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

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To My Family

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List of 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 Centre Le Cancer

UTI: Urinary Tract Infection

VEGF: Vascular Endothelian Growth Factor

WBCs: 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% are 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 anti-tumoral 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 behavior [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.

Review of Literature

Pathology of cancer bladder

The wall of the urinary bladder consists of four layers (Figure 1).

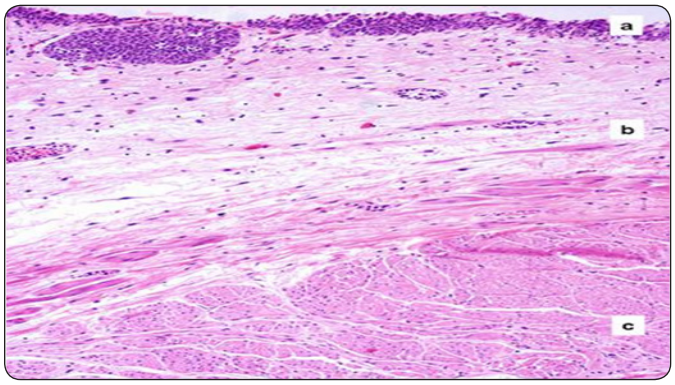

Figure 1: *Normal urinary bladder histology: urothelium.*

a. Lamina propria

b. With muscularis mucosae and submucosa; and muscularis propria (detrusor muscle)

c. Courtesy of Cristina Magi-Galluzzi.

a. Urothelium: The urothelium is the inner most epithelial lining of the bladder. The urothelium is the site of origin for urothelial carcinomas.

b. 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 mucosa [12,13].

c. 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 extra-vesical extension [14].

d. 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 [15] (Figure 2).

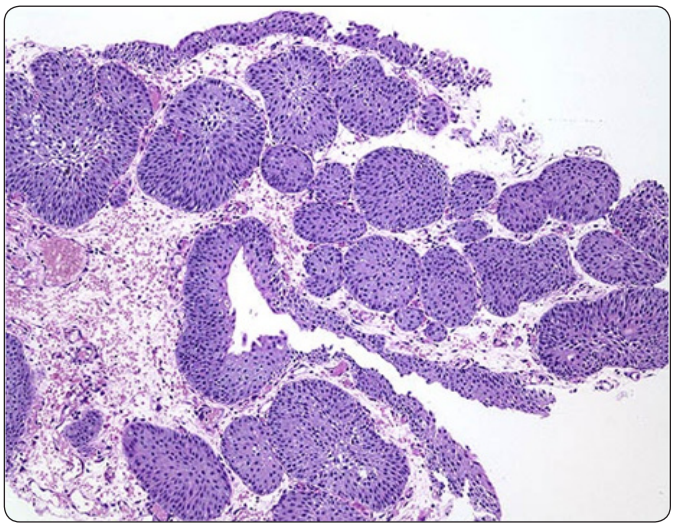

Figure 2: ***Von Brunn's nests.***

Cystitis cystica: Cystitis cystica is similar to Von Brunn's nests except that the center of the nests of urothelium has undergone eosinophilic liquefication. 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 sub mucosal nodule [16] (Figure 3).

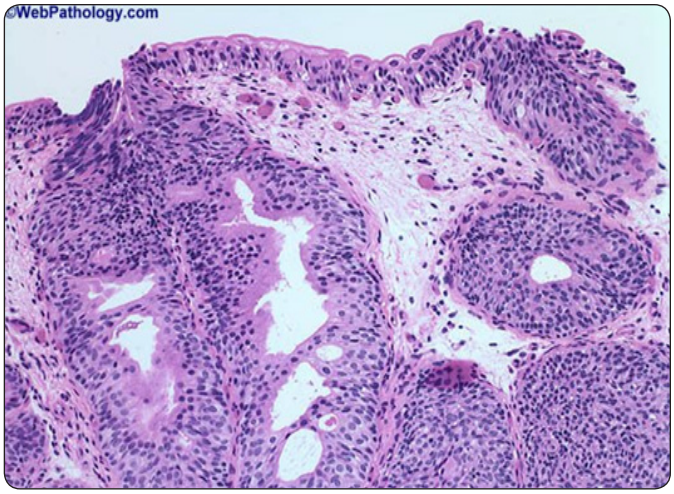

Figure 3: *Cystitis cystica.*

Cystitis glandularis: Cystitis glandularis is similar to cystitis cystica except that the transitional cells have undergone glandular metaplasia. It appears histologically as sub mucosal nests of columnar epithelial cells surrounding a central liquefied region of cellular degeneration [17]. 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 [18] (Figure 4).

