Correlation of Tumor-Infiltrating Neutrophils and Tumor-Infiltrating Lymphocytes with Early Recurrence in Patients with Non-Muscle Invasive Bladder Urothelial Carcinoma (Short-Term Prospective Cohort Study)

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Research Article

Keywords: Tumor Infiltrating Neutrophils, Tumor-Infiltrating Lymphocytes, Neutrophil/ Lymphocyte ratio, Non Muscle invasive Bladder Cancer, Recurrence, Progression

Posted Date: March 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1443290/v1

Abstract

Background

Tumor-infiltrating neutrophils (TINs) and lymphocytes (TILs) play essential roles in promoting or combating various neoplasms, the prognostic value of TILs, TINs and neutrophil to lymphocyte ratio (NLR) in bladder cancer (BC) is still unconcluded.

Aim

To investigate the association between TINs and CD8 + T-lymphocytes, as well as NLR with progressive non-muscle invasive urothelial bladder carcinoma (NMIBC).

Methods

Patients with newly diagnosed NMIBC underwent transurethral resection of bladder tumor (TURBT) followed by a standard BCG regimen, follow-up cystoscopy was performed every 3-months for the first 2-years. Tumor characteristics and histopathological examination were documented. As regards immunohistochemical evaluation, the tumors categorized into two main groups: high or low, according to their TINs, and CD8 + T-lymphocytes immunohistochemically expression and NLR. Kaplan-Meier estimates were used to compare the recurrence-free survival.

Results

104 patients with NMIBC were included; Ta and T1 represented 23.1% and 76.9% of patients respectively. High-grade (HG) and low-grade (LG) were 53.8% and 46.2%, respectively. Tumor recurrence and progression were correlated to a higher stage and grade ($p = 0.05$). Regardless stage and grade; recurrence was more likely to occur in high TINs and TILs groups, while progression was more likely in high TILs and NLR ($p < 0.05$). In Ta LG tumours, the higher TINs and NLR were associated with higher recurrence rate. In T1 (LG) tumour; low TINs, high TILs, and low NLR were associated lower recurrence and progression rate, In T1 HG tumours; high TINs and NLR were associated clinical and statistically lower recurrence rate. Kaplan-Meier survival analysis showed that TINs and TILs were significantly related to the short recurrence free survival (RFS) ($p = 0.03$, $p = 0.001$).

Conclusion

TINs, CD8 + lymphocytes and NLR are important prognostic predictors in patients with NMIBC. Recurrence was more likely to occur in the high TINs and TILs groups, while higher TILs and NLR were correlated to tumour progression.

Introduction

Bladder urothelial cancer is one of the most common genitourinary malignancies characterized by high prevalence and recurrence rate [1]. BC includes NMIBC and muscle invasive bladder cancer (MIBC). NMIBC defined as a superficial cancer confined to the mucosa, represent the most common newly diagnosed type and includes a non-invasive papillary lesion (Ta), carcinoma in situ (CIS), or invasive lesion confined to lamina propria (T1) [2]. According to the guidelines, Patients with NMIBC are treated by TURBT followed by immediate instillation of chemotherapy with standard and maintaining intravesical therapy. In spite of second resection and intravesical therapy, the majority of them experience tumor recurrence and progression [3], probably due to implantation of tumor cells from another site and subsequent proliferation, regrowth of tumor cells of previously resected tumors in case of incomplete TURBT or growth of new cancers [3, 4]. Different outcomes are observed in patients with the same grade, stage, and treatment strategy, for improving the accuracy and prediction of recurrence and progression, risk based stratification could help for better treatment strategy. Highlighting of the importance to identify the effective markers to estimating the risk of progression and response to alternative treatments [5]

The relationship between inflammation and malignant transformation has been studied for several tumors, concluding that the oncological outcome is affected by the host response through systemic inflammation [6]. Cancer-related inflammation is an essential contributor to tumor initiation, growth, and progression [7]. Cytokines and inflammation associated chemical mediators are essential factors in inflammation-mediated tumor recurrence and progression [8.9]. In addition, tumor microenvironment with its associated inflammatory cells, TINs and TILs are strongly associated with changes in tumor behavior [10–15]. TINs were typically pro-tumor and were strongly associated with poorer prognosis in the majority of cancers [15]. CD8 + lymphocytes have been found to be associated with better clinical response in the majority of carcinomas [16–21]. In bladder cancer, the prognostic value of TILs, TINs and neutrophil to lymphocyte ratio (NLR) is still unconcluded. The main aim of this study is to investigate the association between TINs, CD8 + TILs and NLR in the tumor microenvironment with early recurrence and progression in patients newly diagnosed with NMIBC

