

Comparison of hematological markers between testicular torsion and epididymo-orchitis in acute scrotum cases

Akut skrotumda testis torsiyonu ve epididimo-orşit arasındaki hematolojik belirteçlerin karşılaştırılması

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Özet

Amaç: Bu çalışmada, akut skrotum ile başvuran hastalarda testis torsiyonu (TT) ve epididimo-orşit (EO) arasındaki ayırıcı tanıyı belirlemede hematolojik sonuçların yararını araştırmayı ve TT tanısı için prediktif değerini belirlemeyi amaçladık.

Gereç ve Yöntemler: Akut skrotuma bağlı şikayetler ile enstitümüzün üroloji kliniklerine veya acil servisine başvuran 98 olguyu retrospektif olarak inceledik. Çalışmaya TT'ye bağlı orşiektomi veya detorsiyon uygulanan 32 hasta ve EO'lu 48 hasta alındı. Kontrol grubu 80 sağlıklı erkekten oluşuyordu. Gruplar yaş, beyaz kan hücresi (WBC), ortalama trombosit hacmi (MPV), nötrofil/lenfosit oranı (NLR), monosit/lenfosit oranı (MLR) ve trombosit/lenfosit oranı (PLR) gibi hematolojik parametreler açısından karşılaştırıldı.

Bulgular: TT grubunu diğer gruplardan ayırt etmede WBC, MPV ve NLR istatistiksel olarak anlamlı bulundu, sırasıyla; (AUC = 0.732, %95 CI: 0.647-0.816 ve p <0.001), (AUC = 0.720, %95 CI: 0.615-0.825 ve p <0.001), (AUC = 0.629, %95 CI: 0.519-0.739 ve p = 0.024). TT grubu içindeki detorsiyon ve orşiektomi alt grupları arasındaki karşılaştırmada, ilki istatistiksel olarak daha düşük monosit sayısına (p = 0,005) ve MLR düzeyine (p = 0,038) sahipti.

Sonuç: Hematolojik parametrelerin; yani, tam kan sayımı analizinden kolaylıkla belirlenebilen WBC, MPV ve NLR, TT'yi tahmin etmek için Doppler ultrasonografiye benzer şekilde yüksek duyarlılığa ve özgüllüğe sahipti. Ayrıca TT cerrahisinde orşiektomi veya detorsiyon kararında güçlük çekilen olgularda monosit ve MLR düzeylerinin faydalı olabileceğini düşünüyoruz.

Anahtar Kelimeler: akut skrotum, testis torsiyonu, epididimo-orşit, ortalama trombosit hacmi, nötrofil/lenfosit oranı

Abstract

Objective: In this study, we aimed to investigate the benefit of hematological results in determining the differential diagnosis between testicular torsion (TT) and epididymo-orchitis (EO) in patients presenting with acute scrotum, and to determine its predictive value for the diagnosis of TT.

Material and Methods: We retrospectively analyzed 98 patients who applied to our institute's urology clinics or emergency service with complaints of acute scrotum. Thirty-two patients who underwent orchiectomy or detorsion due to TT and 48 patients with EO were included in the study. The control group consisted of 80 healthy men. Groups were compared in terms of hematological parameters such as age, white blood cell (WBC), mean platelet volume (MPV), neutrophil/lymphocyte ratio (NLR), monocyte/lymphocyte ratio (MLR), and platelet/lymphocyte ratio (PLR).

Results: WBC, MPV and NLR were found to be statistically significant in distinguishing TT group from other groups, respectively; (AUC = 0.732, 95% CI: 0.647-0.816 and p<0.001), (AUC = 0.720, 95% CI: 0.615-0.825 and p<0.001), (AUC = 0.629, 95% CI: 0.519-0.739 and p=0.024). In the comparison between the detorsion and orchiectomy subgroups within the TT group, the first had a statistically lower monocyte count (p=0.005) and MLR level (p=0.038).

Conclusion: Hematological parameters; that is, WBC, MPV and NLR, which can be easily determined from complete blood count analysis, had high sensitivity and specificity similar to Doppler ultrasonography to predict TT. In addition, we think that monocyte and MLR levels may be beneficial in patients who have difficulty in the decision of orchiectomy or detorsion in TT surgery.

Keywords: acute scrotum; epididymo-orchitis; mean platelet volume; neutrophil/lymphocyte ratio; testicular torsion.

