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ARTYKUŁ ORYGINALNY / *ORIGINAL ARTICLE*

Predictors of high platelet reactivity during aspirin treatment in patients with type 2 diabetes

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Abstract

Background: Diabetes mellitus type 2 (DM2) is associated with high platelet reactivity both in patients who do not receive antiplatelet drugs and in those treated with acetylsalicylic acid (ASA). The pathomechanism of this phenomenon has not been fully understood.

Aim: 1. To evaluate variability of platelet reactivity in patients with DM2 treated with oral antidiabetic drugs and receiving chronic ASA therapy. 2. To identify independent predictors of high platelet reactivity during ASA therapy in patients with DM2.

Methods: We studied 171 patients with DM2 treated with oral antidiabetic drugs and receiving long-term treatment with 75 mg of ASA daily, selected among the participants of the prospective AVOCADO study. Platelet function was simultaneously evaluated using 4 methods: 1. measurement of serum thromboxane B_2 (TXB_2) concentration; 2. measurement of urinary 11-dehydrothromboxane B_2 (11-dhTXB_2) concentration; 3. VerifyNow® automated analyser; 4. PFA-100® automated analyser. High platelet reactivity was defined as at least 3 of the following criteria: 1. serum TXB_2 concentration in the upper quartile; 2. urinary 11-dhTXB_2 concentration in the upper quartile; 3. value ≥ 550 aspirin reaction units (ARU) by VerifyNow®; 4. collagen-epinephrine closure time (CEPI-CT) below median of readings other than 300 s by PFA-100®. In all patients, DM2 control was evaluated, insulin resistance was measured using HOMA-IR, and routine laboratory tests were performed, including full blood count, renal function parameters, and inflammation markers.

Results: Mean patient age was 67.8 years, and median duration of DM2 was 5 years. We found poor agreement between different tests of platelet function. ARU ≥ 550 (VerifyNow®) was found in 14.0% of patients, and CEPI-CT below median of readings other than 300 s (PFA-100®) was found in 32.8% of patients. Our criteria of high platelet reactivity were met by 9.9% of patients. In multivariate logistic regression analysis, independent predictors of high platelet reactivity despite ASA therapy included chronic heart failure, current smoking, and higher leukocyte count.

Conclusions: 1. Patients with DM2 are characterised by large variability of platelet reactivity, with little agreement between various methods. 2. Smoking, chronic heart failure, and subclinical inflammation may be associated with high platelet reactivity in patients with DM2 treated with ASA.

Key words: acetylsalicylic acid, aspirin resistance, type 2 diabetes mellitus

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INTRODUCTION

Type 2 diabetes mellitus (DM2) is a lifestyle disease which becomes an epidemic of the 21st century, as warned by the World Health Organisation. Patients with DM2 are at a high risk of cardiovascular (CV) disease, and the risk of coronary death in this population is 2–3 times higher compared to subjects with normal glucose tolerance [1]. Acetylsalicylic acid (ASA) therapy is a mainstay of drug treatment of atherosclerosis, resulting in a significant reduction of CV morbidity and mortality. Data from the Antithrombotic Trialists' Collaboration metaanalysis of 195 studies with more than 135,000 participants, including nearly 5000 patients with diabetes, suggest

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that ASA may be less effective for prevention of CV events in diabetics compared to subjects with normal glucose tolerance [2]. Laboratory tests in patients with diabetes show high platelet reactivity despite treatment with ASA, which is often termed aspirin or ASA resistance [3].

Despite multiple studies, criteria of ASA resistance have not been clearly defined, and the pathogenesis of this phenomenon is still a subject of debate. Numerous methods are available for laboratory evaluation of platelet function. Platelet plug formation is a complex process involving various pathways of platelet activation, with multiple agonists and transmitters, and available laboratory methods evaluate only specific aspects of platelet function, which explains poor agreement between various tests [4, 5]. In addition, no clear cut-off values for ASA resistance have been established for most tests. Thus, it seems that comprehensive evaluation of the effect of ASA therapy on platelet function requires the use of multiple tests.

Despite this lack of clear definitions, ASA resistance found in laboratory tests correlates with an increased risk of CV events. In a metaanalysis of 20 studies that included 2930 patients with CV disease, Krasopoulos et al. [6] found a 4-fold increase in the risk of acute coronary syndrome, 6-fold increase in mortality, and nearly 4-fold increase in the risk of any CV event among patients with ASA resistance compared to those sensitive to ASA.

In the diabetic population, the rate of ASA resistance is higher compared to subjects with normal glucose tolerance, ranging from 13% to 45% depending on the test used and the criteria of ASA resistance [7–9]. Previous studies showed that poor response to ASA is related to poor glucose and lipid control in diabetes [9–12]. Some studies also indicate a relation between excessive platelet activation and insulin resistance [8]. However, the precise pathomechanism underlying development of poor response to ASA in patients with DM2 has not been fully understood.

