European Review for Medical and Pharmacological Sciences 2021; 25: 3791-3797

The significance of red cell distribution width and homocysteine values in STEMI patients undergoing PCI in the population of Bosnia and Herzegovina

M. MARKOVIĆ BORAS[1], I. BRIZIĆ[2], I. MIKULIĆ[1]

[1]Department of Laboratory Medicine, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina
[2]Department of Cardiology, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

Abstract. – OBJECTIVE: We aimed to confirm whether serum on admission homocysteine level (HCY) and red cell distribution width (RDW) value are independent risk factors for MACE incidence in patients with STEMI myocardial infarction treated by percutaneous coronary intervention (PCI), as well as the possibility of their joint assessment in order to enhance the risk stratification for major adverse cardiac events (MACE).

PATIENTS AND METHODS: A total of 80 patients with acute myocardial infarction (AMI) were included in the study and tested for blood HCY and RDW values. Patients were followed up for six months after discharge and evaluated for MACE occurrence.

RESULTS: The RDW value was significantly associated with HCY level (r=0.267, p=0.026). Univariate logistic regression analysis identified both the RDW and HCY as independent predictors of MACE (OR 2.179; CI 95% 1.250 to 3.797; *p*=0.006 and OR 1.108; CI 95% 1.013 to 1.213; *p*=0.025, respectively), naming RDW as a stronger predictor of unfavorable prognosis in AMI patients. Addition of HCY to RDW value in receiver operating characteristic (ROC) curve analysis increased the area under the curve (AUC) from 0.705 to 0.730 (*p*=0.007), while risk prediction model, which also included traditional risk factors, increased AUC up to 0.806, implying this model as good predictor of MACE both in low-risk and high-risk STEMI patients.

CONCLUSIONS: A high baseline HCY level and RDW value in patients with STEMI undergoing PCI is independently associated with increased risk for MACE outcome. Their joint assessment increases risk prediction ability.

Key Words:
Homocysteine, Red cell distribution width, Acute myocardial infarction, Percutaneous coronary intervention.

Introduction

Despite substantial improvements in reperfusion therapy with percutaneous coronary intervention (PCI), acute myocardial infarction (AMI) remains a life-threatening disease worldwide[1]. Myocardial tissue becomes inflamed and necrotic; it loses contraction and impulse conducting ability with the net result of decreased oxygen distribution and irreversible damage to the heart muscle[2]. It often occurs in patients without traditional risk factors presented (approximately 20% patients)[3]. Despite significant prognostic improvements with newer treatment approaches and strategies in ST-elevation myocardial infarction (STEMI) patients, incidence of major adverse cardiac events (MACE) remains significant, especially in high-risk patients. Therefore, identification of such patients may have a leading role in application of additional treatment approaches providing prognostic benefits during and after the primary PCI[4]. This is particularly significant for patients without traditional cardiovascular risk factors presented. Existing predicting models for risk evaluation of MACE outcome in asymptomatic individuals are based mainly on traditional risk factors, e.g., dyslipidemia, family history, hypertension, age, cigarette smoking, diabetes mellitus, obesity, and physical inactivity[5]. Therefore, any non-invasive and easily accessible marker with high diagnostic specificity and sensitivity would be greatly beneficial for accurate patient risk stratification. This would enable targeted, effective evidence-based therapy[6].

Corresponding Author: Marijana Marković Boras, Ph.D;
e-mail: marijana.markovic.boras@fpmoz.sum.ba

RDW is an easily available biochemical parameter obtained from a complete blood count which indicates the variability in size of circulating erythrocytes. It is elevated in patients with anemia, the presence of iron deficiency or who underwent blood transfusion[7]. In recent years, many clinical researches revealed that the alterations of RDW levels may be associated with the incidence and prognosis in many cardiovascular and cerebrovascular diseases, e.g., RDW measurement can improve risk stratification in such patients[8-10]. However, the molecular mechanism between RDW and cardiovascular disease has not been completely described. In fact, it has been suggested that RDW could be a biomarker of oxidative stress and inflammation which could explain their relationship. Since the initial success rate of PCI is high, long-term follow–up results are most appropriate for evaluating the predictive value of RDW[10]. Most of the previous research has been conducted on Asian and American population. Since RDW values may be different between populations[11], with strong variations between recent studies as well[12], it would also be useful to research the population of Europe.

