

# Relationship between Peripheral Blood Parameters and Stage and Grade of Disease in Patients with Urothelial Cancer of the Urinary Bladder

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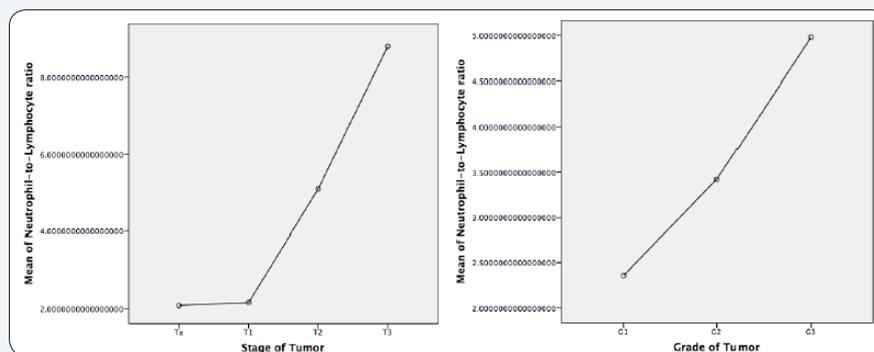
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## Abstract

Various researchers reported relationships between peripheral blood parameters and different solid tumors' stage and prognosis. The current study aims to assess the relationship between peripheral blood parameters and stage and grade of disease in patients with urothelial cancer of the urinary bladder (Figure 1).



**Methods:** 102 patients diagnosed with bladder cancer were enrolled in this prospective cohort study. Preoperative blood samples were taken before the first cystoscopic examination. Patients were grouped as having a non-muscle-invasive (NMIBC) or muscle-invasive urothelial carcinoma (MIBC). The predictive value of various blood parameters (Hb, Total Leucocytic Count (TLC), Neutrophil-Lymphocyte Ratio, and platelet count) for bladder cancer stage and grade were evaluated.

**Results:** 49 patients had NMIBC including 20 (19.6%) with T<sub>0</sub> disease and 29 (28.4%) with T<sub>1</sub> disease. The 53 patients with MIBC, 40 (39.2%) had T<sub>2</sub> disease and 13 (12.7%) had T<sub>3</sub> disease. While, G<sub>1</sub>=22 (21.6 %), G<sub>2</sub>=18 (17.6%) and G<sub>3</sub>=62 (60.8%). TLC was significantly lower in stage T<sub>0</sub> compared to stages T<sub>2</sub> and T<sub>3</sub> ( $p < 0.003$ ), as well as in grades G<sub>1</sub> and G<sub>2</sub> compared to G<sub>3</sub> tumors ( $p < 0.05$ ). Platelet count was not significantly associated with tumor stage ( $p > 0.05$ ), but showed association with tumor grade, specifically G<sub>2</sub> vs. G<sub>3</sub> ( $p = 0.005$ ). The values of neutrophil, lymphocyte count and neutrophil-to-lymphocyte ratio showed statistically significant differences between different tumor stages and grades ( $p < 0.005$ ).

**Conclusions:** Peripheral blood parameters may play a role in predicting stage and grade of urinary bladder cancer and may be used as adjunct to clinical, radiological and pathological findings in disease evaluation.

**Keywords:** Urinary bladder cancer; Peripheral blood parameters; Tumor stage; Tumor grade

**Abbreviations:** CRP: C-reactive Protein; ESR: Erythrocyte Sedimentation Rate; HB: Hemoglobin; HIV: Human Immunodeficiency Virus; IL-1: Interleukin-1; ISUP: International Society of Urologic Pathology; MIBC: Muscle Invasive Bladder Cancer; NF-kB: Nuclear Factor-KappaB; NLR: Neutrophil-to-Lymphocyte Ratio; NMIBC: Non-Muscle Invasive Bladder Cancer; SCC: Squamous Cell Carcinoma; TCC: Transitional Cell Carcinoma; TNF: Tumor Necrosis Factor; TNM: Classification of Malignant Tumor (Tumor, Node, Metastasis); TURBT: Transurethral Resection of the Bladder Tumor; UC: Urothelial Carcinoma; UICC: Union International Contre Le Cancer; UTI: Urinary Tract Infection; VEGF: Vascular Endothelial Growth Factor; WBC: White Blood Cells; WHO: World Health Organization

## Introduction

Bladder cancer is a common tumor in the urinary tract and is the ninth most prevalent cancer around the world [1]. Approximately 90% of bladder cancers are urothelial carcinomas (UC) and 75-85% of UC are non-muscle invasive while 15-25% is invasive. Treatment of UC depends on pathological behavior of the tumor. Non-muscle-invasive tumors can be removed by transurethral resection but recurrence is common [2]. Two main types of bladder cancer are identified: the transitional cell carcinomas (TCC), related to cigarette smoking and most prevalent in Western and industrialized countries, and the squamous cell carcinomas (SCC), which are more frequently seen in some Middle Eastern and African countries, where urinary schistosomiasis is an endemic disease. Rare types of bladder cancer include small cell carcinoma, carcinosarcoma, primary lymphoma, and sarcoma [3]. In the United States, bladder cancer is nearly three times more common among men than woman. In men, it is the fourth most common cancer, after prostate, lung and colorectal cancer, accounting for 5.5% of all cancer cases. In women, it is the eighth most common cancer, accounting for 2.3% of all cancer [4]. In western countries, more than 90% of bladder cancers are transitional cell carcinomas, 80% are papillary and 20% are solid and invasive. Squamous cell carcinoma accounts for only 1% of bladder cancer in England [5] and 3-7% in the United States [6].

The classic presentation of bladder cancer is painless gross hematuria, which is seen in approximately 80-90% of patients. Physical examination results are often unremarkable. Cytology, Cystoscopy and biopsy are the principal diagnostic tests [7]. The host inflammatory response has gained increasing attention in oncology research. Infiltrating cells of the immune system are constituents of virtually all neoplasms [8]. While initially thought to represent an antitumoral response, immune cells, particularly those of the innate immune system, also exhibit effects that promote carcinogenesis and cancer progression [8-9]. Proposed mechanisms include increased supply of growth factors, survival factors, pro-angiogenic factors, extracellular matrix-modifying enzymes (which can facilitate invasion and metastasis) and inductive signals that may lead to epithelial-to-mesenchymal transition [8]. Thus, there is a biological rationale for using NLR, the ratio of circulating neutrophils (immune cells of the innate system) to lymphocytes (immune cells of the adaptive system), as a measure of the systemic host response when evaluating the association between inflammation and cancer outcomes. The prognostic role of NLR has been evaluated in numerous epidemiologic studies of various cancer types. Higher NLR has been found to be consistently associated with more advanced stage and more aggressive tumor behaviour [10-11].

