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Pre-surgery adhesion molecules and angiogenesis biomarkers are differently associated with outcomes in colon and rectal cancer: Results from the ColoCare Study

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Abstract

Background: Cell-to-cell adhesion and angiogenesis are hallmarks of cancer. No studies have examined associations of adhesion-molecules and angiogenesis biomarkers with clinical outcomes in colorectal cancer (CRC).

Methods: In pre-surgery serum from n=426 CRC patients (stage I-III) we investigated associations of CRP, SAA, adhesion molecules (sICAM-1, sVCAM-1), and angiogenesis markers (VEGF-A, and -D) with overall survival (OS), disease-free survival (DFS), and risk of recurrence. We computed hazard ratios (HR) and 95% confidence intervals; adjusted for age, sex, BMI, stage, site, and study site, stratified by tumor site in exploratory analyses.

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Informed consent: Informed consent was obtained from all individual subjects included in this study.

Results: N=65 (15%) were deceased, 59 patients (14%) had a recurrence after a median follow-up of 31 months. We observed significant associations of biomarkers with OS, DFS, and risk of recurrence on a continuous scale and comparing top to bottom tertile, with HRs ranging between 1.19 – 13.92. CRP was associated with risk of death and recurrence in patients in the top tertile compared to patients in the bottom tertile, e.g., risk of recurrence HRQ3-Q1:13.92 (1.72, 112.56). Significant heterogeneity between biomarkers and clinical outcomes was observed in stratified analysis by tumor site for CRP, SAA, sICAM-1, sVCAM-1, and VEGF-D. VEGF-D was associated with a 3-fold increase in risk of death for rectal cancer (HRlog2: 3.26; 95% CI: 1.58–6.70) compared to no association for colon cancer (HRlog2: 0.78; 95% CI: 0.35–1.73; $p_{heterogenity}$ =0.01).

Conclusions: Adhesion molecules and angiogenesis biomarkers are independent prognostic markers for CRC, with differences by tumor site.

Impact: There is need for tailored treatment for colon and rectal cancer.

INTRODUCTION

Colorectal cancer is the second most common incident cancer and the second most common cause of cancer-related death with 147,950 new cases and 53,200 deaths in the U.S. in 2020.(1) Although the rate of recurrences in colorectal cancer patients has been reduced substantially over the past decades, 20–30% of patients develop a post-treatment recurrence, which results in poor survival outcomes and is a major cause of morbidity and mortality.(2) Therefore, it is critical to identify biomarkers for patients at a high risk of developing a recurrence or succumbing to the disease.

Inflammation, cell-to-cell adhesion, and angiogenesis are established hallmarks of cancer development and progression,(3) and have been linked to colorectal cancer.(4,5) Chronic inflammation has consistently been associated with colorectal carcinogenesis by inducing the production of reactive oxygen species that are known to damage macromolecules including DNA.(6,7) Inflammation in the tumor microenvironment promotes carcinogenesis and cancer progression.(8) The tumor itself and cancer treatment (e.g., radiation, chemotherapy) have been presented as drivers of inflammatory processes in cancer patients.(9,10) We and others have previously shown that biomarkers of inflammation are associated with future risk of colorectal cancer.(4,5) The role of biomarkers of inflammation, has been further elucidated in prospective studies in colorectal cancer patients. (11–16) These prior studies, however, have investigated solely inflammation biomarkers and have been based on the assessment of single markers such as C-reactive protein (CRP)(11,17). CRP is a protein synthesized by the liver. It is a systemic biomarker that is secreted in response to inflammation and has been linked to colorectal cancer in previous studies.(17) Serum amyloid A (SAA) is another biomarker of interest. SAA is a protein synthesized in the liver and is produced in response to pro-inflammatory stimuli.(18) There is clinical evidence that SAA is more abundant in colorectal cancer patients compared to cancer-free controls.(19) Both CRP and SAA have been found to predict risk of CRC and survival of other cancers.(4,20) Interleukin-6 (IL-6)(12) is another biomarker that regulates cell proliferation, survival, migration, invasion, metastasis, angiogenesis and inflammation. IL-6 is highly upregulated in many cancers. High IL-6 levels are associated with poor prognosis for

colorectal cancer overall survival and disease-free survival.(21,22) A cytokine that has been measured in the cancer setting is Interleukin- 8 (IL-8). IL-8 is a pro-inflammatory cytokine that is believed to be up regulated in colorectal cancer patients and has been linked to proliferation, angiogenesis and metastasis.(23) More recent studies considered a comprehensive panel of inflammatory biomarkers,(13–16) but did not consider the prognostic impact of adhesion molecules or angiogenesis biomarkers with colorectal cancer.

Angiogenesis is a hallmark of cancer development,(3) which has been previously linked to clinical outcomes in colorectal cancer (CRC) patients.(24) Angiogenic pathways are essential for the growth, proliferation, and invasion of cancer metastases.(24) Vascular Endothelial Growth Factor (VEGF) -A and -D are prominent biomarkers for angiogenesis. VEGF-A signals though the VEGF receptor 2, which is expressed at elevated levels by endothelial cells engaged in angiogenesis.(25) VEGF-A further induces the growth of lymphatic vasculature. VEGF-D is associated with lymph node metastases in colorectal cancer patients and VEGF-D levels are higher in patients with colorectal cancer compared to controls and further correlates with tumor size.(26,27)

Adhesion molecules such as soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble vascular cell adhesion molecule-1 (sICAM-1) modulate interactions between host and cancer cells and are collectively defined as cell adhesion molecules.(28) Loss of cell-to-cell adhesion and anchorage-independent growth are two cancer hallmarks that are both dependent on cell adhesion molecules.(3) sICAM-1 is a member of the immunoglobulin family and is expressed as a surface glycoprotein. In tumor associated fibroblasts, sICAM-1 is increased.(29) Expression is further increased by proinflammatory cytokines. sVCAM-1 participates in the adhesion and transmigration of leukocytes during inflammation.(30,31)Increased levels due to colorectal cancer progression have been reported. (32)Increase of sVCAM-1 is associated with an increased risk of colorectal cancer. (33)Dymicka-Piekarska, 2012 #13345} In a retrospective study (n=46 CRC patients and n=40 controls) statistically significant differences in concentrations of sVCAM-1, sICAM-1, and VEGF between colorectal cancer patients and controls have been reported.(32) All investigated biomarkers (VEGF-A VEGF-D, sICAM-1, and sVCAM-1 were significantly increased in patients with colorectal cancer in comparison to controls ($p<0.001$). However, in this study associations with clinical outcomes were not assessed.(32) None of the previously published prospective studies investigated associations of sVCAM-1 or sICAM-1 with clinical outcomes in colorectal cancer patients.