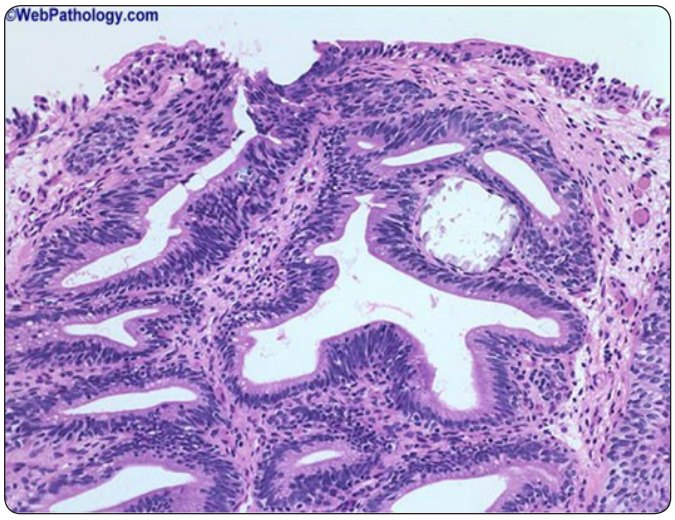

Figure 4: *Cystitis glandularis.*

Atypical hyperplasia: Atypical hyperplasia is similar to epithelial hyperplasia except that there are also nuclear abnormalities and partial derangement of umbrella cell layer [19]. In patients with superficial bladder cancers, the presence of atypical in adjacent urothelium is associated with a 35% to 40% risk of developing invasive disease [20].

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 [21].

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 [22] (Figure 5).

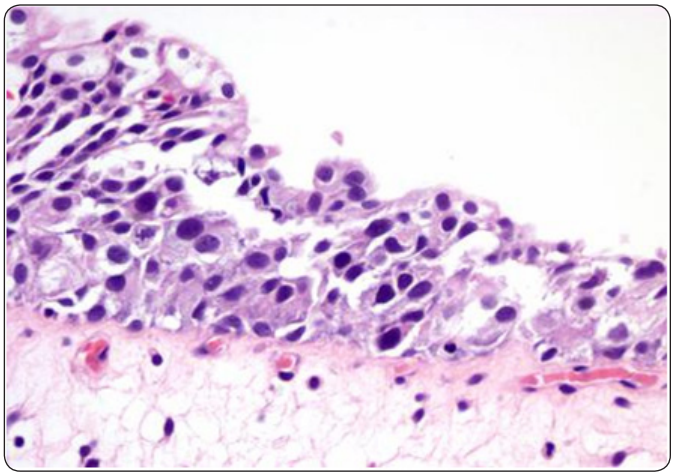

Figure 5: *Carcinoma in situ.*

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 6).

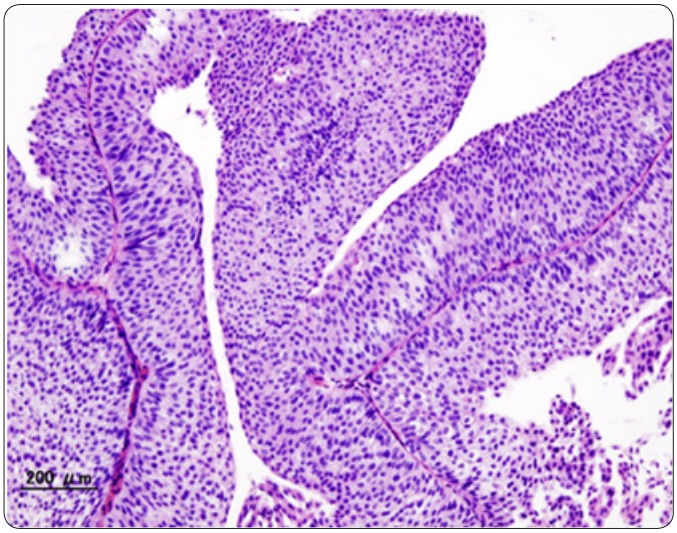

Figure 6: *Bladder urothelial carcinoma.*

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) (Table 1) [23]. 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 [24]. According to this system, urothelial cancer is classified as low-grade and high-grade based upon the degree of nuclear anaplasia and architectural abnormalities.

Table 1: *WHO grading 1973 and 2004.*

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.	

ISUP: International Society of Urologic Pathology.

a. Well-differentiated tumors have a thin fibro vascular 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. [25].

b. Moderately differentiated (low grade urothelial carcinoma) tumors have a wider fibro vascular 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].

c. 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. Marked nuclear pleomorphism is noted, with a high nuclear-cytoplasmic ratio [14].

Staging system: TNM classification approved by the Union International Contre le Cancer (UICC), has been widely accepted (Table 2 & 3).

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 sub epithelial 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 (extra vesical 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