HCY is considered an independent predictor of cardiovascular disease (CVD) and cardiovascular mortality in both low-risk asymptomatic patients and high-risk patients[13]. Whatever the cause, mild and moderate hyperhomocysteinemia (13 to 25 μmol/L) is strongly associated with stroke, coronary artery disease, obstructive CVD[14], restenosis, heart failure and MACE after PCI and with all causes of death in those patients. In general, the effects of HCY in CVD seem to be a consequence of oxidative process in the vascular endothelium resulting in disturbed NO synthesis and vasodilatation[15]. Yet, there is not enough evidence for it to be called a causally related cardiovascular risk factor[13]. Even though the researchers revealed a link between elevated HCY alone and increased mortality, limited number of studies have examined the joint association of HCY and RDW and their risk assessment usefulness[16]. To our knowledge, no such prospective research in patients with STEMI myocardial infarction exist.

Since the data on RDW and HCY as markers of cardiovascular risk are different, inconsistent, and not entirely clear, the aim of this study is to examine their individual and pooled effect on the incidence of MACE in STEMI patients undergoing PCI, as well as to determine the relationship of these two readily available and inexpensive biochemical analytes.

Patients and Methods

This prospective research included 80 patients diagnosed with AMI. Patients were admitted to the Department of Cardiology at the University Clinical Hospital of Mostar (Bosnia and Herzegovina), in the period between October 2016 and December 2018. The AMI diagnosis was made by a cardiologist on the basis of at least two of the three WHO criteria[2]. All patients were diagnosed with STEMI myocardial infarction before performance of PCI as a treatment method. STEMI was confirmed by an electrocardiogram (ECG). Detailed medical history, including usual CVD risk factors, such as hypertension and smoking, was taken for each patient. Hypertension was defined as systolic arterial pressure ≥140 mmHg and/or diastolic arterial pressure ≥90 mmHg or as the use of antihypertensive drugs. Smoking was defined as daily consumption of at least one cigarette. Anemia on admission was defined according to the World Health Organization as the baseline hemoglobin value < 120 g/L in females and < 130 g/L in males[17]. Exclusion criteria were pregnancy, the use of antiepileptic, contraceptive therapy, cancer, and vitamin B_{12} supplementation in the last 6 months. All patients were prospectively followed up for 6 months after discharge. The main endpoints assessed in this study included MACE - defined as all-cause death or non-fatal myocardial infarction.

Patients were divided into two groups: 15 patients (group 2) with MACE and 65 patients without MACE (group 1). All participants in this research were informed about the details of the study. The research did not affect the treatment and hospitalization duration of patients. It was done after the approval by the Ethics Committee of the University Clinical Hospital in Mostar, and in accordance with the Declaration of Helsinki. On admission, after ECG, and before the PCI, venous blood samples were taken into the vacuum tubes. A serum sample used to determine the HCY and other biochemical parameters level was taken with one anticoagulant-free test tube (Sarstedt, Germany). Whole blood sample for complete blood count was taken in a single test tube with EDTA as an anticoagulant (Sarstedt, Germany). Residual parts of the samples were taken for research after the completion of routine treatment ordered by a cardiologist. The level of the HCY (μmol/L) was measured in the serum by a non-competitive immunoassay method on Architect ci8200 Integrated System analyzer (Abbot,

Chicago, IL, USA). Complete blood count was measured by VCS (volume-conductivity-scatter) principle on Sysmex XT2000i. The RDW was calculated by dividing the standard deviation of the mean corpuscular volume (MCV) by the MCV and multiplying by 100 to convert to a percentage value (%)[11].

Statistical Analysis

The statistical analysis was performed by SPSS (SPSS Inc., Chicago, IL, U.S.A.). The Kolmogorov-Smirnov test was used to assess normality of data. Correlations were tested by the nonparametric Spearman correlation. The nonparametric Mann Whitney U test tested the differences between subgroup of patients. Sensitivity and specificity of RDW, as well as its respective optimal cut-off value in prediction of MACE, were expressed by the receiver operating characteristics (ROC) curve. Predictive value of each parameter was calculated by univariate logistic regression analysis, and each statistically significant variable was included in risk prediction model. ROC curves were constructed using the predictive probability as a covariate. AUC were used to evaluate the predictive value of RDW alone, RDW and HCY and risk prediction model for MACE outcome. AUC greater than 0.9 indicated outstanding predictive value. AUC between 0.8 and 0.9 was considered excellent. AUC between 0.7 to 0.8 was considered acceptable, while 0.5 to 0.7 indicated poor prediction value. Finally, AUC less than 0.5 indicated the lack of a prediction value[18]. $p<0.05$ was defined as statistically significant.

