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Evaluation of Hematological Parameters in Testicular Tumors
Testis Tümörlerinde Hematolojik Parametrelerin Değerlendirilmesi

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Abstract
Objective: Recently, many hematological parameters have been frequently investigated as prognostic and predictive biomarkers for malignancies and inflammatory conditions, with the primary examples of these parameters being neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR), platelet-lymphocyte ratio (PLR), and mean platelet volume (MPV). In this study, we aimed to investigate the relationship between hematological parameters and prognosis in patients with a diagnosis of testicular malignancy (TM) by evaluating them in comparison with patients that underwent varicocelectomy as a control group.

Materials and Methods: A total of 187 patients that underwent radical inguinal orchiectomy due to the diagnosis of TM were included in Group 1 and 128 patients of similar age that underwent varicocelectomy using a similar incision in Group 2 as controls. Hematological and biochemical blood results were collected one day before radical orchiectomy. The parameters of NLR (neutrophil/lymphocyte counts), PLR (platelet/lymphocyte counts), and LMR (lymphocyte/ monocyte counts) were expressed as the ratios of indicated blood cell components. Following standard inguinal radical orchiectomy, T staging was performed based on the final histology samples, and clinical N and M staging using imaging modalities.

Results: The mean ages of the patients were 26.5±7.1, and 25.1±8.1 years for Groups 1 and 2, respectively. The neutrophil counts, NLR, and PLR were significantly higher in Group 1, while the lymphocyte count, MPV, and LMR were significantly higher in Group 2 ($p<0.001$ for all). LMR was also significantly higher in patients with T1 stage TM, and NLR in those with >T1 stage TM ($p<0.001$). There was no significant difference between the patients with T1 and >T1 in terms of MPV and PLR.

Conclusion: We determined NLR and LMR as parameters that can be used in the pathological staging of cases with TM. We consider that these hematological parameters have an important place in predicting the prognosis in this patient group.

Keywords: testicular malignancy, neutrophil-lymphocyte ratio, lymphocyte-monocyte ratio, platelet-lymphocyte ratio, hematological parameters

Öz
Amaç: Kanser prognozunu ve inflamatuar durumları öngörmek için hematolojik parametlerinin birçoğu son dönemlerde sıklıkla araştırılmaya başlanmıştır. Hematolojik parametlerden ise özellikle nötrofil-lenfosit oranı (NLO), lenfosit-monosit oranı (LMO), trombosit-lenfosit oranı (PLO) ve ortalama trombosit hacmi (MPV) bu alanda ön plana çıkmaktadır. Biz de çalışmamızda testis malignitesi (TM) tanısı alan hastalarda varikoselektomi yapılan hastalar kontrol grubu alınarak hematolojik parametlerle prognoz arasındaki ilişkiyi araştırmayı hedefledik.

Gereçler ve Yöntemler: Çalışmaya TM tanısı konan ve radikal inguinal orşiektomi uygulanan 187 hasta ve kontrol grubu olarak hem benzer insizyon hem de benzer yaş grubuna sahip varikoselektomi uygulanan 128 hasta dahil edildi. Hematolojik ve biyokimyasal kan sonuçları, radikal orşiektomiden 1 gün önce toplandı. NLO, nötrofil sayısının lenfosit sayısına, PLO, platelet sayısının lenfosit sayısına, LMO ise lenfosit sayısının monosit sayısına bölünmesiyle hesaplandı. Standart inguinal radikal orşiektomi sonrası nihai histolojide T evrelemesi, görüntüleme modaliteleri ile klinik olarak N ve M evrelemeleri belirlendi.

Bulgular: Grup 1'deki hastaların yaş ortalaması 26,5±7,1 yıl, Grup 2'deki hastaların ise 25,1±8,1 yıl olarak gözlenmiştir. Grup 1'de nötrofil sayısı, NLO ve PLO anlamlı saptanırken Grup 2'de ise lenfosit sayısı, MPV ve LMO ($p<0,001$, hepsi için) daha yüksek saptanmıştır. TM T1 evre hastalarda LMO anlamlı düzeyde daha yüksek iken >T1 olan hastalarda ise NLO daha yüksek olarak saptandı ($p<0,001$). MPV ve PLO açısından T1 ve >T1 olan hastalar arasında fark saptanmadı.

Sonuç: TM hastalarının patolojik evrelemesinde NLO ve LMO'larını kullanılabilecek parametreler olarak belirledik. Bu hematolojik parametrelerin TM hastaları için prognozu belirlemede önemli bir yerinin olduğunu düşünmekteyiz.

