关系血液学参数与血糖控制的关系

糖尿病患者

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摘要

背景：糖尿病（DM）与微和宏观并发症是21世纪的全球性 epidemic。研究的目的是确定糖化血红蛋白（HbA1c）为7%或以下和超过7%的患者全血细胞计数（CBC）参数与血糖控制的关系。

方法：该研究包括了178名患者，包括男女两性，年龄超过40岁，来自Health Care Center »Dr Milorad Mika Pavlović«, Indjija, Serbia。为了观察CBC参数与血糖控制之间的可能相关性，将被调查者分为两组，HbA1c £ 7%和HbA1c>7%。我们分析了CBC参数，血糖调节参数，脂质状态，通过标准生物化学方法，进行了人体测量，并通过问卷和电子病历收集了患者数据。

结果：

PMDW参数在HbA1c £ 7%组和HbA1c>7%组之间存在统计学差异（p=0.045）。在所有患者中（单变量线性回归）结果表明，PCT与白细胞（p=0.0005），中性粒细胞（p=0.0046），单核细胞（p=0.003），MPV相关；MPC（p=0.0005），PDW和GLU0（p=0.005），PMDW与HbA1c%（p=0.049），GLU0（p=0.013），HDLC（p=0.001），BMI（p=0.043）相关。

克罗地亚语摘要

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**Introduction**

Diabetes mellitus (DM) is the leading global epidemic of the 21st century with over 422 million diabetics worldwide. The prediction of prevalence of diabetes mellitus patients by 2035 according to the World Health Organization (WHO) is 592 million patients (1). In the Republic of Serbia, 710,000 people or 12.4% of the population suffer from DM, and the increase in the number of patients is estimated to be 15.2% by 2035 (2).

Blood is classified as a connective tissue, with cellular elements suspended in plasma. Elevated blood glucose level in T2DM contributes to disturbance of blood cells and its indices (3). Good glycemic control is the main recommendation in the prevention of the development of diabetic complications. It has been suggested that early normalization of glycemia may inhibit pathological processes that are closely related and induced by hyperglycemia such as increased oxidative stress and glycation of cellular proteins and lipids (4). Therefore, it is crucial to achieve a gradual optimization of HbA1c levels – between 6.5% and 7%, recommended level, as a long-term form of management in order to reduce the incidence of macro and micro-vascular complications in DM patients (5).

Laboratory tests for monitoring diabetes mellitus patients are plasma glucose (random sample), glucose in urine, glycated proteins, glycated hemoglobin (HbA1c), fructosamine, urinary proteins (microalbuminuria, proteinuria), C-peptide, insulin, parameters of lipid status and kidney function (6). Hemoglobin A1c represents the average glycemic value for the last 2–3 months in patients with diabetes. Based on the HbA1c value, an estimated mean glucose (eAG) value can be determined (7, 8).

Parameters obtained from hematologic counters can provide insight into changes that occur in hematological indices such as white blood cells (WBC), red blood cells (RBC), platelets (PLT), and the other parameters. Analysis of these parameters could contribute in the following-up of the development of degenerative complications in DM (9). White blood cells are well established biomarker of inflammation in cardiovascular disease as well as in T2DM and its complications. They could be activated by advanced glycation end products, angiotensin II, oxidative stress in T2DM, induced by hyperglycemia (10).

Recent studies have shown that the number of red blood cells is decreased, its lifespan is reduced in T2DM due to elevated blood glucose (11). In a large prospective study ETDRS, authors have found that decreased Hb and HCT levels are associated with increased risk of T2DM microangiopathy progression. Possible mechanisms combine reduced erythrocyte survival, hypoxemia and decreased erythropoiesis (12).

Activated platelets and leukocytes at the sites of damaged endothelium release vasoactive substances such as leucotrien, serotonin, thromboxane A2 that lead to vasospasm and favourably influence the development of thrombus (13). Additionally, platelets express numerous adhesion molecules and ligands that facilitate interactions among platelets, leukocytes, and endothelium, by direct modulation of the activity of leukocytes (neutrophils, lymphocytes) with phagocytosis and oxidative stress; and endothelium with adhesion molecule and chemokine expression (14).

