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Original Research

Validation of the importance of neutrophil-to-lymphocyte ratio in non-muscle invasive bladder cancer

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Abstract

Background and objective: Since the peritumoral inflammatory reaction is of considerable importance in the oncological prognosis of bladder cancer, the neutrophil-to-lymphocyte ratio (NLR) may be a very practical and accessible biomarker in non-muscle invasive bladder tumors. This study aims to analyze parameters of non-muscle invasive bladder tumors and to identify and validate potential tumor markers with a prognostic role in this condition.

Material and methods: 463 patients diagnosed with non-muscle invasive urothelial tumors, between January 2016 and June 2020, hospitalized at the Urology Clinic in Târgu Mureș, after transurethral resection of bladder tumor (TURBT) were investigated. Inclusion criteria were: non-muscle invasive tumors (Ta, T1, CIS), neutrophil-to-lymphocyte ratio (NLR) determination 2 weeks prior to TURBT and complete follow-up of patients. NLR cut-off was set at 3.

Results: 371 people diagnosed with non-muscle invasive bladder tumors met the inclusion criteria. The mean age of the included patients was 69.96 years (IQR 35–93). 297 (80.05%) were male and 74 (19.95%) were female. 64.95% pTa tumors, 31.26% pT1, 1.07% pTa + CIS, and 2.69% pT1 + CIS, respectively, were identified. Distribution according to Grade: 196 (52.8%) G2, 159 (42.88%) G3 and 16 (4.32%) CIS. 284 (76.5%) patients presented with a single tumor and 87 (23.35%) with multiple tumors; 295 (79.5%) had tumors larger than 3 cm and 76 (20.5%) smaller than 3 cm. 228 (61.4%) presented with recurrence after 3 months after surgery. Overall survival at 5 years was 80.06%. Advanced age at the time of diagnosis correlated with low survival, $p = 0.004$. The mortality rate was higher among men (20.54%) than among women (17.58%). Statistically, there was a positive relationship between the increased ratio of neutrophils and lymphocytes in people diagnosed with non-muscle invasive bladder cancer and increased mortality rate, $p = 0.003$.

Conclusions: The overall survival of patients with non-muscle invasive bladder cancer was negatively influenced by the advanced age of the patients at the time of diagnosis. Increased preoperative NLR was associated with a higher mortality rate in patients with non-muscle invasive bladder cancers. This assay is an accessible, easy to performand useful biomarker in the prognosis of non-muscle invasive bladder tumors.

Keywords

Neutrophil-to-lymphocyte ratio; Non-muscle invasive bladder cancer; Overall survival; Biomarker

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1. Introduction

Bladder cancer is the seventh most common type of cancer among men, and by documenting both sexes, it ranks eleventh in frequency. The incidence is 9.0 per 100,000 people annually in men and 2.2 in women world wide with an incidence rate higher in the European Union, being 19.1 for men and 4.0 among women in 2012 [1, 2]. The global mortality rate per 100,000 people per year is 3.2 for men and 0.9 for women. The incidence and mortality of bladder cancer are influenced by differences in risk factors, methods of diagnosis, and availability of treatment in different countries [3, 4].

Most patients, approximately 75% with bladder tumors present with disease limited to the mucosa (stage Ta, CIS) or submucosa (stage T1). In patients younger than 40 years this percentage is higher. Patients with Ta, T1, or CIS in many cases have a higher prevalence of long-term survival and a lower risk of tumor-specific mortality compared to those of stages T2-4 [5, 6].

The prompt detection of HG/G3 non-muscle-invasive recurrence is crucial and the percentage of tumours missed should be kept as low as possible because a delay in diagnosis and therapy can be life-threatening. A significant variability is observed among pathologists for the diagnosis of CIS; stage T1 vs. Ta tumors and tumor grading in both the 1973 and 2004/2016 classifications. The WHO 2004/2016 classification provides better reproducibility than the 1973 classification [7, 8].

