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RESEARCH ARTICLE

# Evaluation of preoperative serum markers for individual patient prognosis in stage I–III rectal cancer

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**Abstract** Several independent serum biomarkers have been proposed as prognostic and/or predictive markers for colorectal cancer (CRC). To this date, carcinoembryonic antigen (CEA) remains the only recommended serological CRC biomarker. The present retrospective analysis investigates the prognostic value of several serum markers. A total of 256 patients with rectal cancer underwent surgery for curative intent in a university cancer center between January 1988 and June 2007. Preoperative serum was retrospectively analyzed for albumin, alkaline phosphatase (aP), beta-human chorionic gonadotropin, bilirubin, CA 125, cancer antigen 19-9, cancer antigen 72-4 (CA 72-4), CEA, CRP, CYFRA 21-1, ferritin, gamma-glutamyl transpeptidase, glutamate oxaloacetate transanunase, glutamate pyruvate transaminase, hemoglobin, haptoglobin, interleukin-6, interleukin-8, creatinine, lactate-dehydrogenase, serum amyloid A (SAA), and 25-hydroxyvitamin D. Cancer-specific survival (CSS) and disease-free survival (DFS) were estimated. Median follow-up time was 8.4 years. Overall 3- and 5-year CSS was 88.6 and 78.9 %, respectively. DFS rates were 72.8 % (3 years) and 67.5 % (5 years). Univariate analysis of CSS indicated aP, CA 72-4, CEA, and SAA as prognostic factors, while aP, CEA, and SAA were also prognostic with regard to DFS. Multivariate analysis confirmed SAA together with T and N stage as prognostic factors. According to UICC stage, CEA and SAA add prognostic value in stages II and III with regard to DFS and CSS, respectively. The combined use of CEA and SAA is able to identify patients with favorable and poor prognosis. In addition to tumor baseline parameters, routine analysis of SAA together with CEA provided markedly improved prognostic value on CSS and DFS in resected rectal cancer.

**Keywords** Colorectal cancer · Tumor marker · SAA · CEA · Acute-phase proteins · Prognostic factors

Clemens Giessen and Dorothea Nagel contributed equally to this work. Petra Stieber and Christoph Schulz contributed equally to this work.

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## Introduction

With an estimated 447,000 new cases diagnosed in 2012 and approximately 215,000 deaths in Europe, colorectal cancer represents the second highest cancer mortality rate [1].

In patients with resected UICC stages I–III colorectal cancer, serum biomarkers are useful elements of patient maintenance and follow-up care. Also, measurement of serum tumor markers causes minimal inconvenience for patients and is relatively inexpensive compared to novel imaging techniques and interventional procedures.

According to current guideline recommendations, serum carcinoembryonic antigen (CEA) has taken center stage as tumor marker in colorectal cancer (CRC). Its role in clinical routine includes the determination of prognosis before/after (surgical) treatment and for monitoring reasons in the follow-up period. Furthermore, CEA is recommended to evaluate response to (chemotherapy) treatment using CEA as a surveillance marker in metastatic disease [2–6].

Several other serum markers have been explored to add benefit to surveillance and prediction of prognosis of CRC patients: for cancer antigen 19-9 (CA 19-9), a strong association with poor prognosis has been shown for patients with nodal positive CRC who underwent adjuvant chemotherapy [7, 8]. In contrast, CA 19-9 did not provide further prognostic value in nodal negative CRC [9]. Several groups investigated cancer antigen 242 (CA 242) with regard to disease-free survival (DFS) in CRC, and a strong correlation between CA 19-9 and CA 242 has been demonstrated [10]. In addition, cancer antigen 72-4 (CA 72-4) has been evaluated in patients with Dukes A-D CRC. In selected patients, CA 72-4 was associated with recurrent disease and poor prognosis [9, 10]. While elevated serum hCG has been found to be associated with aggressive tumor biology and poor survival in patients with metastatic CRC, it failed to show prognostic value for UICC I–III patients [9, 11]. With CYFRA 21-1 being established in lung cancer patients, limited data on an elevated release of cytokeratin 19 fragments exists in colorectal cancer [12]. Serum amyloid A (SAA), an apolipoprotein belonging to the group of acute phase proteins, has been investigated in both, limited and advanced colorectal cancer [13–15]. Being associated to inflammatory response to tissue damage by malignant disease, it has been found to be a parameter of disease progression and poor outcome in metastatic colorectal cancer [13, 14]. Recently, SAA has found to mediate the process of metastasis by an S100A4-induced inflammatory response [16]. The aim of the present study was to estimate the prognostic value of albumin, alkaline phosphatase (aP), beta-human chorionic gonadotropin (βhCG), bilirubin, cancer antigen 125 (CA 125), CA 19-9, CA 72-4, C-reactive protein (CRP), cytokeratin-19 soluble fragment (CYFRA 21-1), ferritin, gamma-glutamyl transpeptidase (γGT), glutamate oxaloacetate transanunase (GOT), glutamate pyruvate transaminase (GPT), hemoglobin, haptoglobin, interleukin-6 (IL-6), interleukin-8 (IL-8), creatinine, lactate-dehydrogenase (LDH), SAA, and 25-hydroxyvitamin D3 in preoperative serum in addition to patient and tumor baseline characteristics. Since rectal cancer is increasingly considered as a distinguished entity among colorectal cancers with diverging mechanisms of recurrence and metastasis, we focused on patients with rectal cancer only following the REMARK guidelines [17, 18].

## Material and methods

From January 1988 through June 2007, a total of 1,254 patients with colorectal cancer underwent resection for curative intent at the Department of Surgery, Ludwig-Maximilians University (Klinikum Grosshadern). Patients with synchronous metastatic disease ($n$=270), colorectal carcinoma in the previous medical history ($n$=127), missing information on staging or follow-up information ($n$=27), unclear cause of death or death within 30 days ($n$=30), and/or neoadjuvant treatment ($n$=72) were excluded from the analyses. Frozen sera prior to surgery were available in 728 patients. Patients with carcinoma of the colon were analyzed separately ($n$=472). Accordingly, a total of 256 patients with rectal carcinoma meeting the inclusion criteria were available for analysis.

Baseline clinical characteristics of the patients including gender, age, tumor localization, relapse, and follow-up were obtained from patient records and the departmental database. Also, pathological staging according to UICC 2010 classification and histological tumor type (adenocarcinoma or other type), differentiation (grades 1–4), and tumor site (colon, sigma, rectum) were available.

All patients underwent follow-up examinations according to current guideline recommendations including laboratory testing, physical examination, colonoscopy, chest X-ray, abdominal ultrasound scanning, and computed tomography [19]. Patients with rectal cancer classified UICC II and III underwent adjuvant treatment with fluoropyrimidine (5-FU/LV or oral capecitabine) according to current guideline recommendations [19]. In 2009, survival status update was performed by telephone contact with the patients' primary physician. Median follow-up time was 8.4 years as estimated by the reverse Kaplan–Meier method. Written informed consent was available from all patients who underwent surgery. Clinical data was collected in the clinical cancer registry at the Department of General Surgery, University of Munich. Remaining excess serum, not used for scheduled clinical analyses, was used for investigation and merged with anonymized clinical data. None of the analyses required additional blood sampling.

