ORIGINAL ARTICLE

Prevalence of high on-treatment platelet reactivity in patients with chronic kidney disease treated with acetylsalicylic acid for stroke prevention

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KEY WORDS

acetylsalicylic acid, chronic kidney disease, high on-treatment platelet reactivity

ABSTRACT

INTRODUCTION Chronic kidney disease (CKD) is one of risk factors for stroke and may be associated with impaired platelet reactivity.

OBJECTIVES The aim of the study was to evaluate platelet reactivity in patients with CKD treated with acetylsalicylic acid (ASA), using 2 different laboratory methods. Moreover, we searched for factors responsible for the phenomenon of high on-treatment platelet reactivity (HOPR).

PATIENTS AND METHODS A total of 108 patients with CKD and 41 controls without CKD using ASA were enrolled in the study. Platelet function was assessed by impedance aggregometry in whole blood, using a multi-channel platelet function analyzer (Multiplate®; ASPItest). Urinary 11-dehydrotromboxane levels were measured by the AspirinWorks® test.

RESULTS No significant differences were observed in the prevalence of HOPR between patients with and without CKD. Patients with CKD and HOPR measured by ASPItest had higher creatinine levels ($P = 0.05$) and were younger ($P < 0.01$) than patients with CKD without HOPR, while patients with CKD and HOPR measured by AspirinWorks® had lower red blood cell count ($P = 0.05$), hemoglobin ($P = 0.05$), hematocrit ($P = 0.05$), and high-density lipoprotein levels ($P = 0.05$). All patients with HOPR had higher C-reactive protein levels ($P < 0.05$) (AspirinWorks®) and white blood cells ($P < 0.05$) (ASPItest).

CONCLUSIONS The applied methods allowed to detect HOPR in more than one third of CKD patients taking ASA for stroke prevention. The compatibility of both methods for HOPR assessment was confirmed. The study revealed several potential risk factors for HOPR in CKD, including younger age, higher levels of inflammatory markers, dyslipidemia, and lower hematocrit and hemoglobin levels.

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INTRODUCTION Due to its antiplatelet effect, acetylsalicylic acid (ASA) has been widely used in primary and secondary prevention of cardiovascular diseases (CVDs).[1] The mechanism of action of ASA is based on irreversible inhibition of platelet cyclooxygenase 1 (COX-1) and reduction of thromboxane A2 (TXA2) synthesis, which is a strong factor stimulating platelet aggregation and causing vasoconstriction.[2] Up to 60% of patients on ASA exhibit high on-treatment platelet reactivity (HOPR),[3] formerly known as "aspirin resistance." The possible causes of this phenomenon might include drug interactions, alternative platelet activation pathways, non–platelet TXA2 production, genetic polymorphisms, increased platelet turnover,

inappropriate ASA dose, hypercholesterolemia, or cigarette smoking.[4]

It has been demonstrated that chronic kidney disease (CKD), especially end-stage disease, significantly increases the risk of adverse cardiovascular events. It is believed that CVD-related mortality in patients with end-stage renal failure is approximately 15 times higher than in the general population.[5] According to the literature, HOPR might be associated with a significantly higher risk of adverse thrombotic events,[6] especially in patients with CKD.[7,8] However, there are insufficient and ambiguous data on the prevalence of HOPR among these patients or factors associated with this condition.[9,10] Moreover, there has been little research among CKD patients with HOPR assessed by a multi-channel platelet function analyzer and the AspirinWorks® test.[7,11,12]

To our knowledge, no studies on the Polish population have been conducted so far. What is more, despite several years of research, there have been no clear guidelines on whether and when platelet function should be monitored in patients taking ASA for stroke prevention. Finally, the search for risk factors of HOPR might help identify individuals who are at a greater risk for the lack of antiaggregatory activity of ASA. Aspirin is inexpensive and commonly used. However, other antiplatelet drugs are also available, which may be an alternative in the case of resistance to ASA. The aim of this study was thus to assess the prevalence of HOPR in patients with CKD using ASA and to search for factors responsible for this phenomenon.

PATIENTS AND METHODS Study participants were recruited from among patients hospitalized in the Neurological, Nephrological, and Diabetological Departments of Independent Public Clinical Hospital No. 1 in Zabrze as well as outpatients treated in a nephrological clinic and the Fresenius Nephrocare dialysis center in Zabrze, Poland. The study was performed between 2015 and 2017.

The inclusion criteria were as follows: diagnosed CKD (estimated glomerular filtration rate [eGFR] <60 ml/min/1.73 m^2), regular daily ASA intake at a dose of 75 to 150 mg/d (compliance was determined on the basis of medical history; ASA intake was personally controlled by one of the authors 24 hours before the study), diagnosed CVD (coronary heart disease, previous myocardial infarction, previous stroke or transient cerebral ischemia, peripheral artery disease) or high risk of CVD (>10% in the Systematic Coronary Risk Evaluation [SCORE] calculator), no treatment with other antiplatelets, anticoagulants, or nonsteroidal anti-inflammatory drugs (NSAIDs), age above 18 years, and written informed consent for participation in the study.

