

**THROMBOCYTE AND ERYTHROCYTE INDICES IN SEPSIS  
AND DISSEMINATED INTRAVASCULAR COAGULATION****TROMBOCITNI I ERITROCITNI INDEKSI U SEPSI  
I DISEMINOVANOJ INTRAVASKULARNOJ KOAGULACIJI**

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**Summary:** Sepsis is the inflammatory response against infection. The existence of DIC during sepsis indicates a poor prognosis and coagulation abnormalities and thrombocytopenia may exist. The aim of this study was to investigate platelet and erythrocyte indices in sepsis patients with DIC and without DIC. In both groups coagulation tests, platelet count and indices, erythrocyte count and indices were retrospectively analysed. In the sepsis plus DIC patients the prothrombin time and D-dimer values were found significantly higher and fibrinogen, platelet and plateletcrit were found significantly lower than in the sepsis without DIC group. The analysis of mean platelet volume, platelet distribution width, erythrocyte count and indices revealed no significant differences between the two groups. These results showed us that the depression of bone marrow in septic patients with DIC and without DIC did not differ. The activation of the coagulation system might probably be the cause of thrombocyte depletion in DIC.

**Keywords:** sepsis, disseminated intravascular coagulation, platelet (thrombocyte) count, platelet (thrombocyte) indices, erythrocyte indices

**Kratak sadržaj:** Sepsa predstavlja inflamatorni odgovor na infekciju. Prisustvo diseminovane intravaskularne koagulacije (DIK) tokom sepse ukazuje na lošu prognozu, a mogu se javiti i poremećaji u koagulaciji i trombocitopenija. Cilj ove studije bio je da se ispituju trombocitni i eritrocitni indeksi kod pacijenata sa sepsom i DIK, odnosno sepsom bez DIK. U obe grupe retrospektivno su analizirani testovi koagulacije, broj i indeks trombocita i broj i indeks eritrocita. Kod pacijenata sa sepsom i DIK, protrombinsko vreme i vrednosti D-dimera bili su značajno povišeni, dok su fibrinogen, trombociti i trombocitokrit bili značajno niži nego u grupi sa sepsom bez DIK. Prilikom analize srednje zapremine trombocita, širine distribucije trombocita i broja i indeksa eritrocita nisu otkrivene značajne razlike između dve grupe. Ovakvi rezultati ukazuju na to da kod pacijenata sa sepsom i DIK i pacijenata sa sepsom bez DIK nema razlika u depresiji koštane srži. Aktivacija koagulacijskog sistema predstavlja mogući uzrok manjka trombocita u DIK.

**Ključne reči:** sepsa, diseminovana intravaskularna koagulacija, broj trombocita, trombocitni indeksi, eritrocitni indeksi

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**Introduction**

Disseminated intravascular coagulation (DIC) is a thrombohemorrhagic disorder secondary to an underlying clinical condition (1). This is a frequent complication among patients in the intensive care unit and has a high rate of mortality (2). DIC might emerge in patients with sepsis, malignancy, trauma, hepatocellular disorders, vascular abnormalities, placental

abruption, amniotic fluid emboli, preeclampsia, toxicities and major transfusion reactions (3, 4). Sepsis has been defined as the systemic inflammatory reaction against infection and comes first among the causes of DIC (3, 5, 6). Cytokines secreted in sepsis lead to an increase in thrombin formation via release of tissue factor (TF) from the endothelium and monocytes, and disruption of the fibrinolytic system via stimulation of plasminogen activator inhibitor-1 (PAI-I) from the endothelium and platelets (7, 8). The anticoagulant system is disrupted in septic patients due to the low levels of the thrombin inhibitor anti-thrombin III, and activation of Protein C which is the inhibitor of Factor Va and VIIIa (7, 8).

Platelet numbers are mildly decreased and sub-clinical prolongation is observed in coagulation tests along with a picture of DIC characterized by systemic intravascular fibrin deposition, microvascular dysfunction, bleedings and organ failures (9). Low platelet count, low fibrinogen levels, increased fibrin degradation products including D-dimer and prolongation of coagulation tests are used in establishing a diagnosis of DIC in cases of thrombosis and/or bleeding and/or multiorgan failure (3, 10). DIC has a complex pathogenesis which has been reported to include the induction of intravascular platelet activation secondary to thrombin formation (7). It is important to support clinical data with laboratory findings in establishing a diagnosis of DIC. There is no specific routine test assessing persistent thrombin production (7).

