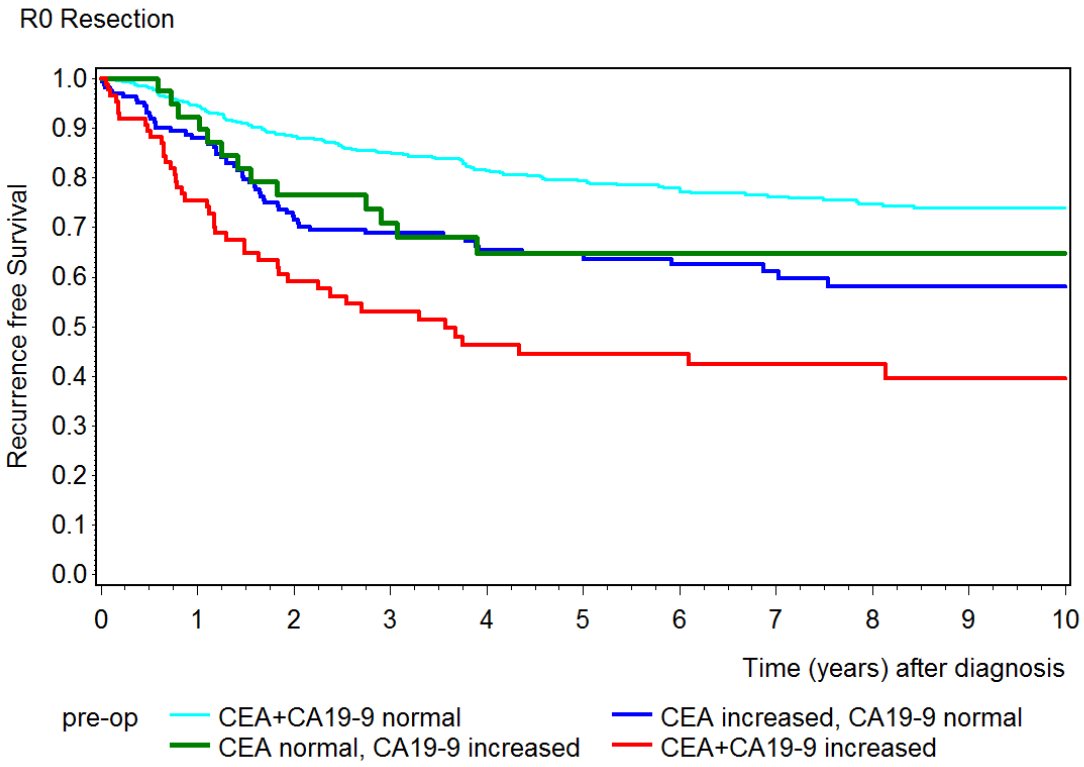

Figure 18: The recurrence-free survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with both preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9). The patient collective was divided into patients with CEA and CA19-9 normal (CEA < 5 ng/ml; CA19-9 < 37 U/ml), CEA increased and CA19-9 normal (CEA ≥ 5 ng/ml; CA19-9 < 37 U/ml), CEA normal and CA19-9 increased (CEA < 5 ng/ml; CA19-9 ≥ 37 U/ml), and CEA and CA19-9 both increased (CEA ≥ 5 ng/ml; CA19-9 ≥ 37 U/ml). Kaplan-Meier estimator. (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

Figure 18 and Table 19 illustrate the recurrence-free survival of the 798 patients who were diagnosed with colorectal cancer and treated at the University Hospital Ulm and had a CEA and CA19-9 value measured prior to primary surgical resection. As the patients who had a microscopic or macroscopic residual tumor (R1/R2) after resection are not representative in this graphic, and due to missing data about either the preoperative tumor marker CEA or CA19-9, 669 of 1467 patients could not be included in this illustration. Out of the remaining 798 patients,

508 patients had both tumor markers below cut-off value, 164 patients had only the tumor marker CEA elevated, 39 patients had only the tumor marker CA19-9 elevated, and 87 patients had both tumor markers increased.

Figure 18 emphasizes that patients with both tumor markers, CEA and CA19-9, below the cut-off value had a lower risk of developing a recurrence followed by patients with either CEA or CA19-9 elevated. Patients with both tumor markers increased had the most incidences of getting a recurrence in the following years after receiving their diagnosis.

The 5-year recurrence-free survival for patients with both tumor markers below the cut-off value is 79%, for patients with only the tumor marker CEA or CA19-9 elevated 65%, and for patients with both tumor markers increased 44%. More differentiated information about the 1- and 3-year survival rate in relation to the tumor markers CEA and CA19-9 can be taken from Table 19.

Table 19: Descriptive statistics of the recurrence-free survival of the patient collective that was diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015 in correlation with both preoperative tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9). The patient collective was divided into patients with CEA and CA19-9 normal (CEA < 5 ng/ml; CA19-9 < 37 U/ml), CEA increased and CA19-9 normal (CEA ≥ 5 ng/ml; CA19-9 < 37 U/ml), CEA normal and CA19-9 increased (CEA < 5 ng/ml; CA19-9 ≥ 37 U/ml), and CEA and CA19-9 both increased (CEA ≥ 5 ng/ml; CA19-9 ≥ 37 U/ml). Modified from (Lakemeyer et al., 2021), CC BY 4.0; https://creativecommons.org/licenses/by/4.0/

		Status		Recurrence-free survival (95% CI)		
Group	**Pat.**	**Dead**	**Alive**	**1 year**	**3 years**	**5 years**
CEA - CA19-9 -	508	111(22%)	397(78%)	95%(92%;96%)	85%(82%;88%)	79%(75%;83%)
CEA + CA19-9 -	164	58 (35%)	106(65%)	88%(82%;92%)	69%(61%;76%)	65%(56%;72%)
CEA - CA19-9 +	39	13 (33%)	26 (67%)	92%(78%;98%)	71%(54%;83%)	65%(47%;78%)
CEA + CA19-9 +	87	43 (49%)	44 (51%)	75%(65%;83%)	53%(41%;64%)	44%(33%;56%)
In total	798	225(28%)	573(72%)			

Note. CI = confidence interval; Pat. = patients; - = below cut-off value; + = increased

4 Discussion

It is estimated that every year about 18.1 million patients are diagnosed with cancer worldwide and about 9.6 million patients die from their disease. Out of all these cases, the incidence for colorectal cancer is 663,000 in men and 570,000 in women, with over 60% of the cases occurring in developed countries, and with a mortality of 608,000 every year (Ferlay *et al.*, 2010, 2019). In Germany the incidence of developing colorectal cancer has doubled since 1970. 2013, 62,400 patients were diagnosed with colon cancer, most of them already in a high tumor stage (Barnes *et al.*, 2016). The publication by Barnes et al. for the Robert Koch Institute describes a 5-year survival rate of 63% for colorectal cancer patients in Germany. A retrospective study by Sudo et al. about the “long-term outcomes after surgical resection in patients with stage IV colorectal cancer” showed a 5-year overall survival of only 19.1% for the 126 examined cases (Sudo *et al.*, 2019). To be able to successfully treat patients for cancer, it is getting increasingly important to diagnose the disease in early stages.