### Marker analysis

All serum analyses were centrally performed at the Institute of Laboratory Medicine, University Hospital of Munich, Germany, strictly according to the manufacturer's instructions and quality control was ensured. Peripheral venous blood was collected in clinical preoperative routine into vacutainers. The tubes were centrifuged within 30 min of collection at 2,500×$g$ for 15 min. Plasma was aliquoted and frozen, and samples stored centrally at −80 °C at the Institute of Laboratory Medicine, University of Munich. All assays were performed in one step in October 2010, and all analyses were conducted while blinded to clinical outcome.

Hemoglobin and white blood cell count (WBC) were estimated prospectively prior to surgery (Sysmex, Norderstedt, Germany). In addition, sera were stored at −80 °C for retrospective analysis of the additional markers: albumin, aP, βhCG, bilirubin, CA 125, CA 19-9, CA 72-4, CEA, CRP, CYFRA 21-1, ferritin, γGT, GOT, GPT, hemoglobin,

haptoglobin, interleukin-6, interleukin-8, creatinine, lactate-dehydrogenase, SAA, and 25-hydroxyvitamin D3.

Tumor markers CA 19-9, CA 72-4, CA 125, CEA, βhCG, and serum 25-hydroxyvitamin D3 were analyzed by electrochemiluminescent immunoenzymometric assays (Elecsys, Roche Diagnostics, Mannheim, Germany). SAA and haptoglobin were determined by nephelometric immunoassay analysis (BN-Prospec-Analyzer GNR 61 and GNR 68, Siemens GmbH/Dade/ Behring, Eschborn, Germany). Measurement of CRP was performed by latex-amplified immunoturbidimetry. LDH, bilirubin, albumin, creatinine, alkaline phosphatase, γGT, GOT, and GPT were estimated by kinetic UV-testing and by photometric testing (AU2700 Beckman Coulter GmbH, Krefeld, Germany). CYFRA 21-1, ferritin, IL-6, and IL-8 were measured by immunological multiparametric chip technique (IMPACT, Roche Diagnostics GmbH, Penzberg, Germany).

## Statistics

Analysis of tumor marker values was carried out by calculating median, quartiles, and range. Furthermore, interactions between age, gender, grading, T stage, N stage, UICC stage, and tumor markers were tested for significance by $Chi^2$ test and Wilcoxon test.

Endpoints for prognostic evaluation were as follows: DFS, defined as the time from surgery until recurrence of tumor (local recurrence or metastases), and CSS, defined as time period from surgery until death from the same cancer. For analysis of survival time, tumor marker values were categorized using the median value as cutoff. Survival times as well as 3- and 5-year survival rates were estimated by means of the Kaplan–Meier method, and the log rank test was used to compare different strata. Variables that showed a significant prognostic value in univariate analysis were included in a multivariate Cox proportional hazards regression model using a backward elimination strategy. For the resulting model, the proportional hazards assumption was tested by including interaction terms of variables with follow-up time. Also, first degree interactions between all variables in the model were tested for significance. Performance of different models was compared by the Akaike Information Criterion (AIC).

Furthermore, calculated models were evaluated by Harrells concordance index [20]. The c-index works as an extension of the area under the receiver operator curve (AUC, ROC) to the case of censored survival data. The c-index is calculated as a percentage of concordant pairs, reflecting a maximum of 1 (optimum discrimination).

All *p values* were calculated two-sided, and $p<0.05$ was considered as statistically significant. Statistical evaluations were carried out using SAS statistical software (SAS Version 9.2, SAS Institute Inc., Cary, NC, USA).

**Table 1** Patient baseline characteristics

| | *n*=256 | |
|---|---|---|
| | *n* | % |
| Sex | | |
| Male | 164 | 64.1 |
| Female | 92 | 35.9 |
| Age | | |
| Median | 64.6 | |
| Range | (30.6–90.7) | |
| T stage of primary | | |
| T1 | 34 | 13.3 |
| T2 | 78 | 30.5 |
| T3 | 135 | 52.7 |
| T4 | 9 | 3.5 |
| N stage of primary | | |
| N 0 | 163 | 63.7 |
| N 1 | 69 | 27.0 |
| N 2 | 24 | 9.4 |
| Grading | | |
| G 1+2 | 159 | 62.1 |
| G 3+4 | 97 | 37.9 |
| UICC stage | | |
| I | 88 | 34.4 |
| II | 75 | 29.3 |
| III | 93 | 36.3 |
| Adjuvant treatment | | |
| Chemotherapy | 13 | 5.1 |
| Radiotherapy | 21 | 8.2 |
| Concomitant chemoradiotherapy | 71 | 27.7 |
| None | 151 | 59.0 |
| Surgical procedures performed | 61 | 23.8 |
| Anterior rectal resection | 132 | 51.6 |
| Deep anterior rectal resection | 38 | 14.8 |
| Abdominoperineal resection | 9 | 3.5 |
| Transanal/endoscopic resection | 8 | 3.1 |
| Sigmoid-rectal resection | 1 | 0.4 |
| Total proctocolectomy | 6 | 2.3 |
| Other | 1 | 0.4 |
| NA | | |

**Table 2** Overall 3- and 5-year cancer-specific survival (CSS) and disease-free survival (DFS)

| *n*=256 | Events | | 3 year survival | | 5 year survival | |
|---|---|---|---|---|---|---|
| | *n* | % | % | 95 % CI | % | 95 % CI |
| CSS | 71 | 27.7 | 88.6 | 83.7–92.0 | 78.9 | 72.8–83.7 |
| DFS | 82 | 32.5 | 72.8 | 66.6–78.0 | 67.5 | 60.9–73.2 |

*CSS* cancer-specific survival, *DFS* disease-free survival, *95% CI* 95 % confidence interval

