Effetcs of platelet function on the haemorrhagic manifestations and mortality in Crimean-Congo haemorrhagic fever

Fazilet Duygu[1], Tugba Sari[2], Hakim Celik[3]

[1]Department of Infectious Diseases and Clinical Microbiology, Abdurrahman Yurtaslan Oncology Training and Research Hospital, Ankara, Turkey;
[2]Department of Infectious Diseases and Clinical Microbiology, Pamukkale University, Faculty of Medicine, Denizli, Turkey.
[3]Department of Physiology, Gaziantep University, Faculty of Medicine , Gaziantep, Turkey

SUMMARY

Crimean-Congo haemorrhagic fever (CCHF) is a viral zoonotic disease which can lead to life-threatening with haemorrhagic manifestations. We aimed here in this study was to evaluate the effect of the platelet count and volume-related indices, such as the mean platelet volume (MPV), platelet distribution width (PDW) which is a measure of platelet anisocytosis and plateletcrit, in the haemorrhagic manifestations and mortality seen in CCHF cases.

We retrospectively examined data derived from 173 patients. The age, gender, alanine transaminase (ALT), aspartate transaminase (AST), platelet counts and MPV, PDW and PCT values upon admission (MPV1, PDW1 and PCT1) and those values measured at the time when the PLT was at the lowest level (MPV2, PDW2 and PCT2), haemorrhagic manifestations and the mortality status of patients diagnosed with CCHF were recorded.

ALT and AST values were higher among the haemorrhagic patients when compared with the others ($p<0.001$), while platelet 1 (PLT1), platelet 2 (PLT2), plateletcrit 1 (PCT1), plateletcrit 2 (PCT2) and platelet distribution width 2 (PDW2) values were significantly lower ($p=0.001$, $p<0.001$, $p=0.002$, $p<0.001$ and $p=0.003$, respectively). A negative correlation was documented between haemorrhage and the PLT1, PLT2, PCT1, PCT2 and PDW2 ($r=-0.255$, $r=-0.415$, $r=-0.241$, $r=-0.377$, $r=-0.223$, respectively); however, there was a positive correlation between haemorrhage and mortality ($r=0.34$).

This was the first study evaluating the platelet functions in CCHF, such as the PLT, PDW and PCT, in CCHF correlated with the mortality and haemorrhagic manifestations. The platelet functions contribute as much to the prediction of haemorrhage and mortality as the PLT. The present study suggests that the PCT and PDW values could be beneficial in anticipating the inclination toward haemorrhage and mortality.

Keywords: Crimean-Congo haemorrhagic fever, blood, platelet, mean platelet volume.

INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) is a disease state caused by *Nairoviruses* of the *Bunyaviridae* family, which are transmitted by the bites of *Hyalomma marginatum* ticks or by direct contact with infected blood or body secretions. The most common clinical signs of CCHF are fever, nausea, headache, diarrhoea, myalgia, petechial rash and haemorrhage [1]. The reported mortality rates of CCHF epidemics and outbreaks vary greatly; however, the average mortality rate is often cited at 5-50% [2-4]. The diagnosis is established through immunological methods, reverse transcription-polymerase chain reactions or virus isolation in the cell culture [5-7].

Infectious diseases can lead to haemorrhag-

Corresponding author
Tugba Sari
E-mail: drtugba82@gmail.com

ic manifestations by means of a constellation of mechanisms, including the induction of thrombocytopenia, depletion of local clotting factors, hyperfibrinolysis and leakage secondary to vessel wall damage [8]. However, the exact underlying pathogenesis behind mortality and haemorrhage in CCHF is yet to be completely understood. All of the previous studies related to CCHF have reported thrombocytopoenia, elevated aspartate transaminase (AST) and alanine transaminase (ALT) levels, and a prolonged activated partial thromboplastin time (aPTT) as ominous prognostic indicators. However, previous studies have yielded distinct findings with regard to the contributions of leukocytosis, lactic dehydrogenase (LDH), creatine phosphokinase (CPK), age and the fibrinogen level as ominous prognostic factors [6-18].

The role platelets play lies primarily in haemostasis; however, previous studies have documented that the platelets constitute an important component of the immune system. Accordingly, platelets contribute to the immune system through various mechanisms, such as engulfing foreign particles, giving off distinctive adhesion molecules, undergoing chemotaxis, triggering complement factors and establishing interactions with microorganisms. The platelet functions can be analysed based on the mean platelet volume (MPV), platelet distribution width (PDW) (which is a measure of platelet anisocytosis) and plateletcrit (PCT) (equivalent to haematocrit with regard to platelets) [19].