The aim of our study was to evaluate variability of platelet reactivity in patients with DM2 treated with oral antidiabetic drugs and receiving chronic ASA therapy, and to identify independent predictors of high platelet reactivity despite ASA therapy in this population.

METHODS

Study group

We included patients aged 30 to 80 years, with at least 6-month duration of DM2, treated with oral antidiabetic drugs and receiving chronic (i.e., for at least 3 months) ASA therapy (75 mg per day) using enteric coated tablets for primary or secondary prevention of CV disease, who were recruited among participants of the prospective Aspirin Versus/Or Clopidogrel in Aspirin-resistant Diabetics inflammation Outcomes (AVOCADO) Study. Exclusion criteria included insulin therapy, treatment with other antiplatelet drugs than ASA, use of anticoagulants (including oral anticoagulants during previous 7 days, and low-molecular-weight or unfractionated heparin during previous 48 h), chronic use of non-steroidal anti-inflammatory drugs (NSAID) other than ASA or taking a NSAID other than ASA during previous 10 days, contraindication to ASA therapy, ASA intolerance, cancer (currently or within previous 5 years), connective tissue disease, active inflammatory process, acute coronary syndrome, worsening of chronic heart failure (HF), acute complications of diabetes and other acute conditions requiring unplanned hospital admission during previous 2 weeks, platelet count below $100 \times 10^3/\mu L$ or above $450 \times 10^3/\mu L$, haemoglobin concentration below 8 g/dL, and surgical procedures within previous 8 weeks.

All patients gave written informed consent for their participation in the study. The study was approved by a local bioethics committee at the Medical University of Warsaw.

Study protocol

Detailed history was taken and physical examination was performed in all patients, and medical records were analysed. In all patients, blood was collected in fasting conditions in the morning hours (8–9 a.m.), 2–3 h since last ASA administration as reported by the patient. To reduce the effect of blood collection on platelet reactivity, first 2 mL of collected blood was allocated for other testing that platelet function. Blood for testing of platelet aggregation (VerifyNow® and PFA-100® analysers) was mixed for 1 min using an automated blood-mixing device. Time delay between blood collection and platelet function testing was at least 20 min (to reduce the effect of blood collection on the test result) but no more than 2 h. Immediately before testing with VerifyNow® and PFA-100® analysed, blood was again mixed for 1 min using an automated blood-mixing device.

In all patients, a laboratory test panel was performed including serum lipid profile, blood glucose, glycated haemoglobin (HbA1c) percentage, full blood count, coagulation profile, and C-reactive protein (CRP), creatinine, urea, and uric acid concentrations. In addition, a portion of the collected blood samples was centrifuged at 1000 g for 15 min and the obtained serum and citrated plasma were frozen at –80°C for determination of thromboxane B_2 (TXB_2) and insulin (serum) and von Willebrand factor (citrated plasma) levels. Collected urine samples were also centrifuged and frozen at –80°C for determination of 11-dehydrothromboxane B_2 (11-dhTXB_2) concentration. Frozen samples (serum, citrated plasma, and urine) were stored at –80°C until patient recruitment was completed, and then were thawed for assays.

Insulin resistance was measured by the homeostasis model assessment as an index of insulin resistance (HOMA-IR). Serum insulin activity was determined by electrochemiluminescence using the Elecsys 2010 analyser (Hitachi High-Technologies Corporation, Japan) and Elecsys Insulin Assay® kits as per manufacturer's instructions (Roche Diagnostics, Germany).

To determine von Willebrand factor functional epitope concentration in citrated plasma, we used von Willebrand Factor Activity Kit® as per manufacturer's instructions (American Diagnostica Inc., USA).

Platelet function testing

Platelet function was simultaneously evaluated using 4 methods: 1. measurement of serum TXB_2 concentration; 2. measurement of urinary 11-dhTXB$_2$ concentration; 3. VerifyNow® automated analyser; 4. PFA-100® automated analyser.

Serum TXB_2 concentration. To obtain serum for TXB_2 concentration determination, blood sample was left to clot for 1 h at 37°C, centrifuged at 1000 g for 15 min and frozen at –80°C. After thawing, serum TXB_2 concentration was measured by ELISA using Thromboxane B_2 EIA Kit® as per manufacturer's instructions (Cayman Chemical Company, USA). Persistent platelet activation despite ASA treatment was defined as serum TXB_2 concentration in the upper quartile.

Urinary 11-dhTXB$_2$ concentration. Urinary 11-dhTXB$_2$ concentration measurements were performed in thawed urine samples using 11-dehydro Thromboxane B_2 EIA Kit® as per manufacturer's instructions (Cayman Chemical Company, USA). We also measured urinary creatinine concentration, and 11-dhTXB$_2$ concentration was indexed for urinary creatinine in mmol. Persistent platelet activation despite ASA treatment was defined as urinary 11-dhTXB$_2$ concentration in the upper quartile.

