



# Diagnostic Value of Chronic Inflammatory Factors in Non-muscle Invasive Bladder Cancer

Nazi Aghaalikhani<sup>1,2</sup>, Nadereh Rashtchizadeh<sup>1,2\*</sup>, Pejman Shadpour<sup>3</sup>, Abdolamir Allameh<sup>4</sup>, Marzieh Mahmoodi<sup>5</sup>

## Abstract

**Objectives:** Chronic inflammation in urothelial tissues can lead to DNA damage and cancer formation. Inflammatory reactions in bladder cancer (BC) probably depend on the stages and grades of tumors. The aim of this research was to find out if there is a relationship between the serum levels of selected inflammatory factors and the pathological grades/stages of tumors in new cases of non-muscle invasive urothelial bladder carcinoma patients.

**Materials and Methods:** Blood samples were collected from 40 newly diagnosed non-muscle invasive urothelial bladder carcinoma patients (before the surgery) and 40 normal individuals without the signs of acute and chronic diseases or cancer. All patients had proven BC and were sampled prior to the initial transurethral resection of bladder tumor and any medical intervention. Finally, the levels of selected inflammatory factors such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- $\alpha$ ) were measured using enzyme-linked immunosorbent assay kits.

**Results:** Based on the results, the levels of IL-6 ( $3.041.32 \pm 0.76$  vs.  $0.76 \pm 0.23$ ), TNF- $\alpha$  ( $18.5 \pm 5.24$  vs.  $10.52 \pm 2.67$ ), and CRP ( $807.09 \pm 564.86$  vs.  $132.08 \pm 53.76$ ) were significantly higher in samples obtained from cancer patients compared to the control group, respectively ( $P < 0.001$ ). In addition, changes in these markers were associated with tumor stages and grades ( $P < 0.001$ ). Eventually, there was a significant increase in the risk of non-muscle invasive bladder cancers (NMIBC) with elevated levels of IL-6 (odds ratio [OR]: 5.93) and TNF- $\alpha$  (OR: 2.39).

**Conclusions:** Overall, the results revealed a desirable relationship between the levels of inflammatory factors and tumor stages/grades in NMIBC cases. These data may suggest that IL-6 and TNF- $\alpha$  are responsible for predisposing epithelial cells to genotoxic agents and cancer development. Finally, IL-6 and TNF- $\alpha$ , together with CRP are valuable inflammatory factors for the diagnosis of new BC cases.

**Keywords:** C-reactive protein, Inflammation, Non-muscle invasive urothelial bladder carcinoma, Interleukin-6, Tumor necrosis factor-alpha

## Introduction

Bladder cancer (BC) is one of the most prevalent urinary malignancies and indicates about 90-95% of urothelial carcinoma (1). Urothelial BCs are divided into two clinically distinct categories of non-muscle and muscle invasive BCs (NMIBCs and MIBCs). Approximately 70% of newly diagnosed urothelial BCs are of NMIBC type at the time of diagnosis. Despite the high recurrence rate of NMIBCs after the standard endoscopic transurethral resection or transurethral resection standing, the survivor rate is more than 90% if treated timely. However, 15% to 25% of BCs are of MIBC type (i.e., T2-T4) upon diagnosis, and nearly 5-25% of NMIBCs are found to progress in invasion (2). It has been reported that chronic inflammation is one of the potential risks of BC (3).

Although inflammation is a host defense mechanism, its protraction can cause damages to the cells and thus lead to various diseases. Virchow assumed that inflammation could induce cancer by stimulating the proliferation of

cells which is due to tissue damage and host response (3). In other words, cancer cells can stimulate the secretion of growth factors by inhibiting the function of the immune cells and thus promoting the proliferation of cancer cells (4,5).

In 20% of cancers, carcinogenesis is associated with infection and chronic inflammation, and the presence of chronic inflammation is involved in tumor growth (6). It has been revealed that inflammation can recruit monocytes, macrophages, and neutrophils, and consequently, cause reactive oxygen generation which per se induces damages in proteins, lipids, and DNA structures (7). The accumulation of these damages can finally pave the way for tumor development and cancer (8), especially urothelial bladder carcinoma (9).