For colon cancer there are already many screening methods for early tumor detection available such as the occult blood test, digital rectal exam, colonoscopy, and computed tomography (Herold, 2015; Schmiegel *et al.*, 2017). First effects of the screening for colorectal cancer may be seen in the United States, as they can account for decreasing numbers of newly diagnosed colorectal cancer cases in the last years (Barnes *et al.*, 2016). Nevertheless, there are still many patients every year getting the diagnosis of colorectal cancer in late stages and, therefore, have a high risk of dying from this disease.

Since 1965, CEA is known as a glycoprotein that can be detected in the blood and in cancer cells of adenocarcinomas (Gold and Freedman, 1965; Hammarström, 1999; Sisik *et al.*, 2013). CA19-9 has been known since 1979, but is used more frequently for early detection of pancreatic carcinomas nowadays (Koprowski *et al.*, 1979). Especially in the last years more studies concentrated on the examination of the behavior not only of the tumor marker CEA, but also of the tumor marker CA19-9 (Chen *et al.*, 2013; Shibutani *et al.*, 2014; Abe *et al.*, 2016;

Kim *et al.*, 2017; Lalosevic *et al.*, 2017; Graziosi *et al.*, 2018; Thomsen *et al.*, 2018). A few other studies expanded the analysis to further tumor markers such as CA50, CA72-4, CA125, CA195, CA242, TK1 and Ferritin (van der Schouw *et al.*, 1992; Yang *et al.*, 2011; Wang *et al.*, 2015; Gao *et al.*, 2018; Ning *et al.*, 2018). Whereas studies calculated a sensitivity of CEA ranging from 65 to 74% in colorectal cancer patients, CA19-9 only had a sensitivity ranging from 26 to 48% (Yakabe *et al.*, 2010; Bagaria *et al.*, 2013; Zhang, Lin and Zhang, 2015). Despite the low sensitivity for CA19-9 on its own, studies detected that CA19-9 correlates with the tumor marker CEA and that it can increase the sensitivity of CEA (Filella *et al.*, 1992; Ueda, Shimada and Urakawa, 1994; Zhang, Lin and Zhang, 2015; Lu *et al.*, 2016; Ozawa *et al.*, 2016; Shin *et al.*, 2019). On the other hand, other studies, such as the one by Bagaria et al. in 2013 disproved the hypothesis and showed no increase in sensitivity, when both tumor markers were analyzed together (Bagaria *et al.*, 2013).

Therefore, nowadays guidelines still only recommend a use of CEA along other screening methods for determining prognosis, surveillance after a curative resection and monitoring treatment, such as chemotherapy or radiation. Due to low sensitivity, the use of CA19-9 on its own for detecting or monitoring the ongoing therapy or follow-up is not recommended (Locker *et al.*, 2006; Duffy *et al.*, 2007, 2014; Sturgeon *et al.*, 2008; Labianca *et al.*, 2010).

The behavior and usefulness of the combination of CEA and CA19-9 for colorectal cancer patients have not yet been investigated enough to make any guideline-oriented recommendations.

4.1 Aim of the study

Between 2000 and 2015 1467 patients were diagnosed and treated for colorectal cancer at the University Hospital Ulm. The aim of the study was to collect data about these patients to provide further information about the value of measuring both tumor markers, CEA and CA19-9, preoperatively.

With the help of the collected data the behavior of the preoperatively measured tumor markers, CEA and CA19-9, in correlation with clinicopathological

parameters were analyzed to find out if both tumor markers are suitable as a diagnostic screening method for colorectal cancer. Furthermore, the function and usefulness of the two markers as a predictor for overall survival and recurrence-free survival was examined by using the Kaplan-Meier estimator. Then, a multivariate analysis was performed to present CEA and CA19-9 as independent parameters for overall survival.

Nevertheless, there remain limitations to this study, such as the remaining bias as this study only included patients treated in a surgical clinic. Since this study is based on a retrospective study, it is recommended to conduct further prospective studies including patients from different faculties.

4.2 Patient collective

This retrospective study included 1467 patients that were diagnosed with colorectal cancer at the University Hospital Ulm from 2000 to 2015.

The patient collective includes 892 men and 575 women. Other studies had a comparable distribution with slightly more men than women (Park *et al.*, 2005). The median age at the time of diagnosis was 67 years with an age range from 20 to 96 years. Takakura et al. investigated a comparable collective in 2015 with a median age of 69 years and an age range from 22 to 92 years (Takakura *et al.*, 2015). On the other hand, the patient collective in the study by Huh et al. showed a lower median age of 58 years in 2014 with an age range of 21 to 86 years (Huh *et al.*, 2014).

This patient collective is almost equally distributed into the UICC classification stages I to IV. 346 patients were diagnosed with a colorectal cancer in stage I, 358 patients had a tumor stage II, 390 patients stage III, and 343 patients stage IV. On the other hand, other studies chose to analyze patients belonging to only one particular tumor stage (Huh *et al.*, 2014; Shibutani *et al.*, 2014; Zhang, Lin and Zhang, 2015; Abe *et al.*, 2016; Ozawa *et al.*, 2016; Sudo *et al.*, 2019).

