Pre-treatment neutrophil-to-lymphocyte ratio as predictor of adverse outcomes in patients undergoing radical cystectomy for urothelial carcinoma of the bladder

T Hermanns*[,1,2,6], B Bhindi[2,3,6], Y Wei[1], J Yu[1], A P Noon[1,2], P O Richard[1,2], J R Bhatt[1,2], A Almatar[1,2], M A S Jewett[1,2], N E Fleshner[1,2], A R Zlotta[2,4], A J Templeton[5] and G S Kulkarni[1,2,3]

[1]Division of Urology, Department of Surgical Oncology, Princess Margaret Cancer Centre, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9; [2]Division of Urology, Department of Surgery, University Health Network, University of Toronto, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9; [3]Institute for Clinical Evaluative Sciences, 2075 Bayview Avenue, Toronto, Ontario, Canada M4N 3M5; [4]Department of Urology, Mount Sinai Hospital, 60 Murray Street, Toronto, Ontario, Canada M5T 3L9 and [5]Division of Medical Oncology, Department of Medicine, Princess Margaret Cancer Centre, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9

Background: An elevated neutrophil-to-lymphocyte ratio (NLR) is associated with poor outcome in various tumours. Its prognostic utility in patients with urothelial carcinoma of the bladder (UCB) undergoing radical cystectomy (RC) is yet to be fully elucidated.

Methods: A cohort of patients undergoing RC for UCB in a tertiary referral centre between 1992 and 2012 was analysed. Neutrophil-to-lymphocyte ratio was computed using complete blood counts performed pre-RC, or before neo-adjuvant chemotherapy where applicable. Time-dependent receiver operating characteristic curves were used to determine the optimal cutoff point for predicting recurrence-free survival (RFS), cancer-specific survival (CSS) and overall survival (OS). The predictive ability of NLR was assessed using Kaplan–Meier analyses and multivariable Cox proportional hazards models. The likelihood-ratio test was used to determine whether multivariable models were improved by including NLR.

Results: The cohort included 424 patients followed for a median of 58.4 months. An NLR of 3 was determined as the optimal cutoff value. Patients with an NLR $\geqslant$ 3.0 had significantly worse survival outcomes (5y-RFS: 53% *vs* 64%, log-rank $P = 0.013$; 5y-CSS: 57% *vs* 75%, log-rank $P < 0.001$; 5y-OS: 43% *vs* 64%, log-rank $P < 0.001$). After adjusting for disease-specific predictors, an NLR $\geqslant$ 3.0 was significantly associated with worse RFS (HR = 1.49; 95% CI = 1.12–2.0, $P = 0.007$), CSS (HR = 1.88; 95% CI = 1.39–2.54, $P < 0.001$) and OS (average HR = 1.67; 95% CI = 1.17–2.39, $P = 0.005$). The likelihood-ratio test confirmed that prognostic models were improved by including NLR.

Conclusions: Neutrophil-to-lymphocyte ratio is an inexpensive prognostic biomarker for patients undergoing RC for UCB. It offers pre-treatment prognostic value in addition to established prognosticators and may be helpful in guiding treatment decisions.

Radical cystectomy (RC) with pelvic lymph node dissection is the standard treatment for muscle-invasive (MI) urothelial carcinoma of the bladder (UCB) and is recommended for patients with non-muscle-invasive (NMI) UCB with high risk of progression (Clark *et al*, 2013). Despite curative intent, disease recurs in a significant proportion of patients and 5-year survival

rates of only 40–60% have consistently been reported (Gakis et al, 2013).

More aggressive treatment options, such as early RC in patients with high-risk NMI UCB or RC in combination with neo-adjuvant chemotherapy (NAC) or adjuvant chemotherapy (AC), have been shown to improve outcomes (Raj et al, 2011; Meeks et al, 2012; Leow et al, 2013; Sternberg et al, 2013). However, employing aggressive strategies unselectively to all patients carries the risk of overtreatment in patients with favourable prognoses. Improved risk stratification will individualise the use of such approaches. At this time, however, risk stratification based on clinicopathological data alone is unlikely to be sufficient for optimal treatment decision-making (Ficarra et al, 2005; Shariat et al, 2007; Canter et al, 2011). Thus, novel prognostic markers are needed to improve stratification, and, eventually outcomes, of patients with UCB.

Inflammation has an important role in the development and progression of many malignancies (Grivennikov et al, 2010; Hanahan and Weinberg, 2011). Putative mechanisms include the increased supply of factors that promote carcinogenesis and tumour progression by cells of the innate immune systems (that is, neutrophils) and decreased antitumoural response by immune cells of the adaptive system (that is, lymphocytes; Hanahan and Weinberg, 2011). The neutrophil-to-lymphocyte ratio (NLR), which can easily be calculated from routine complete blood counts (CBCs) with differentials, is an emerging marker of host inflammation and has been shown to be

an independent prognosticator for a variety of solid malignancies (Proctor et al, 2012; Guthrie et al, 2013; Templeton et al, 2014). However, there is sparse data on the prognostic role of NLR in patients with UCB (Gondo et al, 2012; Demirtas et al, 2013; Krane et al, 2013).