## Aim of the Study

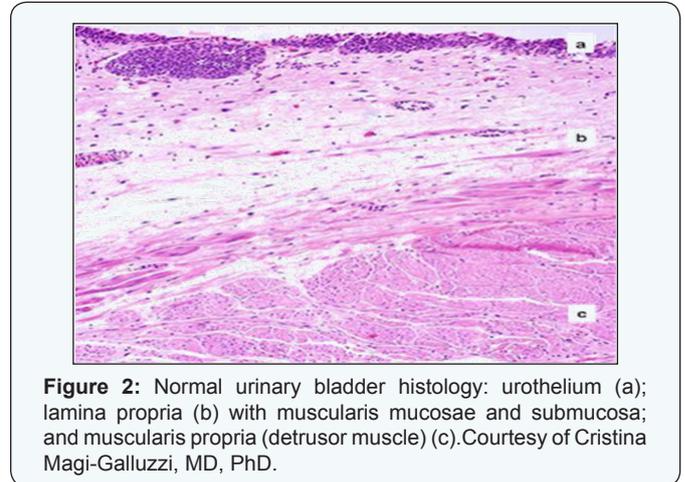
To assess the relationship between peripheral blood parameters and stage and grade of disease in patients with urothelial cancer of the urinary bladder.

## Pathology of Cancer Bladder

### Normal Bladder Urothelium

The wall of the urinary bladder consists of four layers.

**Urothelium:** The urothelium is the innermost epithelial lining of the bladder. The urothelium is the site of origin for urothelial carcinomas (Figure2).



**Figure 2:** Normal urinary bladder histology: urothelium (a); lamina propria (b) with muscularis mucosae and submucosa; and muscularis propria (detrusor muscle) (c). Courtesy of Cristina Magi-Galluzzi, MD, PhD.

**Lamina propria:** The lamina propria is separated from the urothelium by a thin basement membrane that is composed of abundant connective tissue containing vascular and neuronal structures. Fascicles of smooth muscle can be found within the superficial lamina propria, either isolated or forming complete or incomplete muscularis mucosae [12-13].

**Muscularis propria:** The muscularis propria (detrusor muscle) surrounds the lamina propria and consists of thick, irregularly arranged muscle bundles. In small biopsies, fascicles of muscle in the lamina propria may be confused with the larger smooth muscle bundles of the muscularis propria, potentially resulting in an error in tumor staging. Adipose tissue also can be present within the lamina propria and/or muscularis propria. The presence of invasive tumor in fat thus is not always indicative of extravesical extension [14].

**Adventitia or serosa:** The muscularis propria is separated from the surrounding tissues by a serosal layer [13-14].

### Pre-Neoplastic Proliferative Abnormalities

A variety of changes can occur in the urothelium in response to inflammation and irritation or carcinogens. These changes may be proliferative, metaplastic or both.

**Epithelial hyperplasia:** The term epithelial hyperplasia is used to describe non-neoplastic increase in the number of transitional epithelial stratification beyond the normal value of 6 cell layers without nuclear or architectural abnormalities. Increase of about 15 to 20 layers may occur in bilharzial bladder. But the urothelium retains its flat topography without exophytic or endophytic folding. The transitional cells are regular and show normal polarity. The nuclei are relatively small, of uniform size and density.

**Von Brunn 's Nests:** Von Brunn\_s are islands of benign appearing urothelium situated in the lamina propria. These are believed to result from inward proliferation of the basal cells. Brunn's nests may either be solid or contain a central slit like lumen, lined by transitional cells. In the bilharzial urologic series of Zahran and associates, this change was observed in 53.4% of cases (Figure 3).

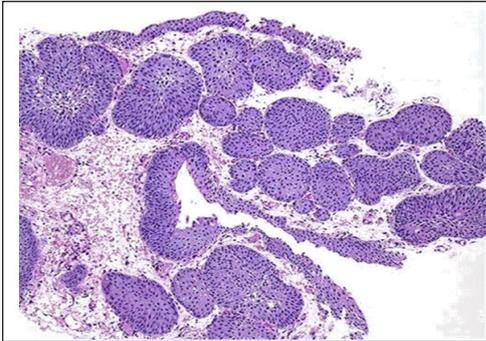


Figure 3: Von Brunn's nests.

**Cystitis cystica:** Cystitis cystica is similar to Von Brunn's nests except that the center of the nests of urothelium has undergone eosinophilic liquefaction. Cystitis cystica is present in 60% of normal bladder autopsies. It should be distinguished from cystitis follicularis, which is non-neoplastic response to chronic bacterial infection. Grossly, it appears as punctuate yellow submucosal nodule (Figure 4).

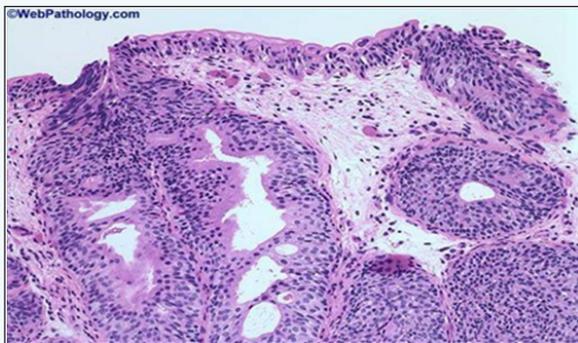


Figure 4: cystitis cystica.

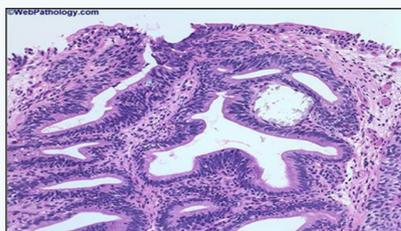


Figure 5: cystitis glandularis.

**Cystitis glandularis:** Cystitis glandularis is similar to cystitis cystica except that the transitional cells have undergone glandular metaplasia. It appears histologically as submucosal nests of columnar epithelial cells surrounding a central liquefied region of cellular degeneration [15]. Two cell types

may be recognized according to cytoplasmic features, namely: an eosinophilic cell type and a mucinous or goblet cell type. Usually one cell type predominates, but at times they occur together in the lesion. The predominance of the mucinous cell type produces glandular structures indistinguishable from those of colonic mucosa. Cystitis glandularis may be a precursor of adenocarcinoma [16] (Figure 5).

**Atypical hyperplasia:** Atypical hyperplasia is similar to epithelial hyperplasia except that there are also nuclear abnormalities and partial derangement of umbrella cell layer [17]. In patients with superficial bladder cancers, the presence of atypia in adjacent urothelium is associated with a 35% to 40% risk of developing invasive disease [18].

**Urothelial metaplasia:** Urothelial metaplasia refers to the bladder lining, often in focal areas, demonstrating a non-transitional epithelial appearance, usually with epidermoid (squamous metaplasia) or glandular (adenomatous metaplasia) development.

**Vesical leukoplakia:** Leukoplakia is characterized by squamous metaplasia with marked keratinization, downward growth of rete pegs (acanthosis), cellular atypia, and dysplasia. It is believed to be a response of the normal urothelium to noxious stimuli and is generally considered a premalignant lesion that may progress to SCC in up to 20% of patients.

**Dysplasia:** It is a term, which denotes epithelial changes that are intermediate between normal urothelium and carcinoma in situ. There are three categories of dysplasia: mild, moderate, and severe. Dysplastic cells have large, round, notched, basely situated nuclei that do not exhibit the normal epithelial polarity. Dysplastic epithelium does not have an increased number of cell layers or mitotic figures [19].

**Carcinoma in situ:** Carcinoma in situ refers to flat areas of epithelium composed of cells with anaplastic features and a disorderly pattern of growth without extension into the bladder lumen or penetration of the basement membrane. The cells show a severe degree of anaplasia, abundant mitotic figures, and a loss of cell cohesiveness. The number of cell layers in carcinoma in situ may be normal or increased with no formation of papillary structures (Figure 6).