The inclusion criteria for the control group were as follows: normal renal function (eGFR ≥60 ml/min/1.73 m^2, albumin-to-creatinine ratio <30 mg/g), regular ASA intake at a dose of 75 to 150 mg/d, diagnosed CVD (coronary heart disease, previous myocardial infarction, previous stroke or transient cerebral ischemia, peripheral vascular disease) or a high risk of CVD (SCORE >10%), no treatment with other antiplatelet drugs or NSAIDs, age above 18 years, and written informed consent for participation in the study.

The exclusion criteria were as follows: use of other antiplatelet drugs (eg, ticlopidine, clopidogrel, dipyridamole) or NSAIDs, irregular intake of ASA, platelet count lower than 100 × 103/μl or higher than 450 × 103/μl, history of hemorrhage, hemoglobin levels lower than 10 mg/dl, and hematocrit levels higher than 50%.

The 10-year risk of fatal CVD according to the SCORE calculator was assessed using the following data: sex, age, systolic blood pressure, total cholesterol, and current smoking status.[13]

Participants with a history of atrial fibrillation had contraindications to or did not consent to oral anticoagulation.

The study was approved by the Bioethics Committee of the Medical University of Silesia (KNW/022/KB1/70/14).

All patients were interviewed and underwent physical examination. Arterial hypertension was diagnosed if patients were treated with antihypertensive drugs or if they had abnormal blood pressure on 2 measurements (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg). Dyslipidemia was recognized if patients were treated with statins or fibrates or if they had abnormal lipid levels (high-density lipoprotein cholesterol [HDL-C] <1.0 mmol/l in men and <1.2 mmol/l in women, low-density lipoprotein cholesterol [LDL-C] ≥3 mmol/l, and/or triglycerides ≥1.7 mmol/l). Coronary heart disease, previous myocardial infarction, and atrial fibrillation were recognized on the basis of medical records. Body mass index (BMI) was calculated by dividing weight in kilograms by height in squared meters, and waist-to-hip ratio (WHR), by dividing waist circumference (cm) by hip circumference (cm). Obesity was recognized when BMI was 30 kg/m^2 or higher, and abdominal obesity, when WHR was higher than 0.8 in women and higher than 1.0 in men.

A 10-ml fasting blood sample was obtained to determine platelet function and additional laboratory parameters (complete blood count, glucose, total cholesterol, LDL-C, HDL-C, triglycerides, glycated hemoglobin A_{1c} [HbA_{1c}], C-reactive protein [CRP], creatinine, and eGFR). Each patient was also asked to provide a urine sample to a disposable container for the measurement of TXA2 metabolites. Each urine sample was centrifuged and frozen at a temperature of –20°C.

Platelet function was assessed by whole blood impedance aggregometry using a multiple platelet function analyzer, Multiplate® (Dynabyte Medical, Munich, Germany), and an enzyme-linked immunosorbent assay [ELISA], AspirinWorks® Test Kit (Corgenix, Inc., Broomfield, Colorado, United States).[14,15] Multiplate® uses multiple electrode aggregation, that is, during each

measurement, a double test is performed. It analyzes platelet aggregation by the attachment of platelets to 2 metal electrodes, which results in a change of electrical impedance. The analyzer allows to perform 5 tests with the use of various activators of platelet aggregation: arachidonic acid (ASPItest), adenosine diphosphate (ADPtest), collagen (COLtest), thrombin receptor activating peptide 6 (TRAPtest), and ristocetin (RISTOtest). In our study, the aggregation was activated using the ASPItest, ADPtest, COLtest, and TRAPtest, according to a previously described protocol.[16] The results were expressed as the area under the curve (AUC) (Supplementary material, *Figures S1* and *S2*).

HOPR was assessed on the basis of the ASPItest. As per the manufacturer's instructions, the AUC lower than 300 AU*min was considered to indicate aspirin sensitivity (ASA responders), and the AUC of 300 AU*min or higher, to indicate HOPR (ASA nonresponders).[17]

The AspirinWorks® Test Kit reflects current COX-1–dependent platelet aggregation and measures the urinary concentration of 11-dehydro -tromboxane B2 (11-dhTXB2), a thromboxane metabolite. In this test, the ELISA method is used with monoclonal antibodies against the metabolites of thromboxane. The test threshold value is 1500 pg/ml. HOPR is diagnosed when the urinary concentration of 11-dTXB2 is higher than 1500 pg/ml.[18,19]

Statistical analysis Statistical analysis was performed using STATISTICA 12.0 (StatSoft Poland). The results were considered significant if the *P* value was 0.05 or lower. Data were expressed as a number and percentage or arithmetic mean and standard deviation. Normal distribution of data was assessed by the Shapiro–Wilk test. The significance of between-group differences was verified by the *t* test, Mann–Whitney test, analysis of variance, and Kruskal–Wallis test. The χ^2 test and post hoc tests were used for comparison of qualitative variables. A multiple logistic regression analysis was performed to identify factors associated with HOPR.

Odds ratios (ORs) and CIs were calculated for the following factors: age ≥60 years, age <60 years, male sex, ASA dose ≤100 mg/d, BMI >25 kg/m^2, BMI >30 kg/m^2, WHR >0.8 in women and >1.0 in men, systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, heart rate ≥70 bpm, presence of each cardiovascular risk factor, each medication taken, red blood cell count (RBC) <3.5 × 106/µl, RBC >4 × 106/µl, hemoglobin <11 g/dl, hemoglobin >14 g/dl, hematocrit <35%, hematocrit >35%, white blood cell count [WBC] >10 × 103/µl, platelet count >300 × 103/µl, HbA_{1c} >6%, fasting glucose >100 mg/dl, total cholesterol >5.2 mmol/l, LDL-C >3.5 mmol/l, HDL-C <1.55 mmol/l, triglycerides >1.7 mmol/l, CRP >5 mg/l, creatinine >1.3 mg/dl, eGFR <60 ml/min/1.73 m^2, and eGFR <15 ml/min/1.73 m^2. Significant differences in the percentage of conflicting results between the 2 methods measuring platelet reactivity were assessed with the McNemar test.

