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Neutrophil-to-lymphocyte ratio as a prognostic factor for patients with urothelial carcinoma of the bladder following radical cystectomy

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Introduction The pre-treatment neutrophil-to-lymphocyte ratio (NLR) has been associated with adverse pathology or survival in a variety of malignancies, including urothelial carcinoma of the bladder (UCB) treated with radical cystectomy (RC). Whether the prognostic value of NLR is retained, or even increased, when measured postoperatively remains to be studied. In this study, we evaluated the association of preoperative and postoperative NLR with oncological outcomes following RC.
Material and methods The NLR was recorded in 132 consecutive patients with UCB treated with open RC: before surgery (NLR1), postoperatively within 2 days (NRL2), between 7 and 15 days after RC before discharge (NLR3), and a few days before recurrence or last available follow-up (NLR4).
Results When assessed by multivariate analysis NLR1 remained independently associated with a significantly increased risk of extravesical disease (pT 3–4) (OR = 1.4, p <0.01) and lymphovascular invasion (LVI) (OR = 1.40, 95% CI 1.09–1.83, p <0.01). NLR4 was independently associated with a significantly increased risk of cancer-specific mortality (CSM) (HR = 1.14, 95%CI 1.03–1.24, p = 0.013). In a postoperative model, NLR3 was found to be an independent predictor of all-cause mortality (ACM) [HR = 1.11, 95% CI 1.02–1.21, p = 0.01].
NLR1 was associated with a significantly increased risk of recurrence in the univariable preoperative model [HR = 1.9, 95%CI 1.00–3.65, p = 0.05], while in the postoperative model NLR4 remained independently associated with a significantly increased risk of recurrence (HR = 1.13, 95%CI 1.04–1.23, p = 0.03).
Conclusions In patients with UCB treated with RC, the NLR is associated with more advanced tumour stage, LVI, lymph node metastasis, and higher CSM. Furthermore, the variation of the NLR after surgery might play a role in predicting higher ACM and recurrence-free survival.

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INTRODUCTION

The neutrophil-to-lymphocyte ratio (NLR) is an easily measured, reproducible, and inexpensive marker of systemic inflammation. It has been hypothesized that the inflammatory cytokines synthesized by the tumour microenvironment alter acute phase reactants and haematological components, including serum neutrophil and lymphocyte counts [1, 2].

As part of the tumour microenvironment, neutrophils and lymphocytes both play prominent regulatory roles in tumour progression. Furthermore, the NLR is a marker of systemic inflammatory response that reflects the balance of the inflammatory system and immune system. The NLR has been associated with oncological outcomes in multiple malignancies, including breast, colorectal, lung, liver, and gastric [3–6]. However, the prognostic role

of the NLR for urological cancers is still not well defined. Urothelial bladder cancers (UCB) can be divided into 2 major disease states with different implications for clinical management [7, 8]. Non-muscle invasive bladder cancers (NMIBCs), which correspond to the bulk of cancer incidence, generally do not pose a significant threat to the life of the patient but do invariably recur, necessitating expensive lifelong cystoscopy and local resection, which generate significant patient discomfort [9] and make bladder cancer the cancer with the highest cost per patient [10]. Importantly, a fraction of high-grade NIMBCs do progress to become invasive. Despite clinical and histological parameters that have been associated with the risk of progression of the disease, new tools and biomarkers for more precise prognostic risk stratification are still needed for incorporation into the standard of care [11]. On the other hand, muscle-invasive bladder cancers (MIBCs) are clinically aggressive, and even after radical cystectomy [12, 13, 14], up to 50% of patients die of their disease. For transitional cell carcinoma (TCC), the evaluation of the NLR might be particularly relevant because inflammation appears to play a critical role in the genesis, progression, and mortality of UCB. Indeed, urothelial carcinoma is one of the few malignancies with a defined role for immunotherapy, e.g. bacillus Calmette-Guerin (BCG).

The accurate prediction of the best treatment option (surgery rather than systemic therapies) is a pivotal issue for clinicians. Biomarkers such as total cholesterol levels [15] and novel biomarkers such as the neutrophil percentage-to-albumin ratio (NPAR) [16] might help in the selection of the most appropriate candidate for therapies to improve outcomes of urological cancers. Again, there are no tools that can be used to distinguish patients with lethal cancers from those that can be cured [11]. NLR evaluation could be helpful in the selection of the best candidate to a specific therapy; however, the exact role of the NLR remains controversial. Current literature differs in study design, sample size, patient selection, timing of blood measurements in relation to surgery or chemotherapy, and NLR kinetics measurements. Thus, there is a need to explore whether the prognostic value of the NLR is retained, or even increased, when measured not only preoperatively but also postoperatively.

In this study, we evaluated the association of preoperative and postoperative NLR with oncological outcomes following RC. Specifically, we assessed the association of the NLR with pathological variables as well as its impact as a predictor of recurrence-free and cancer-specific survival estimates, and all-cause mortality (ACM).

MATERIAL AND METHODS

This study was performed on a prospective, single-centre, single-surgeon cohort of patients with UCB treated with open radical cystectomy (RC) and lymph node dissection.

All included patients undergoing radical treatment provided written informed consent for surgery. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. An institutional review board number was not required due to observational and retrospective nature of the study. The exclusion criteria included patients with infections, inflammatory or autoimmune diseases, a second primary cancer, splenectomy, other bladder cancer subtypes, or haematological or hepatic disorder that potentially altered the neutrophil-lymphocyte ratio, and those with missing information.

The recorded clinicopathological variables included the following: age, sex, Eastern Cooperative Oncology Group (ECOG) performance status, preoperative and postoperative NLR, body mass index (BMI), receipt of BCG therapy, clinical tumour stage, radial surgical margin status, pathological tumour and lymph node stages, presence of lymphovascular invasion (LVI), and receipt of adjuvant chemotherapy. The tumour staging followed the American Joint Committee on Cancer/Union Internationale Contre le Cancer TNM classification.

Blood samples were collected at our hospital and sent to our hospital laboratory for analysis.

The NLR was recorded at the following times:
- before surgery (within 15 days prior to RC, [NLR1]),
- postoperatively (within 2 days [NLR2]),
- between 7 and 15 days after RC before discharge (NLR3),
- a few days before evidence of recurrence or last available follow-up (NLR4),

ΔNLR was calculated as the difference between NLR2 and NLR1 (NLRΔ1) and between NLR2 and NLR3 (NLRΔ2).

