Preoperative Serum C-Reactive Protein and its Prognostic Significance in Patients with Stage III-IV Colorectal Cancer

FRANCO LUMACHI[1], STEFANO M.M. BASSO[2], DAVIDE A. SANTEUFEMIA[3], MARIO ERMANI[1], GIOVANNI LO RE[4] and GIORDANO B. CHIARA[2]

[1]*Department of Surgery, Oncology and Gastroenterology (DiSCOG), University of Padua, School of Medicine, Padova, Italy;*
[2]*Surgery 1 and* [4]*Medical Oncology, S. Maria degli Angeli Hospital, Pordenone, Italy;*
[3]*Medical Oncology, City Hospital, Alghero (SS), Italy*

Abstract. *The molecular mechanism underlying the development of colorectal cancer (CRC) is not yet fully-understood, but there is evidence that inflammation plays a key role. Several circulating tumor and inflammatory markers can be useful for studying patients with CRC. It has been suggested that high serum levels of C-reactive protein (CRP) are associated with elevated risk of various malignancies and that CRP may affect survival of patients with CRC. We analyzed the relationship existing between the stage of the disease and baseline CRP serum levels in a group of 91 patients undergoing surgery for stage III (N=72, 79.1%) and IVa (N=19, 20.9%) CRC. There were 51 (56%) men and 40 (44%) women, with a median age of 66 years. Prior to surgery, all patients underwent quantitative serum CRP measurement. The overall 5-year survival was 37.1±13.0 months. Patients with stage III disease and the sub-group with CRP<3 mg/l (N=43, 47.3%) had a longer survival (p<0.01) than patients with stage IVa and the sub-group with CRP≥3 mg/l (N=48, 52.7%). No relationship between the age of the patients and CRP levels was found (R=–0.005, p=0.96), whilst there was a significant inverse relationship between survival and CRP level (R=–0.37, y=37.5343-0.5868x, p=0.0003). Using multivariate Cox model analysis (forward stepwise method), adjusted for age, CRP and CRC stage were independent parameters related to survival, with a relative risk of 3.5 (95% confidence interval=1.5-8.2) and 8.1 (95% confidence interval=3.0-21.3), respectively. In conclusion, CRP is a sensitive and easily detectable serum marker that can be useful in patients with CRC, allowing their better clinical stratification.*

Colorectal cancer (CRC) accounts for 8-9% of all cancers and is the second most frequent cause of cancer-related death in Western countries (1). The 5-year overall survival for patients with CRC after curative surgical resection is approximately 63-64%, ranging from 83% to 8% for patients with stage IIIa and IV, respectively (2, 3). Cancer progression depends on a complex interaction between tumor characteristics and host response, but the relationship between chronic inflammation, immunity and cancer development has not yet been completely elucidated (4, 5). It has been hypothesized that long-standing inflammation predisposes to CRC development (6). C-Reactive protein (CRP) is a sensitive and non-specific marker of tissue damage and inflammation, and is predominantly produced by hepatocytes under transcriptional control by cytokines, including interleukin-6 (7). Several studies showed that high serum CRP levels are associated with elevated risk of various malignancies and that CRP may affect survival of patients with CRC (8).

The purpose of this study was to analyze the relationship between the stage of the disease and baseline serum CRP levels in patients with advanced CRC.

Patients and Methods

Study population. Ninety-one patients undergoing surgery for histologically confirmed CRC were enrolled in the study. There were 51 (56%) men and 40 (44%) women, with a median age of 66 years (range=47-79 years). Written informed consent was obtained from all the participants. According to the American Joint Committee on Cancer (AJCC) staging system, postoperative staging classified 72 (79.1%) patients as having stage III disease (T1-4, N1-2, M0) and 19 (20.9%) as having stage IVa (T1-4, N1-2, M1a).

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Correspondence to: Professor Franco Lumachi, University of Padua, School of Medicine, Department of Surgery, Oncology and Gastroenterology (DiSCOG), Via Giustiniani 2, 35128 Padova, Italy. Tel: +39 0498211812, Fax: +39 0498214394, e-mail: flumachi@unipd.it

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Table I. *Overall survival (mean±standard deviation) of the four subgroups of patients and respective p-values.*

Parameter	Stage III *vs.* Stage IVa	CRP<3 vs. CRP≥3 mg/l
Number of patients	72 *vs.* 19	43 *vs.* 48
Survival (months)	46.5±1.9 *vs.* 32.1±2.1	44.1±1.7 *vs.* 33.0±2.9
95% CI of the difference between means (months)	13.4-15.4	12.1-10.1
t	28.7	21.9
p	0.0001	0.0001

CRP, C-Reactive protein; 95% CI, 95% confidence interval.