The objective of our investigation was to evaluate the association between pre-treatment NLR and survival in patients undergoing RC for UCB in a cohort of patients from a tertiary care centre.

MATERIALS AND METHODS

Patients and data sources. Using our institutional database, patients who underwent RC between 1 January 1992 and 31 December 2012 were retrospectively identified. Patients were excluded if CBCs with differentials were unavailable for analysis ($n = 14$), or if they had a history of conditions that may have influenced blood cell lines (connective tissue disease: $n = 4$, malignant lymphoma: $n = 3$, leukaemia: $n = 2$, and human immunodeficiency virus infection: $n = 1$). Patients undergoing RC for salvage therapy following failed chemoradiation ($n = 20$) were excluded because of the potential influence of prior chemotherapy on blood cell lines. Patients with non-urothelial cancers ($n = 9$), or for primary prostatic urothelial carcinoma ($n = 5$), were also excluded in order to maintain a homogenous cohort. Electronic hospital chart review was performed to collect

Table 1. Cohort characteristics		Total $n = 424$	NLR $\geqslant 3$ $n = 216$	NLR < 3 $n = 208$	P-value
Patient characteristics					
Age in years		70.1 (60.6–76.3)	71.5 (61.9–77.3)	68.9 (59.5–75.5)	0.086
Female sex, n		99 (23.4)	43 (20.7)	56 (25.9)	0.20
Haemoglobin (g l^{-1})		132.5 (116–145)	125 (109–140)	137.5 (125–148.5)	<0.001[a]
WBC ($\times 10^9$ l^{-1})		7.6 (6.0–9.1)	8.7 (7.05–10.4)	6.6 (5.5–8.2)	<0.001[a]
Platelets ($\times 10^9$ l^{-1})		252 (202–303)	274 (218–329)	236 (193–282.5)	<0.001[a]
Heavy smoking (30 $\geqslant$ pack-years), n		134 (31.6)	64 (30.8)	70 (32.4)	0.72
Charlson comorbidity index[b]		6 (5–7)	6 (5–8)	6 (4.5–7)	0.010[a]
Disease characteristics					
Hydronephrosis, n (%)		119 (28.1)	64 (30.8)	55 (25.5)	0.22
Concurrent CIS, n (%)		190 (44.8)	85 (40.9)	105 (48.6)	0.11
T-stage, n (%)	pT0	25 (5.9)	9 (4.3)	16 (7.4)	0.004[a]
	pTa/T1/T2 CIS	205 (48.4)	87 (41.8)	118 (54.6)	
	pT3/T4	194 (45.7)	112 (53.9)	82 (38.0)	
N-stage, n (%)	pN0	287 (67.7)	144 (69.2)	143 (66.2)	0.68
	pN-pos	116 (27.4)	53 (25.5)	63 (29.2)	
	pNx	21 (4.9)	11 (5.3)	10 (4.6)	
Positive surgical margin, n (%)		333 (7.8)	16 (7.7)	17 (7.9)	0.95
Lymphovascular invasion		143 (33.7)	77 (37.0)	66 (30.6)	0.16
Other treatment characteristics					
Total node count		12 (7–19)	12 (7–19)	13 (8–19)	0.22
NAC/primary chemotherapy[c], n		29 (6.8)	15 (7.2)	14 (6.5)	0.77
Adjuvant chemotherapy[c], n		87 (20.5)	34 (16.4)	53 (24.5)	0.037[a]
Salvage chemotherapy[c], n		55 (13.0)	27 (13.0)	28 (13.0)	>0.99

Abbreviations: CIS = carcinoma in situ; NAC: neo-adjuvant chemotherapy; NLR = neutrophil-to-lymphocyte ratio; WBC = white blood count. All data presented as median (interquartile range) or number (percent).
[a]A significant difference is indicated between the groups NLR $\geqslant$3.0 and <3.
[b]Sum of co-morbidity score (including malignancy) and age score.
[c]Generally, chemotherapy was cisplatin-based. Patients with renal insufficiency received carboplatin-based chemotherapy.

clinical parameters including blood work results. Mortality data were obtained through the Princess Margaret Cancer Centre Cancer Registry. Institutional research ethics board approval was obtained.

Primary study exposure. The date of initiation of treatment for each patient was defined as the date of RC or the date of initiation of NAC for patients who received NAC. All patients were seen for medical assessment before the initiation of treatment. Generally, NLR was calculated using neutrophil and lymphocyte counts from a routine CBC with differentials performed on the same day as these visits (median of 6 days (interquartile range (IQR): 2–10 days) before initiation of treatment). Review of the pre-treatment clinic notes did not reveal any symptoms or signs of infections that may have influenced the NLR.

