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Easily Available Blood Test Neutrophil-To-Lymphocyte Ratio Predicts Progression in High-Risk Non-Muscle Invasive Bladder Cancer

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Abstract

Introduction: The inflammatory response surrounding the tumour has a major importance in the oncologic outcome of bladder cancers. One marker proved to be useful and accessible is NLR (neutrophil-to-lymphocyte ratio). The objective of the study was the analysis of NLR as a prognostic factor for recurrence and progression in pT1a and pT1b bladder cancers.

Material and Methods: Retrospective study, with 44 T1a/T1b bladder cancer patients. Each patient underwent transurethral resection. NLR was considered altered if higher than 3, average follow-up period was of 18 months.

Results: The mean age of the patients included was 73 years (IQR 64 - 77). Most of the patients had NLR<3 (30 patients). In total 29/44 (65.9 %) patients presented recurrence and 15/44 (34.1 %) patients were identified with T2 or higher stage progression during the follow-up period (average 18 months).We found no statistically significant association between NLR>3 and other clinic and pathologic factors. Progression-free survival (PFS) Kaplan-Meier analysis showed a lower PFS in the NLR>3 group, with a p=0.001 value. A total of 64.3% of patients had shown progression in the NLR>3 group and 20% in the NLR<3 group. Mean NLR was 2.67 (IQR 1.88-3.5); 2.50 (IQR 1.89-2.87) in patients that did not present any progression during the follow-up and 3.20 (IQR 1.73-5.80) in those with progression (p=0.09), ROC 0.655. Mean NLR was 2.14 (IQR 1.61-2.77) in patients that did not experience a recurrence during the follow-up and 2.76 (IQR 2.1-4.31) in those with recurrence, ROC 0.671 (p=0.06). Multivariable Cox regression analyses showed that stage T1b and NLR represent independent prognostic factors for PFS.

Conclusion: High Neutrophil-to-Lymphocyte ratio retained a statistically significant value, as an independent prognostic factor for bad prognosis of T1 bladder tumors. NLR represents a biomarker that could support a clinical decision making in case of high-risk on-muscle invasive bladder cancer.

Keywords: *neutrophil-to-lymphocytes ratio, prognostic factors, bladder cancer, microinvasive.*

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Introduction

Bladder carcinoma is the most common malignancy of the urinary tract. In Europe, the highest incidence (ASR = age-standardized rate) is reported in Western (23.6 in males and 5.4 in females) and Southern Europe (27.1 in males and 4.1 in females), followed by Northern Europe (16.9 in males and 4.9 in females). The lowest incidence can be observed in East European countries (14.7 in males and 2.2 in females, respectively). (1)

The tumour, node, metastasis (TNM) classification is widely accepted and used in the daily practice (2). A new histological classification was proposed for the T1 bladder cancers, dividing them into two subgroups, based on the level of invasion in the lamina propria: Ta microinvasive and T1b invasive.

It is well known that inflammation plays an important role in the progression of malignancy. More and more biomarkers are found to be predictive in cancers, but most of them are not yet used in daily clinical practice because of the related high costs, so it is important to investigate and then use easily available biomarkers that one can find in routine blood tests. Such biomarkers were described in the literature, one being the neutrophil-to-lymphocytes ratio (NLR), and the other being the platelet-to-lymphocytes ratio (PLT/LYM) which was found to be predictive for coronary chronic total occlusion in patients admitted with ST-segment elevation myocardial infarction (3). A high NLR can indicate a poor outcome of the disease in urothelial carcinomas (4,5).

So far, several studies have reported NLR as a predictive factor for the presence of lymph node metastases or non-organ confined disease in the vulva squamous cell carcinoma (6) and also in the bladder cancer (7). Neutrophil-to-lymphocytes ratio has been described as a prognostic factor in many other malignancies such as pancreatic, breast and colon (8). In urological carcinomas the importance of NLR has been described in bladder cancer (9), renal cell carcinoma (10) and upper tract urothelial carcinoma (11,12).

Although NLR has been well investigated as a prognostic factor in urothelial carcinomas, as far as we are aware of, it has not been investigated in a sub-group of patients with high-risk disease such as T1 bladder cancer patients.

The aim of the study was to analyze the predictive value of NLR and other prognostic factors involved in the recurrence and progression of pT1a and pT1b bladder tumors.

Material and Methods

We retrospectively included 44 patients that underwent trans-urethral bladder (TURB) resection between January 2011 and December 2013, at the Urology Clinic of Mures County Hospital, out of 100 patients with T1 bladder cancer. The inclusion criteria were stage T1 at the moment of the resection and a complete follow-up time according to guidelines.