VerifyNow® analyser. The VerifyNow® bedside analyser (Accumetrics, USA) allows evaluation of the antiaggregatory activity of antiplatelet drugs. It uses a method similar to light transmission aggregometry (LTA) which is a gold standard for the evaluation of platelet function, with the degree of platelet aggregation assessed based on the measurement of light transmittance using a specific algorithm to express the result in aspirin reaction units (ARU). To evaluate platelet reactivity during ASA treatment, we used VerifyNow® Aspirin Assay cassettes with arachidonic acid as the agonist. Measurements were performed in whole blood collected to vacuum tubes containing 3.2% sodium citrate. Persistent platelet activation despite ASA treatment was defined as ≥ 550 ARU [5].

PFA-100® analyser. The Platelet Function Analyser (PFA)-100® (Siemens, Germany) also allows bedside evaluation of platelet function. In this method, platelet reactivity is evaluated by measurement of the time until formation of the primary haemostatic plug that occludes an aperture in the membrane (closure time). Maximum measurable closure time value is 300 s. To evaluate platelet reactivity during ASA treatment, we used CEPI cassettes with collagen and epinephrine as the agonists. Measurements were performed in whole blood collected to plastic tubes containing 3.8% sodium citrate. Persistent platelet activation despite ASA treatment was defined as collagen and epinephrine closure time (CEPI-CT) below median of readings other than 300 s.

Patient categorisation depending on platelet reactivity as assessed using a combination of four tests

High platelet reactivity despite ASA treatment was defined as persistent platelet activation found in at least 3 of the above 4 tests. Patients showing no persistent platelet activation in any of the 4 tests were categorised as low platelet reactivity group. The remaining patients, i.e. showing persistent platelet activation in 1 or 2 tests were categorised as intermediate platelet reactivity group.

Evaluation of compliance regarding ASA therapy

Only patients with confirmed compliance regarding ASA therapy based on history (self-reported drug use) and serum TXB_2 concentration measurement were included in the study. Based on literature data, the cut-off serum TXB_2 concentration indicating good compliance regarding ASA therapy was defined as 7200 pg/mL [13].

Statistical analysis

Statistical analysis was performed using the SAS® software. Normal distribution of variables was assessed using the Shapiro-Wilk test and visual approaches (histograms and quantile plots). Normally distributed quantitative variables are presented as mean values ± standard deviation (SD), and non-normally distributed variables as medians and interquartile ranges (IQR). Qualitative variables are presented as absolute and relative proportions. Significance of differences between groups was evaluated using the Student *t* test and the Mann-Whitney test, respectively, for quantitative variables and the χ^2 test for qualitative variables. Correlations between quantitative variables were evaluated using the Spearman correlation coefficient (r). Logistic regression analysis was used to evaluate relations between high platelet reactivity and selected prognostic variables, with multivariate models constructed using stepwise regression. For all tests, $p = 0.05$ was considered significant.

RESULTS

Study group characteristics

We analysed 171 patients (mean age 67.8 years, median duration of diabetes 5 years). Obesity (body mass index [BMI] ≥ 30 kg/m^2) was found in 87 (50.9%) patients and overweight (BMI 25–30 kg/m^2) in 63 (36.8%) patients.

Variability of platelet reactivity in the study group

In the overall study group, upper quartiles of serum TXB_2 concentration and urinary 11-dhTXB$_2$ concentration, defining persistent platelet activation despite ASA therapy, were 450 pg/mL and 58.5 ng/mmol creatinine, respectively. The criterion of persistent platelet activation despite ASA therapy by the VerifyNow® test (≥ 550 ARU) was met in 24 (14.0%) patients, and by the PFA-100® test (CEPI-CT below median

Table 1. Number (n) and proportion (%) of patients fulfilling the criteria of persistent platelet activation despite acetylsalicylic acid (ASA) treatment in various tests. Patients with high platelet reactivity during ASA treatment are marked blue, and those with low platelet reactivity are marked grey

No. of tests showing persistent platelet reactivity	N	Per cent	Number (n) and proportion (%) of patients fulfilling the criteria of persistent platelet reactivity		
			In at least 3 tests	In at least 2 tests	In at least 1 test
0	71	41.5%			
1	55	32.2%			100 (58.5%)
2	28	16.4%		45 (26.3%)	
3	12	7.0%	17 (9.9%)		
4	5	2.9%			
Overall	171	100.0%			

of readings other than 300 s, i.e. < 175 s) in 56 (32.8%) patients. We found poor agreement between various tests of platelet function, with significant correlation only between serum TXB_2 concentration and urinary 11-dhTXB_2 concentration ($r = 0.20$; $p = 0.0093$), serum TXB_2 concentration and ARU (VerifyNow®) ($r = 0.40$; $p < 0.0001$), and ARU (VerifyNow®) and CEPI-CT (PFA-100®) ($r = –0.24$; $p = 0.0011$). There were no significant correlations between the results of the remaining platelet function tests. High platelet reactivity based on the combination of 4 tests was found in 9.9% of patients, and low platelet reactivity in 41.5% of patients (Table 1).