The risk factors for T2DM are variable such as hyperglycemia, hypertension, hyperlipidemia and obesity, and fixed like: age, duration of diabetes and pregnancy. The results of the ADDITION study have shown that patients with retinopathy have significantly higher HbA1c as well as systolic and diastolic blood pressure than patients without retinopathy (15).

Early Treatment Diabetic Retinopathy Study (ETDRS) data have shown that elevated serum lipid levels such as total cholesterol, low density lipoprotein cholesterol are associated with an increased risk for micro- and macro-vascular complications in diabetics (16). The low level of HDL cholesterol most commonly exists in patients with type 2 diabetes; and it is one of the risk factors for cardiovascular outcomes (17). Obesity is a major risk factor for the development of insulin resistance and type 2 diabetes mellitus (T2DM) (18).

The aim of the study is to determine possible changes in the parameters of the complete blood count depending on glycoregulation, as well as other risk factors in patients with Type 2 Diabetes mellitus.

**Materials and Methods**

The study was a randomized cross-sectional study aimed at determining the association of the CBC parameters and glycemic control, as well as other risk factors (hypertension, hyperlipidemia, obesity, duration of the disease) in T2DM patients.

**Conclusions:** Based on our study results it may be concluded that some of the parameters of CBC could be useful tool in following glycemic control of diabetics.

**Keywords:** T2DM, CBC parameters, platelets, HbA1c

**Zaključak:** Na osnovu dobijenih rezultata našeg istraživanja može se zaključiti da postoji povezanost između pojedinih hemato-loških parametara i glikoregulacije kod pacijenata sa dijabetesom.

**Ključne reči:** DMT2, parametri KKS, trombociti, HbA1c
All the procedures in the study were conducted according to the rules of the Helsinki Declaration, approved by the Ethical Committee of the Health Care Center »Dr. Milorad Mika Pavlović« in Indjija, Serbia. All patients signed an Informed Consent to participate voluntarily in the study. Patient data are protected and used only for the purpose of the research.

Study Population

The study included a total of 178 patients with type 2 diabetes (T2DM), of both gender, over the age of 40 years, sent from the Diabetes Counseling Center of Health Care Center »Dr Milorad Mika Pavlović«, to the Department of Laboratory Diagnostics. The exclusion criteria from the study were endocrinological diseases affecting the metabolism of glucose and lipids, hematological diseases, systemic diseases, pregnancy, acute diseases, degenerative diseases of the nervous system and malignancy (19). The presence of associated diseases was monitored via an electronic patient card.

To notice the possible correlation between the CBC parameters and T2DM the subjects were divided into two groups based on the glycemic regulation, according to the obtained values for HbA1c. The first group consisted of subjects with HbA1c ≤ 7% (regulated), and the second group with HbA1c > 7% (unregulated).

Questionnaire

The data were collected on the basis of a specially designed questionnaire for this research, which included demographic data, age, gender, smoking status, duration of diabetes, presence of associated illnesses (hypertension, coronary artery disease, dyslipidemia, stroke). The questionnaire was filled in by patients in cooperation with the doctor.

Antropometric measurement

Anthropometric measurements were performed, with the subjects being lightly dressed and barefooted. Body Height (BH) was measured using Martin’s anthropometer with a precision of ± 0.1 cm. Body Weight (BW) was measured using an electronic scale type Omron BF214, with the accuracy of 40 kg to 150 kg ± 1% (20). Measurement of fat percentage in the body was carried out by using the bioelectric impedance method. Body Mass Index (BMI) was determined by the formula: BMI = BW/BH², which is automatically calculated by the scale with the previously entered body weight (BW-kg), body height (BH-m), age and gender.

Blood pressure measurement

Blood pressure measurement (BP) was performed three times after a ten-minute standstill with the Riva-Rocci sphygmomanometer in mmHg and the mean value was estimated. Blood pressure values, greater than or equal to 140/90 mmHg were considered hypertension.