This study was approved by the Non-invasive Clinical Research Ethics Committee of Adıyaman University (Approval number: 2020/11-16). All research was performed in accordance with relevant guidelines/regulations, and informed consent was obtained from all participants.

INTRODUCTION

Acute scrotum is a clinical entity that manifests with sudden onset pain, swelling, and redness in the inguinoscrotal region due to various etiological causes (1,2). Many inguinoscrotal pathologies can cause similar clinical conditions, and therefore testicular torsion (TT), epididymo-orchitis (EO), torsion of testicular appendix or appendix epididymis, inguinal hernia, hydrocele, trauma, idiopathic scrotal edema, varicocele, testicular tumor, and hematological malignancies should be considered for differences in determination of acute scrotum (3). Although TT is not the most frequent cause among the pathologies resulting in acute scrotum, it is the most significant with regard to its consequences. In particular, delayed diagnosis or misdiagnosis leads to permanent ischemic damage and necrosis due to the torsion of the spermatic cord. Due to the potential loss of the testis, testicular torsion should always be considered first in all cases presenting with acute scrotum (1,2).

TT is most frequently confused with EO. Along with the clinical and physical examination findings, demonstration of the blood flow using Doppler ultrasonography (US) and scintigraphy is very useful in the differential diagnosis of TT and EO since the blood flow is reduced in the former and increased in the latter. However, these imaging techniques may not be available at all times or may delay diagnosis due to various reasons. Therefore, there is ongoing research for new rapid, inexpensive, and widely available methods that can be effectively used distinguishing these complications.

In recent years, there has been a growing interest in several inflammatory markers, such as the mean platelet volume (MPV), white blood cell (WBC), red cell distribution width (RDW), platelet distribution width (PDW), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and monocyte/lymphocyte ratio (MLR), which are suggested to be elevated in various diseases (4-6). However, the only a few studies have examined these factors for the differential diagnosis of TT and EO (7-10).

In this report, we wanted to determine the effectiveness of hematological factors as predictors of TT and EO in patients presenting with acute scrotum. In addition, we aimed to determine whether these factors could be predictors of TT.

MATERIAL AND METHODS

Ethics committee approval was received for this study from the ethics committee of Adiyaman University, Faculty of Medicine (Approval number: 2020/11-16). We retrospectively reviewed 98 cases who had presented to the urology clinics or emergency unit of our institute with complaints related to acute scrotum from January 2012 to January 2019. In order to perform differential diagnosis in patients with acute scrotum presenting with symptoms, such as sudden onset pain, swelling, and redness in the inguinoscrotal area, laboratory studies (complete blood count, C-reactive protein, erythrocyte sedimentation rate, etc.) and color Doppler US were requested. The diagnoses of TT and EO were made by the detection of a reduced and increased blood flow, respectively according to the results of the scrotal color Doppler US examination. Patients diagnosed with TT were urgently operated. If the testis preserved its vitality, detorsion was applied, if it lost, orchiectomy was performed.

Blood was collected and examined within 1 hr of collection. A complete blood work-up, including WBC, RDW, PDW, PCT, and MPV was undertaken, and NLR, PLR, and MLR were determined. These factors were analyzed between the groups.

Eighteen cases were excluded from the study due to the time from the onset of scrotal pain and admission to the hospital being longer than six hours or other reasons, including malignancies, hematological problems, immunosuppression, or cerebrovascular and cardiovascular diseases. The control group consisted of healthy men that presented to our urology clinics for any reason without hepatic, renal or hematological disease and with no history of EO, testicular trauma, or scrotal surgery.

Statistical Analysis

Distribution of continuous variables was determined by the Kolmogorov-Smirnov test and homogeneity of variances was determined using a Levene test. Continuous variable analysis was expressed as mean \pm standard deviation (SD) or median (the 1st quartile (Q1) – the 3rd quartile (Q3)) values. The mean differences between more than two independent groups were determined by ANOVA; Kruskal-Wallis test was done for the comparison of continuous variables where

the parametrical test assumptions were not met. If the p-value of these two assumptions was statistically significant, a post-hoc Tukey HSD or Dunn-Bonferroni multiple comparison test was used. The comparison between detorsion and orchietomy groups was done by Student's t-test or Mann-Whitney U test. In order to determine the predictor(s) that best discriminated the TT group from the other EO and control groups, a multinomial logistic regression analysis using backward stepwise was undertaken. Any variable with p-value of <0.25 in the univariable test was accepted as a candidate for the multivariable model. Statistics was done using IBM SPSS Statistics v17 (IBM, NY, USA). A p-value less than 0.05 was considered statistically significant.