Local inflammation is a factor that contributes substantially to the development and progression of malignancy, the local immune response and systemic inflammation having an essential role in this regard [9, 10].

Bladder cancer is closely related to chronic or recurrent local inflammation and an increased number of inflammatory cells at the tumor site. The number of T lymphocytes and natural killer cells is significantly lower in patients with infiltrative bladder tumors, compared to those presenting with superficial urothelial tumors. An increased value of preoperative NLR is related to poorer prognosis. Moreover, some studies have shown that a high value for NLR is associated with a higher recurrence rate and lower overall survival [11, 12].

Since the peritumoral inflammatory reaction is of substantial importance in the oncological prognosis of bladder cancer, theefore, the neutrophil-to-lymphocyte (NLR) ratio may be an extremely practical and appropriate biomarker in non-muscle invasive bladder tumors [13, 14].

2. Aim of the study

The study aims to analyze parameters of non-muscle invasive bladder tumors and to identify and validate potential tumor markers of prognostic value in this condition.

3. Material and methods

A retrospective study is described, involving 463 patients diagnosed with non-muscle invasive urothelial tumors hospitalized at the Urology Clinic in Târgu Mureş, between January 2016 and June 2020, after transurethral resection of bladder tumors (TURBT). The study was conducted in line with the World Medical Association Declaration of Helsinki and was approved by the Ethical Committee of the Mures County Hospital. The inclusion criteria were: non-muscle invasive tumors (Ta, T1, CIS), neutrophil-to-lymphocyte ratio (NLR) determination 2 weeks prior to TURBT and complete follow-up of patients. Both multi-tumors, and unifocal tumors were included. Exclusin criteria: T2 or higher stage tumors and failure to follow-up. Patients with comorbidities associated with elevated inflammatory markers were also excluded from the study. NLR cut-off was set at 3 according to other studies in the literature.

The data collection was performed with the help of the H3 Concept computer system. The data processed included demographic data, such as sex and age, degree of tumor invasion, degree of tumor differentiation and recurrence. Laboratory tests included NLR, tumor size, number of tumors, and overall survival, all centralized in a table in Microsoft Excel program.

Statistical data analysis was performed using the IBM SPSS Statistic Subscription program (version 24.0, IBM Corp., Chicago, IL, USA). Data were considered as significantly different if in the confidence interval set the value $p < 0.05$. The tests used in the statistical analysis process are Chi-square and bivariate correlation.

4. Results

A total of 371 people diagnosed with non-muscle invasive bladder tumors met the inclusion criteria. The mean age of the included patients was 69.96 years (IQR 35–93). 297 (80.05%) were male and 74 (19.95%) were female. 64.95% pTa tumors, 31.26% pT1, 1.07% pTa + CIS, and 2.69% pT1 + CIS, respectively, were identified.

Distribution according to Grade: 196 (52.8%) G2, 159 (42.88%) G3 and 16 (4.32%) CIS. 284 (76.5%) patients presented with a single tumor and 87 (23.35%) with multiple tumors; 295 (79.5%) had tumors larger than 3 cm and 76 (20.5%) were smaller than 3 cm. 228 (61.4%) presented with recurrence after 3 months post-surgery. Overall survival at 5 years was 80.06% (Table 1).

Analyzing the overall survival, there is a positive relationship between advanced age at the time of diagnosis of the patient and low survival rate, $p = 0.004$. The mortality rate was higher among men (20.54%) than among women (17.58%). From a statistical point of view, there was no positive relationship between sex and overall survival, $p = 0.567$.

To study the ratio between neutrophils and lymphocytes (NLR), we divided the patients into two groups: Patients with an NLR less than 3 (low-risk) and NLR greater than 3 (high-risk), respectively.

TABLE 1. Characteristics of 371 patients according to NLR.