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. [58]	NLR	2013	201 (35/166)	2.5	Before RC	Elevated NLR (>2.5) was not associated with overall survival
Hermanns, et al. [59]	NLR	2014	424	3	Before RC	Patients with elevated NLR (≥3) significantly showed more advanced pathologic tumor stage Elevated NLR (≥3) was significantly associated with RFS, OS and CSS
Kaynar, et al. [56]	NLR	2014	299 (192/99)	NA (continuous)	1 day before surgery (TURBT or RC)	Patients with MIBC shoed significantly higher NLR values than those with NMIBC. Also, higher NLR significantly correlated with advanced age, large tumor size and aggressive tumor invasiveness
Potretzke, et al. [60]	NLR	2014	102 (31/71)	NA (continuous)	Before RC	NLR was significant predictor of pathological upstaging after RC; also, patients with pathological upstaging to ≥T3 had significantly greater NLR compared to patients who remained at ≤pT2
Viers, et al. [61]	NLR	2014	899 (392/507)	2.7	Within 90days before RC	Elevated NLR (≥2.7) significantly associated with adverse pathological finding (higher pathological tumor stage, node positive and larger tumor size); increased NLR was independently associated with worse RFS, OS and CSS
Mano, et al. [62]	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; elevated NLR (>2.43) was independent predictor of disease recurrence
Seah, et al. [63]	NLR	2015	26 (0/26)	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. [49]	NLR	2015	385	2.0 (post operative) 2.1 (pre operative)	Within 1 month before RC and within 3 months after RC	Patients with post operative elevated NLR (≥2.0) had higher rates of ≥pT3, LV1 and positive lymph node and elevated post operative NLR (≥2.0) was an independent predictor of OS and CSS; also, patients with Perioperative continuous elevated NLR (2.1->2.0) showed worse OS and CSS compared with other change group

NLR: Neutrophil-to-lymphocyte ratio; TURBT: Transurethral resection of bladder tumor; RC: Radical cystectomy; NACH: Neoadjuvant chemotherapy; NMIBC: Non-muscle invasive bladder cancer; OS: Overall survival; DSS: Disease specific survival; RFS: Recurrence-free-survival; CSS: Cancer specific survival.

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 [26] or to the relatively low histological grade [25] of these tumors is not clear (Figure 7).

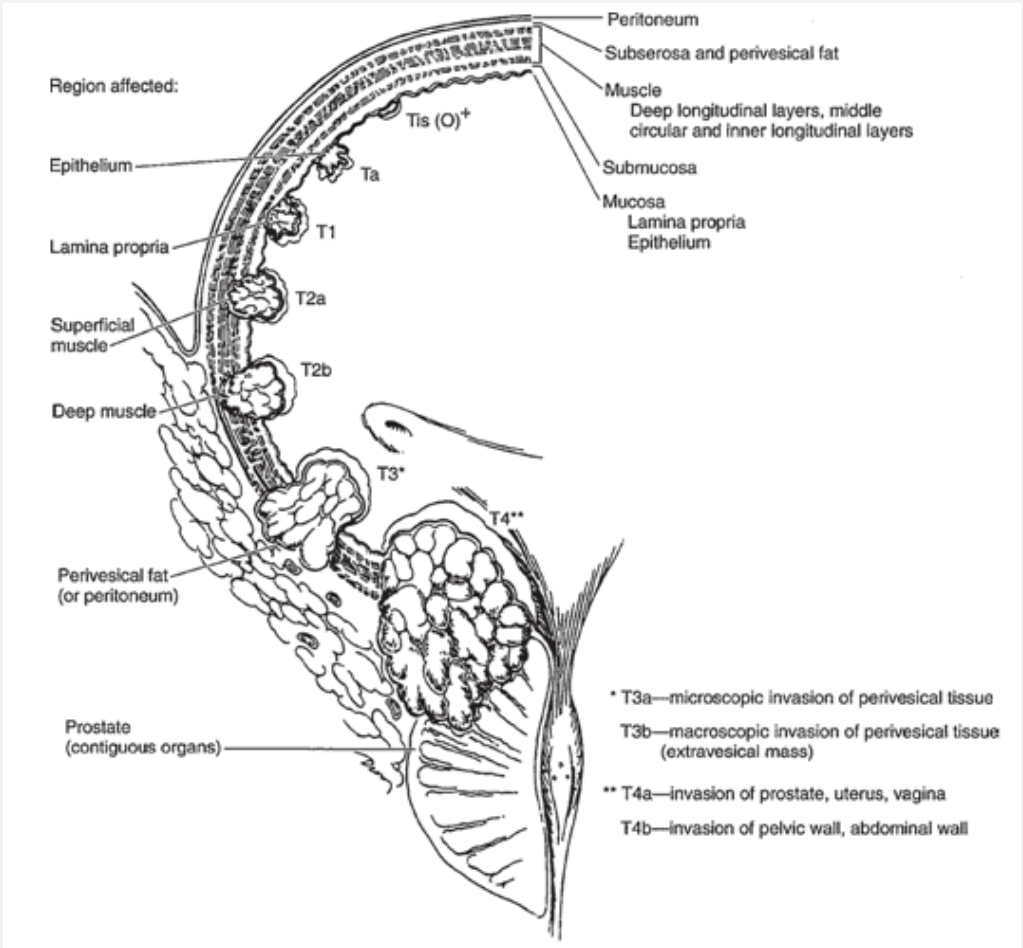

Figure 7: *Staging of bladder cancer.*

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 [27] (Figure 8). Cigarette smoking has also been reported to be significantly associated with an increased risk of bladder SCC [28]. In general, its prognosis is poor because most patients have advanced disease at the time of diagnosis.