There is accumulating evidence on heterogeneity between colon and rectal cancer with regards to risk factors,(34) but also in terms of prognosis,(35) indicating that colon and rectal cancer have distinct etiologies as well as risk and prognostic factors. Some studies have evaluated potential heterogeneity in associations of systemic inflammatory biomarkers by tumor site.(36,37) However, there is no evidence regarding differences in associations by tumor site for angiogenesis biomarkers or adhesion molecules.

The aim of the present study is to assess for the first time associations of pre-surgery concentrations of a broad panel of biomarkers (systemic adhesion molecules and angiogenesis-related biomarkers) in tandem with inflammatory markers with overall survival

(OS), disease-free survival (DFS), and risk of recurrence or risk of distant recurrence in prospectively followed colon and rectal cancer patients.

MATERIALS AND METHODS

Study Setting, Participants and Recruitment

ColoCare (ClinicalTrials.gov Identifier: NCT02328677) is an international, multi-center prospective cohort study among women and men newly diagnosed with a primary invasive colorectal cancer of any stage.(38) ColoCare goals are to investigate biomarkers of cancer recurrence, survival, treatment toxicities and health-related quality of life. The study was initially developed at the Fred Hutchinson Cancer Research Center (Seattle, Washington, USA) in 2007, with subsequent consortium sites at the H. Lee Moffitt Cancer Center and Research Institute (Tampa, Florida, USA) in 2009, National Center for Tumor Diseases and University Hospital of Heidelberg (Heidelberg, Germany) in 2010, Huntsman Cancer Institute (Salt Lake City, Utah, USA) in 2015, Cedars-Sinai Medical Center (Los Angeles, California, USA) in 2017, Washington University School of Medicine (WUSM) (St. Louis, Missouri, USA) in 2017, and the Center for Cancer Research (Memphis, Tennessee, USA) in 2017.

Eligible patients are defined as follows: newly diagnosed colon (ICD-10 C18), rectum, or rectosigmoid cancer (ICD-10 C19/C20); ≥18 years of age at the time of diagnosis, histopathologically confirmed invasive cancer of any stage of disease based on the American Joint Committee on Cancer (AJCC) classification, and able to provide informed consent. ColoCare has been approved by the local institutional review boards serving each site. This study enrolls English speaking patients at all U.S. sites and the German site enrolls German-speaking patients. Patients who died due to postoperative complications (defined as death within 30 days after surgery (n=4) were excluded from this study. The final sample size for this project was n=426 patients.

All questionnaires used in ColoCare are self-administered and can be completed on paper or electronically. The baseline questionnaire includes assessments of demographics (e.g., education, ethnicity, marital status), lifestyle factors known/suspected to be associated with colorectal cancer (e.g., smoking, alcohol use, and physical activity), hormone replacement therapy for women, self-reported anthropometric measures (e.g., height and weight), medical history, family history of cancer, dietary supplement use, and prescription and non-prescription medication use. General and colorectal cancer specific quality of life questionnaires (e.g., SF-12, MDASI, EORTC QLQ-C30) are also included.

For the present study, patients diagnosed with stage I-III CRC who had measurements of inflammatory, cell adhesion, and angiogenesis biomarkers from pre-surgery serum samples were eligible. All analyses in this manuscript are based on data collected between 2010 and 2014 at the ColoCare Study site in Heidelberg (HD), Germany and between 2015 and 2018 at the ColoCare Study site in Salt Lake City, Utah (HCI). The study was approved by the Ethics Committee of the University of Heidelberg and the institutional review board of the University of Utah, and all subjects provided written informed consent.

Biomarkers

Non-fasting blood samples were collected from patients prior to surgery. For patients receiving neoadjuvant chemotherapy, blood samples were collected at least 2 weeks after completion of neoadjuvant chemotherapy. Serum at both study sites was extracted within four hours after blood draw and stored in aliquots at -80ºC until analysis. 500μl of each patient's serum from Heidelberg was shipped on dry ice to the Huntsman Cancer Institute (HCI, Salt Lake City, Utah) for laboratory analysis.

Assays for multiplexed Il-6, IL-8, TNF-α, CRP, SAA, sICAM-1, sVCAM-1, VEGF-A, and VEGF-D have previously been established on the Mesoscale Discovery (MSD) Platform in the Ulrich laboratory at HCI.(39) Assays were run in three batches ranging from 2017–2020. N=37 blinded patient samples and three laboratory quality controls (QCs) were assayed in duplicate on each plate. IL-6, IL-8, and TNF-α were measured using the U-PLEX Pro-inflammatory Combo 1 for the HD cohort and the V-PLEX Proinflammatory Panel 1 for HCI cohort. CRP, SAA, sICAM-1 and sVCAM-1 were measured using the V-PLEX Vascular Injury Plate 2 and VEGF-A and VEGF-D using the V-PLEX Angiogenesis Panel 1 for both cohorts. Samples were run at dilutions of 1:1000 (vascular and adhesion molecules) for the HD cohort and 1:2500 (vascular and adhesion molecules) for the HCI cohort, 1:8 (angiogenesis) and 1:2 (proinflammatory) for both cohorts. The serums had no previous freeze-thaw cycles for the vascular injury and angiogenesis panels and one freeze-thaw cycle for the proinflammatory panels. An MSD sector 2100A was used to read the plates and the data was analyzed using MSD Discovery Workbench 4.0 software (both from MSD, Rockville, MD). Laboratory QCs were generated by spiking varying concentrations of IL-6, IL-8, TNF-α, VEGF-A and VEGF-D into 3 pools of serum from 6 individuals each (3 male and 3 female). Endogenous levels of CRP, SAA, sICAM-1 and sVCAM-1 were used. The pools of serum were purchased from BioIVT (Westbury, NY, USA). Intra-plate coefficients of variation (CVs) were calculated from duplicates from the three QCs. The inter-plate CVs were calculated by using all the QC values from all plates. The inter-plate CVs for HD ranged from 5.1–15.9% and 5.3–13.2% for HCI. The intra-plate CVs ranged from 1.8–7.2% for both sites. Values for IL-6, IL-8 and TNF-α from the UPLEX *versus* VPLEX kits were bridged. The HD values for IL-6, IL-8 and TNF-a are higher than previously reported due to the bridging. (39–44)

Outcome Assessment and Follow-up

Patients were followed up by study staff (in-person and through medical record reviews), and *via* linkages to cancer registry and vital status records. Standardized medical record abstraction, at least 2-years post-diagnosis, was used to obtain detailed information on surgical procedures, treatments (e.g., adjuvant chemotherapy modality, timing and dose), pathologic and molecular features. Vital status was obtained through local medical records, routine follow-up mailings, periodic requests for outside medical records, and state or national cancer and death registries. End of follow-up was defined as the last contact, or date of death, whichever occurred first. Overall survival was defined as death, irrespective of cause. Recurrence was defined as new recurrence of the cancer after complete removal) and classified as either local or distant recurrence. CRC recurrence can be categorized as local, regional, or distant recurrence. Local recurrence includes relapse that occurs at the

site of surgical resection. Regional recurrence occurs at draining lymph nodes and/or lateral pelvic lymph nodes. For the present study local and regional recurrence were combined. Distant recurrence occurs mostly in the liver (up to 50% of metastases), the lung (10–20% of metastases), the peritoneum, the ovaries, the adrenal glands, the bone, or the brain. For n=34 patients, abstraction for recurrence was not yet completed, thus, n=392 (not n=426) patients were eligible for the recurrence-related analyses. Disease-free survival was defined as the occurrence of either recurrence or death, whichever occurred first.