Pre-treatment NLR was collected in every case prior to TURB and was defined as altered if ratio >3. NLR was determined as a ratio between continuous neutrophils and continuous lymphocytes, at the same laboratory for all patients with standard protocol determination. The value was selected based on publications in the literature (13) and ROC curve analysis. NLR, demographic, clinical and pathological data were collected and introduced in an Excel database.

The TNM classification of 2002 American Joint Committee on Cancer – Union for International Cancer Control (AJCC-UICC), the Tumor-Node-Metastasis (TNM) classification and the 1998 WHO/International Society of Urologic Pathology (ISUP) consensus were used for the classification of grading and staging.

All patients underwent TURB with curative

Table 1. Association of neutrophil-to-lymphocytes ratio (NLR) and clinicopathologic characteristics in 44 patients treated with TURB for T1a/T1b bladder cancer

	All patients	Normal NLR	Altered NLR	P value
Total, no. (%)	44	30 (68.2)	14 (31.8)	
Age median (IQR)	73 (64-77)			
Gender, no. (%)				0.66
Male	37 (84.1)	4 (13.4)	3 (21.5)	
Female	7 (15.9)	26 (86.6)	11 (78.5)	
Tumor stage, no. (%)				0.97
pT1a	19 (43.2)	13 (43.4)	6 (42.9)	
pT1b	25 (56.8)	17 (56.6)	8 (57.1)	
Grade, no. (%)				0.66
Low	7 (15.9)	4 (13.4)	3 (21.5)	
High	37 (84.1)	26 (86.6)	11 (78.5)	
Lymphovascular invasion, no. (%)				0.72
Yes	12 (27.3)	9 (30.0)	3 (21.5)	
No	32 (72.7)	21 (70.0)	11 (78.5)	
Concomitant carcinoma in situ, no. (%)				0.87
Yes	8 (18.2)	7 (23.4)	1 (7.2)	
No	36 (81.8)	23 (76.6)	13 (92.8)	
No. Tumors, no. (%)				0.87
1	23 (52.3)	16 (53.3)	7 (50.0)	
2	8 (18.2)	6 (20.0)	2 (14.3)	
3	5 (11.3)	3 (10.0)	2 (14.3)	
4	7 (15.9)	4 (13.3)	3 (21.4)	
5	1 (2.3)	1 (3.4)	0 (0)	
Necrosis, no. (%)				0.96
Yes	3 (6.8)	2 (6.6)	1 (7.2)	
No	41 (93.2)	28 (93.4)	13 (92.8)	
Diameter, no. (%)				0.10
<3 cm	22 (50.0)	18 (60.0)	4 (28.6)	
>3 cm	22 (50.0)	12 (40.0)	10 (71.4)	

intent. The patient follow-up was performed according to EAU Guidelines and institutional protocols (14), and all patients benefited from a second TURB after 6 weeks. The study had the approval of the local Ethical Committee.

Data analysis was performed using STATA 11 statistical software (Stata Corp., College Station, TX, USA). The associations of NLR with category variables were assessed using Fisher's exact test. Kaplan–Meier method was used to estimate recurrence-free survival (RFS) and progression-free survival (PFS); log-rank tests were applied for pair-wise comparison of survival. The receiver-operating characteristic (ROC)