The NLR was analysed both as a continuous variable and as a categorical variable, with a cut-off of 2.7 based on previous studies [17].

Follow-up appointments were scheduled every 3–4 months in the first year, every 6 months in the second year, and annually thereafter, consisting of a physical examination and serum chemistry evaluation.

Tumours were staged according to the 2002 TNM classification, and grading was assigned based on the

1973 World Health Organization grading system. LVI was defined as the presence of nests of tumour cells within an endothelium-lined space [18]. A positive soft-tissue surgical margin was defined as the presence of tumour in stained areas of soft tissue in RC specimens [19].

Categorical variables were presented as number and percentage, and continuous variables as mean ±SD. Group differences for categorical and continuous variables were analysed using the chi-square and Mann-Whitney tests, respectively.

Recurrence-free survival (RFS) (defined as local and/or distant soft tissue recurrence, excluding metachronous upper tract and urethral cancers), cancer-specific mortality (CSM), and ACM were estimated as the time from RC to event using the Kaplan-Meier method. Survival was compared between patients with an NLR <2.7 and those with an NLR ≥2.7 using the log-rank test.

Univariate and multivariate logistic regression and Cox proportional hazard models were used to analyse the association of NLR with extravesical (≥pT3) disease, LVI, lymph node involvement, disease recurrence, and mortality separately between preoperative and postoperative variables. The cut-off for entry of values into the multivariate models was a p-value <0.2.

A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS v. 20 (IBM SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 132 consecutive patients with UCB were treated with open radical cystectomy (RC) and lymph node dissection between July 2013 and December 2016. Patient and tumour characteristics are listed in Table 1. Median age was 74 years (IQR 68–81 years). Median NLR values were 3 (IQR 2.1–4.2), 8.9 (IQR 6.2–13), 4.1 (IQR 3–6.2), and 2.3 (IQR 1.7–3), respectively, for NLR1, NLR2, NLR3, and NLR4 (p <0.05).

Median NLRΔ1 and NLRΔ2 were, respectively, 5.7 (2.6–9.1) and 3.8 (1.07–8.10). Extravesical disease, LVI, and lymph node involvement were found, respectively, in 57 (43.5%), 63 (49.2%), and 20 (18.7%) patients. Median follow-up was 15.9 months (IQR 7.9–26.0 months). During this period, 45 (34.1%) patients had a recurrence of UBC, 60 (45.4%) patients died: 38 (28.8%) of UCB and 22 (16.7%) of other causes. Sixty-four (48.5%) had no evidence of disease at follow-up.

A high NLR1 value was associated with a larger tumour size (p <0.01), a greater likelihood of receiving intravesical therapy (p = 0.04), advanced T stage

Table 1. *Overall patient and tumour characteristics*

	Total (132 patients)
Age, median (IQR)	74 (68–81)
Sex, n (%)	
Female	27 (29.5)
Male	105 (79.5)
BMI kg/m², median (IQR)	26.5 (23.8–29.8)
Neutrophil/lymphocyte ratio before surgery (NLR1), median (IQR)	2.97 (2.1–4.2)
Neutrophil/lymphocyte ratio immediately after surgery (NLR2), median (IQR)	8.87 (6.19–13.03)
Neutrophil/lymphocyte ratio at discharge (NLR3), median (IQR)	4.06 (2.96–6.24)
Neutrophil/lymphocyte ratio at recurrence (NLR4), median (IQR)	2.29 (1.7–3.06)
NLRΔ1, median (IQR)	5.7 (2.6–9.1)
NLRΔ2, median (IQR)	3.8 (1.07–8.10)
ECOG performance status, n (%)	
0	51 (38.6)
1	58 (43.9)
2	20 (15.2)
3	3 (2.3)
Max tumour size, n (%)	
≤2 cm	64 (48.5)
>2, ≤3 cm	0
>3 cm	64 (48.5)
Receipt of intravesical therapy, n (%)	
No vesical therapy	108 (82.4)
Vesical therapy	23 (17.6)
Clinical T stage, n (%)	
≤T2	117 (90)
T3–T4	13 (10)
Pathologic T stage, n (%)	
≤T2	74 (56.5)
T3–T4	57 (43.5)
pN stage, n (%)	
pNx	21 (15.9)
pN0	87 (65.9)
pN1	10 (7.6)
pN2	10 (7.6)
Perineural invasion, n (%)	26 (20.3)
Lymphovascular invasion, n (%)	63 (49.2)
Lymph node involvement, n (%)	20 (18.7)
Positive surgical margin, n (%)	12 (9.1)
Blood transfusion, n (%)	37 (28.2)
Receipt of adjuvant therapy*, n (%)	8 (6.1)
Patients with recurrence of disease, n (%)	45 (34.1)
Follow-up status, n (%):	
Death from other cause	22 (16.7)
Death from bladder cancer	38 (28.8)
No evidence of disease	64 (48.5)
Alive with disease recurrence	8 (6.1)
Follow up time, months, median (IQR)	15.9 (7.9–26.0)
Time to recurrence, months	13.8 (5.4–24.2)

