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XXXI CICLO

The Neutrophil-to-Lymphocyte Ratio as a Prognostic Factor for Patients with Urothelial Carcinoma of the Bladder Following Radical Cystectomy

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Abstract

Introduction: 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 not well studied. In this study, we evaluated the association of preoperative and postoperative NLR with oncological outcomes following RC.

Methods: 132 consecutive patients with UCB treated with open RC were analyzed. NLR was analyzed both as a continuous variable and as a categorical variable using a cut-off of 2.7 based on previous studies. NLR was recorded as followed: before surgery (within 15 days prior to RC, [NLR1]), postoperatively (within 2 days [NLR2], between 7 and 15 days after RC before discharge [NLR3], few days before the evidence of recurrence or last available follow up [NLR4]. ΔNLR was calculated as the difference between NLR2 and NLR1 (NLRΔ1) and between NLR 2 and NLR3 (NLRΔ2). Tumour stage, lymphovascular invasion (LVI) and lymph node involvement were collected. Cancer-specific mortality (CSM), all-cause mortality (ACM) and recurrence-free survival (RFS) were estimated using the Kaplan-Meier method and compared using the log-rank test. Univariable and multivariate logistic regression and Cox proportional hazard models were used to analyze the association of NLR with extravesical disease, LVI, lymph node involvement, recurrence of disease and mortality

Results: During a follow up of 15.9 months, 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 cause. 64 (48.5%) have no evidence of disease at follow-up. When assessed by multivariable analysis NLR1 remained independently associated with a significantly increased risk of extravescical disease (pT 3-4) [OR: 1.4, $p<0.01$] and Lymphovascular invasion [OR: 1.40, $p<0.01$]. NLR4 was independently associated with a significantly increased risk of CSM [HR=1.14, p=0.013]. In a postoperative model, NLR3 was found to be an independent predictor of ACM [HR=1.11, 95%, p=0.01]. NLR1

was associated with a significantly increased risk of recurrence in the univariable preoperative model [HR=1.9, p=0.05] while in the postoperative model, NLR4 remained independently associated with a significantly increased risk of recurrence [HR 1.13, p=0.03].

Conclusions: In patients with UCB treated with RC, NLR is associated with more advanced tumour stage, LVI, lymph node metastasis and higher CSM. Furthermore, the variation of NLR after surgery might play a role to predict higher ACM and RFS.

Introduction

The Neutrofil to Lynfocite ratio in the literature

The neutrophil-to-lymphocyte ratio (NLR) represents an easily measured, reproducible, and inexpensive marker of systemic inflammation.

It has been hypothesized that the synthesis of inflammatory cytokines triggered by the tumor microenvironment alters acute phase reactants and hematologic components, including serum neutrophil and lymphocyte counts [1, 2].

As part of the tumor microenvironment, neutrophils and lymphocytes both play prominent regulatory roles in tumor 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 oncologic outcomes in multiple malignancies, including breast, colorectal, lung, liver and gastric [3-6] however, the prognostic role of the neutrophil to lymphocyte ratio for urological cancers is still not well defined.

The Neutrofil to Lynfocite ratio in Urothelial bladder cancers (UCB)

Urothelial bladder cancers (UCB) can be subdivided into two major disease states with different implications for clinical management [7, 8]. Non-muscle invasive bladder cancers (NMIBCs) correspond to the bulk of cancer incidence. They generally do not pose a significant threat to the life of the patient but do invariably recur, necessitating expensive lifelong cystoscopy and local resection that generate significant patient discomfort and make NMIBC the most expensive of all cancers to clinically manage. Importantly, a fraction of high-grade NIMBCs do progress to become invasive, but no tools are available to prospectively identify these tumors, and surgeons must rely on their clinical judgement and experience to decide when to offer patients definitive therapy. On the other hand, muscle- invasive bladder cancers (MIBCs) are clinically aggressive, and up to 50%

of patients die of their disease. For the urothelial carcinoma (TCC), the evaluation of NLR might be particularly relevant, as inflammation appears to play a critical role in the genesis, progression, and mortality from UCB. Indeed, urothelial carcinoma is one of the few malignancies with a defined role for immunotherapy (eg, bacillus Calmette-Guerin (BCG).

The accurate prediction of the best treatment option (surgery rather than systemic therapies) is a pivotal issue for clinicians. The development of novel biomarkers which might enhance the selection of the most appropriate candidate to therapies would 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. NLR evaluation could be helpful in the selection of the best candidate to a specific therapy, however, the exact role of NLR is still controversial. Current literature differs for study design, sample size, patient's selections, timing of the blood measurements in relation to surgery or chemotherapy and NLR kinetics measurements. Thus, there is a need to explore whether the prognostic value of NLR is retained, or even increased, when measured not only preoperatively but also postoperatively.

Aim of the study

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

Material and Methods

This is a prospective single-centre single-surgeon cohort of 132 consecutive patients with UCB treated with open radical cystectomy (RC) and lymph node dissection between July 2013 and December 2016.

Exclusion criteria of the study were patients with an infection, inflammatory diseases, autoimmune diseases, a second primary cancer, splenectomies, a bladder cancer other than the urothelial cancer subtype, a hematologic or hepatic disorder with the potential to alter the neutrophil-lymphocyte ratio, and missing.