Evidence indicates that cytokines including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) play important roles in the pathogenesis of BC, and increased inflammatory factors have been

Received 17 June 2019, Accepted 20 September 2019, Available online 10 November 2019

<sup>1</sup>Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. <sup>2</sup>Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran. <sup>3</sup>Hasheminejad kidney Center, Hospital Management Research Center, Iran University of Medical Sciences, Tehran, Iran. <sup>4</sup>Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. <sup>5</sup>Faculty of health and nutrition, Bushehr University of Medical Sciences, Bushehr, Iran.

\*Corresponding Author: Nadereh Rashtchizadeh, Tel: +98 41 3364666, Fax: +98 41 34426078, Email: rashtchizadeh@rocketmail.com



shown to induce malignant transformation and promote tumor, invasion, and angiogenesis (10,11). Further, the overexpression of TNF- $\alpha$  is thought to contribute to tumor invasion and metastasis by stimulating the secretion of matrix metalloproteinase-9 as has been observed in the recurrence of BC, in association with angiogenesis (12). Furthermore, IL-6 has been implicated in tumor growth and metastasis. The increased serum levels of IL-6 may be correlated with poor prognosis, metastasis of cancer types such as the ovary and bladder (13,14), higher clinical stages (T2-T4), post-treatment relapse, and the lower survival rates of patients (11).

The C-reactive protein (CRP) is an acute-phase protein, the circulating levels of which become elevated during inflammatory diseases and cancer (15). During the acute phase reaction, pro-inflammatory cytokines (e.g., IL-6 and TNF- $\alpha$ ) stimulate hepatocytes in order to produce large amounts of CRP (16). An increase in the CRP level generally corresponds to the degree of tissue damage. A common CRP measurement is useful for the diagnosis of inflammatory and infectious diseases. As a result, CRP levels >10 mg/L may indicate the presence of acute or chronic inflammatory illnesses or malignancies (17).

In the present study, the serum samples of NMIBC patients with known pathological grades/stages of tumors were examined to find out the possible relationship between the serum inflammatory markers and the progression of the tumors.

## Materials and Methods

### Patients and Blood Specimens

In this cross-sectional study, 40 patients diagnosed as the new cases of primary NMIBC and 40 age/gender-matched healthy subjects were selected within 14 months. The patients were recruited at Hasheminejad Kidney Center, Tehran, Iran. The diagnose of cancer and its possible metastasis to lymph nodes or other areas was initially determined based on bimanual examinations, imaging (e.g., computed tomography scan and magnetic resonance imaging), urine cytology, cystoscopy, and biochemical tests (clinical staging) and then, the review of TURBT, partial cystectomy, or radical cystectomy samples (pathologic staging) according to (18). Finally, the histopathological classification of the stage and grade were performed by an experienced uropathologist in accordance with the 2004 American Joint Committee on Cancer TNM staging system (19) and the 1973 World Health Organization/International Society of Urological Pathology classification system (20), respectively. All participants were informed about the aim of the study and then signed informed satisfaction forms before blood sampling. Next, a questionnaire was used to collect clinical, personal, and demographic information (e.g., gender, age, stage, and grade). However, patients who had a history of chronic diseases (e.g., renal or hepatic dysfunction, acute or chronic blood loss, infection, and immune

system disorders) or other malignancies, immunotherapy, chemotherapy, drug therapy, or prior exposure to blood products were excluded from the study. None of the healthy individuals were suffering from known acute or chronic illnesses and any type of cancer.

To evaluate inflammatory factors, a 5 mL sample of the venous blood was drawn from a patient (before the surgery) and control individuals who had at least 8 hours of fasting. Blood samples were allowed to clot, and then the sera were separated by centrifuging at 1500 g for 15 minutes, and finally, stored in a freezer at -70°C until for the measurement of inflammatory factor levels in the serum samples.

Commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (Sigma-Aldrich, USA) were used for the evaluation of IL-6, TNF- $\alpha$ , and CRP in the serum. All procedures were done based on kit instructions. Finally, the intensity of the color reflecting the factor concentration was determined at 450 nm. For IL-6, the sensitivity of the kit was <2.0 pg/mL and its detection range was 6.25-200 pg/mL. Moreover, the sensitivity of the TNF- $\alpha$  kit and the detection range were 4.4 pg/mL and 2.2-7000 pg/mL, respectively. Eventually, the CRP ELISA kit had a detection range of 31.2-2000 pg/mL and sensitivity of <2.0 pg/mL.