Characteristics	NLR <3			NLR >3		
		mNLR	*p*-value		mNLR	*p*-value
Age (Mean Age)	68.69	2.06	0.118	70.91	6.04	0.308
Sex	F	1.93	0.235	F	6.08	0.219
	M	2.09		M	6.03	
T	pTa	2.02	0.491	pTa	5.71	0.158
	pT1	2.16		pT1	6.51	
	pTa + CIS	2.50		pTa + CIS	7.72	
	pT1 + CIS	1.76		pT1 + CIS	6.63	
Grade	G1	1.76	0.087	G1	3.62	0.008
	G2	2.05		G2	5.67	
	G3	2.10		G3	6.72	
Number of Tumors	1	2.02	0.115	1	6.02	0.007
	2	2.16		2	5.97	
	3	2.03		3	13.64	
	4	2.84		4	4.24	
	7			7	3.49	
Size	<3 cm	1.99	0.062	<3 cm	5.08	0.113
	>3 cm	2.08		>3 cm	6.25	
Recurrence	Yes	1.99	0.342	Yes	6.13	0.374
	No	2.18		No	5.91	

From a statistical point of view, there was no positive relationship between values higher than 3 for NLR and distribution by gender, $p = 0.235$ or values lower than 3 for NLR, $p = 0.219$, respectively.

The average ratio between neutrophils and lymphocytes was higher in the group of deceased people (5.45), compared to those alive (4.04), at the end of the data collection period, with a p value of 0.754 (Fig. 1).

Statistically, there was a positive relationship between the increased ratio of neutrophils and lymphocytes in people diagnosed with non-muscle invasive bladder cancer and increased mortality rate, $p = 0.003$.

5. Discussion

This study aimed to identify prognostic factors for non-muscule invasive bladder tumors, that were significant in terms of overall survival, especially the ratio between neutrophils and lymphocytes and concordance of tumor growth with disease progression. From the point of view of overall survival, the data analyzed show that age at diagnosis is an unfavorable factor ($p = 0.004$).

There was a positive relationship between the increased mortality rate and an increased NLR, so patients with high NLR had a lower overall survival rate.

The significance of the peritumoral inflammatory response in bladder cancer is well-documented. An increased value of preoperative NLR is related to a poorer prognosis. Moreover, some studies have shown that the high value of NLR is associated with a higher recurrence rate and lower overall survival. Our data are in agreement with those published in the literature proving a positive relationship between the increased ratio of neutrophils and lymphocytes in people diagnosed with non-muscle invasive bladder cancer and increased mortality rate, $p = 0.003$ [5–18].

The study, published by Favilla *et al.* [19], described biomarkers in non-muscle invasive bladder cancers, including the ratio of neutrophils to lymphocytes. This study included 178 patients, of whom 148 were men and 30 women. The mean age was 69.27 years and the mean monitoring period was 53 months. Patients with NLR >3 were older and for who a higher rate of recurrence and progression was observed compared to the group of patients with NLR <3. Over a 5-year period, those with NLR <3 had a higher survival rate, 62%, compared to those with an NLR >3, where the survival rate was 49%. Thus, it was concluded that with the help of a inexpensive hematological test, calculating the ratio between neutrophils and lymphocytes, the rate of recurrence and progression of the disease could be estimated [19].

Data from more than 20,000 patients from the Netherlands Cancer Registry revealed statistically significant sex-related differences in stage distribution (Ta vs. T1) at presentation for non-muscle invasive bladder cancer, with women presenting more often with T1 disease [20]. The gender gap in stage at presentation and outcome could be due to biologic differences as well as a diagnostic delay in women. Indeed, there is data reporting no differences in clinical symptoms between the sexes, while the primary diagnostic approach as shown in numerous studies differs. It was reported that a gender gap in the evaluation of hematuria exists. Female gender was associated with a higher risk of receiving symptomatic treatment for hematuria, without further evaluation [21, 22].