In this study the cut-off value for CEA was <5 ng/ml and for CA19-9 <37 U/ml. These cut-off values were derived from the reference levels used by the laboratory of the University Hospital Ulm. Nevertheless, many studies chose different cut-off

values ranging from 3.5 to 10 ng/ml for the tumor marker CEA (Filella *et al.*, 1992; Chapman *et al.*, 1998; Park *et al.*, 2009; Park, Choi and Jun, 2009; Chen *et al.*, 2013) and from 31 to 100 U/ml for CA19-9 (Zheng *et al.*, 2001; Yang *et al.*, 2011; Wang *et al.*, 2015; Zhang, Lin and Zhang, 2015; Abe *et al.*, 2016; Lu *et al.*, 2016; Graziosi *et al.*, 2018). Moreover, only a few studies have examined the behavior of both tumor markers in combination in association with colorectal cancer (Filella *et al.*, 1992; Yang *et al.*, 2011; Chen *et al.*, 2013; Shibutani *et al.*, 2014; Wang *et al.*, 2015; Zhang *et al.*, 2015; Zhang, Lin and Zhang, 2015; Graziosi *et al.*, 2018; Shin *et al.*, 2019). Instead, many studies have only analyzed either CEA or CA19-9 or both, but separately from each other.

4.3 The behavior of the tumor markers CEA and CA19-9 combined in different clinicopathological parameters

This study visualizes the behavior of CEA and CA19-9 combined in correlation with clinicopathological parameters, which are listed in Table 9 to 11 and emphasized in the bar charts in Figure 3 to 12.

CEA and CA19-9 in any combination did not show an association to the parameters gender and age at the time of diagnosis. Here, the distribution of the tumor markers was the same for men and women and for the age at time of the diagnosis under 65 years and 65 years and older. In all groups more than half of the patients had no tumor marker elevated. The most comparable study using both tumor markers in combination by Zhang et al. also showed no association of CEA and CA19-9 to gender and age (Zhang *et al.*, 2015). Other studies only evaluating one tumor marker did not show any association of a high level of tumor marker to the variables either (Lin *et al.*, 2012; Saito *et al.*, 2016; Graziosi *et al.*, 2018).

Compared to obese patients, the group of patients with a body mass index under 25 had slightly more patients with an elevated preoperative CEA value (BMI <25: 26%, BMI ≥25: 20%) and instead fewer patients without any elevated tumor marker (BMI <25: 52%, BMI ≥25: 61%). This result is supported by the studies of Park et al. and Chen et al., who analyzed the correlation of CEA and CA19-9 and the body mass index of colorectal cancer patients. Both studies demonstrated that obese patients had up to 20% lower serum concentrations of the tumor marker

CEA and up to 10% lower serum concentrations of CA19-9 due to higher plasma volumes (Park *et al.*, 2010; Chen *et al.*, 2013).

Adenocarcinomas located in the rectal area did not show a significant association to CEA and CA19-9, since 65% of the patients had no tumor marker elevated. Instead, 49% and 54% of the patients with the tumor located in the right and the left colon had no tumor marker elevated. Nevertheless, they determine a greater number of patients with either only CEA elevated (Right colon: 23%, Left colon: 24%) or both tumor markers increased (Right colon: 21%, Left colon: 18%). Zhang et al. also investigated the combinations of CEA and CA19-9 and the location of the tumor and had a similar result (Zhang, Lin and Zhang, 2015). While in this study both sides of the colon have similar tumor marker elevations, Duffy et al. present a meta-analysis showing that CEA is more often increased in the left colon compared to the other side (Wanebo *et al.*, 1978; Slater, E Papatestas and H Aufses, 1979; Duffy, 2001). This statement is supported by a more recent study by Saito et al. in 2016 (Saito *et al.*, 2016).

The UICC stages and therefore also the size of the primary tumor, the lymph node status and the status of distant metastases (TNM classification) show the most significant association to the tumor markers CEA and CA19-9. While the majority of patients in this patient collective with colorectal cancer in UICC stage I, or rather classified as T1/T2, N0, or M0, had both preoperative tumor markers below the cut-off value, patients diagnosed with higher tumor stages, or rather T3, T4, N1/N2, or M1, had either only the tumor marker CEA or both tumor markers increased. Especially the elevation of both tumor markers CEA and CA19-9 in UICC stage IV, T4, N1/N2, and M1 is very significant in this patient group. Three studies also examined CEA and CA19-9 combined and their association to UICC and TNM stages and had the same outcome as this study (Filella *et al.*, 1992; Zhang *et al.*, 2015; Zhang, Lin and Zhang, 2015).

Comparing the histological grading G1/G2 with G3/G4 and the distribution of tumor marker combinations, only a slight difference can be seen. In both groups a high percentage of patients have no tumor marker elevated (G1/G2: 59%, G3/G4: 47%). Nevertheless, fewer patients with a poorly differentiated tumor have no tumor marker increased, and instead have rather only CA19-9 or both tumor markers elevated. Other studies only analyzed the behavior of either CEA or

CA19-9 in association with the histological grading. A study by Shin et al. support the statement that a higher number of patients with a poorly differentiated tumor had the tumor marker CA19-9 increased (Shin *et al.*, 2019). Then again, two other studies only analyzing the tumor marker CA19-9 did not find any significant difference of CA19-9 in the different histological gradings (Ueda, Shimada and Urakawa, 1994; Park, Choi and Jun, 2009). While this study did not show any difference of the percentage of patients with only CEA increased in both groups, Duffy et al. state in the meta-analysis that higher preoperative serum CEA levels are shown in patients with well-differentiated tumors compared to poorly differentiated tumor tissue (Bhatnagar *et al.*, 1999; Duffy, 2001).

At last, patients whose life status is documented as dead, had more often a preoperative CEA level (21%) or both tumor markers (22%) elevated. This can be traced back to the fact, that many patients who have died in the follow-up period have been primarily diagnosed with colorectal cancer in a more advanced tumor stage. Other studies have not evaluated the life status and the association to tumor markers yet.