Outcome measures. Patients were generally seen at 6–8 weeks after the RC, and otherwise every 3–6 months early on for periodic physical examination, imaging to rule out hydronephrosis or tumour recurrence, and urethroscopy if indicated. Follow-up subsequently became less intensive based on individual physician's practice patterns and clinical suspicion. The outcome measures were recurrence-free survival (RFS), cancer-specific survival (CSS) and overall survival (OS) measured in months from the date of initial treatment.

Statistical analysis. Statistical analyses were performed using SAS v9.3 (SAS Institute Inc, Cary, NC, USA). Clinical characteristics were compared between patients with NLR values above and below the optimal cutoff point (see below) using the Wilcoxon rank-sum test for continuous variables and Pearson's χ^2 test for categorical variables.

In the literature, there is heterogeneity in the NLR cutoff points used (Guthrie *et al*, 2013). Therefore, in order to determine the optimal cutoff point for clinical use, time-dependent receiver-operating characteristic (ROC) curves were created for each outcome measure at 12, 24, 36, 48 and 60 months (Heagerty and Zheng, 2005; Lu and Liu, 2006). NLR values between 1.5 and 6 were considered in 0.5 increments. The optimal NLR value for each outcome at a given time point was identified by minimising the distance from the ROC curve to the top left corner of the ROC plot (and thus optimising both sensitivity and specificity; Perkins and Schisterman, 2006).

Kaplan–Meier analyses with log-rank tests were then used to compare survival outcomes between patients with NLR values above *vs* below the optimal cutoff point. To determine how NLR can influence risk stratification in the pre-treatment and post-cystectomy settings among patients with localised disease (without evidence of nodal disease), we performed additional Kaplan–Meier analyses stratifying by clinical and pathological stage, respectively. In these analyses, patients receiving NAC or AC were excluded to have a more clear impression of how NLR values have an impact on the natural history of disease.

Univariate and multivariable Cox proportional hazards models were built for each survival outcome. Multivariable models adjusted for *a priori* defined patient-related risk factors (age, gender and Charlson comorbidity index), tumour-related variables (pathological T-stage and lymphovascular invasion), treatment-related parameters (year of RC, use of NAC or AC and surgical margin status) and haematologic parameters (haemoglobin and platelet counts). AC was operationalised as a time-varying covariate to address survivor treatment bias (Austin *et al*, 2006). A robust sandwich covariance matrix estimator was used to account for clustering of outcomes by surgeon (Lin and Wei, 1989). The additional value to the models provided by NLR was evaluated using the likelihood-ratio test to compare models for each survival outcome with and without NLR. Statistical model assumptions, including the proportional hazards assumption, were tested (Hess, 1995).

In order to ensure that our use of a cutoff point did not introduce bias (Royston *et al*, 2006), we performed a sensitivity analysis analysing NLR as a continuous variable with log-transformation (because of its skewed distribution). Given that other studies have not included patients receiving NAC (Gondo *et al*, 2012), we also performed a sensitivity analysis excluding such patients. All tests were two-sided with P-values <0.05 considered statistically significant.
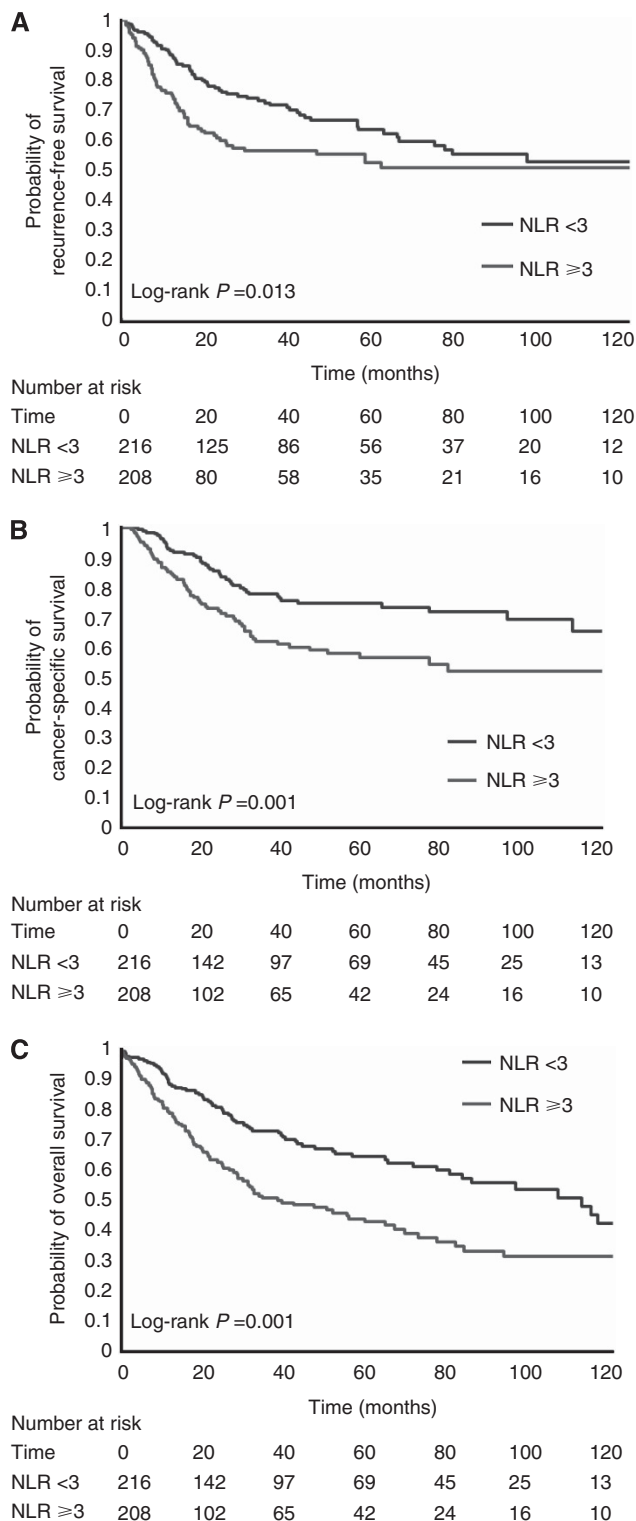
Number at risk