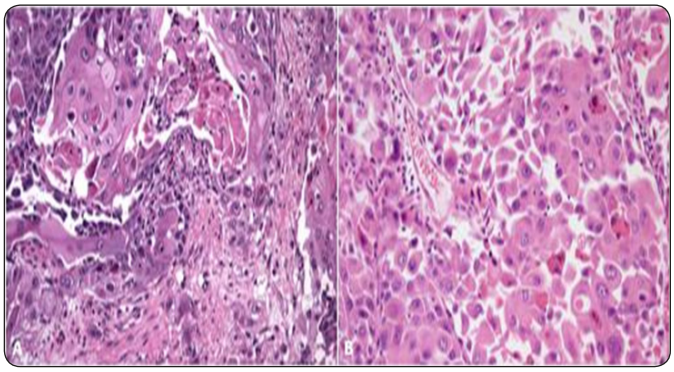

Figure 8: *Squamous cell carcinoma.*

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 non-endemic 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 [29,30].

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 [31]. Others believe that histologic grade influences the tumor stage and clinical outcome [32].

Adenocarcinoma: Adenocarcinomas account for less than 2% of primary bladder cancer [28] (Figure 9). They are classified into three groups: (a) primary vesical; (b) urachal; and (c) metastatic [33]. Adenocarcinomas also occur in intestinal urinary conduits, augmentations, pouches, and uretero sigmoidostomies.

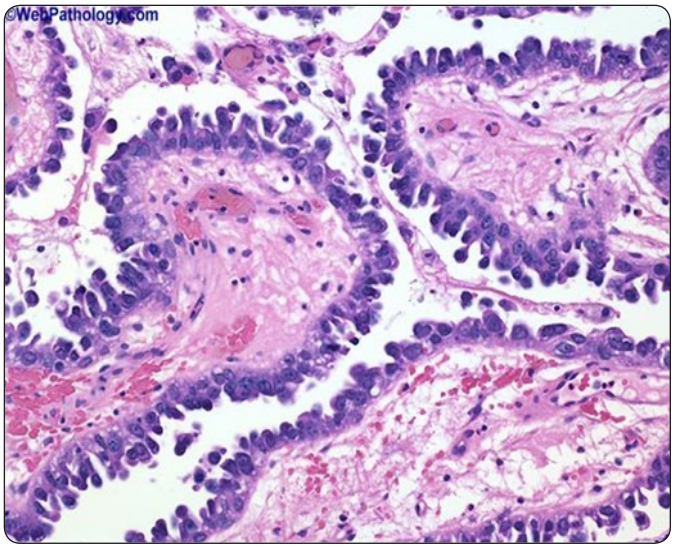

Figure 9: *Adenocarcinoma.*

P**rimary 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 [34]. 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 [17]. 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 [35]. 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 [36].

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 [37].

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 [38].

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 [39].

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 [40]. 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 [41], renal cell carcinoma [42], colorectal cancer [43], gastric cancer [44] and endometrial carcinomas [45].

Neutrophil-to-lymphocyte ratio

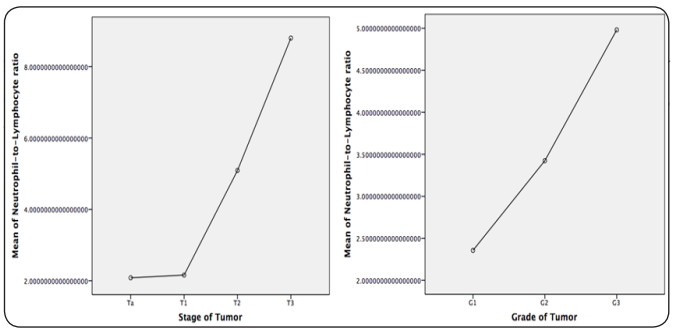

Figure 10: *Mean Plot of neutrophil-to-lymphocyte ratio.*

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 pro-angiogenic factors (VEGF) [46].

The lymphocytic response is the main component in the control of cancer progression. Lymphocytopaenia 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 [47].

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 [48]. Gondo et al. [49] 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 [49].

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.

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

Patients who had pyelonephritis, prostatitis, epididymoorchitis or patients with fever.

1. Hematologic disease (myeloma, leukemia, lymphoma).
2. Secondary malignancy.
3. Patients who had a history of conditions that may have influenced blood cell lines (connective tissue disease, malignant lymphoma, leukemia 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:

a. Name:

b. Gender:

c. Age:

d. Diagnosis, including stage and grade:

e. 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 Sigma Stat 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 inter quartile range. Categorical data were summarized as percentages. All p-values are considered significant when P-values were less than 0.05.