Thrombocytopenia is known to be a poor prognostic indicator of CCHF, and the disease may still pursue a mortal course despite the presence of an adequate number of platelets. This issue propelled us to consider that the platelet function may provide as crucial a contribution as the platelet count. Therefore, the aim of the present study was to evaluate the effect of the platelet count (PLT) and volume-related indices, such as the MPV, PCT and PDW, in the haemorrhagic manifestations and mortality seen in CCHF cases.

PATIENTS AND METHODS

This study included those patients admitted to the Tokat State Hospital in Turkey with complaints of fever, anorexia, weakness, petechial rash and haemorrhage between April 2011 and September 2011 and hospitalized with clinical and laboratory findings compatible with CCHF, whether tick contact was suspected or not were enrolled in this study. In patients pre-diagnosed with CCHF diagnostic criteria were as follows;

Clinical findings: At least two symptoms (fever, headache, myalgia, nausea/vomiting, arthralgia, weakness, haemorrhage) and leukopaenia (<4000/μL)/ thrombocytopaenia (<150 000/μL), elevation of AST, ALT), LDH and CPK.

Supportive findings: Haemorrhagic-purpuric rash and other haemorrhagical symptoms.

Epidemiological history and one or more of the following exposures within the 3 weeks before onset of symptoms: Living in-or travel to endemic area, history of tick exposure, contact with blood or other body fluids of an animal, contact with blood or other body fluids of confirmed CCHF patient, work in a laboratory that handles CCHF specimens.

Suspected case definition: Case meets the clinical and epidemiologic linkage criteria.

Probable case: Case meets the clinical and epidemiologic linkage criteria and meets two supportive findings or case meets the clinical and epidemiologic linkage criteria in endemic areas for CCHF.

Confirmed case: Case meets the clinical + demonstration of viral RNA in blood and tissue samples, specific IgM positivity, four-fold increase in specific IgG titre, epidemiological association with confirmed CCHF patient.

The serum samples of the patients were sent to the National Reference Laboratory for further analysis. The patients with PCR and/or IgM positivity suggestive of CCHF were diagnosed with CCHF. The platelet functions were analysed using an automated blood cell counter (Beckman Coulter Inc., Brea, CA, USA).

We retrospectively examined data derived from 173 confirmed CCHF patients who were hospitalized.

The MPV, PDW and PCT values upon admission (MPV1, PDW1 and PCT1) and those values measured at the time when the PLT was at the lowest level (MPV2, PDW2 and PCT2) were recorded in all the patients. Any haemorrhagic manifestations, such as epistaxis, haematuria and vaginal or gastrointestinal haemorrhage, were also recorded.

The categorical variables, given in counts and percentages, were compared between the groups using Pearson's chi-squared test, and the normally distributed variables were identified using the Kolmogorov-Smirnov test. The continuous variables, presented here in means or medians [interquartile range (IQR)], were compared between the two groups using the two independent sample t test or Mann-Whitney U test. A value of P<0.05 was accepted to imply statistical significance.

A receiver operating characteristic analysis was utilized in order to specify the thresholds associated with the laboratory values with regard to their effects on mortality and the haemorrhagic manifestations. The Statistical Package for the Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all the statistical analyses.

RESULTS

173 patients (83 males and 90 females, 47.9% and 52.1%, respectively) and 11 deaths (6.3%) were identified in this study. Haemorrhaging was observed in 13.8% (n=24) of these patients during hospitalization. When we evaluated the haemorrhaging with regard to the gender of the study participants, the WBC1, WBC2, MPV1, MPV2 and PDW1 values were similar between the haemorrhagic and non-haemorrhagic patients (p>0.05). In addition, there were significantly higher ALT and AST values among the haemorrhagic patients when compared with the others (p<0.001 and p<0.001, respectively).

When we evaluated the PLT1, PLT2, PCT1, PCT2 and PDW2 values, they were significantly lower (p=0.001, p<0.001, p=0.002, p<0.001 and p=0.003, respectively) in the haemorrhagic patients than in the non-haemorrhagic patients. The laboratory values compared with regard to the demographic and clinical features are shown in Table 1. Higher AST and ALT levels (p=0.001 and p<0.001, respectively) and lower PLT, PCT and PDW values (p<0.001, p<0.001 and p=0.003, respectively) were detected in the patients in whom mortality occurred, when compared to the patients that recovered. The lower thresholds for the PCT1 and PCT2 were determined to be ≤0.02 (PCT1 sensitivity = 37.5% and specificity = 92.6%; PCT2 sensitivity = 92.3% and specificity = 82.4%) (Figure 1).