Time	0	20	40	60	80	100	120
NLR <3	216	125	86	56	37	20	12
NLR ⩾3	208	80	58	35	21	16	10

Number at risk

Time	0	20	40	60	80	100	120
NLR <3	216	142	97	69	45	25	13
NLR ⩾3	208	102	65	42	24	16	10

Number at risk

Time	0	20	40	60	80	100	120
NLR <3	216	142	97	69	45	25	13
NLR ⩾3	208	102	65	42	24	16	10

Figure 1. (**A–C**) Kaplan–Meier curves for recurrence-free survival (**A**), cancer-specific survival (**B**) and overall survival (**C**) for patients with an NLR <3 and ⩾3.

RESULTS

The final study cohort consisted of 424 patients with a median follow-up of 58.4 months (IQR: 21.3–94.5 months). The cohort characteristics are described in Table 1. Overall, 138 patients (32.6%) had cancer recurrence and 178 (42%) died, of which 110 (25.9%) died of UCB.

In time-dependent ROC curve analyses, an NLR cutoff point of 3 minimised the distance from the ROC curve to the top left of the plot for 14 out of 15 time points across the three outcome measures (Supplementary Table 1). Given that this cutoff point was among those used by other studies (Guthrie *et al*, 2013), we proceeded to use this as the optimal cutoff point in our study.

There were 216 (50.9%) patients who had an NLR value $\geqslant 3$ (Table 1). These patients had significantly lower haemoglobin values, higher platelet counts and a higher Charlson comorbidity index and were less likely to receive AC. They were more likely to have pT3–4 disease (53.9% *vs* 38%); however, there was no significant difference in pN stage.

In univariate Kaplan–Meier analyses, NLR $\geqslant 3$ *vs* <3 was associated with increased probability of recurrence (5y-RFS: 53% *vs* 64%, log-rank $P = 0.013$, Figure 1A), cancer-specific mortality (5y-CSS: 57% *vs* 75%, log-rank $P < 0.001$, Figure 1B) and overall mortality (5y-OS: 43% *vs* 64%, log-rank $P < 0.001$; Figure 1C).

In univariate Cox models, an NLR $\geqslant 3$ was associated with increased risk of recurrence (hazard ratio (HR) = 1.53, 95% confidence interval (CI) = 1.23–1.89, $P < 0.001$, Table 2),

Table 2. Cox proportional hazard models for recurrence-free survival

Parameter	Unadjusted		Adjusted	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
NLR ($\geqslant 3$ *vs* <3)	1.53 (1.23–1.89)	<0.001	1.49 (1.12–2.00)	0.007
Age	1.01 (1.00–1.02)	0.22	1.00 (0.98–1.03)	0.93
Gender (female *vs* male)	0.96 (0.68–1.34)	0.79	0.87 (0.60–1.27)	0.48
Charlson comorbidity index	1.08 (0.99–1.18)	0.078	1.04 (0.98–1.11)	0.17
Haemoglobin (per 10 units)	0.89 (0.86–0.93)	<0.001	0.97 (0.95–1.00)	0.014
Platelets (per 100 units)	1.24 (1.10–1.38)	<0.001	1.17 (1.10–1.25)	<0.001
T-stage (pT3–4 *vs* pT0–2)	2.75 (1.99–3.80)	<0.001	1.51 (0.97–2.34)	0.067
N-Stage (N + *vs* N0/Nx)	3.01 (2.68–3.38)	<0.001	2.16 (1.82–2.57)	<0.001
Positive surgical margin	2.07 (1.33–3.22)	0.001	1.99 (1.28–3.10)	0.002
Year of radical cystectomy	1.00 (0.97–1.03)	0.93	1.00 (0.97–1.03)	>0.99
Lymphovascular invasion	2.91 (1.88–4.52)	<0.001	1.82 (1.13–2.95)	0.014
NAC/primary chemotherapy	0.93 (0.43–2.01)	0.86	0.90 (0.41–1.97)	0.79
Adjuvant chemotherapy	1.56 (1.21–2.00)	<0.001	0.89 (0.55–1.42)	0.61

Abbreviations: CI = confidence interval; HR = hazard ratio; NAC = neo-adjuvant chemotherapy; NLR = neutrophil-to-lymphocyte ratio.