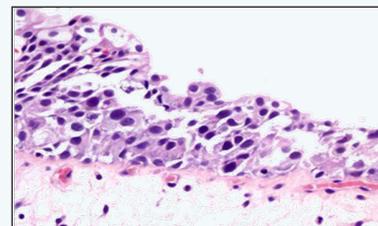


Figure 6: Carcinoma in situ.

### Transitional cell carcinoma

Transitional cell carcinoma differs from normal urothelium by having an increased number of epithelial cell layers with

papillary folding of the mucosa, loss of cell polarity, abnormal cell maturation from the basal to superficial layers, giant cells, nuclear crowding, increased nuclear to cytoplasmic ratio, prominent nucleoli, clumping of chromatin and increased number of mitoses. The significant criteria are the prominent nucleoli, clumping of chromatin, increased cell layers and loss of cell polarity. Urothelial carcinomas demonstrate a variety of patterns of tumor growth, including papillary, sessile, infiltrating, nodular, mixed, and flat intraepithelial growth (carcinoma in situ). Urothelium has great metaplastic potential; therefore, urothelial carcinomas may contain spindle cell, squamous, or adenocarcinomatous elements. These elements are present in about one third of muscle-invasive urothelial bladder cancers, and several may be exhibited in a single cancer. Approximately 70% of bladder tumors are papillary, 10% are nodular, and 20% are mixed [14], (Figure 7).

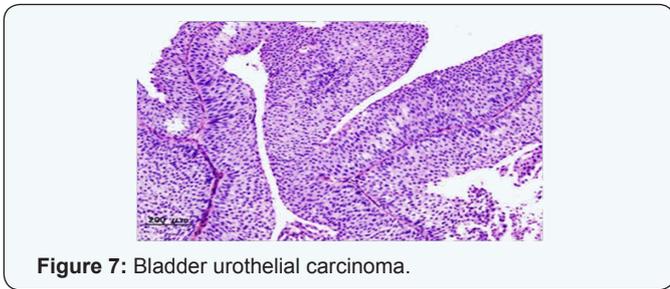


Figure 7: Bladder urothelial carcinoma.

**Tumor grading**

Several systems have been used to grade and classify bladder neoplasms. The system proposed by the World Health Organization (WHO) in 1973 distinguished papillomas from grades I, II, and III papillary transitional cell carcinomas (TCCs) [20]. In 1998, the WHO and ISUP published a consensus classification system for urothelial (transitional cell) neoplasms [14]. The clinical significance of this system was validated by subsequent studies, and in 2004, it was accepted as the standard classification system [21]. According to this system, urothelial cancer is classified as low-grade and high-grade based upon the degree of nuclear anaplasia and architectural abnormalities. "Well-differentiated tumors" have a thin fibrovascular stalk with a thickened urothelium containing more than seven cell layers, with cells exhibiting only slight anaplasia and pleomorphism.

The disturbance of the base-to-surface cellular maturation is mild, and there are only rare mitotic figures. Lesions with this appearance (similar to those formerly called grade 1) are urothelial cancers [22]. "Moderately differentiated (low grade urothelial carcinoma) tumors" have a wider fibrovascular core, a greater disturbance of the base-to-surface cellular maturation, and a loss of cell polarity. The nuclear-cytoplasmic ratio is higher, with more nuclear pleomorphism and prominent nucleoli. Mitotic figures are more frequent. These have been termed low-grade urothelial carcinomas in the new WHO and ISUP classification Murphy and colleagues (2002) point out the difficulties for even experienced practitioners to distinguish between low malignant potential and low-grade carcinoma

lesions as defined in the current classification [14]. Poorly differentiated tumors, named high-grade urothelial carcinoma in the new WHO and ISUP system (old grade 3), have cells that do not differentiate as they progress from the basement membrane to the surface (Table 1). Marked nuclear pleomorphism is noted, with a high nuclear-cytoplasmic ratio [14].

Table 1: WHO grading 1973 and 2004. ISUP: International Society of Urologic Pathology.

2004 WHO/ISUP classification	WHO 1973 Classification
Papillary urothelial neoplasm of low malignant potential	Grade 1
Low-grade papillary urothelial carcinoma	Grade 1; Grade 2
High-grade papillary urothelial carcinoma	Grade 2; Grade 3
Carcinoma in situ	Carcinoma in situ
Abbreviation: ISUP, International Society of Urologic Pathologists.	

TNM classification approved by the Union International Contre le Cancer (UICC), has been widely accepted.

**Staging system**

TNM classification approved by the Union International Contre le Cancer (UICC), has been widely accepted (Table 2), (Figure 8).

Table 2: 2009 TNM classification of urinary bladder cancer.

T - Primary tumor
TX Primary tumor cannot be assessed T0 No evidence of primary tumor
Ta Non-invasive papillary carcinoma Tis Carcinoma in situ: "flat tumor"
T1 Tumor invades subepithelial connective tissue
T2 Tumor invades muscle
T2a Tumor invades superficial muscle (inner half) T2b Tumor invades deep muscle (outer half)
T3 Tumor invades perivesical tissue T3a Microscopically
T3b Macroscopically (extravesical mass)
T4 Tumor invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall
T4a Tumor invades prostate, uterus or vagina T4b Tumor invades pelvic wall or abdominal wall
N - Lymph nodes
NX Regional lymph nodes cannot be assessed N0 No regional lymph node metastasis
N1 Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac or presacral)
N2 Metastasis in multiple lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N3 Metastasis in common iliac lymph node(s)
M - Distant metastasis
MX Distant metastasis cannot be assessed M0 No distant metastasis
M1 Distant metastasis

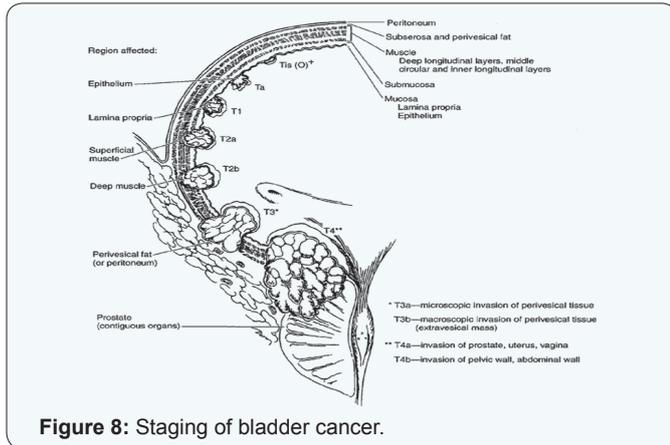


Figure 8: Staging of bladder cancer.

**Squamous cell carcinoma**

Bilharzial SCCs are exophytic, nodular, fungating lesions that are usually well differentiated and have a relatively low incidence of lymph node and distant metastases. Whether the low incidence of distant metastases is due to capillary and lymphatic fibrosis resulting from chronic schistosomal infection [23] or to the relatively low histologic grade [22] of these tumors is not clear.

Non-bilharzial SCCs are usually caused by chronic irritation from urinary calculi, long-term indwelling catheters, chronic urinary infections, or bladder diverticula. As many as 80% of paraplegics with chronic infections and/or indwelling catheters have squamous changes in the bladder, and about 5% develop SCC [24]. Cigarette smoking has also been reported to be significantly associated with an increased risk of bladder SCC [25]. In general, its prognosis is poor because most patients have advanced disease at the time of diagnosis (Figure 9).