RESULTS The study group included 149 patients who regularly took ASA at a dose of 75 to 150 mg/d for primary or secondary stroke prevention. Patients were divided into 2 groups: group 1 including 108 patients with diagnosed CKD stage 3, 4, or 5 (eGFR <60 ml /min/1.73 m^2), and group 2 including 41 controls with normal renal function as defined in the Patients and Methods section.

Group 1 was divided into 2 subgroups: group 1a including 74 patients with CKD stages 3 and 4 (eGFR ≥15 ml/min/1.73 m^2 and <60 ml/min/1.73 m^2), and group 1b including 34 patients with end-stage renal disease (ESRD) (CKD stage 5; eGFR <15 ml/min/1.73 m^2).

Data on clinical characteristics (number of patients, sex, age, BMI, WHR, blood pressure, heart rate, cardiovascular risk factors), laboratory results, and medication use for all study groups are presented in **TABLES 1–3**. The results of platelet function tests are shown in **TABLE 4**. There were no differences in the mean AUC values for ASPItest, COLtest, or TRAPtest between the study groups. The mean AUC values in the ADPtest and the mean urinary concentrations of 11-dTXB2 were significantly higher in controls than in CKD patients.

The prevalence of HOPR for impedance aggregometry and for AspirinWorks® is presented in **TABLE 5**. There was no significant difference in the prevalence of HOPR between the study groups.

The McNemar test confirmed the compatibility of both methods for HOPR assessment (*P* = 0.08). The compatibility was found in 82 patients (55.1%) (sensitivity to ASA observed by both methods in 64 patients [43%], and HOPR, in 18 patients [12.1%]), while the incompatibility was shown in 47 patients (31.5%). Data were missing for 20 patients (13.4%) (the AspirinWorks® test was not done due to anuria) (**TABLE 5**).

Quantitative variables between patients with and without HOPR in individual study groups were compared in **TABLES 6–8** and Supplementary material, *Table S1*. We found that patients with CKD and HOPR on ASPItest had significantly higher creatinine concentrations than patients with CKD without HOPR. In patients with ESRD and HOPR, eGFR was significantly lower than in patients with ESRD without HOPR. Patients with HOPR on ASPItest were also younger and had higher WBC. On the other hand, patients with CKD and HOPR on AspirinWorks® had lower RBC count and hemoglobin, hematocrit, and HDL-C levels. All patients with HOPR on AspirinWorks® had higher CRP concentrations than patients without HOPR.

Risk factors for HOPR were assessed using the multiple logistic regression analysis with the following endpoints: 1) AUC

TABLE 1 Clinical characteristics of patients with chronic kidney disease and controls

Parameter		Group 1 (CKD; n = 108)	Group 1a (CKD stages 3 and 4; n = 74)	Group 1b (CKD stage 5; n = 34)	Group 2 (controls; n = 41)	*P* value	
						1 vs 2	1a vs 1b
Sex, female/male, n (%)		54 (50)/54 (50)	34 (46)/40 (54)	20 (58.8)/14 (41.2)	16 (39)/25 (61)	0.23[a]	0.22[a]
Age, y	Mean (SD)	66.7 (14.7)	69.5 (12.6)	60.5 (17)	65.2 10.2)	0.13[b]	<0.05[c]
	Median (IQR)	70 (18.5)	70.5 (16)	65.5 (24)	64 (13)		
BMI, kg/m^2	Mean (SD)	29.4 (6.3)	30.8 (6.3)	26.4 (5.2)	29.3 (5.3)	0.88[b]	<0.001[c]
	Median (IQR)	28.6 (8.3)	30.1 (8.1)	25.3 (7)	29 (6.7)		
WHR	Mean (SD)	0.96 (0.06)	0.97 (0.07)	0.95 (0.06)	0.99 (0.06)	<0.05[b]	0.36[c]
	Median (IQR)	0.96 (0.1)	0.97 (0.1)	0.95 (0.1)	1 (0.1)		
SBP, mm Hg	Mean (SD)	132.3 (13.2)	133.1 (12.5)	130.3 (14.7)	134.4 (15.6)	0.8[b]	0.9[c]
	Median (IQR)	130 (20)	132.5 (15)	130 (20)	130 (15)		
DBP, mm Hg	Mean (SD)	77 (9.2)	76.7 (10)	77.6 (7.3)	81 (9.2)	<0.05[b]	0.99[c]
	Median (IQR)	80 (10)	80 (10)	80 (5)	80 (10)		
HR, bpm	Mean (SD)	73.7 (8.8)	73.4 (9.2)	74.5 (7.9)	72.7 (8.2)	0.52[b]	0.99[c]
	Median (IQR)	72 (12)	72 (13)	73 (10)	72 (10)		
Diabetes mellitus, n (%)		56 (51.9)	41 (55.4)	15 (44.1)	15 (36.6)	0.99[a]	0.28[d]
Arterial hypertension, n (%)		106 (98.1)	73 (98.6)	33 (97.1)	37 (90.2)	<0.05[a]	0.75[d]
Atrial fibrillation, n (%)		13 (12)	9 (12.2)	4 (11.8)	4 (9.8)	0.92[a]	0.95[d]
Current smoking, n (%)		41 (38)	30 (40.5)	11 (32.4)	21 (51.2)	0.14[a]	0.42[d]
Coronary heart disease, n (%)		43 (39.8)	33 (44.6)	10 (29.4)	9 (22)	<0.05[a]	0.13[d]
Previous myocardial infarction, n (%)		17 (15.7)	12 (16.2)	5 (14.7)	3 (7.3)	0.28[a]	0.84[d]
Previous stroke/TIA, n (%)		17 (15.7)	14 (18.9)	3 (8.8)	23 (56.1)	<0.0001[a]	0.07[d]
Peripheral artery disease, n (%)		7 (6.5)	6 (8.1)	1 (2.9)	1 (2.4)	0.45[a]	0.31[d]
Overweight or obesity (BMI >25 kg/m^2), n (%)		80 (74.8)	62 (83.8)	18 (54.5)	30 (75)	0.98[a]	<0.01[d]