Materials And Methods

Study design:

The study was a prospective cohort study, included patients with newly diagnosed NMIBC from October 2018 throughout June 2020 at two Urology clinics; Suez Canal University and Elkheir-El-Baraka hospitals, Ismailia. Egypt. Excluding patients, whose specimen showed pathological evidence of muscle invasion or received chemotherapy. Data was collected through; medical history, clinical examination, laboratory evaluation [Complete blood count, serum creatinine, urine analysis, urine culture and urine cytology… etc.]. Radiological evaluation includes pelvic-abdominal ultrasound and contrast enhanced CT study, the frequency of follow-up cystoscopies ± TURBTs performed, intravesical therapy, time to recurrence and/or progression, and last follow-up visit were also recorded.

Strategy Of Management And Surveillance:

Patients with NMIBC (Ta, T1 and CIS) underwent digital rectal examination under anesthesia and initial urethrocystoscopy. Tumor characteristics were recorded, including tumor location, size, shape, multiplicity, and associated mucosal abnormality. Complete TURBT was performed accordingly, with en bloc resection of small papillary tumors with the underlying muscle layer and resection of larger tumors in fractions with the underlying muscularis propria. All patients received an early single dose of Epirubcine 50 mg within 6-hour of primary resection. A second look TURBT was performed for all patients with T1, HG disease, in presence of CIS and for more accurate staging, especially in cases with missing detrusor muscle in the initial sampling. The standard regimen of 6-week induction course of BCG (ImmuCyst® 81 mg of freeze-dried preparation made from the Connaught sub-strain of Bacillus Calmette-Guérin) was administered in all patients with T1 tumors, CIS, and Ta HG cancer, followed by maintenance BCG. Follow-up cystoscopy was performed every 3-month for all patients with T1 and HG tumors Ta for the first 2-year, then annually. In patients with LG Ta cancer, the first cystoscopy was performed after 3-month and then annually unless there was documented progression. The tumors were classified by 2017 Union for International Cancer Control (UICC), the TNM staging system and 2004–2016 WHO grading system [2].

Primary endpoints:

The primary endpoints of the study were tumor recurrence and stage progression. Recurrence was defined detection of tumors in the follow up cystoscopies of the same or lower stage and grade documented by histopathological studies. Progression was defined as an increased pathological stage of the primary disease to invade the lamina propria and/or the muscle propria or development of metastatic disease.

Histopathological evaluation:

The samples were fixed with 10% formalin and embedded in paraffin. For each block, sections of 3µm thickness were submitted, mounted to a glass slide, stained with hematoxylin and eosin (H&E) and examined by an independent pathologist. TINs were identified, counted in 4 different High power fields (Hpf) and the average leukocyte count was calculated.

Immunohistochemical staining:

Sections of the selected paraffin blocks were cut into 4 micrometers thick sections for immunohistochemical (IHC) staining. Slides were prepared and incubated with primary anti-CD8 antibody (Anti-CD8 alpha; ab4055, abcam), to detect tumor infiltrating lymphocytes. This was followed by incubations with the appropriate secondary antibody (Anti-rabbit IgG; ab205718, abcam). All slides are lightly counterstained with hematoxylin for 30s prior to dehydration and mounting.

Histopathological and Immunohistochemical scoring

Four tumor fields were selected with a low magnification (×100). Neutrophils and positive CD8 cells were counted with high magnification (×400). TIN was defined as neutrophils that infiltrated into cancer nests or stroma. TILs were defined as CD8 + lymphocytes that infiltrated into cancer nests or stroma.

The median leukocyte count was used as a cutoff point to categorize each case into either a high or low group as follows [20]: the low group is characterized by TIN ≤ 20/ Hpf and TIL ≤ 12/ Hpf, whilst a high group is characterized by TIN ≤ 20/Hpf and TIL ≤ 12/Hpf. In addition, the neutrophil to lymphocyte ratio (NLR) was calculated and categorized the cases into a low or high group: NLR < 1 is low group, while NLR > 1 is higher group.