n – number of patients; IQR – interquartile range; BMI – body mass index; NLR – neutrophil-to-lymphocyte ratio; ECOG – Eastern Cooperative Oncology Group
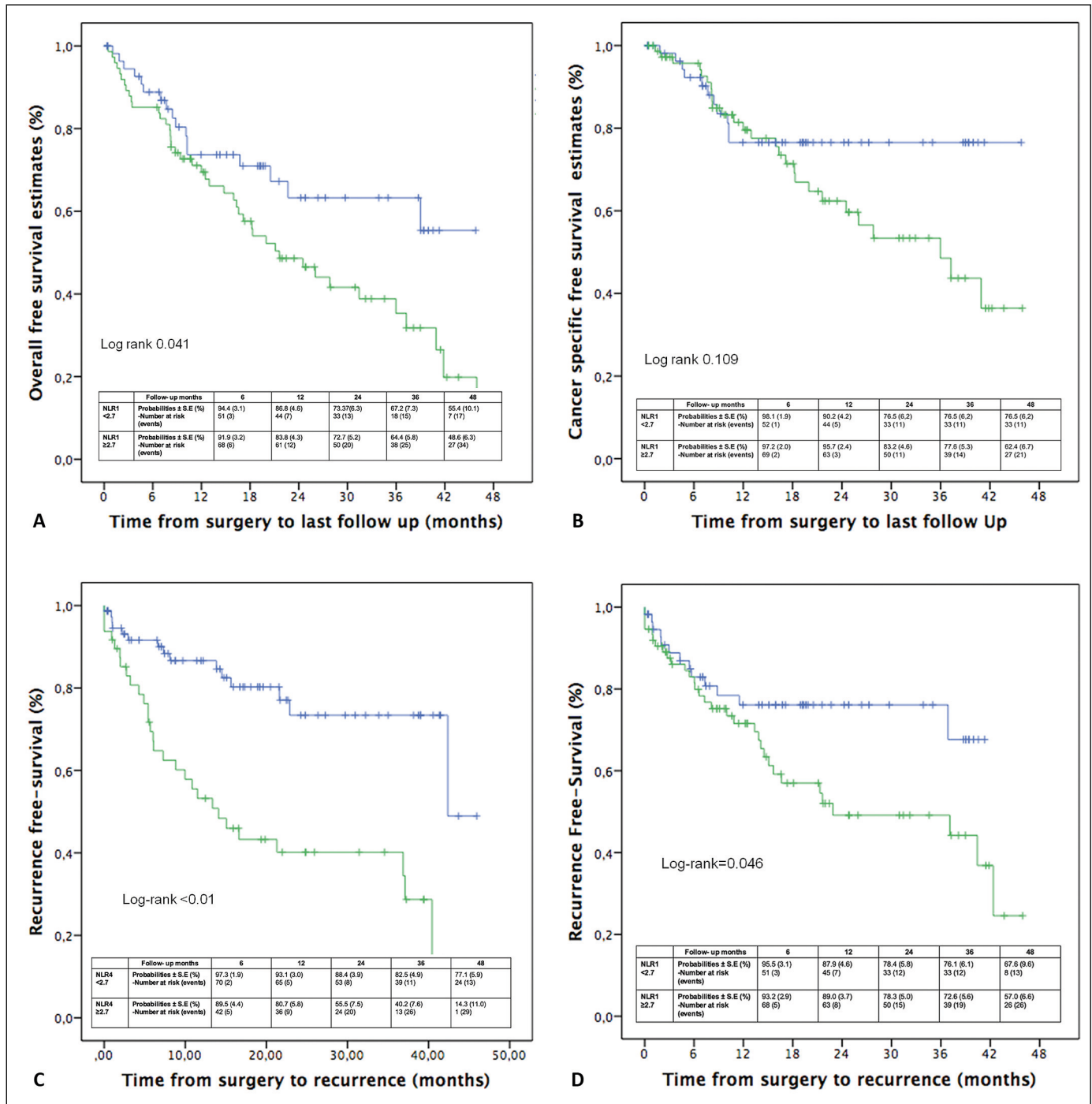
Figure 1. A. *Overall free survival for NLR1 <2.7 (blue) and NLR1 ≥ 2.7 (green).* **B.** *Cancer-specific free survival for NLR1 <2.7 (blue) and NLR1 ≥2.7 (green).* **C.** *Recurrence-free survival for NLR4 <2.7 (blue) and NLR4 ≥2.7 (green).* **D.** *Recurrence-free survival for NLR1 <2.7 (blue) and NLR1 ≥2.7 (green).*

(p <0.01), LVI (p <0.01), positive surgical margin (p = 0.02), a higher likelihood of blood transfusion (p = 0.016), recurrence of disease (p = 0.016), and CSM (p = 0.02) (Supplementary materials, Table S1).
A high NLR2 value was associated with a higher BMI (p <0.01) and greater tumour size (p = 0.04)

(Supplementary materials, Table S2), while a high NLR3 value seemed to have no relation to clinico-pathological characteristics (Supplementary materials, Table S3).
A high NLR4 value was associated with age (p = 0.05), advanced T stage (p = 0.01), lymph node involve-

ment (p = 0.017), positive surgical margin (p = 0.03), a greater likelihood of receiving adjuvant chemotherapy (p = 0.021), recurrence of disease (p <0.01), and CSM (p <0.01) (Supplementary materials, Table S4). When patients were stratified according to NLR1 with a cut-off of 2.7, overall survival and recurrence-free survival were significantly different (p = 0.042 and p = 0.046, respectively) (Figures 1A and 1D). When patients were stratified according to NLR4 with a cut-off of 2.7, recurrence-free survival was significantly different (p <0.01) (Figure 1C).

No difference in cancer-specific survival was found between the groups (Figure 1B).
When the association of NLR1 with extravesical disease and LVI was assessed by multivariate analysis, NLR1 remained independently associated with a significantly increased risk of extravesical disease (pT 3–4) (OR = 1.41, 95% CI 1.11–1.80, p <0.01) and LVI (OR = 1.40, 95% CI 1.09–1.83, p <0.01) (Table 2, Table 3, Table 4).
When the association of NLR4 with CSM and NLR4 was assessed, NLR4 was independently associated

Table 2. *Univariate and multivariate logistic regression predicting extravesical disease (pT 3–4)*

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
Age at surgery	1.00	1.0–1.0	0.73	–	–	–
Sex (female vs male)	0.79	0.3–1.8	0.59	–	–	–
Intravesical therapy	1.23	0.51–3.05	0.64	–	–	–
cT category (cT≥ 2 vs cT <2)	3.35	0.97–11.5	0.05	3.2	1.00–11.5	0.05
NLR1 (continuous)	1.44	1.13–1.85	<0.01	1.41	1.11–1.80	<0.01
NLR1 ≥2.7 vs NLR1 <2.7	4.73	2.15–10.46	<0.01			

OR – odds ratio; CI – confidence interval; NLR – neutrophil-to-lymphocyte ratio

Table 3. *Univariate and multivariate logistic regression predicting lymph node involvement*