Comparison of patient subgroups

Tables 2, 3 and 4 show comparative clinical, anthropometric, and laboratory characteristics of patients in the low, intermediate, and high platelet reactivity groups. Compared to the low platelet reactivity group, patients with high platelet reactivity were characterised by more frequent history of coronary artery disease, chronic HF and smoking, higher triglyceride concentration, higher triglyceride to high density lipoprotein (HDL) cholesterol ratio, higher leukocyte count, and in women also higher BMI. In addition, patients with high platelet reactivity were more frequently treated with metformin compared to patients with low platelet reactivity. To explain this unexpected finding, we compared other parameters between patients treated and not treated with metformin. In comparison to patients not receiving metformin, those treated with metformin were characterised by significantly higher BMI (29.1 ± 4.9 vs. 31.0 ± 4.9 kg/m^2; $p = 0.014$) and were on average 4 years younger (70.6 ± 8.8 vs. 66.2 ± 8.4 years; $p = 0.001$), which may explain the observed relation between high platelet reactivity and metformin treatment, taking into account an association between high platelet reactivity and higher BMI values (in women) and a trend towards younger age among those with high platelet reactivity (Tables 2, 3). For comparison, patients treated with sulphonylurea derivatives had significantly lower BMI (29.8 ± 5.0 vs. 31.6 ± 4.7 kg/m^2; $p = 0.025$) and were non-significantly older (68.4 ± 9.0 vs. 66.1 ± 8.0 years; $p = 0.093$) compared to those not treated with sulphonylurea derivatives.

Due to this association between metformin treatment and variables potentially affecting platelet reactivity, we did not include metformin treatment in the multivariate analysis to avoid bias in data interpretation.

Predictors of high platelet reactivity

In multivariate logistic regression analysis, independent predictors of high platelet reactivity in the overall study group included chronic HF, current smoking, and higher leukocyte count (Table 5).

DISCUSSION

In our study group of patients with DM2 receiving chronic ASA treatment, platelet reactivity varied. Similarly to previous studies, we found little agreement between various platelet function tests [4, 5].

Independent predictors of high platelet reactivity despite ASA treatment in our study group of patients with DM2 included subclinical inflammation as evidenced by increased leukocyte count, chronic HF, and smoking — variables already previously found to be associated with inadequate response to ASA, although available data were from studies in patients with normal glucose tolerance [14].

Inflammation and associated oxidative stress play a major role in the development of ASA resistance. Cells participating in the inflammatory response show increased expression of cyclooxygenase-2 (COX-2), leading to increased generation of thromboxane A_2 (TXA_2) and its precursors, prostaglandins H_2 and G_2 which also may serve as ligands for platelet TXA_2 receptors or be used as a substrate for TXA_2 synthesis by platelet COX-1. Also isoprostanoids formed from arachidonic acid by free oxygen species and other lipid peroxidation products may induce platelet activation and aggregation [14]. In addition, inflammation is usually accompanied by a prothrombotic state, manifested by increased concentrations of tissue factor, fibrinogen, and plasminogen activator inhibitor-1. Activation

Table 2. Clinical parameters in relation to platelet reactivity in the study population