A negative correlation was seen between haemorrhage and the PLT1, PLT2, PCT1, PCT2 and

Table 1 - Comparison of the demographic characteristics and laboratory values of the haemorrhaging and non-haemorrhagic patients.

Age mean±SD	*Haemorrhagic patients (N=149) 47.54±17.3*	*Non-haemorrhagic patients (N=24) 46.83±21.17*	*P >0.05*
Male (N, %)	83 (55,7 %)	12 (50%)	>0.05
WBC1 median (IQR) WBC2 mean±SD	2400 (1900-3200) 2207±992	2500 (1835-3600) 2191±1207	>0.05 >0.05
Plt1 mean±SD Plt2 mean±SD	90.64±38.58 61.08±35.3	60.75±37.97 23.79±15.27	0.001 <0.001
MPV 1mean±SD MPV2 mean±SD	9.18±1.12 9.26±1.05	9.37±0.82 9.37±0.92	>0.05 >0.05
PCT1 mean ±SD PCT2 mean (IQR)	0.08±0.03 0.05±0.03	0.05±0.03 0.05 (0.03-0.07)	0.002 <0.001
PDW1 mean±SD PDW2 mean±SD	16.55±0.81 16.9±0.82	16.63±0.76 16.38±0.93	>0.05 0.003
ALT median (IQR) AST median (IQR)	32.5 (21-49.25) 57.5 (33.75-113)	54 (34.5-96.75) 81.5 (64.5-163.24)	<0.001 <0.001

WBC1: Initial White blood cell count, WBC 2: Lowest White blood cell count, Plt 1: Initial platelet value, Plt 2: Lowest platelet value, MPV 1: Mean platelet volume, MPV 2: Lowest mean platelet value, PCT 1: Initial plateletcrit, PCT 2: Lowest plateletcrit, PDW 1: Initial platelet distribution width, PDW 2: Lowest platelet distribution width, PT: Prothrombine time, aPTT: Active tromboplastine time, SD: Standart deviation, IQR: Interquartile range, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Normal values: WBC: 4. 800-10.800 mm^3, Platelet: 150000-400000 mm^3, MPV 6.5-12 fL, PCT: 0.108-0.282%, PDW: 10-65%, PT: 10-14 sec., APTT: 21-36 sec., AST: 15-37 IU/L, ALT: 30-65 IU/L

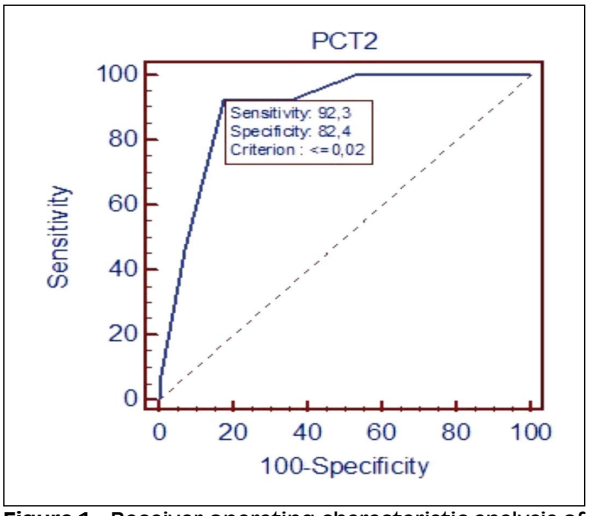

Figure 1 - Receiver operating characteristic analysis of the plateletcrit at the lowest platelet count and mortality.

PDW2 (r=-0.255, r=-0.415, r=-0.241, r=-0.377 and r=-0.223, respectively); however, there was a positive correlation between haemorrhage and mortality (r=0.34).

DISCUSSION

The first CCHF case in Turkey was reported in Tokat province located in Kelkit Valley in 2002, however earlier reports of serologically confirmed cases were available from Turkey and neighboring countries [19]. Since then, CCHF has been endemic in Tokat and in Turkey. Because of this reason the number of cases were significantly high in a very short period of time in our study.