Blood collection

Blood samples were collected after a night time famine of 12–14h. Testing of samples was carried at the Department of Laboratory Diagnostics. The material used to determine CBC 5-part difference and HbA1c was venous blood collected in vacuum tubes with an anticoagulant EDTA (Ethylene Diamine Tetracetic Acid). To determine glucose and lipid status parameters, vacuum tubes were used without anticoagulants, but with gel separator. Hematology method involves the determination of parameters of complete blood count on the hematologic analyzer Advia120 (Siemens-Germany), that uses flowcitometry and VSC technology (volumen, conductivity, light scatter) method (9).

Complete blood count determination included: white blood cells – leukocytes (WBC), neutrophils (neutro), lymphocytes (lympho), monocytes (mono), eosinophils (eos), basophils (baso). The number of red blood cells – erythrocytes (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean cell hemoglobin concentration (MCHC) and red blood cell distribution width (RDW). Number of the platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), mean platelet component (MPC), platelet component distribution width (PCDW), mean platelet dry mass (MPM), platelet dry mass distribution width (PMDW).

The values of glucose were determined by enzymatic methods with glucoso oxidase (GODPAP). Total cholesterol (TC) was analyzed with enzymatic methods using commercially available reagents consisted of cholesterol oxidase, esterase and peroxidase. The end products of TC were measured by spectrophotometry.

The method for determination of triglycerides (TG) is based on enzymatic reaction with glicerol-3-phosphate-oxidase (GPO). High density lipoprotein–cholesterol (HDL-C) values were determined by direct measurement using polymer-polyanion method, and the value of low density lipoprotein-cholesterol (LDL-C) was determined by the Friedewald equation. Index of atherosclerosis (IA) was determined by formula. The HbA1c value was analyzed by the method of immuno-turbidimetric inhibition (TINIA) in hemolized full blood. The target value for good glycemic control was hemoglobin A1c ≤ 7% by ADA (7).
Determination of the biochemical parameters and hemoglobin A1c was done on the biochemical analyzer Advia 1200 (Siemens-Germany). Standardization, calibration, application of control material and sample processing are in accordance with the manufacturer’s instructions (9).

Statistical analyses

The following statistical methods were used in the data analysis. In descriptive statistics we used the mean (arithmetic mean) and measure of variability, standard deviation (SD) for normally distributed data and median or interquartile range (IQR) for data with skewed distribution. The confidence interval (CI 95%) was also examined. Normality of the parameters was examined using boxplots and Kolmogorov-Smirnov tests. Student T-test and binary logistic regression were performed for testing the statistical significance between two groups with normal distribution of data. Binary logistic regression was performed to analyse parameters associated with T2DM in regulated and unregulated group of patients (HbA1c<7, HbA1c>7). For data with skewed distribution Mann-Whitney U-test, the logarithmic scale (log10) and inverse distribution function (IDF) were used.

For association between parameters of glycemic control and CBC 5-part difference parameters, we used univariate linear regression.

To determine connection between regulation of T2DM and CBC–5-part difference, adjusting for confounders, multiple linear regression was applied, and three models have been developed:

Model 1 adjusted for age, gender, duration of diabetes, weight, BMI.

Model 2 adjusted for parameters of complete blood count used in this study.

Model 3 adjusted for parameters of the lipid status.

We also performed multiple linear regression adjusted for all parameters used in this study.

We used SPSS V.21 (IBM) for the descriptive and analytic statistics. The two tailored p value of <0.05 was considered significant.