Clinical parameters	Low platelet reactivity (n = 71)	Intermediate platelet reactivity (n = 83)	High platelet reactivity (n = 17)	P*
Age [years]	68.9 ± 7.5	67.4 ± 9.5	64.9 ± 9.2	0.060
Female gender	37 (52.1%)	39 (47.0%)	7 (41.2%)	0.418
Duration of diabetes [years]	5 (3–10)	5 (3–10)	3 (2-7)	0.299
Diabetes treatment				
Metformin	42 (59.2%)	54 (65.1%)	16 (94.1%)	0.006
Sulphonylurea derivatives	50 (70.4%)	60 (72.3%)	8 (47.1%)	0.068
Sulphonylurea derivatives and metformin	25 (35.2%)	33 (39.8%)	7 (41.2%)	0.646
Acarbose	6 (8.5%)	12 (14.5%)	0 (0%)	0.214
Coexisting conditions				
Hypertension	64 (90.1%)	75 (90.4%)	16 (94.1%)	0.608
Dyslipidaemia	60 (84.5%)	69 (83.1%)	14 (82.4%)	0.827
Metabolic syndrome	62 (87.3%)	74 (89.2%)	16 (94.1%)	0.428
Coronary artery disease	31 (43.7%)	50 (60.2%)	12 (70.6%)	0.046
Previous MI	14 (19.7%)	28 (33.7%)	7 (41.2%)	0.062
Chronic HF	18 (25.4%)	35 (42.2%)	10 (58.8%)	0.008
NYHA class I	7 (9.9%)	5 (6.0%)	2 (11.8%)	
NYHA class II	6 (8.5%)	22 (26.5%)	6 (35.3%)	0.417
NYHA class III	4 (5.6%)	8 (9.6%)	2 (11.8%)	
Previous stroke/TIA	7 (9.9%)	9 (10.8%)	0 (0%)	0.177
CKD stage 3–5	23 (32.4%)	16 (19.3%)	3 (17.6%)	0.231
Current smoking	4 (5.6%)	7 (8.4%)	5 (29.4%)	0.004
Current or past smoking	33 (46.5%)	52 (62.7%)	9 (52.9%)	0.632
Treatment of concomitant diseases				
Beta-blocker	46 (64.8%)	55 (66.3%)	12 (70.6%)	0.650
ACE inhibitor or ARB	59 (83.1%)	65 (78.3%)	12 (70.6%)	0.241
ACE inhibitor	50 (70.4%)	55 (66.3%)	10 (58.8%)	0.356
ARB	12 (16.9%)	15 (18.1%)	4 (23.5%)	0.524
Aldosterone antagonist	2 (2.8%)	11 (13.3%)	1 (5.9%)	0.532
Other diuretics	38 (53.5%)	37 (44.6%)	7 (41.2%)	0.360
Furosemide	7 (9.9%)	16 (19.3%)	1 (5.9%)	0.608
Thiazides	31 (43.7%)	23 (27.7%)	6 (35.3%)	0.530
Calcium antagonist	26 (36.6%)	28 (33.7%)	9 (52.9%)	0.217
Statin	54 (76.1%)	64 (77.1%)	11 (64.7%)	0.339
Fibrate	4 (5.6%)	13 (15.7%)	2 (11.8%)	0.368

Standard deviations or interquartile ranges given in parentheses. *For comparison between high and low platelet reactivity groups; MI — myocardial infarction; HF — heart failure; NYHA — New York Heart Association; TIA — transient ischaemic attack; CKD — chronic kidney disease; ACE — angiotensin-converting enzyme; ARB — angiotensin receptor antagonist

of plasma clotting cascade is in turn associated with increased platelet activation and aggregation, as thrombin is one of the most potent platelet agonists. Inflammation may also lead to increased heamatopoiesis in bone marrow, resulting in increased platelet count. Platelets, by releasing cytokines and growth factors, may in turn contribute to inflammation [15].

Increased platelet activation and aggregation is observed in chronic inflammatory conditions such as end-stage renal failure, rheumatoid arthritis, Crohn disease, ulcerative colitis, and psoriasis [16]. Laboratory response to ASA was also shown to be related to concentrations of inflammation markers such as CRP or interleukin-6 [17, 18].

Table 3. Anthropometric parameters in relation to platelet reactivity in the study population

Anthropometric parameters	Low platelet reactivity (n = 71)	Intermediate platelet reactivity (n = 83)	High platelet reactivity (n = 17)	P*
BMI [kg/m^2]:				
Overall	29.7 ± 4.4	30.7 ± 5.5	31.6 ± 5.3	0.120
Women	29.4 ± 4.4	30.6 ± 5.2	33.9 ± 6.5	**0.025**
Men	29.9 ± 4.4	30.7 ± 5.8	30.0 ± 3.9	0.983
Waist circumference [cm]:				
Overall	100.8 ± 11.7	105.7 ± 14.1	105.8 ± 11.4	0.120
Women	97.7 ± 12.3	101.8 ± 13.6	102.0 ± 13.4	0.406
Men	104.3 ± 10.0	109.2 ± 13.9	108.4 ± 9.6	0.254
WHR:				
Overall	0.95 ± 0.10	0.97 ± 0.09	0.98 ± 0.10	0.324
Women	0.92 ± 0.12	0.92 ± 0.08	0.90 ± 0.05	0.679
Men	0.99 ± 0.06	1.00 ± 0.07	1.04 ± 0.08	0.067
HR [bpm]	70.9 ± 10.5	70.4 ± 9.2	70.7 ± 9.0	0.956
SBP [mm Hg]	142.1 ± 17.7	136.7 ± 18.3	145.5 ± 20.7	0.487
DBP [mm Hg]	80.0 ± 11.5	79.4 ± 11.8	82.1 ± 13.5	0.517

Standard deviations given in parentheses. *For comparison between high and low platelet reactivity groups; BMI — body mass index; WHR — waist-to-hip ratio; HR — heart rate; SBP — systolic blood pressure; DBP — diastolic blood pressure

In our study population, we found no association between platelet reactivity and CRP and fibrinogen concentrations, despite the observed association between platelet reactivity and leukocyte count. This may be related to the fact that exclusion criteria included coexisting acute and chronic inflammatory diseases as well as conditions associated with subclinical inflammation (e.g., exacerbation of chronic HF), and CRP and fibrinogen concentrations in the study group were relatively low (Table 4). The observed association between leukocyte count and platelet reactivity as evaluated using VerifyNow® and PFA-100® analysers may be additionally explained by a direct effect of leukocytes on platelet aggregation. Platelets were shown to interact directly with leukocytes, forming aggregates of platelets and leukocytes [15].