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
	Preoperative					
Age at surgery	0.91	0.86–0.97.	0.01	0.91	0.86–0.97	0.01
Sex (reference: female)	0.62	0.20–1.98	0.42	–	–	–
cT category (cT ≥2 vs cT <2)	1.9	0.69	0.21	3.65	0.42–3.5	0.23
Intravesical therapy (Yes-No)	1.01	0.41–3.32	0.98	–	–	–
NLR 1 (continuous)	0.90	0.70–1.15	0.42	–	–	–
NLR1 ≥2.7 vs NLR1 <2.7	1.72	0.62–4.77	0.29	0.44	0.15–1.25	0.12

OR – odds ratio; CI – confidence interval; NLR – neutrophil-to-lymphocyte ratio

Table 4. *Univariate and multivariate Cox regression predicting lymphovascular invasion*

	Univariate			Multivariate		
	OR	95% CI	p-value	OR	95% CI	p-value
cT category (cT≥ 2 vs cT <2)	2.59	0.75–8.89	0.13	2.29	0.65–8.13	0.20
Age at surgery	1.00	0.96–1.03	0.98	–	–	–
Sex (reference: female)	1.17	0.49–2.76	0.72	–	–	–
Intravesical therapy (Yes-No)	0.75	0.30–1.87	0.54	–	–	–
NLR1 (continuous)	1.45	1.12–1.88	<0.01	1.41	1.09–1.83	<0.01
NLR1 ≥2.7 vs NLR1 <2.7	2.74	1.31–5.74	<0.01	–	–	–

OR – odds ratio; CI – confidence interval; NLR – neutrophil-to-lymphocyte ratio

Table 5. *Univariate and multivariate Cox regression predicting cancer-specific mortality*

	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
Preoperative						
Age at surgery	1.04	1.00–1.08	0.04			
Sex (reference: female)	1.06	0.46–2.41	0.89	–	–	–
ECOG performance status	1.33	0.84–2.12	0.21	–	–	–
Intravesical therapy (Yes-No)	1.2	0.55–2.65	0.62	–	–	–
NLR 1 (continuous)	1.04	0.85–1.27	0.68			
NLR1 ≥2.7 vs NLR1 <2.7	1.76	0.87–3.5	0.11			
Postoperative						
Adjuvant chemotherapy	1.13	0.27–4.80	0.86	–	–	–
NLR2 (continuous)	0.97	0.92–1.02	0.28	–	–	–
NLR3 (continuous)	1.00	0.87–1.15	0.94	–	–	–
NLRΔ1 (continuous)	0.96	0.92–1.02	0.98	–	–	–
NLRΔ2 (continuous)	1.03	0.98–1.08	0.21	–	–	–
NLR4 (continuous)	1.07	1.04–1.12	<0.01	1.14	1.03–1.24	0.013
NLR4 ≥2.7 vs NLR4 <2.7	3.12	1.59–6.10	<0.01	–	–	–
pT3–4 vs pT ≤T2	4.68	2.29–9.56	<0.01	4.34	1.82–10.4	<0.01
Lymph node invasion (pN+ vs pN)	3.34	1.57–7.10	<0.01	2.05	0.90–4.67	0.08

HR – hazard ratio; CI – confidence interval; NLR – neutrophil-to-lymphocyte ratio; ECOG – Eastern Cooperative Oncology Group

with a significantly increased risk [HR = 1.14, 95%CI 1.03–1.24, p = 0.013] (Table 5).

In the univariate analysis NLR1 was found to be a preoperative predictor of ACM (HR = 1.79, 95%CI 1.015–3.14, p = 0.044) (Table 6).

In the postoperative model, NLR3 was found to be an independent predictor of ACM (HR = 1.11, 95%CI 1.02–1.21, p = 0.01) (Table 6). NLR1 was associated with a significantly increased risk of recurrence in the univariate preoperative model (HR = 1.9, 95%CI 1.00–3.65, p = 0.05), while in the postoperative model NLR4 remained independently associated with a significantly increased risk of recurrence (HR = 1.13, 95%CI 1.04–1.23, p = 0.03) (Table 7).

DISCUSSION

In this cohort of patients with urothelial carcinoma of the bladder (UCB), who underwent RC with midterm postoperative follow-up, we found that preoperative and postoperative NLR were associated with advanced pathologic stage at the time of cystectomy, LVI, increased risk for disease recurrence, CSM, and ACM. These findings remained significant after controlling for clinicopathological features, suggesting an independent association of preoperative and postoperative NLR with these adverse outcomes.

Interestingly, our results are in line with prior studies (Supplementary materials, Table S5).

Viers et al., in a study that included 899 patients from a single institution, showed that high NLR is associated with a higher risk of extravesical tumour extension (p = 0.03) and lymph node metastasis (p = 0.02) [17]. They also found that with each unit increase in the NLR, the relative risk of death from all causes and from UCB increased by 3% and 4%, respectively.

Krane et al. [20] reported that an increase in NLR in conjunction with hypoalbuminaemia was associated with a greater risk of extravesical disease and worse OS and CSS in a cohort of 68 patients. However, 15% of their population received neoadjuvant chemotherapy, which may have affected subsequent preoperative NLR values.

Gondo et al. [21] stratified their cohort into risk categories according to tumour size (<3 vs ≥3 cm), the presence of hydronephrosis, haemoglobin level (<11.5 g/dl vs ≥11.5 g/dl), and NLR (<2.5 vs ≥2.5). The 5-year survival rates in the low-, intermediate-, and high-risk groups were 78.2%, 60.7%, and 25.9%, respectively. In multivariate analysis, NLR was an independent prognostic factor for CSS (HR = 1.95, 95%CI 1.04–3.66). Beyond prognostication in RC patients, the NLR may also be useful in identifying

Table 6. *Univariate and multivariate Cox regression predicting all-cause mortality*