**Table 3** Univariate analysis with regard to cancer-specific survival (CSS)

| | Median | *n*= <Median ≥Median | Events | 3-year survival (%) | 95 % CI | 5-year survival (%) | 95 % CI | *p* value |
|---|---|---|---|---|---|---|---|---|
| Albumin (g/dl) | 4.72 | 125 | 39 | 89.0 | 81.7-93.4 | 77.0 | 67.8-83.9 | 0.213 |
| | | 124 | 31 | 87.6 | 80.0-92.5 | 79.8 | 70.9-86.2 | |
| aP (U/l) | 81 | 123 | 25 | 87.5 | 79.8-92.4 | 81.4 | 72.6-87.6 | *0.029* |
| | | 126 | 45 | 89.0 | 81.8-93.5 | 75.6 | 66.4-82.6 | |
| βHCG (mIU/ml) | 0.1 | 149 | 39 | 88.7 | 81.9-93.0 | 80.2 | 72.1-86.2 | 0.606 |
| | | 107 | 32 | 88.3 | 80.3-93.2 | 77.0 | 67.1-84.3 | |
| Bilirubin (mg/dl) | 0.46 | 125 | 35 | 90.8 | 83.9-94.8 | 82.3 | 73.9-88.2 | 0.394 |
| | | 124 | 35 | 85.6 | 77.5-91.0 | 73.9 | 64.2-81.4 | |
| CA 125 (U/ml) | 12.7 | 127 | 32 | 90.3 | 83.2-94.5 | 83.2 | 74.6-89.1 | 0.280 |
| | | 129 | 39 | 86.8 | 79.4-91.7 | 74.7 | 65.7-81.8 | |
| CA 19-9 (U/ml) | 10.6 | 128 | 33 | 88.3 | 81.0-92.9 | 81.6 | 73.1-87.6 | 0.281 |
| | | 128 | 38 | 88.9 | 81.5-93.4 | 76.0 | 66.7-83.0 | |
| CA 72-4 (U/ml) | 1.5 | 127 | 28 | 91.5 | 84.8-95.3 | 86.5 | 78.6-91.7 | *0.017* |
| | | 129 | 43 | 85.7 | 77.9-90.9 | 71.3 | 61.8-78.8 | |
| CEA (ng/ml) | 2.45 | 128 | 27 | 92.2 | 85.6-95.9 | 87.3 | 79.5-92.3 | *0.009* |
| | | 128 | 44 | 84.9 | 77.0-90.2 | 70.6 | 61.1-78.2 | |
| CRP (mg/dl) | 0.32 | 125 | 31 | 90.4 | 83.2-94.5 | 81.3 | 72.5-87.6 | 0.326 |
| | | 124 | 39 | 86.3 | 78.6-91.4 | 75.4 | 66.2-82.5 | |
| CYFRA 21-1 (ng/ml) | 2.46 | 128 | 35 | 89.6 | 82.4-94.0 | 82.0 | 73.4-88.0 | 0.549 |
| | | 128 | 36 | 87.5 | 80.1-92.3 | 75.8 | 66.6-82.8 | |
| Ferritin (μg/l) | 138 | 128 | 37 | 90.6 | 83.6-94.7 | 76.0 | 66.7-83.0 | 0.689 |
| | | 128 | 34 | 86.6 | 79.0-91.6 | 81.8 | 73.3-87.7 | |
| γGT (U/l) | 31 | 125 | 33 | 90.7 | 83.8-94.7 | 78.7 | 69.5-85.3 | 0.672 |
| | | 124 | 37 | 85.8 | 77.9-91.1 | 78.0 | 68.9-84.7 | |
| GOT (U/l) | 23.6 | 124 | 35 | 91.5 | 84.7-95.3 | 83.2 | 74.9-89.0 | 0.360 |
| | | 124 | 34 | 84.9 | 76.7-90.3 | 73.8 | 63.9-81.3 | |
| GPT (U/l) | 14.9 | 126 | 36 | 87.3 | 79.8-92.2 | 80.6 | 72.0-86.8 | 0.982 |
| | | 123 | 34 | 89.3 | 81.9-93.8 | 75.9 | 66.4-83.1 | |
| Hemoglobin (g/dl) | 14.2 | 125 | 30 | 90.5 | 83.5-94.6 | 83.4 | 74.8-89.2 | 0.304 |
| | | 120 | 36 | 86.2 | 78.1-91.5 | 75.2 | 65.6-82.4 | |
| Haptoglobin (g/l) | 1.71 | 121 | 29 | 90.3 | 83.1-94.5 | 85.5 | 77.4-90.9 | 0.118 |
| | | 121 | 39 | 86.6 | 78.8-91.7 | 72.0 | 62.1-79.7 | |
| IL-6 (pg/ml) | 2.8 | 128 | 32 | 92.3 | 85.7-95.9 | 83.6 | 75.2-89.4 | 0.195 |
| | | 128 | 39 | 84.9 | 77.1-90.2 | 74.2 | 64.9-81.3 | |
| IL-8 (pg/ml) | 688 | 128 | 32 | 89.3 | 82.3-93.7 | 83.8 | 75.7-89.4 | 0.115 |
| | | 128 | 39 | 87.7 | 80.1-92.5 | 73.3 | 63.6-80.8 | |
| Creatinine (mg/dl) | 1.03 | 125 | 41 | 88.2 | 80.8-92.8 | 75.9 | 66.8-82.9 | 0.265 |
| | | 124 | 29 | 88.4 | 80.8-93.1 | 81.2 | 72.2-87.5 | |
| LDH (U/l) | 171 | 124 | 37 | 89.7 | 82.5-94.0 | 81.7 | 73.0-87.8 | 0.905 |
| | | 125 | 33 | 86.9 | 79.1-91.9 | 74.9 | 65.5-82.1 | |
| SAA (mg/l) | 5.28 | 120 | 23 | 93.8 | 87.4-97.0 | 86.7 | 78.5-91.9 | *0.002* |
| | | 120 | 44 | 83.0 | 74.6-88.8 | 71.6 | 61.8-79.3 | |
| Vitamin D (ng/ml) | 23 | 127 | 34 | 90.6 | 83.7-94.7 | 81.5 | 72.7-87.7 | 0.804 |
| | | 128 | 37 | 86.5 | 78.9-91.5 | 76.3 | 67.3-83.1 | |
| WBC (G/l) | 7.2 | 118 | 28 | 93.5 | 86.8-96.9 | 83.8 | 74.9-89.8 | 0.215 |
| | | 125 | 38 | 83.4 | 75.1-89.1 | 74.6 | 65.3-81.7 | |

*aP* alkaline phosphatase, *βHCG* beta-human chorionic gonadotropin, *CA* cancer antigen, *CEA* carcinoembryonic antigen, *CRP* C-reactive protein, *CYFRA21-1* cytokeratin-19 soluble fragment, *γGT* gamma-glutamyl transpeptidase, *GOT* glutamate oxaloacetate transanunase, *GPT* glutamate pyruvate transaminase, *IL* interleukin, *LDH* lactate dehydrogenase, *SAA* serum amyloid A, *Vitamin D* 25-hydroxyvitamin D, *WBC* white blood cell

## Results

Between January 1988 and June 2007, a total of 256 patients with rectal cancer underwent surgery for curative intent and were evaluable for marker analysis. The median follow-up time was 8.4 years. With 64.1 %, more male patients were included. Median age was 64.6 years. The majority of patients had T3 (52.7 %) and T2 (30.5 %) primary tumors while T4 (3.5 %) and T1 (13.3 %) were less frequent. Around two thirds of the patients were nodal negative (N0, 63.7 %). Nodal positive status occurred in 27.0 % (N1) and 9.4 % (N2)