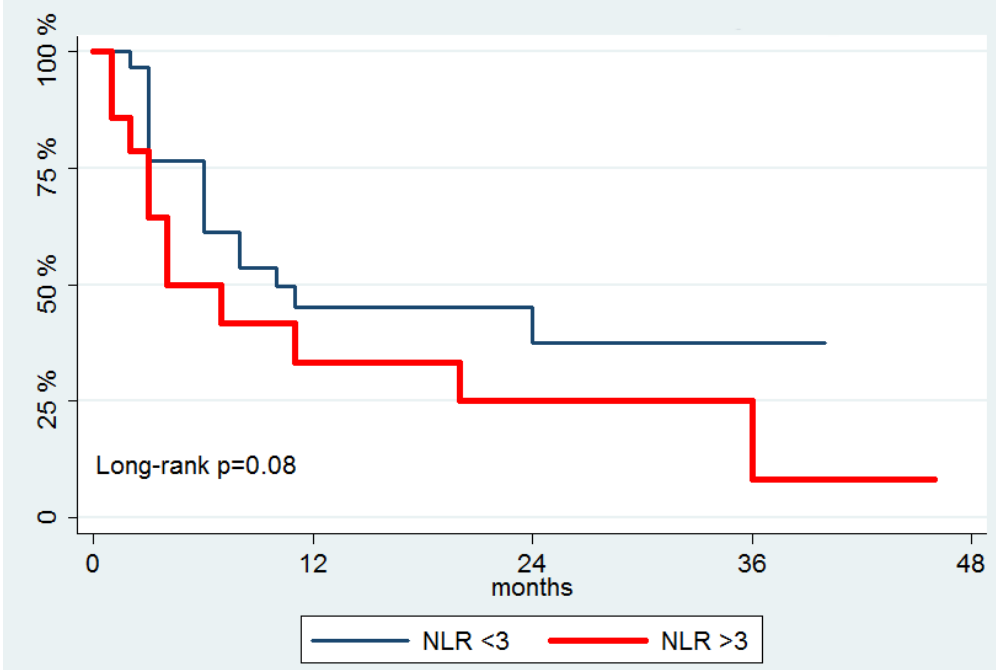

Figure 1. Recurrence free survival according to NLR

curve analysis was used to test the predictive power and to determine cut-off values of NLR. We performed multivariate Cox proportional hazards regression analyses and used backward stepwise elimination to distinguish insignificant co-factors from significant predictors for RFS and PFS. The Cox model was adjusted for age, stage, gender, in situ carcinoma, lymphovascular invasion, necrosis, tumor grade, diameter, number of tumors and NLR. Harrell's concordance index (*c*-index) was used to measure the ordinal predictive power of the model for RFS and PFS. All tests were two-tailed tests and a p-value < 0.05 was considered as statistically significant.

Results

The median age of the patients included was 73 years (IQR 64 - 77). Most of the patients had NLR<3 (30 patients). In total 29/44 (65.9 %) patients presented recurrence and 15/44 (34.1 %) patients were identified with T2 or higher stage progression during the follow-up period (mean 18 months). There was no statistically significant association between NLR>3 and other clinic and pathologic factors. Patient characteristics and the association with NLR are shown in **Table 1**.

We recorded neutrophils and lymphocytes also as a continuous variable, mean neutrophils being 4.05 (IQR 1.83-5.96) and mean lymphocytes being 1.63 (IQR 1.22-2.15). Mean NLR was 2.67 (IQR 1.88-3.5); 2.50 (IQR 1.89-2.87) in patients who did not show any progression during follow-up and 3.20 (IQR 1.73-5.80) in those with progression (p=0.09), ROC 0.655. Mean NLR was 2.14 (IQR 1.61-2.77) in patients who did not experience recurrence during follow-up and 2.76 (IQR 2.1-4.31) in those with recurrence, ROC 0.671 (p=0.06).