Platelets play a central role in the process of coagulation by providing a catalytic surface for coagulation reactions and forming a primary hemostatic plug (11). Release of young platelets from the bone marrow is induced in DIC following the merge of platelets and fibrin network (11). There are studies in the literature indicating a relationship between poor prognosis and thrombocytopenia, and impaired platelet functioning in patients in the intensive care unit (12, 13). Platelet number and indices have been reported as good predictors of 28-days mortality in patients with DIC (10).

In our study, the objective was to investigate the difference in the coagulation markers including prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer and leukocyte count (WBC) as well as platelet and erythrocyte indices in patients with sepsis plus no DIC (Group 1) and patients with both sepsis and DIC (Group 2).

## Material and Methods

PT, aPTT, fibrinogen, D-dimer, thrombocyte, erythrocyte and leukocyte levels were investigated retrospectively in septic patients with or without DIC at the Zonguldak Karaelmas University Education and Research Hospital between 2007 and 2009. The study included 42 sepsis patients without DIC with a mean

age of  $69 \pm 16$  years (range: 26–91) and 18 sepsis patients with DIC with a mean age of  $62 \pm 20$  years (range: 18–85). The diagnostic criteria of the International Sepsis Definitions Conference (14) and ISTH (3, 6) were used in the diagnosis of sepsis and DIC, respectively.

PT, aPTT, fibrinogen were measured with the mechanical method and D-dimer was measured with the optic method using the Amax device (Trinity Biotech Plc, Bray, Co. Wicklow, Ireland). Reference values were 12–18 s,  $<1.21$ , 22.6–35 s, 1.75–4.00 g/L,  $<1.47$  mg/L for PT, INR, aPTT, fibrinogen, and D-dimer respectively. INR was calculated using the  $INR = (\text{Patient PT}/\text{Normal PT})$  ISI formula.

A normal PT value represents the average of the mean normal PT range of the laboratory, whereas the International Sensitivity Index (ISI) is the correction coefficient of thromboplastin in commercial kits calculated according to international reference samples.

Leukocyte count, platelet count (PLT), erythrocyte count (RBC), hemoglobin level (Hb), platelet and erythrocyte indices were measured and calculated using the LH 780 Analyzer (Beckman Coulter, INC. Fullerton, USA) device.

Reference values were  $3.5 \times 10^3$ – $11 \times 10^3$  cells/ $\mu\text{L}$ ,  $130 \times 10^3$ – $450 \times 10^3$  cells/ $\mu\text{L}$ , 0.14–0.52%, 7.4–10.4 fL, 10–17.9%,  $3$ – $5.08 \times 10^6$  cells/ $\mu\text{L}$ , 100–175 g/L, 30.5–46.3%, 78.4–95.9 fL, 27.2–35.5 pg, 325–352 g/L, 11.7–13.4% for WBC, PLT, PCT (plateletcrit level (PLT  $\times$  MPV)), MPV (mean platelet volume), PDW (platelet distribution width), RBC, Hb, HCT (hematocrit (RBC  $\times$  MCV)), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin (Hb/RBC)), MCHC (mean corpuscular hemoglobin concentration (Hb/HCT)), RDW (red cell distribution width), respectively.

In statistical analyses, Kolmogorov-Smirnov test was used to assess the conformity of the values to normal distribution; Mann-Whitney test was used in parameters not conforming to normal distribution, whereas t-test was used in those conforming to a normal distribution.

## Results

Mean, standard deviation, minimum–maximum values and statistical significance of the inter-group differences in PT (s), INR, aPTT (s), fibrinogen (mg/L), D-dimer (mg/L), WBC (cells/ $\mu\text{L}$ ), PLT (cells/ $\mu\text{L}$ ), PCT (PLT  $\times$  MPV), MPV (fL), PDW, RBC (cells/ $\mu\text{L}$ ), Hb (g/L), HCT (RBC – MCV), MCV (fL), MCH (Hb/RBC), MCHC (Hb/Hct), and RDW have been presented in Table I.

Table II demonstrates the distribution of thrombocyte and erythrocyte indices by reference values in Group 1 and Group 2. PT, INR and D-dimer levels were significantly elevated ( $p=0.003$ ,  $p=0.011$ ,  $p=0.012$ ), whereas fibrinogen, PLT and PCT were significantly decreased ( $p=0.004$ ,  $p=0.000$ ,  $p=$

**Table I** Mean, standard deviation, min-max values of the test and statistical significance of inter-group differences.