**Table 4** Univariate analysis with regard to disease-free survival (DFS)

| | Median | *n*= < Median ≥ Median | Events | 3-year survival (%) | 95 % CI | 5-year survival (%) | 95 % CI | *p* value |
|---|---|---|---|---|---|---|---|---|
| Albumin (g/dl) | 4.72 | 123 | 42 | 72.6 | 63.4-79.8 | 65.9 | 56.0-74.0 | 0.529 |
| | | 122 | 39 | 71.9 | 62.7-79.1 | 68.7 | 59.2-76.4 | |
| aP (U/l) | 81 | 119 | 28 | 78.1 | 69.3-84.7 | 76.9 | 67.9-83.7 | *0.006* |
| | | 126 | 53 | 66.6 | 57.3-74.3 | 58.3 | 48.6-66.9 | |
| βHCG (mIU/ml) | 0.1 | 148 | 49 | 72.8 | 64.6-79.5 | 67.4 | 58.6-74.7 | 0.863 |
| | | 104 | 33 | 72.8 | 62.8-80.5 | 67.5 | 56.8-76.0 | |
| Bilirubin (mg/dl) | 0.46 | 121 | 38 | 74.9 | 65.9-81.9 | 70.7 | 61.2-78.2 | 0.451 |
| | | 124 | 43 | 69.4 | 60.2-76.9 | 63.8 | 54.0-72.1 | |
| CA 125 (U/ml) | 12.7 | 126 | 39 | 72.7 | 63.6-79.9 | 69.4 | 59.9-77.1 | 0.645 |
| | | 126 | 43 | 72.8 | 63.9-79.9 | 65.5 | 55.8-73.5 | |
| CA 19-9 (U/ml) | 10.6 | 125 | 38 | 72.3 | 63.3-79.5 | 70.2 | 60.9-77.6 | 0.366 |
| | | 127 | 44 | 73.3 | 64.3-80.3 | 64.9 | 55.2-73.0 | |
| CA 72-4 (U/ml) | 1.5 | 125 | 36 | 77.2 | 68.5-83.8 | 73.0 | 63.8-80.3 | 0.107 |
| | | 127 | 46 | 68.4 | 59.2-76.0 | 61.8 | 52.0-70.1 | |
| CEA (ng/ml) | 2.45 | 126 | 31 | 81.5 | 73.2-87.4 | 77.3 | 68.4-84.0 | *0.003* |
| | | 126 | 51 | 64.2 | 54.8-72.1 | 57.5 | 47.7-66.2 | |
| CRP (mg/dl) | 0.32 | 121 | 36 | 74.0 | 65.0-81.1 | 70.7 | 61.2-78.3 | 0.300 |
| | | 124 | 45 | 70.3 | 61.1-77.7 | 63.9 | 54.1-72.1 | |
| CYFRA 21-1 (ng/ml) | 2.46 | 127 | 42 | 74.5 | 65.7-81.3 | 66.4 | 56.9-74.3 | 0.801 |
| | | 125 | 40 | 71.1 | 61.9-78.5 | 68.8 | 59.3-76.5 | |
| Ferritin (μg/l) | 138 | 125 | 39 | 75.2 | 66.2-82.1 | 71.8 | 62.4-79.2 | 0.594 |
| | | 127 | 43 | 70.5 | 61.5-77.8 | 63.3 | 53.7-71.4 | |
| γGT (U/l) | 31 | 122 | 38 | 75.2 | 66.3-82.1 | 71.6 | 62.1-79.1 | 0.469 |
| | | 123 | 43 | 69.1 | 59.8-76.7 | 63.0 | 53.3-71.2 | |
| GOT (U/l) | 23.6 | 122 | 41 | 74.5 | 65.6-81.4 | 68.6 | 59.2-76.3 | 0.790 |
| | | 122 | 39 | 70.4 | 61.0-77.9 | 66.6 | 56.7-74.7 | |
| GPT (U/l) | 14.9 | 123 | 40 | 72.1 | 63.0-79.3 | 70.1 | 60.9-77.6 | 0.862 |
| | | 122 | 41 | 72.1 | 62.9-79.5 | 64.1 | 54.1-72.5 | |
| Hemoglobin (g/dl) | 14.2 | 123 | 35 | 77.9 | 69.2-84.4 | 72.2 | 62.6-79.7 | 0.165 |
| | | 119 | 44 | 67.8 | 58.2-75.6 | 62.3 | 52.4-70.8 | |
| Haptoglobin (g/l) | 1.71 | 117 | 36 | 74.3 | 65.1-81.4 | 71.1 | 61.6-78.7 | 0.305 |
| | | 121 | 43 | 70.4 | 61.0-77.9 | 63.5 | 53.4-71.9 | |
| IL-6 (pg/ml) | 2.75 | 124 | 37 | 76.9 | 68.1-83.6 | 70.6 | 61.0-78.2 | 0.223 |
| | | 128 | 45 | 68.8 | 59.7-76.3 | 64.5 | 54.9-72.5 | |
| IL-8 (pg/ml) | 688 | 127 | 38 | 77.0 | 68.4-83.5 | 70.8 | 61.5-78.3 | 0.189 |
| | | 125 | 44 | 68.5 | 59.1-76.1 | 64.1 | 54.4-72.3 | |
| Creatinine (mg/dl) | 1.03 | 121 | 43 | 70.8 | 61.6-78.2 | 65.6 | 56.0-73.7 | 0.548 |
| | | 124 | 38 | 73.4 | 64.3-80.5 | 68.9 | 59.3-76.7 | |
| LDH (U/l) | 171 | 124 | 41 | 73.8 | 64.8-80.8 | 68.6 | 59.1-76.3 | 0.757 |
| | | 121 | 40 | 70.5 | 61.1-78.0 | 65.8 | 56.0-74.0 | |
| SAA (mg/l) | 5.28 | 117 | 30 | 78.8 | 70.0-85.3 | 75.5 | 66.2-82.5 | *0.010* |
| | | 119 | 48 | 66.3 | 56.6-74.2 | 59.5 | 49.4-68.2 | |
| Vitamin D (ng/ml) | 23 | 124 | 38 | 73.5 | 64.4-80.6 | 71.0 | 61.5-78.5 | 0.619 |
| | | 127 | 44 | 72.0 | 63.1-79.1 | 64.1 | 54.6-72.2 | |
| WBC (G/l) | 7.2 | 117 | 33 | 76.8 | 67.6-83.8 | 69.9 | 59.9-77.9 | 0.099 |
| | | 123 | 46 | 68.7 | 59.4-76.3 | 64.2 | 54.6-72.4 | |

*aP* alkaline phosphatase, *βHCG* beta-human chorionic gonadotropin, *CA* cancer antigen, *CEA* carcinoembryonic antigen, *CRP* C-reactive protein, *CYFRA21-1* cytokeratin-19 soluble fragment, *γGT* gamma-glutamyl transpeptidase, *GOT* glutamate oxaloacetate transanunase, *GPT* glutamate pyruvate transaminase, *IL* interleukin, *LDH* lactate dehydrogenase, *SAA* serum amyloid A, *Vitamin D* 25-hydroxyvitamin D3, *WBC* white blood cell

of cases. According to local histopathology grading, G1 and G2 were found in 62.1 % of tumors, while G3 and G4 tumors were less frequent (37.9 %). Histopathology staging resulted in UICC I status in 34.4 %, UICC II in 29.3 %, and UICC III in 36.3 % of patients, respectively.