Results

There was no difference between groups in gender, smoking, hypertension, and diabetes mellitus type 2 incidence. Patients in group 2 were older compared to group 1; 65.2±8.6 and 59.0±9.4, respectively ($p=0.023$). Higher prevalence of anemia was observed in MACE group ($p=0.002$) as well as a decreased hemoglobin level ($p=0.045$). Analysis of measured parameters revealed an elevated RDW value in group 2 compared to group 1; 14.4±1.6% and 13.4±0.9%, respectively ($p=0.027$). Significant decrease in vitamin B_{12} level was observed in group 2 ($p=0.044$). Differences in other measured parameters were not observed (Table I).

Correlation analysis presented in Table II revealed moderate positive correlation between RDW and HCY level ($r=0.267$, $p=0.026$), as well as negative correlation with vitamin B_{12} ($r= -0.294$, $p=0.012$) and hemoglobin ($r= -0.330$, $p=0.003$). RDW positively correlated with incidence of anemia ($r=0.324$, $p=0.004$) and with MACE outcome ($r=0.370$, $p=0.001$). There was a negative association between HCY and folic acid

Table I. Baseline characteristics of patients with (group 2) and without (group1) major adverse cardiac events (MACE).

Variable (No;%)	All patients (n=80)	Group 1 (n=65)	Group 2 (n=15)	p
Age (years) (mean ±SD)	60.2±9.6	59.0±9.4	65.2±8.6	0.023
Gender (males (%))	65 (81%)	53 (81%)	12 (80%)	0.891
Smoking (yes (%))	46 (56%)	39 (60%)	7 (47%)	0.267
Hypertension (yes (%))	60 (75%)	43 (73%)	13 (86%)	0.080
Diabetes mellitus type 2(yes (%))	20 (25%)	14 (22%)	6 (40%)	0.179
Anemia (yes (%))	13 (16%)	5 (8%)	8 (53%)	0.002
Parameter (Mean ±SD)				
Hemoglobin- g/L	145.1±15.1	145.6±12.7	133.8±20.2	0.045
RDW- %	13.6±1.1	13.4±0.9	14.4±1.6	0.027
Homocysteine-µmol/L	14.2±6.4	13.2±4.6	18.0±10.2	0.094
Folic acid-nmol/L	15.7±6.5	15.4±5.9	17.9±8.5	0.537
Vitamin B12-pmol/L	256.2±99.0	266.5±104.6	219.2±63.1	0.044
Leukocyte-x10^9/L	10.9±2.8	10.9±2.8	10.9±3.2	0.548
CRP-mg/L	7.8±17.7	6.3±7.4	14.8±30.1	0.939
Fibrinogen-g/L	3.6±0.8	3.4±0.8	3.4±0.9	0.657

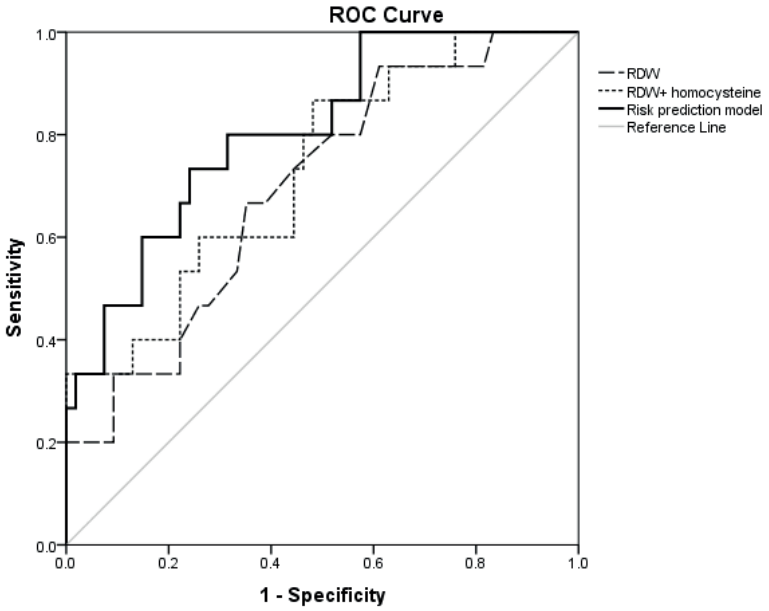

Figure 1. Receiver operating characteristic (ROC) curve for predicting major adverse cardiac events (MACE). For RDW alone, the area under curve (AUC) was 0.705 (95% CI: 0.563-0.846 p=0.014). When homocysteine was added to RDW, the AUC was 0.730 (95% CI: 0.588-0.871 p=0.007). When age, hemoglobin and existence of anemia were added to RDW and homocysteine (Risk prediction model), the AUC was 0.806 (95% CI: 0.687-0.926 p<0.001).

