Elevated serum CRP level as potential biomarker of different stages of bladder cancer

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Abstract

Objectives: The aim of this study was to evaluate serum C-reactive protein (CRP) concentration in patients with bladder cancer, as well as to determine its potential role as biomarker in the differentiation of different stages of the disease.

Methods: The study included 90 patients with bladder carcinoma who were divided into 3 groups: 30 patients with non-invasive, superficial (Ta), 30 patients with superficial (T1), and 30 patients with invasive (T2-T4) bladder cancer. Serum CRP level was determined by laser nephelometry.

Results: Serum CRP levels in T2-T4 group of patients was 8.65 (3.20-18.20) mg/dL and significantly higher compared to the serum CRP level in Ta group of patients (1.55 (0.67 - 3.35) mg/dL; p<0.005), T1 group (1.90 (1.27-7.20) mg/dL, p=0.006) and compared to the control group of patients (1.20 (0.90-2.10) mg/dL; p<0.005). Multiple linear regression revealed that serum CRP level was independently associated with the tumor size (β = 0.376; p<0.001). There was an independent positive association between CRP and high progressing potential of the bladder cancer.

Conclusion: CRP might have a significant role as a biomarker in the diagnosis of this disease, with special attention on its potential role in differentiating different stages of the disease.

Keywords: C-reactive protein, bladder cancer, biomarker, inflammation

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Introduction

C-reactive protein is a widely used systemic biomarker for diagnosing of acute and chronic inflammation. Serum CRP level increases in conditions such as bacterial infections, inflammatory diseases, myocardial infarction, trauma and cancer. This, acute phase protein is produced in the liver as a response to increased level of cytokines after inflammatory stimulation. Cytokines that are able to induce the increase in CRP level are mostly interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor (TNF) [1, 2].

The association between CRP and carcinoma is presented through two possible hypotheses. The first hypothesis states that elevated CRP levels are a result of an underlying cancer or a premalignant state, whereas the second hypothesis states that chronic inflammation and elevated CRP might have a causal role in carcinogenesis [3].

Studies investigating the relationship between genetic polymorphisms and levels of circulating CRP showed that circulating CRP do not cause cancer [4]. A lack of association between elevated CRP levels and increased cancer risk does not, however, invalidate its potential as biomarker in predicting the risk for the occurrence of this disease, and improving staging and treatment allocation in patients diagnosed with cancer. Indeed, studies on general population, showed that those with the highest levels of CRP had a 1.3 times increased risk of developing cancer of any type and 2-fold higher risk of lung cancer [4]. In recent studies it has been shown that patients with a large number of malignant diseases showed significantly higher concentrations of CRP in plasma or serum compared to the control group of
Elevated CRP levels were associated with shorter survival in patients with several types of cancers, including non-Hodgkin lymphoma, lung and esophageal cancer [7]. In patients with prostate cancer CRP levels were significantly associated with poorer prognosis and higher concentrations were found in patients with bone metastases [7]. Several studies have shown that CRP correlates with poor prognosis in patients with carcinoma of the urinary bladder and monitoring of its serum concentration might serve as biomarker in predicting the aggressiveness of this type of cancer and potential impact of therapy [8-10].

The aim of this study was to evaluate serum CRP concentration in patients with bladder cancer, as well as to determine its potential role as biomarker in the differentiation of different stages of the disease.

MATERIALS AND METHODS

Patients
The study was designed as a cross-sectional study. The study included 90 patients, both genders with confirmed bladder cancer. Also, the study included 30 healthy subjects as controls. The patients were divided into three groups: 30 patients with non-invasive, superficial bladder cancer (Ta group), 30 patients with superficial bladder cancer (T1 group), and 30 patients with invasive bladder cancer (T2-T4 group). The control group included 30 apparently healthy volunteers, without manifest signs of urological diseases, who were age and gender matched to the bladder carcinoma groups. Due to the potential impact of inflammatory condition, patients and control subjects with acute or chronic inflammatory diseases, who were diagnosed with Crohn’s disease and ulcerative colitis, pituitary tumors, multiple sclerosis or other malignant neoplasia were excluded from the study. The study was approved by the Clinical Center University of Sarajevo Ethics Committee. All patients and subjects were acquainted with the procedure of the research and signed the required written approval for participation in the project.

Methods
After carrying out diagnostic procedures, which included physical examination, urological examination, ultrasonography (US) of bladder, intravenous urography (IVU), urethrocystoscopy, a definite indication for surgery (transurethral resection - TUR) was given. Complete diagnostic evaluation was conducted at the Department of Urology (transurethral resection-TUR of bladder tumor), Institute of Clinical Chemistry and Biochemistry (biochemical analysis), and the Institute of Radiology (US, IVU) at Clinical Center University of Sarajevo (CCUS). Tissue samples were sent to the Institute of Pathology at CCUS. After histopathological analysis definitive selection of patients was performed. Patients were also divided into groups according to grades which are defined as low-grade and high-grade after histopathological analysis. A group of low-grade is also defined as the group with low metastatic potential, while the group with high grade was defined as high metastatic potential group [11].