Prior to surgery, all patients underwent quantitative serum CRP measurement, using a commercially available human CRP enzyme linked immunosorbent assay (ELISA) kit, which employs a quantitative sandwich enzyme immunoassay technique. The laboratory reference range of CRP was 1-3 mg/l and the population was dichotomized according to CRP<3 mg/l *vs.* CRP≥3 mg/l. Those patients with CRP>10 mg/l, suggesting overt inflammation at baseline, had been previously excluded (9).

Statistical analysis. Continuous variables are summarized as the mean±standard deviation (SD) and comparisons between groups were performed using Student's *t*-test, when required. We used Kaplan–Meier estimate survival curves to analyze the mortality rates of different subgroups. Relative risk (RR) and 95% confidence intervals (95% CI) were also calculated. Log-rank test and Cox regression analysis (forward stepwise method) were used to study how the covariates affect survival. All statistical tests were two-sided. Relationships between parameters were assessed using Pearson's correlation coefficient (R) and the regression line calculation. The differences were considered significant at a *p*-value of less than 0.01.

Results

The age of the patients (67.3±8.4 years) did not differ significantly between men and women (p=0.28). Overall, the mean preoperative serum CRP level was 4.1±3.2 mg/l (median=4 mg/l, range=1-10 mg/l). Patients were followed-up after surgery for at least five years, during which time 43 (47.3%) patients died (stage III=31/72, 43.0%; stage IVa=17/19, 89.5%). The overall survival was 37.1±13.0 months and did not differ according to gender (p=0.52). Patients with stage III disease and the subgroup with CRP<3 mg/l (N=43, 47.3%) had a longer survival (p<0.01) than did patients with stage IVa disease and the subgroup with CRP≥3 mg/l (N=48, 52.7%) (Table I).

No relationship between the age of the patients and CRP level was found (R=–0.005, y=9.1877–0.0023x, p=0.96), whilst there was a significant inverse relationship between survival and CRP level (R=–0.37, y=37.5343–0.5868x, p=0.0003). Using multivariate Cox model analysis (forward stepwise method), adjusted for age, serum CRP level and stage of the disease were independently related to survival, with RR of 3.5 (95% CI=1.5-8.2, p=0.003) and 8.1 (95% CI=3.0-21.3, p<0.001), respectively. Figure 1 shows the 5-year Kaplan–Meier cumulative survival of subgroups of patients.

Discussion

According to the 2014 Surveillance, Epidemiology, and End Results Program database, the number of new cases of CRC in the USA was approximately 44 per 100,000 persons per year and the number of deaths was approximately 16 per 100,000 men and women per year, with an overall 5-year survival of 64.7% (10). In Europe, every year 412,000 people are diagnosed with CRC, and 207,000 patients die from it (11). Unfortunately, the molecular mechanism underlying the development of CRC is not yet fully-understood, but there is evidence that inflammation plays a key role (12). Several circulating tumor and inflammatory markers can be useful for studying patients with CRC, including serum carbohydrate antigen (CA) 19-9, CA 72-4, angioprotein-2 and CRP, with which the Glasgow prognostic score can be calculated (13-16). Some histological findings, such as vascular and lymphatic invasion, overexpression of several molecular markers and gene mutations are also used as prognostic factors (17-19).

C-Reactive protein, so-called for its ability to precipitate the C-polysaccharide of *Streptococcus pneumoniae*, was one of the first non-specific acute-phase proteins of inflammation to be described (7). CRP is a 224-amino-acid protein whose production has been found to be related to survival in patients with cancer by promoting angiogenesis and inducing proinflammatory cytokines from immune cells (20, 21). The evidence for an association between increased CRP and CRC is contradictory, considering that both environmental and genetic factors (*e.g.* human *CRP* gene polymorphism) or individual factors (*e.g.* obesity) may influence baseline serum CRP levels, which vary widely in healthy individuals (20, 22, 23). In patients with CRC, increased CRP levels are also associated with lymph node involvement and larger tumor size, as well as with levels of other markers such as insulin-like growth factor-1 and vascular endothelial growth factor-A (15, 24).