a χ^2 test; **b** *t* test/Mann–Whitney test/Kruskal–Wallis test; **c** Post hoc tests; **d** Bonferroni test

Abbreviations: BMI, body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; HR, heart rate; IQR, interquartile range; SBP, systolic blood pressure; TIA, transient ischemic attack; WHR, waist-to-hip ratio

≥300 AU*min; 2) urinary 11-dTXB2 level ≥1500 pg/ml; 3) AUC ≥300 AU*min or urinary 11-dTXB2 ≥1500 pg/ml; and 4) AUC ≥300 AU*min and urinary 11-dTXB2 levels ≥1500 pg/ml. Data were presented in Supplementary material, *Tables S2-S5*, as OR (95% CI) and the *P* value for the Wald test. No result (–) indicates that the statistical analysis for a given factor in a given group was not possible.

Based on the multiple logistic regression analysis, the parameters associated with a higher probability of HOPR in at least one group were identified. The following risk factors for HOPR were found on ASPItest: age <60 years, hematocrit <35%, hemoglobin <11 g/dl, creatinine >1.3 mg/dl; and on AspirinWorks®: previous stroke / transient ischemic attack (TIA), hematocrit <35%, hemoglobin <11 g/dl, WBC >10 ×103/µl, and CRP >5 mg/l.

DISCUSSION The presence of HOPR in patients treated with ASA significantly increases the risk of adverse cardiovascular events,[6] especially in patients with CKD.[7,11] This seems particularly important in this patient population because CVD-related mortality in patients with ESRD is 15-fold higher than in the general population.[20]

As recommended by most guidelines, 2 laboratory methods were applied in this study to assess platelet reactivity. Impedance aggregometry with the use of the Multiplate® analyzer is a quick method that does not require special blood sample preparation and shows high compatibility with the results obtained by traditional optical aggregometry developed by Gustav VR Born in 1962, which is considered the gold standard for platelet function assessment. It is also one of the methods recommended by the European Society of Cardiology to assess platelet reactivity in patients treated with ASA.[21] Additionally, we evaluated the urinary concentration of 11-dTXB2. It is an intermediate test and uses a completely different measurement method (ELISA). There have been few reports on HOPR evaluation with this method in patients with CKD, probably because its use is limited in ESRD patients with anuria.

Our results correspond to the incidence of HOPR reported by other authors using the same method. Aksu et al[11] found HOPR in 34.1% of patients with CKD stages 3–5, while Kilickesmez et al,[7] in 43.58% of patients with ESRD. In another study, Aksu et al[12] evaluated HOPR in patients with CKD treated with hemodialysis.