and vitamin B_{12}; r=-0.257, p=0.027 and r=-0.246, p=0.036 respectively. Positive association was found between HCY level and MACE outcome (r=0.302, p=0.009), and existence of anemia and MACE (r=0.312, p=0.006) while a negative association was found between hemoglobin level and MACE outcome (r= -0.315, p=0.006). Patients with anemia had decreased eGFR value (r=-0.276; p=0.015). Older age was positively associated with MACE outcome (r=0.254; p=0.023) and female gender (r=0.300; p=0.006), as well as with the presence of anemia (r=0.295; p=0.009) and decrease in hemoglobin level (r=-0.335, p=0.002).

As determined by the univariate logistic regression analysis (Table III), the most significant predictors of MACE were RDW OR 2.179 (95% CI 1.250 to 3.797, p=0.006), HCY OR 1.108 (95% CI 1.013 to 1.213, p=0.025), age OR 1.079 (95% CI 1.008 to 1.154, p=0.028), presence of anemia OR 0.134 (95% CI 0.034 to 0.532, p=0.004) and hemoglobin level OR 0.952 (95%CI 0.915 to 0.989, p=0.013).

Since HCY levels and RDW values are both independent risk factors for MACE, we evaluated the joint effect on the prediction of MACE outcome (Figure 1). The AUC for the RDW value alone was 0.705 (95 %CI 0.563-0.846, p=0.014), and the optimal cutoff value of RDW for discrimination of MACE was 13.65% (sensitivity 67%, specificity 67%). The AUC for the RDW increased up to 0.730 (95% CI 0.588–0.871) with the addition of HCY (p=0.007). The change in AUC was statistically significant when both measures were jointly assessed, suggesting that the combination of RDW with the HCY level contributes to the more accurate prediction of MACE outcome.

Further, after addition of all other significant independent risk factors for MACE, AUC value increased up to 0.806 (95% CI 0.687 to 0.926) and change was statistically significant as well (p<0.001) implying this model an excellent predictor of MACE outcome (Figure 1).

Discussion

Precise estimation of the absolute risk for MACE outcome after PCI is necessary when making treatment recommendations for patients. In the present study, for the first time, we found both that RDW and HCY are independent risk factors for MACE outcome in AMI patients treated with PCI and that their joint assessment significantly increases prediction value. In addition, we found that patients with higher RDW tend to have higher HCY level.

Table II. Correlation analysis.

Parameter	Parameter	*p*	r
RDW	Homocysteine	0.026	0.267
	MACE	0.001	0.370
	Vitamin B12	0.012	-0.294
	Hemoglobin	0.003	-0.330
	Anemia	0.004	0.324
Homocysteine	MACE	0.009	0.302
	Fibrinogen	0.046	0.330
	Vitamin B12	0.036	-0.246
	Folic acid	0.027	-0.257
MACE	Age	0.023	0.254
	Anemia	0.006	0.312
	Hemoglobin	0.002	-0.335

It is well established that chronic inflammation is the foundation of atherosclerosis and its complications. Increasing evidence shows an important relationship among HCY and prothrombotic state, inflammation, and oxidative stress in AMI patients[19]. This was partially confirmed in our paper with positive HCY and fibrinogen correlation. Accumulating evidence demonstrated that elevated HCY level should be regarded as a crucial risk factor for cardiovascular disease and is used as a screening tool of cardiovascular disease in general population[15]. Univariate logistic regression in this study revealed 1.108-time higher probability for MACE outcome for each 1µmol/L increase in HCY level, naming it a good predictor of unfavorable prognosis in AMI patients.

RDW is an indicator of variation in red cell size, and it is usually used for the differential diagnosis of microcytic anemia. RDW values greater than 95th percentile (i.e.,>14%) indicate presence of greater variability in circulating RBC size[20]. Values in this research are lower, but in the upper area of reference range, and recently, those levels were reported to be associated with morbidity and mortality in cardiovascular diseases[11]. Univariate logistic regression in this study revealed 2.179-time higher probability for MACE outcome for each 1% increase in RDW level, naming it an even stronger predictor of unfavorable prognosis in AMI patients. That is in line with previous researches[12,21]. Besides MACE, elevated RDW also independently predicted contrast-induced acute

Table III. Univariate logistic regression analysis for the prediction of major adverse cardiac events (MACE) outcome.

