ORIGINAL ARTICLE

UDC:616.155.2:616.61-008.6
https://doi.org/10.2298/VSP160720374F

Platelet turnover and function in end-stage renal disease

Promet i funkcija trombocita u završnom stadijumu hronične bubrežne insuficijencije

Predrag Filipov*, Dušan Božić[†‡], Romana Mijović*[‡] Gorana Mitić*[‡]

Clinical Centre of Vojvodina, *Centre for Laboratory Medicine, [†]Nephrology and Clinical Immunology Clinic, Novi Sad, Serbia; University of Novi Sad, [‡]Faculty of Medicine, Novi Sad, Serbia

Abstract

Background/Aim. End-stage renal disease (ESRD) is characterized by significant impairment of platelet functions which may cause bleeding or thrombotic complications. The iam of this study was the aim of this study was the assessement of platelet turnover and function and their correlation with inflammatory and procoagulant markers in ESRD patients as well as platelet indicies comparison between ESRD diabetic and ESRD non-diabetic patients. **Methods.** The prospective, observational clinical study included 63 ESRD patients and 30 age and sex matched healthy volunteers. Following laboratory parameters of platelet turnover and function (platelet count, reticulated platelets, platelet indices, whole blood impedance platelet aggregation), inflammatory and procoagulant markers (number of neutrophils, neutrophil to lymphocyte ratio, C-reactive protein, plasma fibrinogen, D dimer, von Willebrand factor) were obtained. **Results.** Platelet turnover (% of reticulated platelets) was significantly higher (3.8 ± 2.3 vs. 2.3 ± 1.3; $p < 0.01$) and platelet aggregation tests induced by thrombin receptor activiting peptide (TRAP) ($p < 0.01$), adenosine diphospate (ADP) ($p < 0.05$), arachidonic acid (ASPI) ($p < 0.05$) and collagen ($p < 0.05$) were markedly increased in the ESRD patients compared to the control group. The comparison of chronic inflammation and procoagulant markers revealed higher values in all patients comparing to the group of healthy subjects ($p < 0.01$ regarding all parameters). There was no difference between the ESRD diabetic and ESRD non-diabetic patients. **Conclusion.** Results point out increased platelet turnover in ESRD as a consequence of platelet activation and consumption induced by clotting system hyperactivity and chronic inflammation. None of the examined parameters do not predict bleeding occurrence.

Key words:
kidney failure, chronic; diabetes mellitus; platelet funcion tests.

Apstrakt

Uvod/Cilj. Završni stadijum hronične bubrežne insuficijencije [nd-stage renal disease (ESRD)] karakteriše prisustvo značajnog oštećenja trombocitnih funkcija koje mogu dovesti do krvarećih i tromboznih komplikacija. Cilj rada bio je procena prometa i funkcionalnosti trombocita i korelacija sa zapaljenskim i prokoagulantnim markerima kod bolesnika sa ESRD, kao i poređenje trombocitnih indeksa između bolesnika kod kojih je šećerna bolest etiološki faktor ESRD i bolesnika sa ESRD druge etiologije. **Metode.** U prospektivno opservaciono kliničko istraživanje uključeno je 63 ispitanika sa ESRD i 30 zdravih osoba usklađenih po starosti i po polu (kontrolna grupa). Određivani su laboratorijski parametri prometa i funkcionalnosti trombocita (broj trombocita, retikulisani trombociti, trombocitni indeksi i agregabilnost trombocita merena impendancom iz pune krvi), zapaljenski i prokoagulantni markeri (broj neutrofila, odnos neutrofila i limfocita, C-reaktivni protein, fibrinogen, D dimer, von Willebrand-ov faktor). **Rezultati.** U grupi ispitanika sa ESRD u poređenju sa grupom zdravih, promet trombocita (% retikulisanih trombocita) je bio statistički značajno veći (3,8 ± 2,3 vs. 2,3 ± 1,3; $p < 0,01$), kao i agregabilnost trombocita indukovana sa trombin receptor-aktivirajućim peptidom (TRAP) ($p < 0,01$), adenozin-difosatom (ADP) ($p < 0,05$), arahidonskom kiselinom (ASPI) ($p < 0,05$) i kolagenom ($p < 0,05$). U ESRD grupi su potvrđene značajno više vrednosti svih zapaljenskih i prokoagulantnih markera u odnosu na zdrave ispitanike ($p < 0,01$, za sve parametre). Nije utvrđena razlika u ispitivanim parametrima između grupe bolesnika kod kojih je šećerna bolest uzročnik ESRD i grupe bolesnika sa ESRD druge etiologije. **Zaključak.** Rezultati pokazuju da je povišen promet trombocita u ESRD posledica njihove aktivacije i potrošnje indukovane hiperaktivnošću koagulacionog sistema i hroničnim zapaljenjem. Nijedan od ispitivanih parametara ne predviđa mogućnost pojave krvarenja.