Adjuvant treatment was administered according to current guideline recommendation in 41.0 % of patients. Chemotherapy, radiotherapy, and concomitant chemoradiotherapy were administered in 5.1, 8.2, and 27.7 % of patients, respectively. The majority of patients underwent deep anterior resection (51.6 %) or anterior rectal resection (23.8 %) (Table 1).

### Overall 3- and 5-year cancer-specific survival and disease-free survival

Within the follow-up period of 5.8 years, a total of 71 cancer-related deaths (27.7 %) occurred. The event rate for DFS was 32 % ($n$=82). The calculated 3- and 5-year CSS was 88.6 and 78.9 %, respectively. Overall 3- and 5-year DFS was 72.8 and 67.5 %, respectively (Table 2).

### Univariate analysis

Evaluation of serum markers according to median values obtained aP, CA 72-4, CEA, and SAA as significantly related to 3- and 5-year CSS. (Table 3) With regard to DFS, only aP, CEA, and SAA were found to be significantly associated with 3- and 5-year DFS rates (Table 4).

### Multivariate analysis

Serum markers that were found to be significant in the univariate analysis were incorporated into different multivariate models. Table 5 shows different models with regard to CSS. Both CEA and SAA provide prognostic value when analyzed together with HR=1.92 and 2.14. AIC was 658 and Harrels c was 0.651. CEA did not reach the level of statistical

**Table 5** Multivariate models with regard to cancer-specific survival (CSS)

| | HR | 95 % CI | $p$ value | AIC | Harrels c 95 % CI |
|---|---|---|---|---|---|
| Model 1 | | | | | |
| CEA | 2.00 | 1.22–3.30 | 0.006 | 665 | 0.596 |
| ≥2.5 vs <2.5 ng/ml | | | | | 0.537–0.655 |
| Model 2 | | | | | |
| SAA | 2.22 | 1.34–3.68 | 0.002 | 663 | 0.606 |
| ≥5.3 vs<5.3 mg/l | | | | | 0.547–0.665 |
| Model 3 | | | | | |
| CEA | 1.92 | 1.17–3.15 | 0.010 | 658 | 0.651 |
| ≥2.5 vs <2.5 ng/ml | | | | | 0.586–0.715 |
| SAA | 2.14 | 1.29–3.54 | 0.003 | | |
| ≥5.3 vs <5.3 mg/l | | | | | |
| Model 4 | | | | | |
| CEA | 1.44 | 0.86–2.41 | 0.17 | 617 | 0.751 |
| ≥2.5 vs <2.5 ng/ml | | | | | 0.696–0806 |
| T stage | 2.68 | 1.71–4.22 | <0.001 | | |
| T4 vs T3, T3 vs T2, T2 vsT1 | | | | | |
| N stage | 2.27 | 1.63–3.17 | <0.001 | | |
| N2 vs N1, N1 vs N0 | | | | | |
| Model 5 | | | | | |
| SAA | 2.22 | 1.33–3.68 | 0.002 | 609 | 0.763 |
| ≥5.3 vs <5.3 mg/l | | | | | 0.710–0.817 |
| T stage | 2.85 | 1.81–4.49 | <0.001 | | |
| T4 vs T3, T3 vs T2, T2 vsT1 | | | | | |
| N stage | 2.35 | 1.70–3.24 | <0.001 | | |
| N2 vs N1, N1 vs N0 | | | | | |
| Model 6 | | | | | |
| CEA | 1.44 | 0.85–2.41 | 0.172 | 611 | 0.780 |
| ≥2.5 vs <2.5 ng/ml | | | | | 0.728–0.831 |
| SAA | 2.22 | 1.33–3.68 | 0.002 | | |
| ≥5.3 vs <5.3 mg/l | | | | | |
| T stage | 2.51 | 1.53–4.12 | <0.001 | | |
| T4 vs T3, T3 vs T2, T2 vsT1 | | | | | |
| N stage | 2.34 | 1.67–3.28 | <0.001 | | |
| N2 vs N1, N1 vs N0 | | | | | |
| Adjuvant Therapy | 1.19 | 0.69–2.06 | 0.522 | | |
| yes vs no | | | | | |

**Table 6** Multivariate models with regard to disease-free survival (DFS)

| | HR | 95 % CI | *p* value | AIC | Harrels c 95 % CI |
|---|---|---|---|---|---|
| Model 1 | | | | | |
| CEA | 2.03 | 1.28–3.22 | 0.002 | 797 | 0.599 |
| ≥2.5 vs <2.5 ng/ml | | | | | 0.545–0.652 |
| Model 2 | | | | | |
| SAA | 1.81 | 1.14–2.85 | 0.011 | 800 | 0.571 |
| ≥5.3 vs<5.3 mg/l | | | | | 0.515–0.627 |
| Model 3 | | | | | |
| CEA | 2.00 | 1.26–3.17 | 0.003 | 793 | 0.632 |
| ≥2.5 vs <2.5 ng/ml | | | | | 0.571–0.692 |
| SAA | 1.77 | 1.12–2.80 | 0.014 | | |
| ≥5.3 vs <5.3 mg/l | | | | | |
| Model 4 | | | | | |
| CEA | 1.45 | 0.90–2.31 | 0.124 | 749 | 0.730 |
| ≥2.5 vs <2.5 ng/ml | | | | | 0.676–0.784 |
| T stage | 2.42 | 1.61–3.65 | <0.001 | | |
| T4 vs T3, T3 vs T2, T2 vsT1 | | | | | |
| N stage | 2.16 | 1.58–2.95 | <0.001 | | |
| N2 vs N1, N1 vs N0 | | | | | |
| Model 5 | | | | | |
| SAA | 1.78 | 1.12–2.81 | 0.014 | 745 | 0.734 |
| ≥5.3 vs <5.3 mg/l | | | | | 0.679–0.789 |
| T stage | 2.52 | 1.67–3.79 | <0.001 | | |
| T4 vs T3, T3 vs T2, T2 vsT1 | | | | | |
| N stage | 2.22 | 1.64–3.02 | <0.001 | | |
| N2 vs N1, N1 vs N0 | | | | | |
| Model 6 | | | | | |
| CEA | 1.45 | 0.90–2.32 | 0.126 | 746 | 0.746 |
| ≥2.5 vs <2.5 ng/ml | | | | | 0.692–0.799 |
| SAA | 1.78 | 1.12–2.81 | 0.015 | | |
| ≥5.3 vs <5.3 mg/l | | | | | |
| T stage | 2.25 | 1.46–3.49 | <0.001 | | |
| T4 vs T3, T3 vs T2, T2 vsT1 | | | | | |
| N stage | 2.15 | 1.56–2.96 | <0.001 | | |
| N2 vs N1, N1 vs N0 | | | | | |
| Adjuvant Therapy | 1.24 | 0.75–2.04 | 0.403 | | |
| yes vs no | | | | | |