	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
Preoperative						
Age at surgery	1.05	1.02–1.08	<0.01	1.04	1.00–1.08	0.013
Sex (reference: female)	0.92	0.49–1.76	0.81	–	–	–
Intravesical therapy (Yes-No)	0.94	0.47–1.87	0.87	–	–	–
ECOG			<0.01			<0.01
1	1.9	0.98–3.70	0.06	1.3	0.62–2.76	0.448
2	1.38	0.57–3.33	0.47	0.78	0.30–2.04	0.62
3	4.8	9.4–17.5	<0.01	3.0	6.6–12.0	<0.01
NLR1 (continuous)	1.09	0.94–1.27	0.24	–	–	–
NLR1 ≥2.7 vs NLR1 <2.7	1.79	1.015–3.14	0.044	1.65	0.93–2.94	0.08
Postoperative						
Adjuvant chemotherapy	1.15	0.35–3.75	0.81	–	–	–
pT3-4 vs pT ≤T2	3.67	2.08–6.47	<0.01	3.9	1.9–7.91	<0.01
Lymph node invasion (pN+ vs pN-)	2.39	1.24–4.63	<0.01	1.38	0.67–2.38	0.38
NLR2 (continuous)	0.98	0.94–1.02	0.37	–	–	–
NLR2 ≥2.7 vs NLR2 <2.7	0.56	0.17–1.8	0.33	–	–	–
NLR3 (continuous)	1.09	1.09–1.17	<0.01	1.11	1.02–1.21	0.01
NLR3 ≥2.7 vs NLR<2.7	1.01	0.53–1.91	0.96	–	–	–
NLRΔ1 (continuous)	0.97	0.93–1.01	0.20	Not significant*		
NLRΔ2 (continuous)	1.05	1.05–1.09	0.028	Not significant*		
NLR4 (continuous)	1.05	1.01–1.09	<0.01	Not significant*		
NLR4 ≥2.7 vs NLR4 <2.7	1.99	1.18–3.34	<0.01	Not significant*		

*Separate models with pT stage, lymph node invasion, and separately NLRΔ1, NLRΔ2, NLR3, or NLR4.
HR – hazard ratio; CI – confidence interval; NLR – neutrophil-to-lymphocyte ratio; ECOG – Eastern Cooperative Oncology Group

patients with non-muscle-invasive UCB who would benefit from early RC.

In a recent study of 424 non-muscle-invasive UCB patients, those with NLR3 had similar survival rates to those treated for muscle-invasive UCB [22].

Lucca et al., in a multicentre study with 4061 patients, found that NLR ≥2.7 was associated with advanced pathological tumour stage (p <0.001), lymph node involvement (p <0.001), lymphovascular invasion (p = 0.008), and positive soft tissue surgical margins (p = 0.001). Furthermore, they found an independent association with both OS (HR = 1.11, 95%CI 1.01–1.22; p = 0.029) and cancer-specific survival (CSS) (HR = 1.21, 95%CI 1.07–1.37, p = 0.003) [23]. Sudol et al., in a cohort of 137 patients who underwent RC, found that elevated NLR was associated with worse OS, higher tumour stage, and higher frequency of positive lymph nodes [24].

Other studies evaluated the predictive ability of NLR for OS, CSS, and progression-free survival (PFS), also in smaller cohorts of patients [25–36]. Some studies were unable to demonstrate the predictive ability of NLR for OS and CSS [31, 38]. Interestingly,

some papers evaluated NLR kinetics for the prediction of oncological outcomes, as in the present study [17, 33].

Unfortunately, the available literature used different NLR cut-off values, ranging between 2.5 and 3.89, so the results were not always comparable.

Another limitation of the available literature is an unclear definition for the timing of the blood test for the NLR count before or after surgery. Indeed, this uncertainty is present in more than the 70% of the available literature. Furthermore, the inclusion criteria are also different in terms of tumour stage (local tumour vs advanced and metastatic patients).

Although evidence suggests a role of the NLR as a prognostic marker in all BC tumour stages, the biological explanation is complex and is yet to be elucidated.

A high NLR reflects both a heightened neutrophil-dependent inflammatory reaction and a decreased, lymphocyte-mediated antitumour immune response, both of which may contribute to aggressive tumour biology, cancer progression, and poor prognosis [5, 38]. For example, circulating neutrophils have been

Table 7. *Univariate and multivariate Cox regression predicting recurrence*

	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
Preoperative						
Age at surgery	1.02	0.98–1.05	0.25	–	–	–
Sex (reference: female)	1.20	0.56–2.60	0.67	–	–	–
Intravesical therapy (Yes-No)	1.00	0.47–2.19	0.97	–	–	–
ECOG			<0.01			<0.01
1	1.23	0.63–2.42	0.54	1.07	0.54–2.11	0.85
2	0.78	0.29–2.09	0.62	0.76	0.25–1.84	0.44
3	3.4	2.76–5.49	<0.01	14.9	1.21–5.69	0.01
cT category (cT ≥2 vs cT <2)	2.8	1.34–5.94	<0.01	2.6	1.21–5.68	0.01
NLR before surgery (continuous)	1.1	0.89–1.25	0.51	–	–	–
NLR1 ≥2.7 vs NLR1 <2.7	1.9	1.00–3.65	0.05	1.66	0.85–3.25	0.14
Postoperative						
Adjuvant chemotherapy	1.99	070–5.62	0.2			
pT3–4 vs pT ≤T2	4.1	2.18–7.71	<0.01	2.7	1.26–5.79	<0.01
Lymph node invasion (pN+ vs pN)	4.6	2.23–9.6	<0.01	2.7	1.26–5.79	0.01
NLR2 (continuous)	1.00	0.96–1.05	0.82	–	–	–
NLR2 ≥2.7 vs NLR<2.7	1.46	0.20–10.68	0.70	–	–	–
NLR3 (continuous)	0.99	0.91–1.09	0.95	–	–	–
NLR3 ≥2.7 vs NLR3 <2.7	0.89	0.44–1.80	0.75	–	–	–
NLRΔ1 (continuous)	0.99	0.95–1.04	0.87	–	–	–
NLRΔ2 (continuous)	1.00	0.96–1.04	0.97	–	–	–
NLR4 (continue)	1.04	1.01–1.07	<0.01	1.13	1.04–1.23	0.03
NLR4 ≥2.7 vs NLR4 <2.7	3.7	1.97–7.06	<0.01	–	–	–

HR – hazard ratio; CI – confidence interval; NLR – neutrophil-to-lymphocyte ratio; ECOG – Eastern Cooperative Oncology Group

shown to produce cytokines, such as tumour necrosis factor, interleukin (IL)-1, and IL-6, and to secrete pro-angiogenic vascular endothelial growth factor [39]. Furthermore, a relative lymphocytopaenia may reflect a lower count of CD4+ T-helper lymphocytes, resulting in a suboptimal lymphocyte-mediated immune response to malignancy. Thus, the NLR may reflect the combined prognostic information of these 2 processes and be a stronger predictor.