### Statistical Analysis

All statistical analyses were done using the Statistical Package for the Social Sciences, version 20.0. Using the Shapiro-Wilk test, the normality of variables was investigated and the results showed that the distribution of the considered variables significantly deviated from normality ( $P<0.05$ ). Therefore, non-parametric tests were used to analyze the data. Additionally, statistical comparisons were performed using Mann-Whitney and Kruskal-Wallis tests in order to compare two and more than two independent variables, respectively. Likewise, post hoc analysis for pairwise comparisons was used for further refinement of Kruskal-Wallis results. The odds ratios (OR) of circulating inflammatory factors for NMIBC were calculated using logistic regression analysis. A statistical significance level was considered at  $P<0.05$ .

## Results

Demographic and clinicopathological characteristics for all patients and the healthy group are provided in Table 1.

In addition, Table 2 presents the mean  $\pm$  standard deviation levels of serum inflammatory factors in NMIBC patients and the normal group. The levels of inflammatory factors were significantly higher in NMIBC patients compared with the normal group ( $P<0.001$ ). In addition, the serum levels of all three factors were higher in patients with NMIBC in the T1 stage compared to those in the Ta stage ( $P<0.001$ ).

Figure 1 displays the relationship between the serum levels of TNF- $\alpha$ , IL-6, and CRP with the grade of the tumor. As shown, there were positive associations between the

**Table 1.** Characteristics of Patients With Newly Diagnosed Non-muscle Invasive Urothelial Bladder Carcinoma and the Healthy Group

Variables	Patient Group (n = 40)	Healthy Group (n = 40)
Age (y)	60.25 ± 15.61	51.12 ± 15.10
Gender, No. (%)		
Male	36 (90)	36 (90)
Female	4 (10)	4 (10)
Stage, No. (%)		
Ta	24 (60)	-
T1	16 (40)	-
Grade, No. (%)		
I	13 (32.5)	-
II	17 (42.5)	-
III	10 (25)	-

levels of inflammatory factors and the grade of the tumor ( $P < 0.05$ ).

Further, a significant increase was found in the risk of NMIBC with elevated levels of IL-6 and TNF- $\alpha$  ( $P < 0.05$ ). Similarly, the logistic regression analysis demonstrated that the high levels of IL-6/TNF- $\alpha$  could have a detrimental effect and increase the risk of NMIBC about 6 and 2.4 folds, respectively (Table 3). In addition, the T1 stage of the tumor, compared to the Ta stage, showed the ORs of 8.7 and 1.67 for IL-6 and TNF- $\alpha$ , respectively ( $P < 0.05$ ). However, CRP was not a considerable risk factor for NMIBC and its stage (OR: 1.2,  $P > 0.05$ ).

**Discussion**

The present study was carried out on a group of patients with newly diagnosed and yet untreated NMIBC to find out if inflammatory factors are linked to the progression of newly diagnosed NMIBC as shown by the pathological grade/stage.

The obtained data showed the increased levels of serum

inflammatory markers in patients further revealing that the levels of inflammatory factors were associated with different pathological stages/grades.

This finding was more evident in NMIBC cases who were diagnosed with T1 stage tumors. However, the association of serum inflammatory markers and the malignancy was relatively lower in cases having Ta stage tumors. This relationship was also indicated in terms of the pathological grades of the tumors.

Previous reports demonstrated that inflammatory diseases can predispose the tissues to cancers leading to the increased risk of the development of various types of cancers. Further, inflammatory cells and cytokines can interact with the micro-environment of all tumors at the early stages (6). Hence, chronic inflammatory reactions can potentiate the progression of the tumors. Furthermore, the two-way interaction of inflammatory factors and tumor progression implies that the growth and development of most tumors can induce inflammation in the tumor micro-environment. Under these circumstances, the production of tumor-associated cytokines can induce the production of acute-phase proteins (21).