Ključne reči:
bubreg, hronična insuficijencija; dijabetes melitus; trombociti, funkcijski testovi.

Correspondence to: Predrag Filipov, Clinical Centre of Vojvodina, Centre for Laboratory Medicine, Hajduk Veljkova 1, 21 000 Novi Sad, Serbia. E-mail: drfilbuzzgroup7@yahoo.com

Introduction

Cardiovascular diseases (CVD) are the most common cause of mortality among patients with chronic kideny disease (CKD). Due to progressive development of atherothrombosis, the risk of CVD in patients with impaired renal function is up to twentyfold higher as compared to healthy subjects [1, 2]. On the other hand, the association of CKD with different clinical forms of hemorrhagic diathesis is well-known [3, 4]. Thrombocytopathia and shortened life span of platelets in the uremic milieu are caused by impaired thrombocytopoiesis, structural and functional disorders and increased platelet consumption ("turnover") due to the activation of haemostatic system and the presence of chronic inflammation in CKD. The above-mentioned changes appear as an increased number of reticulated platelets (platelet with enhanced RNA), among other laboratory findings [5, 6]. Considering the role of primary haemostasis in the process of bleeding and atherothrombosis and the fact that the progressive course of CKD is characterized by the presence of clinically quite opposite but equally represented manifestation of a complex disorder of haemostasis – the paradoxical coexistence of prothrombotic state and bleeding tendency, the evaluation of altered platelet function in conditions of chronic inflammation and activation of the coagulation system could contribute to the elucidation of the pathophysiological mechanisms of atherothrombosis, CVD and bleeding diathesis in chronic kidney disease [7, 8].

With respect to the fact that many of the biological parameters in the diabetes mellitus (DM) and CKD are altered and that both diseases have a high risk of atherothrombosis and CVD, we thought it would be useful to examine whether the changed functionality of primary hemostasis was dominantly influenced by ESRD or by DM as etiological factor [9].

In the absence of a single predictor marker, we hypothesized that the usage of one of the platelet indices (e.g. reticulated platelets, mean platelet volume, plateletcrit and plateled distribution width) in addition to their total number and aggregability, could be used as an auxiliary laboratory method in identification of patients with higher susceptibility to the occurrence of one of the two above-mentioned clinically “opposite” disorders of haemostatic system functionality in CKD-bleeding or thrombosis [10, 11].

Methods

The study was conducted at the Clinical Centre of Vojvodina, Novi Sad in accordance with the Helsinki Declaration, approved by the local Ethics Committee, with the written consent of all the participants who were also interviewed. Observational clinical trial was initiated in November, 2012 and concluded on 31st of March, 2015. It included the total of 93 subjects divided into two groups: Group I included 63 patients with ESRD and with the intention of separate study on DM impact on investigated parameters; it was divided into two subgroups: the first, labelled as IA, consisted of 25 patients having DM as the cause of ESRD while the second one was labelled as IB with 38 patients whose ESRD etiology was based on some other factors (hypertension, glomerulonephritis, polycystic kidney disease, etc.). Group II was the control group included 30 age and sex matched healthy volunteers, non-smokers who did not use any drugs.

The study included both men and women with ESRD (glomerular filtration rate – GFR < 15 mL/min 1.75 m^2, Cockcroft-Gault equation) hospitalized prior to the creation of permanent vascular access for haemodialysis (autologous arteriovenous fistula) without contraindications for surgery. The study excluded patients younger than 18, pregnant women, those who did not give consent to participate in the study, those who had associated malignancy, individuals with liver failure and those with an acute complication of DM.

Blood samples for laboratory investigations were obtained in the morning after overnight fasting by puncture of the cubital vein and were analyzed within 120 minutes.

For the platelet indices determination, the blood samples were collected in vacuum tubes containing K_2EDTA. For the analysis of platelet aggregation, the blood samples were taken in vacuum blood collection tubes containing Li-heparin, and for the determination of fibrinogen, D dimer, vWF Ag and vWAct blood was extracted in the vacuum tube with Na-citrate.

As a part of complete blood count (CBC), number of neutrophils (NoN), neutrophil to lymphocyte ratio (NLR), platelet count (NoPlt), reticulated platelets (% rPlt) and platelet indices – mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) were measured by automated hematology analyser CELL-DYN Sapphire, Abbott Diagnostics, using fluorescent flow cytometry analyzer to provide automated reticulocyte analysis and separate subpopulations of young cells (reticulated platelets are an integral part of reticulocyte essays) of mature blood cells [12].

The degree of platelet aggregation was evaluated using whole blood impedance platelet aggregometer, Multiplate®; Dynabyte, Munich, Germany, in basal conditions – thrombin receptor activating peptide (TRAP) test, and with adenosindiphosphate (ADP), arachidonic acid (ASPI) and collagen as agonists (20 μL of each reagent).

