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Preoperative High Neutrophil–to–Lymphocyte Ratio Is Associated with High-grade Bladder Cancer

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Abstract. *Aim: To evaluate the correlation between the neutrophil–to–lymphocyte ratio (NLR) and histopathological characteristics of bladder cancer. Materials and Methods: A retrospective analysis was performed on a cohort of patients (n=302) who were diagnosed with bladder cancer and underwent transurethral resection of bladder tumor or cystectomy between 2009 and 2016. The pathological outcomes were compared between patients with low NLR and those with high NLR with a cut-off value of 2.5, and a logistic regression analysis was performed to find potential predictors of pathological tumor outcomes. Results: Patients with high-grade disease had significantly higher NLRs compared to those with low-grade disease (median NLR=4.42 vs. 3.42, p<0.001). Univariate analysis suggested that age, neutrophil count and NLR, as a continuous or binary variable, were significantly associated with high-grade disease. Multivariate analysis suggested that age and NLR, as a continuous variable, were predictors of pathologically high-grade disease. Conclusion: Preoperative NLR was found to be associated with pathological tumor grading, but was not associated with pathological tumor staging in patients with bladder cancer.*

Based on recent theories, systemic inflammation induced by tumors might play a critical role in tumor occurrence, progression and metastasis (1). The neutrophil count rises while the lymphocyte count falls under systemic inflammation, which leads to a change in the neutrophil to lymphocyte ratio (NLR) (2). As a marker of systemic inflammation, the NLR has been evaluated as an effective predictive marker for a range of cancer typess, including gastrointestinal (3) and epithelial ovarian (4).

Several studies have suggested that NLR may be a predictor of poor survival in patients with bladder cancer (5-8). However, the link between elevated NLR and poor survival remains controversial. Pathological differences between patients with a low NLR and those with a high NLR might be one important reason for this. In order to investigate associations between pathological differences and NLR, we conducted this study in patients with bladder cancer.

Materials and Methods

Study population. A retrospective analysis was performed to identify patients with bladder cancer at Peking University Cancer Hospital between 2009 and 2016. The inclusion criterion were: a) adult patients aged 18 years or older, male or female; b) diagnosed with bladder cancer for the first time before surgery; c) transurethral resection of the bladder tumor or radical cystectomy; d) bladder urothelial carcinoma was confirmed by pathology report with complete grading and staging information. The exclusion criterion were: a) other types of bladder cancer, such as squamous carcinoma, metastatic cancer; b) factors potentially influencing the NLR, such as infection and leukocytosis.

Data collection. The data for basic patient information, laboratory results and pathological results were collected from the electronic patient records. All hematological parameters were calculated prior to operations. The pathological staging and histological grading of the bladder cancer were based on the American Joint Committee TNM Staging System for Bladder Cancer (seventh edition) (9). Data obtained from the patients' routine tests before surgery included the white blood cell count (WBC), neutrophil count and lymphocyte count. The NLR was defined by dividing the neutrophil count by the lymphocyte count and the NLR cut-off value was 2.5 from previous publications, with an abnormal value defined as >2.5 (6).

Statistical analysis. Descriptive statistics were used to summarize patients' characteristics. Categorical variables are presented as numbers and percentages and continuous variables as median and interquartile range (IQR). Patients were divided into two groups by pathological tumor grading, low grade and high grade. The *t*-test was used to compare the continuous variables between patients with low-grade disease and those with high-grade disease. Chi-square test

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was used to compare the categorical variables between these two groups. Patients were then divided into two groups with a cut-off NLR value of 2.5 and the *t*-test was used to compare the continuous variables between those with a low NLR and those with a high NLR. Chi-square test was used to compare the categorical variables between these two groups. Lastly, we performed logistic regression analysis to determine the potential predictors for pathological outcomes in patients with bladder cancer. An initial assessment of predictors was performed using univariate analysis, and only those variables with significance or clinical implications were included in the multivariate analysis. In order to avoid multi-colinearity of continuous and binary NLR, only NLR as a continuous variable was retained in the multivariate analysis. Statistical analysis was performed using Stata 14 for windows (StataCorp, College Station, TX, USA). All tests were two-sided and a value of $p<0.05$ was considered statistically significant.

Results

Patient characteristics. Three hundred and two patients with bladder cancer were enrolled in our analysis from March 2009 through December 2016, including 144 (47.68%) with low-grade disease and 158 (52.32%) with high-grade disease. Patients' clinicopathological characteristics stratified by pathological tumor grading are shown in Table I. The median age was 66 years, and patients with a high-grade disease were significantly older than those with low-grade disease (69 *vs.* 64 years, $p<0.01$). There were 66 (21.85%) female patients and 236 (78.15%) male patients; there was no significant difference in gender distribution between those with low-grade disease and those with high-grade disease ($p=0.481$).