TABLE 2 Laboratory tests in patients with chronic kidney disease and controls

Parameter		Group 1 (CKD; n = 108)	Group 1a (CKD stages 3 and 4; n = 74)	Group 1b (CKD stage 5; n = 34)	Group 2 (controls; n = 41)	*P* value	
						1 vs 2	1a vs 1b
Creatinine, mg/l	Mean (SD)	303.7 (247.2)	166.9 (81.9)	601.6 (223.4)	91.3 (25.3)	<0.0001	<0.0001
	Median (IQR)	183.4 (277.1)	137.5 (81.5)	525.1 (380)	82 (34)		
eGFR, ml/min/1.73 m^2	Mean (SD)	30.2 (18.2)	40.1 (12.8)	8.7 (3.4)	81.4 (18.5)	<0.0001	<0.0001
	Median (IQR)	29.9 (35.8)	43.2 (21.6)	8.5 (5.2)	76.4 (26.5)		
RBC, 10^6/µl	Mean (SD)	4.1 (0.7)	4.3 (0.6)	3.6 (0.6)	4.9 (1.7)	<0.0001	<0.0001
	Median (IQR)	4.1 (1.1)	4.3 (0.9)	3.5 (0.7)	4.7 (0.5)		
Hematocrit, %	Mean (SD)	37.4 (5.8)	39.1 (5.5)	33.7 (4.6)	42.1 (4.8)	<0.0001	<0.0001
	Median (IQR)	36.5 (8.9)	39.1 (7.8)	32.9 (5.2)	42.5 (6.4)		
Hemoglobin, g/dl	Mean (SD)	12.5 (2)	13.1 (1.9)	11.2 (1.6)	14.2 (1.7)	<0.0001	<0.0001
	Median (IQR)	12.4 (3.2)	13.2 (2.9)	10.9 (1.8)	14.1 (2.1)		
WBC, 10^3/µl	Mean (SD)	7.6 (2.6)	7.6 (2.6)	7.5 (2.7)	8.1 (2.7)	0.33	0.99
	Median (IQR)	7.2 (2.9)	7.3 (2.7)	7 (3.2)	8 (44)		
Platelets, 10^3/µl	Mean (SD)	224 (59.4)	225.2 (58)	221.3 (63.1)	249.3 (77.1)	0.09	0.99
	Median (IQR)	221 (63)	225 (63)	216 (51)	235.5 (81)		
Total cholesterol, mmol/l	Mean (SD)	4.6 (1.1)	4.7 (1.2)	4.3 (1)	4.7 (1)	0.32	0.19
	Median (IQR)	4.5 (1.4)	4.6 (1.3)	4.2 (1.2)	4.7 (1.7)		
LDL-C, mmol/l	Mean (SD)	2.5 (0.9)	2.6 (1)	2.3 (0.7)	2.7 (0.7)	0.1	0.6
	Median (IQR)	2.4 (1)	2.4 (1)	2.4 (0.8)	2.6 (1.3)		
HDL-C, mmol/l	Mean (SD)	1.2 (0.5)	1.2 (0.5)	1.2 (0.5)	1.3 (0.4)	0.64	0.99
	Median (IQR)	1.2 (0.5)	1.2 (0.4)	1.1 (0.7)	1.2 (0.5)		
Triglycerides, mmol/l	Mean (SD)	1.9 (1)	1.8 (0.8)	2.1 (1.4)	1.7 (0.8)	0.26	0.99
	Median (IQR)	1.6 (0.9)	1.6 (0.9)	1.6 (1)	1.5 (0.8)		
Fasting glucose, mg/dl	Mean (SD)	112.7 (35.7)	115.3 (37.5)	106.6 (30.7)	120.5 (43.2)	0.24	0.99
	Median (IQR)	101.5 (39.3)	101.6 (46.2)	101.5 (20.1)	108.4 (40.5)		
HbA_{1c}, %	Mean (SD)	6.2 (1.4)	6.3 (1.5)	6 (1.3)	6.4 (1.6)	0.68	0.99
	Median (IQR)	6 (1.8)	6.1 (1.8)	5.7 (2.1)	6 (1.5)		
CRP, mg/l	Mean (SD)	11.6 (27.3)	12.4 (30.1)	8.5 (8.8)	6.8 (8.6)	0.68	0.99
	Median (IQR)	3.1 (8.2)	2.9 (7.3)	5.5 (9.6)	3.7 (5.9)		

a *t* test/Mann–Whitney test/Kruskal–Wallis test; **b** Post hoc tests

Abbreviations: CRP, C-reactive protein; eGFR, glomerular filtration rate; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; RBC, red blood cell count; WBC, white blood cell count; others, see TABLE 1

The frequency of HOPR was higher before than after hemodialysis (51.9% vs 50%).[12] In our study, HOPR was assessed before hemodialysis.

In our study, the prevalence of HOPR in CKD was not significantly different between patients with and without CKD (based on Multiplate® or AspirinWorks®). Other authors found a higher prevalence of HOPR in patients with CKD (CKD stage 3, 22.4%; CKD stages 4 and 5, 48.2%) compared with those without CKD (21.2%).[11] Authors using other methods[9,10,22] also found a significantly higher prevalence of HOPR in patients with CKD than in individuals with normal eGFR values, which is in contrast to our results.

However, we found a significant correlation between serum creatinine concentrations and HOPR in patients with CKD (TABLE 6). This is in line with the results of other studies, in which both impedance aggregometry[11] and other laboratory methods were used.[10] We also observed a significant correlation between HOPR and eGFR in patients with ESRD, which is consistent with the study of Blann et al.[22] Most other authors[10,11,22] found a weak correlation between HOPR and eGFR values.

Only a few authors assessed platelet reactivity by measuring 11-dTXB2 levels, especially in patients with CKD.[17,23,24] Lopez et al,[18] who also applied this method, found increased platelet reactivity in 14.8% of patients with diabetes mellitus and in 28.7% of those with acute coronary syndrome (vs 8.4% in controls). The urinary concentration of 11-dTXB2 in patients participating in the HOPE study (Heart Outcomes Prevention Evaluation) was evaluated by Eikelboom et al.[23] Those authors found higher urinary 11-dTXB2 concentrations in patients with previous myocardial infarction, and those who died due to cardiovascular causes when compared with the control group. These results partially correspond to our results. Of course, this method was used only in patients with residual urine output.