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

NLR was recorded as followed:

- before surgery (within 15 days prior to RC, [NLR1]),
- postoperatively (within 2 days [NLR2],
- between 7 and 15 days after RC before discharge [NLR3],
- few days before the evidence of recurrence or last available follow up [NLR4],

ΔNLR was calculated as the difference between NLR2 and NLR1 (NLRΔ1) and between NLR 2 and NLR3 (NLRΔ2).

NLR was analysed both as a continuous variable and as a categorical variable using a cut-off of 2.7 based on previous studies [9].

Postoperative follow-up was at least every 3–4 mo in year 1, every 6 mo in year 2, and annually thereafter. Follow-up visits consisted of a physical examination and serum chemistry evaluation.

Tumors were staged according to the 2002 TNM classification. Tumor grade was assigned according to the 1973 World Health Organization grading system. LVI was defined as the presence of nests of tumor cells within an endothelium-lined space [10]. A positive soft-tissue surgical margin was defined as the presence of tumor in stained areas of soft tissue in RC specimens [11].

Data for categorical variables were presented as number and percentage, and data for continuous variables as mean ± SD. Group differences for categorical and continuous variables were analyzed using 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 all-cause mortality (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 ≥2.7 with the log-rank test.

Univariable 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. Entry values into the multivariable models were 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).

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Results

Patient and tumor characteristics are listed in Table 1.

Median age was 74 years (IQR 68-81years). 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 (IQR 7.9-26.0 months). During this timespan, 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 cause. 64 (48.5%) have no evidence of disease at follow up.

A high NLR1 was associated with a bigger tumour size ($p<0.01$), a greater likelihood of receiving intravescical therapy ($p=0.04$), advanced T stage ($p <0.01$), lymphovascular invasion ($p<0.01$) positive surgical margin ($p = 0.02$), a greater likelihood of blood transfusion ($p=0.016$), recurrence of disease ($p=0.016$) and cancer specific death ($p=0.02$) (table 2)

A high NLR2 was associated with an BMI ($p=<0.01$) and tumor size ($p=0.04$) (table 3), while a high NLR3 seems to have no relation with clinic-pathological characteristics (table 4).

A high NLR4 was associated with age ($p=0.05$) advanced T stage ($p= 0.01$), lymph node involvement ($p=0.017$), positive surgical margin ($p=0.03$), a greater likelihood of receiving adjuvant chemotherapy ($p = 0.021$), recurrence of disease ($p<0.01$), and cancer specific death ($p<0.01$) (table 5).

On survival analysis, when patients were stratified according to NLR 1 with a cut of 2.7, overall survival and recurrence-free survival were significantly different ($p= 0.042$ and $p= 0.046$), (fig 1 and 3). When patients were stratified according to NLR 4 with a cut of 2.7, recurrence-free survival

was significantly different ($p < 0.01$) (fig 4). No difference in cancer-specific survival was found between groups (fig 3).

When assessed by multivariable analysis, NLR1 remained independently associated with a significantly increased risk of extravescical disease (pT 3-4) [OR: 1.41 95% CI 1.11-1.80 $p<0.01$] and lymphovascular invasion [OR: 1.40, 95% CI 1.09-1.83, $p<0.01$] (Table 6 -8).

When assessed the association of NLR with CSM, NLR4 was independently associated with a significantly increased risk [HR=1.14, 95% CI 1.03-1.24 p=0.013] (table 9).

At univariable analysis NLR1 was found to be a preoperative predictor for ACM [HR=1.79, 95% CI 1.015-3.14, p=0.044] (table 10).

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 10). 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].

Table 1. Overall Patients and tumor Characteristics

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

Table 2. 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**
Age, Median (IQR)	73 (65-79.25)	76 (68-82)	0.09
Sex, no. (%) Female Male	 9 (16.7) 45 (83.3)	 17 (23.0) 57 (77)	0.38
BMI, kg/m2 (IQR)	27 (24.1-30.0)	25.9 (23.0-29.5)	0.28
ECOG performance status, no. (%) 0 1 2 3	 22 (40.7) 26 (48.1) 5 (9.3) 1 (1.9)	 26 (35.1) 31 (41.9) 15 (20.3) 2 (2.7)	0.38
Max tumor size <=2 cm >3 cm	 35 (67.3) 17 (32.7)	 28 (38.9) 44 (61.1)	**<0.01**
Receipt of Intravescical therapy: No vescical therapy Vescical therapy	 39 (73.6) 14 (26.4)	 65 (87.8) 9 (12.2)	**0.04**
Clinical T stage, no. (%) <=2 T3-T4	 50 (94.3%) 3 (5.7%)	 63 (86.3%) 10 (13.7%)	**<0.01**
Pathologic T stage, no. (%) <=2 T3-T4	 41 (77.4) 12 (22.6)	 31 (41.9) 43 (58.1)	**<0.01**
pN stage pNx pN0 pN1 pN2	 6 (11.3) 40 (7.5) 2 (3.8) 5 (9.4)	 15 (21.2) 43 (60.6) 8 (11.3) 5 (7.0)	0.16
Perineural invasion no. (%)	9 (17.0)	16 (22.5)	0.44
Lymphovascular invasion, no (%)	19 (35.8)	43 (60.6)	**<0.01**
Lymph node involvement, no (%)	7 (14.9)	13 (23.2)	0.28
Positive surgical margin, no. (%)	1 (1.9%)	10 (13.5)	**0.02**
Blood transfusion	9 (17)	27 (36.5)	**0.016**
Receipt of adjuvant therapy*, no. (%)	3 (5.7)	5 (6.8)	0.80
Patients with recurrence of disease	13 (22.4)	32 (43.2)	**0.016**
Follow up status: Death for other cause Death for bladder cancer Non evidence of disease Alive with disease recurrence	 6 (10.3) 11 (19.0) 39 (67.2) 2 (3.4)	 16 (21.6) 27 (36.5) 25 (33.8) 6 (8.1)	**0.02**
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