One possible mechanism explaining the prognostic values of inflammatory factors in cancer patients is such an increase in inflammatory factor levels associated with angiogenesis and DNA damage that, in turn, can lead to tumor growth (22). Therefore, the detection of inflammatory markers using sensitive assays is always anticipated to link inflammation and cancer (15).

IL-6 is a multi-functional cytokine that can facilitate both carcinogenesis and tumor progression (23). It was assumed that changes in serum IL-6 could be implicated as a biochemical marker in the differential diagnosis of bladder tumors with major differences in the pathological grade/stage.

Moreover, an elevation in the levels of IL-6 in high

**Table 2.** Serum Levels of Inflammatory Factors in Terms of the Tumor Stage in Newly Diagnosed Non-muscle Invasive Bladder Cancer Patients

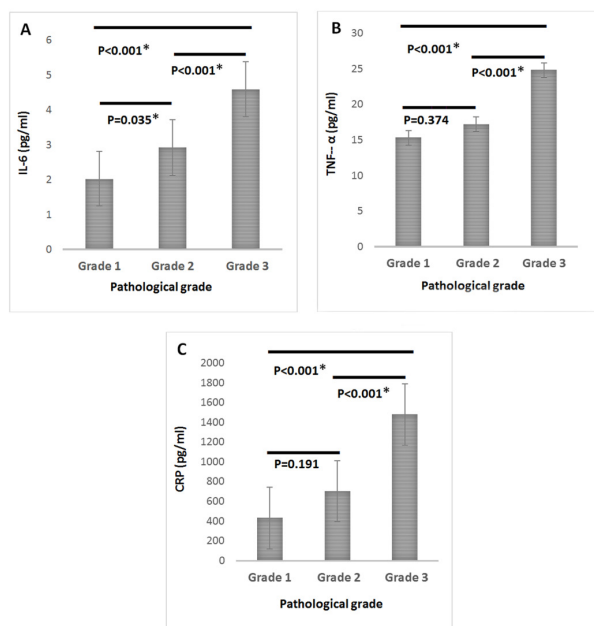
Serum Factors	Healthy Group (n=40)	Cancer Group (n=40)	P-value	Stage Ta (n=24)	Stage T1 (n=16)	P Value
IL-6 (pg/mL)	0.76±0.23	3.04 ± 1.32	<0.001*	2.19±0.62	4.32±1.08	<0.001*
TNF- $\alpha$ (pg/mL)	10.52±2.67	18.5 ± 5.24	<0.001*	15.30±0.78	23.29±5.60	<0.001*
CRP (pg/mL)	132.08±53.76	807.09±564.86	<0.001*	407.27±117.39	1406.80±436.27	<0.001*

Note. IL: Interleukin; TNF: Tumor necrosis factor; CRP: C-reactive protein; \*A statistically significant level was defined in  $P < 0.05$ .

**Table 3.** ORs and 95% CIs for the Association Between Inflammatory Factors and Pathological Stages (Ta/T1) and Non-muscle Invasive Bladder Cancer Risk

Serum Factors	Groups		P Value	Stages		P Value
	Control (n=40)	Patient (n=40)		Ta (n=24)	T1 (n=16)	
IL-6 (pg/mL)	ORs (95% CI, Lower-Upper)			ORs (95% CI, Lower-Upper)		
	5.93 (2.61-13.52)		<0.001	8.72 (2.18-34.84)		0.002*
TNF- $\alpha$ (pg/mL)	2.39 (1.63-3.51)		<0.001	1.67 (1.21-2.32)		0.002*
CRP (pg/mL)	1.24 (0.85-1.80)		0.262	1.20 (0.00-45.81)		0.992

Note. IL: Interleukin; TNF: Tumor necrosis factor; CRP: C-reactive protein; OR: Odds ratio; CI: Confidence interval; \*A statistically significant level was defined at  $P < 0.05$ .



**Figure 1.** The Pathological Grades and the Levels of Inflammatory Factors in Newly Diagnosed Non-muscle Invasive Bladder Cancer Patients. (A) The pathological grades and the level of IL-6 in patients, (B) The pathological grades and the level of TNF- $\alpha$  in patients, and (C) The pathological grades and the level of CRP in patients. \* Sign represents  $P < 0.05$  significant between the patient group and controls. Note. IL: Interleukin; TNF: Tumor necrosis factor; CRP: C-reactive protein.

grade (grade 3)/T1 stage compared to low-grade (grade 1)/Ta stage patients indicates the importance of IL-6 as a surrogate marker for the diagnosis of the malignancy in this cancer.