Statistical Analysis

Means and standard deviations (SD) were calculated for continuous variables (e.g., age, body mass index (BMI)) and compared across study sites. Frequency and percentages were computed for categorical variables (e.g., sex, tumor stage, tumor site, neoadjuvant, and adjuvant treatment; Table 1).

Since biomarker concentrations were right skewed, log-2 transformation was applied to prevent heteroscedasticity. We utilized the Extreme Studentized Deviate (ESD) test, to identify and exclude influential outliers for any of the investigated biomarkers.(45) The following outliers were excluded from analysis: Il-6 (n=2), IL-8 (n=3), TNF-alpha (n=1), VEGF-A (n=6), VEGF-D (n=6), sICAM-1 (n=8), sVCAM-1 (n=2). We did not identify any outliers for CRP or SAA.

Median and interquartile range (IQR) were calculated for systemic biomarkers: CRP, SAA, VEGF-A, VEGF-D, TNF-α, IL-6, IL-8, sICAM-1, and sVCAM-1 for each of the study sites (Supplementary Table 1).

Cox proportional hazard models were used to compute OS, DFS, risk of recurrence, and risk of distant recurrence: hazard ratios (HR) and 95% confidence intervals (95% CI) using log2-transformed biomarker concentrations and study site-specific tertiles based on the distribution in controls. A continuous probit score, generating a rank for each participant in each cohort by biomarker concentrations, was used to test for trend across tertiles.

We conducted stratified analyses comparing (1) tumor sites: colon *versus* rectal, and (2) study sites: HCI *versus* HD. The following covariates were considered as potential confounders: age, sex, body mass index (BMI), tumor stage, tumor site, and adjuvant treatment. The final model included age (continuous), sex (male/female), tumor site (colon/rectal), tumor stage (I/II/III), study site (HCI/HD), and BMI (continuous). We did not observe significant differences in a model additionally adjusting for adjuvant treatment.

We further investigated associations of biomarkers specifically with risk of distant recurrence (n=44 events). The sample size was too small to investigate associations with local-regional recurrences (n=15 events).

A prior study has reported poor clinical outcomes for patients with high levels of CRP,(13) thus we decided to specifically investigate associations of high-level CRP (>10mg/l) with clinical outcomes in colorectal cancer patients.

Heterogeneity in associations between biomarkers and clinical outcomes stratified by aforementioned factors (tumor site and study site) was assessed using likelihood-ratio tests for the comparison of the model fit for logistic regression models with and without corresponding interaction terms. All data were analyzed using SAS version 9.4 statistical software. All tests were two-sided, with a p value < 0.05 considered statistically significant.

Ethical guidelines

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Data Availability Statement

The ColoCare Study data is available from the corresponding author on reasonable request and as described on the ColoCare website (https://uofuhealth.utah.edu/huntsman/labs/colocare-consortium/). Our data sharing procedures have been updated and are available online (https://uofuhealth.utah.edu/huntsman/labs/colocare-consortium/data-sharing/new-projects.php). For any additional questions please contact the ColoCare Study Administrator Team (colocarestudy_admin@hci.utah.edu).

RESULTS

A total of n=426 patients were included. Table 1 presents baseline characteristics of the overall study population. In Supplementary Table 1 we present the baseline biomarker for patients recruited at HCI and patients recruited in HD. Most patients underwent surgery (99%) and have been diagnosed with tumor stage III (47%). Overall, after a median follow-up time of 31 months n=65 (15%) patients were deceased and n=59 (15%) patients had a recurrence.

Pre-surgery biomarkers and overall survival

A total of n=65 patients were deceased. Associations of biomarkers with OS are described in Table 2 (tertiles) and Supplementary Table 2 (continuous scale). Doubling of sICAM-1 was associated with a four-fold increase in risk of death (HR_{log2}: 4.05; 95% CI: 2.35–6.98; p<0.0001). Comparing the top to the bottom tertile we observed a 4-fold increase in risk for sICAM-1 ($HR_{Q3\text{-}Q1}$: 4.12; 95% CI: 1.94–8.77; p_{trend}= 0.001). Similarly, doubling of sVCAM-1 was associated with a more than three-fold increase in risk of death (e.g., HR_{log2}: 3.53, 95% CI: 1.82–6.85); respectively a 2.5 fold increase in risk of death comparing the top to the bottom tertile ($HR_{Q3\text{-}Q1}$: 2.51; 95% CI: 1.29–4.88; p_{trend}= 0.004). Doubling of CRP and SAA was associated with an increased risk of death, e.g., doubling of CRP was associated with a 24% increase in risk of death (HR_{log2}: 1.24; 95% CI: 1.11–1.40; p=0.0002), and a 3-fold increase in risk comparing the top to the bottom tertile ($HR_{Q3\text{-}Q1}$: 3.06; 95% CI: 1.55–1.06; p_{trend}= 0.001).

In stratified analysis by tumor site, we observed significant heterogeneity in the associations of CRP and VEGF-D with death: e.g., doubling of VEGF-D was associated with 3-fold increase in death of rectal cancer (HR_{log2}: 3.26; 95% CI: 1.58–6.70) and no statistically

significant association was observed with risk of death of colon cancer (HR_{log2}: 0.78; 95% CI: 0.35–1.73; $p_{heterogeneity}$<0.01). Similarly for CRP, colon cancer patients in the top tertile had a 7-fold increase in risk of death for colon cancer: $HR_{Q3\text{-}Q1}$:7.71 (1.70, 35.04) compared to patients in the bottom tertile. For patients with rectal cancer in the top tertile we observed a two-fold increase of risk of death compared to patients to the bottom tertile ($HR_{Q3\text{-}Q1}$:2.20 (0.96, 5.02)), although not statistically significant ($p_{heterogeneity}$=0.03). We did not observe statistically significant heterogeneity in associations across study sites (e.g., SAA: $HR_{log2, HCI}$: 1.24; 95% CI: 1.04–1.47; $HR_{log2, HD}$: 1.17; 95% CI 0.98–1.39; $p_{heterogeneity}$ >0.78; Supplementary Table 3).

