Associations Between Mean Platelet Volume and Various Factors in Type 2 Diabetes Patients: A Single-Center Study from Poland

Karolina Marta Gacuta, Olga Martyna Koper-Lenkiewicz, Anna Justyna Milewska, Magdalena Ćwiklińska-Dworakowska, Joanna Matowicka-Karna, Joanna Kamińska

Corresponding Authors: Karolina Marta Gacuta, e-mail: karolina.marta.gacuta@gmail.com, Olga Martyna Koper-Lenkiewicz, e-mail: o.koper@wp.pl

Background: Thromboembolic episodes, which are largely mediated by blood platelets, are prevalent chronic complications of diabetes. The mean platelet volume (MPV) serves as a marker for in vivo platelet activation. This study aimed to assess the factors influencing MPV in 106 patients with type 2 diabetes, compared with 59 non-diabetic individuals at a single center in Poland.

Material/Methods: We performed linear regression analysis, with MPV as the dependent variable and factors such as age, sex, thrombopoiesis-influencing cytokines, blood pressure, body mass index, glycosylated hemoglobin percentage, platelet count, large platelet count, lipid profile parameters, creatinine concentration, estimated glomerular filtration rate, treatment modalities, and comorbidities as independent variables. MPV was measured using the ADVIA 2120 hematology analyzer, with a reference range of 7-12 fL.

Results: The analysis revealed that in patients with type 2 diabetes, an increase in platelet count by 1×10^3/μL resulted in a decrease in MPV by 0.05 (P<0.001), while an increase in large platelet count by 1×10^3/μL led to an increase in MPV by 0.18 (P<0.001). Additionally, patients taking β-blockers or insulin had lower MPVs by 0.77 (P=0.008) and 5.63 (P<0.001), respectively, compared with those not on these medications.

Conclusions: This study delineates the relationship between MPV, platelet parameters, and treatment modalities in type 2 diabetes, paving the way for further research to elucidate underlying mechanisms and potential clinical applications.

Keywords: Diabetes Mellitus, Type 2 • Mean Platelet Volume • Platelet Activation • Platelet Count

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Background

Type 2 diabetes is one of the major health problems of the 21st century [1]. Patients with diabetes face a considerably higher risk of developing coronary heart disease, chronic kidney disease, vision loss, peripheral arterial disease, and cerebrovascular accident. This is primarily due to the significant complications that affect both small and large blood vessels, commonly observed in individuals with diabetes [2]. The most common chronic complication of diabetes is thromboembolic episodes [3]. Platelets play a significant role in the development of thrombosis [4-6]. During platelet activation, active substances are released from alpha-granules, which additionally intensify their activation [4]. The mean platelet volume (MPV) is a well-established marker of platelet activation [5,7]. Research has revealed elevated MPV values among individuals with type 2 diabetes [8-11]. According to Lippi et al [2], MPV could serve as a valuable prognostic indicator for cardiovascular complications in patients with type 2 diabetes. Additionally, a higher MPV value was found to be independently linked to an increased risk of type 2 diabetes [12].

The initial stages of the formation and maturation of blood platelets in the bone marrow can affect their morphological parameters [13]. The hormone that stimulates the process of thrombopoiesis is a thrombopoietin (TPO) [14,15]. Bosco et al [16] and Larsen et al [17] revealed significantly increased TPO levels in patients with type 2 diabetes than in individuals with normal glucose tolerance. In patients with type 2 diabetes, the secretion of TPO is increased, probably due to elevated concentration of interleukin 6 (IL-6), which affects the synthesis of this hormone by hepatocytes [16,18]. Measurement of plasma IL-6 levels showed an increased concentration in diabetic patients compared with that of healthy control participants [19]. TPO exerts a stronger effect on megakaryocytes in the presence of IL-6. Additionally, IL-6 stimulates the growth of megakaryocytes via the IL-6 receptors present on their surface, which increases nuclear ploidy and contributes to an increase in the size of megakaryocytes and the shedding of larger cytoplasmic fragments [15,20]. The increase in the MPV value in a blood morphology test reflects this process [20].

Activation of blood platelets leads to the translocation of P-selectin from alpha granules of blood platelets to their surface, which enables them to roll along the surface of the endothelium [5]. Then, P-selectin presented on the surface of the blood platelets is released into the bloodstream, forming its soluble form (sP-selectin) [5]. An additional source of sP-selectin is Weibel-Palade bodies in endothelial cells [5]. sP-selectin has a long half-life and additionally enhances the procoagulant properties of blood platelets [5]. Studies showed that in patients with type 2 diabetes, both the expression of sP-selectin on the surface of platelets [16] and the concentration of sP-selectin [21,22] were significantly higher than that in individuals with normal glucose metabolism. Studies revealed that patients with type 2 diabetes have a significantly higher concentration of sCD40L than healthy individuals [23-25]. Receptor membrane ligand CD40L (CD154) is found on various cell surfaces, including B and T lymphocytes, natural killer cells, basophils, monocytes/macrophages, mastocytes, endothelial cells, dendritic cells, and platelets [26-28]. The interaction of its soluble form (sCD40L) with its receptor CD40 significantly increases the inflammation of the endothelium via the enhancement of IL-6 production, which also affects thrombocytopenia [4,26]. Additionally, sCD40L increases the expression of P-selectin [28].

Literature data indicate that besides TPO, IL-6, sCD40L, and sP-selectin [4,13,15,20,26,28], other factors can influence blood platelet activation and the MPV value [20]. Various factors have been identified, such as the percentage of glycated hemoglobin (HbA1c), fasting and postprandial blood glucose concentration, diabetes duration, platelet morphological parameters (platelet distribution width and large platelet ratio), retinopathy, and microalbuminuria, which have been indicated as factors influencing the MPV value in patients with type 2 diabetes [2,8,29,30]. Moreover, patients with type 2 diabetes have a significantly higher concentrations of lipid profile parameters, creatinine, and body mass index (BMI) than do healthy controls [23,31]. Other reported factors influencing the MPV value include age, sex, platelet count, inflammatory conditions, applied treatment, hormone profile, race, and lifestyle [20]. Therefore, it is suspected that all the above-mentioned parameters may have an influence on the MPV value also in patients with diabetes.