Smoking induces inflammation and oxidative stress, which explains its association with high platelet reactivity. Smoking was shown to be associated with increased non-platelet TXA_2 synthesis by COX-2, and thus increased urinary 11-dhTXB_2 concentrations [17, 19].

Chronic HF is also associated with chronic subclinical inflammation. In severe HF, increased platelet reactivity may also result from venous congestion, a component of the classic Virchow's triad. In addition, overactivation of the sympathetic nervous system and the renin–angiotensin–aldosterone system along with endothelial dysfunction, all seen in HF, may also lead to increased platelet reactivity. This explains a high rate of inadequate response to ASA observed in these patients. Among 88 patients with NYHA class II–IV HF and left ventricular ejection fraction (LVEF) < 40%, treated with 325 mg of ASA per day, as many as 57% of patients showed ASA resistance despite very stringent criteria adopted in that study (poor response to ASA seen in at least 4 of 5 tests used) [20]. Also other studies showed increased platelet reactivity in patients with both acute and chronic HF, regardless of its aetiology (ischaemic vs. non-ischaemic) [21, 22].

In our study group, we also found a borderline significant association between high platelet reactivity during ASA treatment and younger age. A similar association was previously reported by Maree et al. [23] in a group of 131 patients with stable CV disease treated with 75 mg of ASA per day. In that study, ASA resistance was related to pharmacokinetic factors [23], as reduced ASA bioavailability in younger patients may result from increased activity of esterases that hydrolyse ASA. Plasma esterase activity, including that of ASA esterase, was shown to be decreased in older subjects with additional risk factors, in particular coexisting chronic inflammatory conditions [24]. Higher activity of ASA esterase was found in patients with DM2 compared to healthy volunteers [25]. Thus, younger patients with DM2 may be characterised by higher ASA esterase activity, contributing to more rapid ASA hydrolysis and the development of ASA resistance.

In the study population, we found that high platelet reactivity was related to triglyceride concentrations and triglyceride to HDL cholesterol ratio, but these associations were lost in multivariate analysis. We found no association between platelet reactivity and glycaemic control, total cholesterol concentration, and low-density lipoprotein (LDL) cholesterol concentration. Previous studies reported associations between

Table 4. Laboratory parameters in relation to platelet reactivity in the study population

Laboratory parameters	Low platelet reactivity (n = 71)	Intermediate platelet reactivity (n = 83)	High platelet reactivity (n = 17)	P*
Insulin resistance parameters				
HOMA-IR	3.12 (2.21–4.94)	4.19 (2.90–7.44)	4.03 (2.21–9.95)	0.324
Insulin [μIU/mL]	10.37 (7.85–18.66)	15.50 (9.90–22.11)	13.84 (8.54–22.57)	0.241
Glucose control parameters				
Fasting glycaemia [mg/dL]	116 (103–136)	120 (107–141)	120 (104–156)	0.417
HbA1c [%]	6.2 (6.0–6.7)	6.6 (6.0–7.4)	6.4 (6.2–7.1)	0.294
Lipid control parameters				
TG [mg/dL]	122.8 ± 47.9	141.6 ± 68.9	180.0 ± 88.6	0.019
HDL cholesterol [mg/dL]	50.5 ± 13.8	48.4 ± 12.8	45.5 ± 8.7	0.065
TG/HDL ratio	2.26 (1.75–3.28)	2.74 (1.75–4.07)	3.45 (2.41–4.52)	0.006
TC [mg/dL]	166.5 ± 34.7	163.5 ± 34.8	176.3 ± 43.0	0.320
LDL cholesterol [mg/dL]	89.8 ± 29.2	87.6 ± 29.8	94.8 ± 36.8	0.551
Other laboratory parameters				
PLT [$10^3/\mu$L]	225.3 ± 60.9	228.5 ± 64.4	236.1 ± 41.6	0.491
MPV [fL]	9.9 ± 1.3	9.8 ± 1.1	9.8 ± 1.4	0.715
WBC [$10^3/\mu$L]	6.44 ± 1.26	6.97 ± 1.81	7.98 ± 2.33	0.017
RBC [$10^6/\mu$L]	4.65 ± 0.46	4.65 ± 0.45	4.72 ± 0.40	0.584
Haemoglobin [g/dL]	13.9 ± 1.2	13.9 ± 1.2	14.3 ± 1.5	0.204
Haematocrit [%]	41.7 ± 3.4	41.7 ± 3.4	42.3 ± 4.1	0.518
Fibrinogen [mg/dL]	436.1 ± 102.6	430.6 ± 115.4	437.7 ± 104.9	0.955
vWF activity [%]	129.8 (99.0–161.2)	140.4 (102.9–199.5)	159.7 (102.9–197.6)	0.244
APTT [s]	29.3 ± 3.0	28.7 ± 3.9	30.7 ± 6.6	0.398
INR	0.96 (0.92–1.01)	0.97 (0.92–1.01)	0.96 (0.94–1.04)	0.343
hsCRP [mg/dL]	2.7 (1.5–4.1)	2.9 (1.4–4.9)	2.8 (1.7–5.9)	0.422
GFR [mL/min/1.73 m^2]	68.7 (57.8–86.4)	82.4 (63.2–97.1)	78.0 (66.9–94.8)	0.258
Urea [mg/dL]	41.6 ± 12.2	39.9 ± 10.5	39.1 ± 10.8	0.436
UA [mg/dL]	5.9 ± 1.4	5.6 ± 1.4	5.7 ± 1.5	0.749