Results

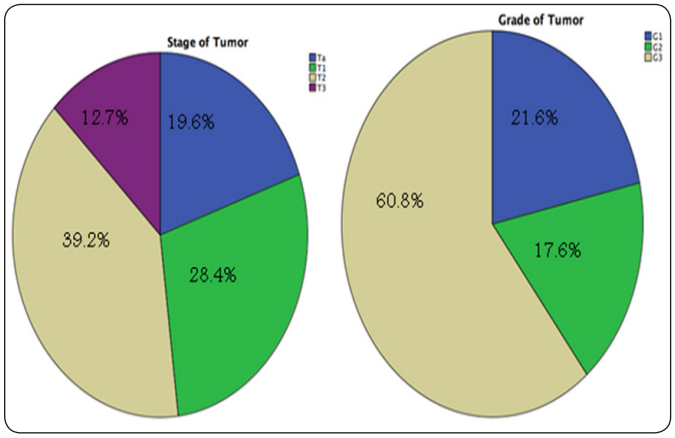

Figure 11: Descriptive Statistics of stage and grade of tumor.

A total of 102 patients were included in the study, 49 NMIBC and 53 MIBC, prospectively. 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 (Figure 11).

The value of hemoglobin showed no statistically significant associations (p>0.05) in comparison between groups (Table 4) (Figure 12).

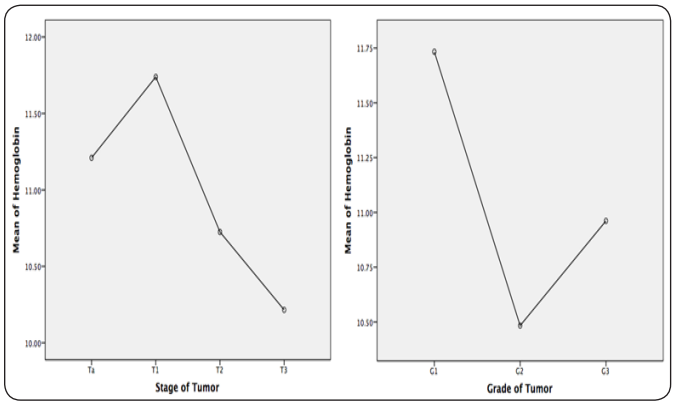

Figure 12: Mean Plot of hemoglobin.

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

Dependent Variable		(1) Stage of Tumor	(J) Stage of Tumor	Mean Difference (1--1)	Std. Error	Sig.
Hemoglobin	Scheffe	Ta	T1	-0.52897	0.56285	0.829
			T2	0.485	0.53032	0.841
			T3	0.99462	68989	0.559
		T1	Ta	0.52897	56285	829
			T2	1.01397	0.47228	0.21
			T3	1.52358	0.64634	0.143
		T2	Ta	-0.485	0.53032	841
			TI	-1.01397	0.47228	0.21
			T3	0.50962	0.61822	878
		T3	Ta	-0.99462	0.66969	0.559
			T'l	-1.52358	0.64634	0.143
			T2	-0.50962	0.61822	0.878
	Dunnettt (2-sided)[9]	Ta	T3	0.99462	0.58989	0.303
		T1	T3	1.52358'	0.54634	0.048
		12	T3	0.50962	0.61822	0.694
Dependent Variable		**(I) Grade of Tumor**	**(J) Grade of Tumor**	**Mean Difference -14**	**Std. Error**	**Sig.**
Hemoglobin	Scheffe	G1	G2	1.24985	0.62145	0.138
			G3	0.77189	0.48524	0.287
		G2	G1	-1.24985	0.52145	138
			G3	-0.47796	0.52352	0.66
		G3	G1	-0.77189	0.48524	0.287
			G2	0.47796	0.52352	0.66
	Dunnettt (2-sided)[9]	G1	G3	0.77189	0.48524	0.212
		G2	G3	-0.47796	0.52352	0.587

*. The mean difference is the significant at the 0.05 level.

a. Dunnett t-test treat one group as control and compare all other groups against it.

The value total leucocytic 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) (Figure 13).

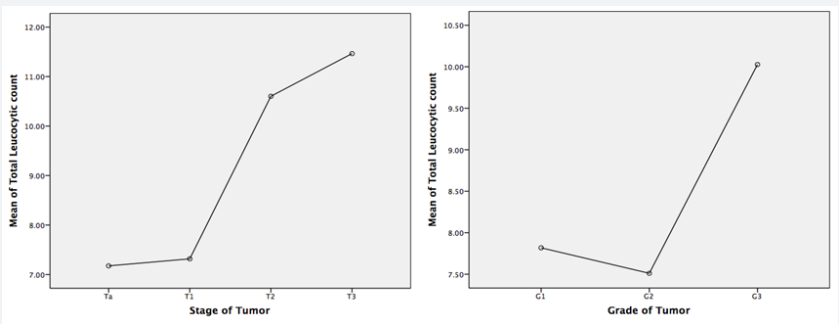

Figure 13: Mean Plot of leukocytic count.