The analysis of factors influencing platelet activation in patients with type 2 diabetes can have significant importance because this knowledge may contribute to reducing the occurrence of thromboembolic episodes in these patients [8,29]. Therefore, in this study, we aimed to compare the factors associated with MPV in 106 patients with type 2 diabetes mellitus with that of healthy control participants at a single center in Poland.

Material and Methods

Ethics Statement

The study was approved by the Bioethics Committee of the Medical University in Białystok (approval number APK.002.207.2022). All participants gave their written informed consent for inclusion before they participated in the study. The study participants were provided with the document to read and given an opportunity to ask questions...
about the study. Consents were recorded by a physician from the Department of Diabetology, Endocrinology, and Internal Diseases at the Provincial Integrated Hospital named after J. Śniadecki in Białystok.

**Type 2 Diabetes Patients**

The study group consisted of 106 patients diagnosed with type 2 diabetes (50 men and 56 women, median age 70 years, ranging from 40 to 91 years) who were hospitalized in the Department of Diabetology, Endocrinology, and Internal Diseases at the Provincial Integrated Hospital named after J. Śniadecki in Białystok. The exclusion criteria for the type 2 diabetes group included malignancy in medical history, blood transfusion, pregnancy or delivery in the last 6 months, hormone replacement therapy, autoimmune diseases, and acute inflammation. Table 1 presents the clinical and demographical characteristics of the study group.

**Control Group**

The control group consisted of 59 volunteers with normal glucose metabolism (24 men and 35 women, median age 57 years, ranging from 34 to 87 years). The exclusion criteria for the control group included family health history of diabetes (parent, brother, or sister with type 2 diabetes), impaired glucose tolerance, BMI >25 kg/m², gestational diabetes, polycystic ovary syndrome, treatment with oral antidiabetic drugs, insulin, antithrombotic drugs, antihypertensive drugs, lipid-lowering agents, diuretics, and proton pump inhibitors.

**Platelets Morphological Parameters Evaluation**

Morphological parameters of blood platelets – platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), and large platelet count (LPLT) – were determined in venous blood collected on K$_3$-ethylenediaminetetraacetic acid (EDTA-K$_3$) tubes (S-Monovette, SARSTEDT, 2.7 mL) using kits based on the sandwich enzyme-linked immunosorbent assay technology (ELISA). TPO concentration was measured using an ELISA Quantikine Human TPO kit (Cat#: DTP00B; R&D Systems Europe Ltd., Abingdon, England). IL-6 concentration was measured using ELISA Quantikine Human IL-6 Immunoassay kit (Cat#: D6050; R&D Systems Europe Ltd.). sP-selectin concentration was measured using ELISA Quantikine Human P-Selectin Immunoassay kit (Cat#: DPSE00; R&D Systems Europe Ltd.). sCD40L concentration was measured using a human sCD40L ELISA kit (Cat#: 650 120 096; Diaclone SAS, Besançon, France). Experiments were conducted following the manufacturers’ instructions. According to the manufacturers’ specifications, samples were not diluted before analysis.

**Percentage of HbA1c Evaluation**

The percentage of HbA1c was evaluated in whole blood collected into tubes with EDTA-K$_3$ (S-Monovette, SARSTEDT, 2.7 mL) on the biochemical analyzer Architect ci8200 (Abbott Diagnostic, Germany). The determination of HbA1c percentage was performed using the ARCHITECT ci 8200 biochemical analyzer, using the MULTIGENT test. Blood underwent preliminary processing with a denaturing reagent, leading to erythrocyte lysis. The hemoglobin released from within the RBCs underwent degradation by a proteolytic enzyme, pepsin. The resulting hemolysate underwent further processing to determine the concentrations of total hemoglobin and glycosylated hemoglobin. A colorimetric method was utilized to determine the concentration of total hemoglobin. For this purpose, the hemolysate was subjected to the action of an alkaline solution of nonionic substances, causing a reduction in surface tension. This led to the conversion of hemoglobin to hematin, giving the solution a green color. Subsequently, the absorbance of the sample was measured, and the concentration of total hemoglobin was read from the calibration curve of this parameter. The concentration of glycosylated hemoglobin was determined using an immunoturbidimetric method, employing the inhibition of micro-particle agglutination. Initially, an alkaline solution of a nonionic detergent was added to the hemolysate, causing the conversion of hemoglobin to alkaline hematin. This resulted in appropriate values according to the theory of Mie light scattering. Data obtained at low-angle detection indicate cell volume, while measurements provided during high-angle detection are associated with the refractive index, which depends on the presence of particles in the blood platelets. The laboratory’s normal reference range for MPV is 7-12 fl, PLT is 130-350×10$^3$/μL, PCT is 0.12-0.36%, and PDW is 40-60%.

**TPO, IL-6, sP-Selectin, and sCD40L Evaluation**

TPO, IL-6, sP-selectin, and sCD40L concentrations were evaluated in blood collected into tubes without anticoagulant (S-Monovette, SARSTEDT, 2.7 mL) using kits based on the sandwich enzyme-linked immunosorbent assay technology (ELISA). TPO concentration was measured using an ELISA Quantikine Human TPO kit (Cat#: DTP00B; R&D Systems Europe Ltd., Abingdon, England). IL-6 concentration was measured using ELISA Quantikine Human IL-6 Immunoassay kit (Cat#: D6050; R&D Systems Europe Ltd.). sP-selectin concentration was measured using ELISA Quantikine Human P-Selectin Immunoassay kit (Cat#: DPSE00; R&D Systems Europe Ltd.). sCD40L concentration was measured using a human sCD40L ELISA kit (Cat#: 650 120 096; Diaclone SAS, Besançon, France). Experiments were conducted following the manufacturers’ instructions. According to the manufacturers’ specifications, samples were not diluted before analysis.
Table 1. Demographic, clinical, and routine laboratory data, coexisting diseases, and medications of patients with type 2 diabetes.