Measurements of serum C-reactive protein levels
Due to the possible influence of surgery and diagnostic procedures on the serum CRP concentration, blood sampling for biochemical analysis was performed before any procedures, during first examination of the patients at the Urology Clinic, Clinical Center University of Sarajevo. The blood sample was collected during the routine biochemical tests that are part of the protocol in diagnosing the disease. Serum CRP concentration was determined by means of particle enhanced immunonephelometry with the use of BN II analyzer at the Institute of Clinical Chemistry and Biochemistry, University of Sarajevo Clinics Centre. CardioPhase high-sensitivity CRP (DADE BEHRING) was used as a diagnostic reagent. CardioPhase hsCRP consists of a suspension of polystyrene particles coated with mouse monoclonal antibodies to CRP. Reference interval for CRP with the use of this method is from 0 to 5 mg/l.

Statistical analysis
Data are reported as median and interquartile range (IQR). Data distribution was determined by Shapiro-Wilk test. Since CRP was highly skewed, data were analyzed with Kruskal-Walls or Mann-Whitney U Test. Associations between continuous variables were tested with Spearman’s rank correlation analysis. The specificity and sensitivity of CRP as potential progression marker of disease was investigated by ROC curve. Binary logistic regression was performed to show how changing variable can influence on progression of the disease. p values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS 13.0 statistical software system.

Results
The median serum CRP concentration in T1 and T2-T4 group was 1.90 (1.27-7.20) mg/dL and 8.65 (3.20-18.20) mg/dL respectively and were significantly higher compared to the serum CRP concentration in control group (1.20 (0.90 to 2.10)) mg/dL(p <0.005) (Figure 1). However, median serum CRP concentration in T2 group of patients was not significantly different compared to the control group (p = 0.745). Serum CRP level in T2-T4 group was 8.65 (3.20-18.20) mg/dL and significantly higher compared to the Ta group (1.55 (0.67 to 3.35) mg/dL (p <0.005)) and T1 group (1.90
The serum C-reactive protein (CRP) concentration was measured in patients with different stages of bladder cancer and compared to control subjects. There was no significant difference in serum CRP concentration between Ta and T1 group of patients (p = 0.092) (Figure 1).

As presented in Figure 2, there was a statistically significant positive correlation between tumor size and serum concentration of CRP in patients with bladder cancer (p<0.01). Multiple linear regression revealed that serum CRP level was independently associated with the tumor size (β = 0.376, p<0.001). Our results revealed independent positive association between CRP and high progressing potential of bladder carcinoma (p=0,016)(Table 1).

ROC curve analysis showed that CRP levels in serum are important indicators for diagnosis of high bladder cancer gradus with a sensitivity of 66,1% and specificity of 66,7% (Figure 3, Table 2).

**Figure 1. Serum C-reactive protein levels in control subjects and in patients with different stages of bladder carcinoma**

* compared to the control group, ** compared to the Ta group, *** compared to the T1 group

**Figure 2. Correlation between tumor size and serum CRP level in patients with bladder cancer**

**Figure 3. ROC curve of serum CRP level in the diagnosis of high gradus patients with bladder cancer**

<table>
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<th>Negative predictive value (%)</th>
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<td>CRP (mg/dL) (cut off –2.15)</td>
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<td>66.1</td>
<td>66.7</td>
<td>79.6</td>
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</table>
Discussion

Previous studies have shown that CRP concentrations may be associated with the progression of carcinomatous disease [3, 4]. Inflammatory conditions contribute to the risk of developing colon cancer or bladder cancer. It is known that CRP is one of the acute phase proteins, and belongs to the innate immune response that increases after infection, trauma, burns, myocardial tissue and during inflammatory processes [10, 12, 13].

Increased levels of CRP also reflect chronic inflammatory conditions directly, but also indirectly contribute to pathogenesis and progression of malignancies [3, 13]. It was shown that the preoperative levels of CRP in patients during last stages of colorectal cancer were significantly higher compared to the levels in patients with early stage of the disease. In addition, elevated levels of CRP and IL-6 in patients with colorectal cancer were associated with tumor stage, with the tumor recurrence, as well as with reduced survival rates [14].

The results of our study have shown that the serum CRP level in T2-T4 group of patients (patients with infiltrating tumor) was significantly higher compared to control group, Ta group, and compared to the T1 group. Also, there was a significant difference in the serum CRP level between T1 and control group, whereas no statistically significant difference in CRP concentration between T1 and Ta groups, as well as between Ta and the control group was observed.