Statistical Analysis

The statistical analysis was conducted using SPSS 22.0.0.0 for Windows. Data were presented in tables and figures as appropriate. Quantitative data were expressed as mean and standard deviation, whereas qualitative data were expressed as number and percentage. Comparisons were performed using chi square for qualitative data and T test for quantitative data. Significance was considered at a p value of < 0.05. Kaplan-Meier estimates were used to compare the RFS. P < 0.05 for the difference was considered as significant

Results

A total of 104 patients newly diagnosed with NMIBC were included in this study. The median age was 66.4 years (range 40:85 years). The male to female ratio was 9.4:1 patients, 54% of patients were smokers. Positive family history of bladder cancer was documented in 3.8% of patients. 50% of patients had a tumour size greater than 3 cm. Multiple tumours were detected in 53.8% of patients. Regarding pathological stage, 80 (76.9%) and 24 (23.1%) of patients were categorized as T1 and Ta respectively. HG and LG tumors detected in 53.8% and 46.2% of the patients respectively. Concomitant CIS with T1 tumours was present in 1.9% of the patients. We categorized our study population into two main groups: high or low, according to their TINs, TILs and NLR status. Table 1

Table 1
Demographic data and pathological characters of the study population

Variable			Number	%
Gender	Male		94	90.4
	Female		10	9.6
Smoking	Positive		56	53.8
Family History	Positive for BC		4	3.8
Tumor characteristics	Size	> 3	52	50
		< 3	52	50
	Number	Single	48	46.2
		Multiple	56	53.8
	Pathologic Stage	Ta	24	23.1
	CIS (Concomitant T1) *	T1	80	76.9
		CIS	2	1.9
	Grade	Low	48	46.2
		High	56	53.8
	TINs	Low (< 20/Hpf)	38	36.5
		High (> 20/Hpf)	66	63.5
	TILs	Low (< 12/Hpf)	50	48.1
		High (12/Hpf)	54	51.9
	NLR	Low (1)	50	48.1
		High (1)	54	51.9

TINs; Tumor infiltrating neutrophils, TILs; Tumor infiltrating lymphocytes, NLR; Neutrophil to Lymphocyte ratio, CIS; carcinoma in situ, Hpf; high power field

During the Follow-up; the recurrence and progression rate were significantly higher in pT1 group. Recurrences occurred in 20 patients (18 patients in the T1 group and 2 patients in the Ta group), whereas progression occurred in 20 patients in the T1 group only. The rate of recurrence and progression was significantly higher in HG tumours (p = 0. 05). **Table 2**

Table (2) Recurrence and progression according to tumour stage and grade:

Oncologic outcome		No event	Recurrence.19.23%	Progression.19.23%
Tumor Stage	**Ta**	22	2	0
	T1	42	18	20
p-value			0.028	0.001
Tumor Grade	**Low**	37	6	5
	High	27	14	15
p-value			0.029	0.013

We investigate the association of TINs, TILs and NLR with the clinical- pathological characters in patients with NMIBC. The results revealed that TILs was more frequently high among non-smokers (p = 0.006). Intergroup comparison displayed no associations of TINs, TILs and NLR with neither gender, nor number of masses (p > 0.05). Table 3

Table 3
The association of TINs, TILs, and NLR with clinical-pathological features of bladder cancer patients

Variable		TINs			TILs			NLR		
		High	**Low**	**p. value**	**High**	**Low**	**p. value**	**High**	**Low**	**p. value**
Gender	**Female**	6	4	0.61	4	6	0.25	3	2	0.54
	Male	60	34		62	32		24	23	
Smoking	**No**	36	12	0.094	40	8	0.006	14	10	0.28
	Yes	30	26		26	30		13	15	
Mass number	**Multiple**	32	16	0.44	28	20	0.34	14	10	0.82
	Single	34	22		38	18		13	15	

TINs; Tumor infiltrating neutrophils, TILs; Tumor infiltrating lymphocytes, NLR. Neutrophil to Lymphocyte ratio.

Regardless of tumor stage and grade; recurrence is more likely to occur in high TINs and TILs groups in comparison to their lower counterparts (p 0.05), while progression is more likely in association with high TILs and NLR (p < 0.05). Table 4

Table 4

Cross tabulation between TINs, TILs, NLR status, recurrence, and progression in NMIBC.