TABLE 3 Medication use in patients with chronic kidney disease and controls

Medication		Group 1 (CKD; n = 108)	Group 1a (CKD stages 3 and 4; n = 74)	Group 1b (CKD stage 5; n = 34)	Group 2 (controls; n = 41)	*P* value	
						1 vs 2	1a vs 1b
ASA	75 mg/d	27 (25)	18 (24.3)	9 (26.5)	20 (48.8)	<0.01	0.08
	150 mg/d	81 (75)	56 (75.7)	25 (73.5)	21 (51.2)		
Diuretics		79 (73.1)	59 (79.7)	20 (58.8)	23 (56.1)	<0.05	<0.05
ACEIs		31 (28.7)	20 (27)	11 (32.4)	22 (53.7)	<0.001	0.56
ARBs		15 (13.9)	11 (14.9)	4 (11.8)	4 (9.8)	0.69	0.67
Calcium antagonists		70 (64.8)	46 (62.2)	24 (70.6)	15 (36.6)	<0.01	0.4
β-Blockers		81 (75)	53 (71.6)	28 (82.4)	26 (63.4)	0.16	0.23
α-Blockers		42 (38.9)	30 (40.5)	12 (35.3)	13 (31.7)	0.42	0.61
Clonidine		21 (19.4)	6 (8.1)	15 (44.1)	3 (7.3)	0.12	<0.001
Statins		56 (51.9)	44 (59.5)	12 (35.3)	27 (65.9)	0.12	<0.05
Fibrates		8 (7.4)	7 (9.5)	1 (2.9)	3 (7.3)	0.98	0.22
PPIs		41 (38)	24 (32.4)	17 (50)	4 (9.8)	<0.001	0.08
Oral hypoglycemics		17 (15.9)	13 (17.8)	4 (11.8)	10 (24.4)	0.24	0.43
Insulin		40 (37.4)	29 (39.7)	11 (32.4)	7 (17.1)	<0.05	0.47
Calcium carbonate		44 (40.7)	24 (32.4)	20 (58.8)	1 (2.4)	<0.0001	<0.0001
Allopurinol		43 (39.8)	35 (47.3)	8 (23.5)	13 (31.7)	0.36	<0.05

Data are presented as number (percentage) of patients.

a χ^2 test; **b** Bonferroni test

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid; PPI, proton-pump inhibitors; others, see **TABLE 1**

TABLE 4 Results of platelet function assessment by a multi-channel platelet function analyzer (ASPItest, COLtest, ADPtest, TRAPtest) and AspirinWorks® Test Kit (urinary 11-dTXB2 levels)

Platelet reactivity test		Group 1 (CKD; n = 108)	Group 1a (CKD stages 3 and 4; n = 74)	Group 1b (CKD stage 5; n = 34)	Group 2 (controls; n = 41)	*P* value			
						1 vs 2[a]	1a vs 1b[b]	1a vs 2[b]	1b vs 2[b]
ASPItest, AU*min	Mean (SD)	299.9 (226.5)	270.4 (159.7)	363.2 (321.2)	327.4 (221)	0.4	0.94	0.78	0.99
	Median (IQR)	242 (219)	226 (188)	260 (308)	284 (270)				
COLtest, AU*min	Mean (SD)	454.9 (238.9)	442.5 (221.2)	481.2 (274.4)	474.7 (208.8)	0.56	0.99	0.99	0.99
	Median (IQR)	425.5 (290)	432 (287.5)	398 (333)	439 (248)				
ADPtest, AU*min	Mean (SD)	431.8 (289.6)	452.3 (292.4)	387.8 (282.7)	582.1 (297.8)	<0.01	0.48	0.05	<0.01
	Median (IQR)	320 (352)	361 (340)	278 (352)	546 (476)				
TRAPtest, AU*min	Mean (SD)	1005.3 (469.9)	1031.8 (444.3)	946.4 (524.5)	1111.4 (406.9)	0.18	0.83	0.99	0.26
		1030.5 (644)	1036 (627)	987 (665)	1123 (533)				
11-dTXB2, pg/ml	Mean (SD)	865.1 (1299.3)	909.3 (1390.9)	738.2 (1007.2)	1269.2 (1162)	<0.05	0.99	0.12	<0.05
	Median (IQR)	333 (1139.3)	359.3 (1103.9)	164.1 (1430.4)	944.5 (1924.3)				

a *t* test/Mann–Whitney test/Kruskal–Wallis test; **b** Post hoc tests

Abbreviations: 11-dTXB2, 11-dehydrotromboxane B2; ADP, adenosine diphosphate; ASPI, aspirin; COL, collagen; TRAP, thrombin receptor activating peptide 6; others, see **TABLE 1**

Our study showed compatibility between the methods used for assessing platelet reactivity. This constitutes a strength of our study because there have been only a few studies using at least 2 methods for such an assessment in patients with CKD.

We observed significantly higher CRP levels in patients with HOPR (AspirinWorks®), which is consistent with observations of other authors.[7,10,11] This may indicate the importance of inflammation as a factor contributing to HOPR. Furthermore, patients with CKD and HOPR (AspirinWorks®) had significantly lower values of RBC, hemoglobin, hematocrit, and HDL-C when compared with patients without HOPR. Tanrikulu et al[9] found similar correlations, using the VerifyNow® Aspirin assay.[9] Other authors[7,11,12] also observed lower hemoglobin levels in patients with HOPR.