In most clinical studies, an increased level of IL-6 has been reported in patients with cancer. It seems that the serum level of IL-6 in cancer patients increases as a late-stage event during tumor development (24).

Andrews et al also reported a significant rise in the levels of IL-6 and its soluble receptor in patients with metastatic BC compared to the control group in a stage-related status, suggesting that IL-6 is a strong/independent marker in BC recurrence/survival (13).

Similar results were also reported for other cancer types. In this regard, it was found that the IL-6 level was higher in patients with small cell lung cancer when compared to the controls. In addition, a good relationship was established between the serum levels of IL-6 and the stage of cancer which encouraged the researchers to introduce the high levels of IL-6 as an independent prognostic factor for small cell lung cancer (25). In another study, a significant association was found between IL-6 levels and the poor prognosis/short overall survival of cancer patients. These findings suggested that the high levels of IL-6 could act as an independent prognostic biomarker for the overall survival of patients with prostate, non-small cell lung, and gastrointestinal cancers (26-28).

Moreover, a substantial association was observed between the increased levels of IL-6 and tumor metastasis in patients with colorectal/prostate cancers (29).

TNF- $\alpha$ , which is also known as an important

inflammatory factor, plays a key role in the early stages of tumor growth and is considered as one of the main cancer-associated inflammatory factors (30). An increase in the levels of TNF- $\alpha$  was reported in patients with cancer, especially those at advanced stages and with poor prognosis (31).

Our results showing a significant increase in serum TNF- $\alpha$  levels in NMIBC patients compared to the controls is in agreement with other reports. Additionally, changes in TNF- $\alpha$  was variable in terms of the tumor stage and grade.

It was reported that the TNF- $\alpha$  level increased in patients with BC. In addition, it was shown that patients at T3/T4 advanced stages had higher TNF- $\alpha$  compared to those at early stages (T1/T2), suggesting that the TNF- $\alpha$  level might be involved in the progression of BC (32). In supporting this finding, Cai et al concluded that TNF- $\alpha$  could induce the development of breast cancer through the activation of TNFR1-mediated nuclear factor kappa B and/or p38/signal transducer and activator of transcription 3 signaling pathways and thus the upregulation of hepatitis B X-interacting protein (HBXIP). Interestingly, HBXIP itself can trigger the expression of TNFR1 and thus lead to positive feedback for TNF- $\alpha$ -mediated cancer progression (33). The higher levels of TNF- $\alpha$  were also observed in patients with renal cell cancer and its levels were suggested as a biomarker for the recognition of cancer at the early stages (34).

According to a recent report, the serum CRP significantly increased in upper tract urothelial cancer (UTUC) patients. Moreover, an association was found

between the CRP elevation and the clinical symptoms of cancer, representing that the serum CRP can be considered as a complementary test for the detection of low-grade UTUC (35). Wu et al demonstrated that the higher level of CRP was associated with the significantly elevated risk of colorectal cancer among Chinese men and suggested that the CRP level could be a potential biomarker for disease occurrence/development (36). In another study, the elevated plasma level of CRP was introduced as a risk factor for ovarian/lung cancers due to the distinguished role of the chronic inflammation in carcinogenesis (37-39). Furthermore, the results of a study showed that serum CRP levels in patients with T2-T4 stages were higher compared to patients with Ta-T1 stages and healthy controls. Thus, the researchers concluded that CRP is an indicative factor for tumor development and its diagnosis (40). In this regard, it was indicated that the elevated CRP level is an independent predictor for the survival of patients with kidney, lung, prostate, and BCs (41-44).

In accordance with previous studies, the findings of the current study revealed that CRP levels increased in NMIBC patients and its level was associated with the tumor stage/grade.

Due to the synthesis and release of CRP under the influence of IL-6, the obtained results were understandable. The increased serum level of CRP can be suggested as a promising diagnostic marker for the new cases of NMIBC (45). Hence, CRP changes should be monitored in combination with other inflammatory factors (35).