Anahtar kelimeler: testis kanseri, nötrofil-lenfosit oranı, lenfosit-monosit oranı, trombosit-lenfosit oranı, hematolojik parametreler

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Introduction

Testicular malignancy (TM) is the most common solid organ cancer in men aged 15-35 years and constitutes 1-1.5% of all male cancer cases [1]. Patient outcomes are very favorable with early diagnosis which increases the importance of diagnosis and treatment of testicular malignancies in their early-stages. The methods used in diagnosing TM are as follows: physical examination, imaging techniques, analysis of laboratory parameters, and tumor markers. In the literature, besides the studies showing the relationship between some hematological parameters with inflammation and tumorigenesis, the close relationship between the inflammatory response with tumorigenesis and invasion has also been demonstrated [2]. Inflammation plays a role in every step of carcinogenesis by various mechanisms [3]. Changes in the systemic inflammatory response can be evaluated using hematological parameters. Indeed, changes in C-reactive protein (CRP) levels, and the neutrophil-lymphocyte ratios (NLRs) are indicators of systemic inflammatory response in many malignancies [4]. The association of high NLR value with poor prognosis in some urological cancers has been shown in previous studies [5]. NLR is not only a marker of systemic inflammatory manifestations, but also a valuable predictor in many malignancies [6]. There is an increasing interest in easy-to-obtain hematological parameters, such as NLR to predict cancer prognosis and inflammatory conditions. NLR, LMR, PLR, and MPV can be used as prognostic predictive factors in many clinical conditions [7]. In the current study, we retrospectively examined these parameters that can be easily evaluated in clinical practice in patients diagnosed with TM in comparison with a control group of similar age that underwent varicocelectomy. Thus, we aimed to investigate the effects of these hematological parameters on the prognosis of TM.

Materials and Methods

Following the approval of Bakirkoy Dr. Sadi Konuk Training and Research Hospital Institutional Review Board (2022/86), we retrospectively reviewed the cancer registry database of our institution and identified patients diagnosed with TM between January 2012 and June 2021. The study included 187 patients that were diagnosed with TM based on physical examination, laboratory, and scrotal ultrasonography findings, who underwent radical inguinal orchiectomy, and 128 control subjects of similar age that underwent varicocelectomy through the inguinal incision. Patients with testicular stromal tumors, infectious or inflammatory symptoms and conditions, hematological diseases or other malignancies, cardiovascular diseases, end-stage renal disease, cerebrovascular disease, or diabetes mellitus, smokers, patients using corticosteroids or β-agonists, and those with missing preoperative data (complete blood count and tumor markers) were excluded.

In patients with TM, contrast-enhanced thoracoabdominal computed tomography (CT) was performed and serum tumor marker tests [alpha fetoprotein (AFP), lactate dehydrogenase (LDH), and beta-human chorionic gonadotropin (beta-HCG)] were carried out for staging after radical inguinal orchiectomy. After standard inguinal radical orchiectomy, T staging was performed on final histology samples, and clinical N and M staging was made using imaging modalities. Hematological and biochemical blood test results were collected one day before radical orchiectomy as part of our routine preoperative evaluation. Tumor markers were collected at one week and one month after radical orchiectomy to determine the final definitive staging.

Venous blood samples were taken into tubes containing ethylenediaminetetraacetic acid for laboratory tests. The neutrophil count, lymphocyte count, mean erythrocyte volume, and erythrocyte distribution width were measured using the Beckman Coulter DXH 780 device, and AFP, beta-hCG, and LDH were analyzed using Beckman Coulter DxI 800. NLR (neutrophil/ lymphocyte counts), PLR (platelet/lymphocyte counts, and LMR (lymphocyte/monocyte counts) were expressed as the ratios of indicated blood cell components.

Statistical Analysis

Categorical data were presented as numbers and percentages. Normally distributed numerical data were shown as mean and standard deviation, and non-normally distributed numerical data as median (interquartile range) values. The Kolmogorov-Smirnov test was used to test the normality of the distribution of numerical data. Student's t-test was conducted to compare normally distributed numerical data. The frequencies of categorical variables were compared with the Pearson chi-square and Fisher's exact tests. A p value below 0.05 was considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences version 21 (IBM SPSS Statistics; IBM Corp., Armonk, NY, USA).

Results

In this study, data of 187 patients with TM (Group 1) and 128 controls that underwent varicocelectomy (Group 2) were evaluated. The mean ages of the patients in Groups 1, and 2 were 26.5±7.1, and 25.1±8.1 years, respectively. The mean body mass indexes of the patients in Groups 1, and 2 were 23.4±6.8 kg/m^2 and 24.4±8.1 kg/m^2 respectively without any statistically significant intergroup differences. The median beta-HCG, AFP, and LDH values of the patients with TM were determined as 26.6 mIU/ml, 32.3 IU/ml, and 181 U/L, respectively. The demographic data of the patients are summarized in **Table 1**.