Pre-surgery biomarkers and disease-free survival

A total of n=82 patients had either a recurrence or were deceased with an average follow-up time of 16 months. Associations of biomarkers with DFS are shown in Table 3 (tertiles) and Supplementary Table 4 (continuous scale). Doubling of CRP was associated with a 19% increase in risk of recurrence or death (HR_{log2}: 1.19; 95% CI: 1.08–1.32; p=<0.001). We observed an almost 3-fold increase in risk for patients in the top tertile compared to the bottom tertile ($HR_{Q3\text{-}Q1}$: 2.70; 95% CI: 1.50–4.89; p_{trend}= 0.0007), indicating a linear relationship. Similarly, doubling of SAA was associated with a 18% risk of recurrence or death (HR_{log2}: 1.18; 95% CI: 1.06–1.32; p=0.0025). Doubling of sICAM-1 was associated with a more than two-fold increase in risk of death or recurrence (HR_{log2}: 2.19; 95% CI: 1.37–3.50; p=0.001) and a 2.5-fold increase in risk comparing top to bottom tertile (e.g., $HR_{Q3\text{-}Q1}$: 2.55; 95% CI: 1.40–4.64; p_{trend}= 0.002).

We did observe statistically significant heterogeneity in risk associations between colon and rectal cancer comparing the top to the bottom tertile for CRP, SAA, sICAM-1 and sVCAM-1 (CRP: $p_{heterogeneity}$=0.04; SAA: $p_{heterogeneity}$=0.03; sICAM-1: $p_{heterogeneity}$=0.02; sVCAM-1: $p_{heterogeneity}$=0.03), but not on the continuous scale. For VEGF-D we did not observe statistically significant heterogeneity in risk associations between colon and rectal cancer ($p_{heterogeneity}$>0.07); doubling of VEGF-D was associated with 2-fold increase in risk of death or recurrence in rectal cancer (HR_{log2}: 2.20; 95% CI: 1.14–4.23) and a 18% risk reduction for death or recurrence in colon cancer (HR_{log2}: 0.82; 95% CI: 0.36–1.83; $p_{heterogeneity}$=0.07; Table 3), although not statistically significant. We did not observe heterogeneity in associations of biomarkers with DFS across study sites ($p_{heterogeneity}$>0.09), with the exception of the associations of IL-6 with DFS, e.g., $HR_{log2,HCI}$: 1.86; 95% CI: 1.21–2.88; p=0.005 and $HR_{log2,HD}$: 1.06; 95% CI: 0.91–1.23; p=0.45; $p_{heterogeneity}$=0.01; Supplementary Table 3). This heterogeneity could be due to different measurement platforms for the samples from Heidelberg (U-PLEX) and HCI (V-PEX).

Pre-surgery biomarkers and risk of recurrence

A total of n=59 patients had a recurrence with a median follow-up time of 16 months. Associations of biomarkers with risk of recurrence are presented in Table 4 (tertiles) and in Supplementary Table 5 (continuous). Doubling of CRP was associated with a 19% increase in risk of recurrence (HR_{log2}: 1.19; 95% CI: 1.06–1.33; p=0.0023). We observed an almost 3-fold increase in risk of recurrence for patients in the top tertile compared to the bottom tertile ($HR_{Q3\text{-}Q1}$: 2.88; 95% CI: 1.40–5. 49; p_{trend}= 0.003). Similarly, doubling of SAA was

associated with a 20% risk of recurrence (HR_{log2}: 1.20; 95% CI: 1.06–1.36; p<0.001) and a 2.4-fold increase in risk comparing the top to the bottom tertile ($HR_{Q3\text{-}Q1}$: 2.40; 95% CI: 1.14–5.04; p_{trend}= 0.02).

We did not observe heterogeneity in risk associations between colon and rectal cancer on the log2 transformed scale ($p_{heterogeneity}$>0.10) or comparing top to bottom tertile ($p_{heterogeneity}$ > 0.06); except for IL-6, comparing top to bottom tertile between colon and rectal cancer patients ($p_{heterogeneity}$=0.002). We did not observe heterogeneity in associations of biomarkers with disease-free survival across study sites ($p_{heterogeneity}$>0.09; Supplementary Table 3).

Pre-surgery biomarkers and risk of recurrence in patients diagnosed with distant recurrences

A total of n=44 patients had a distant recurrence. Associations of biomarkers with risk of a distant recurrence are presented in Table 5 (tertiles) and Supplementary Table 6 (continuous). Doubling of CRP was associated with a 25% increase in risk of distant recurrence (HR_{log2}: 1.25; 95% CI: 1.10–1.43; p<0.001) and a 3.4 fold increase in risk comparing the top tertile to the bottom tertile ($HR_{Q3\text{-}Q1}$: 3.42; 95% CI: 1.50–7.76; p_{trend}= 0.002). Similarly, doubling of SAA was associated with a 27% risk of distant recurrence (HR_{log2}: 1.27; 95% CI: 1.10–1.46; p=0.0045) and a 3.5-fold increase in risk comparing the top to the bottom tertile ($HR_{Q3\text{-}Q1}$: 3.53; 95% CI: 1.44–8.649; p_{trend}= 0.003).

We did not observe heterogeneity in risk associations between colon and rectal cancer for any biomarker ($p_{heterogeneity}$>0.18); except for IL-6 comparing top to bottom tertile in colon and rectal cancer patients ($p_{heterogeneity}$=0.002). There was no heterogeneity in associations of biomarkers with risk of recurrence across study sites ($p_{heterogeneity}$>0.09; Supplementary Table 7), except for the association of IL-6 with distant recurrence ($p_{heterogeneity}$=0.04).

Sensitivity analysis

The proportional hazard assumption was fulfilled for all analysis using categorical exposures (tertiles). With a few exceptions, all models using continuous biomarkers (log2-transformed) fulfilled the proportional hazards assumption.(46) For example, the proportional hazard assumption was not fulfilled for associations of sVCAM-1 in rectal cancer patients: rectal cancer: sVCAM-1 (p = 0.04) as well as colon cancer: VEGF-A (p = 0.03). Regarding the risk of recurrence in rectal cancer patients only the associations of sVCAM-1 was statistically significant. For the analysis of risk of distant recurrence in colon cancer patients VEGF-A (p= 0.045) as well as VEGF-D (p= 0.02) showed statistical significance.

We excluded patients with blood drawn more than 100 days before surgery (n=18) for sensitivity analysis on the continuous scale (log-2 biomarker) and did not observe substantial differences in the results. For example: overall survival for patients with colon cancer CRP (excluding n=18 with blood drawn >100 days before surgery: HR: 1.42 (1.17, 1.72) was similar for the results of CRP (including n=18 with blood drawn >100 days before surgery): HR: 1.35 (1.15,1.58).

In additional sensitivity analysis, we adjusted for adjuvant treatment. We observed comparable results for OS, DFS and risk of recurrence (Supplementary Table 8).