Table 3. Patients and tumor characteristics according to NLR2 <2.7 and NLR2 ≥2.7

	NLR2 <2.7 (immediately after surgery) (4 patients)	**NLR2 ≥2.7 (immediately after surgery) (123 patients)**	**p**
Age, Median (IQR)	82.5 (72-91)	75 (68-81)	0.80
Sex, no. (%) Female Male	 0 4 (100)	 26 (21.1) 97 (78.9)	0.3
BMI, kg/m2 (IQR)	24 (23.5-24.5)	26.6 (23.6-29.7)	**<0.01**
ECOG performance status, no. (%) 0 1 2 3	 1 (25) 3 (75) 0 0	 47 (38.2) 53 (43.1) 20 (16.3) 3 (2.4)	0.6
Max tumor size <=2 cm >3 cm	 4 (100) 0	 57 (47.9) 62 (52.1)	**0.04**
Receipt of Intravescical therapy: No vescical therapy Vescical therapy	 3 (75) 1 (25)	 100 (82) 22 (18)	0.75
Clinical T stage, no. (%) <=2 T3-T4	 4 (100) 0	 108 (89.3) 13 (10.7)	0.48
Pathologic T stage, no. (%) <=2 T3-T4	2 (50) 2 (50)	69 (56.6) 53 (43.4)	0.79
pN stage pNx pN0 pN1 pN2	 1 (25) 2 (50) 0 1 (25)	 19 (16) 82 (68.9) 10 (8.4) 8 (6.7)	0.47
Perineural invasion no. (%)	1 (25)	24 (20.2)	0.81
Lymphovascular invasion, no (%)	2 (50)	60 (50.4)	0.99
Lymph node involvement, no (%)	1 (33.3)	18 (18)	0.5
Positive surgical margin, no. (%)	0	12 (9.8)	0.5
Blood transfusion	1 (25)	34 (27.9)	0.9
Receipt of adjuvant therapy*, no. (%)	0	7 (5.7)	0.6
Patients with recurrence of disease	1 (25)	42 (34.1)	0.70
Follow up status: Death for other cause Death for bladder cancer Non evidence of disease Alive with disease recurrence	 2 (50) 1 (25) 1 (25) 0	 20 (16.3) 36 (29.3) 60 (48.8) 7 (5.7)	0.35
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

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

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

Table 6: univariable and multivariable logistic regression predicting extravescical disease (pT 3-4)

	Univariable			Multivariable		
	OR	**95% CI**	**p**	**OR**	**95% CI**	**p**
Age at surgery	1,00	1.0-1.0	0.73	-	-	-
Sex (female vs male)	0.79	0.3-1.8	0.59	-	-	-
Intravescical 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**			

Table 7: univariable and multivariable logistic regression predicting Lymph node involvement

	Univariable			Multivariable		
	OR	**95% CI**	**p**	**OR**	**95% CI**	**p**
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
Intravescical Therapy (Yes-No)	1.01	0.41-3.32	0.98	-	-	-
NLR 1 (continuous)	0.90	0.70-1.15	0.42	-	-	-
NLR 1 ≥2.7 vs NLR1 <2.7	1.72	0.62-4.77	0.29	0.44	0.15-1.25	0.12

Table 8: Univariable and Multivariable Cox regression predicting Lymphovascular invasion

	Univariable			**Multivariable**		
	HR	**95% CI**	**p**	**OR**	**95% CI**	**p**
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	-	-	-
Intravescical 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**	-	-	-

Table 9: Univariable and Multivariable Cox regression predicting cancer specific mortality

	Univariable			**Multivariable**		
	HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
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	-	-	-
Intravescical Therapy (Yes-No)	1.2	0.55-2.65	0.62	-	-	-
NLR1(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	-	-	-
NLR Δ 1 (continuous)	0.96	0.92-1.02	0.98	-	-	-
NLR Δ 2 (continuous)	1.03	0.98-1.08	0.21	-	-	-
NLR 4 (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

Table 10. Univariable and multivariable Cox regression predicting all cause mortality (ACM)