Results

From a total of 178 patients with HbA1c≤7 was 94 (52.82%) and 84 (49.19%) with HbA1c>. In total number of patients 66 male (37.08%) and 112 female (62.92%), age between 40 and 75 years were included in the study. According to gender there were 32 men (48.48%) with HbA1c≤7 and 34 (51.52%) with HbA1c>7; 62 women (55.36%) with HbA1c≤7, and 50 (44.64%) with HbA1c>. Of the total number of participants there were 65 smokers (36.52%). When we included number of comorbidities (hypertension, angina pectoris, irregular heartbeat, stroke) participants were divided in three groups: without comorbidities (33 HbA1c≤7, 25 HbA1c>7), one comorbidity (39 HbA1c≤7, 36 HbA1c>7), two comorbidities (22 HbA1c≤7, 23 HbA1c>7) (Table I). According to the level of HbA1c (%). participants were divided in two groups, regulated (HbA1c≤7) and unregulated group (HbA1c>7). There were no statistical difference for two groups HbA1c≤7 and HbA1c>7 in terms of age, anthropometric measurements and some parameters of CBC, except for duration of T2DM (DD) (HbA1c≤7 7±4.31, CI95%=0.0279; HbA1c>7 9±5.03, CI95%=0.03; T-test p=0.00115, bin.log.reg. p=0.003); fasting glucose (HbA1c≤7 8.1±1.42, CI95%=0.009; HbA1c>7 9.2±3.07, CI95%=0.02; T-test p=0.0005, bin.log.reg. p=0.0005); postprandial glucose (HbA1c≤7 9.4±2.57, CI95%=0.02; HbA1c>7 11.5±3.94, CI95%=0.027; T-test p=0.0005, bin.log.reg. p=0.0005); PDMW (HbA1c≤7 0.8±0.07, CI95%=0.0044; HbA1c>7 0.81±0.07, CI95%=0.0005, T-test p=0.045, bin.log.reg. p=0.058); HDL-C (HbA1c≤7 1.2±0.35, CI95%=0.002; HbA1c>7 1.18±0.27, CI95%=0.002; T-test p=0.0067, 0.0067).

Table I Type 2 Diabetes mellitus patients’ demographic data, smoking status, number of comorbidities comparing those with HbA1c ≤7 and HbA1c > 7 in all patients.

<table>
<thead>
<tr>
<th></th>
<th>HbA1c≤7 (n=94)</th>
<th>HbA1c&gt;7 (n = 84)</th>
<th>T-test</th>
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<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
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<tr>
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<tr>
<td>Male (M)</td>
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<tr>
<td>Female (F)</td>
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<tr>
<td>Smoking status</td>
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<tr>
<td>Disease0</td>
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<td>56.9</td>
<td>25</td>
</tr>
<tr>
<td>Disease1</td>
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<td>52</td>
<td>36</td>
</tr>
<tr>
<td>Disease2</td>
<td>22</td>
<td>48.89</td>
<td>23</td>
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Table II: Type 2 Diabetes mellitus patients’ duration of disease, glycoregulation parameters, PMDW, HDL-C comparing those with HbA1c ≤ 7 and HbA1c > 7 in all patients.

<table>
<thead>
<tr>
<th></th>
<th>HbA1c ≤ 7 (n=94)</th>
<th>HbA1c &gt; 7 (n= 84)</th>
<th>T-test</th>
<th>Bin. Log. Reg.</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>mean/median</td>
<td>SD or IQR</td>
<td>95%CI</td>
<td>mean/median</td>
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<td>DD</td>
<td>7</td>
<td>4.311</td>
<td>0.0279</td>
<td>9</td>
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<td>GLU0</td>
<td>8.1</td>
<td>1.421</td>
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<td>GLU2</td>
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<td>0.0166</td>
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<tr>
<td>PMDW</td>
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<td>0.067</td>
<td>0.0004</td>
<td>0.81</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.2</td>
<td>0.351</td>
<td>0.0023</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Legend: hba1c %, Glycated hemoglobin in %; DD, duration of the disease in year; GLU0, fasting glucose in mmol/L; GLU2, post-prandial glucose in mmol/L; PMDW, Platelet Dry Mass Distribution Width in pg; HDL-C, high density lipoprotein–cholesterol in mmol/L.