<table>
<thead>
<tr>
<th>Type 2 diabetes patients</th>
<th>Coexisting diseases n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Hypertension 90 (85%)</td>
</tr>
<tr>
<td>Sex: female/male</td>
<td>Hyperlipidemia 22 (21%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>Obesity 68 (64%)</td>
</tr>
<tr>
<td></td>
<td>Chronic renal failure 34 (32%)</td>
</tr>
<tr>
<td>BP systolic, mmHg</td>
<td>Coronary artery disease 63 (59%)</td>
</tr>
<tr>
<td>BP diastolic, mmHg</td>
<td>Mitral valve regurgitation 12 (13%)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>Cardiac arrhythmias 34 (32%)</td>
</tr>
<tr>
<td></td>
<td>Polyneuropathy 13 (12%)</td>
</tr>
<tr>
<td>Laboratory parameters</td>
<td>Stroke 10 (9%)</td>
</tr>
<tr>
<td>WBC, 10⁹/μL</td>
<td>Diabetic foot 16 (15%)</td>
</tr>
<tr>
<td>RBC, 10⁶/μL</td>
<td>Atherosclerosis of the lower limbs 30 (28%)</td>
</tr>
<tr>
<td>HGB, g/dL</td>
<td>Secondary anemia 9 (8%)</td>
</tr>
<tr>
<td>HCT, %</td>
<td>Taking drugs N (%)</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>Acetylsalicylic acid 64 (60%)</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>Fibrates 21 (20%)</td>
</tr>
<tr>
<td>MCHC, g/dL</td>
<td>Statins 42 (40%)</td>
</tr>
<tr>
<td>CHCM, g/dL</td>
<td>Beta-blockers 74 (70%)</td>
</tr>
<tr>
<td>RDW, fl</td>
<td>ACE inhibitors 85 (80%)</td>
</tr>
<tr>
<td>HDW, g/dL</td>
<td>ARBs 11 (10%)</td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>Calcium channel blockers 40 (38%)</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>Diuretics 77 (73%)</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>Proton pump inhibitors 15 (14%)</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>Insulin 99 (93%)</td>
</tr>
<tr>
<td>INR</td>
<td>Oral antidiabetic drugs 51 (48%)</td>
</tr>
</tbody>
</table>
| PT, sec.                 | **SI conversion factors:** to convert WBC to ×10⁹/L, multiply by 1.0; RBC to ×10¹²/L multiply by 1.0; HGB to g/L multiply by 10.0; HCT to proportion of 1.0 multiply by 0.01; MCHC to g/L multiply by 10.0; CHCM to g/L multiply by 10.0; TC to mmol/L multiply by 0.0259; HDL-C to mmol/L multiply by 0.0259; LDL-C to mmol/L multiply by 0.0259; TG to mmol/L multiply by 0.0113; fibrinogen to μmol/L multiply by 0.0294; D-dimer to mmol/L multiply by 5.476; creatinine to μmol/L multiply by 88.4; eGFR to ml/sec multiply by 0.0167**
| Fibrinogen, mg/dL        | **ACE** – angiotensin-converting enzyme; **ARBs** – angiotensin receptor blockers; **BMI** – body mass index; **BP** – blood pressure; **CHCM** – mean hemoglobin concentration in the red blood cell; **eGFR** – estimated glomerular filtration rate; **HCT** – hematocrit; **HDL-C** – high-density lipoprotein; **HDW** – standard deviation of hemoglobin concentration in red blood cells; **HGB** – hemoglobin concentration; **INR** – prothrombin time in the form of the International Normalized Ratio; **LDL-C** – low-density lipoprotein; **MCH** – mean mass of hemoglobin in a red blood cell; **MCHC** – mean concentration of hemoglobin in a red blood cell; **MCV** – mean red blood cell volume index; **PT** – prothrombin time; **RBC** – red blood cell count; **RDW** – red blood cell volume distribution range; **TC** – total cholesterol; **TG** – triglyceride; **WBC** – white blood cell count. Results are presented as the median and interquartile range (25th and 75th percentiles) or as numbers and percentages.
a change in the color of the solution, which became more intense with higher concentrations of hemoglobin in the tested sample. In the next step, a polymer consisting of multiple copies of HbA1c immunoreactive sites was added. This caused latex particles to aggregate due to the presence of monoclonal anti-HbA1c antibodies. The binding sites on the latex particles competed with HbA1c molecules, leading to the inhibition of the agglutination process and a decrease in absorbance due to reduced light scattering. The final step in the determination of the HbA1c percentage involved calculating the ratio of HbA1c concentration to the total hemoglobin after applying a conversion factor. This result correlates with the National Glycohemoglobin Standardization Program-certified high-performance liquid chromatography method.