Previous studies(13) have excluded patients with CRP levels >10mg/l which are indicative of an acute immune response. We sought to specifically investigate the associations of CRP levels >10mg/l with OS, DFS, and risk of recurrence. Patients with CRP >10mg/l had an almost three-fold increase in risk of mortality or recurrence compared to patients with CRP ≤ 10mg/l: e.g., $HR_{log2,\ OS}$: 2.75; 95% CI: 1.57–4.79; p<0.0001; Supplementary Table 9, consistent across study site (Supplementary Table 10) and tumor site (Supplementary Table 11). We have performed additional analyses to investigate correlations between the investigated biomarkers. Correlations ranged between r=0.31 (sICAM-1 and TNF-alpha) up to r=0.74 (CRP and SAA). We have performed additional analysis to test for the independence of the associations for CRP as the main biomarker as it showed the strongest correlations. Data were comparable: e.g., rectal cancer: overall-survival: $CRP_{\text{no additional adjustment}}$: HR:1.19 (1.01, 1.39), $CRP_{\text{adjusted for all biomarkers}}$: HR: 1.25 (1.02,1.53). We have further performed sensitivity analyses additional adjusting for NSAID use one months prior to surgery. We observed comparable results. E.g., CRP_{OS}: rectal cancer $_{\text{not adjusted for NSAID}}$: HR: 1.17 (1.00,137) compared to rectal cancer $_{\text{adjusted for NSAID}}$: HR: 1.19 (1.01, 1.39).

DISCUSSION

In this study we examine associations of a comprehensive panel of systemic biomarkers of inflammation, cell-to-cell adhesion, and angiogenesis, all measured prior to surgery, with clinical outcomes in prospectively followed colon and rectal cancer patients. This is the first study to date, to evaluate the association of adhesion molecules and angiogenesis biomarkers with clinical outcomes and exploring differences in associations across tumor site.

We observed statistically significant associations of CRP with OS, risk of recurrence, and DFS. CRP is an acute phase protein that is a marker for acute inflammation and infection. There is strong and consistent evidence for a direct association of CRP with clinical outcomes in CRC patients.(13,16) The present study confirmed previous results, as we have shown that pre-surgical concentrations of CRP are associated with OS, DFS, and risk of recurrence. Notably, we observed statistically significant heterogeneity in the associations of CRP with colon and rectal cancer comparing top to bottom tertiles. This is in line with two previous retrospective studies showing an association of CRP with colon, but not rectal cancer.(5,37) The lack of an association with rectal cancer could reflect a distinct alternative biological pathway for the development of rectal cancer. In agreement with previous studies(13,47) we have shown that elevated concentrations of CRP (>10mg/l) were associated with a 4-fold increase in risk for OS, DFS, and risk of recurrence compared to patients with CRP <=10mg/l, although independent of tumor site.

We observed statistically significant associations of SAA with OS, DFS, and risk of recurrence. SAA is an acute phase protein that is mainly synthesized in the liver and induces inflammation. A previous population-based study linked SAA to 26% increase in risk of being diagnosed with colorectal cancer.(4) SAA has further been linked to OS in a variety

of solid tumors (e.g., urinary system or gastrointestinal cancers) including colorectal cancer.(48) There is *in vitro* evidence that SAA can stimulate tumor invasion by influencing the adhesion of tumor cells to platelets.(49) Additionally, SAA can facilitate tissue infiltration by monocytes and neutrophils and further enable recruitment of myeloid cells to the liver.(50) This cellular remodeling of the liver leads to a pro-metastatic niche that is supportive of colonization of disseminated tumor cells in the liver,(51) which is the most common organ for colorectal cancer metastasis.(52)

sICAM-1 and sVCAM-1 were statistically significantly associated with death among both colon and rectal cancer patients. Both biomarkers belong to the family of cell-adhesion molecules that shed from the cell surface in to the systemic circulation. There is growing evidence on the relationship between circulating cell adhesion molecules with Epithelial Mesenchymal Transition (EMT), which is linked to poor prognosis in cancer patients.(53) The prognostic relevance of adhesion molecules such as sICAM-1, sVCAM-1 has been previously shown in colorectal and breast cancer and has been linked to their impact on leukocyte trafficking events.(29) For example, sICAM-1 in the serum of women with breast cancer was significantly higher compared to healthy women.(54) Elevated plasma levels of sICAM-1 are related to inflammation, infections, and numerous neoplasms, including gastric, pancreatic, prostate, colon, and other cancers.(55) The biological mechanisms underlying the association of sICAM-1 and sVCAM-1 with increased risk of mortality in the present study are not completely understood. However, cell adhesion is a key mediator of cancer progression and facilitates immune evasion.(56) This notion is supported by *in vitro* evidence that proinflammatory biomarkers such as sICAM-1 influence the adhesion of human colon carcinoma cells in the systemic circulation to the lung endothelium and, thereby, promote metastasis.(55) Dietary intake of naturally occurring inhibitors of cell-adhesion molecules, such as flavonols (e.g., quercetin) (57) have been linked to reduced colorectal cancer risk,(58) likely *via* inhibition of cell-to-cell adhesion.(59) Although there are as yet no data in humans, there is *in vivo* evidence that quercetin induces apoptosis in KRAS-mutant colorectal cancer cells.(59) This is supported by a pre-clinical study showing that alternate consumption of quercetin and beta- glucan reduces mortality rates in mice with colorectal cancer.(60)

In stratified analysis by tumor site, we observed significant heterogeneity in the association of VEGF-D with OS with a higher risk of death for patients diagnosed with rectal cancer compared to no association in patients with colon cancer. A similar pattern of heterogeneity was observed for DFS and risk of recurrence, although not statistically significant. VEGF-D is a secreted protein that can promote remodeling of blood vessels and lymphatics in development and disease and has been linked to angiogenesis in colorectal cancer previously.(61) Angiogenesis is an essential process for tumor and cancer cell growth, invasion and dissemination, and angiogenesis blockade is an active area of clinical and translational cancer research.(62) The observation of a suggestive protective effect of VEGF-D on prognosis in colon cancer is unexpected. Given the wide range of confidence intervals this result need to be interpreted with caution. However, there have been previous meta-analysis that have linked VEGF-D to worse clinical outcomes in colorectal cancer,(63) to date no stratified analysis have been performed to explore differences between colon and rectal cancer. Tyrosine-kinase inhibitors (TKI) like sorafenib and sunitinib neutralize

angiogenesis biomarkers such as VEGF-D and prevent tumorigenesis. In two recent phase I/II clinical trials it has been shown that particularly patients with locally advanced rectal cancer respond well to sorafenib in combination with standard therapy.(64) This result is in line with the observed associations of VEGF-D, particularly in rectal cancer patients in the present study.