TABLE 5 Prevalence of high on-treatment platelet reactivity assessed by Multiplate® (ASPItest) and AspirinWorks® Test Kit (urinary 11-dTXB2 levels)

Platelet reactivity test	Group 1 (CKD; n = 108)	Group 1a (CKD stages 3 and 4; n = 74)	Group 1b (CKD stage 5; n = 34)	Group 2 (controls; n = 41)	*P* value			
					1 vs 2[a]	1a vs 1b[b]	1a vs 2[b]	1b vs 2[b]
HOPR (ASPI test) (AUC ≥300 AU*min)	40 (37.4)	25 (34.2)	15 (44.1)	19 (46.3)	0.32	0.38	0.85	0.32
HOPR (AspirinWorks®; 11-dTXB2) (≥1500 pg/ml)	20 (22.5)	14 (21.2)	6 (26.1)	15 (36.6)	0.09	0.22	0.39	0.63
HOPR (ASPItest or AspirinWorks®)	52 (48.2)	35 (47.3)	17 (50)	24 (58.5)	0.26	0.79	0.25	0.46
HOPR (ASPItest and AspirinWorks®)	8 (7.4)	4 (5.4)	4 (11.8)	10 (24.4)	<0.05	0.24	0.67	0.52

Data presented as number (percentage) of patients.

a χ^2 test; **b** Bonferroni test; **c** McNemar test for all the examined patients (n = 149)

Abbreviations: HOPR, high on-treatment platelet reactivity; others, see TABLES 1 and 4

TABLE 6 Comparison of quantitative variables between patients with and without high on-treatment platelet reactivity measured by ASPItest in individual study groups (Mann–Whitney test)

Variable	Whole study group	Group 1 (CKD)	Group 1a (CKD stages 3 and 4)	Group 1b (CKD stage 5)	Group 2 (controls)
Age	<0.01↓	<0.01↓	<0.01↓	0.1	0.8
BMI	0.36	0.65	0.07	0.23	0.33
WHR	0.7	0.49	0.75	0.7	0.07
SBP	0.63	0.84	0.86	0.6	0.27
DBP	0.34	0.33	0.19	0.83	0.89
HR	0.1	0.12	0.96	0.06	0.73
RBC	0.62	0.14	0.16	0.65	0.46
Hematocrit	0.63	0.1	0.17	0.47	0.39
Hemoglobin	0.72	0.12	0.07	0.61	0.38
WBC	<0.05↑	0.37	0.4	0.6	<0.05↑
Platelets	0.09	0.48	0.25	0.9	0.07
Total cholesterol	0.39	0.23	0.31	0.65	0.81
Triglycerides	0.42	0.51	0.41	0.99	0.87
LDL-C	0.24	0.11	0.08	0.42	1
HDL-C	0.2	0.45	0.29	0.91	0.21
HbA_{1c}	0.74	0.1	0.46	0.15	0.08
Fasting glucose	0.77	0.59	0.44	0.27	0.09
CRP	0.84	0.93	0.72	0.91	0.9
Creatinine	0.41	0.05↑	<0.05↑	0.33	0.51
eGFR	0.67	0.06	0.24	<0.05↓	0.74

↑ – Higher values in patients with HOPR

↓ – Lower values in patients with HOPR

Abbreviations: see TABLES 1, 2, 4, and 5

Using the multiple logistic regression analysis, we found younger age and low hematocrit and hemoglobin levels to be risk factors for HOPR in ASPItest. For HOPR assessed by AspirinWorks®, the risk factors included previous stroke/TIA, low hematocrit and hemoglobin levels, higher WBC count and higher CRP levels. Other authors observed the following risk factors: female sex,[10,11] hemodialysis,[10] low HDL-C levels,[10] high LDL-C levels,[24] current smoking,[24] and elevated mean platelet volume.[11] Some authors did not find an association between HOPR and platelet count or age.[25-28] Many studies that did not consider renal function reported a greater risk of HOPR among women,[6,29,30] which was not confirmed in our study.

HOPR is associated with a significantly higher incidence of adverse thrombotic events,[6]

TABLE 7 Comparison of quantitative variables between patients with and without high on-treatment platelet reactivity measured by AspirinWorks® Test Kit (urinary 11-dTXB2 levels) in individual study groups (Mann–Whitney test)

Variable	Whole study group	Group 1 (CKD)	Group 1a (CKD stages 3 and 4)	Group 1b (CKD stage 5)	Group 2 (controls)
Age	0.19	0.05	0.53	<0.05↓	0.99
BMI	0.1	0.22	1	0.35	0.26
WHR	0.59	0.58	0.82	0.54	0.94
SBP	0.09	0.26	0.75	0.28	0.2
DBP	0.36	0.29	0.7	0.29	0.59
HR	0.14	0.53	1	0.49	0.07
RBC	0.5	<0.05↓	0.1	0.12	0.46
Hematocrit	0.54	<0.05↓	0.06	0.19	0.7
Hemoglobin	0.41	<0.05↓	<0.05↓	0.17	0.71
WBC	0.35	0.76	0.55	1	0.12
Platelets	0.6	0.59	0.92	0.69	0.27
Total cholesterol	0.23	0.15	0.81	0.2	0.61
Triglycerides	0.87	0.5	0.15	0.11	0.42
LDL-C	0.79	0.35	0.24	0.66	0.96
HDL-C	<0.01↓	<0.05↓	0.15	0.08	<0.05↓
HbA_{1c}	0.31	0.83	0.51	0.85	0.19
Fasting glucose	0.52	0.62	0.22	0.99	0.51
CRP	<0.05↑	0.08	0.5	0.29	0.08
Creatinine	0.27	0.42	0.42	0.71	0.36
eGFR	0.41	0.37	0.39	0.97	0.86