**Lipid Profile Parameters and Creatinine Evaluation**

Lipid profile parameters and creatinine concentrations were evaluated in blood collected into tubes without anticoagulant (S-Monovette, SARSTEDT, 2.7 mL) on the biochemical analyzer Architect ci8200. The concentrations of total cholesterol were determined using an enzymatic method. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that already present in the sample, is then oxidized by cholesterol oxidase to cholesterol-4-ene-3-one and hydrogen peroxide. Hydrogen peroxide combines with hydroxybenzoic acid and 4-aminantipyrin to form a chromophore (quinonimine dye) that is measured at a wavelength of 500 nm. The concentration of triglycerides was determined using an enzymatic method with glycerolphosphate oxidase. Triglycerides are enzymatically hydrolyzed by lipase to free fatty acids and glycerol. Glycerol is phosphorylated by adenosine triphosphate and glycerol kinase to form glycerol-3-phosphate and adenosine diphosphate. Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate by glycerol phosphate oxidase to form hydrogen peroxide. In the color reaction catalyzed by peroxidase, hydrogen peroxide reacts with 4-aminantipyrine and 4-chlorophenol to produce a red dye. The absorbance value of this dye is directly proportional to the concentration of triglycerides in the tested sample. The concentration of high-density lipoprotein cholesterol (HDL-C) was determined using a direct method, without the need for off-analyzer pre-treatment or centrifugation. This method uses 2 reagents and relies on the properties of a unique detergent. It involves accelerating the reaction of cholesterol oxidase with non-HDL esterified cholesterol, and selectively dissolving HDL cholesterol using an appropriate detergent. In the case of the first reagent, the non-HDL esterified cholesterol undergoes an enzymatic reaction, and the resulting hydrogen peroxide is consumed in a peroxidase-DSBmT (N,N-bis (4-sulphobutyl)-m-toluidine-disodium) reaction, leading to the formation of a colorless product. The second reagent consists of a detergent (capable of dissolving HDL cholesterol), cholesterol esterase, and a color-developing coupling agent, enabling the formation of color indicative of the quantitative content of HDL cholesterol. The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. The concentration of creatinine was determined using the Jaffe creatinine method. In an alkaline pH environment, creatinine in the sample reacts with picrate to create a creatinine-picrate complex. The rate at which the absorbance at 500 nm increases, as a result of the formation of this complex, is directly proportional to the concentration of creatinine present in the sample.

**Statistical Analysis**

The obtained results were analyzed with STATISTICA 13.3 PL software (StatSoft Inc., Tulsa, OK, USA), GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA), and STATA 17 (StataCorp LLC, College Station, TX, USA). Since the data for platelets morphological parameters results and TPO, IL-6, sP-selectin, and sCD40L concentration results did not follow a normal distribution, the Mann-Whitney U test was utilized. This test serves as the counterpart to the t test, which is used for quantitative variables with a normal distribution [32]. Empirical research has shown that the Mann-Whitney U test generally exhibits greater statistical power than the t test. The t test demonstrates higher power only when the distribution is relatively symmetric [33]. Correlation coefficients were obtained by applying Pearson or Spearman rank correlations. Values for continuous variables are presented as median with interquartile range, if not otherwise stated. The association between variables was tested with Pearson or Spearman rank correlations and linear regression analysis, in which MPV was entered as the dependent variable and the following were the independent variables: IL-6, TPO, sP-selectin, and sCD40L, as well as age, sex, blood pressure, BMI, HbA1c percentage, PLT, LPLT, total cholesterol concentration, HDL-C and LDL-C cholesterol concentration, triglyceride concentration, creatinine concentration, estimated glomerular filtration rate (eGFR), treatment used, and coexisting diseases. Moreover, using logistic regression analysis, it was checked whether the values of platelet morphological parameters (PLT, MPV, PCT, PDW, LPLT) were associated with type 2 diabetes. Regression analysis models were created using backward stepwise regression. Differences were considered significant for 2-tailed P<0.05.

**Results**

**Platelets Morphological Parameters Results**

The median MPV in patients with type 2 diabetes was significantly higher (9.4 fL, interquartile range 8.3-10.9 fL) than that...
of the control group (8.5 fl, interquartile range 8.0-9.3 fl), 
P=0.0017 (Figure 1A). The median PLT in patients with type 2 diabetes was higher (238×10^3/μL, interquartile range 182-287×10^3/μL) than that of the control group (236×10^3/μL, interquartile range 204-272×10^3/μL); however, the difference was not significant, 
P=0.6833 (Figure 1B). The median LPLT in patients with type 2 diabetes was significantly higher (7.5×10^10/ μL, interquartile range 5.1-11.0×10^10/μL) than that of the control group (6.0×10^10/μL, interquartile range 4.1-7.1×10^10/μL), 
P=0.0014 (Figure 1C). The median PCT in patients with type 2 diabetes was higher (0.22%, interquartile range 0.18-0.28%) than that of the control group (0.21%, interquartile range 0.18-0.23%); however, the difference was not significant, 
P=0.1081 (Figure 1D). The median PDW in patients with type 2 diabetes was significantly higher (54.4 fL, interquartile range 48.9-61.2 fl) than that of the control group (53.1 fl, interquartile range 47.2-56.5 fl), 
P=0.0349 (Figure 1E). The presence of increased platelet volume, as indicated by significantly higher MPV, LPLT, and PDW values, collectively, suggested elevated platelet activity among the patients with diabetes in the present study group.

TPO, IL-6, sP-Selectin, and sCD40L Concentration Results
The median concentration of TPO in patients with type 2 diabetes was higher (76 pg/mL, interquartile range 51-104 pg/ mL) than that of the control group (72 pg/mL, interquartile range 55-93 pg/mL); however, the difference was not significant (P=0.7210; Figure 2A). The median concentration of IL-6 in patients with type 2 diabetes was significantly higher (7.4 pg/μL, interquartile range 6.4-9.5 pg/mL) than that of the control group (5.8 pg/μL, interquartile range 5.5-6.1 pg/mL), 
P<0.0001 (Figure 2B). The median concentration of sP-selectin in patients with type 2 diabetes was significantly higher (128 ng/mL, interquartile range 87-161 ng/mL) than that of the control group (91 ng/mL, interquartile range 73-118 ng/mL), 
P=0.0008 (Figure 2C). The median concentration of sCD40L in patients with type 2 diabetes was significantly higher (96 ng/ mL, interquartile range 73-121 ng/mL) than that of the control group (68 ng/mL, interquartile range 52-90 ng/mL), 
P=0.0010 (Figure 2D). The significant elevation in IL-6, sP-selectin, and sCD40L concentrations in patients with type 2 diabetes suggested that these molecules may contribute to the procoagulant properties of blood platelets. Notably, IL-6 is currently included in routine laboratory testing, making it a readily accessible parameter.