This is the first prospective study to link VEGF-D to increased risk of death specifically in patients with rectal cancer. We have identified one study that reported increased expression of VEGF-D in rectal cancer tissue compared to rectal polyps and normal rectal tissue suggesting that tumor-promoting processes are induced by VEGF-D.(65)

We did not observe statistically significant associations of TNF-alpha or IL-8 with any of the clinical outcomes investigated.

This study has several strengths and limitations. This is the first study in colorectal cancer patients investigating associations of a large panel of adhesion molecules, angiogenesis biomarkers, and inflammatory biomarkers with OS, DFS, and risk of recurrence in colorectal cancer patients, though our sample size was limited for some of the stratified analysis. A limitation of this and previous studies is that participants provided a single blood sample. However, studies reported that a single measure accurately captures the short-term variability of most of the analyzed biomarkers also in long time stored samples.(66,67) A number of statistical tests were conducted; therefore, some reported observations may be due to chance. However, all statistical analyses were hypothesis driven.

In conclusion, our data provide first evidence that systemic concentrations of angiogenesis biomarkers and adhesion molecules may contribute to an increased risk of recurrence and death with significant differences between colon and rectal cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Baseline characteristics of patients recruited into the ColoCare Study.

Characteristics	Study Population
Sex (n, %)	
Female	162 (38.03%)
Male	264 (61.97%)
Age at recruitment (years, median, SD)	62.68 (12.59)
Body Mass Index (BMI, m^2, median, SD)	27.52 (5.19)
Neoadjuvant treatment (n, %)	
No	313 (73.82%)
Yes	111 (26.18%)
Surgery	
No	4 (0.94%)
Yes	422 (99.06%)
Adjuvant treatment (n, %)	
No	259 (62.11%)
Yes	158 (37.89%)
Tumor stage (n, %)	
I	85 (19.95%)
II	142 (33.33%)
III	199 (46.71%)
Tumor site (n, %)	
Colon	210 (49.30%)
Rectum	216 (50.70%)
NSAID ** **use in the past month**	
No	234 (66.67%)
Yes	117 (33.33%)
Vital status (n, %)	
Alive	358 (84.63%)
Follow-up time (months, median, SD)	31.79 (16.29)
Deceased	65 (15.37%)
Follow-up time (months, median, SD)	15.39 (13.22)
Recurrence *	
None	333 (84.95%)
Follow-up time (months, median, SD)	31.84 (16.62)
Yes	59 (15.05%)
Follow-up time (months, median, SD)	15.96 (11.18)
Local/Regional Recurrence (n, %)	15 (25.42%)
Distant Recurrence (n, %)	44 (74.85%)

* For n=34 patients information on recurrence is not available. The population at risk of recurrence is n=392.

** NSAID:Non-steroidal anti-inflammatory drugs.

Table 2.

Associations of inflammatory biomarker, adhesion molecules, and angiogenesis biomarkers with overall survival in tertiles (hazard ratio and 95% confidence interval) and stratified by tumor site (colon *versus* rectal cancer)

	Overall Survival		Colon Cancer		Rectal Cancer		
	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	$p_{heterogeneity}$
CRP	355 (58; per tertile)		181 (26; per tertile)		174 (32; per tertile)		
T1	ref		ref		ref		
T2	1.49 (0.72, 3.10)		4.54 (0.94, 21.93)		0.77 (0.29, 2.00)		
T3	3.06 (1.55, 6.06)	0.001	7.71 (1.70, 35.04)	0.004	2.20 (0.96, 5.02)	0.07	0.03
SAA	354 (57: per tertile)		181 (26; per tertile)		173 (31; per tertile)		
T1	ref		ref		ref		
T2	1.29 (0.61, 2.74)		1.63 (0.47, 5.67)		1.05 (0.39, 2.77)		
T3	2.10 (1.05, 4.20)	0.03	3.56 (1.15, 11.03)	0.02	1.51 (0.60, 3.79)	0.36	0.47
VEGF-A	360 (58; per tertile)		185 (26; per tertile)		175 (32; per tertile)		
T1	ref		ref		ref		
T2	1.37 (0.70, 2.69)		2.30 (0.78, 6.77)		1.07 (0.44, 2.61)		
T3	1.51 (0.78, 2.91)	0.22	1.78 (0.64, 4.92)	0.32	1.55 (0.64, 3.78)	0.32	0.46
VEGF-D	359 (58; per tertile)		184 (26; per tertile)		175 (32; per tertile)		
T1	ref		ref		ref		
T2	0.92 (0.47, 1.80)		0.84 (0.30, 2.35)		1.13 (0.46, 2.77)		
T3	1.40 (0.72, 2.73)	0.33	0.82 (0.30, 2.19)	0.69	2.46 (0.93, 6.49)	0.08	0.16
sICAM-1	355 (58; per tertile)		184 (26; per tertile)		174 (32; per tertile)		
T1	ref		ref		ref		
T2	2.25 (1.00, 5.04)		6.45 (1.62, 25.66)		1.45 (0.54, 3.89)		
T3	4.12 (1.94, 8.77)	0.001	8.68 (2.40, 31.44)	0.0007	2.99 (1.12, 7.99)	0.02	0.11
sVCAM-1	355 (58; per tertile)		181 (26; per tertile)		174 (32; per tertile)		
T1	ref		ref		ref		
T2	0.85 (0.41, 1.79)		1.41 (0.34, 5.81)		0.69 (0.28, 1.70)		
T3	2.51 (1.29, 4.88)	0.004	4.07 (1.16, 14.29)	0.009	2.18 (0.92, 5.20)	0.11	0.21
IL-6	370 (58; per tertile)		174 (24: per tertile)		196 (34; per tertile)		
T1	ref		ref		ref		

	Overall Survival		Colon Cancer		Rectal Cancer		
	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	$p_{heterogeneity}$
T2	1.82 (0.93, 3.57)		2.08 (0.73, 5.95)		1.69 (0.70, 4.10)		
T3	2.15 (1.05, 4.42)	0.04	2.35 (0.72, 7.68)	0.14	1.79 (0.72, 4.43)	0.21	0.85
IL-8	370 (58; per tertile)		174 (24: per tertile)		196 (34; per tertile)		
T1	ref		ref		ref		
T2	0.80 (0.40, 1.61)		0.74 (0.24, 2.29)		0.89 (0.37, 2.17)		
T3	1.29 (0.66, 2.50)	0.40	1.46 (0.46, 4.61)	0.43	1.18 (0.53, 2.65)	0.69	0.76
TNF-α	370 (58; per tertile)		174 (24: per tertile)		196 (34; per tertile)		
T1	ref		ref		ref		
T2	1.77 (0.90, 3.46)		1.88 (0.54, 6.56)		1.51 (0.66, 3.42)		
T3	1.85 (0.96, 3.56)	0.06	2.53 (0.79, 8.07)	0.11	1.36 (0.58, 3.20)	0.42	0.40

*adjusted for age at recruitment (continuous), sex (male/female), body mass index (continuous), tumor stage (I, II, III), tumor site (colon/rectum), and study site (HCI/HD). P values <0.05 are statistically significant.