Pathological results suggested most patients had pTa (n=168) or pT1 disease (n=102), accounting for 55.63% and 33.77% respectively. There was no significant difference in pathological T-stage distribution between those with low-grade disease and those with high grade disease ($p=0.23$). All the patients with lymph node metastasis (pN1, pN2) had high-grade disease. Median WBC count was 7.28×10^9/l, neutrophil count was 5.21×10^9/l, lymphocyte count was 1.43×10^9/l and median NLR was 3.83. No significant difference was detected in WBC count in a comparison of patients with low-grade disease and those with high-grade disease. However, those with high-grade disease had a significantly higher neutrophil count ($p=0.003$), significantly lower lymphocyte count (1.30×10^9/l *vs.* 1.53×10^9/l, $p<0.001$) and significantly higher NLR value (4.42 *vs.* 3.42, $p<0.001$) compared to patients with low-grade disease. There were significantly more patients with a high NLR in the high-grade disease group (58.59%), and more patients with a low NLR in the low-grade disease group (66.67%) ($p<0.001$). Patients with high-grade disease had a significantly longer hospitalization time (9.5 days *vs.* 9 days, $p=0.017$).

Pathological outcomes according to NLR. The pathological outcomes according to the NLR group are shown in Table II. More patients had a NLR higher than 2.5 (75.17%). Within those with low-grade disease, 50 (34.72%) patients had low NLR and 94 (65.28%) patients had higher NLR. For high-grade diseases, 25 (15.82%) patients had lower NLR and 133 (84.18%) patients had a higher NLR. There were significantly more high-grade disease patients in the high NLR patient cohort ($p<0.001$). There was no significant difference in the distribution of pathological T-stage ($p=0.311$), muscle invasive bladder cancer ($p=0.841$) or pathological N-stage ($p=0.181$) by NLR.

Predictors of pathological high-grade bladder cancer. Univariate and multivariate logistic regression analysis for predictors associated with bladder cancer pathological tumor grading are presented in Table III. From univariate analyses, age ($p<0.001$), neutrophil count ($p=0.004$), NLR both as a continuous ($p<0.001$) and binary ($p<0.001$) variable were significantly associated with high-grade disease. Multivariate analyses showed that only age ($p<0.001$) and NLR as a continuous variable ($p=0.001$) were statistically significantly associated with tumor grade.

Discussion

More and more studies have found an intimate connection between inflammation and cancer (1, 3, 4). The hypothesis for this association was based on inflammatory mediators and cytokines released in inflammatory reactions leading to cell damage and gene mutation. These factors are considered to be important elements for tumorigenesis and also help create a microenvironment promoting cancer cell proliferation and metastasis (10, 11). Alongside this, inflammation may also accelerate cancer progression by increasing vascular permeability, and up-regulating lymphatic infiltration and stromal invasion at metastatic sites (2). The NLR increases during inflammatory reactions in which the neutrophil granulocyte count increases while the lymphocyte count decreases and thus it could be an effective predictor in cancer. Compared to other assays, the NLR can easily be obtained from peripheral blood tests before treatment, providing a cheap and convenient measure.

Use of the NLR has been proven to be effective in gastrointestinal cancer (3), and a high NLR might also be a prognostic factor in patients with bladder cancer, indicating a worse oncological outcome, thus providing appropriate prognostic information for these patients (12-16). However, the link between elevated NLR and poor survival was unclear, as was the effect of pathological differences on NLR. Hence we conducted this study to compare the pathological outcomes in bladder cancer according to preoperative NLR and to examine whether NLR is predictive of pathological characteristics of the disease.

Our study found that compared to patients with low-grade bladder cancer, those with high-grade disease had a higher

Table I. *Clinicopathological characteristics stratified by pathological tumor grading.*

Characteristic	Total	Pathological tumor grade		p-Value
		Low	High	
Patients, n (%)	302 (100)	144 (47.68)	158 (52.32)	
Median age (IQR), years	66 (59-75)	64 (56-71)	69 (61-77)	<0.001
Gender, n (%)				0.481
Female	66 (21.85)	34(51.52)	32 (48.48)	
Male	236(78.15)	110(46.61)	126 (53.39)	
T-Stage, n (%)				0.230
pTis	2 (0.66)	1 (50)	1 (50)	
pTa	168 (55.63)	90 (53.57)	78 (46.43)	
pT1	102 (33.77)	39 (38.24)	63 (61.67)	
pT2	24 (7.95)	12 (50)	12 (50)	
pT3	4 (1.32)	1 (25)	3 (75)	
pT4	2 (0.66)	1 (50)	1 (50)	
N-Stage, n (%)				0.009
pN0	292 (96.69)	144 (49.32)	148 (50.68)	
pN1	4 (1.32)	0 (0)	4 (100)	
pN2	6 (1.99)	0 (0)	6 (100)	
Median WBC (IQR), $\times 10^9$/l	7.28 (6.21-9.16)	7.08 (6.06-8.93)	7.52 (6.32-9.24)	0.054
Median neutrophils (IQR), $\times 10^9$/l	5.21 (4.18-7.05)	4.93 (3.92-6.76)	5.57 (4.44-7.15)	0.003
Median lymphocytes (IQR), $\times 10^9$/l	1.43 (1.09-1.8)	1.53 (1.16-1.90)	1.30 (1.04-1.64)	<0.001
Median NLR (IQR)	3.83 (2.50-5.56)	3.42 (2.16-4.91)	4.42 (3.00-6.31)	<0.001
NLR, n (%)				<0.001
≤2.5	75 (24.83)	50 (66.67)	25 (33.33)	
>2.5	227 (75.17)	94 (41.41)	133 (58.59)	
Median hospitalization (IQR), days	9 (6-15)	9 (6-14)	9.5 (6-18)	0.017