	Sepsis without DIC (Group 1) n=42	Sepsis with DIC (Group 2) n=18	P
PT (s)	16.12±5.44 (9.60–32.20)	21.78±8.23 (13.50–41.00)	<b>p=0.003</b>
INR	1.37±0.48 (0.81–2.89)	1.89±0.72 (1.11–3.47)	<b>p=0.011</b>
aPTT (s)	43.7±22.39 (17.90–98.60)	58.92±34.45 (20.93–159.30)	P=0.056
Fibrinogen (g/L)	3.23±1.34 (1.53–5.87)	1.92±1.06 (0.38–4.6)	<b>p=0.004</b>
D-Dimer (mg/L)	2.04±2.40 (0.22–9.61)	7.52±10.60 (0.48–43.05)	<b>p=0.012</b>
WBC (cells/μL)	14.954±8.468 (×10 <sup>3</sup> ) (2.100–36.550)	13.793±10.162 (×10 <sup>3</sup> ) (2.230–46.200)	p=0.649
PLT (cells/μL)	188.58±132.20 (×10 <sup>3</sup> ) (33.33–624.50)	57.62±38.39 (×10 <sup>3</sup> ) (23.0–171.0)	<b>p=0.000</b>
PCT (PLT × MPV)	0.170±0.109 (0.028–0.435)	0.050±0.031 (0.020–0.130)	<b>p=0.000</b>
MPV (fL)	9.196±1.428 (6.900–13.000)	8.887±1.021 (7.200–11.030)	p=0.410
PDW	16.77±0.98 (13.20–18.50)	16.97±0.93 (15.10–18.53)	p=0.474
RBC (Cells/μL)	3.487±0.665 (×10 <sup>6</sup> ) (2.150–5.020)	3.326±0.760 (×10 <sup>6</sup> ) (2.150–5.493)	p=0.415
Hb (g/L)	101.8±18.5 (67.5–147.0)	96.3±20.3 (69.7–152.0)	p=0.307
HCT (RBC × MCV)	30.26±5.38 (20.50–42.00)	28.98±6.13 (19.87–45.83)	p=0.422
MCV (fL)	87.1±5.0 (78.3–104.0)	87.6±5.6 (74.0–96.8)	p=0.718
MCH (Hb/RBC)	29.34±1.84 (25.30–35.00)	29.33±2.83 (20.43–33.13)	p=0.981
MCHC (Hb/Hct)	33.69±0.86 (31.60–35.10)	33.43±1.78 (27.63–35.37)	p=0.568
RDW	15.62±2.38 (9.50–20.93)	17.0±3.43 (13.70–25.40)	p=0.078

**Table II** Distribution of thrombocyte and erythrocyte indices (WBC, PLT, PCT, MPV, PDW, RBC, Hb, HCT, MCV, MCH, MCHC, RDW) according to reference values in the groups of patients with sepsis and DIC and sepsis without DIC (Group 1).

	Sepsis without DIC (Group 1) n=42			Sepsis with DIC (Group 2) n=18		
	Decreased	Normal	Elevated	Decreased	Normal	Elevated
WBC	2 4.8%	13 31%	27 64.3%	2 11.1%	5 27.8%	11 61.1%
PLT	16 38.1%	24 57.1%	2 4.8%	17 94.4%	1 5.6%	0
PCT	19 45.2%	23 54.8%	0	18 100%	0	0
MPV	5 11.9%	32 76.2%	5 11.9%	2 11.1%	15 83.3%	1 5.6%
PDW	0	38 90.5%	4 9.5%	0	16 88.9%	2 11.1%
RBC	9 21.4%	33 78.6%	0	4 22.2%	13 72.2%	1 5.6%
Hb	20 47.6%	22 52.4%	0	12 66.7%	6 33.3%	0
HCT	22 52.4%	20 47.6%	0	13 72.2%	5 27.8%	0
MCV	1 2.4%	39 92.9%	2 4.8%	1 5.6%	15 83.3%	2 11.1%
MCH	3 38.1%	39 57.1%	0	1 5.6%	17 94.4%	0
MCHC	4 9.5%	38 90.5%	0	4 22.2%	13 72.2%	1 5.6%
RDW	1 2.4%	3 7.3%	37 90.2%	0	0	18 100%

0.000) in Group 2 compared to Group 1. aPTT was higher in Group 2 compared to Group 1, however the difference was not statistically significant ( $p=0.056$ ). No significant differences were found in the levels of MPV, PDW ( $p=0.410$ ,  $p=0.474$ ), WBC ( $p=0.649$ ), RBC ( $p=0.415$ ), Hb ( $p=0.307$ ) and HCT, MCV, MCH, MCHC, RDW ( $p=0.422$ ,  $p=0.718$ ,  $p=0.981$ ,  $p=0.568$ ,  $p=0.078$ ) between Group 1 and Group 2.