Confounding Factors Results
Potential confounding variables in our models included the age and sex of the patients. The analysis revealed a significant difference in age between the study and control groups (P<0.0001), but they were sex-matched (P=0.1820). However, both age and sex were considered during the creation of the linear regression models and were assumed to have no statistically significant impact (P>0.05). During the creation of the logistic regression models, we assumed age to be statistically significant (P<0.001).

Correlation Coefficient Results
In the whole group of type 2 diabetes patients, we correlated MPV with platelets morphological parameters, TPO, IL-6, sP-selectin, and sCD40L concentrations, results of routine laboratory tests, and demographic and clinical data of patients. In type 2 diabetes patients, a negative correlation between MPV and PLT was found (r=-0.3701; 
P=0.0003; Figure 3A), which indicated that also in diabetes patients, a relatively stable platelet mass (platelet count × MPV) was established. Moreover, MPV positively correlated with LPLT (r=0.4212; 
P=0.0001; Figure 3B), which indicated LPLT as another parameter useful for evaluating platelet activity in type 2 diabetes patients. MPV did not correlate with patient sex, age, PCT, PDW, TPO, IL-6, sP-selectin, and sCD40L levels, BMI, HbA1c percentage, white blood cell count, total cholesterol, HDL-C, LDL-C, triglyceride concentration, creatinine concentration, eGFR, international normalized ratio, prothrombin time, fibrinogen, D-dimers, or systolic and diastolic blood pressure values.

Linear Regression Analysis Results for MPV
Univariate linear regression analysis showed that MPV in patients with type 2 diabetes was affected by PLT, LPLT, and D-dimer concentrations. The following was shown in patients with type 2 diabetes: (1) With an increase in PLT by 10×10^3/μL, the MPV increased by 0.05 (decreased by 0.05). (2) With an increase in LPLT by 1×10^10/μL, the MPV increased by β=0.15. (3) With an increase in D-dimer concentration by 100 μg/mL, the MPV increased by 100×β=0.02 (Table 2, Figure 4A-4C).

Multivariate linear regression analysis showed that the MPV in type 2 diabetes patients was affected by PLT, LPLT, β-blockers, and insulin. The following was found in patients with type 2 diabetes (with other parameters fixed): (1) With an increase in PLT by 10×10^3/μL, MPV increased by 10×β=-0.05 (decreased by 0.05). (2) With an increase in LPLT by 1×10^10/μL, the MPV increased by β=0.18. (3) In patients taking β-blockers, MPV is higher by β=-0.77 (lower by 0.77 in relation to those not taking these drugs). (4) In patients taking insulin, the MPV was higher by β=-5.63 (lower by 5.63 compared to those not taking these drugs) (Table 2). The coefficient of determination R^2 was 0.46, which means that the created multivariate linear regression model explains 46% of the variability of the dependent variable. The association of MPV with β-blockers and insulin intake provide information on the potential benefits of diabetes therapy regarding cardiovascular diseases.
Figure 1. Platelet morphological parameters result in type 2 diabetes patients compared with those in the control group. (A) The median mean platelet volume (MPV) in patients with type 2 diabetes was significantly higher compared to the control group ($P=0.0017$). (B) The median PLT in patients with type 2 diabetes did not differ from that of the control group ($P=0.6833$). (C) The median LPLT in patients with type 2 diabetes was significantly higher than that of the control group ($P=0.0014$). (D) The median PCT in patients with type 2 diabetes did not differ from that of the control group ($P=0.1081$). (E) The median PDW in patients with type 2 diabetes was significantly higher than that of the control group ($P=0.0349$). LPLT – large platelet count; MPV – mean platelet volume; PCT – plateletcrit; PDW – platelet distribution width; PLT – platelet count. Statistical significance: * $P \leq 0.05$, ** $P \leq 0.01$. The figure was created with the use of the GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA).
Analysis of PLT residual values did not show a normal distribution. Therefore, PLT values had to be log-transformed to obtain a normal distribution. The univariate linear regression analysis showed that PLT in patients with type 2 diabetes was influenced by white blood cell count (WBC), concentration of TPO, sP-selectin, sCD40L, and systolic blood pressure. In the whole group of patients with type 2 diabetes, the following was found: (1) With an increase in WBC by $1 \times 10^3/\mu$L, PLT increased $e^{1.034}$ times (increase by 3.4%). (2) With an increase in TPO concentration by 10 pg/mL, PLT increased $e^{0.972}$ times (decrease by 2.8%); ie, it will decrease $1/e^{0.972}$ times. (3) With an increase in the concentration of sP-selectin by 10 ng/mL, PLT increased $e^{1.02}$ times (increase by 2%). (4) With an increase in sCD40L concentration by 10 ng/mL, PLT increased $e^{0.971}$ times (decrease by 2.9%); ie, it will decrease $1/e^{0.971}$ times. (5) With an increase in systolic blood pressure by 10 mm Hg, PLT increased $e^{1.055}$ times (increase by 5.5%). (6) In patients taking insulin, PLT was higher $e^{0.539}$ times; ie, $1/e^{0.539}$ times lower than in those not taking these drugs (Table 3).