Test		Recurrence (n)			Progression (n)		
		-ve	**+ve**	**P**	**-ve**	**+ve**	**P**
TINs	**Low**	25	6	0.031	25	4	0.079
	High	39	14		39	16	
TILs	**Low**	19	4	0.012	19	6	0.049
	High	45	16		45	14	
NLR	**Low**	33	10	0.90	33	8	0.02
	High	31	10		31	12	

TINs: Tumor infiltrating neutrophils, TILs: Tumor infiltrating lymphocytes, NLR: neutrophil to lymphocyte ratio.

However, when taking into consideration the tumor stage and grade of the tumour, in Ta LG tumour the higher TINs and NLR were associated with higher recurrence rate. On the other hand, In T1 LG tumour; low TIN, high TILs, and low NLR were associated lower recurrence and progression rate, In T1 HG tumours high TIN and high NLR were associated clinical and statistically lower recurrence rate. Table 5

Table 5
The correlation between TINs, TILs, NLR expressions and tumour recurrence, and progression as regards stage and grade of non-muscle invasive balder cancer.

Grade		Test		Recurrence (n)			Progression (n)		
				-ve	+ve	p.value	-ve	+ve	P.value
Ta	Low Grade	TINs	Low	4	0	0.03	4	0	-
24 Pt	22 Pt		High	16	2	0.001	16	0	-
		TILs	Low	2	2	0.50	2	0	-
			High	18	0	0.001	18	0	-
		NLR	Low	10	0	0.001	10	0	-
			High	10	2	0.004	10	0	-
	High Grade	TINs	Low	0	0	-	0	0	-
	2 Pt		High	2	0	-	2	0	-
		TILs	Low	2	0	-	2	0	-
			High	0	0	-	0	0	-
		NLR	Low	0	0	-	0	0	-
			High	2	0	-	2	0	-
T1	Low Grade	TINs	Low	11	0	0.001	11	0	0.001
80 Pt	26 Pt		High	6	4	0.60	6	5	0.90
		TILs	Low	5	4	0.90	5	1	0.08
			High	12	0	0.001	12	4	0.01
		NLR	Low	13	0	0.001	13	1	0.001
			High	4	4	0.60	4	4	0.8
	High Grade	TINs	Low	10	10	0.80	10	4	0.06
	54 Pt		High	15	4	0.001	15	11	0.4
		TILs	Low	10	6	0.30	10	5	0.10
			High	15	8	0.07	15	10	0.40
		NLR	Low	10	10	0.80	10	5	0.10
			High	15	4	0.001	15	10	0.40

Histopathological and immunohistochemical examination of tissue sections prepared from the tumors revealed that in NMIBC, there was predominantly of CD8 + TIL with few TIN. On the other hand, in MIBC, most of the inflammatory cells within tumor nests were neutrophils, with few CD8 + T-lymphocytes. Figure 1

Kaplan-Meier survival analysis showed that TINs and TILs were related to the short RFS (p = 0.03 and 0.001 respectively), while and NLRs showed no statistically significant relation to the short RFS (p = 031). Figure 2

Univariate analysis showed that tumor sizes, grades, TINs, TILs, and NLR were statically significant association with overall survival in NMIBC ($P < 0.05$), the tumour size, grade, TINs, NLR were unfavorable predictors. TILs showed a better prognosis. Multivariate analysis showed that TIN and NLR were only independent prognostic predictors in NMIBC ($P < 0.05$). Table 6

Table 6
Univariate and Multivariate analysis of the association between variables and recurrence survival among the study population.

Clinical and pathological feature	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age	0.881	0.364–1.741	0.712	-	-	-
Gender	1.61	0.851–2.141	0.521	-	-	-
Smoking	1.44	0.624–1.802	0.912	-	-	-
Medical morbidities	1.79	0.873–2.24	0.077	-	-	-
LUTS	1.32	0.472–1.211	0.421	-	-	-
Size	3.84	1.427–4.863	0.002*	1.01	0.47–1.63	0.218
Mass number	1.97	0.874–2.747	0.240	1.17	0.874–1.747	0.240
Site	1.82	0.701–2.537	0.312	1.22	0.517–1.74	0.312
Pathology Stage	2.74	0.917–4.120	0.338	0.874	0.218–1.77	0.338
Grade	4.21	1.748–6.241	0.001*	1.74	0.624–2.14	0.117
TIN	4.012	1.660–7.220	< 0.001*	3.11	1.44–5.280	0.012*
TIL	0.367	0.125–0.772	< 0.001*	0.714	0.125–0.772	0.321
NLR	4.71	1.85–10.745	0.012*	3.301	2.02–6.47	0.001*
P < 0.05 = considered statistical significant.						