Variable	OR	95% CI	*p*-value
Age (years) (mean ±SD)	1.079	1.008-1.154	0.028
Gender (males (%))	0.906	0.221-3.716	0.891
Smoking (yes (%))	0.491	0.138-1.748	0.272
Diabetes mellitus 2	2.222	0.681-7.255	0.186
Anemia (yes (%))	0.134	0.034-0.532	0.004
Parameter			
Hemoglobin- g/L	0.952	0.915-0.989	0.013
RDW-%	2.179	1.250-3.797	0.006
Homocysteine-µmol/L	1.108	1.013-1.213	0.025
Folic acid-nmol/L	1.040	0.955-1.131	0.367
Vitamin B12-pmol/L	0.994	0.986-1.001	0.100
Leukocyte -x10^9/L	1.035	0.870-1.230	0.699
CRP-mg/L	1.033	0.989-1.079	0.143
Fibrinogen-g/L	1.062	0.348-3.240	0.915

kidney injury, stent restenosis and bleeding[12]. Considering these findings, and particularly as there is no additional cost, evaluation of RDW before PCI in patients with AMI may improve risk stratification, thus is strongly recommended.

Moderate association between RDW and HCY was observed in our study and was confirmed with negative correlation of both parameters with folic acid and vitamin B_{12} level - molecules that directly affect both HCY level and RDW value. That is in line with a recently published paper, where researchers suggested that RDW can predict HCY levels among the healthy adults. They identified that RDW positively correlated with HCY[16] in patients with physiological level of folic acid and vitamin B_{12}.

Potential mechanisms of association of RDW with MACE outcome and with HCY level are not fully understood yet. Previous studies indicated that folic acid and vitamin B_{12} deficiencies influence the morphology of erythrocyte, thereby altering the RDW value[22]. However, in this research, folic acid and B_{12} levels are within respected reference ranges thus that explanation is not very probable. Oxidative stress and subclinical inflammation may be possible pathophysiologic mechanisms which explain this phenomenon, as indicated by a moderate HCY correlation with inflammatory marker fibrinogen in this research. There is growing evidence that elevated levels of HCY tend to increase oxidation in the body, increased HCY levels may raise oxidative stress and inflammation, and decrease the production and bioavailability of nitric oxide[16]. In conclusion, accumulated HCY is associated with enhanced oxidative stress. Unfortunately, high oxidative stress can reduce the lifespan of red blood cells and promote immature erythrocytes from bone marrow into the peripheral circulation[23], which is then associated with increased RDW value. Mentioned changes jointly contribute to negative prognosis in AMI patients, and significantly increase risk for MACE occurrence after PCI, which is indicated by a significant increase in AUC value after adding HCY level to RDW value in ROC curve analysis. Thus, this kind of assessment refers to HCY and RDW as novel markers for MACE risk prediction, which can be useful, especially in patients without traditional risk factors presented, e.g., low-risk population. This study seems to suggest the superiority of RDW over HCY for MACE risk prediction in AMI population of Bosnia and Herzegovina. Adding other traditional risk factors (presence of anemia, hemoglobin, and age) to this model significantly increases AUC area and MACE risk prediction, pointing efficiency of presented model for high- risk population as well.

Conclusions

This investigation suggests that the combined assessment of RDW and HCY can help cardiologists improve MACE risk assessment and identify high-risk patients at an earlier stage, when suitable interventions can influence a better outcome in such patients. To our knowledge, this is one of the first prospective preliminary research addressing the value of HCY and RDW taken together as predictive biomarkers for MACE outcome in a clinical setting in population with very high prevalence of CVD. Considering potential links between them and ability to improve risk assessment, further multicenter researches involving a greater number of patients and longer follow up should be of great value.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction. Lancet 2017; 389: 197-210.
2) Mendis S, Thygesen K, Kuulasmaa K, Giampaoli S, Mähönen M, Ngu Blackett K, Lisheng L. Writing group on behalf of the participating experts of the WHO consultation for revision of WHO definition of myocardial infarction. World health organization definition of myocardial infarction: 2008–09 revision. Int J Epidemiol 2011; 40: 139-146.
3) Ali RA, Asadollah M, Hossien RA. The role of unknown risk factors in myocardial infarction. Cardiol Res 2010; 1: 15-19.
4) Isik T, Kurt M, Tanboga IH, Ayhan E, Gunaydin ZY, Kaya A, Uyarel H. The impact of admission red cell distribution width on long-term cardiovascular events after primary percutaneous intervention: a four-year prospective study. Cardiol J 2016; 23: 281-288.
5) De Goma EM, Knowles JW, Angeli F, Budoff MJ, Rader DJ. The evolution and refinement of traditional risk factors for cardiovascular disease. Cardiol Rev 2012; 20: 118-129.
6) Li N, Zhou H, Tang Q. Red blood cell distribution width: a novel predictive indicator for cardiovascular and cerebrovascular diseases. Dis Markers 2017; 2017: 7089493.