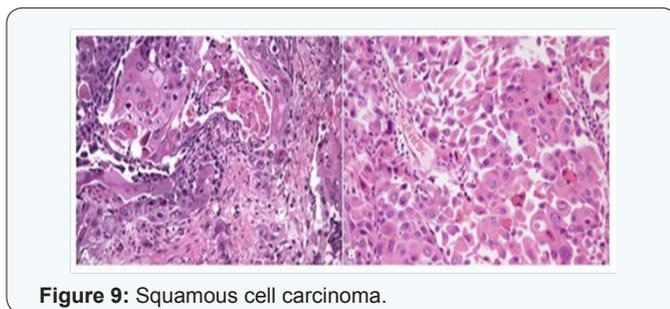


Figure 9: Squamous cell carcinoma.

**Verrucous squamous cell carcinoma**

This is a rare variant of squamous cell carcinoma of the bladder, accounting for less than 5% of cases. Most cases are associated with *S. haematobium* infection; few cases have been reported from nonendemic areas. The tumor has an indolent growth pattern and spreads by direct extension. It does not metastasize, although it may develop foci of invasive SCC [26-27].

**Grading of squamous cell carcinoma**

The tumor is generally graded as well, and moderately

or poorly differentiated, depending upon the extent of keratinization and nuclear pleomorphism. However, the grading system is not universally reproducible, as some authors believe that there is no direct correlation between the aggressiveness and the tumor grade [28]. Others believe that histologic grade influences the tumor stage and clinical Outcome [29].

**Adenocarcinoma**

Adenocarcinomas account for less than 2% of primary bladder cancer [25], (Figure 9). They are classified into three groups: (a) primary vesical; (b) urachal; and (c) metastatic [30]. Adenocarcinomas also occur in intestinal urinary conduits, augmentations, pouches, and ureterosigmoidostomies (Figure 10).

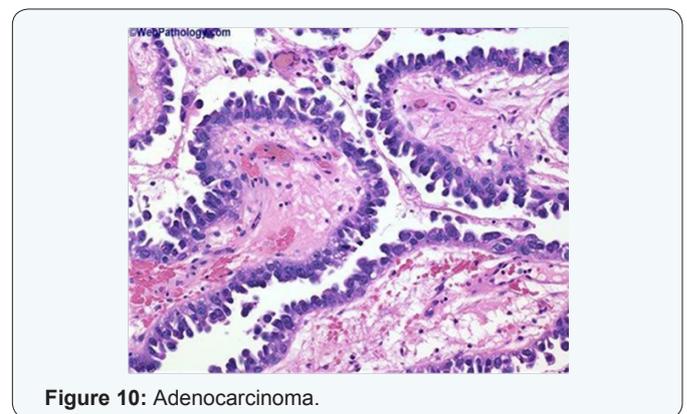


Figure 10: Adenocarcinoma.

**Primary vesical adenocarcinoma**

Adenocarcinomas usually arise in the bladder base area or in the dome, but they can occur anywhere. It is the most common type of cancer in exstrophic bladders. These tumors develop in response to chronic inflammation and irritation [31]. All histologic variants of enteric adenocarcinoma occur in the bladder. Most adenocarcinomas are poorly differentiated and invasive. They are more commonly associated with cystitis glandularis than with carcinoma in situ.

**Urachal carcinoma**

Urachal carcinomas are extremely rare tumors that arise outside the bladder, and they are usually adenocarcinomas, although they may be primary TCCs or SCCs and, rarely, even sarcomas. Urachal carcinomas have a sharp demarcation between the tumor and the adjacent bladder epithelium, with the tumor being located in the bladder wall beneath the normal epithelium. They may appear with a bloody or mucoid discharge from the umbilicus or produce a mucocele, occurring as a palpable mass. Tumors invading the bladder lumen may produce mucus in the urine. Patients with urachal carcinomas have a worse prognosis than do those with primary bladder adenocarcinomas [15]. Histologically, these tumors exhibit wider and deeper infiltration of the bladder wall than expected, compromising the results of partial cystectomy. Urachal carcinomas metastasize to iliac and inguinal lymph nodes, omentum, liver, lung and bone.

**Metastatic adenocarcinoma**

One of the most common forms of adenocarcinoma of the bladder is metastatic (or invasive) adenocarcinoma [32]. The primary sites for these tumors include the rectum, stomach, endometrium, breast, prostate, and ovary.

**Values of peripheral blood parameters in NMIBC and MIBC**

**Hemoglobin (Hb):** The most common primary symptom of bladder cancer is painless hematuria (80-90% of patients) and gross hematuria more commonly predicts bladder cancer than does microscopic hematuria [33]. The anemia of cancers is caused by stimulation of the cellular immune system and inflammatory changes, which stimulate the production of chemicals called cytokines and affect both red cell production and survival. Several cytokines, including tumor necrosis factor (TNF), Interferon Gamma and Interleukin-1 (IL-1), can suppress bone marrow production (erythropoiesis) by affecting red cell production [34].

**White blood cells and the platelets counts:** White blood cells (WBCs), also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders [35]. The most frequent systemic alterations detected in patients with malignant solid tumors are leukocytosis and neutrophilia. These hematological conditions are significantly correlated with advanced disease and, consequently, with poor prognosis. Leukocytosis is a condition often encountered in a clinical setting, usually caused by an increase in the number of neutrophils, which represent 50 to 60% of total leukocytes [36]. Platelets, also called thrombocytes, are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clotting blood vessel injuries [37]. Thrombocytosis is commonly observed in neoplastic diseases. Elevated levels of platelet counts may be associated with tumor progress. Various studies have showed that thrombocytosis is associated with poor prognosis in ovarian cancer renal cell carcinoma [38], colorectal cancer [39], gastric cancer [40], and endometrial carcinomas [41].

**Neutrophil-to-lymphocyte ratio:** In medicine neutrophil to lymphocyte ratio (NLR) is used as a marker of subclinical

inflammation. It is calculated by dividing the number of neutrophils by number of lymphocytes, usually from peripheral blood sample [42]. The neutrophil and lymphocyte counts play important roles in systemic inflammation. The neutrophil count is increased by anti-apoptotic markers that affect tumor growth and progression (NF-κB), growth factors, and proangiogenic factors (VEGF) [43].

The lymphocytic response is the main component in the control of cancer progression. Lymphocytopenia leads to a decrease in the cellular immune response. While some studies have reported that decreased T-cell activity inside the tumor speeds up primary tumor progression, other studies have shown a link between lymphocytes and the cell-mediated response with respect to tumor infiltration. Additionally, a low level of lymphocytic infiltration at tumor margins indicates a poor prognosis. The fact that the NLR indicates only part of the inflammatory response somewhat limits its value as a marker. However, the NLR is still a convenient, inexpensive, and reproducible parameter that demonstrates the link between inflammation and tumor development.

Neutrophil to lymphocyte ratio is associated with different types of cancer. Increased pretreatment NLR is associated with poor prognosis in colorectal, gastric, and ovarian cancer; malignant mesothelioma; and renal cell carcinoma [44-45] in their study, stated that, NLR threshold value <2.5 and ≥2.5 as an independent prognostic factor, likewise tumor size, hydronephrosis, Hb levels, and the combination of these factors, could stratify disease-specific survival (DSS) risks in bladder cancer patients treated with radical cystectomy .