The values of plasma fibrinogen (FBG), D dimer, von Willebrand factor activity (vWF Act) and von Willebrand factor antigen (vWF Ag) were determined using ACL analyzer, Instrumentation Laboratory Assays, Italy.

The value of blood glucose, urea, creatinine and C-reactive protein (CRP) was determined by using automatic biochemical analyzer Architect c8000, Abbott Diagnostics.

Body weight and height were measured and body mass index (BMI) was calculated using the formula: BMI = Body weight (kg)/height (m) ^{2}.

Data distribution was tested by the Kolmogorov-Smirnov test. Normally distributed data was presented as the mean ± SD and as the median (25th, 75th percentile) if not normally distributed. Two-sided unpaired *t* test was used for comparison of means between the groups and Mann-Whitney test was used to compare the median values between groups if data was not normally distributed. Correlations between various parameters were determined by Pearson's correlation analysis. Categorical variables were compared by χ^2 test. A *p*-value < 0.05 was con-

sidered to be statistically significant. Statistical software used for the statitical anaysis was MedCalc® Ver. 12.1.3 (MedCalc software, Mariakerke, Belgium).

Results

There was no statistically significant difference between the group of patients with ESRD and healthy control regarding age, gender and BMI. Blood glucose, urea and creatinine concentration were significantly higher in the patients' group.

The comparison of demographic characteristics examined in the defined IA and IB subgroups of patients revealed no differences. Blood glucose concentration was significantly higher in the Group IA DM ESRD while urea and creatinine were not (Table 1).

Table 1

Baseline characteristics and biochemical parameters (blood glucose, urea and creatinene concentration) of study population

Parameter	Group I ESRD (n = 63)	Group II CG (n = 30)	I A DM ESRD (n = 26)	I B non-DM ESRD (n = 37)	*p*-value	*p**-value
Age (years)	60.9 ± 11.7	56 ± 11.1	62.1 ± 10.9	60.0 ± 12.3	ns	ns
Male, n (%)	47 (74.6)	21 (70)	18 (69.2)	29 (78.4)	ns	ns
Female, n (%)	16 (25.4)	9 (30)	8 (30.8)	8 (21.6)	ns	ns
BMI (kg/m²)	26.7 ± 5.4	25.1 ± 3.2	25.6 ± 3.9	27.4 ± 6.2	ns	ns
Blood glucose (mmol/L)	6.4 ± 3.1	5.1 ± 0.5	8.1 ± 4.0	5.2 ± 1.4	< 0.01	< 0.01
Urea (mmol/L)	28.1 ± 10.3	5.0 ± 1.2	29.0 ± 7.6	27.5 ± 11.9	< 0.01	ns
Creatinine (µmol/L)	686.4 ± 312.2	79.7 ± 17.6	618.5 ± 198.4	734.2 ± 367.2	< 0.01	ns

The data are expressed as mean ± SD; sample size – n (%); ESRD – end-stage renal disease; CG – control group; DM-diabetes mellitus; non-DM – non diabetes mellitus; BMI – body mass index; *p*-value, difference between Group I ESRD/Group II CG; *p-value, difference between subgroup IA DM ESRD/subgroup IB non-DM ESRD.**

Also, there was no difference in platelet count (NoPlt) and platelet indices (MPV, PDW, PCT) between the two groups, but the value of reticulated platelets (% rPlt) was significantly higher in the ESRD group than in the healthy volunteers group (3.8 ± 2.3 vs. 2.3 ± 1.3; $p < 0.01$) (Table 2).

Furthermore, the platelet aggregation tests induced by TRAP, ADP, ASPI and collagen were significantly higher in the ESRD patients compared to the control group. The comparison of chronic inflammation and procoagulant markers revealed significantly higher values of fibrinogen (5.0 ± 1.4 vs. 3.3 ± 0.6 g/L; $p < 0.01$), the number of neutrophils (5.4 ± 2.3 vs. 3.2 ± 1.1 × 10^9/L; $p < 0.01$), NLR [3.2 (2.3–4.9) vs. 1.5 (1.2–1.9); $p < 0.01$], CRP [15.1 (3.5–42.4) vs. 0.9 (0.4–1.4) mg/L]; $p < 0.01$], D dimer [738.0 (355.0–1070.5 ng/mL) vs. 192.0 (118.3–246.3); $p < 0.01$], vWF Ag (215.8 ± 78.3 vs. 142.5 ± 41.6%; $p < 0.01$) and vWF Act (170.0 ± 57.9 vs. 120.6 ± 36.7%; $p < 0.01$) in the observed group compared to the group of healthy subjects.

Considering IA and IB subgroups, only the number of platelets in the subgroup of the DM ESRD was higher compared to the IB non-DM ESRD subgroup but this differencedid not reach statistical significance (277.3 ± 108.9 vs. 228.0 ± 108.9; $p = 0.08$). Interestingly, the values of all other examined parameters were not statistically significantly different.

