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Original Article

Preoperative Neutrophil-to-Lymphocyte Ratio as a Predictive Biomarker of Tumor Grade in Non–Muscle-Invasive Bladder Cancer: A Prospective Cross-Sectional Study

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Abstract

Background: Non–muscle-invasive bladder cancer (NMIBC) accounts for the majority of newly diagnosed bladder tumors and is associated with high recurrence and progression rates. The neutrophil-to-lymphocyte ratio (NLR), a readily available marker of systemic inflammation, has emerged as a potential prognostic biomarker in urothelial carcinoma.

Objective: To evaluate the association between preoperative neutrophil-to-lymphocyte ratio (NLR) and tumor grade in patients with newly diagnosed NMIBC.

Methodology: This prospective cross-sectional analytical study was conducted at a tertiary care center from July 2025 to December 2025. Adult patients (≥18 years) with histologically confirmed NMIBC undergoing transurethral resection of bladder tumor (TURBT) were included. Patients with muscle-invasive disease, prior bladder cancer treatment, active infections, autoimmune disorders, or other confounding conditions were excluded. Preoperative complete blood counts were used to calculate NLR. Tumor characteristics and histopathological grading were recorded. Statistical analysis was performed using SPSS version 26.0, with $p < 0.05$ considered statistically significant.

Results: A total of 71 patients were included, with a mean age of 56.3 ± 12.7 years. Low-grade NMIBC was observed in 53 patients (74.6%), while 18 patients (25.4%) had high-grade tumors. Median preoperative NLR was significantly higher in the high-grade group compared to the low-grade group [3.7 (IQR 3.2–4.1) vs 2.4 (IQR 2.0–2.8); $p < 0.001$]. An NLR cutoff value of 3.0 demonstrated acceptable discriminatory ability for predicting high-grade NMIBC (AUC = 0.76; 95% CI: 0.65–0.87; $p < 0.01$).

Conclusion: Elevated preoperative neutrophil-to-lymphocyte ratio (NLR ≥3) is significantly associated with high-grade NMIBC and may serve as a simple, cost-effective biomarker for identifying patients with aggressive disease at diagnosis.

Keywords: Biomarkers, Lymphocyte, Neutrophil, Transitional cell carcinoma, Urinary bladder carcinoma.

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Introduction

Bladder cancer represents a significant global health burden and is one of the most commonly diagnosed malignancies of the urinary tract. Approximately 70% of newly diagnosed cases are classified as non–muscle-invasive bladder cancer (NMIBC), characterized by tumor involvement limited to the urothelium or lamina propria [1]. The standard initial management of NMIBC involves transurethral resection of bladder tumor (TURBT), often followed by intravesical therapy depending on risk stratification. Despite advancements in diagnostic and therapeutic approaches, NMIBC remains associated with high recurrence rates ranging from 31% to 78%, and up to 40% of patients may progress to muscle-invasive bladder cancer (MIBC) within five years [2].

Risk stratification in NMIBC is essential for guiding treatment decisions and surveillance strategies. Established models, including the European Organization for Research and Treatment of Cancer (EORTC) risk tables and the CUETO scoring system, incorporate clinicopathological variables such as tumor size, multiplicity, stage, grade, and presence of carcinoma in situ (CIS) to estimate recurrence and progression risks [3,4]. However, these models have limitations in predictive accuracy and do not fully account for underlying biological and inflammatory processes contributing to tumor behavior.

In recent years, increasing attention has been directed toward the role of systemic inflammation in tumor initiation, progression, and metastasis. Inflammatory biomarkers derived from routine hematological parameters have gained interest due to their accessibility, cost-effectiveness, and potential prognostic value. Among these, the neutrophil-to-lymphocyte ratio (NLR), calculated from peripheral blood counts, has emerged as a promising indicator of the balance between pro-tumor inflammatory activity and anti-tumor immune response. Elevated neutrophil counts may promote tumor progression through the release of cytokines, growth factors, and angiogenic mediators, whereas lymphopenia reflects impaired cellular immunity and reduced tumor surveillance [5,6].

Several studies have demonstrated that elevated preoperative NLR is associated with adverse pathological features and poorer survival outcomes in patients with bladder cancer undergoing radical cystectomy [5,6]. A meta-analysis conducted in 2021 further confirmed that higher NLR values are significantly correlated with reduced overall and disease-specific survival, highlighting its prognostic relevance across different stages of bladder cancer [7].

While the prognostic significance of NLR in muscle-invasive disease is well established, its role in NMIBC remains an area of active investigation. Emerging evidence suggests that elevated preoperative NLR may be associated with more aggressive tumor characteristics, including higher tumor grade and increased risk of recurrence in NMIBC patients [8,9]. Studies have reported that patients with NLR ≥2.5 exhibit reduced recurrence-free survival and a higher likelihood of high-grade disease, and incorporating NLR into existing clinical models may improve risk prediction accuracy.