The neutrophil, lymphocyte, monocyte, platelet, MPV, NLR, LMR, and PLR values of the patients in Groups 1 and 2 were evaluated to compare hematological parameters. The neutrophil count, NLR, and PLR were statistically significantly higher in Group 1, while the lymphocyte count, MPV, and LMR were statistically significantly higher in Group 2 ($p<0.001$ for all). The data on comparison of hematological parameters are shown in **Table 2**.

Considering the pathological data of the patients with TM, seminoma was the most common histological type, seen at a rate of 58.3%, followed by mixed germ cell tumors. The tumor stage was T1 in 49.2%, T2 in 45.5%, and T3 in 5.3% of the patients. Lymph node metastasis was not observed in 48.1% of the patients. During the follow-up, metastases outside the lymph node were detected in 11.2% of the patients. **Table 3** presents

Table 1. Comparison of demographic data between the study groups and summary of tumoral data of Group 1

Parameters (mean ± SD)	Group 1 (n = 187)	Group 2 (n = 128)	p
Age (year)	26.5 ± 7.1	25.1 ± 8.1	0.126
Body mass index (kg/m^2)	23.4 ±6.8	24.4 ±8.1	0.187
Follow-up duration (month)	51.6 ± 22.1	48.3 ± 20.9	0.217
Tumor size (cm)	5.1 ± 2.4	-	-
Beta-HCG$^+$ (mlU/ml)	3.7 (26.6)	-	-
AFP$^+$ (lU/ml)	3.6 (32.3)	-	-
LDH$^+$ (U/L)	254 (181)	-	-

Group 1: patients with testicular malignancy; Group 2: control group; SD: standard deviation; HCG: human chorionic gonadotropin; AFP: alpha fetoprotein; LDH: lactate dehydrogenase

Table 2. Comparison of hematological data between the study groups

Neutrophil count (x10^{3}cells/mm^3)	6.2 ±2	4.1 ± 1.6	**<0.0001**
Lymphocyte count (x10^{3}cells/mm^3)	1.9 ± 0.8	2.3 ± 0.6	**<0.0001**
Monocyte count (x10^{3}cells /mm^3)	0.6 ± 0.2	0.6 ± 0.3	0.519
Platelet count (x10^{3}cells /mm^3)	364.7 ± 74.7	248.9 ± 61.9	0.06
MPV (fL)	8.4 ± 1.5	9.3 ± 1.7	**<0.0001**
NLR	3.8 ± 2.7	1.8 ± 0.7	**<0.0001**
LMR	3.2 ± 1.6	4.3 ± 1.7	**<0.0001**
PLR	156.9 ±66.3	110.4 ± 32.2	**<0.0001**

Group 1: patients with testicular malignancy; Group 2: control group; SD: standard deviation; MPV: mean platelet volume; NLR: neutrophil-lymphocyte ratio, LMR: lymphocyte-monocyte ratio; PLR: platelet-lymphocyte ratio

Table 3. Summary of pathological and follow-up results of Group 1

Parameters (mean ± SD)	Group 1 (n = 187)
Pathology result (n; %)	
Seminoma	109 (58.3)
Mixed germ cell tumor	62 (33.2)
Choriocarcinoma	3 (1.6)
Yolk sac tumor	1 (0.5)
Embryonal carcinoma	7 (3.7)
Teratoma	5 (2.7)
Tunica Vaginalis invasion (n; %)	25 (13.4)
Rete testis invasion (n; %)	63 (33.7)
Lymphovasculer invasion (n; %)	121 (64.7)
Tunica Albuginea Invasion (n; %)	72 (38.5)
T stage (n; %)	
T1	92 (49.2)
T2	85 (45.5)
T3	10 (5.3)
Clinical stage (n; %)	
1A	41 (21.9)
1B	50 (26.7)
2A	52 (27.8)
2B	12 (6.4)
2C	7 (3.7)
3A	5 (2.6)
3B	11 (5.8)
3C	9 (4.8)
Lymph node stage (n; %)	
0	92 (49.2)
N1	53 (28.3)
N2	27 (14.4)
N3	17 (9.1)
Metastasis (n; %)	21 (11.2)

the data on the pathological and follow-up results of the patients with TM.

The relationship between the hematological data and T stages was compared. LMR was statistically significantly higher in the patients with T1 stage TM, while NLR was statistically significantly higher in those with >T1 pathologies. There was no significant difference between the T1 and >T1 cases in terms of MPV and PLR. The hematological data of the patients and their comparisons are given in **Table 4**.