Table 3.

Associations of inflammatory biomarker, adhesion molecules, and angiogenesis biomarkers with disease-free survival in tertiles (hazard ratio and 95% confidence interval) and stratified by tumor site (colon *versus* rectal cancer)

	Disease-free Survival		Colon Cancer		Rectal Cancer		
	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	$p_{heterogeneity}$
CRP	320 (73; per tertile)		165 (29; per tertile)		155 (44; per tertile)		
T1	ref		ref		ref		
T2	1.34 (0.69, 2.58)		3.18 (0.63, 16.15)		1.22 (0.57, 2.60)		
T3	2.70 (1.50, 4.89)	0.0007	9.63 (2.20, 42.20)	0.0004	1.73 (0.84, 3.56)	0.14	0.04
SAA	320 (73; per tertile)		165 (29; per tertile)		155 (44; per tertile)		
T1	ref		ref		ref		
T2	1.24 (0.64, 2.39)		2.94 (0.72, 12.01)		1.00 (0.46, 2.14)		
T3	2.04 (1.10, 3.76)	0.02	7.37 (2.06, 26.28)	0.0007	1.20 (0.56, 2.58)	0.63	0.03
VEGF-A	325 (74; per tertile)		169 (29; per tertile)		156 (45; per tertile)		
T1	ref		ref		ref		
T2	1.09 (0.61, 1.94)		1.07 (0.41, 2.84)		1.16 (0.56, 2.41)		
T3	1.29 (0.73, 2.29)	0.38	1.18 (0.49, 2.84)	0.71	1.37 (0.63, 2.95)	0.42	0.72
VEGF-D	624 (74; per tertile)		168 (29; per tertile)		156 (45; per tertile)		
T1	ref		ref		ref		
T2	0.91 (0.51, 1.64)		0.65 (0.25, 1.67)		1.21 (0.56, 2.61)		
T3	1.36 (0.77, 2.42)	0.30	0.67 (0.27, 1.66)	0.39	2.30 (1.05, 5.02)	0.04	0.05
sICAM-1	320 (73; per tertile)		165 (29; per tertile)		155 (44; per tertile)		
T1	ref		ref		ref		
T2	1.40 (0.73, 2.71)		4.74 (1.18, 18.97)		0.92 (0.43, 1.98)		
T3	2.55 (1.40, 4.64)	0.002	6.78 (1.96, 23.44)	0.002	1.69 (0.80, 3.56)	0.17	0.02
sVCAM-1	320 (73; per tertile)		165 (29; per tertile		155 (44; per tertile)		
T1	ref		ref		ref		
T2	0.68 (0.37, 1.24)		1.23 (0.36, 4.21)		0.56 (0.27, 1.14)		
T3	1.23 (0.70, 2.13)	0.42	2.72 (0.90, 8.20)	0.04	0.79 (0.38, 1.64)	0.42	0.03
IL-6	342 (75; per tertile)		160 (29; per tertile)		182 (46: per tertile)		
T1	ref		ref		ref		

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	Disease-free Survival		Colon Cancer		Rectal Cancer		
	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	$p_{heterogeneity}$
T2	2.57 (1.38, 4.77)		6.56 (2.07, 20.82)		1.66 (0.76, 3.63)		
T3	2.77 (1.43, 5.37)	0.003	4.44 (1.21, 16.23)	0.03	2.22 (1.04, 4.76)	0.04	0.92
IL-8	342 (75; per tertile)		160 (29; per tertile)		182 (46: per tertile)		
T1	ref		ref		ref		
T2	0.88 (0.48, 1.60)		0.96 (0.35, 2.64)		0.94 (0.44, 2.04)		
T3	1.16 (0.66, 2.03)	0.59	1.45 (0.53, 3.99)	0.42	1.12 (0.56, 2.24)	0.75	0.92
TNF-α	342 (75; per tertile)		160 (29; per tertile)		182 (46; per tertile)		
T1	ref		ref		ref		
T2	1.29 (0.73, 2.29)		1.30 (0.44, 3.79)		1.12 (0.56, 2.24)		
T3	1.71 (0.98, 2.98)	0.06	2.49 (0.93, 6.65)	0.06	1.31 (0.64, 2.70)	0.46	0.30

*adjusted for age at recruitment (continuous), sex (male/female), body mass index (continuous), tumor stage (I, II, III), tumor site (colon/rectum), and study site (HCI/HD). P values <0.05 are statistically significant.

Table 4.

Associations of inflammatory biomarker, adhesion molecules, and angiogenesis biomarkers with risk of recurrence in tertiles (hazard ratio and 95% confidence interval) and stratified by tumor sites (colon *versus* rectal cancer)

	Recurrence-free survival		Colon Cancer		Rectal Cancer		
	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	$p_{heterogeneity}$
CRP	320 (25; per tertile)		165 (20; per tertile)		155 (32; per tertile)		
T1	ref		ref		ref		
T2	1.82 (0.82, 4.00)		5.87 (0.64, 54.26)		1.61 (0.66, 3.91)		
T3	3.32 (1.58, 6.95)	**0.001**	14.93 (1.91, 116.96)	**0.002**	2.10 (0.87, 5.09)	0.10	0.06
SAA	320 (25; per tertile)		165 (20; per tertile)		155 (32; per tertile)		
T1	ref		ref		ref		
T2	1.45 (0.65, 3.26)		2.59 (0.42, 15.77)		1.22 (0.49, 3.07)		
T3	2.48 (1.18, 5.23)	**0.01**	10.37 (2.20, 48.95)	**0.001**	1.34 (0.53, 3.41)	0.54	**0.02**
VEGF-A			169 (20; per tertile)		156 (33; per tertile)		
T1	ref		ref		ref		
T2	1.31 (0.65, 2.62)		1.19 (0.37, 3.82)		1.57 (0.64, 3.83)		
T3	1.57 (0.79, 3.41)	0.20	1.27 (0.43, 3.73)	0.67	1.79 (0.72, 4.46)	0.22	0.51
VEGF-D	325 (33; per tertile)		168 (20; per tertile)		156 (33; per tertile)		
T1	ref		ref		ref		
T2	0.94 (0.48, 1.83)		0.60 (0.20, 1.81)		1.42 (0.58, 3.46)		
T3	1.11 (0.55, 2.25)	0.79	0.41 (0.12, 1.38)	0.14	2.21 (0.84, 5.84)	0.11	**0.02**
sICAM-1	320 (52; per tertile)		165 (20; per tertile)		155 (32; per tertile)		
T1	ref		ref		ref		
T2	1.84 (0.85, 3.86)		5.58 (1.04, 29.96)		1.33 (0.56, 3.17)		
T3	2.40 (1.11, 4.87)	**0.03**	6.68 (1.47, 30.36)	**0.01**	1.39 (0.54, 3.57)	0.49	**0.04**
sVCAM-1	320 (52; per tertile)		165 (20; per tertile)		155 (32; per tertile)		
T1	ref		ref		ref		
T2	0.68 (0.35, 1.31)		0.97 (0.26, 3.57)		0.57 (0.25, 1.29)		
T3	0.82 (0.42, 1.61)	0.55	1.30 (0.38, 4.41)	0.60	0.59 (0.24, 1.46)	0.20	0.12
IL-6	342 (55; per tertile)		160 (21; per tertile)		182 (34; per tertile)		
T1	ref		ref		ref		