Table 3. Cox proportional hazard models for cancer-specific survival

Parameter	Unadjusted		Adjusted	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
NLR ($\geqslant 3$ *vs* <3)	1.88 (1.52–2.33)	<0.001	1.88 (1.39–2.54)	<0.001
Age	1.01 (0.99–1.04)	0.19	1.00 (0.97–1.04)	0.88
Gender (female *vs* male)	1.17 (0.95–1.46)	0.14	1.10 (0.87–1.40)	0.41
Charlson Comorbidity Index	1.16 (1.06–1.28)	0.002	1.16 (1.09–1.24)	<0.001
Haemoglobin (per 10 units)	0.87 (0.82–0.91)	<0.001	0.96 (0.92–0.99)	0.013
Platelets (per 100 units)	1.28 (1.14–1.44)	<0.001	1.19 (1.03–1.37)	0.019
T-stage (pT3–4 *vs* pT0–2)	3.12 (2.18–4.47)	<0.001	1.65 (1.06–2.56)	0.026
N-stage (N + *vs* N0/Nx)	3.21 (2.14–4.81)	<0.001	2.26 (1.38–3.70)	0.001
Positive surgical margin	1.98 (1.03–3.82)	0.041	1.61 (0.79–3.31)	0.19
Year of radical cystectomy	0.98 (0.92–1.04)	0.50	0.96 (0.90–1.03)	0.30
Lymphovascular invasion	3.09 (1.98–4.82)	<0.001	1.91 (1.10–3.34)	0.023
NAC/primary chemotherapy	1.47 (0.66–3.25)	0.34	1.33 (0.59–2.98)	0.50
Adjuvant chemotherapy	1.36 (1.01–1.83)	0.041	0.95 (0.49–1.86)	0.89

Abbreviations: CI = confidence interval; HR = hazard ratio; NAC = neo-adjuvant chemotherapy; NLR = neutrophil-to-lymphocyte ratio.

Table 4. Cox proportional hazard models for overall survival

Parameter	Unadjusted		Adjusted	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
NLR (⩾3 vs <3)	1.80 (1.48–2.20)	<0.001	1.67[a] (1.17–2.39)	0.005
Age	1.03 (1.02–1.05)	<0.001	1.01 (0.99–1.03)	0.20
Gender (female vs male)	0.95 (0.81–1.11)	0.48	0.89 (0.73–1.07)	0.21
Charlson Comorbidity Index	1.28 (1.18–1.39)	<0.001	1.23 (1.16–1.31)	<0.001
Haemoglobin (per 10 units)	0.85 (0.82–0.87)	<0.001	0.93 (0.89–0.97)	<0.001
Platelets (per 100 units)	1.21 (1.11–1.31)	<0.001	1.21 (1.14–1.28)	<0.001
T-stage (pT3–4 vs pT0–2)	2.44 (1.58–3.77)	<0.001	1.41 (0.82–2.42)	0.21
N-stage (N+ vs N0/Nx)	2.22 (1.67–2.94)	<0.001	1.59 (1.14–2.22)	0.006
Positive surgical margin	2.00 (0.99–4.04)	0.054	1.83 (0.94–3.60)	0.078
Year of radical cystectomy	0.97 (0.93–1.01)	0.15	0.95 (0.92–0.98)	0.004
Lymphovascular invasion	2.65 (2.06–3.41)	<0.001	2.08 (1.18–3.68)	0.012
NAC/primary chemotherapy	1.44 (0.67–3.06)	0.35	1.31 (0.81–2.13)	0.28
Adjuvant chemotherapy	0.91 (0.62–1.33)	0.61	0.84 (0.48–1.49)	0.55

Abbreviations: CI = confidence interval; HR = hazard ratio; NAC = neo-adjuvant chemotherapy; NLR = neutrophil-to-lymphocyte ratio.
[a]HR represents average HR across follow-up time. However, the HR varies as a function of time (see Figure 2 for details).

cancer-specific mortality (HR = 1.88, 95% CI = 1.52–2.33, $P < 0.001$, Table 3) and overall mortality (HR = 1.80, 95% CI = 1.48–2.20, $P < 0.001$, Table 4).