4.4 Preoperative serum CEA and CA19-9 as a predictor for overall survival

While there have been many studies about the connection of CEA and CA19-9 in colorectal cancer patients to the overall survival, most of them analyzed CEA and CA19-9 separately. Only recently, the first studies appeared addressing CEA and CA19-9 in combination. Guidelines already recommend using the tumor marker CEA to determine the prognosis along other prognostic factors (Duffy *et al.*, 2007, 2014; Bolocan *et al.*, 2012). This study supports this statement. Figure 13 emphasizes the overall survival of patients with a serum CEA level below and above the cut-off value. It clearly shows that patients with an increased CEA have a much lower overall survival compared to the patients with a normal CEA value. Many other studies could present a similar outcome using the same cut-off value 5 ng/ml for the tumor marker CEA (Ueda, Shimada and Urakawa, 1994; Zheng *et al.*, 2001; Shibutani *et al.*, 2014; Uratani *et al.*, 2015; Wang *et al.*, 2015; Graziosi *et al.*, 2018; Thomsen *et al.*, 2018; Yamamoto *et al.*, 2019). On the other hand,

studies that examined a patient collective with a colorectal cancer in UICC stage IV could not show a correlation between CEA and the overall survival (Ishizuka *et al.*, 2001; Zhang *et al.*, 2015; Abe *et al.*, 2016; Ozawa *et al.*, 2016). In patients with a stage IV colorectal cancer, Ishizuka et al. only had a significant difference between the overall survival curves when setting the cut-off value at 150 ng/ml for the preoperative serum CEA level.

Further, Figure 14 supports the assumption that patients with a CEA value over 200 ng/ml have an even shorter survival outcome. Here, the 5-year survival rate was 69% for patients with a normal CEA value, 44% with a CEA value equal or above 5 ng/ml and below 200 ng/ml, and 7% for patients with a CEA value equal or above 200 ng/ml. There is no other study showing the same kind of division of the tumor marker CEA. Park et al. examined different cut-off values for preoperative CEA in 2005 and was able to demonstrate a significantly worse overall survival in patients with a cut-off value of 17 ng/ml (Park *et al.*, 2005).

Until now, there have been considerably many studies about the overall survival in correlation to the tumor marker CA19-9. Nevertheless, guidelines still do not recommend the use of preoperative CA19-9 for determining a prognosis (Locker *et al.*, 2006; Duffy *et al.*, 2007; Bolocan *et al.*, 2012). Figure 15 shows the overall survival in correlation with a preoperative serum CA19-9 value below and above the cut-off value. It presents a very similar outcome as there is for CEA. Patients with an increased preoperative CA19-9 have a significantly poorer overall survival than patients with a normal CA19-9. Many other studies were able to present the same results using slightly different cut-off values between 31 and 37 U/ml (Ueda, Shimada and Urakawa, 1994; Zheng *et al.*, 2001; Park, Choi and Jun, 2009; Shibutani *et al.*, 2014; Wang *et al.*, 2015; Zhang *et al.*, 2015; Lu *et al.*, 2016; Ozawa *et al.*, 2016; Graziosi *et al.*, 2018; Thomsen *et al.*, 2018; Shin *et al.*, 2019). Despite the slightly different cut-off values, the key message stays the same that CA19-9 is a good predictor for overall survival in colorectal cancer patients. Again, only two studies did not show a correlation between CA19-9 and the overall survival of colorectal cancer patients. Both of them only analyzed data of patients who had a stage IV colorectal cancer (Ishizuka *et al.*, 2001; Abe *et al.*, 2016). Even though Abe et al. used an even higher cut-off value of 50 U/ml, the Kaplan-Meier curve still did not show a significant difference (p-value = 0,1192).

Already in 1994, Ueda et al. analyzed and confirmed that a CA19-9 value over 160 U/ml results in an even shorter survival outcome. After 1.25 years, all patients had died with a CA19-9 value over 160 U/ml (Ueda, Shimada and Urakawa, 1994). Ishizuka et al. only found a significant difference in overall survival when setting the cut-off value much higher to 200 U/ml (Ishizuka *et al.*, 2001). Lu et al. analyzed the overall survival with different cut-off values for CA19-9, setting them to 35 U/ml, 100 U/ml and 200 U/ml. In this study, no significant difference appeared between the different cut-off values. Already a cut-off value of 35 U/ml showed a significantly shorter survival for patients with colorectal cancer (Lu *et al.*, 2016). Nevertheless, it is to mention that Ishizuka et al. and Lu et al., both, used a patient collective that only included stage IV colorectal cancer. This study significantly shows that patients in any stage with a CA19-9 level equal or above 200 U/ml have an even shorter survival, with a 5-year survival of only 8%, than patients with a moderately high CA19-9 level equal or above 37 U/ml and below 200 U/ml, with a 5-year survival rate of 38%. The best outcome still had patients with no increase of CA19-9, with a 5-year survival rate of 66%.

Unlike this study, a couple of studies analyzed the differences between overall survival in different tumor stagings. Takakura et al. reported a significant difference in survival only between stage II and III for CEA with a cut-off value of 5 ng/ml. For CA19-9 with a cut-off value of 37 U/ml, all stages showed a significantly shorter survival when CA19-9 was elevated (Takakura *et al.*, 2015). Shin et al. only analyzed CA19-9 and presented a survival difference in stage II, III, and IV. Only stage I did not show a significant difference in survival in patients with a normal or increased CA19-9 (Shin *et al.*, 2019).

Only a few studies analyzed the overall survival in correlation with CEA and CA19-9 combined. This study illustrates a much shorter survival for patients with both tumor markers elevated, followed by patients with either CEA or CA19-9 elevated. The best outcome had patients with both tumor markers below cut-off value. A different result had Thomsen et al. and Shin et al. in their study. Patients with both tumor markers elevated and patients with only CA19-9 elevated had a significantly shorter survival compared to patients with an elevated CEA or both tumor markers below the cut-off value (Thomsen *et al.*, 2018; Shin *et al.*, 2019). Graziosi et al. only formed two groups including patients with both tumor markers elevated and

with either CEA or CA19-9 elevated. Here, patients with both tumor markers increased had a significantly lower survival (Graziosi *et al.*, 2018). Shibutani et al. also chose to include patients with either CEA or CA19-9 elevated into one group. There was no significant difference in survival between patients with both tumor markers below cut-off value and patients with only one tumor marker elevated. On the other hand, patients with both tumor markers elevated had a significantly shorter survival (Shibutani *et al.*, 2014).