**Table 7** CEA levels according to UICC stage and cancer-specific survival (CSS)

| | No. | Events | 3-year survival (%) | 95 % CI | 5-year survival (%) | 95 % CI | *p* value |
|---|---|---|---|---|---|---|---|
| CEA <2.5 ng/ml Stage I | 61 | 6 | 98.1 | 87.4–99.7 | 94.0 | 82.4–98.0 | <0.001 |
| CEA ≥2.5 ng/ml Stage I | 27 | 0 | 100.0 | . | 100.0 | . | |
| CEA <2.5 ng/ml Stage II | 25 | 6 | 91.2 | 69.0–97.7 | 86.4 | 63.3–95.4 | |
| CEA ≥2.5 ng/ml Stage II | 50 | 18 | 84.3 | 69.7–92.2 | 74.0 | 57.8–84.8 | |
| CEA <2.5 ng/ml Stage III | 42 | 15 | 84.3 | 68.3–92.6 | 78.5 | 61.4–88.7 | |
| CEA ≥2.5 ng/ml Stage III | 51 | 26 | 77.0 | 62.2–86.6 | 51.1 | 35.5–64.7 | |

**Table 8** CEA levels according to UICC stage and disease-free survival (DFS)

| | No. | Events | 3-year survival (%) | 95 % CI | 5-year survival (%) | 95 % CI | *p* value |
|---|---|---|---|---|---|---|---|
| CEA <2.5 ng/ml Stage I | 61 | 7 | 93.1 | 82.7–97.4 | 88.8 | 76.6–94.9 | <0.001 |
| CEA ≥2.5 ng/ml Stage I | 26 | 2 | 92.3 | 72.6–98.0 | 92.3 | 72.6–98.0 | |
| CEA <2.5 ng/ml Stage II | 25 | 8 | 69.5 | 46.3–84.2 | 69.5 | 46.3–84.2 | |
| CEA ≥2.5 ng/ml Stage II | 49 | 18 | 65.3 | 49.4–77.2 | 58.9 | 42.3–72.2 | |
| CEA <2.5 ng/ml Stage III | 40 | 16 | 71.4 | 54.3–83.1 | 65.2 | 47.5–78.2 | |
| CEA ≥2.5 ng/ml Stage III | 51 | 31 | 48.3 | 33.7–61.6 | 38.3 | 24.3–52.2 | |

significance in model 4 indicating a strong association with T and N stage. In contrast, SAA remains significant independently of T and N stage with HR=2.22, AIC of 609, and Harrels c of 0.763 (model 5). Adjuvant therapy was not significant in the multivariate analysis (model 6). With regard to disease recurrence (DFS), comparable results were obtained in the multivariate models (Table 6). CEA was also strongly associated to T and N stage and did not reach the level of statistical significance (model 4). In contrast, SAA provided prognostic value with HR=1.78, AIC of 745, and Harrels c=0.734 (model 5). Adjuvant therapy did not turned out to be a significant confounder for DFS in the multivariate model 6.

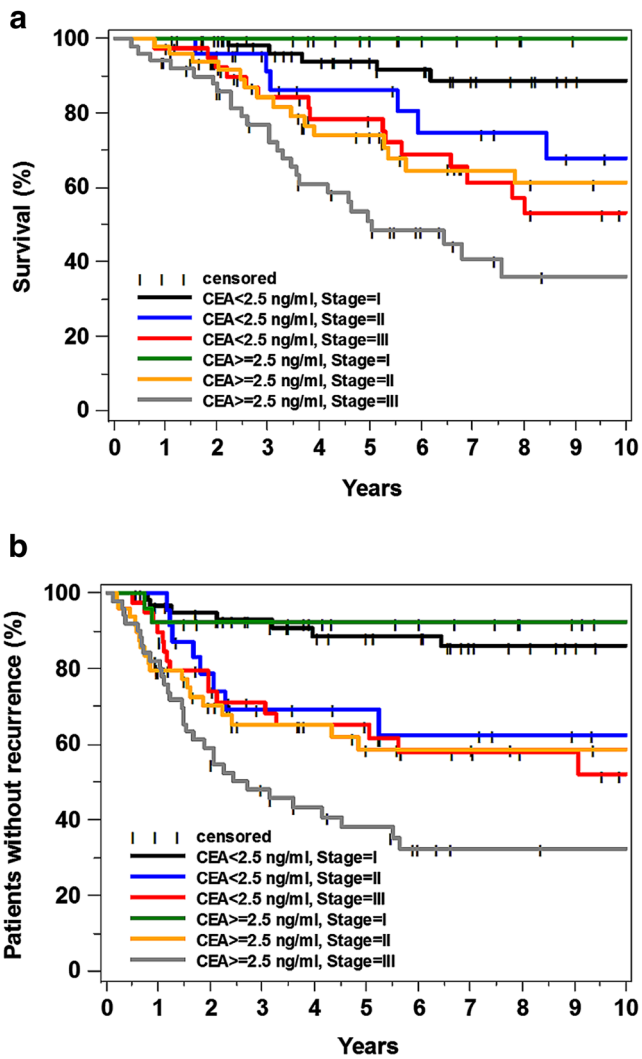


**Fig. 1** Kaplan-Meier plots showing CEA according to UICC stage and CSS (**a**) and DFS (**b**), respectively

## Kaplan–Meier survival analysis

Furthermore, Kaplan–Meier survival analysis was performed for CEA and SAA levels above and below the median and was analyzed according to UICC stage. Tables 7 and 8 show CEA levels according to UICC stage with regard to CSS and DFS. Within stage I patients, CEA does not provide prognostic value for 3- and 5-year CSS and DFS, respectively. In the analysis of stage II patients, CEA levels <2.5 ng/ml are associated to higher 3- and 5-year CSS rates, while DFS remains comparable between the two groups. Among patients with stage III rectal cancer, preoperative CEA levels <2.5 ng/ml were found to be associated with favorable 3- and 5-year CSS rates compared to patients with CEA levels >2.5 ng/ml. Furthermore, patients with elevated CEA levels had lower 3- and 5-year DFS rates. Figures 1a and b summarize these findings in Kaplan–Meier plots showing CEA according to UICC stage and CSS and DFS, respectively.

Tables 9 and 10 show SAA levels according to UICC stage and 3- and 5-year CSS and DFS rates. In patients with stage I rectal cancer, SAA levels <5.3 mg/l are not associated with favorable 3- and 5-year CSS and DFS rates. Among patients with stage II disease, elevated SAA levels provide prognostic value indicating patients with unfavorable 3- and 5-year CSS rates. With regard to 3- and 5-year DFS, this finding is even pronounced. In the analysis of stage III patients, SAA levels <5.3 mg/l were found to be associated with favorable 3- and 5-year CSS, while this finding was less strongly in 3- and 5-year

**Table 9** SAA levels according to UICC stage and cancer-specific survival (CSS)

| | No. | Events | 3-year survival (%) | 95 % CI | 5-year survival (%) | 95 % CI | *p* value |
|---|---|---|---|---|---|---|---|
| SAA <5.3 mg/l Stage I | 51 | 1 | 97.9 | 85.8–99.7 | 97.9 | 85.8–99.7 | <0.001 |
| SAA ≥5.3 mg/l Stage I | 30 | 3 | 100.0 | . | 100.0 | . | |
| SAA <5.3 mg/l Stage II | 19 | 5 | 94.4 | 66.6–99.2 | 88.5 | 61.4–97.0 | |
| SAA ≥5.3 mg/l Stage II | 51 | 18 | 82.4 | 67.7–90.9 | 71.8 | 55.4–83.1 | |
| SAA <5.3 mg/l Stage III | 50 | 17 | 89.2 | 76.0–95.4 | 74.1 | 58.0–84.8 | |
| SAA ≥5.3 mg/l Stage III | 39 | 23 | 70.9 | 53.6–82.7 | 51.4 | 34.3–66.1 | |

DFS. Figures 2a and b summarize these findings in Kaplan–Meier plots showing SAA according to UICC stage and CSS and DFS, respectively.