Multivariate linear regression analysis showed that PLT in type 2 diabetes patients was affected by WBC, sP-selectin concentration, and intake of aspirin, insulin, and oral antidiabetic drugs. The following was found in patients with type 2 diabetes (with other parameters fixed): (1) With an increase by 10 ng/mL, PLT increased $e^{1.02}$ times (increase by 2%).

Figure 2. Comparison of TPO, IL-6, sP-selectin, and sCD40L concentration results in type 2 diabetes patients and the control group. (A) The median TPO concentration in patients with type 2 diabetes did not differ from that of the control group ($P=0.7210$). (B) The median IL-6 concentration in patients with type 2 diabetes was significantly higher than that of the control group ($P=0.0010$). (C) The median sP-selectin concentration in patients with type 2 diabetes was significantly higher than that of the control group ($P=0.0008$). (D) The median sCD40L concentration in patients with type 2 diabetes was significantly higher than that of the control group ($P=0.0010$). IL-6 – interleukin 6; sCD40L – soluble form of CD40L; sP-selectin – soluble form of selectin P; TPO – thrombopoietin. Statistical significance: *** $P<0.001$, **** $P<0.0001$. The figure was created with the use of the GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA).
in WBC by $1 \times 10^3/\mu L$, PLT increased $e^b=1.041$ times (increased by 4.1%). (2) With an increase of sP-selectin by 10 ng/mL, PLT increased $e^b=1.041$ times (increased by 4.1%). (3) In patients taking aspirin, PLT was $e^b=1.293$ times higher than in those not taking these drugs (29% higher). (4) In patients taking insulin, PLT was higher $e^b=0.255$ times; ie, $1/e^b=3.92$ times lower than in those not taking these drugs. (5) In patients taking oral antidiabetic drugs, PLT was $e^b=1.238$ times higher than in those not taking these drugs (24% higher) (Table 3). The coefficient of determination $R^2$ was 0.32, which means that the created multivariate linear regression model explains 32% of the variability of the dependent variable. The association between PLT and aspirin, insulin, and oral antidiabetic drug intake offers insights into the advantages of diabetes treatment in relation to platelet function, taking into account the well-known phenomenon of negative correlation between platelet count and MPV.

**Logistic Regression Analysis Results**

Univariate logistic regression analysis showed that MPV, LPLT, and age increased the likelihood of type 2 diabetes. The analysis showed the following: (1) With an increase in MPV by 1 fl, the chance of diabetes increased OR=$1.621$ times (increased by 62%). (2) With an increase in PLT by $1 \times 10^3/\mu L$, the chance of developing diabetes increased OR=$1.225$ times (increased by 23%). (3) With an increase in age of 1 year, the chance of developing diabetes increased OR=$1.070$ times (increased by 7%) (Table 4).
Figure 4. Linear regression analysis results for MPV. (A) With an increase in PLT, the MPV value decreased ($P=0.005$). (B) With an increase in LPLT, the MPV value increased ($P<0.001$). (C) With an increase in D-dimer concentration, the MPV value increased ($P=0.026$). LPLT – large platelet count; PLT – platelet count; MPV – mean platelet volume. The figure was created with the use of the STATISTICA 13.3 PL software (StatSoft Inc., Tulsa, OK, USA).
Table 3. Univariate and multivariate logistic regression analysis results for PLT logarithm in patients with type 2 diabetes.

<table>
<thead>
<tr>
<th>No.</th>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>MPV</td>
<td>1.621</td>
<td>(1.224; 2.147)</td>
<td>0.001</td>
</tr>
<tr>
<td>2.</td>
<td>LPLT</td>
<td>1.225</td>
<td>(1.083; 1.385)</td>
<td>0.001</td>
</tr>
<tr>
<td>3.</td>
<td>Age</td>
<td>1.070</td>
<td>(1.038; 1.103)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Multivariate logistic regression analysis:

<table>
<thead>
<tr>
<th>No.</th>
<th>Variable</th>
<th>exp(β)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PLT</td>
<td>1.0070</td>
<td>(1.001; 1.013)</td>
<td>0.014</td>
</tr>
<tr>
<td>2.</td>
<td>MPV</td>
<td>2.7293</td>
<td>(1.764; 4.224)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3.</td>
<td>PDW</td>
<td>1.0506</td>
<td>(1.019; 1.083)</td>
<td>0.002</td>
</tr>
<tr>
<td>4.</td>
<td>Age</td>
<td>1.0834</td>
<td>(1.045; 1.124)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4. Univariate and multivariate linear regression analysis results for PLT logarithm in patients with type 2 diabetes.

<table>
<thead>
<tr>
<th>No.</th>
<th>Variable</th>
<th>exp(β)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>WBC</td>
<td>1.034</td>
<td>(1.012; 1.057)</td>
<td>0.003</td>
</tr>
<tr>
<td>2.</td>
<td>TPO</td>
<td>0.997</td>
<td>(0.995; 0.999)</td>
<td>0.023</td>
</tr>
<tr>
<td>3.</td>
<td>sP-selectin</td>
<td>1.022</td>
<td>(1.005; 1.040)</td>
<td>0.019</td>
</tr>
<tr>
<td>4.</td>
<td>sCD40L</td>
<td>0.997</td>
<td>(0.995; 0.999)</td>
<td>0.011</td>
</tr>
<tr>
<td>5.</td>
<td>BP systolic</td>
<td>1.005</td>
<td>(1.001; 1.010)</td>
<td>0.013</td>
</tr>
<tr>
<td>6.</td>
<td>Insulin</td>
<td>0.539</td>
<td>(0.297; 0.980)</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Multivariate linear regression analysis:

<table>
<thead>
<tr>
<th>No.</th>
<th>Variable</th>
<th>exp(β)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>WBC</td>
<td>1.041</td>
<td>(1.012; 1.071)</td>
<td>0.006</td>
</tr>
<tr>
<td>2.</td>
<td>sP-selectin</td>
<td>1.002</td>
<td>(1.0000; 1.004)</td>
<td>0.019</td>
</tr>
<tr>
<td>3.</td>
<td>Aspirin</td>
<td>1.293</td>
<td>(1.050; 1.591)</td>
<td>0.016</td>
</tr>
<tr>
<td>4.</td>
<td>Insulin</td>
<td>0.255</td>
<td>(0.117; 0.558)</td>
<td>0.001</td>
</tr>
<tr>
<td>5.</td>
<td>Oral antidiabetic drugs</td>
<td>1.238</td>
<td>(1.011; 1.516)</td>
<td>0.039</td>
</tr>
</tbody>
</table>

BP – blood pressure; CI – confidence interval; sCD40L – soluble form of CD40L; sP-selectin – soluble form of P-selectin; TPO – thrombopoietin; WBC – white blood cell count.