↑ – Higher values in patients with HOPR

↓ – Lower values in patients with HOPR

Abbreviations: see TABLES 1, 2, 4, and 5

especially in patients with ESRD.[7] In our study, we assessed only "laboratory resistance" to aspirin (ie, measured by laboratory methods) and not clinical resistance (occurrence of thrombotic events in patients taking ASA for the prevention of stroke or other CVD). Some authors found that HOPR is common in stroke patients and is associated with an increased risk of stroke recurrence.[31]

We did not find an association between the ASA dose in CKD patients and HOPR assessed by both impedance aggregometry and AspirinWorks®. The available data are ambiguous, although numerous authors have shown that lower doses of ASA promote resistance to this drug.[32-34] Some investigators have demonstrated that an increase in the ASA dose reduces the incidence of HOPR.[35-37]

We did not find an association between the occurrence of HOPR and the use of other drugs in patients with CKD. Some authors found a relationship between ASA sensitivity and such drugs as proton pump inhibitors,[38,39] NSAIDs,[40] β-blockers,[27] angiotensin-converting enzyme inhibitors,[22,41] angiotensin II receptor antagonists,[42] statins,[38,43] or glucose-lowering drugs.[23,44]

Study limitations According to the literature, patient noncompliance is an important factor limiting the effect of ASA. In our patients, compliance was determined on the basis of a careful medical history. Additionally, the intake of ASA was personally controlled by one of the authors 24 hours prior to the study. Secondly, we assessed only laboratory resistance to ASA, and not clinical. Moreover, the number of patients with previous stroke was higher in the control group. As we found the association between HOPR (assessed by AspirinWorks®) and previous stroke in all patients, it might have interfered with the results (a nonsignificant difference in the prevalence of HOPR between patients with and without CKD). We did not observe such an association between HOPR and previous stroke when impedance aggregometry was used (ASPItest). Finally, the sample size of patients with ESRD was rather small (n = 34), and it might have influenced the statistical analysis. The number of controls (n = 41) was adjusted to the number of patients with ESRD.

Conclusions The applied methods allowed a detection of HOPR in more than one third of patients with CKD taking ASA for stroke prevention. The prevalence of HOPR was similar between patients with and without CKD. However, serum creatinine concentrations were higher and eGFR was lower in patients with CKD and HOPR. The study revealed several potential risk factors for HOPR in CKD such as younger age, higher levels of inflammatory markers (WBC and CRP), dyslipidemia (lower HDL-C

TABLE 8 Comparison of quantitative variables between patients with and without high on-treatment platelet reactivity measured by the ASPItest and AspirinWorks® Test Kit (urinary 11-dTXB2 levels) in individual study groups (Mann–Whitney test)

Variable	Whole study group	Group 1 (CKD)	Group 1a (CKD stages 3 and 4)	Group 1b (CKD stage 5)	Group 2 (controls)
Age	0.23	0.26	1	<0.05↓	0.99
BMI	0.11	0.24	0.88	0.06	0.22
WHR	0.84	0.74	0.91	0.81	0.38
SBP	0.22	0.66	0.58	0.93	0.12
DBP	0.94	0.81	0.53	0.15	0.34
HR	0.97	0.65	0.48	0.81	0.37
RBC	0.52	0.07	0.21	0.11	0.22
Hematocrit	0.59	0.10	0.28	0.13	0.51
Hemoglobin	0.69	0.10	0.36	<0.05↓	0.36
WBC	0.07	0.53	0.43	0.99	<0.05↑
Platelets	0.25	0.56	0.73	0.78	0.34
Total cholesterol	0.21	0.15	0.26	0.62	0.92
Triglycerides	0.20	0.17	0.14	0.89	0.89
LDL-C	0.44	0.18	0.51	0.11	0.81
HDL-C	0.41	0.56	0.16	0.39	0.62
HbA_{1c}	0.96	0.34	0.18	0.63	0.13
Fasting glucose	0.76	0.81	0.44	0.31	0.18
CRP	0.28	0.26	0.33	0.79	0.71
Creatinine	0.80	0.33	0.65	<0.05↑	0.77
eGFR	<0.001↓	0.27	0.27	0.14	0.8

↑ – Higher values in patients with HOPR

↓ – Lower values in patients with HOPR

Abbreviations: see TABLES 1, 2, 4, and 5

concentrations), lower hematocrit value, and lower hemoglobin concentration.

To our knowledge, our study is the first to assess the phenomenon of HOPR in Polish patients with CKD who take ASA for CVD prevention. Further research is needed to establish clear guidelines on monitoring and diagnosis of HOPR, as well as its risk factors, in this group of patients.

SUPPLEMENTARY MATERIAL Supplementary material is available with the article at www.pamw.pl.

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CONTRIBUTION STATEMENT BŁ-R conceived the concept for the study. BŁ-R and MH contributed to study design. MH, BŁ-G, MŚ, and BM were involved in data collection. EN and BŁ-G analyzed the data. BŁ-R coordinated funding for the project. BM and MŚ were involved in literature search. All authors edited and approved the final version of the manuscript.

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