Discussion

Tumor microenvironment inflammation plays an important role in both tumor recurrence and progression. Neutrophils within tumor nests can behave as an anti-tumor (N1) phenotype; by inducing cytotoxicity, tumor rejection and anti-tumor immune memory or Pro-tumour phenotype (N2) which promotes tumour progression through enhancing angiogenesis, invasion, metastasis and immunosuppression [22–24].

Moreover, lymphocytes located around tumor cells have dual regulatory roles in both anti-tumor immune responses, by inhibiting tumor growth and tumor progression or by creating a microenvironment, which stimulates tumor outgrowth and protection the tumour from the immunological events [20, 24]. The present study investigated the association between TINs and CD8 + T lymphocytes with early recurrence in patients newly diagnosed with non-muscle invasive bladder urothelial carcinoma.

Our study demonstrated that TINs had pro-carcinogenic effects on tumor recurrence and progression, as by using Kaplan-Meier survival analysis, it showed that TIN were statistically significantly related to the short RFS ($p = 0.03$). This finding was agreeing with that previously reported in malignancy[15] as regards; UB carcinoma [20], renal cell carcinoma [25], non-small cell lung cancer [26], and esophageal carcinoma [27], suggesting the associations between the presence of TINs and poor prognosis. Moreover, in the present study, regarding the prognosis, high level of TINs was significantly associated with early recurrence not progression. At the early stage of carcinoma development, neutrophils show anti-tumor activity which causes cell lysis and stimulates T cell mediated immunity through enhancing the CD4 + and CD8 + T lymphocyte proliferation and activation. Under certain conditions, neutrophils may release certain endogenous mediators, growth-stimulating signals and matrix-degrading proteases during carcinogenesis [24].

Regarding TILs, we found that in patients with NMIBC, the group with recurrent disease had higher of CD8 + lymphocytes than the group without recurrent disease. In the present study, recurrence as well as progression were statistically significantly higher in the high CD8 + TILs group in comparison to low CD8 TILs group ($p = 0.012$ and 0.049 respectively). Kaplan-Meier survival analysis showed that TILs were statistically significantly related to the short RFS ($p = 0.001$). This finding is consistent with that reported in Liu et al. [20] they showed that low CD8 + TILs were an independent favorable prognostic factor in NMIBC, However, the association between TILs and recurrence RFS of NMIBC patients was non-significant in their study. Krpina et al, [19] reported that the abundant CD8 + TILs were related to higher risks of recurrence. On the other hand, it has been reported that patients with progressive BC, who showed higher numbers of CD8 + TIL had better disease-free survival than did patients with similar-staged of BC and fewer numbers of CD8 TIL [28]. Such conflicting data necessitate larger sample studies to confirm such results and to highlight the possibility of other factors in determining the fate of CD8 TIL in tumour biology.

Neutrophil to lymphocyte ratio (NLR) reflects the immune response against tumor cells, with both its innate (neutrophils) and adaptive (lymphocytes) immune responses, with a high NLR value indicates a potent inflammatory reaction, which in turn, correlates with decreased tumor-specific immunity [28, 29]. It has been reported that NLR was considered as a prognostic marker in urothelial bladder cancer, as well as metastatic and advanced disease [29]. In addition, Morizawa et al. [30] showed that NLR was significantly associated with recurrence-free survival, cancer-specific survival, and overall survival. Moreover, Krane et al. [31] reported that the patients with elevated NLR before radical cystectomy have worse overall survival than patients without elevated NLR. Other studies reported that a low NLR was related to better response to neoadjuvant chemotherapy [32, 33] and there was a significant relationship

between NLR and both pathological response and survival [34]. In the present study, we found that NLRs showed no statistically significant relation to short RFS and recurrence, but only high NLR is associated with an increased risk of tumour progression in NMIBC. Again, such discrepancy in the results highlight the complexity of inflammatory response in urothelial carcinogenesis and that other factors could potentiate or attenuate the role of inflammatory cells as an anti-tumor or pro-tumor cells.