IQR, Interquartile range; NLR, neutrophil-to-lymphocyte ratio; WBC, white blood cells.

neutrophil count and lower lymphocyte count, and accordingly a higher NLR. Defining 2.5 as a NLR cut-off value, compared with patients with a low NLR, those with high NLR had high-grade disease. Regression analysis suggested NLR was a predictor of high-grade disease, while the WBC count failed to predict high-grade disease. This might be the reason why high NLR groups had worse survival in other studies (7, 14, 17).

In a recent study, Celik *et al*. found that the NLR might act as a significant biomarker in the staging of bladder cancer (12). They analyzed 222 patients with bladder cancer, including 59 cases with low-grade disease and 103 with high-grade, and found the NLR between these two groups was significantly different. However, in our study, we did not find the difference in NLR value according to pathological staging (non-muscle-invasive *vs*. muscle-invasive), instead, we found a significant difference in NLR according to the pathological grading of bladder cancer. This means the value of NLR is yet to be clarified. Larger scale and well-designed studies are required before NLR becomes an effective biomarker in the clinic.

The limitation of this study includes its retrospective design and limited sample size from a single institution. In

Table II. *Pathological outcomes according to the neutrophil-to-lymphocyte ratio (NLR).*

Characteristic	NLR, n (%)		*p*-Value
	≤2.5	>2.5	
Total patients	75 (24.83)	227 (75.17)	
Pathological tumor grade			<0.001
Low	50 (34.72)	94 (65.28)	
High	25 (15.82)	133 (84.18)	
T-Stage			0.311
pTis	1 (50)	1 (50)	
pTa	47 (27.98)	121 (72.02)	
pT1	20 (19.61)	82 (80.39)	
pT2	4 (16.67)	20 (83.33)	
pT3	2 (50)	2 (50)	
pT4	1 (50)	1 (50)	
Muscle invasion			0.841
No (pTis, pTa, pT1)	68 (25.00)	204 (75.00)	
Yes (pT2, pT3, pT4)	7 (23.33)	23 (76.67)	
N-Stage			0.181
pN0	75 (25.68)	217 (74.32)	
pN1	0 (0)	4 (100)	
pN2	0 (0)	6 (100)	

Table III. *Predictors of pathological tumor grading by logistic regression analysis.*

Variable	Pathological tumor grade		Univariate			Multivariate		
	Low	High	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value
Median age (IQR), years	64 (56-71)	69 (61-77)	1.04	1.02-1.06	<0.001	1.04	1.02-1.06	<0.001
Gender, n (%)			1.22	0.70-2.10	0.481	1.21	0.67-2.18	0.519
Female	34 (51.52)	32 (48.48)						
Male	110 (46.61)	126 (53.39)						
T-Stage, n (%)			1.24	0.93-1.64	0.137	1.16	0.86-1.56	0.344
pTis	1 (50)	1 (50)						
pTa	90 (53.57)	78 (46.43)						
pT1	39 (38.24)	63 (61.67)						
pT2	12 (50)	12 (50)						
pT3	1 (25)	3 (75)						
pT4	1 (50)	1 (50)						
Muscle invasion, n (%)			1.05	0.49-2.23	0.907			
No (pTis, pTa, pT1)	130 (47.79)	142 (52.21)						
Yes (pT2, pT3, pT4)	14 (46.67)	16 (53.33)						
Median WBC (IQR), $\times 10^9$/l	7.08 (6.06-8.93)	7.52 (6.32-9.24)	1.11	1.00-1.23	0.057	0.98	0.85-1.13	0.776
Median neutrophils (IQR), $\times 10^9$/l	4.93 (3.92-6.76)	5.57 (4.44-7.15)	1.19	1.06-1.33	0.004			
Median NLR (IQR)	3.42 (2.16-4.91)	4.42 (3.00-6.31)	1.29	1.15-1.44	<0.001	1.28	1.11-1.47	0.001
NLR, n (%)			2.83	1.64-4.89	<0.001			
≤2.5	50 (66.67)	25 (33.33)						
>2.5	94 (41.41)	133 (58.59)						

95% CI, 95% Confidence interval; IQR, interquartile range; OR, odds ratio; WBC, white blood cells.

addition, we did not obtain the survival data for these patients and thus cannot establish a link of NLR with survival.

In conclusion, our study indicated that preoperative NLR was associated with pathological tumor grade, but not associated with pathological tumor staging in patients with bladder cancer. A high preoperative NLR could be helpful in predicting the pathological grade of bladder cancer.

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