**Some Clinical Studies on the Prognostic Value of NLR in UC of the Bladder**

**Patients and methods**

The study design is a prospective cohort study includes 102 patients who were diagnosed with urothelial cancer of the urinary bladder between October 2015 and April 2016. The study was done at the Department of Urology, Cairo University. Preoperative blood sample were taken before the first cystoscopic examination (complete blood picture with differential). The stage and grade of the disease were recorded from pathology reports (Table 3).

**Table 3:** Clinical studies on the prognostic value of NLR in UC of the bladder.

Study	Marker	Publication year	Number of patients(NMIBC/MIBC)	Threshold	Assessment period	Main findings
Demirtas, et al. [40]	NLR	2013	201(35/166)	2.5	Before RC	Evaluated NLR (>2.5) was not associated overall survival
Harmanns, et al. [41]	NLR	2014	424	3	Before RC	Patients with elevated NLR (>3) significantly showed more advance pathologic tumor stage. Elevated NLR (>3) was significantly associated with RFS, OS and CSS

Kaynar, et al. [42]	NLR	2014	291(192/99)	NA (continuous)	1day before surgery (TURBT or RC)	Patients with MIBC showed significantly higher NLR value then those with NMIBC. Also higher NLR significantly correlated with advanced age, larger tumor size, and aggressive tumor invasiveness
Potretzke, et al. [43]	NLR	2014	102(31/71)	NA (continuous)	Before RC	NLR was significant predictor of the pathological upstaging after RC; also, patients with pathological upstaging to >pT3 had a significantly greater NLR compare to patients who remained at <pT2
Viers, et al. [44]	NLR	2014	899(392/507)	2.7	Within 90 days RC	Evaluated NLR (>2.5) was significantly associated with adverse pathologic funding (high pathologic tumor stage, node positive and large tumor size); increased NLR (continuous) independently associated with worse RFS, OS, and CSS
Mano, et al. [47]	NLR	2015	107(107/0)	2.41 (for progression ) 2.43 (for recurrence)	Before TURBT	Elevated NLR (>2.41) showed more pT1 tumors and was significantly associated with disease progression; evaluated NLR (>2.43) was independent predictor of disease recurrence
Seah, et al. [48]	NLR	2015	26(26/0)	NA	Before NACH, during NACH and after RC	Significant NLR decrease from before NACH to before RC was observed in patients with pathological response after NACH and RC
Kang, et al. [45]	NLR	2015	385	2.0 (Postoperative) 2.1 (preoperative)	Within 1 month before RC Within 3 months after RC	Patients with elevated postoperative NLR (>2.0) had higher rates of >pT3, LVI, and positive lymph node and elevated postoperative NLR (>2.0) was an independent predictor of OS and CSS; also patients with preoperative continuous elevated NLR (2.1->2.0) showed worse OS and CSS compared with other change groups
<p>NLR: Neutrophil-to-Lymphocyte Ratio, TURBT: Transurethral Resection of Bladder Tumor; RC: Radical Cystectomy, NACH: Neo Adjuvant Chemotherapy, NMIBC: Non muscle Invasive Bladder Cancer; OS: Overall Survival, DSS: Disease Specific Survival, RFS: Recurrence -Free Survival, and CSS: Cancer Specific Survival.</p>						

**Inclusion criteria**

Patients who were diagnosed with urothelial cancer of the urinary bladder and admitted to the Urology Department at Kasr Alainy Hospitals.

**Exclusion criteria**

1. Patients who had pyelonephritis, prostatitis, epididymoorchitis or patients with fever.
2. Hematologic disease (myeloma, leukemia, lymphoma).
3. Secondary malignancy.
4. Patients who had a history of conditions that may have influenced blood cell lines (connective tissue disease, malignant lymphoma, leukaemia and HIV infection).

**Methodology in Details**

Preoperative blood samples were taken before the first cystoscopic examination from patients who diagnosed with urothelial cancer of the urinary bladder to make assessment relationship between peripheral blood parameters and stage and grade of the disease in patients with urothelial cancer of urinary bladder. Patients were grouped as having a non-muscle-

invasive or muscle-invasive urothelial carcinoma. The stage and grade of the disease were recorded from pathology reports.

A standard form was designed and included the following:

**1-Name:**

**2-Gender:**

**3-Age:**

**4-Diagnosis, including stage and grade:**

**5- Peripheral blood parameters:** hemoglobin (Hb), white blood cells (WBCs), neutrophil count, lymphocyte count, neutrophil-to-lymphocyte ratio and platelets count.

**Statistical Methods**

Data management and analysis were performed using SigmaStat program; version 3.5 (Systat Software, Inc., USA). The graphs were done using Microsoft Excel 2007. The numerical data were statistically presented in terms of range, mean, standard deviation, median and interquartile range. Categorical data were summarized as percentages. All p-values are considered significant when P-values were less than 0.05.

**Results**

A total of 102 patients were included in the study, 49 NMIBC and 53 MIBC, respectively. The majority of the patients were male (97 of 102, 95.1%), with a median age of 59 years of the NMIBC patients, 20 (19.6%) had Ta disease and 29 (28.4%) had

T1 disease. Of the MIBC patients, 40 (39.2%) had T2 disease and 13 (12.7%) had T3 disease. While, G1=22 (21.6 %), G2=18 (17.6%) and G3=62 (60.8%). Full blood count samples were taken prior to TURBT surgery. The value of hemoglobin showed no statistically significant associations ( $p>0.05$ ) in comparison between groups. (Table 4).

**Table 4:** Shows the value of hemoglobin for bladder cancer patients according to stage and grade of tumor.

Dependant variable		(I) Stage of tumor	(J) Stage of tumor	Mean Difference (I-J)	Std. Error	Sig.
Hemoglobin	Scheffe	Ta	T1	-.5287	.56285	.829
			T2	.48500	.53032	.841
			T3	.99462	.68989	.559
		T1	Ta	.52897	.56285	.829
			T2	1.01397	.47228	.210
			T3	1.52358	.64634	.143
		T2	Ta	-.48500	.53032	.841
			T1	-1.01397	.47228	.210
			T3	.50962	.61822	.878
	T3	Ta	-.99462	.68989	.559	
		T1	-1.52358	.64634	.143	
		T2	-.50962	.61822	.878	
	Dunnett T(2-sided) <sup>a</sup>	Ta	T3	.99462	.68989	.303
		T1	T4	1.52358*	.64634	.048
		T2	T5	.50962	.61822	.694
Dependant variable		(I) Grade of tumor	(J) Grade of tumor	Mean Difference (I-J)	Std. Error	Sig.
Hemoglobin	Scheffe	G1	G2	1.24985	.62145	.138
			G3	.77189	.48524	.287
		G2	G1	-1.24985	.62145	.138
			G3	-.47716	.52352	.660
		G3	G1	-.77189	.48524	.287
			G2	.47796	.52352	.660
	Dunnett T(2-sided) <sup>a</sup>	G1	G3	.77189	.48524	.212
		G2	G3	-.47796	.52352	.587
* The mean difference is significant at the 0.051 level						
a. Dunnett t-tests treat one group as a control, and compare all the groups against it						

The value total leukocytic count showed a highly statistically significant association with tumor stage ( $p<0.003$ ) and a significant association ( $p<0.05$ ) with tumor grades G1 and G2 versus G3 (Table 5). Platelet count was not significantly associated with tumor stage ( $p>0.05$ ), but an association was

seen between platelet count and tumor grade, specifically G2 vs. G3 ( $p=0.005$ ) (Table 6). The value of neutrophil count showed a highly significant ( $p<0.005$ ) association with both tumor grade and stage, with neutrophils counts increasing with higher tumor grade and stage (Table 7). The value of lymphocytic count showed

a highly significant ( $p < 0.005$ ) association with tumor grade and stage, with mean lymphocytic count decreasing with increasing tumor grade and stage (Table 8). The value of neutrophil-to-lymphocyte ratio also showed a highly significant ( $p < 0.005$ ) association with tumor grade and stage (Table 9) (Figure 11-17).