4.5 Prognostic factors for overall survival

In this study the univariate analysis identified age at diagnosis, body mass index, location of the tumor, UICC classification, histological grading, tumor markers CEA and CA19-9 in combination, and the status of resection as significant parameters associated with a poorer overall survival (p-value < 0,05). Other studies report very similar results with only a few variations. The study by Wang et al. analyzed CEA, CA19-9 and CA242 in a univariate analysis. Here, prognostic factors for overall survival were very similar to this study with tumor size, lymph node invasion, UICC classification, grading, CEA, CA19-9, CA242 and all three tumor markers combined (Wang *et al.*, 2015). Further, Uratani et al. presented tumor size, grading, venous invasion, CA19-9 and body mass index as significant predictors in overall survival. Here, the tumor marker CEA was not significant (p-value = 0,066) (Uratani *et al.*, 2015). Tokunaga et al. and Shin et al. also listed parameters associated with a poorer prognosis. All of them are already mentioned above and go along with an advanced tumor stage (Tokunaga *et al.*, 2015; Shin *et al.*, 2019).

In this study, a multivariate analysis was performed identifying the parameters male, age at diagnosis, body mass index below 25 kg/m^2, UICC stages III and IV, histological grading 3 and 4, CEA and/or CA19-9 increased and status of resection R1/R2 as significant independent factors associated with poorer overall survival in colorectal cancer patients (p-value < 0,05). All other studies revealed similar independent prognostic factors. Wang et al. presented tumor size, lymph node invasion, grading, and the three tumor markers CEA, CA19-9 and CA242 combined and Uratani et al. a bigger tumor size (T3-4), grading 3 and 4, an elevated CA19-9, and a body mass index below 20 kg/m^2 as prognostic factors

(Uratani *et al.*, 2015; Wang *et al.*, 2015). Similar results were listed by Tokunaga et al., Shin et al., and Park et al. (Park *et al.*, 2005; Tokunaga *et al.*, 2015; Shin *et al.*, 2019).

Despite minor variations in the multivariate analysis in different studies, the overall survival tends to be associated with an advanced UICC stage, grading and elevated tumor markers. Only this study included CEA and CA19-9 combined in the univariate and multivariate analysis. But almost all studies could prove an association of the preoperative serum CEA and CA19-9 separately as an independent predictor for overall survival.

4.6 Preoperative serum CEA and CA19-9 as a predictor for recurrence-free survival in patients with R0-resection

After presenting the overall survival of patients with colorectal cancer, this study analyzed the recurrence-free survival in correlation with CEA and CA19-9 combined in Figure 18 and Table 19.

The Kaplan-Meier curves show that patients with both preoperative tumor markers increased have a significantly reduced recurrence-free survival, followed by patients with either CEA or CA19-9 elevated. Patients with both tumor markers below cut-off value had the best outcome. Shin et al. and Chen et al. showed similar results in CEA and CA19-9 combined in association to recurrence-free survival in colorectal cancer patients (Chen *et al.*, 2013; Shin *et al.*, 2019). Whereas Zhang et al. and Shibutani et al. both present a much shorter recurrence-free survival for patients with both tumor markers increased, patients with only one or no tumor marker increased show a similar outcome in terms of recurrence-free survival (Shibutani *et al.*, 2014; Zhang *et al.*, 2015). Yang et al. made a similar statement in 2011 but had chosen a slightly different distribution of the two tumor markers. Here patients with either only one or both tumor markers increased were included in one group and showed a significantly shorter recurrence-free survival compared to the patients with no tumor markers increased (Yang *et al.*, 2011). In this study the 5-year recurrence-free survival rate for patients with both tumor markers below cut-off value was 79%, for patients with either CEA or CA19-9 increased 65%, and for patients with both tumor markers increased 44%.

Compared to these results, Shin et al. presents a similar result with a 5-year recurrence-free survival rate of 85% in patients with both tumor markers below cut-off value, 73% in patients with an increased CEA and 71% in patients with an increased CA19-9. Shin et al. did not mention the 5-year recurrence-free survival rate for patients with both tumor markers elevated (Shin *et al.*, 2019).

5 Summary

For this study information was collected of 1487 patients that were diagnosed with colorectal cancer and treated at the University Hospital Ulm from 2000 to 2015. Thereafter, 20 patients were excluded from the study because the tumor was not an adenocarcinoma. This study focused on the measured tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) prior to primary resection. Further clinicopathological characterizations, such as the patient's general information, information about the primary tumor diagnosis, and information about the detection of the first local recurrence or metastases, were collected from the data base provided by the Department of General and Visceral Surgery and the Cancer Retrieval Evaluation and Documentation System (CREDOS) and listed in Microsoft Excel for Mac 2011. Then, the correlation of both tumor markers in combination and the clinicopathological parameters was evaluated, the overall and recurrence-free survival in association with CEA and CA19-9 was analyzed and a multivariate analysis for overall survival was performed.

CEA and CA19-9 only showed significant elevations in advanced tumor stages and poorly differentiated tumors. Here, most patients either had only CEA or both tumor markers elevated.

Then, preoperative serum CEA and CA19-9 in combination showed a significant influence on the overall and recurrence-free survival. CEA and CA19-9 separately had a similar prognostic value on the overall survival. In both, CEA and CA19-9, a higher cut-off value of over 200 led to an even lower overall survival. Using both tumor markers together, the results were even more significant. Patients with both tumor markers elevated had the shortest overall survival followed by patients with either CEA or CA19-9 elevated. The best prognosis had patients with both tumor markers below the cut-off value.

In addition, the multivariate analysis identified CEA and CA19-9 either separately or both elevated as an independent parameter associated with a significantly poorer life expectancy. Further variables that are associated with a shorter survival

were male patients, age at diagnosis, a low body mass index (BMI), Union for International Cancer Control (UICC) stages III and IV, a poorly differentiated tumor (histological grading G3/G4), and a residual tumor after resection (R1/R2).

Further, CEA and CA19-9 both elevated resulted in a significantly poorer recurrence-free survival, followed by patients with either CEA or CA19-9 increased. Patients with both tumor markers below cut-off value had the best long-term outcome.

These results were compared with other publications focusing on the behavior of CEA and CA19-9, either separately or in combination, in association with colorectal cancer.

The following conclusions were drawn:

I. The levels of the tumor markers CEA and CA19-9 only showed a correlation to advanced tumor stages and poorly differentiated cells. Therefore, preoperative CEA and CA19-9 in combination is not suitable as a screening method.

II. Preoperative CEA and CA19-9 separately are both suitable as a predictor for overall survival. Further, evaluating CEA and CA19-9 in combination can be even more significant in predicting overall survival.