RESULTS

The study included 32 patients with TT, 48 patients with EO, and 80 healthy men. Comparison of demographic and laboratory values between groups is shown in Table 1. WBC, neutrophil, monocyte, NLR, MLR levels of TT and EO groups were significantly higher than the control group (p <0.001 and p <0.001, respectively). The MPV level for the TT group was lower than the controls (p <0.001). The area under the ROC curve (AUC) for WBC, MPV, NLR were found to be statistically significant in distinguishing the TT group from other groups, respectively; (AUC = 0.732, 95% CI: 0.647-0.816 and p <0.001), (AUC = 0.720, 95% CI: 0.615-0.825 and p <0.001), (AUC = 0.629,

95% CI: 0.519-0.739 and p = 0.024) (Table 2, Figure 1). In distinguishing TT from the remaining groups, the best cut-off point for WBC was determined to be 9.42. Based on this value, WBC had a sensitivity and specificity of 81.3%, and 69.5%, a PPV of 39.4%, and NPV of 93.8%, and its diagnostic accuracy rate was determined as 71.8%. For MPV, the best cut-off value was found to be 7.591, at which this parameter had a sensitivity and specificity of 65.6% and 76.3%, PPV of 40.4%, NPV of 90.1%, diagnostic accuracy rate of 74.2% in predicting TT. For NLR, the best cut-off value was found to be 4.031, at which this parameter had a sensitivity and specificity of 53.1% and 77.1%, PPV of 36.2%, NPV of 87.1%, diagnostic accuracy rate of 72.4% in predicting TT (Table 3).

WBC had a higher cut-off value than 9.42 in distinguishing the control and TT groups, which increased the probability of TT by 15.859 times, independent of the remaining factors (95% CI: 3.848-65.355) (p<0.001). MPV also had a lower cut-off value than 7.591 in distinguishing the control and TT groups, which increased the probability of TT by 6.263 times, independent of the remaining factors (95% CI:1.713-22.903) (p=0.006). In addition, TT was seen at a higher rate among patients with an NLR level greater than 4.031 (OR = 9.003, 95% CI: 1.318-61.485 and p = 0.025) (Table 4). In the comparison between the detorsion and orchietomy sub-groups within the TT group, the former had a statistically lower monocyte count (p = 0.005) and MLR level (p = 0.038) (Table 5).

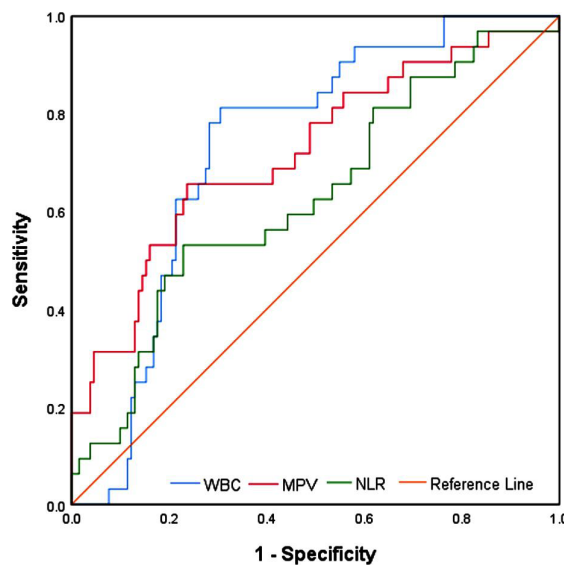


Figure 1. ROC curve for WBC, MPV, and NLR in distinguishing testicular torsion group