In order to evaluate the association between platelet turnover and function with inflammatory and procoagulant markers in the ESRD patients and their relationship with diabetes mellitus as the most frequent etiological factor of chronic kidney disease correlation analysis was carried out. We found a significant positive correlation between platelet count and number of neutrophils (r = 0.391, $p < 0.01$), serum fibrinogen (r = 0.440, $p < 0.01$) and CRP concentration (r = 0.264; $p < 0.05$) and the absence of correlation of any other platelet indices with inflammatory and procoagulant markers in the ESRD patients. Furthermore, in the same group, there was a significant positive correlation between enhanced platelet aggregability and the number of neutrophils [with TRAP (r = 0.387, $p < 0.01$), ASPI (r = 0.321, $p < 0.05$), ADP (r = 0.366, $p < 0.01$) and collagen (r = 0.281, $p < 0.05$) as agonists] and between the increased platelet aggregation and concentration of inflammatory markers i.e. fibrinogen [with ASPI (r = 0.309, $p < 0.05$), ADP (r = 0.260, $p < 0.05$) and collagen (r = 0.290, $p < 0.05$)], and CRP [with ASPI (r = 0.294, $p < 0.05$) and ADP (r = 0.302, $p < 0.05$) as inductors] (Figure 1).

In the DM ESRD subgroup a significant positive correlation between platelet count and the number of neutrophils (r = 0.485, $p < 0.05$) and CRP (r = 0.444, $p < 0.05$), and between platelet aggregation and fibrinogen concentration [ADP (r = 0.400, $p < 0.05$) and collagen (r = 0.415, $p < 0.05$)] was found. In the non-DM ESRD subgroup positive correlations between platelet count and serum fibrinogen (r = 0.356, $p < 0.05$) and between platelet aggregability and CRP [ASPI (r = 0.392, $p < 0.05$)] were present.