Cantiello *et al.* [23] found that a single score based on NLR, PLR (platelet to lymphocyte ratio), and LMR (lymphocyte to monocyte ratio) cutoffs, denoted as SIM (systemic inflammatory markers), can predict recurrence and progression in this

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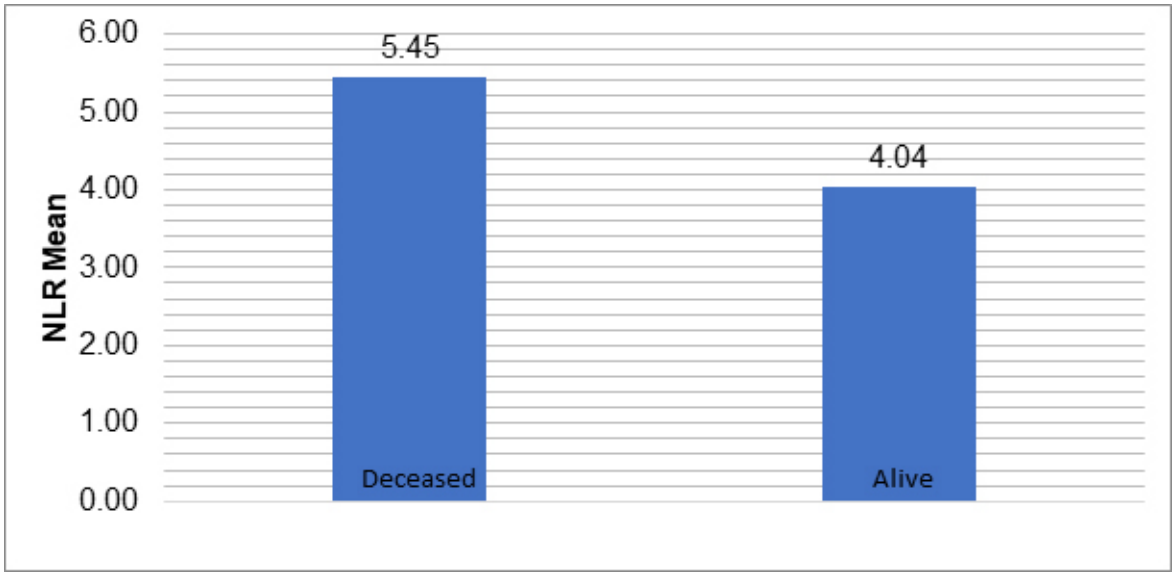

FIG. 1. Mean NLR ratio for deceased and survivors during the data collection period.

patient population. A higher SIM score is related to worse outcomes, even after adjusting for pathological variables such as tumor size, CIS, and multifocality. Furthermore, inclusion of this score in clinical decision analysis seems to be of clinical benefit over individual components in the predictive model [23].

Ferro *et al.* [24] concluded in a retrospective study involving 1045 patients with primary T1 HG/G3, that baseline basophil counts may predict recurrence in BCG-treated HG/G3 T1 bladder cancer patients.

In the era of numerous genetic prognostic factors in bladder cancer [25–28], there is an urgent need for easily accessible, affordable, and widely interpretable biomarkers for current practice. The Neutrophil-to-lymphocyte Ratio proves to have all these benefits [29–31].

6. Conclusions

The overall survival of patients with non-muscle invasive bladder cancer is negatively influenced by the advanced age of patients at the time of diagnosis.

The increased neutrophil-to-lymphocyte ratio (NLR) preoperatively is associated with a higher mortality rate in patients with non-muscle invasive bladder cancers.

NLR is an accessible, easy to determineand useful biomarker in prognosis for non-muscle invasive bladder tumors.

Author contributions

PHD, MO and BD—designed the research study. BD, RT, BBN, VAO—performed the research. PHD and BD—wrote the article. RT and BBN—analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee Nr 9573/05.07.2021.

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Conflict of interest

The authors declare no conflict of interest.

References

[1] Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder Cancer Incidence and Mortality: a Global Overview and Recent Trends. European Urology. 2017; 71: 96–108.

[2] Burger M, Catto JWF, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, *et al.* Epidemiology and risk factors of urothelial bladder cancer. European Urology. 2013; 63: 234–241.