We recognize that our study has several limitations. It included few patients, from a single institution, and with an intermediate follow-up duration. Unfortunately, information about perioperative transfusion, drugs, and courses of neoadjuvant chemotherapy were not included. Furthermore, inflammation-based scores, like the NLR, consist of parameters that can be affected by infection, chronic disease, and other similar factors not necessarily associated with cancer. Although the influence of confounding factors may be minimal in this series of surgical candidates who had good performance status and normal body temperature, we were unable to preclude these aspects. Data of C-reactive protein-levels as well as proinflammatory cytokines were not available. Thus, further prospective, well-controlled clinical studies are needed to confirm if haematological parameters and cytokines are a result of tumour growth and an underlying cause of mortality.

We acknowledge the relatively arbitrary cut-off point used for the Kaplan-Meier analyses in our study based on previous literature; nevertheless, this threshold allows our data to be contextualized in light of previously published analyses, which also dichotomized the NLR.

It is unclear whether our findings in patients undergoing RC are generalizable to all bladder cancer patients. Further studies are thus warranted in patients with low-intermediate risk NMIBC or different histology subtype.

CONCLUSIONS

In patients with UCB treated with RC, a high preoperative NLR is associated with more advanced

tumour stage, lymph node involvement, and worse survival.

Identifying patients at higher risk for recurrence may help develop additional therapies to surgery (like neoadjuvant or adjuvant therapies) to improve survival outcomes or establish individualised follow-up protocols.

Future multicentric studies are needed to evaluate the clinical utility of NLR. Investigations into these relationships, including measuring proinflammatory cytokines, may provide further insight into the carcinogenesis and progression to extravesical or systemic disease. These provide interesting and potentially targetable areas for future systemic therapies. The advantages of the NLR as a prognostic biomarker are its availability and low cost. Thus, for the future, it may be useful in preoperative patient risk stratification, including consideration for clinical trial enrolment, patient counselling, predictions models, and clinical decision-making for more extensive surgery (e.g. more extensive lymph node dissection) and/or perioperative chemotherapy or radiotherapy.

CONFLICTS OF INTEREST

Øyvind Ulvik has acted as a consultant for Olympus. The other authors have nothing to declare.

ETHICS APPROVAL

All included patients undergoing radical treatment provided written informed consent for surgery. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. An institutional review board number was not required due to the observational and retrospective nature of the study.

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SUPPLEMENTARY MATERIALS

Table S1. *Patients and tumor characteristics according to NLR1 <2.7 and NLR1 ≥2.7*

	NLR1 <2.7 (before surgery) (54 patients)	NLR1 ≥2.7 (before surgery) (74 patients)	p-value
Age, median (IQR)	73 (65–79.25)	76 (68–82)	0.09
Sex, n (%)			0.38
Female	9 (16.7)	17 (23.0)	
Male	45 (83.3)	57 (77)	
BMI kg/m^2, median (IQR)	27 (24.1–30.0)	25.9 (23.0–29.5)	0.28
ECOG performance status, n (%)			0.38
0	22 (40.7)	26 (35.1)	
1	26 (48.1)	31 (41.9)	
2	5 (9.3)	15 (20.3)	
3	1 (1.9)	2 (2.7)	
Max tumor size			<0.01
≤2 cm	35 (67.3)	28 (38.9)	
>3 cm	17 (32.7)	44 (61.1)	
Receipt of Intravescical therapy:			0.04
No vescical therapy	39 (73.6)	65 (87.8)	
Vescical therapy	14 (26.4)	9 (12.2)	
Clinical T stage, n (%)			<0.01
≤T2	50 (94.3%)	63 (86.3%)	
T3–T4	3 (5.7%)	10 (13.7%)	
Pathologic T stage, n (%)			<0.01
≤T2	41 (77.4)	31 (41.9)	
T3–T4	12 (22.6)	43 (58.1)	
pN stage			0.16
pNx	6 (11.3)	15 (21.2)	
pN0	40 (7.5)	43 (60.6)	
pN1	2 (3.8)	8 (11.3)	
pN2	5 (9.4)	5 (7.0)	
Perineural invasion, n (%)	9 (17.0)	16 (22.5)	0.44
Lymphovascular invasion, n (%)	19 (35.8)	43 (60.6)	<0.01
Lymph node involvement, n (%)	7 (14.9)	13 (23.2)	0.28
Positive surgical margin, n (%)	1 (1.9%)	10 (13.5)	0.02
Blood transfusion	9 (17)	27 (36.5)	0.016
Receipt of adjuvant therapy*, n (%)	3 (5.7)	5 (6.8)	0.80
Patients with recurrence of disease	13 (22.4)	32 (43.2)	0.016
Follow up status:			0.02
Death for other cause	6 (10.3)	16 (21.6)	
Death for bladder cancer	11 (19.0)	27 (36.5)	
Non evidence of disease	39 (67.2)	25 (33.8)	
Alive with disease recurrence	2 (3.4)	6 (8.1)	
Follow up time, months	16.1 (7.3–26.6)	16.1 (8.2–26.4)	0.93
Time to recurrence	15.9 (5.5–25.6)	12.3 (4.5–22.5)	0.82

n – number of patients; IQR – interquartile range; BMI – body mass index; NLR – neutrophil-to-lymphocyte ratio; ECOG – Eastern Cooperative Oncology Group

Table S2. *Patients and tumor characteristics according to NLR2 <2.7 and NLR2 ≥2*