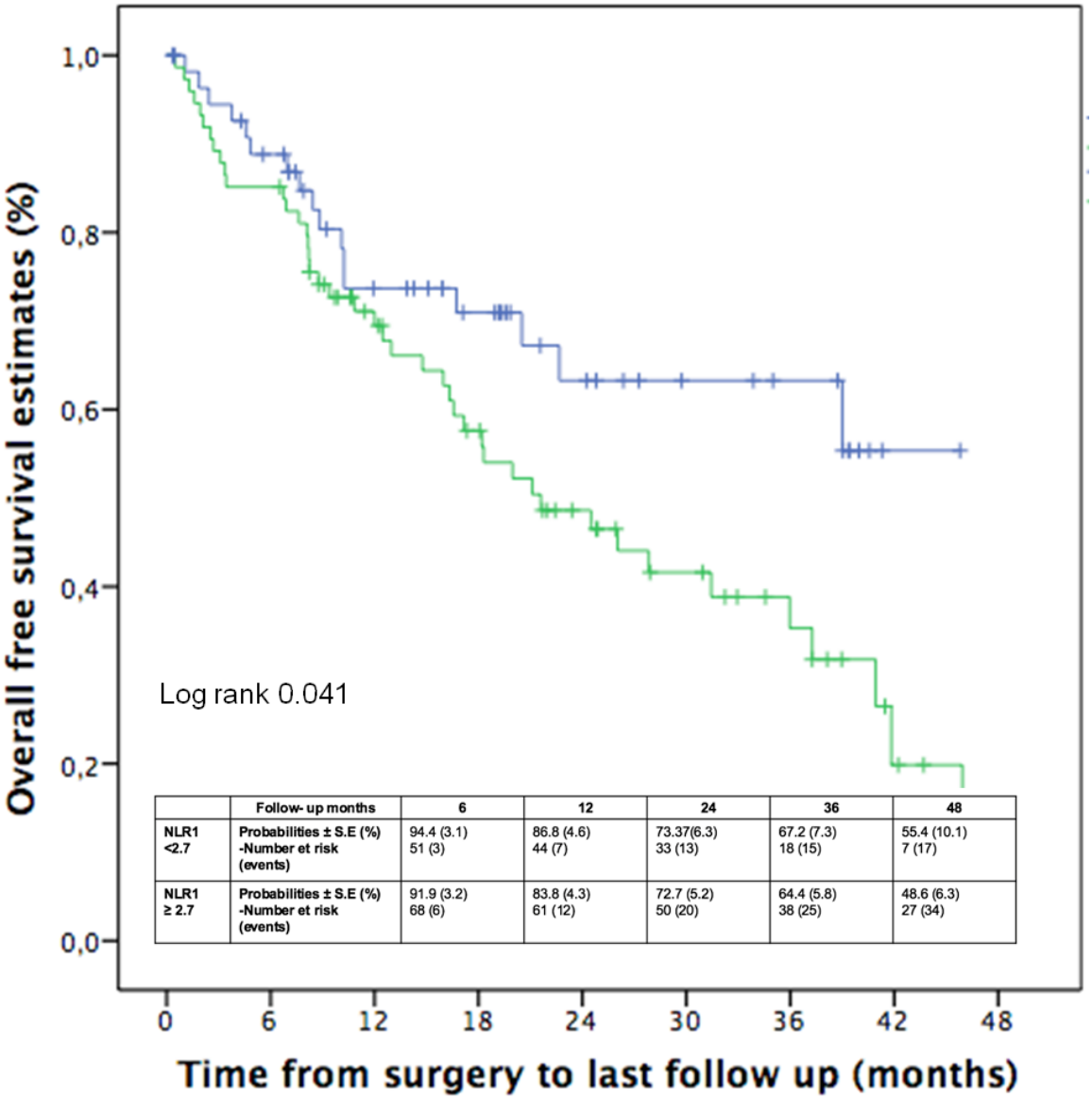
	Univariable			**Multivariable**		
	HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
Preoperative						
Age at surgery	1.05	1.02-1.08	**<0.01**	1.04	1.00-1.08	**0.013**
Sex (reference: female)	0.92	0.49-1.76	0.81	-	-	-
Intravescical Therapy (Yes-No)	0.94	0.47-1.87	0.87	-	-	-
ECOG			**<0.01**			**<0.01**
1	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	-	-	-
NLR 2 ≥2.7 vs NLR2 <2.7)	0.56	0.17-1.8	0.33	-	-	-
NLR 3 (continuous)	1.09	1.09-1.17	**<0.01**	1.11	1.02-1.21	**0.01**
NLR 3 (≥2.7 vs NLR<2.7)	1.01	0.53-1.91	0.96	-	-	-
NLR Δ1 (continuous)	0.97	0.93-1.01	0.20	**Not significant ***		
NLR Δ2 (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 pTstage, Lymph node invasion and separately NLR Δ 1, NLR Δ 2, NLR3 or NLR 4.