With SAA and CEA being significantly associated with CSS and DFS, these two markers were also investigated together. First, the two markers were correlated and did not show a strong correlation with $R=0.23$ (Spearman correlation coefficient). Furthermore, the patients were divided into four groups according to CEA and SAA levels below and above the median level, respectively (Tables 11 and 12). Patients with both markers below the median level had favorable 3- and 5-year CSS and DFS rates, respectively. In contrast, patients with both markers elevated above the median level showed poor 3- and 5-year DFS. With regard to CSS, this effect was found very strong in 5-year CSS (61.2 %) and weaker in a 3-year CSS rate of 80.2 %. In the group of patients with elevated CEA and SAA levels below the median, the 3- and 5-year CSS and DFS rates were found comparable to patients with elevated SAA and CEA levels below the median. Figures 3a and b illustrate the intermediate survival date of the two patient groups with one elevated serum marker and one below the median level.

## Discussion

Current guidelines recommend routine testing of CEA as the tumor marker of choice in the preoperative setting. Also, postoperative measurement should be performed every 3 to 6 months in patients with stage II or III disease [2, 5]. In the light of many other tumor markers evaluated in stages I–III disease, only CEA has demonstrated prognostic value in large analyses [21, 22]. In the present study, we evaluated an extended set of tumor markers along with other serum markers that have been associated with prognostic value in CRC.

In the analysis of 256 patients who underwent curative rectum resection, SAA provided prognostic value for DFS and CSS in both uni- and multivariate analysis. With CEA being strongly associated to T and N stage, CEA did not reach the level of statistical significance. Therefore, CEA and SAA were explored according to UICC stage, where SAA provided prognostic value in stage II patients with regard to both CSS and DFS. In stage III rectal cancer patients, CEA was found as a strong discriminant with regard to DFS, while SAA was found as a string prognostic serum marker with regard to CSS. In addition, the combined use of both markers was able to

**Table 10** SAA levels according to UICC stage and disease-free survival (DFS)

| | No. | Events | 3-year survival (%) | 95 % CI | 5-year survival (%) | 95 % CI | *p* value |
|---|---|---|---|---|---|---|---|
| SAA <5.3 mg/l Stage I | 50 | 3 | 94.0 | 82.5–98.0 | 94.0 | 82.5–98.0 | <0.001 |
| SAA ≥5.3 mg/l Stage I | 30 | 4 | 96.7 | 78.6–99.5 | 87.8 | 66.5–96.0 | |
| SAA <5.3 mg/l Stage II | 18 | 4 | 82.4 | 54.7–93.9 | 74.9 | 45.6–89.9 | |
| SAA ≥5.3 mg/l Stage II | 51 | 21 | 57.6 | 42.1–70.4 | 57.6 | 42.1–70.4 | |
| SAA <5.3 mg/l Stage III | 49 | 23 | 61.7 | 46.3–73.9 | 56.4 | 40.6–69.4 | |
| SAA ≥5.3 mg/l Stage III | 38 | 23 | 53.7 | 36.4–68.2 | 42.0 | 25.8–57.4 | |

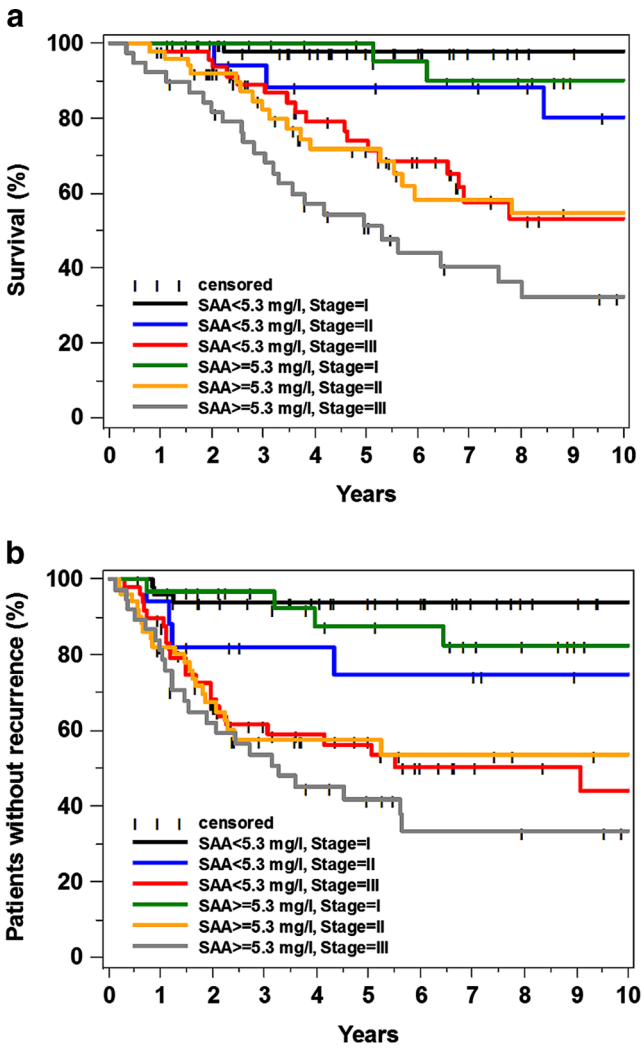


**Fig. 2** Kaplan-Meier plots showing SAA according to UICC stage and CSS (**a**) and DFS (**b**), respectively

separate between patients with favorable prognosis (both markers below median level) and poor prognosis (both markers elevated).

The finding of SAA being associated with unfavorable prognosis may be explained by a well-known association between inflammatory response to tissue damage caused by rectal cancer and the release of SAA. Routine testing of SAA therefore provides additional prognostic information of both recurrence and cancer-specific mortality in patients with stage II and stage III rectal cancer. Furthermore, nephelometric immunoassay analysis of SAA implicates low cost and low effort for standard clinical laboratory. Considering its nonspecific increase due to inflammatory processes, a possible advantage can be seen especially in patients with CEA values below the median of healthy individuals [15]. It might be concluded, that SAA represent a suitable biomarker for CRC independently of other inflammation-associated parameters like CRP or WBC and is therefore beneficial especially in the preoperative scenario [23]. Therefore, the combined use of CEA together with SAA may be used for risk-adapted follow-up care and intensified monitoring of high-risk patients.