As regards tumour stage and grade, our study showed that there was a significant correlation between higher stage and grade and increased tumour recurrence and progression and this findings constancy with the previous studies [3, 5]. In Ta LG, the rate of recurrence was significantly correlated to higher (TINs, TILs and NLR), this results was in agreement with others. [19, 20] The Ta HG did not show any progression, or recurrence that limits the ability to statistically analyze the corresponding association. In T1 LG tumour: low TINs, high TILs, and low NLR were associated lower recurrence and progression rate, and clinically the recurrent rate was high in high TINs (4/10), low TILs (4/9) and high NLR (4/8). In T1 HG tumours: high TINs and high NLR were associated clinical and statistically lower recurrence rate. while, clinically in T1 HG higher recurrent rate was seen low TINs (10/20) and NLR (10/20), this finding was agreeing with that previously reported in UB carcinoma [20], and others carcinomas [15, 25–27]

The major limitations in this study were the limited numbers of the study population and the short term follow up. Further studies with large sample size are needed to demonstrate the correlation between tumour infiltration immunological cells and the clinical-pathological features, with long term follow up are required to demonstrate the prognostic effect of such immunological cells in tumour behavior

Summary And Conclusion

The current study highlights the significance of inflammatory cells within tumor environment of early urothelial carcinoma. In our study, recurrence and progression of non-muscle invasive urothelial carcinoma were statistically significant higher in tumors with both high tumor infiltrating CD8 + lymphocytes and TINs, which highlight the importance of inflammatory process not only in combating tumors, but also in the progression of tumors. However, further large scale prospective multicenter studies with prolonged follow up are recommended to confirm our results, before IHC staining of CD8 positive tumor infiltrating T lymphocytes can be included in the routine clinical workup of urothelial carcinoma patients.

Abbreviations

BC	bladder cancer
CIS	carcinoma in situ
HG	High-grade
Hpf	High power field
LG	low-grade
MIBC	muscle invasive urothelial bladder carcinoma
NMIBC	non-muscle invasive urothelial bladder carcinoma
NLR	neutrophil to lymphocyte ratio
RFS	recurrence free survival
TILs	Tumor-infiltrating lymphocytes
TINs	Tumor-infiltrating neutrophils
TURBT	Transurethral resection of bladder tumor

Declarations

Ethics approval and consent to participate: All procedures performed were in accordance with the Helsinki declaration and its later amendments (2004). The institutional research and ethics committee had reviewed and approved the study [Suez Canal University with IRB no (3857)]. Each patient in this study had signed an informed consent according to the Ethics Committee of faculty of medicine, Suez Canal University Hospital.

Consent for Publication: Written informed consent was obtained from the patients for publication of this study and any accompanying images. A copy of the written consent is available for review upon request.

Availability of data and materials: The datasets generated and/or analyzed during the current study are not publicly available due to limitations of ethical approval involving the patient data and anonymity but are available from the corresponding author upon reasonable request.

***Conflict of interest*:** None of the contributing authors has any conflict of interest, including specific financial interests or links and affiliations related to the subject or material discussed in the manuscript.

Funding: NO funding agency, all clinic-pathological work in this study were funded by the authors.

Author Contributions:

All authors (E. Shalaby, O. Salem, A. El Nashar, A. El-Tobgy), made significant contributions to the reported work, including design, study design, implementation, data acquisition, analysis and interpretation. Also,

in all these areas; took part in the writing, revision or critical review of the article; gave final approval of the version to be published; agreed upon the review to which the article was submitted.

Acknowledgments: The authors are particularly grateful to Dr Wael Abdou Hassan (Lecturer in Pathology) for his advice and support in the revision of the pathological parts of the study. Mr. M Saad for his technical skills in the preparation of histopathologic slides.