Table 1. Demographical and laboratory measurements regarding for groups

Variable	Controls (n=80)	Epididymo-orchitis (n=48)	Testicular torsion (n=32)	p-value
Age	25.2±3.5 ^a	43.4±15.5 ^{a,b}	21.2±9.9 ^b	<0.001†
WBC	7.3 (6.2-8.0) ^{a,c}	12.6 (8.6-16.8) ^a	11.4 (9.7-4.3) ^c	<0.001‡
RDW	12.4 (11.5-13.4)	12.9 (11.6-14.2)	12.3 (11.6-13.1)	0.336‡
Platelet count	246.6 (206.0-278.8) ^d	251.1 (201.8-318.3)	266.2 (228.5-322.3) ^d	0.047‡
PDW	19.8 (18.3-20.7)	19.2 (18.3-20.4)	18.6 (17.6-20.2)	0.184‡
PCT	0.20 (0.17-0.27)	0.21 (0.16-0.24)	0.19 (0.17-0.25)	0.959‡
MPV	8.9 (7.8-9.8) ^c	7.8 (7.2-9.7)	7.2 (6.5-8.4) ^c	<0.001‡
PLR	103.7 (82.3-160.5)	126.4 (92.8-172.2)	127.3 (86.4-175.0)	0.099‡
Neutrophil	4.3 (3.1-5.2) ^{a,c}	8.9 (5.9-12.5) ^a	8.6 (5.3-11.6) ^c	<0.001‡
Lymphocyte	2.4 (1.6-2.6)	1.9 (1.4-2.6)	2.1 (1.8-2.9)	0.229‡
NLR	1.8 (1.2-2.7) ^{a,c}	4.6 (3.0-7.7) ^a	4.1 (1.9-5.9) ^c	<0.001‡
Monocyte	0.5 (0.4-0.6) ^{a,c}	0.8 (0.7-1.1) ^a	0.7 (0.5-0.8) ^c	<0.001‡
MLR	0.2 (0.2-0.3) ^{a,c}	0.5 (0.3-0.6) ^a	0.4 (0.2-0.4) ^c	<0.001‡

WBC: White blood cell, RDW: Red cell distribution width, PDW: Platelet distribution width, PCT: Platelet Crit, MPV: Mean platelet volume, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio,

† One-Way ANOVA, data were shown as mean ± SD, ‡ Kruskal Wallis test, data were expressed as median (Q₁-Q₃),

a: Controls vs Epididymo-orchitis (p<0.001), **b:** Epididymo-orchitis vs Testicular torsion (p<0.001),

c: Controls vs Testicular torsion (p<0.001), **d:** Controls vs Testicular torsion (p=0.043).

Table 2. The results of ROC curve analyses for laboratory measurements in distinguishing testicular torsion

Variable	AUC	95% CI		p-value
		Lower limit	Upper limit	
WBC	0.732	0.647	0.816	<0.001
RDW	0.528	0.421	0.635	0.623
Platelet count	0.626	0.520	0.732	0.028
PDW	0.590	0.481	0.700	0.114
PCT	0.516	0.406	0.627	0.775
MPV	0.720	0.615	0.825	<0.001
PLR	0.564	0.446	0.682	0.261
Neutrophil	0.689	0.590	0.788	<0.001
Lymphocyte	0.535	0.418	0.651	0.543
NLR	0.629	0.519	0.739	0.024
Monocyte	0.626	0.516	0.737	0.027
MLR	0.604	0.507	0.702	0.068

WBC: White blood cell, RDW: Red cell distribution width, PDW: Platelet distribution width, PCT: Platelet Crit, MPV: Mean platelet volume, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, AUC: Area under the curve, CI: Confidence interval.

Table 3. The best cut-off points for laboratory measurements and diagnostic performance in order to discriminate testicular torsion group

Variable	Cut-off point	Sensitivity	Specificity	PPV	NPV	Accuracy
WBC	>9.42	81.3%	69.5%	39.4%	93.8%	71.8%
Platelet count	>245.5	75.0%	48.9%	26.4%	88.9%	54.0%
MPV	<7.591	65.6%	76.3%	40.4%	90.1%	74.2%
Neutrophil	>7.437	62.5%	75.6%	38.5%	89.2%	73.0%
NLR	>4.031	53.1%	77.1%	36.2%	87.1%	72.4%
Monocyte	>0.526	84.4%	42.7%	26.5%	91.8%	51.0%
MLR	>0.362	53.1%	70.2%	30.4%	86.0%	66.8%

WBC: White blood cell, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte- to-lymphocyte ratio, PPV: Positive predictive value, NPV: Negative predictive value.

Table 4. The results of multi-nominal logistic regression analysis

Variable	Odds ratio	95% Confidence interval		Wald	p-value
		Lower limit	Upper limit		
Control vs TT					
Age	1.001	0.911	1.100	0.001	0.982
WBC>9.42	15.859	3.848	65.355	14.632	<0.001
MPV<7.591	6.263	1.713	22.903	7.691	0.006
NLR>4.031	9.003	1.318	61.485	5.026	0.025
EO vs TT					
Age	0.828	0.759	0.903	18.330	<0.001
WBC>9.42	1.495	0.291	7.680	0.232	0.630
MPV<7.591	4.152	1.040	16.580	4.062	0.044
NLR>4.031	0.505	0.109	2.328	0.768	0.381

WBC: White blood cell, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, TT: Testicular torsion, EO: Epididymo-orchitis.