Multivariate logistic regression analysis showed that MPV, LPLT, PDW, and age increased the likelihood of type 2 diabetes. The analysis showed the following (with other parameters fixed): (1) With an increase in PLT by 10×10^3/μL, the chance of diabetes increased exp(β)=1.071 times (increased by 7%). (2) With an increase in MPV by 1 fL, the chance of developing diabetes increased OR=2.7293 times (increased by 273%). (3) With an increase in PDW by 1 fL, the chance of developing diabetes increased OR=1.051 times (increased by 5%). (4) With an increase in age of 1 year, the chance of developing diabetes increased OR=1.083 times (increased by 8%) (Table 4). These results indicate that increased MPV, PLT, PDW, and age are likewise associated with type 2 diabetes.
Discussion

The present study revealed that MPV and PLT are associated with type 2 diabetes diagnosis. We also found that MPV in patients with type 2 diabetes was affected by PLT, LPLT, β-blockers, and insulin treatment. The coefficient of determination $R^2$ was 0.46, which means that the created multivariate linear regression model explains 46% of the variability of the dependent variable. Factors such as patient’s age, sex, cytokines influencing MPV value at the stage of thrombopoiesis, blood pressure, BMI, degree of lipid and carbohydrate metabolism compensation, kidney function, and coexisting diseases did not have any influence on MPV value in the present cohort of type 2 diabetes patients.

Type 2 diabetes is a known risk factor for micro- and macrovascular complications, such as stroke, cardiovascular diseases, and thromboembolism [34]. Thrombotic events are a major cause of mortality in these patients. It is reported that patients with type 2 diabetes have a 2- to 4-fold higher risk of recurrent atherothrombotic events and vascular complications, such as heart attack, stroke, and peripheral artery disease, than do patients without diabetes [35,36]. Platelet hyperactivity and endothelial dysfunction are the main factors responsible for the development of prothrombotic and inflammatory states in diabetes patients [36]. Other factors increasing the risk of thrombosis include a high BMI, hypertension, dyslipidemia, smoking, or chronic kidney disease [36-38]. Thus, exploring the factors that can influence platelet activation in patients with type 2 diabetes is important because it may lead to a reduction in the incidence of thromboembolic events in these individuals.

In this context, our study, which analyses various factors that could influence MPV, a widely accepted laboratory indicator of platelet activation, holds significant value.

The literature emphasizes that MPV and PLT should be assessed together because there is a negative correlation between these 2 parameters, which means that as one of these values increases, the other decreases. This link has been well-documented in the literature and is present in various populations [20,39]. The relationship mentioned above was also confirmed in the present cohort of type 2 diabetes patients.

In the present study, univariate linear regression analysis demonstrated that, in patients with type 2 diabetes, MPV was influenced by PLT, LPLT, and D-dimer concentrations. Hwang et al suggest that the reason behind the inverse correlation between MPV and PLT is linked to the ability to maintain hemostasis by keeping a consistent platelet mass [40]. The positive association of MPV with LPLT, namely platelets larger than 20 fl, is in line with the results of Grove et al [30]. Research has suggested that the presence of LPLT in the blood can be associated with an increased risk of thrombotic and vascular complications. Platelets of larger size possess denser granules, exhibit increased secretion of serotonin and β-thromboglobulin, and produce higher levels of thromboxane A2 than do smaller platelets [22]. Studies have found that LPLT are more pro-thrombotic, meaning that they have a greater tendency to form clots than do normal-sized platelets [10]. Multivariate logistic regression analysis showed that both MPV and PLT are associated with type 2 diabetes. Thus, it could be concluded that analysis of MPV and LPLT together can provide additional information about platelet function and the risk of thrombotic events in patients with type 2 diabetes.

Recent studies show there is a relationship between D-dimer levels and MPV, indicating that in type 2 diabetes patients, increased platelet activation can reflect increased fibrinolytic activity [41,42]. D-dimers are small protein fragments produced during fibrinolysis and are well-established markers of venous thrombosis formation in the general population [42]. Previous studies indicated that increased D-dimer concentration was found in individuals with prediabetes [43], type 2 diabetes patients [42], or even first-degree relatives of type 2 diabetes patients [44]. Cheng et al [45] showed that patients with type 2 diabetes who have high plasma concentrations of D-dimer are at an increased risk of cardiovascular disease events, even after adjusting for cardiovascular risk factors and treatments. The authors concluded that by measuring D-dimer concentrations, it may be possible to improve the current risk stratification criteria for these patients [45]. Nevertheless, it is important to note that measuring D-dimer concentration exhibits limited specificity, as increases can be detected in patients with conditions such as disseminated intravascular coagulation syndrome, malignancies, pregnancy, liver diseases, inflammatory processes, and heart diseases, as well as in patients who have undergone surgery or experienced trauma [46,47]. Additionally, elevated D-dimer concentration can be falsely elevated in patients with higher triglyceride concentrations [47].