7) Parizadeh SM, Jafarzadeh-Esfehani R, Bahreyni A, Ghandehari M, ShafieeM, RahmaniF, Parizadeh MR, Seifi S, Ghayour-Mobarhan M, Ferns GA, Avan A, Hassanian SM. The diagnostic and prognostic value of red cell distribution width in cardiovascular disease; current status and prospective. Biofactors 2019; 45: 507-516.
8) Tonelli M, Sacks F, Arnold M, Moye L, Davis B, Pfeffer M. Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease. Circulation 2008; 117: 163-168.
9) Chen PC, Sung FC, Chien KL, Hsu HC, Su TC, Lee YT. Red blood cell distribution width and risk of cardiovascular events and mortality in a community cohort in Taiwan. Am J Epidemiol 2010; 171: 214-220.
10) Yao HM, Sun TW, Zhang XJ, Shen DL, Du YY, Wan YD, Zhang JY, Li L, Zhao LS. Red blood cell distribution width and long-term outcome in patients undergoing percutaneous coronary intervention in the drug-eluting stenting era: a two-year cohort study. PLoS One 2014; 10: 9: e94887.
11) Danese E, Lippi G, Montagnana M. Red blood cell distribution width and cardiovascular diseases. J Thorac Dis 2015; 7: 402-411.
12) Bao D, Luo G, Kan F, Wang X, Luo J, Jiang C. Prognostic value of red cell distribution width in patients undergoing percutaneous coronary intervention: a meta-analysis. BMJ Open 2020; 10: e 033378.
13) Mallikethi-Reddy S, Briasoulis A, Akintoye E, Afonso L. Novel biomarkers with potential for cardiovascular risk reclassification. Biomarkers 2017; 22: 189-199.
14) Prajapati P, Panjwani SJ. Serum homocysteine level as a risk factor for acute coronary syndrome. IJASEAT 2016; 2: 19-20.
15) Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J 2015; 14: 6.
16) Peng YF, Pan GG. Red blood cell distribution width predicts homocysteine levels in adult population without vitamin B12 and folate deficiencies. Int J Cardiol 2017; 227: 8-10.
17) WHO Scientific Group on Nutritional Anemias and World Health Organization (1968). Nutritional anaemias: report of a WHO scientific group. World Health Organ Tech Rep Ser 405: 5-37.
18) Hosmer DW, Lemeshow S. Applied logistic regression. John Wiley and Sons, 2000
19) Marković Boras M, Čaušević A, Brizić I, Mikulić I, VasiljM, Jelić-Knezović N. A relation of serum homocysteine, uric acid and C-reactive protein level in patients with acute myocardial infarction. Med Glas (Zenica) 2018; 15: 101-108.
20) Montagnana M, Cervellin G, Meschi T, Lippi G. The role of red blood cell distribution width in cardiovascular and thrombotic disorders. Clin Chem Lab Med 2011; 50: 635-641.
21) Fatemi O, Paranilam J, Rainow A, Kennedy K, Choi J, Cutlip D, Pencina M, Berger PB, Cohen DJ, Kleiman NS. Red cell distribution width is a predictor of mortality in patients undergoing percutaneous coronary intervention. J Thromb Thrombolysis 2013; 35: 57-64.
22) He L, Gao C, Wang Y, Wang H, Zhao H. Red cell distribution width and homocysteine act as independent risk factors for cardiovascular events in newly diagnostic essential hypertension. Oncotarget 2017; 8: 102590-102599.
23) Ghaffari S. Oxidative stress in the regulation of normal and neoplastic hematopoiesis, Antioxid Redox Signal 2008; 10: 1923-1940.