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

Dependent Variable		(I) Stage of Tumor	(.3) Stage of Tumor	Mean Difference (@A)	Std. Error	Sig.
Total Leucocytic	Scheffe	Ta	Ti	-0.14224	0.90617	0.999
			T2	-3.42750’	0.8538	0.002
			T3	-428962’	1.1107	0.003
		T1	Ta	_14224	_90617	_999
			T2	-3.28526-	0.76036	0.001
			T2	-4.14737-	104059	0.002
		T2	Ta	3.42750-	0.8538	0.002
			Ti	328526-	0.76036	0.001
			T3	-0.86212	0.99532	0.861
		T3	Ta	4_28962’	1_11070	0.003
			Ti	4.1473T	1.04059	0.002
			T2	0.86212	0.99532	0.861
	Dunnett t (2-sided)	Ta	T3	-4.28962’	1.1107	0.001
		T1	T3	-4.14737’	1.04059	0
		T2	T3	-0.86212	0.99532	0.664
Dependent Variable		**(I) Grade of Tumor**	**(3) Grade of Tumor**	**Mean Difference (1-1)**	**Std. Error**	**Sig.**
Total Leucocytic count	Scheffe	G1	G2	0.30707	1.0818	0.961
			G3	-2 20E27-	0.84469	0.037
		G2	G1	- 31)707	1.0818	0.961
			G3	-2.51531’	0.91133	0.025
		3	G1	2.20827’	0.84469	0.037
			G2	2.51534’	0.91133	0.025
	Dunnettt (2-sided)$	G1	G3	-2.2082T	0.84469	0.02
		G2	G3	-2.51534’	0.91133	0.014

*. The mean difference is the significant at the 0.05 level.

a. Dunnett t-test treat one group as control and compare all other groups against it.

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) (Figure 14).

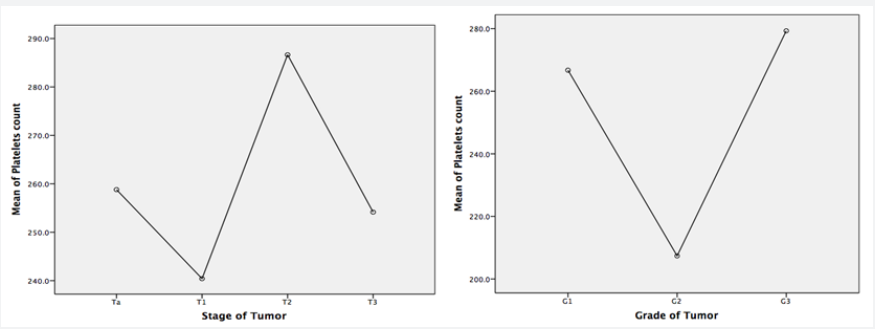

Figure 14: Mean Plot of platelets count.

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

Dependent Variable		(I) Stage of Tumor	(.I.) Stage of Tumor	Mean Difference (1-J)	Std. Error	Sig.
Platelets count	Scheffe	Ta	T1	18.3852	24.1195	900
			T2	-27.85	22.7256	0.683
			T3	4.6452	29.5634	999
		T1	Ta	-18.3862	24.1195	0.9
			T2	-46.2362	20.2386	0.164
			T3	-13.7401	27.6974	0.97
		T2	Ta	27.85	22.7255	0.683
			T1	45.2352	20.2385	0.154
			T3	32.4962	26.4924	0.582
		T3	Ta	-4.6452	29.5634	0.999
			T1	13.7401	27.5974	0.97
			T2	-32.4962	25A924	0.682
	Dunnettt (2-sided),	Ta	T3	4.6462	29.5634	0.996
		T1	T3	-13.7401	27.6974	0.901
		T2	T3	32.4962	25.4924	0.421
Dependent Variable		**(I) Grade of Tumor**	**(..1) Grade of Tumor**	**Mean Difference (1-J)**	**Std. Error**	**Sig.**
Platelets count	Scheffe	G1	G2	59.3384	25.5719	0.073
			G3	-12.5792	19.9€70	0.82
		G2	G1	-59.3384	25.5719	0.073
			G3	-71.9176’	21.5424	0.005
		G3	G1	12.5792	19.967	0.82
			G2	71_9176	21.5424	0.005
	Dunnettt (2-sided)$	G1	G3	-12.5792	19.967	0.773
		G2	G3	-71.9176’	21.5424	0.002

“. The mean difference is significant at the 0.05 level.

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

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) (Figure 15).

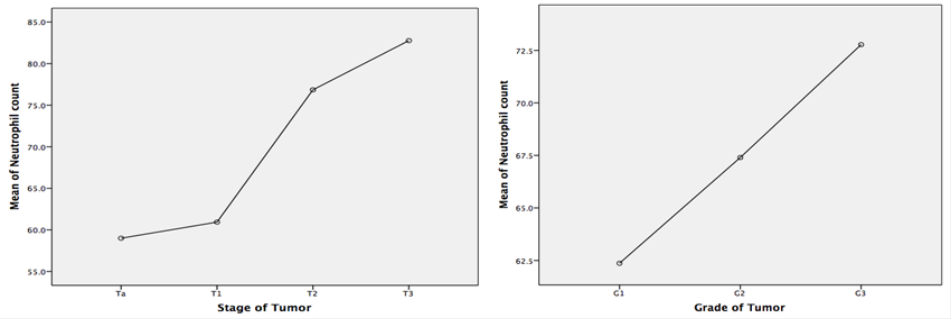

Figure 15: Mean Plot of neutrophil count.