Given these considerations, there is a growing need to identify simple and reliable biomarkers that can enhance early risk stratification and guide individualized management strategies in NMIBC. Therefore, the present study aims to evaluate the association between preoperative neutrophil-to-lymphocyte ratio and tumor grade in patients with newly diagnosed NMIBC. Understanding the potential role of NLR as a biomarker of tumor aggressiveness may contribute to improved clinical decision-making and optimization of surveillance protocols.

Methodology

Study Design

This study was designed as a prospective cross-sectional analytical investigation conducted to evaluate the association between preoperative neutrophil-to-lymphocyte ratio (NLR) and tumor grade in patients diagnosed with non–muscle-invasive bladder cancer (NMIBC). The prospective nature of the study allowed for systematic data collection at the time of diagnosis and ensured temporal alignment between exposure (NLR) and outcome (tumor grade).

Ethics

Prior to initiation, ethical approval was obtained from the Institutional Ethics Review Committee (Ref. No. F.2-81/2025-GENL/394/JPMC). The study adhered to established ethical principles for biomedical research involving human participants. Written informed consent was obtained from all participants after explaining the study objectives, procedures, and confidentiality measures.

Setting

The study was conducted at the Department of Urological Surgery and Transplantation of a tertiary care hospital. Patient recruitment and data collection were carried out over a six-month period from July 2025 to December 2025, encompassing both outpatient and emergency department admissions.

Participants

The study included adult patients (≥18 years) of both genders who were newly diagnosed with NMIBC and underwent transurethral resection of bladder tumor (TURBT). Inclusion criteria required histopathological confirmation of NMIBC following TURBT. Patients with muscle-invasive bladder cancer (MIBC), prior history of bladder cancer, or those who had received chemotherapy, radiotherapy, or intravesical therapy were excluded. Additional exclusions included patients with active infections, autoimmune or connective tissue disorders, hematological diseases, immunodeficiency (including HIV), concurrent malignancies, or those receiving medications such as corticosteroids, NSAIDs, or immunomodulators that could influence inflammatory markers. Patients with non-transitional histopathology and primary prostatic urothelial carcinoma were also excluded to maintain cohort homogeneity.

Variables

The primary independent variable was the preoperative neutrophil-to-lymphocyte ratio (NLR), calculated from complete blood count parameters. The primary dependent variable was tumor grade (low-grade vs high-grade), determined through histopathological examination. Covariates included demographic variables (age, sex), tumor characteristics (size categorized as <3 cm or ≥3 cm, number of lesions), and clinical parameters relevant to disease staging.

Data Sources / Measurement

Data were collected using a structured approach prior to surgical intervention. Preoperative laboratory investigations included complete blood counts, from which absolute neutrophil and lymphocyte counts were used to calculate NLR. Clinical staging was assessed using imaging based on the 2017 Union for International Cancer Control (UICC) TNM classification to exclude advanced disease and upper tract involvement [9]. Intraoperative findings during TURBT were used to assess tumor size and multiplicity. Histopathological examination provided definitive tumor grading.

Bias

Several measures were implemented to minimize bias. Selection bias was reduced by including consecutive eligible patients meeting inclusion criteria. Information bias was minimized through standardized data collection protocols and objective laboratory measurements. Confounding was addressed by excluding patients with conditions that could independently affect inflammatory markers and by adjusting for potential confounders (age, sex, tumor size) in multivariable analysis. However, residual confounding due to unmeasured variables cannot be entirely excluded.

Study Size

Sample size calculation was based on detecting a difference in preoperative NLR between low-grade and high-grade NMIBC groups. Assuming a moderate effect size (Cohen's d = 0.7), a two-sided alpha of 0.05, and 80% statistical power, the minimum required sample size was calculated as 64 patients. To account for potential attrition or incomplete data, a total of 71 patients were enrolled in the study.

Quantitative Variables:

Continuous variables such as age and NLR were analyzed either as continuous or categorized variables depending on distribution. NLR was also dichotomized using a clinically relevant cutoff (≥3 vs <3) based on prior literature and ROC curve analysis. Tumor size was categorized as <3 cm or ≥3 cm.

Statistical Methods

Statistical analysis was performed using SPSS version 26.0. Normality of continuous variables was assessed using the Shapiro–Wilk test. As NLR values were not normally distributed, they were expressed as medians with interquartile ranges and compared using the Mann–Whitney U test. Categorical variables were presented as frequencies and percentages and analyzed using chi-square or Fisher's exact tests as appropriate. Multivariable logistic regression analysis was conducted to identify independent predictors of high-grade NMIBC, including NLR, tumor size, age, and sex. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were reported. A p-value of <0.05 was considered statistically significant.