bin.log.reg. p = 0.0111 (Table I). In total number of patients for univariate linear regression, (n=178) it is shown that HCT was associated with DD (p = 0.001); MPC (p = 0.015), fasting glucose (p = 0.049), BW (p = 0.0005); PCT was associated with WBC (p = 0.0005), neutrophiles (p = 0.046), monocytes (p = 0.003); PMDW was associated with fasting glucose (p = 0.013), HbA1c (p = 0.049), HDL-C (p = 0.001), BW (p = 0.043). In T2DM patients with HbA1C ≤ 7 for univariate linear regression, (n=94) it is shown that HCT was associated with fasting glucose (p = 0.003); PCT was associated with WBC (p = 0.0005), neutrophiles (p = 0.008), lymphocytes (p = 0.014), monocytes (p = 0.033). In T2DM patients with HbA1C>7 for univariate linear regression, (n=84) it is shown that HCT was associated with DD (p = 0.02), MPC (p = 0.016), PDW (p = 0.019), BW (p = 0.0005); PCT was associated with WBC (p = 0.0005), monocytes (p = 0.0005), RBC (p = 0.027); PLT (p = 0.0005); MPM was associated with HDL-C (p = 0.002); PMDW was associated with HDL-C (p = 0.001). In multiple linear regression for all patients, in model 1 HCT was associated with duration of the disease (DD) of T2DM (p = 0.027), with body weight (p = 0.0005) and BMI (p = 0.045); PCT was associated with BMI (p = 0.027). In model 2 HCT was associated with MPV (p = 0.002) and MPC (p = 0.017); MPM was associated with PMDW (p = 0.0005). In model 3 HCT was associated with HDL-C (p = 0.001), MPM with HDL-C (p = 0.024) and PMDW with HDL-C (p = 0.031). In multiple linear regression for regulated patients, in model 1 HCT was associated with age (p = 0.037). In model 2 MPM was associated with PMDW (p = 0.0005). In model 3 HCT was associated with HDL-C (p = 0.0005), PCT was associated with triglycerides (p = 0.008). In multiple linear regression for unregulated patients, in model 1 HCT was associated with BW (p = 0.001). In model 2 HCT was associated with MPV (p = 0.014), PCT was associated with WBC (p = 0.01), neutrophiles (p = 0.002), lymphocytes (p = 0.003), monocytes (p = 0.006), eosinophiles (p = 0.036) and with MPC (p = 0.003); PMDW was associated with RBC (p = 0.005), with MCH (p = 0.036) and with MPM (p = 0.0005). In model 3 HCT was associated with HDL-C (p = 0.007); MPM with HDL-C (p = 0.019) and PMDW with HDL-C (p = 0.008). In multiple linear regression adjusted for all parameters, HCT was associated with MPV (p = 0.002), MPC (p = 0.021); MPM was associated with HGB (p = 0.025); PMDW was associated with DD (p = 0.03) (Data are not shown in tables). For other parameters there were no statistical significances.

Discussion

Diabetes mellitus is one of the most common multisystemic diseases with microangiopathic and macroangiopathic complications. In T2DM hyperglycemia disturbs hematological indices and together with well recognised risk factors could lead to its degenerative complications. In our study there was no correlation between WBC and parameters of glycoregulation. However, other studies have shown association between WBC and impaired glucose control. Chronic inflammatory state in DM due to insulin action on the adipose tissue, muscles and liver promote differentiation and maturation of WBC via proinflammatory cytokines (10).

In a study conducted by Irace C et al. (12) hemoglobin, hematocrit and whole blood viscosity were significantly lower in subjects with retinopathy compared with subjects without microvascular complication, retinopathy. Possible mechanisms for decreased RBC indices in T2DM are structural modifications of erythrocytes membrane, changes of surface electric charge, erythrocyte aggregation, that...
could lead to the shorter lifespan of RBC. According to Malandrino N et al. (21). RDW was elevated in the patients with macro and microvascular complications in diabetes. However, in Duman E et al. (22) study the correlation between RDW and peripheral arterial disease was not found.

There is a correlation between hematocrit (HCT) and duration of diabetes (DD), body weight (BW) in our study in the patients with HbA1c>7 group (unregulated diabetes group).

Insulin resistance is commonly present in T2DM and associated with a disorder of platelet function due to insulin action. The complete coagulation cascade is dysfunctional in T2DM (13).

Studies of Jindal et al. (23) and Kodiatte et al. (24) have shown that platelet indices, MPV, especially PDW, differ in DM patients with and without microvascular complications. An increase in MPV may point to a possible indicator of the development of atherosclerosis associated with DM and vascular complications (23, 24).