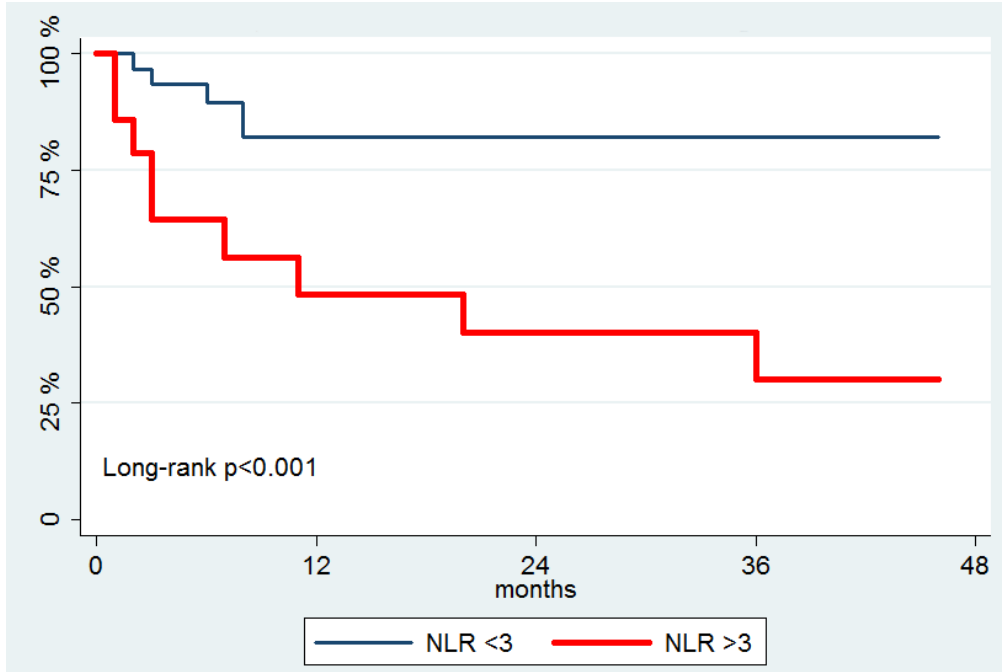

Figure 2. Progression free survival according to NLR

Recurrence-free survival Kaplan-Meier analysis showed a slight decrease in the case of patients with NLR>3 of RFS, but did not retain a statistically significant value even if 12 out of 14 patients with NLR>3 had a recurrence during follow-up (see **Fig. 1**).

Progression-free survival Kaplan-Meier analysis showed a higher PFS in the NLR>3 group, with a value p=0.001. A total of 64.3 % of patients had progression in the NLR>3 group and 20 % in the NLR<3 group (see **Fig. 2**).

For the evaluation of predictive factors for recurrence and progression, a multivariable Cox proportional hazards regression analysis has been performed and adjusted for relevant prognostic factors, such as: age, gender, T1a/T1b staging , in situ carcinoma, lymphovascular invasion, necrosis, tumor grading, tumor diameter, number of tumors and NLR.

The multivariable Cox regression model for predicting recurrence showed, just as an independent predictive factor diameter>3 with a HR of 4.03 (see **Table 2**). The Harrell's C index, predictive power of the model for recurrence, was 72.9 with no addition of NLR to the prognostic model.

Univariable Cox regression analysis showed that necrosis (p=0.05), and NLR (p<0.001) are prognostic factors for progression. Multivariable Cox regression model for predicting progression showed that T1b stage and NLR are independent prognostic factors for the aggressive behaviour of these tumors. The Harrell's C index in the case of progression was 79.4 without NLR and the predictive power increase with 2 points by adding NLR to the model (p=0.02).

Table 2. Univariable and multivariable Cox regression analyses predicting recurrence and progression of 44 patients treated with TURB for T1a/T1b bladder cancer.

		Recurrence-free survival						Progression-free survival					
		Univariable			Multivariable			Univariable			Multivariable		
		HR	95 % CI	p value	HR	95 % CI	p value	HR	95 % CI	p value	HR	95 % CI	p value
Age		0.99	0.95-1.04	0.95	1.00	0.94-1.06	0.85	0.99	0.93-1.06	0.87	0.98	0.92-1.06	0.75
Gender		1.57	0.54-4.56	0.39	2.90	0.64-13.14	0.16	0.71	0.19-2.56	0.60	1.42	0.21-9.42	0.71
No. Tumors	1							Ref.					
	2	2.3	0.90-5.90	0.08	2.81	0.91-8.67	0.07	0.82	0.16-4.13	0.81	1.23	0.18-8.33	0.82
	3	0.87	0.25-3.07	0.83	0.37	0.08-1.73	0.20	2.30	0.57-9.26	0.23	1.91	0.32-11.32	0.47
	4	1.60	0.51-5.05	0.41	0.61	0.15-2.45	0.49	2.40	0.60-10.20	0.20	1.02	0.18-5.79	0.97
	5	1.14	0.14-8.88	0.89	0.22	0.01-2.93	0.25	2.38	-	1	3.61	-	-
Grade		1.49	0.51-4.34	0.46	2.35	0.60-9.16	0.21	3.14	0.40-24.17	0.27	4.19	0.35-49.02	0.25
Lymphovascular invasion		0.85	0.36-2.02	0.72	1.36	0.42-4.34	0.59	0.66	0.18-2.37	0.52	1.47	0.23-9.10	0.67
Carcinoma in situ		0.63	0.21-1.84	0.40	0.51	0.13-1.96	0.32	0.30	0.03-2.31	0.24	0.12	0.01-1.41	0.09
Necrosis		2.13	0.49-9.16	0.31	1.33	0.23-7.58	0.74	4.64	0.99-21.73	**0.05**	5.21	0.71-37.88	0.10
Diameter		1.39	0.65-2.95	0.38	4.03	1.23-13.17	**0.02**	2.86	0.89-9.15	0.07	5.04	0.96-33.40	0.09
Stage		1.30	0.61-2.77	0.48	2.7	0.95-7.70	0.06	1.67	0.55-5.01	0.35	4.71	1.08-20.42	**0.03**
NLR		1.87	0.88-3.97	0.10	1.67	0.66-4.20	0.27	4.75	1.58-14.24	**<0.001**	4.57	1.21-17.13	**0.02**
Harrell C index					72.9						79.4		
Harrell C Index with NLR					72.9						81.4		