	NLR2 <2.7 (immediately after surgery) (4 patients)	NLR2 ≥2.7 (immediately after surgery) (123 patients)	p-value
Age, median (IQR)	82.5 (72–91)	75 (68–81)	0.80
Sex, n (%)			0.3
Female	0	26 (21.1)	
Male	4 (100)	97 (78.9)	
BMI kg/m^2, median (IQR)	24 (23.5–24.5)	26.6 (23.6–29.7)	<0.01
ECOG performance status, n (%)			0.6
0	1 (25)	47 (38.2)	
1	3 (75)	53 (43.1)	
2	0	20 (16.3)	
3	0	3 (2.4)	
Max tumor size			0.04
≤2 cm	4 (100)	57 (47.9)	
>3 cm	0	62 (52.1)	
Receipt of Intravescical therapy:			0.75
No vescical therapy	3 (75)	100 (82)	
Vescical therapy	1 (25)	22 (18)	
Clinical T stage, n (%)			0.48
≤T2	4 (100)	108 (89.3)	
T3–T4	0	13 (10.7)	
Pathologic T stage, n (%)			0.79
≤T2	2 (50)	69 (56.6)	
T3–T4	2 (50)	53 (43.4)	
pN stage			0.47
pNx	1 (25)	19 (16)	
pN0	2 (50)	82 (68.9)	
pN1	0	10 (8.4)	
pN2	1 (25)	8 (6.7)	
Perineural invasion, n (%)	1 (25)	24 (20.2)	0.81
Lymphovascular invasion, n (%)	2 (50)	60 (50.4)	0.99
Lymph node involvement, n (%)	1 (33.3)	18 (18)	0.5
Positive surgical margin, n (%)	0	12 (9.8)	0.5
Blood transfusion	1 (25)	34 (27.9)	0.9
Receipt of adjuvant therapy*, n (%)	0	7 (5.7)	0.6
Patients with recurrence of disease	1 (25)	42 (34.1)	0.70
Follow up status:			0.35
Death for other cause	2 (50)	20 (16.3)	
Death for bladder cancer	1 (25)	36 (29.3)	
Non evidence of disease	1 (25)	60 (48.8)	
Alive with disease recurrence	0	7 (5.7)	
Follow up time, months	15.7 (9.4–26.4)	16.1 (8.1–26.5)	0.19
Time to recurrence	15.7 (7.9–26.4)	13.8 (13.8–24.8)	0.2

n – number of patients; IQR – interquartile range; BMI – body mass index; NLR – neutrophil-to-lymphocyte ratio; ECOG – Eastern Cooperative Oncology Group

Table S3. *Patients and tumor characteristics according to NLR3 <2.7 and NLR3 ≥2.7*

	NLR3 <2.7 (at discharge) (25 patients)	NLR3 ≥2.7 (at discharge) (104 patients)	p-value
Age, median (IQR)	73 (63.5–82)	75 (68–81)	0.20
Sex, n (%)			0.89
Female	5 (20)	22 (21.2)	
Male	20 (80)	82 (78.8)	
BMI kg/m², median (IQR)	24.9 (24–28.1)	28 (23.6–29.8)	0.17
ECOG performance status, n (%)			0.17
0	7 (28)	41 (39.4)	
1	16 (64)	42 (40.4)	
2	2 (8)	18 (17.3)	
3	0	3 (2.9)	
Max tumor size			0.96
≤2 cm	12 (50)	50 (49.5)	
>3 cm	12 (50)	51 (50.5)	
Receipt of Intravescical therapy:			0.38
No vescical therapy	19 (76)	86 (83.5)	
Vescical therapy	6 (24.0)	17 (16.5)	
Clinical T stage, n (%)			0.68
≤T2	23 (92)	91 (89.2)	
T3–T4	2 (8)	11(10.8)	
Pathologic T stage, n (%)			0.63
≤T2	13 (52)	59 (57.3)	
T3–T4	12 (48)	44 (42.7)	
pN stage			0.71
pNx	4 (16.7)	17 (16.8)	
pN0	18 (75)	66 (65.3)	
pN1	1 (4.2)	9 (8.9)	
pN2	1 (4.2)	9 (8.9)	
Perineural invasion, n (%)	4 (16.7)	22 (21.8)	0.58
Lymphovascular invasion, n (%)	11 (45.8)	51 (50.5)	0.68
Lymph node involvement, n (%)	2 (10)	18 (21.4)	0.2
Positive surgical margin, n (%)	1 (4)	10 (9.7)	0.36
Blood transfusion	6 (24)	30 (29.1)	0.60
Receipt of adjuvant therapy*, n (%)	1 (4.0)	7 (6.8)	0.6
Patients with recurrence of disease	10 (40)	35 (33.7)	0.55
Follow up status:			0.76
Death for other cause	3 (12.0)	19 (18.3)	
Death for bladder cancer	9 (36.0)	29 (27.9)	
Non evidence of disease	12 (48.0)	49 (47.1)	
Alive with disease recurrence	1 (4.0)	7 (6.7)	
Follow up time, months	17.3 (6.7–28.8)	15.9 (8.2–25.9)	0.48
Time to recurrence	16.7 (5.6–28.0)	12.4 (4.3–22.8)	0.68

n – number of patients; IQR – interquartile range; BMI – body mass index;
NLR – neutrophil-to-lymphocyte ratio; ECOG – Eastern Cooperative Oncology Group

Table S4. *Patients and tumor characteristics according to NLR4 <2.7 and NLR4 ≥2.7*

	NLR4 <2.7 (at recurrence or last follow up) (77 patients)	NLR4 ≥2.7 (at recurrence or last follow up) (48 patients)	p-value
Age, median (IQR)	74 (65–81)	76 (69–81)	0.05
Sex, n (%)			0.99
Female	16 (20.8)	10 (20.8)	
Male	61 (79.2)	38 (79.2)	
BMI kg/m², median (IQR)	27.3 (23.9–29.9)	25.5 (23.4–29.0)	0.25
ECOG performance status, n (%)			0.45
0	33 (42.9)	14 (29.2)	
1	32 (41.6)	24 (50)	
2	10 (13.0)	9 (18.8)	
3	2 (2.6)	1 (2.1)	
Max tumor size			0.39
≤2 cm	39 (52.7)	21 (44.7)	
>3 cm	35 (47.3)	26 (55.3)	
Receipt of Intravescical therapy:			0.58
No vescical therapy	62 (81.6)	41 (85.4)	
Vescical therapy	14 (18.4)	7 (14.6)	
Clinical T stage, n (%)			0.22
≤T2	70 (92.1%)	40 (85.1%)	
T3–T4	6 (7.9%)	7 (14.9%)	
Pathologic T stage, n (%)			0.01
≤T2	51 (67.1)	21 (43.8)	
T3–T4	25 (32.9)	27 (56.2)	
pN stage			0.017
pNx	11 (14.9)	9 (19.1)	
pN0	58 (78.4)	26 (55.3)	
pN1	3 (4.1)	5 (10.6)	
pN2	2 (2.7)	7 (14.9)	
Perineural invasion, n (%)	15 (20.3)	10 (21.3)	0.89
Lymphovascular invasion, n (%)	31 (41.9)	27 (57.4)	0.09
Lymph node involvement, n (%)	5 (7.9)	12 (31.6)	<0.01
Positive surgical margin, n (%)	4 (5.3)	8 (16.7)	0.03
Blood transfusion	19 (25)	16 (33.3)	0.31
Receipt of adjuvant therapy*, n (%)	1 (1.3)	5 (10.4)	0.021
Patients with recurrence of disease	15 (19.5)	29 (60.4)	<0.01
Follow up status:			<0.01
Death for other cause	14 (18.2)	7 (14.6)	
Death for bladder cancer	13 (16.9)	25 (52.1)	
Non evidence of disease	48 (62.3)	12 (25)	
Alive with disease recurrence	2 8 (2.6)	4 (8.3)	
Follow up time, months	16.9 (7.5–27.7)	15.9 (7.6–24.8)	0.53
Time to recurrence	14.9 (6.6–24.6	11.2 (3.5–24.1)	0.96