III. CEA and CA19-9 in combination are independent prognostic markers for survival. Therefore, a more aggressive therapy can be considered in patients diagnosed with advanced colorectal cancer, however low their CEA and CA19-9 levels are, given their superior prognosis.

IV. Preoperative CEA and CA19-9 in combination are suitable for predicting recurrence-free survival.

6 References

1. Abe S, Kawai K, Ishihara S, Nozawa H, Hata K, Kiyomatsu T, Tanaka T, Watanabe T: Prognostic impact of carcinoembryonic antigen and carbohydrate antigen 19-9 in stage IV colorectal cancer patients after R0 resection. *Journal of Surgical Research* 205: 384–392 (2016)
2. Bagaria B, Sood S, Sharma R, Lalwani S: Comparative study of CEA and CA19-9 in esophageal, gastric and colon cancers individually and in combination (ROC curve analysis). *Cancer biology & medicine* 10: 148–157 (2013)
3. Barnes B, Kraywinkel K, Nowossadeck E, Schönfeld I, Starker A, Wienecke A, Wolf U: Bericht zum Krebsgeschehen in Deutschland 2016. *Robert Koch-Institut* 16–77 (2016)
4. Berg E L, Magnani J, Warnock R A, Robinson M K, Butcher E C: Comparison of L-selectin and E-selectin ligand specificities: The L-selectin can bind the E-selectin ligands Sialyl Lexand Sialyl Lea. *Biochemical and Biophysical Research Communications* 184: 1048–1055 (1992)
5. Bhatnagar J, Tewari H B, Bhatnagar M, Austin G E: Comparison of carcinoembryonic antigen in tissue and serum with grade and stage of colon cancer. *Anticancer research* 19: 2181–2187 (1999)
6. Bolocan A, Ion D, Ciocan D N, Paduraru D N: Prognostic and predictive factors in colorectal cancer. *Chirurgia* 107: 555–563 (2012)
7. Chapman M A S, Buckley D, Henson D B, Armitage N C: Preoperative carcinoembryonic antigen is related to tumour stage and long-term survival in colorectal cancer. *British Journal of Cancer* 78: 1346–1349 (1998)
8. Chen W, Liu Q, Tan S-Y, Jiang Y-H: Association between carcinoembryonic antigen, carbohydrate antigen 19-9 and body mass index in colorectal cancer patients. *Molecular and Clinical Oncology* 1: 879–886 (2013)
9. Comprehensive Cancer Center Ulm (CCCU) *Kolorektales Karzinom*. Available at: https://www.uniklinik-ulm.de/comprehensive-cancer-center-ulm-cccu/fuer-patienten-und-angehoerige/krebserkrankungen/kolonkarzinom.html (28.09.2018).

10. Duffy M J: Carcinoembryonic antigen as a marker for colorectal cancer: Is it clinically useful? *Clinical Chemistry* 47: 624–630 (2001)
11. Duffy M J, van Dalen A, Haglund C, Hansson L, Holinski-Feder E, Klapdor R, Lamerz R, Peltomaki P, Sturgeon C, Topolcan O: Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. *European Journal of Cancer* 43: 1348–1360 (2007)
12. Duffy M J, Lamerz R, Haglund C, Nicolini A, Kalousová M, Holubec L, Sturgeon C: Tumor markers in colorectal cancer, gastric cancer and gastrointestinal stromal cancers: European group on tumor markers 2014 guidelines update. *International Journal of Cancer* 134: 2513–2522 (2014)
13. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin D M: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer* 127: 2893–2917 (2010)
14. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin D M, Pineros M, Znaor A, Bray F: Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International journal of cancer* 144: 1941–1953 (2019)
15. Filella X, Molina R, Grau J J, Piqué J M, Garcia-Valdecasas J C, Astudillo E, Biete A, Bordas J M, Novell A, Campo E, Ballesta A M: Prognostic Value of CA 19.9 Levels in Colorectal Cancer. *Annals of Surgery* 216: 55–59 (1992)
16. Gao Y, Wang J, Zhou Y, Sheng S, Quian S Y, Huo X: Evaluation of Serum CEA, CA19-9, CA72-4, CA125 and Ferritin as Diagnostic Markers and Factors of Clinical Parameters for Colorectal Cancer. *Scientific Reports* 8: 2732 (2018)
17. George P K, Loewenstein M S, O'Brien M J, Bronstein B, Koff R S, Zamcheck N: Circulating CEA Levels in Patients with Fulminant Hepatitis. *Digestive Diseases and Sciences* 27: 139–142 (1982)
18. Gold P, Freedman S O: Specific Carcinoembryonic Antigens of the Human Digestive System. *Journal of Experimental Medicine* 122: 467 - 81 (1965)
19. Gonzalez-Pons M, Cruz-Correa M: Colorectal Cancer Biomarkers: Where Are We Now? *BioMed Research International* 2015: 149014 (2015)
20. Graziosi L, Elisabetta M, Rebonato A, Donini A: Preoperative Serum

Markers Prognostic Evaluation in Colon Cancer Patients. *Journal of Cancer Science & Therapy* 10: 22–26 (2018)

21. Hammarström S: The carcinoembryonic antigen (CEA) family: Structures, suggested functions and expression in normal and malignant tissues. *Seminars in Cancer Biology* 9: 67–81 (1999)
22. Henne-Bruns D, Dürig M, Kremer B: *Chirurgie*. 3. Edition. Thieme pp. 396-406 (2008)
23. Herold, G: *Innere Medizin*. Cologne, Germany: Herold, pp. 493-495 (2015)
24. Huh J W*, Lee W Y, Park Y A, Cho Y B, Yun S H, Kim H C, Chun H-K*: Prognostic factors associated with primary cancer in curatively resected stage IV colorectal cancer. *Journal of Cancer Research and Clinical Oncology* 140: 435–441 (2014)
25. Ishizuka D, Shirai Y, Sakai Y, Hatakeyama K: Colorectal carcinoma liver metastases: Clinical significance of preoperative measurement of serum carcinoembryonic antigen and carbohydrate antigen 19-9 levels. *International Journal of Colorectal Disease* 16: 32–37 (2001)
26. Kim N H, Lee M Y, Park J H, Park D I, Sohn C I, Choi K, Jung Y S: Serum CEA and CA19-9 Levels are Associated with the Presence and Severity of Colorectal Neoplasia. *Yonsei Med Journal* 58: 918-924 (2017)
27. Kim S, Park B K, Seo J H, Choi J, Choi J W, Lee C K, Chung J B, Park Y, Kim D W:Carbohydrate antigen 19-9 elevation without evidence of malignant or pancreatobiliary diseases. *Scientific Reports* 10: 1–9 (2020)
28. Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P: Colorectal carcinoma antigens detected by hybridoma antibodies. *Somatic Cell Genetics* 5: 957–971 (1979)
29. Labianca R, Nordlinger B, Beretta G D, Brouquet A, Cervantes A: Primary colon cancer: ESMO clinical practice guidelines for diagnosis, adjuvant treatment and follow-up. *Annals of Oncology* 21: 70–77 (2010)
30. Lakemeyer L, Sander S, Wittau M, Henne-Bruns D, Kornmann M, Lemke J: Diagnostic and Prognostic Value of CEA and CA19-9 in Colorectal Cancer. *Diseases* 9: 21 (2021)
31. Lalosevic M S, Stankovic S, Stojkovic M, Markovic V, Dimitrijevic I, Lalosevic J, Petrovic J, Brankovic M, Markovic A P, Krivokapic Z: Can