**Table 5:** Shows the value of total leucocytic count for bladder cancer according to stage and grade of tumor.

Dependant variable		(I) Stage of tumor	(J) Stage of tumor	Mean Difference (I-J)	Std. Error	Sig.
Total Leucocytic count	Scheffe	Ta	T1	-.14224	.90617	.999
			T2	-3.42750*	.85380	.002
			T3	-4.28962*	1.11070	.003
		T1	Ta	.14224	.90617	.999
			T2	-3.28526*	.76036	.001
			T3	-4.14737*	1.04059	.002
		T2	Ta	3.42750*	.85380	.002
			T1	3.28526*	.76036	.001
			T3	-.86212	.99532	.861
	T3	Ta	4.28962*	1.11070	.003	
		T1	4.14737*	1.04059	.002	
		T2	.86212	.99532	.861	
	Dunnett T(2-sided) <sup>a</sup>	Ta	T3	-4.28962*	1.11070	.001
		T1	T4	-4.14737*	1.04059	.000
		T2	T5	-.86212	.99532	.664
Dependant variable		(I) Grade of tumor	(J) Grade of tumor	Mean Difference (I-J)	Std. Error	Sig.
Total Leucocytic count	Scheffe	G1	G2	.30707	1.08180	.961
			G3	-2.20287*	.84469	.037
		G2	G1	-.30707	1.08180	.961
			G3	-2.51534*	.91133	.025
		G3	G1	2.20827*	.84469	.037
			G2	2.51534*	.91133	.025
	Dunnett T(2-sided) <sup>a</sup>	G1	G3	-2.20827*	.84469	.020
G2		G3	-2.51534*	.91133	.014	

\*. The mean difference is significant at the 0.051 level

a. Dunnett t-tests treat one group as a control, and compare all the groups against it

**Table 6:** Shows the value of platelets count for bladder cancer patients according to stage and grade of tumor.

Dependant variable		(I) Stage of tumor	(J) Stage of tumor	Mean Difference (I-J)	Std. Error	Sig.
Platelets count	Scheffe	Ta	T1	18.3862	24.1195	.999
			T2	-27.8500	22.7256	.683
			T3	4.6462	29.5634	.999
		T1	Ta	-18.3862	24.1195	.900
			T2	-46.2362	20.2386	.164
			T3	-13.7401	27.6974	.970
		T2	Ta	27.8500	22.7256	.683
			T1	46.2362	20.2386	.164
			T3	32.4962	26.4924	.628
		T3	Ta	-4.6462	29.5634	.999
			T1	13.7401	27.6974	.970
			T2	-32.4962	26.4924	.682
	Dunnett T(2-sided) <sup>a</sup>	Ta	T3	4.6462	29.5634	.996
		T1	T4	-13.7401	27.6974	.901
		T2	T5	32.4962	26.4924	.421
Dependant variable		(I) Grade of tumor	(J) Grade of tumor	Mean Difference (I-J)	Std. Error	Sig.
Platelets count	Scheffe	G1	G2	-30707	1.08180	.073
			G3	-2.20287*	.84469	.820
		G2	G1	-.30707	1.08180	.073
			G3	-2.51534*	.91133	.005
		G3	G1	2.20827*	.84469	.820
			G2	2.51534*	.91133	.005
	Dunnett T(2-sided) <sup>a</sup>	G1	G3	-2.20287*	.81169	.773
		G2	G3	-2.51534*	.91133	.002
* The mean difference is significant at the 0.051 level						
a. Dunnett t-tests treat one group as a control, and compare all the groups against it						

**Table 7:** Shows the value of neutrophil count for bladder cancer patients according to stage and grade.

Dependant variable		(I) Stage of tumor	(J) Stage of tumor	Mean Difference (I-J)	Std. Error	Sig.
Neutrophile count	Scheffe	Ta	T1	-1.9379	1.9011	.792
			T2	-17.8500*	1.7912	.000
			T3	-23.7692*	2.3302	.000
		T1	Ta	1.9379	1.9011	.792
			T2	-15.9121*	1.5952	.000
			T3	-21.8313*	2.1831	.000
		T2	Ta	17.8500*	1.7912	.000
			T1	15.9121*	1.5952	.000
			T3	-5.9192	2.0881	.051
	T3	Ta	23.7692*	2.3302	.000	
		T1	21.8313*	2.1831	.000	
		T2	5.9192	2.0881	.051	
	Dunnett T(2-sided) <sup>a</sup>	Ta	T3	-23.7692*	2.3302	.000
		T1	T4	-21.8313*	2.1831	.000
		T2	T5	-5.9192*	2.0881	.014
Dependant variable		(I) Grade of tumor	(J) Grade of tumor	Mean Difference (I-J)	Std. Error	Sig.
Neutrophile count	Scheffe	G1	G2	-5.0364	3.3667	.331
			G3	-10.4106*	2.6288	.001
		G2	G1	5.0364	3.3667	.331
			G3	-5.3742	2.8362	.171
		G3	G1	10.4106*	2.6288	.001
			G2	5.3742	2.8362	.171
	Dunnett T(2-sided) <sup>a</sup>	G1	G3	-10.4106*	2.6288	.000
		G2	G3	-5.3742	2.8362	.116
*- The mean difference is significant at the 0.051 level a- Dunnett t-tests treat one group as a control, and compare all the groups against it						

**Table 8:** Shows the value of lymphocytic count for bladder cancer patients according to stage and grade of tumor.

Dependant variable		(I) Stage of tumor	(J) Stage of tumor	Mean Difference (I-J)	Std. Error	Sig.
Neutrophile count	Scheffe	Ta	T1	-2.2466	1.7336	.643
			T2	12.0250*	1.6335	.000
			T3	17.1808*	2.1249	.000
		T1	Ta	2.2466	1.7336	.643
			T2	14.2716*	1.4547	.000
			T3	19.4273*	1.9908	.000
		T2	Ta	-12.0250*	1.6335	.000
			T1	-14.2716*	1.4547	.000
			T3	5.1558	1.9042	.069
	T3	Ta	-17.1808*	2.1249	.000	
		T1	-19.4273*	1.9908	.000	
		T2	-5.1558	1.9042	.069	
	Dunnett T(2-sided) <sup>a</sup>	Ta	T3	17.1808*	2.1249	.000
		T1	T4	19.4273*	1.9908	.000
		T2	T5	5.1558*	1.9042	.020
Dependant variable		(I) Grade of tumor	(J) Grade of tumor	Mean Difference (I-J)	Std. Error	Sig.
Neutrophile count	Scheffe	G1	G2	3.5268	2.8522	.468
			G3	8.4707*	2.2271	.001
		G2	G1	-3.5268	2.8522	.468
			G3	4.9439	2.4028	.126
		G3	G1	-8.4707*	2.2271	.001
			G2	-4.9439	2.4028	.126
	Dunnett T(2-sided) <sup>a</sup>	G1	G3	8.4707*	2.2271	.000
		G2	G3	4.9439	2.4028	.081
*- The mean difference is significant at the 0.051 level a- Dunnett t-tests treat one group as a control, and compare all the groups against it						

**Table 9:** Shows the value of Neutrophil-to-Lymphocyte ratio for bladder cancer patients according to stage and grade of tumor.