Standard deviations or interquartile ranges given in parentheses. *For comparison between high and low platelet reactivity groups; HOMA-IR — homeostasis model assessment-insulin resistance; HbA1c — haemoglobin A1c; TG — triglycerydes; HDL — high-density lipoprotein, TC — total cholesterol; LDL — low-density lipoprotein; PLT — platelet count; MPV — mean platelet volume; WBC — white blood cell count; RBC — red blood cell count; vWF — von Willebrand factor; APTT — activated partial thromboplastin time; INR — international normalized ratio; hsCRP — high-sensitivity C-reactive protein; GFR — glomerular filtration rate; UA — uric acid

Table 5. Predictors of high platelet reactivity during acetylsalicylic acid treatment

Dependent variable	Explanatory variables	Odds ratio	95% CI	P
High platelet reactivity	Age [per 5 years]	0.733	0.533–1.009	0.057
	Chronic HF	**3.750**	**1.192–11.800**	**0.024**
	Current smoking	**3.891**	**1.052–14.391**	**0.042**
	WBC [$10^3/\mu$L]	**1.363**	**1.042–1.783**	**0.024**

CI — confidence interval; HF — heart failure; WBC — white blood cell count

inadequate response to ASA with parameters of metabolic control of diabetes, i.e. HbA1c percentage, glycaemia, and triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol concentrations [9–12]. Analysis of the available literature indicates, however, that these parameters were found to be independent predictors of ASA resistance mostly

in patients with poorly controlled diabetes, and in some cases in studies that included both diabetic patients and subjects with normal glucose tolerance. Such study group characteristics allow for an increased range of the analysed parameters, and thus it is easier to demonstrate significance of the observed differences. In addition, with higher glucose, triglyceride, and cholesterol concentration it is more likely that the induced platelet function disturbances will be severe enough to be reflected in laboratory tests. In our study, we evaluated a more homogeneous group of patients with relatively good diabetes control, which may explain the lack of association between glucose and lipid control parameters and platelet reactivity.

In our study group, we found no association between platelet reactivity and insulin resistance by HOMA-IR. In a single study that reported an association between insulin resistance and platelet reactivity in patients with DM2 treated with ASA, mean values of both HOMA-IR (10.3 ± 2.9 and 5.6 ± 0.7, respectively, in the ASA resistant and ASA sensitive groups) and waist circumference (124 ± 7 and 109 ± 3 cm, respectively, in the ASA resistant and ASA sensitive groups) were much higher compared to our study population [8].

In summary, in patients with short-lasting and relatively well controlled DM2 treated with oral antidiabetic drugs, independent predictors of high platelet reactivity despite ASA treatment were shown to be similar compared to subjects with normal glucose tolerance.

Limitations of the study

Our study had some important limitations. First, we did not use LTA which is considered a gold standard for the evaluation of platelet function. However, this method has multiple limitations including lack of standardisation and low reproducibility of the results, is time-consuming and requires the use of platelet-rich plasma. As stated by the Polish Cardiac Society and the European Society of Cardiology (ESC) Working Group on Thrombosis, serum TXB_2 concentration measurements and the VerifyNow® test are considered equally reliable to LTA and recommended for evaluation of platelet reactivity during ASA treatment [3].

Second, compliance regarding ASA treatment was evaluated based on patient self-reports and serum TXB_2 concentration measurements. An optimal approach would involve directly observed administration of an ASA dose, followed by testing performed after a specified uniform time in all participants.

Third, we evaluated only selected variables and did not take into account some important clinical risk factors including microvascular complications (retinopathy, microalbuminuria), some macrovascular complications (peripheral arterial disease, asymptomatic carotid artery disease), as well as neuropathic and mixed aetiology complications, such as diabetic foot. However, a reliable confirmation of the coexistence of most of these clinical conditions would require significant extension of the diagnostic work-up in our patients (e.g. with fundoscopy, microalbuminuria testing, determination of the ankle-brachial index, and carotid ultrasonography).