Upon adjusting for confounders using multivariable models, NLR remained significantly associated with increased risk of recurrence (HR = 1.49, 95% CI = 1.12–2, $P = 0.007$, Table 2), cancer-specific mortality (HR = 1.88, 95% CI = 1.39–2.54, $P < 0.001$, Table 3) and overall mortality (average HR = 1.67, 95% CI = 1.17–2.39, $P = 0.005$, Table 4). Of note, the proportional hazards assumption was satisfied for the models for RFS and CSS but not for OS. This would suggest that the HR for the association between NLR and OS is not constant but varies as a function of time. Therefore, the HR presented in Table 4 represents an average value across the study period. As others have presented such findings (Lipscombe *et al*, 2013), Figure 2 shows how the HR changes as a function of time from initial treatment (with the corresponding model that includes an NLR × time interaction term shown in Supplementary Table 2). Neutrophil-to-lymphocyte ratio has the strongest association with increased risk of overall mortality early on and then gradually decreases. This association remained statistically significant up to ∼50 months from the start of treatment.

Associations between NLR and increased risk of adverse survival outcomes remained statistically significant when NLR was analysed as a log-transformed continuous variable, as well as in the sensitivity analysis excluding patients who received NAC (data not shown). Using the likelihood-ratio test and comparing multivariable Cox models with and without NLR, it was determined that NLR significantly improved models for RFS ($P = 0.013$), CSS ($P = 0.001$) and OS ($P = 0.003$).

Lastly, we performed exploratory analyses to assess the potential prognostic impact of using NLR when risk stratifying patients into two scenarios. For these analyses, patients receiving NAC or AC were excluded to better reflect the natural history of disease and avoid confounding from adjunctive treatment. The first scenario assessed patients without *clinical* evidence of nodal disease (cN0), where risk stratification may guide initial management in the pretreatment setting. We sought to determine whether NLR further stratified patients beyond their clinical stage, analysing its impact
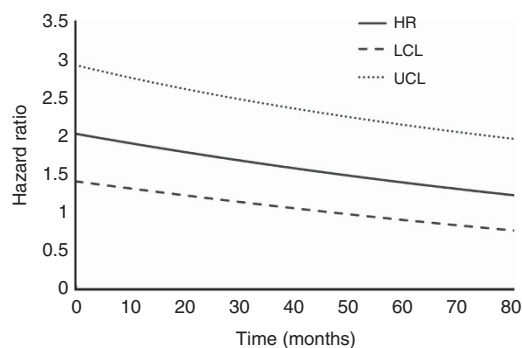

Figure 2. Instantaneous hazard ratio *vs* time with corresponding confidence intervals for OS LCL = lower 95% confidence limit, UCL = upper 95% confidence limit.

on clinically NMI UCB *vs* MI UCB disease subgroups. In these Kaplan–Meier analyses (Figures 3A–C), NLR added valuable prognostic information. Patients with clinical NMI UCB appeared to separate into two groups, with those with clinical NMI UCB and NLR ⩾3 manifesting survival outcomes comparable to clinical MI UCB. The second scenario was the postoperative setting among patients who were pN0 (where a decision must be made regarding the use of AC). NLR further stratified patients within pT-stage categories (Figures 4A–C). Notably among patients with organ-confined (pT0–pT2) disease, NLR identified a subset of patients who were at increased risk of adverse oncologic outcomes.