In the study Kilicli-Camur N et al. (25) authors suggest that MPV is higher in patients with an acute myocardial infarction (AMI) and is also associated with higher mortality following MI. These findings raise the hypothesis of the potential importance of MPV in the underlying pathophysiology of cardiovascular disease (25, 26).

However, in our study, there is no statistically significant difference in the values of MPV among the groups. The explanation for this difference is perhaps a better regulation of glycemia with less vascular micro and macroangiopathies in T2DM patients in our study and later diagnosis of disease and its complications in the other studies.

Our results show that in the HbA1c≤7% group there is a correlation between fasting glucose and platelets. Additionally, PCT is associated with WBC, neutrophiles, lymphocytes and monocytes. Similar results were also obtained in the Levent Demirtas study (10). However, the results of other studies have not shown the correlation between platelets and other platelet indices and the regulation of T2DM (27, 28).

The results of our study have shown that there is a statistically significant difference in the values of PMDW between regulated and unregulated T2DM patients (Table II), as well as association of PCT with fasting glucose and PLT, PCT with postprandial glucose in unregulated group of diabetic patients. There is also a correlation of RDW and PMDW with HbA1c and fasting glucose when we adjusted the analyses for all examined confounders. We have found also a correlation between PCT and triglyceride in the group of regulated patients.

UKPD and KPDS50 studies indicated that the development of microvascular complications, especially retinopathy was closely related to the level of fasting glucose, the presence of hyperglycemia for more than 6 years and elevated blood pressure. Findings indicate the need for good glyceregulation and blood pressure control in order to reduce microvascular complications, like retinopathy progression (29). It has also been observed that the values of fasting and postprandial glucose are statistically higher in the group with unregulated versus those with regulated DM, in all observed patients (Table II). Data obtained from the pan European research indicate that low HDL-cholesterol is common in people with diabetes (17). The whole group of patients who were divided according to HbA1c values had significant differences in HDL-cholesterol: lower values in the HbA1c>7 group than those with HbA1c≤7 (Table II) (30). There is a connection between HDL cholesterol with fasting and postprandial glucose as well as HbA1c in all patients. In the group of patients with HbA1c>7 HDL-cholesterol is associated with platelet indices (MPW, MPC, PDW, MPM, PMDW).

Overweight and prevalence of obesity are rapidly increasing in many countries. The World Health Organization recognized that there is a »global obesity epidemic« with the increased prevalence of T2DM. Both diseases have a combined background in genetic factors, environments, society changes – modernization 0 which lead to excessive nutrition and sedentary lifestyle. These factors are important, but it is difficult to provide consistent evidence of their effects (18).

In our study, we found that BW is associated with HDL-C and hematocrit, in a group of subjects with unregulated T2DM.

In patients with unregulated glycemia the hematological parameters such as platelets, WBC, PCT, MPM, PMDW are increased but the HCT is decreased in our study.

The limitations of this study are: the study was conducted in a population in which there was increased number of patients with T2DM, metabolic syndrome and obesity. There are also other factors, confounders that influence the occurrence of T2DM which could not be examined in this study.

Based on our study results it may be concluded that some of the parameters of CBC could be useful tool in following up T2DM and potential markers of its complications, such as platelets, WBC, PCT, MPM, PMDW, HCT. The relationship among other risk factors such as lipoproteins, anthropometric measurements and development of T2DM and its complications is well recognized, and studied in multiple studies. Our findings suggest that hematological indices are accessible, useful, simple, inexpensive laboratory parameters which could be utilised in health care centers. Therefore, general practitioners are the first line for diagnosis and follow-up of T2DM patients who use these indices in daily practice.
In order to confirm the results of our study there is a need for the future, larger size study.

Acknowledgments. We want to thank for his cooperation our data miner and analyst MSc Zoran Vukusic. This work is based and supported with resources and facilities of Department of Laboratory Diagnostics, Health Care Center »Dr Milorad Mika Pavlović, Indjija, Serbia.

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Conflict of interest statement

The authors state that they have no conflicts of interest regarding the publication of this article.

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Received: April 4, 2018
Accepted: July 4, 2018