Table 11: Univariable and Multivariable Cox regression predicting recurrence

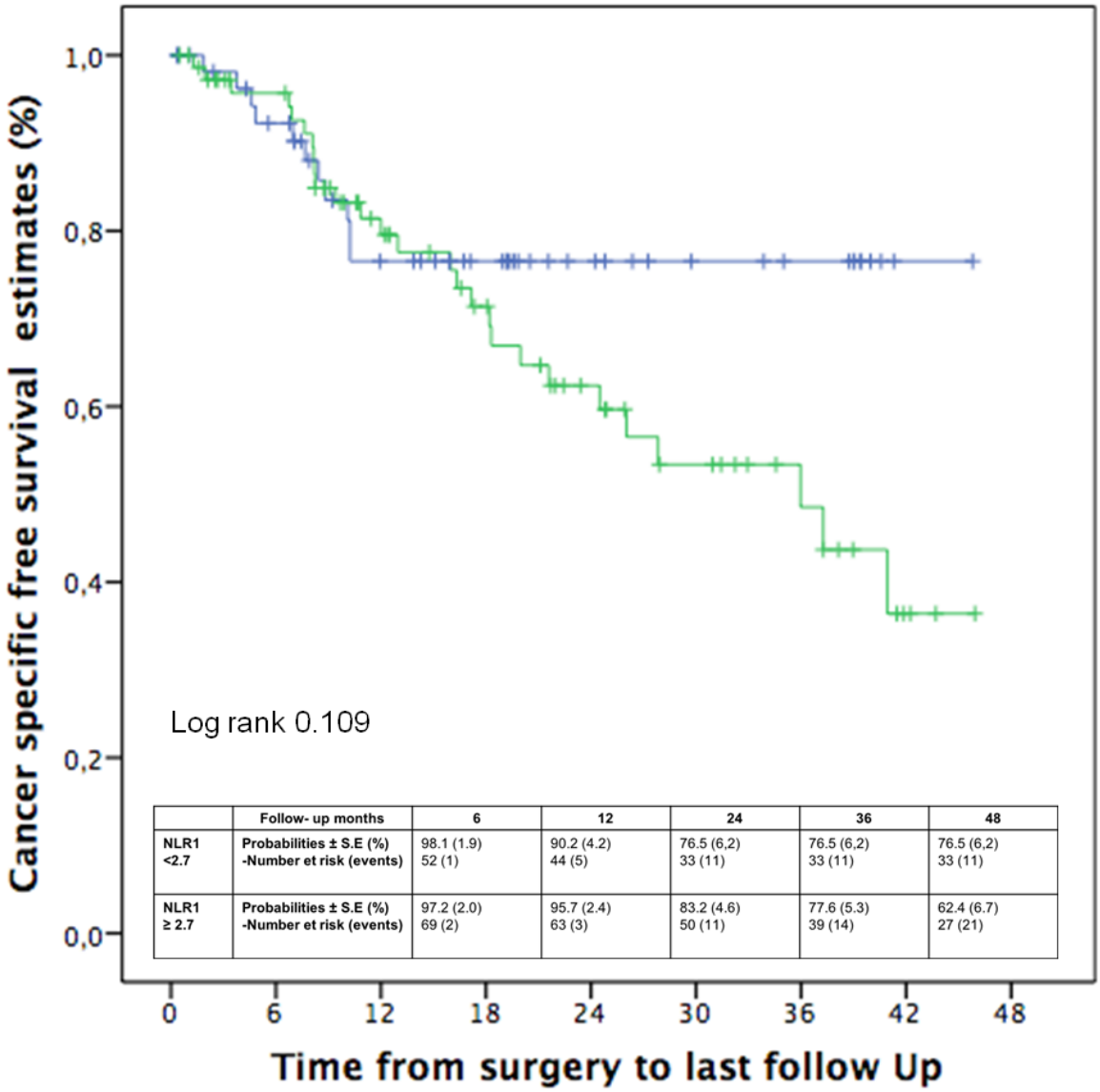
	Univariable			**Multivariable**		
	HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
Preoperative						
Age at surgery	1.02	0.98-1.05	0.25	-	-	-
Sex (reference: female)	1.20	0.56-2.60	0.67	-	-	-
Intravescical Therapy (Yes-No)	1.00	0.47-2.19	0.97	-	-	-
ECOG			**<0.01**			**<0.01**
1	1.23	0.63-2.42	**0.54**	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≥2vs 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	-	-	-
NLR 3 (continuous)	0.99	0.91-1.09	0.95	-	-	-
NLR 3 ≥2.7 vs NLR3 <2.7)	0.89	0.44-1.80	0.75	-	-	-
NLR Δ 1 (continuous)	0.99	0.95-1.04	0.87	-	-	-
NLR Δ 2 (continuous)	1.00	0.96-1.04	0.97	-	-	-
NLR 4 (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**	-	-	-

Fig1. Overall Free survival for NLR 1<2.7 (blue) and NLR1 ≥ 2.7 (green).