Fourth, in the context of the observed effect of chronic HF on platelet reactivity, evaluation of the relation between platelet function and objective parameters of left ventricular function, e.g. LVEF, would be valuable. Unfortunately, such data were not available for all patients, and the study protocol did not include echocardiographic examination.

We studied patients receiving ASA for both secondary and primary prevention of CV disease. Patients were recruited for the AVOCADO study in 2008–2010. In the recent years, indications for the use of ASA for primary prevention have been questioned. Studies clearly indicate a mortality reduction with ASA treatment in patients with established CV disease, but no benefits of ASA in primary prevention in diabetic patients were shown [26]. In contrast to the current Polish Diabetes Society guidelines, which allow ASA treatment for primary prevention in diabetic patients with additional CV risk factors, the most recent 2012 ESC guidelines recommend the use of ASA in diabetic patients only for the secondary prevention of CV disease [1, 27].

The AVOCADO study tested not only ASA low dose, but also ASA double dose (150 mg) and clopidogrel (Areplex®, Adamed, Poland) usage in DM2. These results are publised in other papers [28–30].

CONCLUSIONS

Patients with DM2 are characterised by large variability of platelet reactivity, with little agreement between various methods. Smoking, chronic HF, and subclinical inflammation may be associated with high platelet reactivity in patients with DM2 treated with ASA.

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Czynniki predykcyjne wysokiej reaktywności płytek krwi w trakcie leczenia kwasem acetylosalicylowym u pacjentów z cukrzycą typu 2

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Streszczenie

Wstęp: Cukrzyca typu 2 (DM2) wiąże się z wysoką reaktywnością płytek krwi zarówno u pacjentów nieleczonych antyagregacyjnie, jak i u chorych przewlekle przyjmujących kwas acetylosalicylowy (ASA). Patomechanizm tego zjawiska nie został dostatecznie poznany.

Cel: Celami pracy były: ocena zróżnicowania reaktywności płytek krwi w grupie pacjentów z DM2 leczonych doustnymi lekami przeciwcukrzycowymi i przyjmujących przewlekle ASA oraz identyfikacja niezależnych czynników predykcyjnych wysokiej reaktywności płytek krwi w trakcie terapii ASA u chorych na DM2.

Metody: Analizą objęto 171 pacjentów z DM2 leczonych doustnymi lekami przeciwcukrzycowymi i przyjmujących przewlekle ASA w dawce 75 mg/d., wyselekcjonowanych spośród uczestników prospektywnego badania AVOCADO. Funkcję płytek krwi oceniano jednocześnie za pomocą 4 metod: pomiaru stężeń tromboksanu B_2 (TXB_2) w surowicy; pomiaru stężeń 11-dehydrotromboksanu B_2 (11-dhTXB_2) w moczu; automatycznego analizatora VerifyNow®; automatycznego analizatora PFA-100®. Wysoką reaktywność płytek krwi zdefiniowano jako spełnienie co najmniej 3 z 4 poniższych kryteriów: stężenia TXB_2 powyżej górnego kwartyla; stężenia 11-dhTXB_2 powyżej górnego kwartyla; wartość ARU (*Aspirin Reaction Units*) ≥ 550 w teście VerifyNow®; czas okluzji (CEPI-CT) poniżej mediany wartości innych niż 300 s w teście PFA-100®. U wszystkich pacjentów oceniono wyrównanie DM2, określono wskaźnik insulinooporności (HOMA-IR) i wykonano podstawowe badania laboratoryjne (m.in. morfologia, parametry funkcji nerek i wykładniki stanu zapalnego).

Wyniki: Średnia wieku w badanej grupie wynosiła 67,8 roku, a mediana czasu trwania DM2 — 5 lat. Zaobserwowano słabą zgodność między poszczególnymi testami oceniającymi funkcję płytek krwi. Wartość ARU ≥ 550 (VerifyNow®) stwierdzono u 14,0% pacjentów, a CEPI-CT poniżej mediany wartości innych niż 300 s (PFA-100®) — u 32,8% osób. Przyjęte w badaniu kryterium wysokiej reaktywności płytek krwi spełniało 9,9% chorych. W wieloczynnikowej analizie regresji logistycznej niezależnymi czynnikami predykcyjnymi wysokiej reaktywności płytek krwi mimo terapii ASA były: przewlekła niewydolność serca, aktualny nikotynizm i wyższa liczba leukocytów.

Wnioski: Pacjentów z DM2 charakteryzuje duże zróżnicowanie reaktywności płytek krwi. Zgodność wyników między poszczególnymi metodami jest niewielka. Palenie tytoniu, przewlekła niewydolność serca i subkliniczny proces zapalny mogą się wiązać z wysoką reaktywnością płytek krwi u chorych na DM2 leczonych ASA.

Słowa kluczowe: kwas acetylosalicylowy, aspirynooporność, cukrzyca typu 2

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