	Recurrence-free survival		Colon Cancer		Rectal Cancer		
	HR (95% CI)	**p_{trend}**	**HR (95% CI)**	**p_{trend}**	**HR (95% CI)**	**p_{trend}**	**$p_{heterogeneity}$**
T2	1.92 (0.95, 3.85)		6.28 (1.64, 23.94)		1.03 (0.40, 2.59)		
T3	2.12 (1.02, 4.40)	**0.04**	3.72 (0.80, 17.23)	0.10	1.75 (0.76, 4.04)	0.20	**0.01**
IL-8	342 (55; per tertile)		160 (21; per tertile)		182 (34; per tertile)		
T1	ref		ref		ref		
T2	0.82 (0.41, 1.67)		0.92 (0.29, 2.93)		0.83 (0.32, 2.12)		
T3	1.14 (0.60, 2.17)	0.68	1.54 (0.47, 5.05)	0.45	1.11 (0.50, 2.48)	0.79	0.93
TNF-α	342 (55; per tertile)		160 (21; per tertile)		182 (34; per tertile)		
T1	ref		ref		ref		
T2	1.13 (0.60, 2.16)		1.06 (0.35, 3.25)		1.01 (0.45, 2.27)		
T3	1.26 (0.66, 2.43)	0.55	1.35 (0.45, 3.99)	0.59	1.16 (0.50, 2.66)	0.65	0.69

*adjusted for age at recruitment (continuous), sex (male/female), body mass index (continuous), tumor stage (I, II, III), tumor site (colon/rectum), and study site (HCI/HD). P values in bold are statistically significant.

Table 5.

Associations of inflammatory biomarker, adhesion molecules, and angiogenesis biomarkers with risk of distant recurrence in tertiles (hazard ratio and 95% confidence interval) and stratified by tumor site (colon *versus* rectal cancer).

	Risk of distant recurrence		Colon Cancer		Rectal Cancer		
	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	HR (95% CI)	p_{trend}	$p_{heterogeneity}$
CRP	306 (38; per tertile)		161 (16; per tertile)		145 (22; per tertile)		
T1	ref		ref		ref		
T2	1.18 (0.45, 3.11)		4.43 (0.44, 44.19)		1.02 (0.32, 3.29)		
T3	3.42 (1.50, 7.76)	0.002	13.92 (1.72, 112.56)	0.004	2.09 (0.77, 5.70)	0.16	0.10
SAA	306 (38; per tertile)		161 (16; per tertile)		145 (22; per tertile)		
T1	ref		ref		ref		
T2	1.71 (0.63, 4.65)		1.99 (0.27, 14.61)		1.73 (0.52, 5.78)		
T3	3.53 (1.44, 8.64)	0.003	8.12 (1.68, 39.25)	0.005	2.13 (0.66, 6.86)	0.21	0.10
VEGF-A	311 (39; per tertile)		165 (16; per tertile)		146 (23; per tertile)		
T1	ref		ref		ref		
T2	1.08 (0.47, 2.46)		1.05 (0.31, 3.56)		1.41 (0.44, 4.55)		
T3	1.62 (0.75, 3.53)	0.21	0.94 (0.27, 3.26)		2.32 (0.78, 6.94)	0.12	0.15
VEGF-D	310 (39; per tertile)		164 (16; per tertile)		146 (23; per tertile)		
T1	ref		ref		ref		
T2	0.80 (0.36, 1.80)		0.56 (0.15, 2.13)		1.38 (0.46, 4.09)		
T3	1.07 (0.47, 2.40)	0.87	0.53 (0.15, 1.94)	0.33	1.74 (0.54, 5.57)	0.35	0.06
sICAM-1	306 (38; per tertile)		161 (16; per tertile)				
T1	ref		ref		ref		
T2	2.47 (1.00, 6.14)		4.01 (0.71, 22.80)		2.34 (0.78, 7.00)		
T3	2.73 (1.10, 6.74)	0.03	5.22 (1.10, 24.85)	0.03	1.45 (0.41, 5.12)	0.53	0.08
sVCAM-1	306 (38; per tertile)		161 (16; per tertile)		145 (22; per tertile)		
T1	ref		ref		ref		
T2	0.77 (0.35, 1.67)		1.20 (0.29, 5.07)		0.63 (0.23, 1.71)		
T3	0.89 (0.40, 1.97)		1.39 (0.34, 5.67)	0.64	0.49 (0.16, 1.52)	0.19	0.09
IL-6	328 (41; per tertile)		157 (18; per tertile)		171 (23; per tertile)		
T1	ref		ref		ref		

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	Risk of distant recurrence		Colon Cancer		Rectal Cancer		
	HR (95% CI)	**p_{trend}**	**HR (95% CI)**	**p_{trend}**	**HR (95% CI)**	**p_{trend}**	**$p_{heterogeneity}$**
T2	1.56 (0.71, 3.43)		4.93 (1.29, 18.87)		0.54 (0.14, 2.01)		
T3	1.68 (0.74, 3.80)	0.21	2.64 (0.51, 13.73)	0.19	1.33 (0.52, 3.37)	0.59	0.002
IL-8	328 (41; per tertile		157 (18; per tertile)		171 (23; per tertile)		
T1	ref		ref		ref		ref
T2	0.63 (0.27, 1.46)		0.84 (0.24, 2.97)		0.49 (0.13, 1.81)		
T3	1.08 (0.52, 2.24)	0.83	1.78 (0.49, 6.40)	0.37	0.94 (0.37, 2.41)	0.89	0.45
TNF-α	328 (41; per tertile		157 (18; per tertile)		171 (23; per tertile)		
T1	ref		ref		ref		
T2	0.93 (0.43, 2.01)		0.98 (0.29, 3.33)		0.71 (0.24, 2.07)		
T3	1.15 (0.55, 2.42)	0.74	1.58 (0.48, 5.22)	0.47	0.83 (0.30, 2.27)	0.66	0.40

*adjusted for age at recruitment (continuous), sex (male/female), body mass index (continuous), tumor stage (I, II, III), tumor site (colon/rectum), and study site (HCI/HD). P values <0.05 are statistically significant.