Discussion

Recently, it has been increasingly discussed whether inflammatory markers among hematological parameters can be independent as prognostic factors in cancer patients. Among these parameters, NLR, PLR, and LMR have been extensively investigated, and their relationships with the development and progression of malignancies have been emphasized [4]. Immune mechanisms including the promoter role of neutrophils as inflammatory markers in tumorigenesis and the presence of antitumoral activity of lymphocytes have been associated with malignancies. It has been stated that platelets can prevent the death of cancer cells by natural killer cells and secrete angiogenic and tumor growth factors to induce cancer growth, progression, and metastasis [8,9]. Many recent studies have shown the role and usability of these inflammatory parameters in urinary system malignancies, such as prostate, bladder, and kidney cancers [10-13]. However, despite the relative predictive values of NLR, PLR, LMR, and MPV in other malignancies, its value in TM is not yet fully understood, which may be due to its lower prevalence. In this study, we aimed to define the potential relationship between hematological parameters based on preoperative complete blood count analysis and TM in patients that underwent radical orchiectomy by comparing them to patients that underwent varicocelectomy performed from the same site through a similar incision as the control group.

Table 4. Comparison of hematological parameters between the patients with T1 and >T1 pathologies in the testicular malignancy group

	T1 (n = 92) Mean± SD (median)	>T1 (n = 95) Mean± SD (median)	p
MPV	8.7 ± 1.6 (8.7)	8.1 ± 1.3 (7.9)	0.113
NLR	2.8 ± 1.8 (2.3)	4.7 ± 3.1 (4)	**<0.001**
LMR	3.7 ± 1.8 (3.3)	2.6 ± 1.3 (2.3)	**<0.001**
PLR	149 ± 93.7 (118.4)	164 ±78.6 (153.6)	0.06

SD: standard deviation; MPV: mean platelet volume; NLR: neutrophil-lymphocyte ratio, LMR: lymphocyte-monocyte ratio; PLR: platelet-lymphocyte ratio

MPV is not just a marker reflecting platelet counts. It is defined as an index of bioactive platelets that are activated and participate in the inflammatory process [14]. Yun et al. [15] reported that reduced MPV showed poor prognosis in renal cell cancer. However, Sahin et al., [16] stated that MPV could not be used as a prognostic factor in patients with stage T1-T2-T3 TM. In our study, when the control and TM groups were compared, MPV was found to be statistically significantly lower in patients with TM, but without any statistically significant difference between stage T1 and >T1 cases in terms of MPV values. Although MPV was lower in patients with TM, we do not think that it can be used as a prognostic factor in this malignancy.

In a meta-analysis of 100 studies including 40,559 patients, Templeton et al., [6] showed a strong association between NLR and the presence of more than 20 solid tumors. Although this analysis includes a very heterogeneous group of malignancies, it reflects the essential role of NLR in inflammation and immune suppression in cancer biology. Yuksel et al. [17] compared preoperative NLR values between patients with localized testis germ cell tumors and those with varicoceles as controls and found that NLR was significantly higher in patients with TM. Jankovich et al. [18] compared NLR values between patients with T1 and >T1 pathologies and determined higher NLR values in those with >T1 cancer. In our study, in which we also included the patients that underwent varicocelectomy in the control group, we found the NLR value to be statistically significantly higher in the patients with TM. In addition, the patients with a >T1 pathology result had significantly higher NLR values. Based on these findings, we can state that NLR is increased in patients with TM compared to healthy individuals and in those with stage >T1TM.

In a meta-analysis of 17 studies including 5,552 patients, Li et al., [19] determined that high preoperative LMR was associated with a favorable prognosis and concluded that it could be a potential prognostic biomarker in patients with urological cancer. However, in the literature, studies investigating the relationship between TM and LMR are very limited in number. Herraiz-Raya et al. [20] found that a LMR value of >3 in patients with TM indicated smaller tumor volume and lower cancer stage. In our study, lower LMR values were found in the patients with TM than in the varicocele control group. We also determined that LMR values statistically significantly differed between the patients with T1 and >T1 pathological stages. These results suggest that LMR decreases in patients with TM and in those with a higher pathological stage.

In their meta-analysis, Li et al., [19] detected lower PLR values in healthy individuals than in patients with urological tumors except those with bladder cancer. In addition, the authors observed that survival rates were lower in patients with higher PLR values. In another study where TM and varicocele groups were compared, Sahin et al., [16] could not find any significant difference between both groups in terms of PLR values. In the current study, we detected a statistically significantly higher PLR values in TM group compared to the healthy varicocele group, but we did not find a statistically significant difference between cases in stages T1 and >T1 regarding PLR values. Therefore, we consider that increased PLR can be used as a parameter in patients with TM, but it cannot provide information about staging.

The limitations of our study include its retrospective and single-center design, limited sample size, and absence of a prognostic predictive analysis.

Conclusion

Previous studies have mostly concerned with NLR, and only few have examined LMR and PLR. In light of our results, we can state that while hematological parameters in general can be used as prognostic, and predictive markers in patients with TM, NLR and LMR can be used in the pathological staging of these patients. Larger and randomized controlled studies are needed to obtain more conclusive results.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of University of Health Sciences, Dr. Sadi Konuk Training and Research Hospital (approval date and number: 07.03.2022/86).
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