There are some typical biochemical and hematological changes in CCHF patients. Thrombocytopenia, leukopenia, AST, ALT, LDH, and CPK elevation, aPTT and PT prolongation, INR elevation, and a decrease in fibrinogen are observed in these patients [4, 13]. Numerous studies have been shown that, low platelet counts, prolongation of aPTT and PT, and elevated INR values were found to be significant markers used in both the diagnosis and follow-up of CCHF cases. Our clinical and laboratory results are consistent with those previously reported with regard to CCHF disease. A high WBC count was found to be significant only in the study conducted by Swanepoel et al., but not in the other studies and in our study [8,11-15].

A PLT count <20x10^9/mL has been reported to be an indicator of a poor prognosis The platelet functions, such as the PLT, PDW and PCT, in CCHF correlated with the mortality and haemorrhagic manifestations in our study which was compatible with previously reported results in the literature [6, 8, 12-17, 20, 21].

Yilmaz et al. found that the PDW could be used to determine the disease severity [21]. In addition, Onguru et al. evaluated the correlations between mortality and the coagulation-related parameters in CCHF, such as proteins C and S, antithrombin III, activated protein C resistance and D-dimer results [17]. Reporting no correlations in the former, they announced the existence of an association between mortality and several parameters, including the platelet count, prothrombin time, aPTT, international normalized ratio and fibrinogen levels, and that the traditional coagulation parameters were sufficient for monitoring during the diagnosis and follow-up.

There are three major components of haemostasis: primary haemostasis, secondary haemostasis and fibrinolysis [21]. Primary haemostasis can be evaluated via a complete or full blood count, and a test to provide data regarding the PLT and platelet volume, morphology and maturity has been evaluated [21, 22]. Modern blood counters can be used for the rapid measurement of these parameters, including the MPV, PDW and PCT.

A sizable number of compounds contributing to inflammation, coagulation, thrombosis and atherosclerosis are secreted from activated platelets, including chemokines, cytokines and coagulation factors. Previous clinical trials have indicated that platelets are a pivotal component in the evaluation of the inflammatory response, and the aforementioned factors play roles in aggregation, adhesion and thrombus generation [23]. The platelet volume increases upon activation, with larger platelets documented to possess thrombotic potential and induce inflammatory processes [24].

The platelet size is dictated by progenitor cells, such as megakaryocytes, and some studies have suggested that cytokines like interleukin (IL)-3 and IL-6 stimulate the megakaryocytes at the chromosomal level, thus augmenting the production of much more reactive and voluminous platelets [25]. The MPV indicates platelet activation, and it is an important marker predicting the

function, morphology and maturity of the platelets. The MPV is provided by a complete blood count, creating no further costs for its measurement [26]. For reliable MPV measurement, the potential influence of anticoagulant or standardizing the time delay between sampling and analysis [27]. The MPV level has been shown to increase during inflammatory disease states, including ankylosing spondylitis, rheumatoid arthritis and infectious diseases like pulmonary tuberculosis [28, 29]. Ekiz et al. reported that significant increase in MPV was observed in patients with CCHF compared with healthy controls but the MPV levels were found to be normal in this study [16]. At this point, we considered that these two opposite issues were related to CCHF itself, which is an infectious disease characterized by thrombocytopenia, and cancelled each other out, sustaining a normal MPV range.

The limit at which platelet transfusion should be commenced remains to be elucidated. Our clinical experiences yield conflicting data, ranging from cases of haemorrhage with PLTs >50,000 to others with PLTs <20,000 without any overt haemorrhage. Representing a more precious indicator in terms of haemorrhage risk when compared to the PLT, a PCT value <0.1% dictates the implementation of a thrombocyte transfusion [30].

The present study found correlations between a decreasing PTC and haemorrhage and mortality during the CCHF follow-up. We consider that the PCT value may prove useful in the anticipation of the platelet transfusion timing in patients with CCHF. Similar to the concept of the erythrocyte distribution range, the PDW represents an index indicating the heterogeneity of the platelet volumes. An evaluation of the PWD along with the MPV provides a better estimation of the platelet volume distribution. For example, it has been shown that the PDW is greater in patients with activated platelets when compared with healthy subjects [31].

The PDW, similar to the MPV, has been reported to increase in patients with platelet activation when compared with healthy subjects. Moreover, it was suggested that the PDW acted more specifically when compared to the MPV. Contrarily, the MPV and PDW are generally measured at the lower limits during thrombocytopenic states caused by bone marrow failure. We believe that use of the MPV and PDW in combination is likely to yield a more accurate prediction of the coagulation activation [32].