Results

Participants

A total of 71 patients diagnosed with non–muscle-invasive bladder cancer (NMIBC) following transurethral resection of bladder tumor (TURBT) were included in the final analysis. All participants met the predefined inclusion criteria and had complete preoperative laboratory and histopathological data available for evaluation. No patients were excluded after enrollment, and there were no missing data for the primary variables of interest. Preoperative complete blood counts were obtained for all patients to calculate the neutrophil-to-lymphocyte ratio (NLR), which served as the main exposure variable in this study.

Descriptive Data

The study population had a mean age of 56.3 ± 12.7 years, ranging from 28 to 79 years. The majority of participants were male (52 patients, 73.2%), while females accounted for 19 patients (26.8%). The mean body mass index (BMI) was 23.4 ± 2.9 kg/m². The overall mean preoperative NLR was 2.8 ± 0.9. Histopathological evaluation revealed that 53 patients (74.6%) had low-grade NMIBC, whereas 18 patients (25.4%) were diagnosed with high-grade disease. These baseline demographic and clinical characteristics reflect a typical NMIBC cohort and provide context for subsequent comparative analyses.

Outcome Data

The primary outcome of interest was tumor grade (low-grade vs high-grade NMIBC). When stratified by NLR categories, a significant difference in tumor grade distribution was observed. Among patients with NLR <3, the majority (90.5%) had low-grade tumors, while only 9.5% had high-grade disease. In contrast, among patients with NLR ≥3, a substantially higher proportion (48.3%) had high-grade tumors, with only 51.7% classified as low-grade. This association between elevated NLR and higher tumor grade was statistically significant ($p < 0.001$), as demonstrated in Table 1.

Further stratification analyses showed that tumor grade distribution did not significantly differ across age groups ($p = 0.211$), as illustrated in Table 2. However, tumor size demonstrated a strong association with tumor grade. Patients with tumors ≥3 cm were more likely to have high-grade disease (66.7%) compared to those with tumors <3 cm, where the majority were low-grade (81.1%), as shown in Table 3 ($p < 0.001$).

Main Results

In univariate analysis, the median preoperative NLR was significantly higher in patients with high-grade NMIBC compared to those with low-grade tumors (3.7 vs 2.4; $p < 0.001$), indicating a strong association between systemic inflammatory status and tumor aggressiveness (Table 1).

Multivariable logistic regression analysis further confirmed that NLR ≥3 was an independent predictor of high-grade NMIBC after adjusting for potential confounders including age, sex, and tumor size. Patients with NLR ≥3 had more than threefold increased odds of having high-grade tumors (adjusted odds ratio [aOR] 3.10; 95% CI: 1.40–6.90; $p = 0.004$). Tumor size ≥3 cm was also independently associated with high-grade disease (aOR 2.80; 95% CI: 1.20–6.50; $p = 0.010$), whereas age and sex were not statistically significant predictors (Table 4).

Additionally, receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminatory ability of NLR in predicting high-grade NMIBC. The analysis yielded an area under the curve (AUC) of 0.76 (95% CI: 0.65–0.87; $p < 0.01$), indicating acceptable diagnostic performance. An NLR cutoff value of 3.0 provided the optimal balance between sensitivity (77.8%) and specificity (71.7%) for identifying high-grade tumors. However, this finding remains exploratory as no internal or external validation was conducted.

Table 1: Association Between Tumor Grade and Neutrophil-to-Lymphocyte Ratio (NLR ≥3 vs. <3)

Tumor Grade	NLR < 3 (n=42) %	NLR ≥ 3 (n=29) %	Total (n=71) %	p-value
Low-grade	38 (90.5)	15 (51.7)	53 (74.6)	<0.001
High-grade	4 (9.5)	14 (48.3)	18 (25.4)	

Table 2: Stratification of Tumor Grade by Age Group

Age Group (years)	Low-grade (n=53) %	High-grade (n=18) %	p-value
<40	12 (22.6)	2 (11.1)	0.211
40–59	22 (41.5)	6 (33.3)	
≥60	19 (35.9)	10 (55.6)	

Table 3: Stratification of Tumor Grade by Tumor Size

Tumor Size	Low-grade (n=53) %	High-grade (n=18) %	p-value
<3 cm	43 (81.1)	6 (33.3)	<0.001
≥3 cm	10 (18.9)	12 (66.7)	