n – number of patients; IQR – interquartile range; BMI – body mass index;
NLR – neutrophil-to-lymphocyte ratio; ECOG – Eastern Cooperative Oncology Group

Table S5. *A non systematic review of the literature on paper dealing with NLR and bladder cancer*

Study	Patients	Study region	Research time	Multi centre	Stage	Surgery	NLR cut-off	NLR Kinetics	Timing of L/N evaluation — Before surgery	Timing of L/N evaluation — After surgery	Median follow-up (months)	End point	HR/OR	p-value
Hermanns et al. [22]	424	Canada	1992–2012	No	Mix (organ confined-metastatic)	RC	3	Not evaluated	Median of 6 days before	N/A	58.4	OS / CSS	1.67 / 1.88	0.005 / <0.001
Krane et al. [20]	68	United States of America	2005–2011	No	OC	RC	2.5	Not evaluated	Not clear the timing	N/A	25	OS / CSS	2.49 / 2.68	N/A / N/A
Gondo et al. [21]	189	Japan	2000–2009	No	OC	RC	2.5	Not evaluated	Not clear the timing	N/A	25.1	CSS	1.95	0.039
Viers et al. [17]	899	United States of America	1994–2005	No	OC	RC	2.7	Evaluated	Within 3 mo from RC	Within 3 mo of RC	130.8	OS / CSS	1.69 / 1.57	<0.001 / <0.001
Lucca et al. [23]	4061	Europe		Yes	OC	RC	2.7	Not evaluated			42	OS / CSS	1.11 / 1.21	0.029 / 0.003
Potretzke et al. [25]	102	United States of America	2002–2012	No	OC	RC	N/A	Not evaluated	Within 100 days	N/A	N/A	OC vs NOC	1.50	0.02
Demirta G et al. [26]	201	Turkey	1999–2013	No	OC	RC	2.5	Not evaluated	Not clear the timing	N/A	37.22	N/A	N/A	N/A
Bhindi et al. [27]	418	Canada	1992–2012	No	OC	RC	N/A	Not evaluated	A week before treatment initiation	N/A	40	OS / CSS / PFS	1.56 / 1.47 / 1.52	0.004 / 0.001 / 0.002
Can et al. [28]	182	Turkey	2001–2011	No	OC	RC	2.57	Not evaluated	Not clear the timing	N/A	45	Pathological stage	2.78	0.004
Ozcan et al. [29]	286	Turkey	1990–2013	No	OC	RC	≥2.5	Not evaluated	Not clear the timing	N/A	8	PFS	1.965	0.022
Zhang et al. [30]	124	China	2009–2009	Yes	Mix (organ confined-metastatic)	RC	≥4	Not evaluated	Not clear the timing	N/A	N/A	OS	NS	NS
Ozyalvacli et al. [31]	166	Turkey	2008–2013	Yes	OC	surgery	≥2.43	Not evaluated	Not clear the timing	N/A	24.2	PFS	2.43	<0.001
Kaynar et al. [32]	291	Turkey	2009–2013	Yes	OC	RT	>2.5	Not evaluated	The day before surgery	N/A	N/A	N/A	N/A	N/A
Kang et al. [33]	385	Republic of Korea	1999–2012	No	OC	RC	≥2.0	Evaluated	Within 1 month before RC	During the early recovery and 3 to 4 months after surgery	38	OS (Postop.) / CSS (preop.) / CSS (post op.)	0.021 / 1.2 / 1.18	<0.001 / 0.019 / 0.021

Table S5. *Continued*

Study	Patients	Study region	Research time	Multi centre	Stage	Surgery	NLR cut-off	NLR Kinetics	Timing of L/N evaluation before surgery	Timing of L/N evaluation after surgery	Median follow-up (months)	End point	HR/OR	p -value
Bambury et al. [34]	129	United States of America	2008–2013	No	Locally advanced/ metastatic	Perioperative or metastatic setting	2.5 and 3	Not evaluated	Unknown	N/A	N/A	OS	1.03	<0.01
Mano et al. [35]	107	Israel	2003–2010	No	OC	TURBT	Disease progression 2.41 and recurrence 2.43	Not evaluated	Not clear the timing	N/A	40	PFS Recurrence	3.52 1.75	0.012 0.034
Lee et al. [36]	226	United Kingdom	2011–2013	No	OC	RC	>3.89	Not evaluated	60 days of TURBT	N/A	N/A	MIBC	8.244	<0.01
Yoshida et al. [37]	302	Japan	1995–2013	Yes	OC	RC*	N/A	Evaluated	1 month before Surgery	1–3 months after RC	6.8 years	OS CSS	NS NS	NS NS

*Neoadjuvant chemotherapy in 20 (6.6%)
OC – organ confined; NOC – non-organ-confined; N/A – not applicable; NS – not significant; RC – radical cystectomy; RT – radical radiotherapy; OS – overall survival; CSS – cancer specific survival; PFS – progression free survival; TURBT – transurethral resection of bladder tumor; RT –; HR – hazard ratio; OR – odds ratio; MIBC – muscle invasive bladder cancer