Additionally, the results of our study indicated that inflammatory factors (i.e., IL-6 and TNF- $\alpha$ ) were significantly associated with the increased ORs of NMIBC. Based on the finding of this study, the circulating levels of IL-6 and TNF- $\alpha$  were significantly associated with NMIBC risk although the association highly relied on the stage of cancer. Regarding IL-6, a stronger association was found with the risk of NMIBC compared to TNF- $\alpha$ . According to previous studies, it is known that pro-inflammatory cytokines such as IL-6 and TNF- $\alpha$  play an important role in tumor initiation and progression.

Evidence shows that using multiple biomarkers related to the inflammatory reaction is a correct approach for applying such markers in the diagnosis or prognosis of cancers.

Accordingly, it is of great importance to realize that chronic inflammation may not only be a host defense mechanism for BC progression but also can predispose to potential cancer-causing agents. Therefore, determining some inflammatory factors using non-invasive methods can be useful for understanding the contribution of inflammation to cancer development.

However, more studies including a larger population are needed to confirm our results and determine the cut-off levels for circulating cytokines regarding diagnosing cancer.

In conclusion, the results showed a good relationship

between immuno-inflammatory biomarkers and the pathological stages/grades of developing bladder tumors. This may suggest that IL-6 and TNF- $\alpha$  are responsible for predisposing epithelial cells to genotoxic agents and cancer development. Thus, inflammatory factors could simultaneously be considered as risk factors and useful markers in the diagnosis of new BC cases.

#### Conflict of Interests

None declared.

#### Ethical Issues

This study was approved by the Committee for Ethics in Research and the Institutional Ethics Committee of Tabriz University of Medical Sciences (Reg. No. TBZMED.REC.1394.315).

#### Financial Support

Drug Applied Research Center of Tabriz University of Medical Sciences supported the study.

#### Acknowledgments

The authors would like to thank the Drug Applied Research Center, Tabriz University of Medical Sciences, and the Hasheminejad Kidney Center for supporting this study. Finally, we thank all patients and healthy individuals who participated in this study.

#### References

- Babjuk M, Böhle A, Burger M, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. *Eur Urol*. 2017;71(3):447-461. doi:10.1016/j.eururo.2016.05.041
- Shadpour P, Emami M, Haghani S. A comparison of the progression and recurrence risk index in non-muscle-invasive bladder tumors detected by narrow-band imaging versus white light cystoscopy, based on the EORTC scoring system. *Nephrourol Mon*. 2016;8(1):e33240. doi:10.5812/numonthly.33240
- Leibovici D, Grossman HB, Dinney CP, et al. Polymorphisms in inflammation genes and bladder cancer: from initiation to recurrence, progression, and survival. *J Clin Oncol*. 2005;23(24):5746-5756. doi:10.1200/jco.2005.01.598
- Brandau S, Suttman H. Thirty years of BCG immunotherapy for non-muscle invasive bladder cancer: a success story with room for improvement. *Biomed Pharmacother*. 2007;61(6):299-305. doi:10.1016/j.biopha.2007.05.004
- Kawai K, Miyazaki J, Joraku A, Nishiyama H, Akaza H. Bacillus Calmette-Guerin (BCG) immunotherapy for bladder cancer: current understanding and perspectives on engineered BCG vaccine. *Cancer Sci*. 2013;104(1):22-27. doi:10.1111/cas.12075
- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;454(7203):436-444. doi:10.1038/nature07205
- Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420(6917):860-867. doi:10.1038/nature01322
- Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med*. 2010;49(11):1603-1616. doi:10.1016/j.freeradbiomed.2010.09.006
- Sawicka E, Lisowska A, Kowal P, Długosz A. [The role of oxidative stress in bladder cancer]. *Postepy Hig Med Dosw (Online)*. 2015;69:744-752. doi:10.5604/17322693.1160361
- Abdel-Latif MM, Duggan S, Reynolds JV, Kelleher D. Inflammation and esophageal carcinogenesis. *Curr Opin Pharmacol*. 2009;9(4):396-404. doi:10.1016/j.coph.2009.06.010
- Chen MF, Lin PY, Wu CF, Chen WC, Wu CT. IL-6 expression regulates tumorigenicity and correlates with prognosis in bladder cancer. *PLoS One*. 2013;8(4):e61901. doi:10.1371/journal.pone.0061901
- Feng C, Guan M, Ding Q, et al. Expression of pigment epithelium-