The PDW values measured upon admission were normal, whereas the levels measured at the time of the lowest platelet count (PDW2) were found to be lower. The decrease in the PDW, measured to be normal at the onset of the disease, in a parallel manner to the deterioration of the disease, suggests that the PDW2 may be associated with haemorrhage and mortality, and that it can be used in predicting thrombocyte activation as a prognostic indicator during the follow-up of this disease.

Sharifi-Mood et al. reported that high-dose methylprednisolone is effective in the treatment of patients with CCHF and its effect on thrombocyte activation. Further investigation is necessary in order to determine the efficacy of corticosteroid and its effect on outcome [33].

CONCLUSION

Haemorrhage is one of the most important reasons for mortality in CCHF cases. The platelet functions contribute as much to the prediction of haemorrhage and mortality as the PLT. The present study suggests that the PCT and PDW values could be beneficial in anticipating the inclination toward haemorrhage and mortality, beginning from the onset of the disease and from the time when the PLT begins to decrease, respectively. We suggest that certain parameters, like the PCT and PDW, which are included in the CBC test and do not incur additional costs to measure, may be utilized in the follow-up of CCHF patients, and that further studies are likely to help elucidate this issue.

ACKNOWLEDGEMENTS

The authors would like to thank all health workers of Tokat State Hospital for assistance.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

[1] Drosten C., Minnak D., Emmerich P., Schmitz H., Reinicke T. Crimean-Congo hemorrhagic fever in Kosovo. J. Clin. Microbiol. 40, 3, 1122-1123, 2002.

[2] Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J. Med. Entomol*. 22, 15, 4, 307-417, 1979.