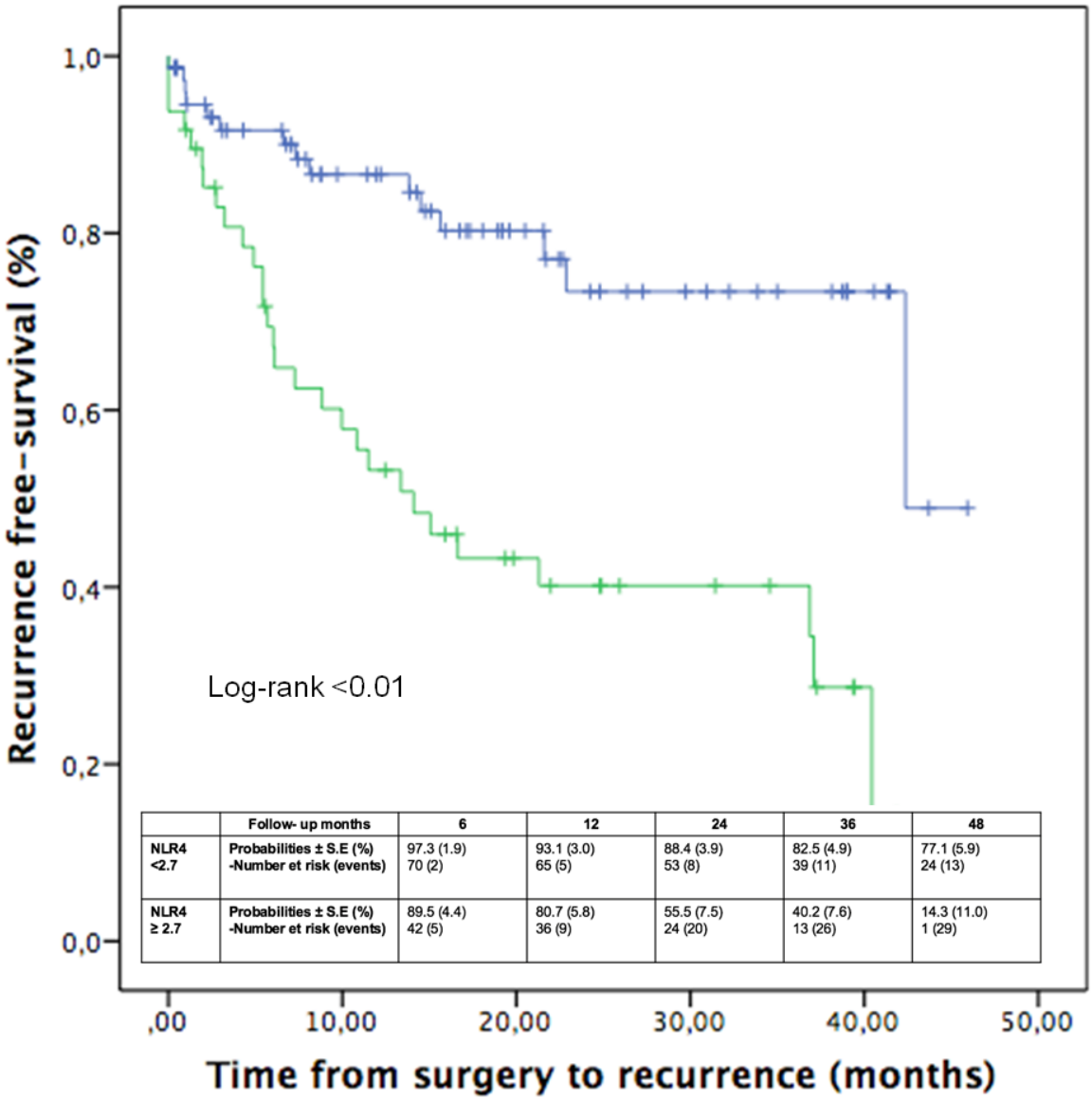
	Follow- up months	6	12	24	36	48
NLR1 <2.7	Probabilities ± S.E (%) -Number et risk (events)	94.4 (3.1) 51 (3)	86.8 (4.6) 44 (7)	73.37(6.3) 33 (13)	67.2 (7.3) 18 (15)	55.4 (10.1) 7 (17)
NLR1 ≥ 2.7	Probabilities ± S.E (%) -Number et risk (events)	91.9 (3.2) 68 (6)	83.8 (4.3) 61 (12)	72.7 (5.2) 50 (20)	64.4 (5.8) 38 (25)	48.6 (6.3) 27 (34)

Fig 2. Cancer specific free survival for NLR 1<2.7 (blue) and NLR1 ≥ 2.7 (green)