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Figures

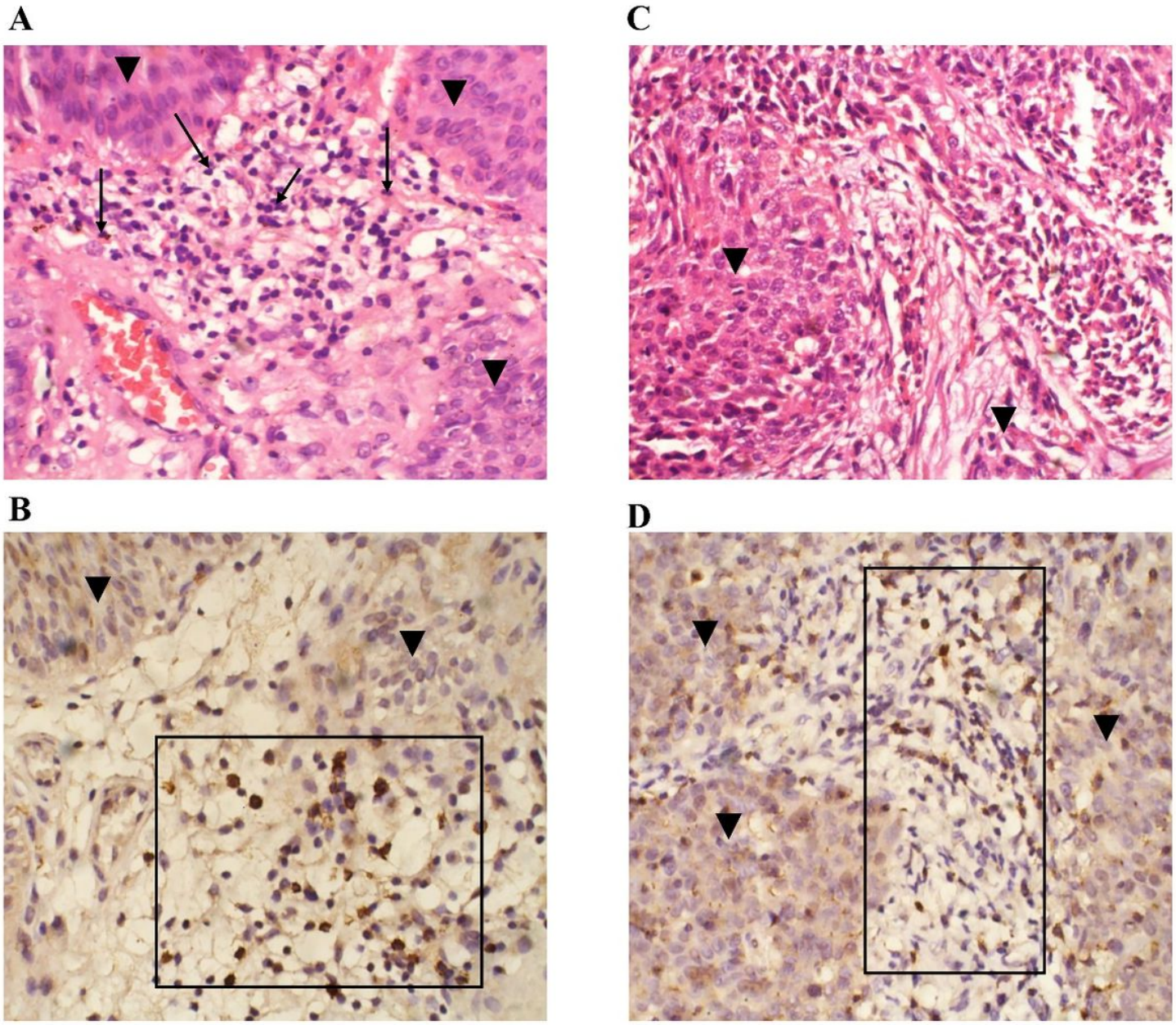

Figure 1

Tumor associated neutrophils and CD8+ lymphocytes in non-muscle invasive (A and B) and muscle-invasive urothelial carcinoma (C and D).