Table 5. Demographical and laboratory measurements regarding for testicular torsion sub-groups

Variable	Detorsion (n=23)	Orchiectomy (n=9)	p-value
Age	20.8±8.8	22.0±12.9	0.769†
WBC	11.0 (7.9-13.7)	13.0 (11.1-15.4)	0.145‡
RDW	12.3 (11.7-12.8)	13.8 (11.2-15.9)	0.433‡
Platelet count	263.0 (222.7-309.0)	273.4 (231.1-432.5)	0.386‡
PDW	18.8 (18.1-20.3)	17.9 (16.9-19.7)	0.133‡
PCT	0.19 (0.17-0.25)	0.20 (0.17-0.25)	0.837‡
MPV	7.3 (6.5-8.8)	6.7 (5.9-7.4)	0.170‡
PLR	126.2 (107.0-168.9)	142.8 (73.3-222.6)	0.681‡
Neutrophil	8.6 (5.3-10.5)	8.1 (6.4-12.5)	0.681‡
Lymphocyte	2.2 (1.8-2.6)	2.0 (1.7-3.6)	0.621‡
NLR	4.0 (1.9-5.9)	4.9 (2.0-6.2)	0.773‡
Monocyte	0.6 (0.5-0.8)	1.0 (0.7-1.3)	0.005‡
MLR	0.3 (0.2-0.4)	0.4 (0.3-0.9)	0.038‡

WBC: White blood cell, RDW: Red cell distribution width, PDW: Platelet distribution width, PCT: Platelet Crit, MPV: Mean platelet volume, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, † Student's t test, data were shown as mean ± SD, ‡ Mann Whitney U test, data were expressed as median (Q1 - Q3).

DISCUSSION

Acute scrotum is used to refer to all pathologies of scrotal organs that require urgent medical or surgical treatment. The two most important pathologies resulting in acute scrotum manifestation are TT and EO, which need to be clearly differentiated since urgent surgical treatment is indicated for the former. Failure to diagnose TT is among major medicolegal issues in Turkey (11). Although TT can be seen at any age, it is more common in males below the age of 25 years (3). In this report, the mean age in the TT group was 21.2 ± 9.9 years. The mean age was lower in the control and TT groups.

In acute scrotum cases, early differential diagnosis is vital for avoiding unnecessary surgical interventions, as well as not overlooking the diagnosis of important conditions, such as TT. Although differential diagnosis is made mostly based on clinical findings, the use of scrotal color Doppler US is currently the most common approach with a sensitivity ranging from 63% to 86% and specificity of 97 to 100% (8). However, there is ongoing research for new rapid, inexpensive, and convenient diagnostic methods due to the operator-dependent nature of US, the long procedure time (30 to 40 minutes) even at well-equipped hospitals, and diagnostic complications in certain cases (12).

Since pathologies are known to lead to acute scrotum involve inflammatory processes, hematological factors associated with systemic inflammation have been studied to facilitate diagnosis. Several studies have documented an elevated leukocyte count as a predictor of inflammation in patients diagnosed with TT (13).

Bitkin et al. reported a significantly increased leukocyte count in both EO and TT groups in comparison with a control population, but noted that this parameter did not have a predictive value for the differential diagnosis of these two conditions (8). Similarly, Yucel et al. reported increased leukocytes in the TT and EO cohorts compared to the control group. Additionally, they found that the monocyte count alone showed significant differences that could be used to distinguish between TT and EO (7). In our study, we found that the leukocyte levels of TT and EO groups were significantly higher compared to the control group. In distinguishing

TT from the remaining groups, the best cut-off point for WBC was determined to be 9.42. Based on this value, WBC had a sensitivity and specificity of 81.3%, and 69.5%, a PPV of 39.4%, and NPV of 93.8%, and its diagnostic accuracy rate was determined as 71.8%. WBC had a higher cut-off value than 9.42 in distinguishing the control and TT groups, which increased the probability of TT by 15.859 times, independent of the remaining factors.