[3] Nichol S.T. Bunyaviruses. In: Lippincott, Williams

& Wilkins, Philadelphia. Knipe, D.M., Howley, P.M. (Eds.); p. 1603-1633, 2001.
[4] Whitehouse C.A. Crimean-Congo hemorrhagic fever. *Antiviral Res.* 64, 3, 145-160, 2004.
[5] Van Gorp E.C.M., Suharti C., Ten Cate H., Dolmans W.M.V., Van der Meer J.W. Ten Cate J.W., Brandjes D.P.M. Infectious diseases and coagulation disorders. *J. Infect. Dis.* 180, 1, 176-186, 1999.
[6] Leblebicioglu H., Sunbul M., Barut S., et al. Multi-center prospective evaluation of discharge criteria for hospitalized patients with Crimean-Congo Hemorrhagic Fever. *Antiviral Res.* 133, 9-13, 2016.
[7] Pittalis S., Meschi S., Castilletti M.C., et al. Crimean-Congo haemorrhagic fever: an enemy at the gates. *Infez. Med.* 17, 3, 133-140, 2009.
[8] Çevik M.A., Erbay A., Bodur H., et al. Clinical and laboratory features of Crimean-Congo hemorrhagic fever: predictors of fatality. Int. J. Infect. Dis. 12, 4, 374-379, 2008.
[9] Hatipoglu C.A., Bulut C., Yetkin M.A., et al. Evaluation of clinical and laboratory predictors of fatality in patients with Crimean-Congo haemorrhagic fever in a tertiary care hospital in Turkey. Scand. J. Infect. Dis. 42, 6-7, 516-521, 2010.
[10] Yesilyurt M., Gul S., Ozturk B., et al. The early prediction of fatality in Crimean Congo hemorrhagic fever patients. Saudi Med. J. 32, 7, 742-743, 2011.
[11] Swanepoel R., Gill D.E., Shepherd A.J., et al. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev. Infect. Dis.*11, 4, S794-S800, 1989.
[12] Bakir M., Ugurlu M., Dokuzoguz B., et al. Crimean-Congo haemorrhagic fever outbreak in Middle Anatolia: a multicentre study of clinical features and outcome measures. J. Med. Microbiol., 54, 4, 385-389, 2005.
[13] Ergonul O., Celikbas A., Baykam N., Eren S., Dokuzoguz B. Analysis of risk-factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited. Clin. Microbiol. Infect. 12, 6, 551-554, 2006.
[14] Fisgin N.T., Tanyel E., Doganci L., Tulek N. Risk factors for fatality in patients with Crimean-Congo haemorrhagic fever. Trop. Doct. 39, 3, 158-160, 2009.
[15] Ozturk B., Tutuncu E., Kuscu F., Gurbuz Y., Sencan I., Tuzun H. Evaluation of factors predictive of the prognosis in Crimean-Congo hemorrhagic fever: new suggestions. Int. J Infect. Dis. 16, 2, 89-93, 2012.
[16] Ekiz F., Gürbüz Y., Başar Ö., et al. Mean platelet volume in the diagnosis and prognosis of Crimean-Congo Hemorrhagic Fever. Clin. Appl. Thromb. Hemost. 19, 4, 441-444, 2013.
[17] Onguru P., Dagdas S., Bodur H., et al. Coagulopathy parameters in patients with Crimean-Congo hemorrhagic fever and its relation with mortality. J. Clin. Lab. Anal. 24, 3, 163-166, 2010.
[18] Sari T., Hatipoglu C.A. A case of Crimean-Congo haemorrhagic fever with normal laboratory findings. *Infez. Med.*, 22, 3, 227-229, 2014.
[19] Yilmaz H., Yilmaz G., Menteşe A., et al. Prognostic impact of platelet distribution width in patients with Crimean-Congo hemorrhagic fever. J. Med. Virol. 88, 11, 1862-1866, 2016.
[20] Eren S. H., Zengin S., Büyüktuna S.A., Gözel M.G. Clinical severity in forecasting platelet to lymphocyte ratio in Crimean-Congo hemorrhagic fever patients. J. Med. Virol. 65, 10, 1100-1104, 2016.
[21] Lippi G., Favaloro E.J., Franchini M., Guidi G.C. Milestones and perspectives in coagulation and hemostasis. Semin. Thromb. Hemost. 35, 01, 009-022, 2009.
[22] Harrison P., Goodall A.H. "Message in the platelet" - more than just vestigial mRNA! *Platelets* 19, 6, 395-404, 2008.
[23] Coppinger J.A., Cagney G., Toomey S., et al. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. *Blood* 103, 6, 2096-2104, 2004.
[24] Bath P.M., Butterworth R.J. Platelet size: measurement, physiology and vascular disease. *Blood Coagul. Fibrinolysis Int. J. Haemost Thromb.* 7, 2, 157-161, 1996.
[25] Debili N., Masse J.M., Katz A., Guichard J., Breton-Gorius J., Vainchenker W. Effects of the recombinant hematopoietic growth factors interleukin-3, interleukin-6, stem cell factor, and leukemia inhibitory factor on the megakaryocytic differentiation of CD34+ cells. *Blood* 82, 1, 84-95, 1993.
[26] Thompson C.B., Jakubowski J.A., Quinn P.G., Deykin D., Valeri C.R. Platelet size and age determine platelet function independently. *Blood* 63, 6, 1372-1375, 1984.
[27] Varol E. Diagnostic and Prognostic Value of Mean Platelet Volume in Patients With Crimean-Congo Hemorrhagic Fever. *Clin. Appl. Thromb. Hemost.* 19, 4, 460, 2013.
[28] Kisacik B., Tufan A., Kalyoncu U., et al. Mean platelet volume (MPV) as an inflammatory marker in ankylosing spondylitis and rheumatoid arthritis. *Joint Bone Spine* 75, 3, 291-294, 2008.
[29] Tozkoparan E., Deniz O., Ucar E., Bilgic H., Ekiz K. Changes in platelet count and indices in pulmonary tuberculosis. *Clin. Chem. Lab. Med.* 45, 8, 1009-1013, 2007.
[30] Mohr R., Martinowitz U., Golan M., Ayala L., Goor D.A., Ramot B. Platelet size and mass as an indicator for platelet transfusion after cardiopulmonary bypass. *Circulation* 74, 5, 2, 153-158, 1986.
[31] Vagdatli E., Gounari E., Lazaridou E., Katsibourlia E., Tsikopoulou F., Labrianou I. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia* 14, 1, 28, 2010.
[32] Jackson S.R., Carter J.M. Platelet volume: laboratory measurement and clinical application. *Blood Rev.* 1, 7, 2, 104-113, 1993.
[33] Sharifi-Mood B., Alavi-Naini R., Metanat M., Mohammadi M., Shakeri, A., Amjadi A. Efficacy of high-dose methylprednisolone in patients with Crimean-Congo haemorrhagic fever and severe thrombocytopenia. *Tropical Doct.* 43, 2, 49-53, 2013.