Multivariate linear regression analysis showed that MPV was affected by PLT and LPLT as well as β-blockers and insulin intake. A study conducted by Tsujimoto et al [48] revealed that patients with diabetes mellitus taking β-blockers had a significantly lower cardiovascular disease rate than did individuals not taking β-blockers. In addition, the treatment with β-blockers was found to have a preventative effect on deaths related to cardiovascular disease in patients with type 2 diabetes [48]. To the best of our knowledge, there is currently no available data in the literature regarding the correlation between platelet function and the use of β-blockers in individuals with type 2 diabetes. Multiple mechanisms can account for the suppression of platelet activation caused by β-blockers. One potential explanation is that β-blockers can exhibit their antplatelet effect by chemically interacting with the cell membrane of platelets, which results in membrane stabilization and reduced
sensitivity to agonists. An additional mechanism involves the inhibition of β2-receptors on platelets by nonselective β-blockers. This inhibition has the potential to influence the levels of adenosine 3′,5′-cyclic monophosphate (cAMP) within platelets, resulting in decreased calcium availability and consequently reducing platelet activation. In addition, platelet activation is triggered through multiple pathways in the presence of hypertension, and a decrease in blood pressure by β-blockers can subsequently lead to a reduction in platelet aggregation [49].

The available literature provides very limited and conflicting information on the correlation between platelet morphological parameters and insulin therapy in patients with type 2 diabetes [39,50-53]. Administering insulin therapy to individuals with type 2 diabetes mellitus can cause unexpected increases in platelet reactivity in vivo [50]. On the contrary, Alay et al [51] observed that an increase in MPV was seen only in the oral antidiabetic drugs group, but not in the insulin therapy group [51]. Their results are in line with those obtained by Vernekar et al and Sahpaz et al [52,53]. On the other hand, Serbas et al [39] did not find a significant difference in MPV values between patients taking oral antidiabetic drugs and those taking insulin. Nevertheless, in healthy humans, platelet function can be directly regulated by insulin through the functional insulin receptor present on platelets [54]. Insulin acts in opposition to platelet agonists such as collagen, adenosine diphosphate, adrenaline, and platelet-activating factor. This occurs as a result of insulin’s activation of inhibitory G protein via insulin receptor substrate-1 [55]. In the present study, we showed that type 2 diabetes patients taking β-blockers with insulin had lower MPV values than did those not taking these drugs. Thus, it could be hypothesized that taking β-blockers together with insulin by patients with type 2 diabetes could reduce the risk of thromboembolism complication in these patients. Undoubtedly, our findings may provide new insights into the relationship between MPV and the treatment of patients with type 2 diabetes. However, further studies are needed to elucidate the mechanisms by which β-blockers and insulin taking together influence MPV.

Some researchers propose that the reason for the enlarged size of platelets in diabetes is due to osmotic swelling caused by elevated blood glucose levels or elevated levels of certain glucose metabolites [22]. It is noteworthy in our study that there was no correlation observed between MPV and HbA1c percentage, which is in line with the results of Hekimsoy et al [9] and Wojiszel et al [56]. Contrary results were obtained by Demirtunc et al [57], Kodiaette et al [29], Lippi et al [2], and Ulutus et al [8]. The reasons why the present study did not identify HbA1c percentage levels directly associated with MPV could have been due to the other studies’ inclusion of a younger population of diabetic individuals [2,8,29,57], the dominance of male [2,29] or female [57] individuals, or exclusion of individuals with hemoglobin concentration below the laboratory reference values and receiving aspirin treatment, compared with in our study group [29]. The lack of association between MPV and HbA1c is in accordance with evidence that platelet activation in patients with diabetes does not depend on glycemic control [9]. Another explanation of the absence of a correlation between MPV and HbA1c could be due to some evidences suggesting that platelet activation in individuals with diabetes is not influenced by glycemic control [58]. Therefore, further research is required to gain insight in the complex interactions between glycemic control, platelet function, and other contributing factors in order to understand the connection between MPV and the percentage of HbA1c in individuals with type 2 diabetes.

This study had limitations. One limitation was the small number of type 2 diabetes patients included. As a result, we did not conduct a prospective power analysis, which is commonly carried out in large cooperative studies involving hundreds of participants, especially when proposing a new diagnostic or therapeutic method in the study’s conclusion. Our objective was more modest, and we focused on investigating the factors that are associated with MPV in patients with type 2 diabetes. Another limitation of the study was that patients with hemoglobin concentrations below the laboratory’s reference values were included in the analysis, which could be a reason for increased MPV values, due to reactive thrombocytosis [8]. Also, the use of EDTA as an anticoagulant can influence MPV values because of platelet swelling in a time-dependent manner [59]. Additionally, we did not gather data on atrial fibrillation, obstructive sleep apnea, and seasonal variations, all of which have been reported to have associations with MPV [12]. Finally, there is also still uncertainty regarding the most precise methodology for measuring MPV, which poses a limitation to its use as a marker [59]. Therefore, to validate our study results, it is important to address all the aforementioned limitations by including a larger cohort of patients and establishing a control group.

**Conclusions**

The first main finding of our study is the presence of a well-documented negative correlation between MPV and PLT, as reported in the literature in various populations, also in type 2 diabetes patients. The second finding provided by our study is the association of both MPV and PLT with type 2 diabetes diagnosis. The third and probably the most interesting finding is the association of MPV with PLT, LPLT, β-blockers, and insulin treatment. Exploring factors that influence platelet activation, measured by MPV evaluated within routine cell blood count, could be important in reducing the incidence of thromboembolic events in patients with type 2 diabetes. In this context, the present study
adds a piece of knowledge potentially useful for the improved treatment of patients with type 2 diabetes. However, further research is needed to better understand the underlying mechanisms and clinical implications of these observations.

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Declaration of Figures’ Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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