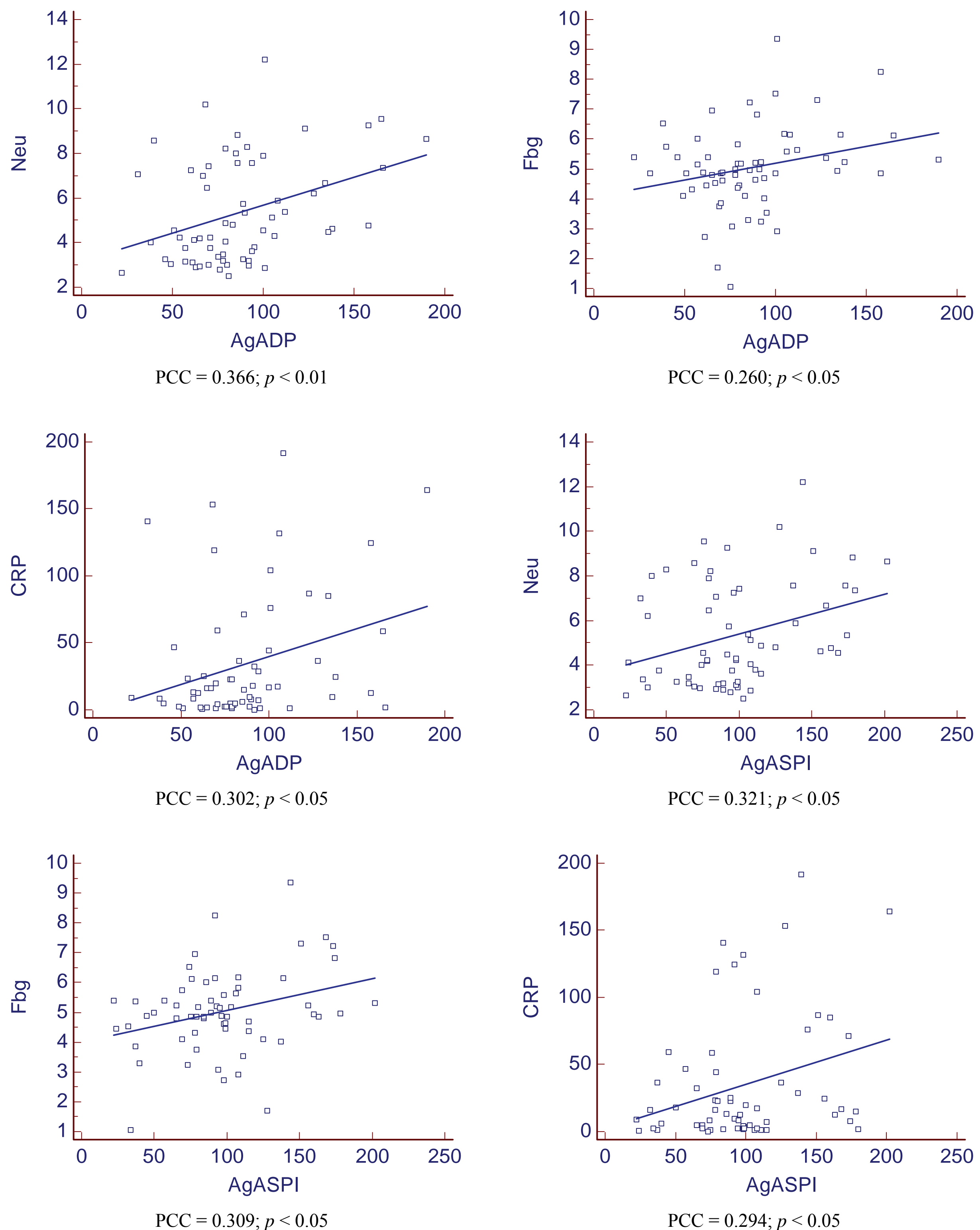

Fig. 1 – Scatter plots showing the corellation between adenosindiphospate (ADP) and arachidonic acid (ASPI) induced platelet agreggation (Ag) and the number of neutrophils (NEU), fibrinogen (Fbg) and C-reactive protein (CRP) in end-stage renal disease (ESRD) patients.
PCC – Perason's correlation coefficient; *p*-value.

Table 2

Values od platelets indicies, platelet aggregation and chronic inflamation and procoagulant activity markers in Group I ESRD and Group II CG, and in subgroup IA DM ESRD and subgroup IB non-DM ESRD

Parameter	Group I ESRD (n = 63)	Group II CG (n = 30)	*p*-value	I A DM ESRD (n = 26)	I B non-DM ESRD (n = 37)	*p**-value
NoPlt (× 10^9/L)	248.3 ± 110.8	233.6 ± 53.7	ns	277.3 ± 108.9	228 ± 108	ns (*p*=0.08)
rPlt (%)	3.8 ± 2.3	2.3 ± 1.3	<0.01	3.3 ± 1.9	4.2 ± 2.6	ns
MPV (fL)	8.2 ± 1	8.3 ± 0.9	ns	8.1 ± 1.0	8.3 ± 1.1	ns
PTC (%)	0.2 ± 0.1	0.2 ± 0.03	ns	0.2 ± 0.1	0.2 ± 0.1	ns
PDW(fL)	16.6 ± 4	16.3 ± 1.6	ns	16 ± 0.7	17 ± 5.1	ns
AgTRAP (%)	115.8 ± 35	98.5 ± 23.4	<0.01	111.0 ± 37	119.2 ± 33.7	ns
AgASPI (%)	98 ± 41.9	82 ± 22.7	<0.05	93.4 ± 45.6	101.3 ± 39.3	ns
AgADP (%)	87.4 ± 34	76.5 ± 18.3	<0.05	85.4 ± 36	88.8 ± 33.0	ns
AgCollagen (%)	65.5 ± 31	51.8 ± 13.3	<0.05	70.6 ± 31.1	62 ± 30.8	ns
aPTT (R)	0.9 ± 0.1	0.9 ± 0.1	ns	0.9 ± 0.1	0.9 ± 0.1	ns
PT (R)	1.03 ± 0.1	1.05 ± 0.1	<0.05	1 ± 0.1	1 ± 0.1	ns
Fbg (g/L)	5 ± 1.4	3.3 ± 0.6	<0.01	5.4 ± 1.1	4.8 ± 1.5	ns
D dimer (ng/mL)	738 (355–1,070.5)	192 (118.3–246.3)	<0.01	748.5 (465–903)	707 (344.8–1,107.5)	ns
vWF Ag (%)	215.8 ± 78.3	142.5 ± 41.6	<0.01	219.4 ± 76.1	213.3 ± 80.7	ns
vWFAct (%)	170 ± 57.9	120.6 ± 36.7	<0.01	174.4 ± 62	167 ± 55.5	ns
NoN (× 10^9/L)	5.4 ± 2.3	3.2 ± 1.1	<0.01	5.8 ± 2.4	5 ± 2.3	ns
NLR	3.2 (2.3–4.9)	1.5 (1.2–1.9)	<0.01	4.1 (2.2–5.3)	3 (2.3–4.3)	ns
CRP (mg/L)	15.1 (3.5–42.4)	0.9 (0.4–1.4)	<0.01	13.7 (4.9–44.4)	16.1 (2.1–39.1)	ns