Gunes et al. showed a significantly increased platelet count in the TT group when compared to the controls, which is consistent with our findings (9). However, we were not able to determine a statistical difference for the TT and EO groups with regards to the WBC count, RDW, platelet count, and PDW or PCT levels.

MPV, NLR, PLR, and MLR have been reported to be indicators of inflammatory response (9), and they can be preoperatively determined by a complete blood count analysis. These markers are inexpensive, easy to calculate, and practical, and therefore they have been widely adopted in clinical use. Bitkin et al. and Cicek et al. reported significantly higher MPV levels in TT cases compared to healthy individuals (8,14). In contrast, Gunes et al. (9) and Yucel et al. (7) did not find any changes in MPV between TT and control groups. In the current study, the MPV for the TT group was significantly reduced compared to controls. For MPV to distinguish the TT group from other groups, the best cut-off value was determined as 7.591, at which MPV had a sensitivity and specificity of 65.6% and 76.3%, PPV of 40.4%, NPV of 90.1%, and diagnostic accuracy rate of 74.2%.

Güneş et al. examined 75 subjects with TT versus 56 healthy controls. The authors reported that NLR had a sensitivity and specificity of 84% and 92%, respectively for predicting TT. In addition, PLR had a sensitivity and specificity of 51% and 89%, respectively for TT prediction (9). In another study, Bitkin et al. did not find any statistical difference for the TT and controls in terms of PLR; however, the comparison of the TT and EO groups presented significant differences (8). Yucel et al. showed that there was no change between the TT and EO groups in terms of NLR and PLR, but that these two parameters were significantly elevated compared to

the controls. Furthermore, they reported that MLR had a sensitivity and specificity of 55% and 73%, respectively, for predicting TT (7).

Zhu et al. reported in evidence from a systematic review and meta-analysis identified that WBC, PLT and NLR were different between TT, EO patients and healthy controls, and they can be critical factors for TT diagnosis. They reported that TT patients had higher WBC and NLR than healthy controls. Meanwhile, TT patients had lower NLR and PLT compared to EO patients. WBC is an useful parameter for diagnosing both TT and EO, but it cannot be used in differentiating the two diseases. They reported that NLR is beneficial parameter for differential diagnosis between TT and EO, that PLT can also be utilised in differential diagnosis among young patients (15).

In our study, the NLR of the TT and EO groups were higher compared to the controls. The best cut-off for NLR was 4.031 in distinguishing TT from the other groups. At this value, the sensitivity, specificity, PPV, NPV, and diagnostic accuracy of NLR were 53.1%, 77.1%, 36.2%, 87.1%, and 72.4%, respectively. In the present study, in the comparison between the detorsion and orchiectomy sub-groups within the TT group, the former had a significantly monocyte count and MLR level than the latter. We believe that these parameters can be helpful in cases that present with difficulties in the differentiation between orchiectomy and detorsion to determine the necessity of TT surgery.

Limitations of the study mostly related to the retrospective nature of the study and that the data were collected from a single center database. Therefore, the number of patients is low

Furthermore, we did not evaluate some of the acute phase reactants, such as serum amyloid A and procalcitonin since they are not routinely examined in every patient due to their high cost.

CONCLUSION

In this study, we found that the hematological parameters such as WBC, MPV and NLR, which can be readily determined from a complete blood count, had high sensitivity and specificity for TT prediction, similar to those of Doppler US. Therefore, we recommend the use of these parameters in the diagnosis of TT. Fur-

thermore, we consider that monocytes and MLR levels can be helpful in cases that present with difficulties in the decision of orchiectomy or detorsion in TT surgery. Nevertheless, prospective, randomized, multi-centered studies and large-scale meta-analyses are necessary to confirm the safe clinical utility of these parameters.

Conflict of Interest

The authors declare to have no conflicts of interest.

Financial Disclosure

The authors declared that this study has received no financial support.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

The study was approved by the Non-invasive Clinical Research Ethics Committee of Adiyaman University (Approval number: 2020/11-16) and written informed consent was received from all participants. The study protocol conformed to the ethical guidelines of the Helsinki Declaration.

Author Contributions

Conception and design; CB, AÇ, Data acquisition; CB, AÇ, Data analysis and interpretation; CB, AÇ, Drafting the manuscript; CB, AÇ, Critical revision of the manuscript for scientific and factual content; CB, AÇ, Statistical analysis; CB, AÇ, Supervision; CB, AÇ.

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