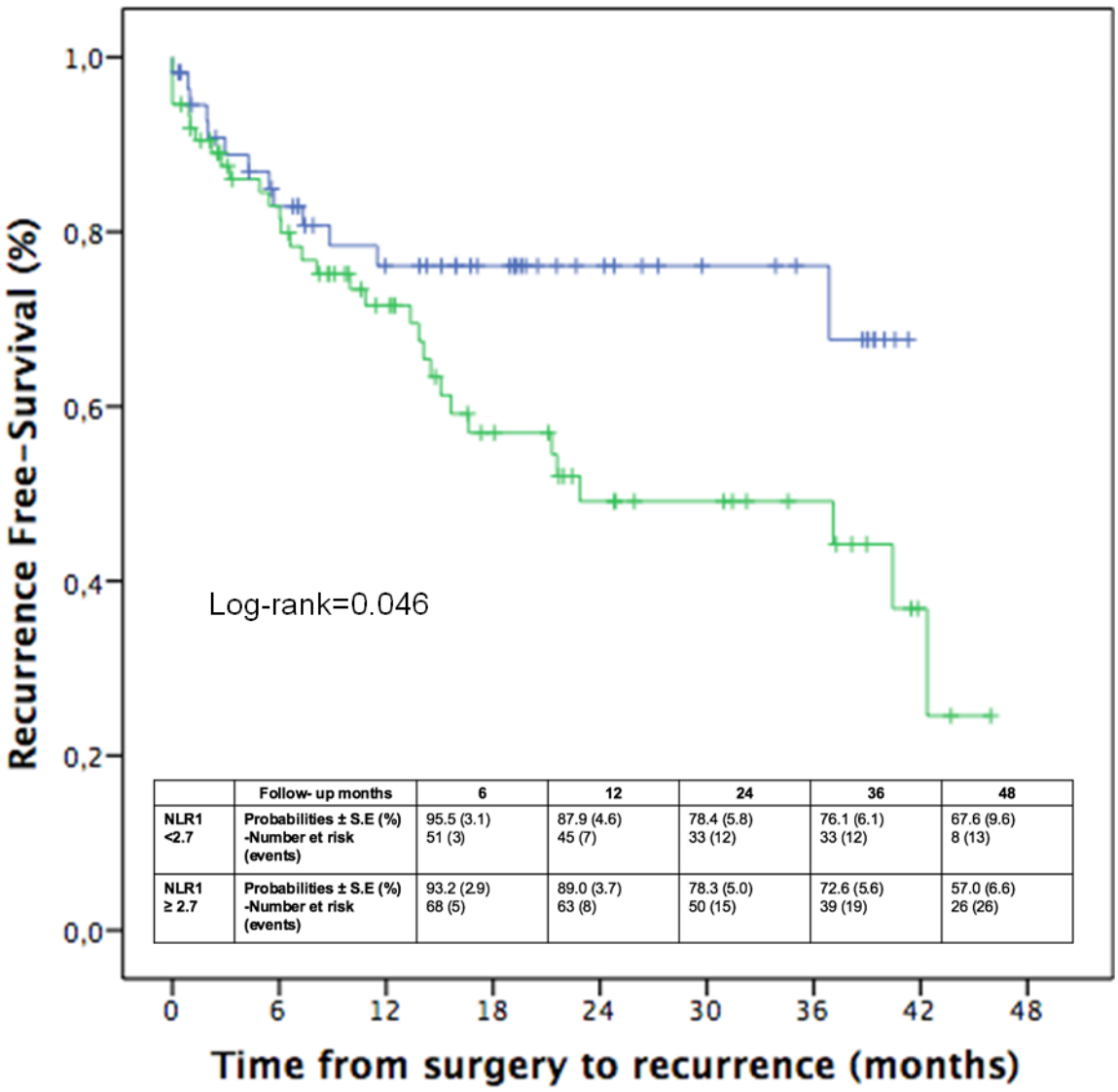
	Follow- up months	6	12	24	36	48
NLR1 <2.7	Probabilities ± S.E (%) -Number et risk (events)	98.1 (1.9) 52 (1)	90.2 (4.2) 44 (5)	76.5 (6,2) 33 (11)	76.5 (6,2) 33 (11)	76.5 (6,2) 33 (11)
NLR1 ≥ 2.7	Probabilities ± S.E (%) -Number et risk (events)	97.2 (2.0) 69 (2)	95.7 (2.4) 63 (3)	83.2 (4.6) 50 (11)	77.6 (5.3) 39 (14)	62.4 (6.7) 27 (21)

Fig 3. Recurrence free survival for NLR4 <2.7 (blue) and NLR4 ≥ 2.7 (green)



	Follow- up months	6	12	24	36	48
NLR4 <2.7	Probabilities ± S.E (%) -Number et risk (events)	97.3 (1.9) 70 (2)	93.1 (3.0) 65 (5)	88.4 (3.9) 53 (8)	82.5 (4.9) 39 (11)	77.1 (5.9) 24 (13)
NLR4 ≥ 2.7	Probabilities ± S.E (%) -Number et risk (events)	89.5 (4.4) 42 (5)	80.7 (5.8) 36 (9)	55.5 (7.5) 24 (20)	40.2 (7.6) 13 (26)	14.3 (11.0) 1 (29)

Fig 4. Recurrence free survival for NLR 1<2.7 (blue) and NLR1 ≥ 2.7 (green)



	Follow- up months	6	12	24	36	48
NLR1 <2.7	Probabilities ± S.E (%) -Number et risk (events)	95.5 (3.1) 51 (3)	87.9 (4.6) 45 (7)	78.4 (5.8) 33 (12)	76.1 (6.1) 33 (12)	67.6 (9.6) 8 (13)
NLR1 ≥ 2.7	Probabilities ± S.E (%) -Number et risk (events)	93.2 (2.9) 68 (5)	89.0 (3.7) 63 (8)	78.3 (5.0) 50 (15)	72.6 (5.6) 39 (19)	57.0 (6.6) 26 (26)

Discussion

In this cohort of patients with UCB undergoing RC with middle term postoperative follow-up, we found that preoperative and postoperative NLR were associated with advanced pathologic stage at the time of cystectomy, lymphovascular invasion as well as increased risk for disease recurrence, CSM, and ACM. These findings remained significant after controlling for clinicopathologic features, suggesting an independent association of preoperative and postoperative NLR with these adverse outcomes. Interestingly, our results are in line with privies studies (table 12).

In a study that included 899 patients from a single institution, Viers et al [9] showed that a high NLR is associated with a higher risk of extravesical tumor extension ($p = 0.03$) and lymph node metastasis ($p = 0.02$). 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 [12]found that an increase in NLR in conjunction with hypoalbuminemia 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 [13] stratified their cohort into risk categories according to tumor size (<3 vs 3 cm), presence of hydronephrosis, hemoglobin level (11.5 g/dl vs >11.5 g/dl), and NLR (<2.5 vs 2.5). The 5-yr 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, NLR may also be useful in identifying 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 NLR 3 had similar survival rates compared to those treated for muscle-invasive UCB [14].

Lucca et al in a multicenter study with 4061 patients found that NLR ≥2.7 was associated with advanced pathological tumor stages ($p< 0.001$), lymph node involvement ($p< 0.001$),

lymphovascular invasion (p= 0.008), and positive soft tissue surgical margins (p= 0.001). Furthermore, in their study found an indipendent 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) [15].