- derived factor in bladder tumour is correlated with interleukin-8 yet not with interleukin-1 $\alpha$ . *J Huazhong Univ Sci Technolog Med Sci.* 2011;31(1):21-25. doi:10.1007/s11596-011-0144-6
13. Andrews B, Shariat SF, Kim JH, Wheeler TM, Slawin KM, Lerner SP. Preoperative plasma levels of interleukin-6 and its soluble receptor predict disease recurrence and survival of patients with bladder cancer. *J Urol.* 2002;167(3):1475-1481.
  14. Scambia G, Testa U, Benedetti Panici P, et al. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. *Br J Cancer.* 1995;71(2):354-356. doi:10.1038/bjc.1995.71
  15. Heikkilä K, Ebrahim S, Lawlor DA. A systematic review of the association between circulating concentrations of C reactive protein and cancer. *J Epidemiol Community Health.* 2007;61(9):824-833. doi:10.1136/jech.2006.051292
  16. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest.* 2003;111(12):1805-1812. doi:10.1172/jci18921
  17. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. *Crit Rev Clin Lab Sci.* 2011;48(4):155-170. doi:10.3109/10408363.2011.599831
  18. Babjuk M, Burger M, Compérat E, et al. Non-Muscle-Invasive Bladder Cancer. *European Association of Urology*; 2017.
  19. Magers MJ, Lopez-Beltran A, Montironi R, Williamson SR, Kaimakliotis HZ, Cheng L. Staging of bladder cancer. *Histopathology.* 2019;74(1):112-134. doi:10.1111/his.13734
  20. Pan CC, Chang YH. The 2004 World Health Organization/International Society of Urological Pathology classification system for non-muscle-invasive bladder cancer. *Urol Sci.* 2013;24(3):96-100. doi:10.1016/j.urols.2013.06.006
  21. Chechlinska M, Kowalewska M, Nowak R. Systemic inflammation as a confounding factor in cancer biomarker discovery and validation. *Nat Rev Cancer.* 2010;10(1):2-3. doi:10.1038/nrc2782
  22. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet.* 2001;357(9255):539-545. doi:10.1016/s0140-6736(00)04046-0
  23. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol.* 2015;16(5):448-457. doi:10.1038/ni.3153
  24. Lippitz BE, Harris RA. Cytokine patterns in cancer patients: a review of the correlation between interleukin 6 and prognosis. *Oncoimmunology.* 2016;5(5):e1093722. doi:10.1080/2162402x.2015.1093722
  25. Wójcik E, Jakubowicz J, Skotnicki P, Sas-Korczyńska B, Kulpa JK. IL-6 and VEGF in small cell lung cancer patients. *Anticancer Res.* 2010;30(5):1773-1778.
  26. Silva EM, Mariano VS, Pastrez PRA, et al. High systemic IL-6 is associated with worse prognosis in patients with non-small cell lung cancer. *PLoS One.* 2017;12(7):e0181125. doi:10.1371/journal.pone.0181125
  27. Nakashima J, Tachibana M, Horiguchi Y, et al. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. *Clin Cancer Res.* 2000;6(7):2702-2706.
  28. Vainer N, Dehlendorff C, Johansen JS. Systematic literature review of IL-6 as a biomarker or treatment target in patients with gastric, bile duct, pancreatic and colorectal cancer. *Oncotarget.* 2018;9(51):29820-29841. doi:10.18632/oncotarget.25661
  29. Akimoto S, Okumura A, Fuse H. Relationship between serum levels of interleukin-6, tumor necrosis factor-alpha and bone turnover markers in prostate cancer patients. *Endocr J.* 1998;45(2):183-189. doi:10.1507/endocrj.45.183
  30. Ueda T, Shimada E, Urakawa T. Serum levels of cytokines in patients with colorectal cancer: possible involvement of interleukin-6 and interleukin-8 in hematogenous metastasis. *J Gastroenterol.* 1994;29(4):423-429. doi:10.1007/bf02361238