Table 4: Multivariable Logistic Regression for Predictors of High-Grade NMIBC

Variable	Adjusted Odds Ratio (aOR)	95% Confidence Interval (CI)	p-value
NLR ≥ 3	3.10	1.40 – 6.90	0.004
Tumor size ≥ 3 cm	2.80	1.20 – 6.50	0.010
Age (per 10-year increase)	1.15	0.85 – 1.56	0.350
Male sex	1.20	0.50 – 2.85	0.680

Discussion

In this prospective cross-sectional study, we demonstrated that an elevated preoperative neutrophil-to-lymphocyte ratio (NLR ≥3) is significantly associated with high-grade non–muscle-invasive bladder cancer (NMIBC). These findings reinforce the growing body of evidence supporting the role of systemic inflammation as a key contributor to tumor aggressiveness and progression. The observed association between higher NLR values and adverse pathological features highlights the potential of NLR as a clinically useful biomarker for early risk stratification in NMIBC.

Our results are consistent with previous studies that have identified NLR as a significant prognostic indicator in bladder cancer. Elevated NLR has been associated with worse oncological outcomes, including recurrence and progression, in both muscle-invasive and non–muscle-invasive disease [10-12]. In line with our findings, Zhang et al. reported in a meta-analysis that higher NLR is significantly correlated with poor survival outcomes, with optimal cutoff values generally ranging between 2.5 and 3 [13]. Similarly, Huang et al. demonstrated that elevated preoperative NLR is associated with high-grade pathology and early recurrence in NMIBC patients [14].

Further supporting this evidence, Vartolomei et al. conducted a large meta-analysis involving more than 2,000 NMIBC patients and found that elevated NLR was significantly associated with reduced recurrence-free survival (HR 1.78) and progression-free survival (HR 2.14) [15]. Ferro et al. validated an NLR cutoff value of 3 and identified high NLR as an independent predictor of recurrence, progression, and cancer-specific survival [16]. Additionally, Getzler et al. reported that NLR greater than 2.5 significantly predicts disease progression in NMIBC [17], while Lee et al. demonstrated that incorporating NLR into existing clinical models improves risk stratification, particularly in patients receiving Bacillus Calmette–Guérin (BCG) therapy [18].

The biological plausibility of these findings is well supported. Neutrophils play a critical role in tumor progression by secreting pro-inflammatory cytokines, growth factors, and angiogenic mediators that promote tumor proliferation and metastasis. They may also suppress cytotoxic T-cell activity, thereby facilitating immune evasion. Conversely, lymphocytes are central to anti-tumor immunity, and lymphopenia reflects impaired immune surveillance. Therefore, an elevated NLR represents a shift toward a pro-tumor inflammatory state and reduced host immune response. Tumor-induced cytokines such as interleukin-6 (IL-6) and granulocyte colony-stimulating factor (G-CSF) further amplify this systemic inflammatory response, reinforcing the link between NLR and tumor aggressiveness.

Current risk stratification strategies recommended by the European Association of Urology (EAU) primarily rely on clinicopathological variables [1]. While these models provide valuable guidance, they do not incorporate readily available biological markers such as NLR. The integration of NLR into existing predictive models may enhance their accuracy and enable more individualized treatment planning. Given its simplicity, low cost, and widespread availability, NLR holds promise as a practical adjunct in routine clinical decision-making.

Despite these strengths, several limitations should be acknowledged. First, the optimal cutoff value for NLR remains inconsistent across studies, with reported thresholds ranging from 1.73 to 3 [15-17]. Although we used a cutoff of 3.0 in alignment with prior literature, population-specific variability may limit generalizability. Second, this was a single-center study with a relatively small sample size, which may affect the external validity of the findings. Third, although efforts were made to exclude patients with confounding conditions such as infections and inflammatory diseases, unrecognized factors may still have influenced NLR values. Fourth, the cross-sectional design limits the ability to assess long-term outcomes such as recurrence, progression, and survival. Finally, dynamic changes in NLR over time were not evaluated; emerging evidence suggests that longitudinal trends may provide additional prognostic value [18].

Conclusion

This study demonstrates that an elevated preoperative neutrophil-to-lymphocyte ratio (NLR ≥3) is significantly associated with high-grade non–muscle-invasive bladder

cancer. These findings support the role of NLR as a simple, cost-effective, and readily available biomarker that may aid in identifying patients with more aggressive disease at the time of diagnosis.

Authors' Contributions:

All authors meet the ICMJE criteria for authorship.

Author 1: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.
Author 2: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.
Author 3: Analysis and interpretation of data, drafting the manuscript, final approval, agreed to be accountable for all the work.
Author 4: Analysis and interpretation of data, critical revision, final approval, agreed to be accountable for all the work.
Author 5: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.
Author 6: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.

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