[3] Chavan S, Bray F, Lortet-Tieulent J, Goodman M, Jemal A. International Variations in Bladder Cancer Incidence and Mortality. European Urology. 2014; 66: 59–73.

[4] Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC. Association between smoking and risk of bladder cancer among men and women. The Journal of the American Medical Association. 2011; 306: 737–745.

[5] Compérat E, Larré S, Roupret M, Neuzillet Y, Pignot G, Quintens H, *et al.* Clinicopathological characteristics of urothelial bladder cancer in patients less than 40 years old. Virchows Archiv. 2015; 466: 589–594.

[6] Sylvester RJ, Rodríguez O, Hernández V, Turturica D, Bauerová L, Bruins HM, *et al.* European Association of Urology (EAU) Prognostic Factor Risk Groups for Non-muscle-invasive Bladder Cancer (NMIBC) Incorporating the WHO 2004/2016 and WHO 1973

Classification Systems for Grade: An Update from the EAU NMIBC Guidelines Panel. European Urology. 2021; 79: 480–488.

[7] Soukup V, Čapoun O, Cohen D, Hernández V, Babjuk M, Burger M, *et al.* Prognostic Performance and Reproducibility of the 1973 and 2004/2016 World Health Organization Grading Classification Systems in Non-muscle-invasive Bladder Cancer: a European Association of Urology Non-muscle Invasive Bladder Cancer Guidelines Panel Systematic Review. European Urology. 2017; 72: 801–813.

[8] Witjes JA, Moonen PMJ, van der Heijden AG. Review pathology in a diagnostic bladder cancer trial: effect of patient risk category. Urology. 2006; 67: 751–755.

[9] Molinaro R, Corbo C, Livingston M, Evangelopoulos M, Parodi A, Boada C, *et al.* Inflammation and Cancer: in Medio Stat Nano. Current Medicinal Chemistry. 2018; 25: 4208–4223.

[10] Gakis G. The role of inflammation in bladder cancer. Advances in Experimental Medicine and Biology. 2014; 816: 183–196.

[11] Pfannstiel C, Strissel PL, Chiappinelli KB, Sikic D, Wach S, Wirtz RM, *et al.* The Tumor Immune Microenvironment Drives a Prognostic Relevance that Correlates with Bladder Cancer Subtypes. Cancer Immunology Research. 2019; 7: 923–938.

[12] Mártha O, Balan D, Porav-Hodade D, Drágus E, Vartolomei MD, Chibelean CB, *et al.* The role of neutrophil to lymphocyte ratio in patients with pTa non-muscle invasive bladder cancer. Revista Romana De Medicina De Laborator. 2020; 28: 29–38.

[13] Vartolomei MD, Porav-Hodade D, Ferro M, Mathieu R, Abufaraj M, Foerster B, *et al.* Prognostic role of pretreatment neutrophil-to-lymphocyte ratio (NLR) in patients with non-muscle-invasive bladder cancer (NMIBC): a systematic review and meta-analysis. Urologic Oncology: Seminars and Original Investigations. 2018; 36: 389–399.

[14] Luo F, Ma C, Wu J, Li J. Prognostic Value of the Neutrophil-to-Lymphocyte Ratio in Nonmuscle-Invasive Bladder Cancer Treated with GreenLight Laser Vaporization. Photobiomodulation, Photomedicine, and Laser Surgery. 2019; 37: 312–317.

[15] Yürük E, Saitz TR, Gönültaş S, Şerefoğlu EC, Müslümanoğlu AY. Neutrophil-to-lymphocyte ratio is elevated in recurringnonmuscle invasive bladder cancer. Turkish Journal of Medical Sciences. 2017; 47: 234–239.

[16] Getzler I, Bahouth Z, Nativ O, Rubinstein J, Halachmi S. Preoperative neutrophil to lymphocyte ratio improves recurrence prediction of non-muscle invasive bladder cancer. BMC Urology. 2018; 18: 90.