Other studies evaluated the predictive ability of NLR for OS, CSS and PFS also in smaller cohort of patients [16-27]. Only few studies were unable to demonstrate the predictive ability of NLR for OS and CSS [21, 28]. Interestingly, a few paper valuated the NLR kinetics in the prediction of oncological outcomes, as also shown by the present study [9, 24].

Unfortunately, available literature empirically used a differed NLR cutoff, ranging between 2.5 to 3.89 making results not always comparable.

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

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

A high NLR reflects both a heightened neutrophil-dependent inflammatory reaction and a decreased, lymphocyte-mediated, antitumor immune response. Both of these factors may contribute to aggressive tumor biology, cancer progression, and poor prognosis [5, 29]. For example, circulating neutrophils have been shown to produce cytokines, such as tumor necrosis factor, interleukin (IL)-1, and IL-6, and to secrete the pro-angiogenic vascular endothelial growth factor [30]. Furthermore, a relative lymphocytopenia 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 two processes and be a stronger predictor.

We recognize that our study has several limitations. There are a relatively limited number of patients from a single institution with a intermediate follow-up duration. Unfortunately, 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 hematologic parameters and cytokines are an end result of tumour growth and an underlying cause of mortality.

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

Table 12: 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		Median follow-up (mo)	End point	HR/OR	p Value
									before surgery	after surgery				
Hermanns et al [14]	424	Canada	1992 - 2012	No	Mix (organ confined-metastatic)	RC	3	Not Evaluated	Median of 6 days before	NA	58.4	OS CSS	1.67 1.88	0.005 <0.001
Krane et al [12]	68	USA	2005 - 2011	No	OC	RC	2.5	Not Evaluated	Not clear the timing	NA	25	OS CSS	2.49 2.68	NA NA
Gondo et al [13]	189	Japan	2000 - 2009	No	OC	RC	2.5	Not Evaluated	Not clear the timing	NA	25.1	CSS	1.95	0.039
Viers et al [9]	899	USA	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 [15]	4061	Europe		yes	OC	RC	2.7	Not Evaluated			42	OS CSS	1.11 1.21	0.029 0.003
Potretzke et al [16]	102	USA	2002 - 2012	No	OC	RC	N/A	Not Evaluated	Within 100 days	NA	NA	OC vs NOC	1.50	0.02
Demirta G et al [17]	201	Turkey	1999 - 2013	No	OC	RC	2.5	Not Evaluated	Not clear the timing	NA	37.22	NA	NA	NA
Bhindi et al [18]	418	Canada	1992 - 2012	No	OC	RC	NA	Not Evaluated	A week before treatment initiation	NA	40	OS CSS PFS	1.56 1.47 1.52	0.004 0.001 0.002
Can et al [19]	182	Turkey	2001-2011	No	OC	RC	2.57	Not Evaluated	Not clear the timing	NA	45	pathological stage	2.78	p=0.004
Ozcan et al [20]	286	Turkey	1990 - 2013	No	OC	RC	>=2,5	Not Evaluated	Not clear the timing	NA	8	PFS	1.965	0.022

Study	Patients	Study region	Research time	Multi centre	Stage	Surgery	NLR cut-off	NLR Kinetics	Timing of L/N evaluation		Median follow-up (mo)	End point	HR/OR	p Value
									before surgery	after surgery				
Zhang et al [21]	124	China	2009 - 2009	Yes	Mix (organ confined-metastatic)	RC	>=4	Not Evaluated	Not clear the timing	NA	NA	OS	NS	NS
Ozyalvacli et al [22]	166	Turkey	2008 - 2013	Yes	OC	surgery	≥2.43	Not Evaluated	Not clear the timing	NA	24.2	PFS	2.43	<0.001
Kaynar et al [23]	291	Turkey	2009 - 2013	Yes	OC	RT	>2,5	Not Evaluated	The day before surgery	NA	NA	NA	NA	NA
Kang et al [24]	385	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 (pre op.) CSS (post op.)	0.021 1.2 1.18	<0.001 0.019 0.021
Bambury et al [25]	129	USA	2008 - 2013	No	Locally advanced/ metastatic	Perioperative or metastatic setting	2,5 and 3	Not Evaluated	Unknown	NA	NA	OS	1.03	<0.01
Mano et al [26]	107	Israel	2003 - 2010	No	OC	TURV	disease progression 2.41 and recurrence 2.43	Not Evaluated	Not clear the timing	NA	40	PFS Recurrence	3.52 1.75	0.012 0.034
Lee et al [27]	226	UK	2011 - 2013	No	OC	RC	>3.89	Not Evaluated	60 days of TURBT	NA	NA	MIBC	8.244	<0.01
Yoshida et al [28]	302	Japan	1995 - 2013	Yes	OC	RC*	NA	Evaluated	1 Month before Surgery	1 - 3 Months after RC	6.8 yr	OS CSS	NS NS	NS NS

OC: organ confined, NOC: nonorgan-confined NS: not Significant, RC: radical cistectomy; OS: overall survival, CSS: cancer specific survival, PFS: progression free survival, TURBT: trasuretral resection of the bladder.

* Neoadjuvant chemotherapy in 20 (6.6%)

Conclusions

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

Advantages of 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, patients counselling, predictions models and clinical decision-making for more extensive surgery (like more extensive lymph node dissection) and/or perioperative chemo- or radio-therapy.

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