preoperative CEA and CA19-9 serum concentrations suggest metastatic disease in colorectal cancer patients? *Hellenic Journal of Nuclear Medicine* 20: 41–45 (2017)

32. Lin P-C, Lin J-K, Lin C-C, Wang H-S, Yang S-H, Jiang J-K, Lan Y-T, Lin T-C, Li A F-Y, Chen W-S, Chang S-C: Carbohydrate antigen 19-9 is a valuable prognostic factor in colorectal cancer patients with normal levels of carcinoembryonic antigen and may help predict lung metastasis. *International Journal of Colorectal Disease* 27: 1333–1338 (2012)
33. Locker G Y, Hamilton S, Harris J, Jessup J M, Kemeny N, Macdonald J S, Somerfield M R, Hayes D F, Bast Jr R C: ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *Journal of Clinical Oncology* 24: 5313–5327 (2006)
34. Lu Z, Peng J, Wang Z, Pan Z, Yuan Y, Wan D, Li B: High preoperative serum CA19-9 level is predictive of poor prognosis for patients with colorectal liver oligometastases undergoing hepatic resection. *Medical Oncology* 33: 1–7 (2016)
35. Nakayama T, Watanabe M, Teramoto T, Kitajima M: Slope analysis of CA19-9 and CEA for predicting recurrence in colorectal cancer patients. *Anticancer Research* 17: 1379–1382 (1997)
36. Ning S Wei W, Li J, Hou B, Zhong J, Xie Y, Liu H, Mo X, Chen J Zhang L: Clinical significance and diagnostic capacity of serum TK1, CEA, CA 19-9 and CA 72-4 levels in gastric and colorectal cancer patients. *Journal of Cancer* 9: 494–501 (2018)
37. Ozawa T, Ishihara S, Kawai K, Nozawa H, Yamaguchi H, Kitayama J, Watanabe T:Prognostic Significance of Preoperative Serum Carbohydrate Antigen 19-9 in Patients With Stage IV Colorectal Cancer. *Clinical Colorectal Cancer* 15: e157–e163 (2016)
38. Park I J, Kim H C, Yu C S, Yoo J H, Kim J C: Cutoff Values of Preoperative s-CEA Levels for Predicting Survivals after Curative Resection of Colorectal Cancer. *Journal of Korean Medical Science* 20: 624–627 (2005)
39. Park I J, Choi G-S, Lim K H, Kang B M, Jun S H: Serum carcinoembryonic antigen monitoring after curative resection for colorectal cancer: Clinical significance of the preoperative level. *Annals of Surgical Oncology* 16:

3087–3093 (2009)

40. Park I J, Choi G-S, Jun S H: Prognostic Value of Serum Tumor Antigen CA19-9 after Curative Resection of Colorectal Cancer. *Anticancer research* 29: 4303–8 (2009)
41. Park J S, Choi G-S, Jang Y S, Jun S H, Kang H: Influence of obesity on the serum carcinoembryonic antigen value in patients with colorectal cancer. *Cancer Epidemiology Biomarkers and Prevention* 19: 2461–2468 (2010)
42. Robert Koch-Institut, Zentrum für Krebsregisterdaten, Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V.: Krebs in Deutschland für 2013/2014. *Robert Koch-Institut* 11 (2017)
43. Saito G, Sadahiro S, Okada K, Tanaka A, Suzuki T, Kamijo A: Relation between Carcinoembryonic Antigen Levels in Colon Cancer Tissue and Serum Carcinoembryonic Antigen Levels at Initial Surgery and Recurrence. *Oncology* 91: 85–89 (2016)
44. Schmiegel W, Buchberger B, Follmann M, Graeven U, Heinemann V, Langer T, Nothacker M, Porschen R, Rödel C, Rösch T, Schmitt W, Wesselmann S, Pox C: S3-Leitlinie - Kolorektales Karzinom', *Zeitschrift fur Gastroenterologie* 55: 1344–1498 (2017)
45. van der Schouw Y T, Verbeek A L M, Wobbes T, Segers M F G, Thomas C M G: Comparison of four serum tumour markers in the diagnosis of colorectal carcinoma. *British journal of cancer* 66: 148–54 (1992)
46. Shibutani M, Maeda K, Nagahara H, Ohtani H, Sakurai K, Toyokawa T, Kubo N, Tanaka H, Muguruma K, Ohira M, Hirakawa K: Significance of CEA and CA19-9 combination as a prognostic indicator and for recurrence monitoring in patients with stage II colorectal cancer. *Anticancer Research* 34: 3753–3758 (2014)
47. Shin J K, Kim H C, Lee W Y, Yun S H, Cho Y B, Huh J W, Park Y A, Chun H-K: High preoperative serum CA 19-9 levels can predict poor oncologic outcomes in colorectal cancer patients on propensity score analysis. *Annals of Surgical Treatment and Research* 96: 107 (2019)
48. Sisik A, Kaya M, Bas G, Basak F, Alimoglu O: CEA and CA 19-9 are Still Valuable Markers for the Prognosis of Colorectal and Gastric Cancer Patients. *Asian Pacific Journal of Cancer Prevention* 14: 4289–4294 (2013)