## Discussion

Sepsis is an important cause of DIC (5, 9). Cytokines released in sepsis lead to induction of the coagulation pathway and the disruption of fibrinolytic as well as anticoagulant systems (7, 8). Coagulation abnormalities and thrombocytopenia often emerge in severe sepsis and a clinical picture ranging from minor alterations to DIC and disseminated microvascular thrombosis might be observed (14). DIC is a clinical picture including a dramatic activation of the coagulation pathway, fibrin formation, consumption of coagulation factors and platelets, and disseminated hemorrhages (11).

There are literature studies on the use of anticoagulant therapy in sepsis and DIC (8, 16, 17). Franchini has reported replacement with fresh frozen plasma, restoration therapy with anticoagulants and anticoagulant pathways and treatment approaches aimed at prevention of microvascular fibrin accumulation (15). Bernard, on the other hand, has suggested that protein C therapy decreases the mortality in patients with increased risk of bleeding and severe sepsis (8). It has been reported that duration of life was prolonged and marked morphological protection was obtained following the administration of tissue factor pathway inhibitor (TFPI) in baboons infected with lethal *e. coli* (17).

Thrombocytopenia and impaired platelet functioning have been reported to be associated with poor prognosis (12) and platelet functioning to be correlated with disease severity (18) in intensive care unit patients. It has been demonstrated that thrombocytopenia is a risk factor for premature death (19) and that it is associated with an increase in mortality rate in septic patients (20). Kitchens et al. have reported that thrombocytopenia does not protect the patients from thrombosis (4). The increased thrombin production has been reported to be the cause of platelet activation in the pathogenesis of DIC (7). Currently, there are no specific routine tests evaluating thrombin production (7). Kunishima et al. (21) have suggested a relationship between thrombocytopenia and platelet consumption by demonstrating an increase in the platelet membrane glycoprotein glyocalin concentration which is a marker of platelet destruction in patients with DIC (21, 22).

In our study, thrombocyte number and PCT were significantly lower in the group of septic patients

with DIC compared to those without DIC, and no significant differences were determined in MPV and PDW values between the groups. Decreased PCT level is associated with the decrease in thrombocyte count, rather than the thrombocyte volume. Kim et al. have reported a lower platelet count and PCT, and higher MPV and PDW in patients with significant DIC compared to those without significant DIC and in deceased patients compared to those who survived (11). Yilmaz et al. (23) have demonstrated that platelet count and PCT were decreased in 0.5 hours in endotoxemic dogs and that this decrease persisted until 24 hours, whereas MPV and PDW were increased. Akarsu et al. (24) have determined thrombocytopenia and increased levels of MPV and PDW in patients with neonatal sepsis. Kikuchi et al. (25) have determined no differences in RDW in the elderly patients with DIC with an underlying etiology of sepsis, pneumonia, pyelonephritis and inflammatory diseases compared to those without DIC, however, authors have reported higher MPV and PDW values.

The reduction in thrombocyte levels in patients with DIC in our study is compatible with the results of literature studies. No significant differences were determined between the groups in terms of the thrombocyte volume and thrombocyte shape. However, MPV and PDW were increased in 11.9% and 9.5% of the septic patients without DIC, and 5.6% and 11.1%, respectively, of those with DIC according to the reference values.

In our study, no significant differences were determined between the WBC, RBC, Hb and erythrocyte indices of septic patients with or without DIC. Hb and HCT levels were decreased in 47.6% and 52.4% of the septic patients without DIC and 66.7% and 72.2% of those with DIC, respectively, according to the reference values. RDW was elevated in 90.2% of the sepsis without DIC patients and in all the patients with sepsis and DIC. Rates of anisocytosis were increased in both groups. In their study, Esper et al. (26) have demonstrated that RDW was higher in the group consisting of patients with sepsis, severe sepsis and septic shock.

## Conclusion

Relying on the study results, we suggest that bone marrow functions do not differ in sepsis and DIC; however, the coagulation pathway is more active leading to consumption of platelets in DIC compared to sepsis, as demonstrated by the decreased fibrinogen, increased D-dimer as well as prolonged PT levels. Further studies should be performed to investigate the markers of platelet degradation in order to provide an early diagnosis of DIC in septic patients and to determine anticoagulation treatment strategies.

## Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.



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