Recently, the association of acute-phase proteins like SAA and CRP with carcinogenesis has been described by several studies [24, 25]. In contrast with these reports, elevated CRP was not associated with an increased risk of recurrent CRC in the present analysis. Furthermore, elevated SAA levels were also not associated with increased WBC. Unfortunately, we were not able to evaluate the lymphocyte to monocyte ratio that has been recently shown to identify high-risk CRC patients [26]. Recently, the analysis of the HORIZON II trial also found (pro-)inflammatory parameters IL-6, IL-8, CRP, and ICAM-1 to be of prognostic value for PFS and OS [27]. Unfortunately, SAA was not estimated in the analysis.

In the present study, clinical and histopathological parameters also turned out to be significant in uni- and multivariate analysis. T status and N status at time of diagnosis were found to be highly significant for CSS and DFS. This result is well in line with several trials addressing individual patient outcome in colorectal cancer [22, 28, 29].

There are several limitations given in the present analysis: first, only data from a single but large university cancer center were included. Also, the time frame of surgery ranges from January 1988 and June 2007 incorporating progress in both surgical techniques and neo/adjuvant treatment for stages I–III CRC. We therefore adjusted results obtained for SAA and CEA for adjuvant therapy and time of surgery and found both factors independent of CSS and DFS outcome. It is also important to

**Table 11** Combined analysis of CEA and SAA levels with regard to cancer-specific survival (CSS)

| | No. | Events | 3-year survival (%) | 95 % CI | 5-year survival (%) | 95 % CI | *p* value |
|---|---|---|---|---|---|---|---|
| CEA <2.5 ng/ml SAA <5.3 mg/l | 64 | 9 | 96.7 | 87.6–99.2 | 93.0 | 82.5–97.3 | <0.001 |
| CEA ≥2.5 ng/ml SAA <5.3 mg/l | 56 | 14 | 90.3 | 78.3–95.8 | 79.3 | 64.8–88.3 | |
| CEA <2.5 ng/ml SAA ≥5.3 mg/l | 56 | 16 | 86.1 | 73.0–93.1 | 83.8 | 70.0–91.6 | |
| CEA ≥2.5 ng/ml SAA ≥5.3 mg/l | 64 | 28 | 80.2 | 67.7–88.3 | 61.2 | 47.1–72.7 | |

**Table 12** Combined analysis of CEA and SAA levels with regard to disease-free survival (DFS)

| | No. | Events | 3-year survival (%) | 95 % CI | 5-year survival (%) | 95 % CI | *p* value |
|---|---|---|---|---|---|---|---|
| CEA <2.5 ng/ml SAA <5.3 mg/l | 63 | 13 | 83.4 | 71.4–90.7 | 81.5 | 69.0–89.3 | <0.001 |
| CEA ≥2.5 ng/ml SAA <5.3 mg/l | 54 | 17 | 73.5 | 59.3–83.4 | 68.4 | 53.4–79.4 | |
| CEA <2.5 ng/ml SAA ≥5.3 mg/l | 55 | 16 | 81.0 | 67.4–89.3 | 73.7 | 58.8–83.9 | |
| CEA ≥2.5 ng/ml SAA ≥5.3 mg/l | 64 | 32 | 53.5 | 39.9–65.3 | 47.0 | 33.5–59.5 | |

note that the release of acute-phase proteins like SAA may have been influenced by other inflammatory diseases that may have rarely been disregarded prior to the surgical intervention. Furthermore, we were not able to evaluate further serum marker measurements at different time points, especially in patients with recurrent or metastatic disease.

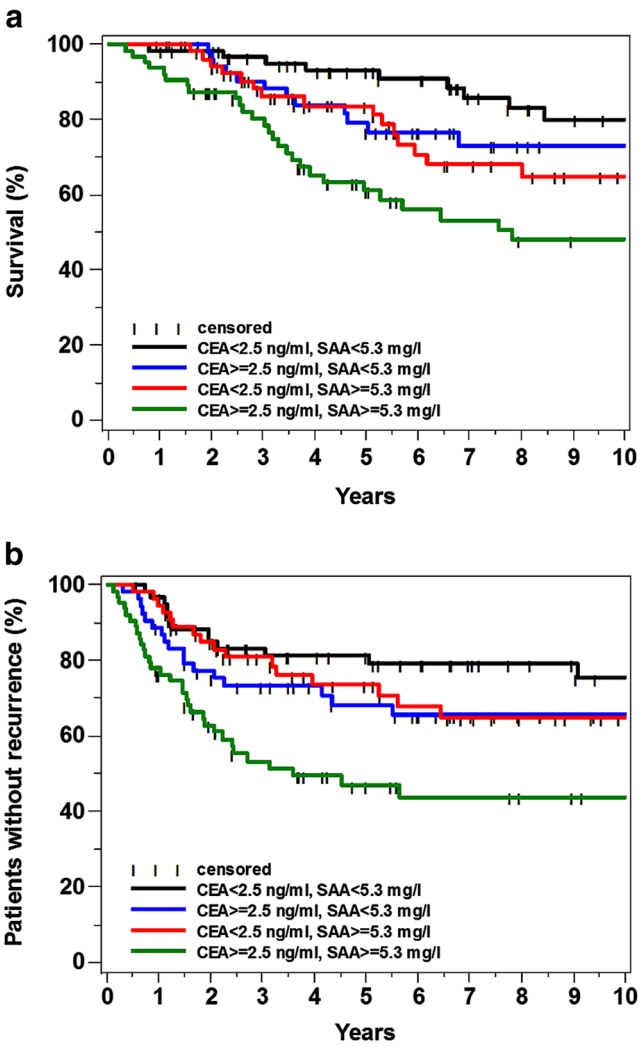


**Fig. 3** Overall survival (Fig. 3a) and disease-free survival (Fig. 3b) in four different patient groups according to CEA and SAA levels

Currently, other serum markers are intensively explored in the setting of prognostic factors for CRC. Circulating levels of 25-hydroxyvitamin D levels have evoked promising results in large, prospective analyses indicating protective effects on overall survival in patients with high plasma levels [30, 31]. In contrast, the present analysis failed to show a significant association with improved DFS or CSS in patients with higher levels of 25-hydroxyvitamin D.

Moreover, CA 19-9 is widely regarded as an important tumor marker in gastrointestinal cancer. However, to this date its role for screening, staging, and treatment monitoring for CRC cannot be recommended due to insufficient data [2]. Incorporating preoperative measurement of CA 19-9, our analysis also failed to show an association with DFS or CSS.

In conclusion, the measurement of SAA connotes additive prognostic value to CEA with regard to DFS and CSS in stages II and III rectal cancer. Since there is no universal tumor marker for colorectal cancer of any stage, a differentiated use including clinical and histopathological factors is warranted. Therefore, the combined use of both markers is able to provide additive prognostic information in patients with resected rectal cancer and is able to identify patients with favorable and poor prognosis. In this regard, prospective evaluation including markers like SAA in both limited and metastatic CRC is strongly encouraged.

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**Conflicts of interest** None

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