The data are expressed as mean ± SD or median (25th,75th percentile); n-sample size; ESRD – end-stage renal disease; CG – control group; DM – diabetes mellitus; non-DM – non diabetes mellitus; *p*-value, difference between Group I ESRD/Group II control group; *p-value, difference between subgroup IA DM ESRD/sub-group IB non-DM ESRD; NoPlt – number of platelets; rPlt – reticulated platelets; MPV – mean platelet volume; PCT – plateletcrit; PDW – platelet distribution width; AgTRAP – thrombin receptor activating peptide aggregation test; AgADP – adenosindiphosphate aggregation test; AgASPI – arachidonic acid aggregation test; AgCollagen – collagen aggregation test; vWF Ag – von Willebrand factor antigen; vWF Act – von Willebrand factor activity; NoN – number of neutrophils; NLR – neutrophil to lymphocyte ratio; CRP – C-reactive protein.**

Discussion

The results indicate several conclusions: first, platelet activity in ESRD is significantly altered and manifested by the increase of reticulated platelets and platelet aggregation; second, the values of inflammatory and procoagulant markers are significantly increased in the ESRD patients compared to the control group of healthy volunteers; third, in ESRD there is a positive correlation between the platelet count, elevated platelet aggregation and values of proinflammatory markers; fourth, the values and correlation of the examined parameters did not differ in sub-groups of patients with regard to the chronic renal failure (CRF) etiological factors; fifth, enhanced reticulated platelets (or augmented platelet turnover) and increased platelet function (or aggregability) in ESRD seem to be more associated with chronic inflammation and procoagulant state rather than with diabetes mellitus as an individual etiological factor of CKD; sixth, neither platelet turnover and function nor inflammatory and procoagulant markers do not predict the likelihood of bleeding in ESRD.

The presence of increased reticulated platelets – "young platelets" with elevated density of granules and RNA content, but similar volume with "mature platelets", is a reliable indicator of enhanced "turnover" or consumption of platelets and increased megakaryocytopoietic activity in the patients with CRF. The above-mentioned fact may be considered important as the absence of thrombocytopenia and lack of changes in platelet volume parameters (MPV, PCT, PDW) in the patients with ESRD compared to the healthy subjects in our study may be explained by the sustained balance between the shortened lifespan and increased level of platelet degranulation (which also represents the indicator of their activation) on one hand, and, on the other hand, the process of sufficiency thrombocytopoiesis i.e. increased "output" of young platelets as a compensatory mechanism aimed at maintaining homeostasis [13, 14]. Previously published studies of platelet function in ESRD are contradictory and refer to patients undergoing dialysis treatment – haemodialysis or chronic ambulatory peritoneal dialysis [15]. According to them, the measurement of platelet aggregation as the gold standard for testing platelet function using platelet rich plasma (PRP) showed that induced platelet aggregation was either reduced or enhanced. Also, the tests of platelet aggregation using whole blood and determining the platelet activation markers by flow cytometry were inconclusive [16, 17].

In order to avoid the above-mentioned imperfections, we tested platelet functionality in the patients with ESRD who had not yet begun regular dialysis treatment, using the method of whole blood impedance platelet aggregometry which had much better reproducibility [18]. Increased platelet aggregation in our study with conventional inductors (TRAP, ADP and collagen) may be associated with the increased representation of "immature" reticulated platelets subpopulation in the platelet total mass whose haemostatic potential is sig-

nificantly increased during a very short period of time. This is caused by the presence of mRNA, granular endoplasmic reticulum and ribosomes of megakaryocytic origin and the ability of nucleic synthesis of numerous thrombogenic proteins, glycoprotein platelet membrane, α granule proteins and enzymes, fibrinogen, P-selectin, vWF, GP IIb/IIIa inhibitors and cyclooxygenase-1 (COX-2) [19–21]. Elevated platelet aggregation with arachidonic acid could be further explained by stimulated formation of platelet TxA2 and/or TxA2 generated from the "processing" of excess arachidonic acid (formed as a consequence of endothelial cells damage and decreased binding to albumin in ESRD) by platelet cyclooxygenase-1 (COX-1). This mechanism could "outbalance" the pharmacological effect of possible aspirin use [22–24].

The proportional association between indicators of reduced renal function and increased values of inflammatory and procoagulant markers is already known [25, 26]. Our findings of increased FBG and CRP concentration, NoN, NLR, D dimer, vWF Act and vWF Ag in the patient group are in full conformity with numerous statements regarding the presence of a hypercoagulable state in CKD that occurs due to a complex of disorders of haemostatic balance with reduced fibrinolytic potential and the presence of procoagulant stimulation of multi-causal origin in the background [27, 28].

The endothelial cell damage caused by uremic toxins triggers a complex haemostatic system functionality disorder which affects primary haemostasis and the reactive adaptive response of the body in the form of chronic inflammation and oxidative stress that generates "spreading" of secondary haemostasis functionality disorder, fibrinolytic processes and system of natural inhibitors [29–31].

We considered that a separate study of the relationship of platelet turnover and their functionality with markers of stimulated coagulation activities, especially in the sub-group of patients where DM is the cause of CRF, was justified from the standpoint of the total, additionally negative impact of etiological factors on functionality of all haemostatic system components, scaling up proatherogenic potential and risk for CVD [32].