49. Slater G, Papatestas A E, Aufses Jr A H: Preoperative Carcinoembryonic Antigen Levels in Colorectal Carcinoma. *Archives of surgery* 114: 52–53 (1979)
50. American Cancer Society: Colorectal Cancer Facts & Figures 2014-2016. *Atlanta: American Cancer Society* (2014)
51. Steinberg W: The clinical utility of the CA 19-9 tumor-associated antigen. *The American journal of gastroenterology* 85: 350–355 (1990)
52. Sturgeon C M, Duffy M J, Stenman U-H, Lilja H, Brünner N, Chan D W, Babaian R, Bast R C, Dowell B, Esteva F J, Haglund C, Harbeck N, Hayes D F, Holten-Andersen M, Klee G G, Lamerz R, Looijenga L H Molina R, Nielsen H J, Rittenhouse H, Semjonow A, Shih L-M, Sibley P, Sölétormos G, Stephan C, Sokoll L, Hoffmann B R, Diamandis E P: National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. *Clinical Chemistry* 54 (2008)
53. Sudo M, Furuya S, Shimizu H, Nakata Y, Iino H, Shiraishi K, Akaike H, Hosomura N, Kawaguchi Y, Amemiya H, Kawaida H, Inoue S, Kono H, Ichikawa D: Long-term outcomes after surgical resection in patients with stage IV colorectal cancer: A retrospective study of 129 patients at a single institution. *World Journal of Surgical Oncology* 17: 1–8 (2019)
54. Takakura Y, Ikeda S, Imaoka Y, Urushihara T, Itamoto T: An elevated preoperative serum carbohydrate antigen 19-9 level is a significant predictor for peritoneal dissemination and poor survival in colorectal cancer. *Colorectal Disease* 17: 417–425 (2015)
55. Thomsen M, Skovlund E, Sorbye H, Bolstad N, Nustad K J, Glimelius B, Pfeiffer P, Kure E H, Johansen J S, Tveit K M, Christoffersen T, Guren T K: Prognostic role of carcinoembryonic antigen and carbohydrate antigen 19-9 in metastatic colorectal cancer: A BRAF-mutant subset with high CA 19-9 level and poor outcome. *British Journal of Cancer* 118: 1609–1616 (2018)
56. Tokunaga R, Sakamoto Y, Nakagawa S, Miyamoto Y, Yoshida N, Oki E, Watanabe M, Baba H: Prognostic nutritional index predicts severe complications, recurrence, and poor prognosis in patients with colorectal cancer undergoing primary tumor resection. *Diseases of the Colon and*

Rectum 58: 1048–1057 (2015)

57. Ueda T, Shimada E, Urakawa T: The clinicopathologic features of serum CA 19-9-positive colorectal cancers. *Surgery Today* 24: 518–525 (1994)
58. Uratani R, Toiyama Y, Shimura T, Mori K, Fujikawa H, Hiro J, Ohi M, Inoue Y, Tanaka K, Araki T, Mohri Y, Kusunoki M: Preoperative Lower Body Mass Index Correlates with Poorer Prognosis in Patients Undergoing Curative Laparoscopic Surgery for Colorectal Cancer. *Anticancer Research* 35: 5639–5648 (2015)
59. Wanebo H J, Rao B, Pinsky C M, Hoffmann R G, Stearns M, Schwartz M K, Oettgen H F: Preoperative carcinoembryonic antigen level as a prognostic indicator in colorectal cancer. *The New England journal of medicine* 299: 448–451(1978)
60. Wang J, Wang X, Yu F, Chen J, Zhao S, Zhang D, Yu Y, Liu X, Tang H, Peng Z: Combined detection of preoperative serum CEA, CA19-9 and CA242 improve prognostic prediction of surgically treated colorectal cancer patients. *International Journal of Clinical and Experimental Pathology* 8: 14853–14863 (2015)
61. WHO (2016) *ICD-10 Version:2016*. Available at: http://apps.who.int/classifications/icd10/browse/2016/en (28.09.2018)
62. WHO (2018) *Body Mass Index - BMI*. Available at: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi (28.09.2018)
63. Yakabe T, Nakafusa Y, Sumi K, Miyoshi A, Kitajima Y, Sato S, Noshiro H, Miyazaki K: Clinical significance of CEA and CA19-9 in postoperative follow-up of colorectal cancer. *Annals of Surgical Oncology*, 17: 2349–2356 (2010)
64. Yamamoto M, Saito H, Uejima C, Tanio A, Tada Y, Matsunaga T, Sakamoto T, Honjo S, Ashida K, Fujiwara Y: Prognostic value of combined tumor marker and controlling nutritional status (CONUT) score in colorectal cancer patients. *Yonago Acta Medica* 62: 124–130 (2019)
65. Yang X-Q, Chen C, Wang F-B, Peng C-W, Li Y: Preoperative serum carcinoembryonic antigen, carbohydrate antigen19-9 and carbohydrate antigen 125 as prognostic factors for recurrence-free survival in colorectal

cancer. *Asian Pacific Journal of Cancer Prevention* 12: 1251–1256 (2011)

66. Yu Z, Chen Z, Wu J, Li Z, Wu Y: Prognostic value of pretreatment serum carbohydrate antigen 19-9 level in patients with colorectal cancer: A meta-analysis. *PLOS ONE* 12: 1–12 (2017)
67. Zhang L-N, OuYang P-Y, Xiao W-W, Yu X, You K-Y, Zeng Z-F, Xu R-H, Gao Y-H:Elevated CA19-9 as the Most Significant Prognostic Factor in Locally Advanced Rectal Cancer Following Neoadjuvant Chemoradiotherapy. *Medicine* 94: e1793 (2015)
68. Zhang S-Y, Lin M, Zhang H-B: Diagnostic value of carcinoembryonic antigen and carcinoma antigen 19-9 for colorectal carcinoma. *International journal of clinical and experimental pathology* 8: 9404–9 (2015)
69. Zheng C X, Zhan W H, Zhao J Z, Zheng D, Wang D P, He Y L, Zheng Z Q:The prognostic value of preoperative serum levels of CEA and CA19-9 and CA72-4 in patients with colorectal cancer. *World Journal of Gastroenterology* 7: 431–434 (2001)

Acknowledgements

The Acknowledgements section was removed for data privacy protection reasons.

Curriculum vitae

The Curriculum vitae section was removed for data privacy protection reasons.