In NMIBC, the majority of inflammatory cells (Black box) between tumor nests (Arrowheads) are lymphocytes, with few neutrophils identified (Black arrows) (A). Immunohistochemical examination for CD8 protein reveal that majority of cells are CD8 positive lymphocytes between tumor nests (Arrow heads) (B) (H&E and IHC, 400x)

On the other hand, in MIBC, the majority of inflammatory cells (Black box) between tumor nests (Arrow heads) are neutrophils, with few lymphocytes (C). Immunohistochemical examination for CD8 protein

reveal that few cells are CD8 positive lymphocytes between tumor nests (Arrow heads) (H&E and IHC, 400x)

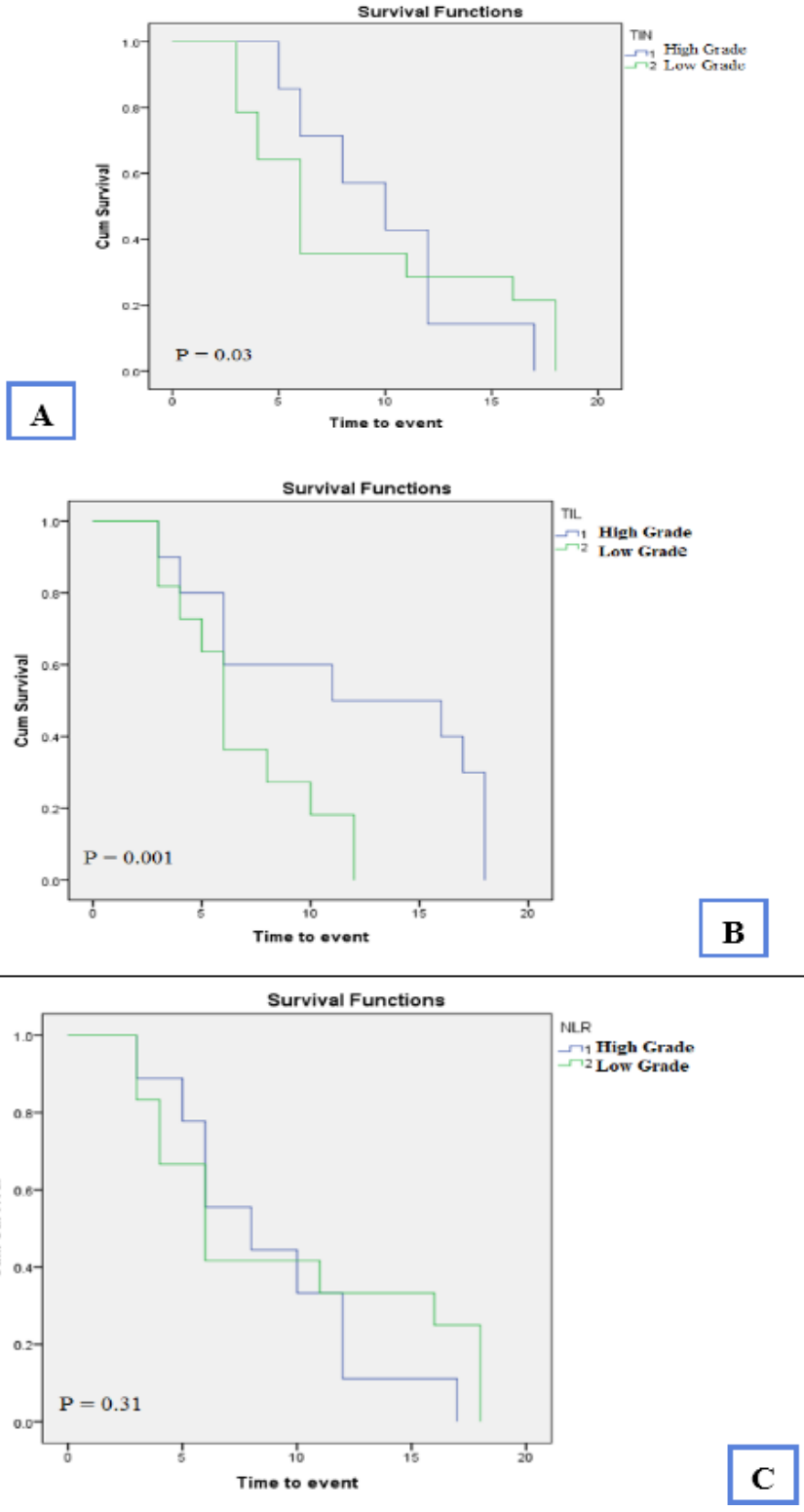

Figure 2

Kaplan-Meier survival analysis of TINs, TILs and NLR with recurrent free survival of patients with NMIBC. (A)TINs (B) TILs (C) NLR.