The theoretical question is the impact of etiological factor as the provoker of kidney disease and CKD *per se* and their dominance in the development of hemostatic system disorders. Some authors state that the risk of venous thromboembolism (VTE) increases with the degree of renal and hemostatic system impairment and that comorbidity has a "modelling" role [33]. It is also known that DM (type-2, type-1) is the most common etiologic factor of CKD in the form of diabetic nephropathy or advanced CKD, as well as combined effect of DM and CKD in the process of atherothrombosis and cardiovascular diseases [34]. In presented results, the influence of etiological factor(s) on the investigated parameters is absent, which can be explained by the small sample size and the lack of differentiation types of diabetes.

It is worth mentioning that distinguishing of the impact of the combined and superimposed metabolic dysfunction, oxidative stress, inflammation and pathological signalling mechanisms on the molecular base of prothrombotic state was beyond our everyday capabilities and in practice routinely used clinical – laboratory diagnostic procedures and methods [35–37].

Limitations of this study include a small number of respondents determined by the CKD incidence in our general population, the fact that it was observational and that it did not exclude the impacts of confounding factors (smoking, hypertension, hyperlipidemia, cardiovascular comorbidities and the use of various drugs) in the ESRD patients [38].

Conclusion

Results and correlation of the studied parameters point out increased platelet turnover in ESRD as a consequence of platelet activation and consumption induced by clotting system hyperactivity and chronic inflammation i.e. prothrombotic state and that it was nonsignificantly influenced by etiological factors. The presence of increased reticulated platelets is a reliable sign of their increased consumption and preserved megakaryocytopoiesis in ESRD. Although none of the examined parameters did not indicate the likelihood of bleeding in ESRD, in everyday clinical practice in the absence of more precise method, both whole blood platelet aggregation (indicator of platelet functionality) and reticulated platelets (indicator of increased platelet turnover) could be used as auxiliary laboratory methods in timely identification of patients with higher susceptibility to the occurrence of one of the two extremes, clinically "opposite" and therapeutically completely differently managed disorders of hemostatic system functionality in CRD – bleeding or thrombosis. Within this statement, the need for further testing of rational use of antiplatelet therapy in CRF in prevention of CVD is also imposed.

Conflict of interest

The authors reports no conflict of interest. The authors alone are responsible for the content and writing of the paper.

R E F E R E N C E S

1. *Levin A.* Clinical epidemiology of cardiovascular disease in chronic kidney disease prior to dialysis. Semin Dial 2003; 16(2): 101−5.
2. *Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C.* Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004; 351(13): 1296−305.
3. *Remuzzi G.* Bleeding in renal failure. Lancet 1988; 1(8596): 1205−8.
4. *Özkan G, Ulusoy Ş.* Bleeding diathesis in hemodialysis patients. INTECH 2013: doi: 10.5772/52926.
5. *Escolar G, Diaz-Ricart M, Cases A.* Uremic platelet dysfunction: past and present. Curr Hematol Rep 2005; 4(5): 359−67.
6. *Shlipak MG, Fried LF, Crump C, Bleyer AJ, Manolio TA, Tracy RP*, et al. Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. Circulation 2003; 107(1): 87−92.