[17] D'Andrea D, Moschini M, Gust K, Abufaraj M, Özsoy M, Mathieu R, *et al.* Prognostic Role of Neutrophil-to-Lymphocyte Ratio in Primary Non-muscle-invasive Bladder Cancer. Clinical Genitourinary Cancer. 2017; 15: e755–e764.

[18] Balan D, Martha O, Chibelean CB, Tataru S, Voidezan S, Sin A, *et al.* Comparison of 10-year overall survival between patients with G1 and G2 grade Ta bladder tumors. Medicine. 2018; 97: e0522.

[19] Favilla V, Castelli T, Urzì D, Reale G, Privitera S, Salici A, *et al.* Neutrophil to lymphocyte ratio, a biomarker in non-muscle invasive bladder cancer: a single-institutional longitudinal study. International Brazilian Journal of Urology. 2016; 42: 685–693.

[20] Mungan NA, Kiemeney LA, van Dijck JA, van der Poel HG, Witjes JA. Gender differences in stage distribution of bladder cancer. Urology. 2000; 55: 368–371.

[21] Santos F, Dragomir A, Kassouf W, Franco E, Aprikian A. Urologist referral delay and its impact on survival after radical cystectomy for bladder cancer. Current Oncology. 2015; 22: e20–e26.

[22] Noon AP, Albertsen PC, Thomas F, Rosario DJ, Catto JWF. Competing mortality in patients diagnosed with bladder cancer: evidence of undertreatment in the elderly and female patients. British Journal of Cancer. 2013; 108: 1534–1540.

[23] Cantiello F, Russo GI, Vartolomei MD, Farhan ARA, Terracciano D, Musi G, *et al.* Systemic Inflammatory Markers and Oncologic Outcomes in Patients with High-risk Non-muscle-invasive Urothelial Bladder Cancer. European Urology Oncology. 2018; 1: 403–410.

[24] Ferro M, Di Lorenzo G, Vartolomei MD, Bruzzese D, Cantiello F, Lucarelli G, *et al.* Absolute basophil count is associated with time to recurrence in patients with high-grade T1 bladder cancer receiving bacillus Calmette–Guérin after transurethral resection of the bladder tumor. World Journal of Urology. 2020; 38: 143–150.

[25] Minoli M, Kiener M, Thalmann GN, Kruithof-de Julio M, Seiler R. Evolution of Urothelial Bladder Cancer in the Context of Molecular Classifications. International Journal of Molecular Sciences. 2020; 21: 5670.

[26] Jalanko T, de Jong JJ, Gibb EA, Seiler R, Black PC. Genomic Subtyping in Bladder Cancer. Current Urology Reports. 2020; 21: 9.

[27] Li Q, Wang H, Peng H, Huang Q, Huyan T, Huang Q, *et al.* MicroRNAs: Key Players in Bladder Cancer. Molecular Diagnosis & Therapy. 2019; 23: 579–601.

[28] Wu Z, Zhu K, Liu Q, Liu Y, Chen L, Cui J, *et al.* Profiles of Immune Infiltration in Bladder Cancer and its Clinical Significance: an Integrative Genomic Analysis. International Journal of Medical Sciences. 2020; 17: 762–772.

[29] Tang X, Du P, Yang Y. The clinical use of neutrophil-to-lymphocyte ratio in bladder cancer patients: a systematic review and meta-analysis. International Journal of Clinical Oncology. 2017; 22: 817–825.

[30] Tan YG, Eu EWC, Huang HH, Lau WKO. High neutrophil-to-lymphocyte ratio predicts worse overall survival in patients with advanced/metastatic urothelial bladder cancer. International Journal of Urology. 2018; 25: 232–238.

[31] Kawahara T, Kato M, Tabata K, Kojima I, Yamada H, Kamihira O, *et al.* A high neutrophil-to-lymphocyte ratio is a poor prognostic factor for castration-resistant prostate cancer patients who undergo abiraterone acetate or enzalutamide treatment. BMC Cancer. 2020; 20: 919.