Dependant variable		(I) Stage of tumor	(J) Stage of tumor	Mean Difference (I-J)	Std. Error	Sig.
Neutrophile to Lymphocyt count	Scheffe	Ta	T1	-.075808465273281	.695634799502813	1.000
			T2	-3.006686383367512*	.655432372967403	.000
			T3	-6.716438861318103*	.852643631109964	.000
		T1	Ta	.75808465273281	.695634799502813	1.000
			T2	-2.930877918094232*	.583704185155539	.000
			T3	-6.640630396044823*	.798824224661986	.000
		T2	Ta	3.006686383367512*	.655432372967403	.000
			T1	2.930877918094232*	.583704185155539	.000
			T3	-3.709752477950591*	.764070653252143	.000
	T3	Ta	6.716438861318103*	.852643631109964	.000	
		T1	6.640630396044823*	.798824224661968	.000	
		T2	3.709752477950591*	.764070653252143	.000	
	Dunnett T(2-sided) <sup>a</sup>	Ta	T3	-6.716438861318103*	.852643631109964	.000
		T1	T4	-6.64063039604423*	.798824224661968	.000
		T2	T5	-3.709752477950591*	.764070653252143	.000
Dependant variable	(I) Grade of tumor	(J) Grade of tumor	Mean Difference (I-J)	Std. Error	Sig.	
Neutrophile to Lymphocyt count	Scheffe	G1	G2	-1.067505753932009	.987141129985743	.559
			G3	-2.624337691239761*	.770777986499315	.004
		G2	G1	-1.067505753932009	.987141129985743	.559
			G3	-1.556831937307751	.831590924387040	.179
		G3	G1	-2.624337691239761*	.770777986499315	.004
			G2	-1.556831937307751	.831590924387040	.179
	Dunnett T(2-sided) <sup>a</sup>	G1	G3	-2.624337691239761*	.770777986499315	.002
		G2	G3	-1.556831937307751	.831590924387040	.122
* - The mean difference is significant at the 0.051 level						
a- Dunnett t-tests treat one group as a control, and compare all the groups against it						

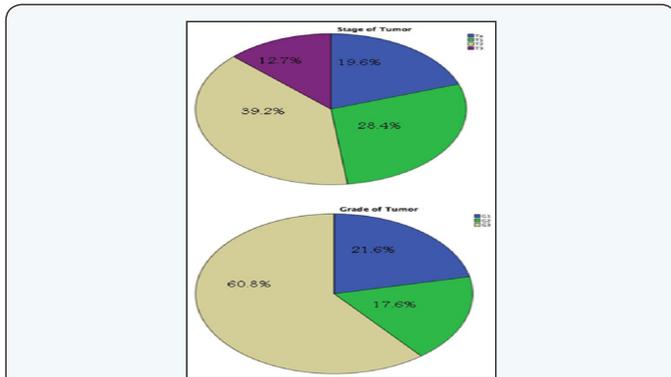


Figure 11: Descriptive Statistics of stage and grade of tumor.

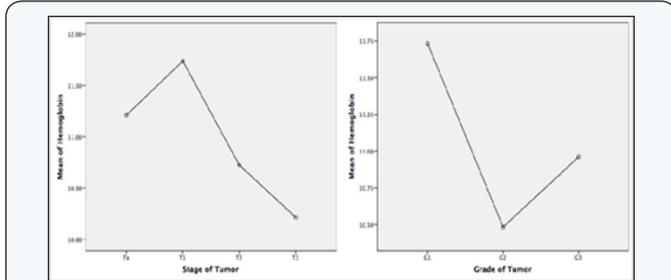


Figure 12: Mean Plot of hemoglobin.

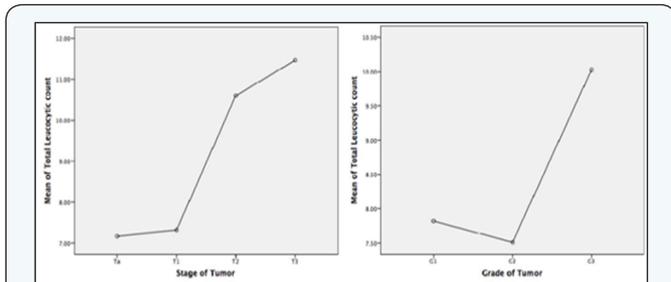


Figure 13: Mean Plot of leukocytic count.

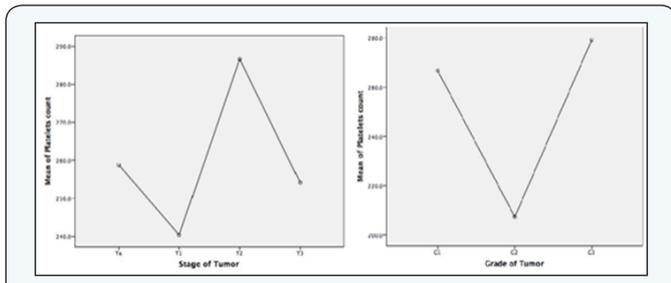


Figure 14: Mean Plot of platelets count.

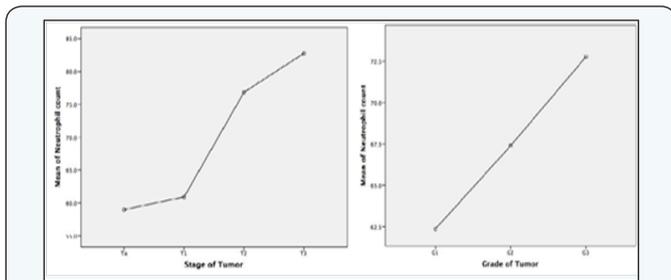


Figure 15: Mean Plot of neutrophil count.

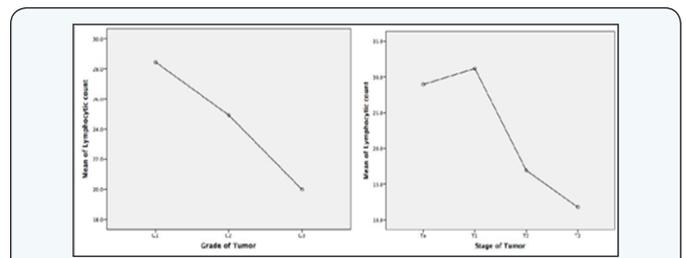


Figure 16: Mean Plot of lymphocytic count.

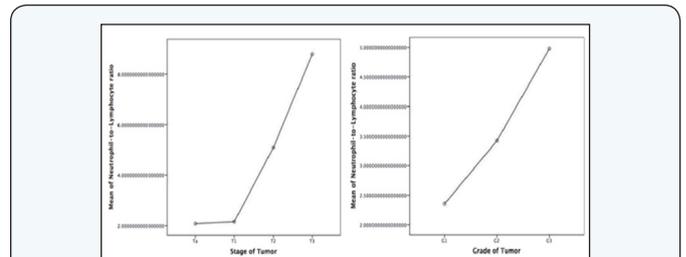


Figure 17: Mean Plot of neutrophil-to-lymphocyte ratio.