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

Dependent Variable		(I) Stage of Tumor	(J) Stage of Tumor	Mean Difference (1-3)	Std. Error	Sig.
Neutrophil count	Scheffe	Ta	T1	-1.9379	1_9011	0.792
			T2	-17.8500'	1.7912	0
			T3	-23.7692"	2_3302	0
		T1	Ta	1.9379	1_9011	/92
			T2	-15.9121'	1_5952	0
			T3	-2t8313'	2_1831	0
		T2	Ta	17.8500'	1.7912	0
			T1	15.9121'	1_5952	0
			T3	-5.9192	2_0881	0.051
		T3	Ta	23.7692-	2_3302	0
			T1	21.8313-	2_1831	0
			T2	5.9192	2_0881	0.051
	Dunnettt (2-sided)$	Ta	T3	-23.7692-	2.702	0
		T1	T3	-21.8313'	2_1831	0
		T2	T3	-5.9192"	2_0881	0.014
Dependent Variable		**(I) Grade of Tumor**	**(0) Grade of Tumor**	**Mean Difference(1-1)**	**Std. Error**	**Sig.**
Neutrophil count	Scheffe	G1	G2	-5.0364	3.3567	0.331
			G3	-10.4106'	2.6288	0.001
		G2	G1	5.0364	3.3667	0.331
			G3	-5.3742	2_8362	_171
		G3	G1	10.4106'	2.6288	0.001
			G2	5.3742	2.8362	0.171
	Dunnettt (-sided)s	G1	G3	-10.4106'	2.6288	0
		G2	G3	-5.3742	2_8362	116

". The mean difference is significant at the 0205 level_

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

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) (Figure 16).

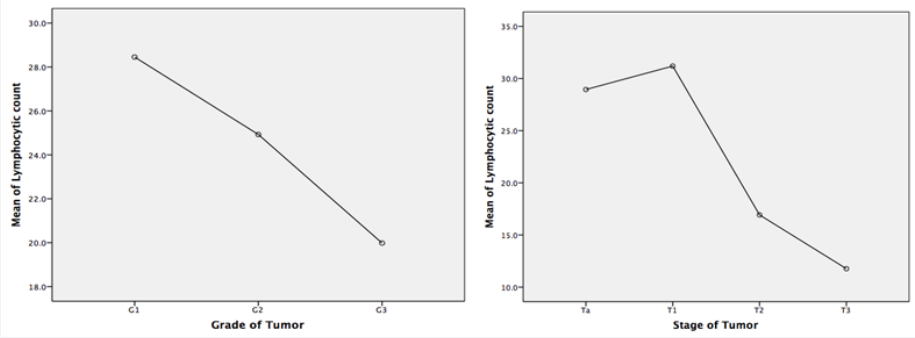

Figure 16: Mean Plot of lymphocytic count.

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

Dependent Variable		(0) Stage of Tumor	(J) Stage of Tumor	Mean Difference -14	Std. Error	Sig.
Lymphocytic count	Scheffe	Ta	T1	-2.2466	1.7336	0.643
			T2	12.0250-	t6335	0
			T3	17.1808’	21249	0
		Ti	Ta	21466	1.7336	0.643
			T2	14.2716’	1A547	0
			T3	19.4273’	1.9908	0
		T2	Ta	-12.0250’	1.6335	0
			Ti	-14.2716’	1.4547	0
			T3	5.1558	1.9042	0.069
		T3	Ta	-17.1808’	2.1249	0
			T1	-19.4273’	1.9908	0
			T2	-5.1558	1.9042	0.069
	Dunnettt (2-sided)9	Ta	T3	17.1808’	2.1249	0
		Ti	T3	19.4273’	1.9908	0
		T2	T3	5.1558-	L9042	0.02
Dependent Variable		**(1) Grade of Tumor**	**(J) Grade of Tumor**	**Mean Difference (1-J)**	**Std. Error**	**Sig.**
Lymphocytic count	Scheffe	G1	G2	3.5268	2.8522	.468
	Dunnettt (2-sided),	G2	G3	8.470r	2.2271	.001
		G3	G1	-3.5268	2.8522	0.468
		G1	G3	4.9439	2.4028	.126
		G2	G1	-8.4707’	2.2271	0.001
			G2	-4.9439	2.4028	.126
			G3	8.470T	2.2271	.000
			G3	4.9439	2.4028	.081

*. The mean difference is significant at the 0.05 level.

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

Discussion

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 (Kumar R et al, 2015) [50]. 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 [51].

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 Dennett 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 [52].

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 [37]. 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 (<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 leucocytic 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-Muhammad, et al. who examined the relationship between leucocytic 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 [53] about the rare condition of para neoplastic syndrome of bladder cancer. Para neoplastic 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 para neoplastic syndromes have been reported in metastatic transitional cell carcinoma including hyper calcemia, 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 [54] (Table 9). 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 [55].