CI: confidence interval, HR: hazard ratio, NLR: neutrophil-to-lymphocytes ratio

Discussion

In the present study, we assessed the importance of an easily available biomarker in predicting the aggressive behaviour of bladder tumors. Even if the inclusion criteria referred only to patients with highly aggressive non-muscle invasive bladder cancer, we believe that in this specific sub-group of bladder cancer patients it is very important to correlate all the data available for good management. Moreover, routine blood tests are always available and at lower costs. From our result, we can see that patients with NLR higher than 3 have a higher risk experiencing progression in the next 2 years (4.5 higher). Including NLR together with age, gender, number of tumors, grade, lymphovascular invasion, association with concomitant CIS, necrosis, diameter and stage in a predictive model, we reached an accuracy of 81.4 points. Basically, our results confirm that these are the most important prognostic factors in bladder cancer management. Adding NLR to the model increased the accuracy with 2 points for predicting PFS. If we consider that one out of five T1 bladder cancer patients will undergo cystectomy in the next 2 years (15,16), it is important to look at the NLR when planning a personalized follow-up for these high-risk patients.

Recently, a prospective study assessed the importance of second TURB in T1b patients because there is a tree times higher risk of progression (17). This is why all our patients underwent routinely re-TURB, but even in this case, there is a high risk of progression in this sub-group of patients. Rouprêt et al. showed in a French multi-centric cohort that T1b stage is predictive for progression and recurrence, but a subsequent transurethral resection of bladder tumor was not performed (18). Instead, we showed that NLR and T1b stage are independent predictive factors for progression, as recurrence rates did not differ, perhaps because of second TURB.

Our results are also in line with Viers et al. who showed an association between pre-treatment NLR and risks of disease recurrence, death from bladder cancer and overall survival after radical cystectomy (19). In this study, NLR was determined 90 days prior to surgery. Moreover, almost half of T1 patients had an altered NLR, but the cutoff was set at 2.7.

Favilla et al. investigated NLR>3 in a cohort of patients with non-muscle invasive bladder cancer and found it predictive for recurrence, but not for progression. On the contrary, we found NLR to be an independent predictive factor for progression in T1 bladder cancer patients. Except for us, they included only 31 patients with T1 tumors representing only 22.5 % of the studied population. In terms of progression, they found as independent predictors only the T1 stage, high grade, concomitant CIS and smoking status.

It is still unclear how high NLR influences poor outcome of cancer patients, one theory promotes inflammation and immune response, including increased neutrophils and decreased lymphocytes (20). Another theory suggested by De Larco, states that tumor cells interact with their microenvironment and enhance local inflammation by releasing different cytokines and interleukins (21).

Other studies explored the role of inflammation parameters in the prognosis of urinary cancers, but the results were not consistent. The modified Glasgow Prognostic Score (mGPS), a systemic inflammation marker, was found as a prognostic marker for recurrence in patients with urothelial bladder (22). On the other hand, bladder epithelium and bladder cancer cells express CD44 that binds glycosaminoglycans (GAG) such as chondroitin sulphate (CS). In an experimental study, Ferro et al. showed that when treating human bladder cancer cell lines HT-1376 with CS in combination with either gemcitabine (GEM) or mitomycin-C (MMC), it induced ap-

optosis by activating caspases 9&3 and inhibits cell growth by enhancing the antitumor activity of the two chemotherapy drugs (23).

Due to its retrospective design, our study is limited, but still, our cohort is a contemporary one that included only high-risk patients. The low number of patients is due to the low incidence of high-risk patients even in a tertiary care hospital like ours, also most of the patients identified had to be excluded because of lack of follow-up and proper post operatory management. As stated by other authors, it is difficult to follow up patients with bladder cancer as only few of them benefit from long-term management according to guidelines (24,25). To validate our results, it is important to run prospective studies or at least multi-centric retrospective ones in order to assure the inclusion of the highest population of T1 bladder cancer patients.

In conclusion, high neutrophil-to-lymphocyte ratio retained a statistically significant value, as an independent prognostic factor for a worse prognosis of T1 bladder tumors. NLR represents an easily available biomarker that should be included in daily practice as it can support a proper clinical decision making in case of high-risk non-muscle invasive bladder cancer.

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