DISCUSSION

The host inflammatory response has gained increasing attention in oncology research. Infiltrating cells of the immune system are constituents of virtually all neoplasms (Hanahan and Weinberg, 2011). While initially thought to represent an antitumoural response, immune cells, particularly those of the innate immune system, also exhibit effects that promote carcinogenesis and
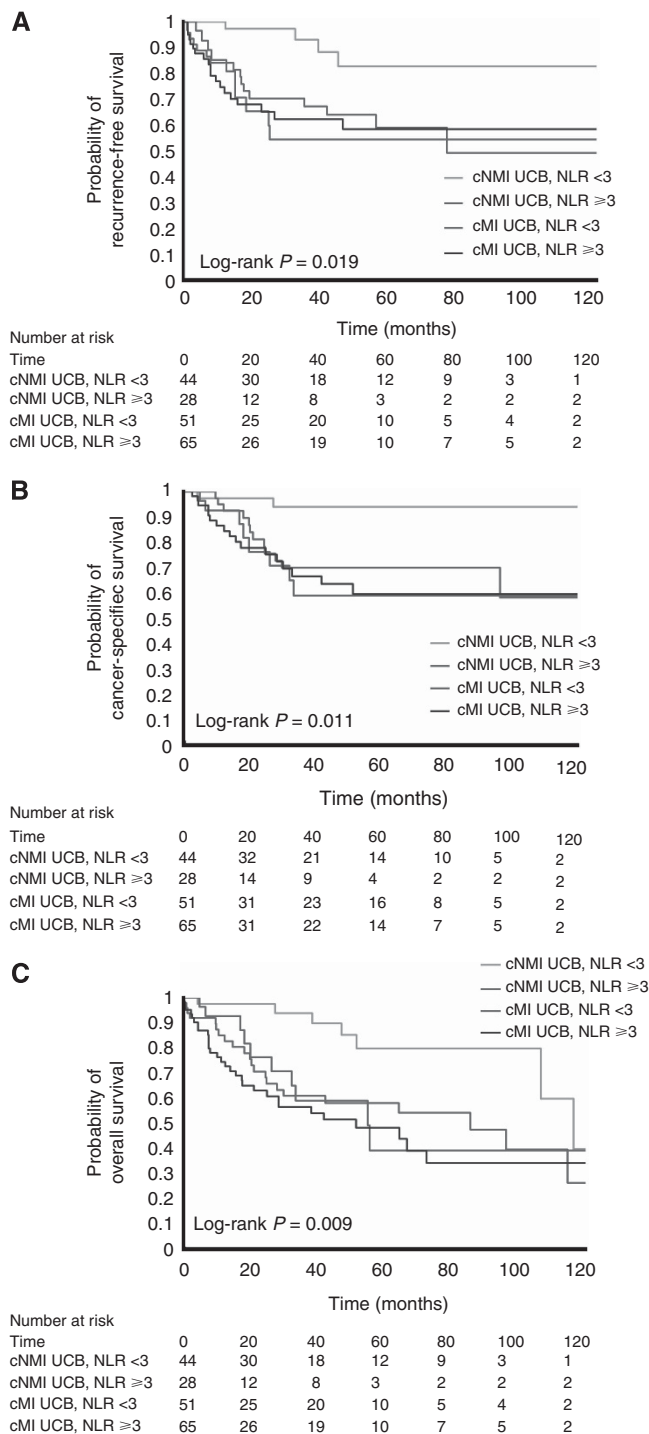
Figure 3. (**A–C**) Kaplan–Meier curves for recurrence-free survival (**A**), cancer-specific survival (**B**) and overall survival (**C**) for patients without *clinical* evidence of nodal disease (cN0) and clinically NMI bladder cancer (BC) or MI BC and an NLR <3 or ≥3, respectively.

Figure 4. (**A–C**) Kaplan–Meier curves for recurrence-free survival (**A**), cancer-specific survival (**B**) and overall survival (**C**) for patients with organ-confined bladder cancer (pT0–2 pN0) or non-organ-confined bladder cancer (pT3–4 pN0) and an NLR <3 or ≥3, respectively.

cancer progression (Grivennikov *et al*, 2010; Hanahan and Weinberg, 2011). 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 (Hanahan and Weinberg, 2011). 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
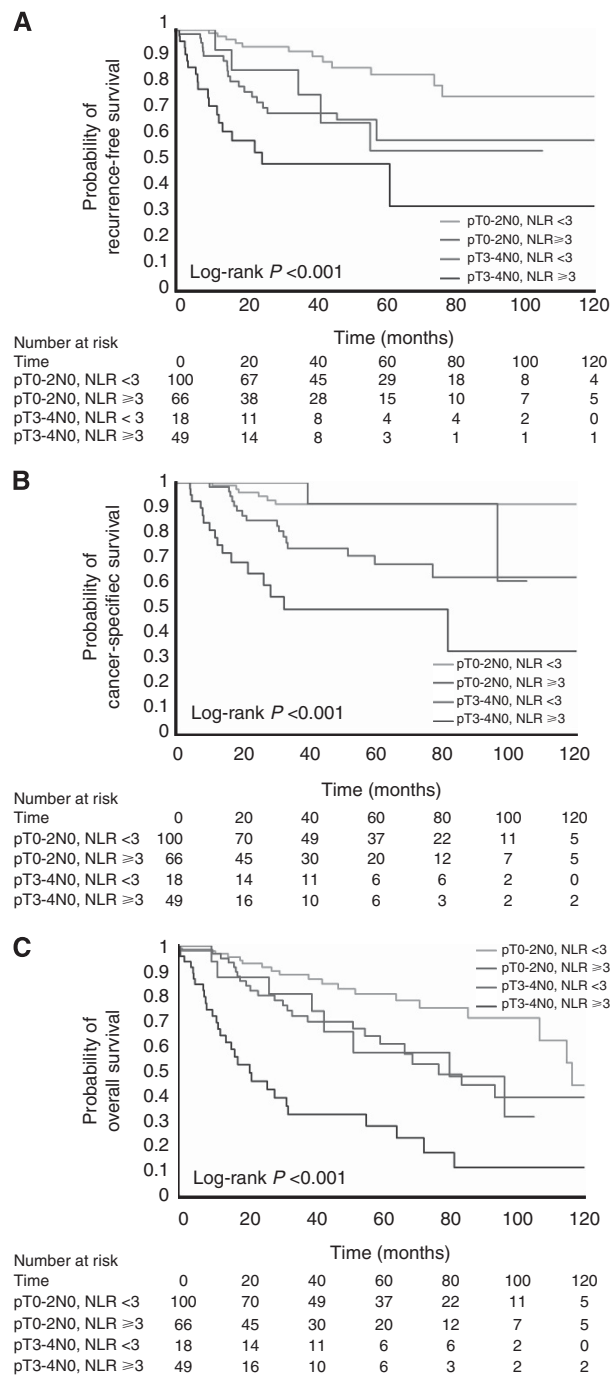
of the systemic host response when evaluating the association between inflammation and cancer outcomes.