7. *Boccardo P, Remuzzi G, Galbusera M.* Platelet Dysfunction in Renal Failure. Semin Thromb Hemost 2004; 30(5): 579−89.
8. *Astrup T.* The haemostatic balance. Thromb Diath Haemorrh 1958; 2(3-4): 347−57.
9. *Kakouros N, Rade JJ, Kourliouros A, Resar JR.* Platelet function in patients with diabetes mellitus: From a theoretical to a practical perspective. Int J Endocrinol 2011; 2011: 742719.
10. *Gafter U, Bessler H, Malachi T, Zevin D, Djaldetti M, Levi J.* Platelet count and thrombopoietic activity in patients with chronic renal failure. Nephron 1987; 45(3): 207−10.
11. *Hoffmann JJ, van den Broek NM, Curvers J.* Reference intervals of reticulated platelets and other platelet parameters and their associations. Arch Pathol LabMed 2013; 137(11): 1635−40.
12. *Hoffmann JJ.* Reticulated platelets: analitical aspects and clinical utility. Clin Chem Lab Med 2014; 52(8): 1107−17.
13. *Leader A, Pereg D, Lishner M.* Are platelet volume indices of clinical use? A multidisciplinary review. Ann Med 2012; 44(8): 805−16.
14. *Schoorl M.* Activation of platelets and coagulation during haemodialysis. Ned Tijdschr Klin Chem Labgeneesk 2016; 41: 17−27.
15. *Darlington A, Ferreiro JL, Ueno M, Suzuki Y, Bhaloo D, Capranzano P*, et al. Haemostatic profiles assessed by thromboelastography in patients with end-stage renal disease. Thromb Haemost 2011; 106(1): 67−74.
16. *Małyszko J, Małyszko JS, Myśliwiec M, Buczko W.* Hemostasis in chronic renal failure. Rocz Akad Med Bialymst 2005; 50: 126−31.
17. *van Bladel ER, de Jager RL, Daisy W, Cornelissen L, Gaillard CA, Boven LA*,et al. Platelets of patients with chronic kidney disease demonstrate deficient platelet reactivity in vitro. BMC Nephrol 2012; 13(1): 127.
18. *Ho SJ, Gemmel R, Brighton TA.* Platelet function testing in uraemic patients. Hematology 2008; 13(1): 49−58.
19. *Abe Y, Wada H, Tomatsu H, Sakaguchi A, Nishioka J, Yabu Y, Onishi K.* A simple technique to determine thrombopoiesis level using immature platelet fraction (IPF). Thromb Res 2006; 118(4): 463−9.
20. *Zwaginga J.* Hemodialysis, erythropoietin and megakaryocytopoiesis: Factors in uremic thrombocytopathy and thrombophilia. J Thromb Haemost 2004; 2(8): 1272−4.
21. *Grove EL.* Antiplatelet effect of aspirin in patients with coronary artery disease. Dan Med J 2012; 59(9): B4506.
22. *Dusse LM, Freitas LG.* Clinical applicability of reticulated platelets. Clin Chim Acta 2015; 439: 143−7.
23. *Sirolli V, Ballone E, Garofalo D, Merciaro G, Settefrati N, Di Mascio R*, et al. Platelet activation markers in patients with nephrotic syndrome. A comparative study of different platelet function tests. Nephron 2002; 91(3): 424−30.
24. *Kilickesmez KO, Kocas C, Abaci O, Okcun B, Gorcin B, Gurmen T.* Follow-up of aspirin-resistant patients with end-stage kidney disease. Int Urol Nephrol 2013; 45(4): 1097−102.
25. *Jalal D, Chonchol M, Targher G.* Disorders of hemostasis associated with chronic kidney disease. Semin Thromb Hemost 2010; 36(1): 34−40.
26. *Keller C, Katz R, Cushman M, Fried LF, Shlipak M.* Association of kidney function with inflammatory and procoagulant markers in a diverse cohort: A cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis (MESA). BMC nephrology 2008; 9: 9.
27. *Stenvinkel P.* Inflammation in end-stage renal disease: The hidden enemy. Nephrology (Carlton) 2006; 11(1): 36−41.
28. *Forbes SH, Ashman N, Yaqoob MM.* The role of platelets in the prognosis of renal disease. OA Nephrology 2013; 1(2): 17.
29. *Adams M, Irish A, Watts G, Oostryck R, Dogra GK.* Hypercoagulability in chronic kidney disease is associated with coagulation activation but not endothelial function. Thrombosis Res 2008; 123(2): 374−80.
30. *Casserly LF, Dember LM.* Thrombosis in End-Stage Renal Disease. Semin Dial 2003; 16(3): 245−56.
31. *Małyszko J, Małyszko JS, Myśliwiec M.* Endothelial cell injury markers in chronic renal failure on conservative treatment and continuous ambulatory peritoneal dialysis. Kidney Blood Press Res 2004; 27(2): 71−7.
32. *Krentz AJ.* Diabetes, the kidney and vascular disease: A complex relationship. Pract Diab Int 2006; 23(1): 1−3.
33. *Wattanakit K, Cushman M.* Chronic kidney disease and venous thromboembolism: Epidemiology and mechanisms. Curr Opin Pulm Med 2009; 15(5): 408−12.
34. *Fox CS, Larson MG, Leip EP, Culleton B, Wilson PW, Levy D.* Predictors of new-onset kidney disease in a community-based population. JAMA 2004; 291(7): 844−50.
35. *Libetta C, Sepe V, Esposito P, Galli F, Dal Canton A.* Oxidative stress and inflammation: Implications in uremia and hemodialysis. Clin Biochem 2011; 44(14): 1189−98.
36. *Turkmen K, Guney I, Yerlikaya FH, Tonbul HZ.* The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. Ren Fail 2012; 34(2): 155−9.
37. *Glorieux G, Cohen G, Jankowski J, Vanholder R.* Platelet/Leukocyte activation, inflammation, and uremia. Semin Dial 2009; 22(4): 423−7.
38. *Würtz M, Wulff LN, Grove EL, Kristensen SD, Hvas AM.* Influence of renal function and platelet turnover on the antiplatelet effect of aspirin. Thromb Resh 2012; 129(4): 434−40.

Received on July 20, 2016.
Revised on September 13, 2016.
Accepted on October 24, 2016.
Online First December, 2016.