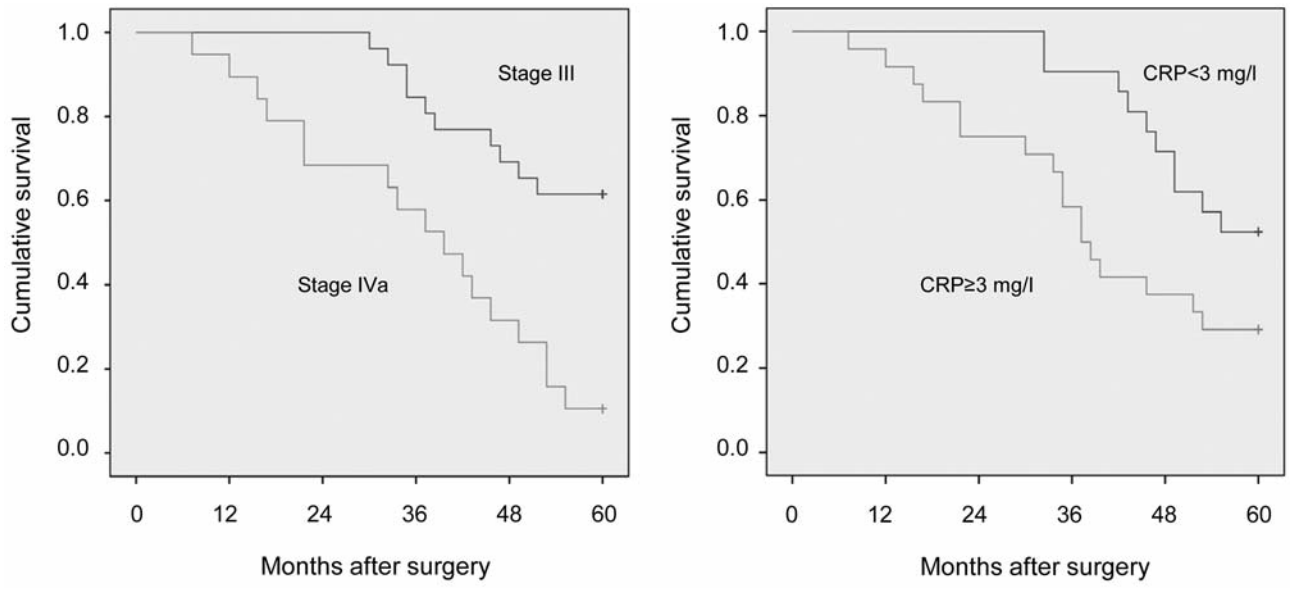

Figure 1. *Kaplan–Meier survival curves for patients with stage III and IVa colorectal cancer and for subgroups with C-reactive protein (CRP) <3 mg/l and ≥3 mg/l.*

The association between CRP and an increased risk of CRC and cancer-related mortality has long been observed. In a European multi-centric study, after adjustment for stage, the relative excess risk of death of patients with CRC was 1.59 (25). A systematic review showed that the RR for CRC per one unit change in natural log-transformed CRP level was 1.12 (95% CI=0.86-1.30), with a weak difference between men and women (26). According to the results obtained by the European Prospective Investigation into Cancer and Nutrition group, the risk is significantly increased in men but not in women (RR=1.74 *vs*. 10.6) and in colonic cancer but not in rectal cancer (RR=1.36 *vs*. 1.02), independently of obesity, dyslipidemia and insulin resistance (27). In a more recent study, the estimated adjusted RR of having CRC in patients with elevated serum CRP was 1.37 (28). We found a higher RR of CRC of 3.5, and independent of disease stage, in the subgroup of patients with CRP>3 mg/l. These results are similar to those reported in the U.S. National Health and Nutrition Examination Survey in which patients with CRC and clinically raised baseline serum CRP had a significantly greater risk of death (hazard ratio=2.36-2.47), independent of excess body fat (29). The survival of patients who undergo surgery for stage IV CRC is low, since the median survival time is approximately 22 months and the 2-year and 5-year survival is 36% and 11%, respectively (30). In our sub-group of stage IVa CRC, the 5-year survival was 10.5% and, as expected, the RR of being affected by cancer was 8.6 compared to that for patients with stage III disease.

In conclusion, since inflammation is one of the multifactorial causes of cancer, the availability of a non-specific but sensitive, easily detectable and inexpensive serum marker such as CRP can be useful in patients with CRC, allowing their better clinical stratification.

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