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

Dependent Variable		(I) Stage of Tumor	(J) Stage of Tumor	Mean Difference (I-J)	Std. Error	Sig.
Neutrophil -to-Lymphocyte ratio	Scheffe	Ta	T1	-0.075808465	63	1
					- 695 479951)2	
					613	
			T2	-3.005585383367512’	0.655432373	0
					403	
			T3	-5.715438851318103’	. 852543531109	0
					964	
		T1	Ta	0.075808465	0.6956348	1000
					613	
			T2	-2.930877918094232’	-5.83704E+11	0
					539	
			T3	-6-640630396044823’	0.798824225	0
					968	
		T2	Ta	4	0.655432373	0
				3.006686383	403	
			Ti	0.930877918	-5.83704E+11	0
				232-	539	
			T3	-3.07614E-14	1.64071E+11	0
					143	
		T3	Ta	5.715438851318103-	0.852643531	0
					9C.4	
			T1	6.640630396044823-	0.798824225	0
					966	
			T2	3/09752477950591’	7.641E+11	0
					143	
	Dunnettt (2-sided)[9]	Ta	T3	-6.716438861318103’	-8.526E+11	0
					9b4	
		T1	T3	-6.640630396044823’	0.7988242	0
					968	
		T2	T3	-3/09752477950591’	-7.541E+11	0
					143	
Dependent Variable		**(I) Grade of Tumor**	**(J) Grade of Tumor**	**Mean Difference OM**	**Std. Error**	**Sig.**
Neutrophil-to-Lymphocyte ratio	Scheffe	G1	G2	-1.057505754	0.98714113	0.559
			G3	-2.524337591239751’	0.770777986	0.004
		G2	G1	1.057505754	0.98714113	0.559
			G3	-1.555831937	0.831590924	0.179
		G3	G1	2.524337591239751’	70777985499315	0.004
			G2	1.555831937	0.831590924	0.179
	Dunnettt (2-sided),	G1	G3	-2.524337591239751’	0.770777986	0.002
		G2	G3	-1.555831937	0.831590924	122

*. The mean difference is significant at the 0.05 level.

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

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 NIMBC group, 149 (77.6 %) of them have NLR ≤ 2.5 and 43 (22.4 %) have NLR > 2.5. Also, in MIBC, 67 (67.7 %) of them have NLR ≤ 2.5 and 32 (32.3 %) have NLR > 2.5. The mean NLR in the NMIBC group was 2.4 ± 0.1 (range 0.08-6.49, 95 % CI 1.52–2.71) and in the MIBC 2.9 ± 0.2 (range 0.08-16.72, 95% CI 1.67-2.97). In terms of NLR, there was a statistically significant difference between the NMIBC and MIBC groups (p=0.028) [56].

Another study [57], examined 198 patients: NMIBC (n=162), MIBC (n=36) and the values of NLR were found as 4.14 ± 2.76 and 3.36 ± 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

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 leucocytic 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. 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). The values of neutrophil count, lymphocyte count and neutrophil-to-lymphocyte ratio all showed highly statistically significant differences (p<0.005) between tumor stage and grade groups.

Conclusion

In conclusion, it would appear that the systemic inflammatory response parameters were strongly associated with stage and grade in patients with bladder cancer. The role of the systemic inflammatory response in determining disease-specific survival in patients with bladder cancer is worthy of further study to establish its value as a prognostic factor. NLR is an independent predictor of muscle-invasive disease and may provide a simple, cost-effective and easily measured marker for MIBC. It can be performed at the time of diagnostic cystoscopy, thereby assisting in the planning of further treatment and follow-up, including tumor resection and provision of intra vesical therapy.

Arab summary

This proactive study to assess the relationship between the ocean and the stage of the disease and the degree of blood parameters in patients with cancer of the urothelium in the urinary bladder.

The study was conducted at the Palais-Aini hospital - Urology department on 102 patients with bladder cancer and the candidate for the work of binoculars and take a sample.

Blood samples were taken before the first diagnostic endoscope to find a relationship between the parameters of peripheral blood and the stage and the degree of urinary bladder cancer.

Patients were divided into two groups according to the results of the sample (urothelium cancer is penetrating the muscular wall and cancer urothelium infiltrated the muscular wall of the bladder).

In this study, we examined the predictive values of bladder cancer. Our results confirmed the following values:

Hemoglobin is not important and there are no significant differences in the comparison between groups. Total the total number of white blood cells showed significant differences and that it is important to compare the grades and stages of the disease.

Platelets value did not show any significant change in the comparison between the stages of the disease, while high moral value shown in the comparison between the second and third degrees of illness.

Neutrophil values, the number of lymphocytes and the percentage of neutrophils to lymphocytes, all of which showed significant differences statistically significant differences in the comparison between groups.

In short, it seems that the systemic inflammatory response is the stage and the degree of independent prognostic factor in

patients with bladder cancer. The role of systemic inflammatory response in identifying the disease - specific survival in patients with bladder cancer deserves further study to establish its value as a prognostic.

Neutrophils - to - lymphocyte ratio is an independent predictor of piercing muscle diseases it can be considered effective and simple in terms of cost and ease of measurement of disease penetrating the bladder cancer. And it can be implemented and its work at the time of the diagnostic cystoscopy, which helps in planning for further treatment and follow-up, including lumpectomy Ooajra drug injection into the urinary bladder.

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