Our results showed a statistically significant positive correlation between CRP serum level and tumor size in patients with bladder cancer. The method of multiple linear regression analysis demonstrated a statistically significant independent positive correlation between tumor size and serum concentrations of CRP in patients with bladder cancer.

Gakis et al. [8] reported that increased CRP levels in patients with invasive bladder cancer indicates a poorer prognosis. Within the postoperative algorithm, this group of authors investigates the disease stage, grade disease, positive margins and serum CRP levels.

Sejima et al. [9] showed that the higher preoperative CRP levels were independent predictors of poorer prognosis in patients who underwent radical cystectomy because of bladder cancer. Results of our study showed that CRP level in serum is important indicator for diagnosis of high grades of bladder cancer with a sensitivity of 66.1% and specificity of 66.7%.

Binary logistic regression showed an independent positive association of CRP with a high progressing potential of bladder cancer, from which it follows that, if the value of CRP increase by one, the probability that cancer goes into the stage of progressing with a high potential, increases by 1.19 times.

Relationships between CRP and cancer can be mediated by several possible mechanisms: First, tumor growth can cause tissue inflammation, which is reason for increasing CRP levels. Second, CRP is sometimes an indicator of an immune response to tumor antigens. Third, there are data that cancer cells can increase the production of inflammatory proteins, which could increase CRP levels in patients with cancer. There are cancerous cells which can express CRP. Cancer cell lines have been shown to secrete IL6 and IL8, which have role in production of CRP. These mechanisms suggested that increased CRP level is a response to the neoplastic process. That’s why the CRP concentrations could be a marker for identifying people with cancer at an early stage when treatment could be more effective. Furthermore, CRP is an important marker for chronic inflammation, and it is known that chronic inflammation has an important etiological role in cancer [5].

Several authors have investigated the effect of CRP on carcinogenesis and the course of disease in a variety of cancers [4, 13]. It was shown that high levels of CRP are bad sign for survival the patients with post castrated resistant prostate cancer treated with docetaxel [15]. Park et al. [16] reported that preoperative high CRP might be an independent predictive factor for poorer prognosis in renal cancer.

Steffens et al. [17] reported that patients with CRP >10 mg/L have 2.48% more chance of dying of renal cancer compared to patients whose CRP was <4 mg/L. Allin et al. [3] in a study that included 63,500 patients with different cancers: lung, breast and colorectal cancers and the results have shown that patients with CRP >3 mg/L are 80% more likely to die earlier than patients whose CRP was <1 mg/L. Aref et al. [5] found a positive correlation between the CRP level and the tumor stage in patients with non-small cell lung cancer, while Lee et al. [18] demonstrated a weak relationship between tumor diameter and higher level of CRP.

In tumor tissue, cancer cells are implanted into the microenvironment resembling chronic inflammation. Also, tumor cells and the microenvironment containing leukocytes, lymphocytes and macrophages act as mediators via cytokines and chemokines. All these changes reflect an inflammatory condition. This microenvironment may contribute to carcinogenesis through the induction of genomic instability, epigenetic changes and subsequent inappropriate gene expression, which consequently leads to enhanced proliferation, resistance to apoptosis, neovascularization and spreading of cancer cells. Cancer cells have the effect of the microenvironment via increased regulation of inflammatory pathways producing proinflammatory mediators such as cytokines, chemokines, cyclooxygenase-2 (COX-2),
prostaglandins, inducible NO synthase and NO. All of the above mentioned facilitates the promotion of tumor and its progression. Tumor microenvironment may confer resistance to the host immune response and to the effects of cytostatics. Elevated CRP is probably a secondary response of the consequent tumor necrosis, local tissue damage or related inflammation in patients with malignant diseases [13].

CRP is produced by hepatocytes as a systemic response to cytokines, particularly interleukin-6 (IL-6), released from leukocytes within the tumor microenvironment. Interleukin-6 helps CRP binding to phospholipids of tumor cells, activation of the classical path C1Q the complement system and the very act opsonization, which may result in lysis of tumor cells. Thus, CRP is not only an epiphenomenon or response to tumor microenvironment but also eradicating cancer cells [13]. Since that the results of this study showed significantly higher levels of CRP in patients with T2-T4 stage compared to Ta, T1 and control group of patients and significantly higher CRP levels in T1 compared to Ta and control group, we believe that CRP reflects the progression of tumors. This is supported by the fact that the corresponding results of our study showed a significant positive correlation between its serum concentration and tumor size.

**Conclusion**

Based on our results it may be suggested that CRP might play a significant role as biomarker in the diagnosis of this disease, and in monitoring of its progression. The assumption may also be that CRP is involved in the pathogenesis of this disease, and contributes to its progression. Another possible reason for the CRP increase with tumor invasion is possible proinflammatory activity of tumor cells and tumor microenvironment and associated necrosis or other tissue damage.

**Declaration of interest**

The authors declare no conflict of interest for this study.

**References**