  31. Raziuddin S, Masihuzzaman M, Shetty S, Ibrahim A. Tumor necrosis factor alpha production in schistosomiasis with carcinoma of urinary bladder. *J Clin Immunol.* 1993;13(1):23-29. doi:10.1007/bf00920632
  32. Raziuddin S, Masihuzzaman M, Shetty S, Ibrahim A. Tumor necrosis factor alpha production in schistosomiasis with carcinoma of urinary bladder. *J Clin Immunol.* 1993;13(1):23-29. doi:10.1007/bf00920632
  33. Cai X, Cao C, Li J, et al. Inflammatory factor TNF- $\alpha$  promotes the growth of breast cancer via the positive feedback loop of TNFR1/NF- $\kappa$ B (and/or p38)/p-STAT3/HBXIP/TNFR1. *Oncotarget.* 2017;8(35):58338-58352. doi:10.18632/oncotarget.16873
  34. Yoshida N, Ikemoto S, Narita K, et al. Interleukin-6, tumour necrosis factor alpha and interleukin-1beta in patients with renal cell carcinoma. *Br J Cancer.* 2002;86(9):1396-1400. doi:10.1038/sj.bjc.6600257
  35. Beig-Mohammadi H, Masoudian N, Tabibi A, Allameh A. Diagnostic value of a combined C-reactive protein and haptoglobin test in new cases of upper tract urothelial carcinoma. *Journal of Basic Research in Medical Sciences.* 2016;3(4):28-33.
  36. Wu J, Cai Q, Li H, et al. Circulating C-reactive protein and colorectal cancer risk: a report from the Shanghai Men's Health Study. *Carcinogenesis.* 2013;34(12):2799-2803. doi:10.1093/carcin/bgt288
  37. Chaturvedi AK, Caporaso NE, Katki HA, et al. C-reactive protein and risk of lung cancer. *J Clin Oncol.* 2010;28(16):2719-2726. doi:10.1200/jco.2009.27.0454
  38. Vaguliènė N, Žemaitis M, Miliauskas S, Urbonienė D, Šitkauskienė B, Sakalauskas R. Comparison of C-reactive protein levels in patients with lung cancer and chronic obstructive pulmonary disease. *Medicina (Kaunas).* 2011;47(8):421-427.
  39. Li J, Jiao X, Yuan Z, Qiu H, Guo R. C-reactive protein and risk of ovarian cancer: a systematic review and meta-analysis. *Medicine (Baltimore).* 2017;96(34):e7822. doi:10.1097/md.00000000000007822
  40. Lepara Z, Junuzović D, Kulovac B, et al. Elevated serum CRP level as potential biomarker of different stages of bladder cancer. *Folia Med Fac Med Univ Sarav.* 2014;49(2):81-86.
  41. Lamb GW, McArdle PA, Ramsey S, et al. The relationship between the local and systemic inflammatory responses and survival in patients undergoing resection for localized renal cancer. *BJU Int.* 2008;102(6):756-761. doi:10.1111/j.1464-410X.2008.07666.x
  42. Scott HR, McMillan DC, Forrest LM, Brown DJ, McArdle CS, Milroy R. The systemic inflammatory response, weight loss, performance status and survival in patients with inoperable non-small cell lung cancer. *Br J Cancer.* 2002;87(3):264-267. doi:10.1038/sj.bjc.6600466
  43. McArdle PA, Qayyum T, McMillan DC. Systemic inflammatory response and survival in patients with localised prostate cancer: 10-year follow-up. *Urol Int.* 2010;84(4):430-435. doi:10.1159/000313364
  44. Saito K, Kihara K. Role of C-reactive protein in urological cancers: a useful biomarker for predicting outcomes. *Int J Urol.* 2013;20(2):161-171. doi:10.1111/j.1442-2042.2012.03121.x
  45. Saito K, Kihara K. C-reactive protein as a biomarker for urological cancers. *Nat Rev Urol.* 2011;8(12):659-666. doi:10.1038/nrurol.2011.145

**Copyright** © 2021 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.