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 tumour behaviour (Guthrie *et al*, 2013; Templeton *et al*, 2014). However, data regarding the association of NLR and prognosis for UCB after RC are still scarce. To date, only three small studies have been published in this population (Gondo *et al*, 2012; Demirtas *et al*, 2013; Krane *et al*, 2013). Gondo *et al* (2012) were the first to describe an association between higher

NLR (>2.5) and CSS in a cohort of 189 patients undergoing RC. Demirtas *et al* (2013) (201 patients) reported no association between NLR (>2.5) and OS, whereas Krane *et al* (2013) (68 patients) found that an elevated NLR (>2.5) was an independent predictor of extravesical disease and worse OS. In the latter study, however, 10 patients received NAC and it is unclear how their calculated NLR based on immediate preoperative blood work may have been affected.

To the best of our knowledge, our study has the largest sample size investigating the independent prognostic ability of NLR in patients undergoing RC for UCB. It is the first of its kind to show that pre-treatment NLR is an independent prognostic factor for RFS, CSS and OS. Among patients receiving NAC, we used CBCs collected before the initiation of chemotherapy to eliminate this potential confounder. In addition, a sensitivity analysis excluding patients receiving NAC was performed to confirm robustness of the findings.

There is heterogeneity in reported thresholds used to define an elevated NLR in the literature (range 2–7.7; Templeton *et al*, 2014). This may reflect variations in the host response for different disease sites and stages, or may reflect the different approaches used when determining cutoff values. Not all studies used an accepted method for cutoff point determination, and in some instances the rationale for the cutoff point decision was not described (Templeton *et al*, 2014). All three previously mentioned studies in the RC population used an optimal NLR cutoff point of 2.5 (Gondo *et al*, 2012; Demirtas *et al*, 2013; Krane *et al*, 2013). Gondo *et al* (2012) used the cutoff point that generated the lowest *P*-value in Kaplan–Meier analyses. It is unclear, however, whether this cutoff point was associated with optimal sensitivity and specificity for adverse oncologic outcomes in their study population. One of the other studies chose 2.5 as their cutoff point for consistency with Gondo *et al* (2012) (Krane *et al*, 2013), whereas the third study did not elaborate on the rationale for their cutoff point value (Demirtas *et al*, 2013).

We used time-dependent ROC curves to determine the optimal cutoff point for NLR. Whereas ROC curves are conventionally used for binary outcomes to identify points of optimal sensitivity and specificity, this approach was adapted for survival analyses (Heagerty and Zheng, 2005; Lu and Liu, 2006). In our study, 3 was determined optimal cutoff point. We felt that it was important to identify an *a priori* optimal cutoff point both for practical purposes, and to minimise bias. Even so, there is likely a continuous association between NLR and risk of adverse oncologic outcomes. This warranted a sensitivity analysis using NLR as a log-transformed continuous variable to ensure that we did not introduce any cutoff point bias (Royston *et al*, 2006).

Lastly, our exploratory analyses indicate that NLR may better risk-stratify patients in the pre- and postoperative settings in order to guide treatment strategies. In patients with clinically NMI UCB, there is a high risk of under-staging and a high risk of disease progression to MI UCB (Shariat *et al*, 2007; Thomas *et al*, 2012; Chamie *et al*, 2013). The NLR may be helpful to identify patients most likely to benefit from early RC. Similarly, NLR may improve postoperative risk stratification to guide the use of AC. However, this was not the primary objective of this study and further work is needed to identify the clinical scenarios in which NLR may be helpful.

There are limitations to our study. First, this is a retrospective, single-institution observational study. Second, our study included patients across a long recruitment period, during which practice patterns might have changed. We addressed this by including year of cystectomy in the multivariable model. Third, we did not measure NLR after RC and therefore cannot investigate whether post-RC improvement of NLR has a predictive value. Finally, we are unable to determine whether the outcomes following NAC or AC are different among those patients with high *vs* low NLR because of the limited number of patients receiving NAC or AC in our cohort.

In conclusion, NLR is an inexpensive haematologic test based on commonly measured parameters that predicts RFS, CSS and OS in patients with UCB undergoing RC, independent of well-established patient-related and tumour-related predictors. Whereas our results suggest that NLR may have a role as a prognostic biomarker in the pre-RC and post-RC settings, further studies are needed to maximise the clinical utility of NLR.

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