### Discussion

Bladder cancer is a common tumor in the urinary tract and is the ninth most prevalent cancer around the world. Approximately 90% of bladder cancers are urothelial carcinomas (UC) and 75-85% of UC are non-muscle invasive, while 15-25% are muscle-invasive. Treatment of UC depends on pathological behavior of the tumor and optimum management of bladder cancer is guided by accurate staging, for which pathological analysis is the gold standard [2]. There is increasing evidence that host inflammatory responses play a critical role in carcinogenesis, with inflammatory cells and innate immune system signaling molecules being involved in tumor progression [46]. This systemic inflammatory response leads to changes in relative levels of circulating leukocytes, providing a means to measure this response, in addition to circulating acute-phase proteins, e.g., C-reactive protein, fibrinogen, ferritin, albumin, etc [47]. In this present study, we examined the predictive values of peripheral blood parameters for bladder cancer.

We found that hemoglobin level generally did not differ significantly ( $p > 0.05$ ) between groups of tumor stage and grade; yet hemoglobin level was significantly lower in T3 patients compared to patients with T1 disease ( $p < 0.05$ ) by using Dunnett test. Probable explanation for this study finding is what's called anemia of chronic disease. Anemia of chronic disease is a blood disorder that refers to anemia found in people with certain long-term medical conditions, such as cancers. It is a common problem for cancer patients and sometimes results from the therapies used to suppress or control tumors. Approximately one-third of cancers have anemia [48]. The anemia of cancers is caused by stimulation of the cellular immune system and inflammatory changes, which stimulate the production of chemicals called cytokines and affect both red cell production and survival. Several cytokines, including tumor necrosis factor

(TNF), Interferon Gamma and Interleukin-1 (IL-1), can suppress bone marrow production (erythropoiesis) by affecting red cell production [34]. Patients with higher-stage bladder cancer may be more likely to be affected by these mechanisms.

In the study by Celik et al, the authors retrospectively evaluated the data collected from 639 patients who underwent TURBT surgery and The primary efficacy endpoint was the effect of preoperative anemia status on cancer-specific and overall survival. Independent t-test and chi-square analyses were performed to assess the effects of anemia on oncologic outcomes. Survival was estimated by using the Kaplan-Meier test. There were 118 (36.9%) and 202 (63.1%) patients in the anemia (Group-1) and non- anemia groups (Group-2), respectively. The median follow-up duration was 68 months. Anemia was associated with decreased overall survival ( $p < 0.001$ ). Comparison between cancer-specific survival of two groups did not show any statistically significant difference ( $p = 0.17$ ). Preoperative anemia status of bladder cancer patients according to World Health Organization classification is associated with decreased overall survival, but not with cancer-specific survival.

Total leukocytic counts in our cohort of patients were significantly associated with tumor stage and grade ( $p < 0.003$ ) when comparing between stages Ta and T2, T3 and ( $p < 0.05$ ) when comparing between grades G1 and G2, G3. Our results don't agree with a previous study by Al-Muhammadi et al who examined the relationship between leukocytic count and bladder cancer. Their results showed no significant associations between TLC and tumor stage or grade, and this may be attributed to the fact that the majority of their patients were low-stage. The value of platelets count showed insignificantly changed ( $p > 0.05$ ) in comparison between stages, but a highly significant ( $p < 0.005$ ) in comparison between G2 and G3 of tumor. In contrast, there was an inconsistency between our results and what was stated by [49] about the rare condition of paraneoplastic syndrome of bladder cancer. Paraneoplastic syndromes are defined as non-metastatic systemic effects that accompany malignant disease. These syndromes may occur in up to 10-15% of malignancies. A few paraneoplastic syndromes have been reported in metastatic transitional cell carcinoma including hypercalcemia, thrombocytosis, eosinophilia, and leukemoid reaction. Leukemoid reaction has rarely been reported in patients with primary bladder carcinomas.

The values of neutrophil count, lymphocyte count and neutrophil-to-lymphocyte ratio all showed a highly significant association with tumor stage and grade ( $p < 0.005$ ) in comparison between groups. Our results confirmed that NLR can be an independent predictor of MIBC. NLR was first proposed as a simple index to assess the systemic inflammatory response in critically ill patients. It has the advantage over other markers of inflammation on the basis of low cost and ease of access, given that it comprises components of the routine full blood count assay, and can easily be performed prior to cystoscopy or TURBT surgery.

The association between NLR and invasive disease is complex and remains to be elucidated. A high NLR is likely to reflect an increased neutrophil-dependent inflammatory reaction and decreased lymphocyte-mediated anti-tumor immune response [50]. Our results agree with previous studies that have examined the relationship between NLR and bladder cancer staging. The consensus finding in these studies was elevated NLR in MIBC as compared to NMIBC. A study was conducted in Turkey on 216 patients who underwent transurethral resection of the bladder tumor, in the NMIBC group, 149 (77.6 %) of them have (range 0.08-16.72, 95 % CI 1.67-2.97).

In terms of NLR, there was a statistically [51]. Another study [52], examined 198 patients: NMIBC (n=162), MIBC (n=36) and the values of NLR were found as  $4.14 \pm 2.76$  and  $3.36 \pm 2.88$ , respectively ( $p = 0.03$ ) and they concluded that if the NLR value is greater than 3.96, Ta-T1 tumors are likely to be invasive by 50%. Furthermore, the aim of our study was to assess the relationship between peripheral blood parameters and stage and grade of urinary bladder cancer and differentiate muscle-invasive bladder cancer from non-muscle invasive disease. The groups were separated into NMIBC (Ta/T1) and MIBC (T2+). It is noted that muscle-invasive, or T2+ disease does include a wide range of disease. This can include cancer that has invaded the muscularis propria (T2), up to adjacent structures (T4), such as the prostate, vagina or pelvic wall, and it is likely that the systemic inflammatory response increases with tumor stage. There are limitations to our study. First, this is a single-institution observational study. Second, while patients with concurrent inflammatory conditions (e.g. infection, hematological disorder) were omitted, the confounding effect of these cannot be completely excluded. Finally, we wished to expand the study by measuring additional markers of inflammation in this population such as C-reactive protein (CRP) and ESR.

### Summary and Conclusion

The current study is a prospective cohort study to assess the relationships between peripheral blood parameters and stage and grade of disease in patients with urothelial cancer of the urinary bladder. The study was conducted in Kasr Alainy hospital, Urology department on 102 patients diagnosed with bladder cancer. Preoperative blood samples were taken before the first cystoscopic examination to assess the relationship between peripheral blood parameters and stage and grade of the cancer of urinary bladder. Patients were grouped as having a non-muscle-invasive or muscle-invasive urothelial carcinoma according to pathology reports. In this present study, we examined the predictive values of various blood parameters for bladder cancer stage and grade. Our results confirmed that hemoglobin level had no significant association ( $p > 0.05$ ) with stage or grade groups. Total leukocytic count was significantly lower ( $p < 0.003$ ) in stage Ta compared to stages T2 and T3, and a significantly lower ( $p < 0.05$ ) in grades G1 and G2 compared to G3 tumors.



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