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ORIGINAL ARTICLE

The utility of inflammation and platelet biomarkers in patients with acute coronary syndromes

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KEYWORDS

Acute coronary syndrome;
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Abstract *Introduction:* Thrombotic and inflammatory mechanisms are involved in the pathophysiology of acute coronary syndrome (ACS). The aim of the study was the evaluation of inflammation (white blood cells count/WBC, C-reactive protein/CRP, interleukin-6/IL-6) and platelet (platelet count/PLT, mean platelet volume/MPV, large platelet/LPLT, beta-thromboglobulin/ β -TG) biomarkers in the groups of ACS patients depending on the severity of signs and symptoms and compared to controls without coronary artery disease.

Materials and methods: The study group included 93 patients categorized into 3 subgroups depending on the severity of signs and symptoms of ACS. PLT, MPV, LPLT, and WBC were determined on hematological analyzer, IL-6 and β -TG were measured using the ELISA method.

Results: In the whole group of ACS patients WBC, CRP, IL-6, MPV, and β -TG were significantly higher as compared to controls. Analyzing the inflammation and platelet biomarkers depending on the severity of signs and symptoms in comparison to controls, statistically significant

Abbreviations: ACS, acute coronary syndrome; ACC, diagnostic accuracy; ALT, alanine transaminase; AST, aspartate transaminase; AUC, area under the ROC curve; BP, blood pressure; CRP, C-reactive protein; cTnI, cardiac troponin I; ECG, echocardiogram; eGFR, estimate glomerular filtration rate; F, female; HCT, hematocrit; HDL, high-density lipoprotein cholesterol; HGB, hemoglobin; IFN- γ , interferon gamma; IL-6, interleukin-6; LBBB, Left Bundle Branch Block; LDL, low-density lipoprotein cholesterol; L-PLT, large platelet; M, male; MI, myocardial infarction; MPV, mean platelet volume; NPV, negative predictive value; NS, not statistically significant; NSTEMI, non-ST-segment elevation myocardial infarction; PAF, platelet activating factor; PLT, platelet count; PPV, positive predictive value; RBC, red blood cell count; ROC, Receiver operator characteristic; SE, Standard Error; sP-selectin, soluble form of P-selectin; STEMI, ST-segment elevation myocardial infarction; TCH, total cholesterol; TG, triglycerides; TNF- α , tissue necrosis factor alfa; UA, unstable angina; WBC, white blood cells count; β -TG, β -thromboglobulin.

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differences for above-mentioned parameters were also found. There were no significant differences between the advancement of coronary artery changes and inflammation as well as platelet parameters, except for CRP concentrations. The AUCs for all inflammation parameters tested were similar, however the highest AUCs showed WBC and CRP. Among platelet parameters the highest AUC revealed β -TG.

Conclusion: Markers of inflammation and platelet activation may be associated to myocardial ischemia and myocardial injury. WBC, CRP and IL-6 as inflammation parameters and MPV and β -TG as platelet biomarkers may be useful indicators of the presence of coronary artery disease.

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1. Introduction

Acute coronary syndrome (ACS), the initial presentation of coronary artery disease, is the umbrella term utilized for the clinical signs and symptoms of myocardial ischemia (Go et al., 2014; Heeschen et al., 2005). Depending on the sensitivity of signs and symptoms ACS may be classified as non-ST-segment elevation myocardial infarction (NSTEMI), ST-segment elevation myocardial infarction (STEMI), or unstable angina (UA) (Heeschen et al., 2005). ACS can lead to adverse cardiovascular events such as sudden cardiac death (Wang et al., 2014). Therefore strategies concerning the prevention of acute coronary events belong to the most important public health goals and identifying subjects at increased risk of ACS, who may benefit from preventative procedures, nowadays is a major challenge (Arbab-Zadeh et al., 2012).

Atherosclerosis is a chronic inflammatory arterial disease developing over decades, characterized by an impaired lipid metabolism and imbalanced immune response, which lead to the subendothelial lipoprotein retention and activation of endothelial cells with continuous migration of leukocytes and smooth muscle cells to the inflamed intima. Acute occlusive atherothrombotic complications, such as myocardial infarctions (MI) and strokes, result from the formation of vulnerable atherosclerotic plaque (Hristov and Weber, 2015).

Acute myocardial infarction is a typical sterile inflammation, in which hyperglycemia, increased concentrations of inflammatory cytokines, platelet activation, as well as leukocytosis are recognized. Macrophages and mast cells, which are deeply involved in atherosclerotic plaque formation, generate pro-inflammatory cytokines, such as IL-1, IL-6, TNF- α , and IFN- γ . Moreover mast cells, also release vasoactive and angiogenic compounds, pro-inflammatory mediators, such as arachidonic acid metabolites, histamine, platelet activating factor (PAF), and proteolytic enzymes, via the accumulation in the arterial intima and adventitia during atherosclerotic plaque progression (Spinas et al., 2014).

Repair of the vascular damage and preservation of the patency of narrow capillaries is a complex mechanism, in which platelets are key regulatory players. In atherosclerosis platelets contribute to the endothelial dysfunction as well as the rupture of the vulnerable plaque (Nording et al., 2015). The interaction of platelets with endothelial cells leads to an excessive platelet activation, which results in shorter half-life and increased platelet turnover. It may influence the platelet count (PLT), mean platelet volume (MPV) as well as the percentage of large platelets (LPLT) (Pal et al., 2014), which are

defined as platelets above 12 fL. Specific proteins are released during platelet activation. The first platelet-specific protein, isolated in 1975 and released during platelet aggregation, is beta-thromboglobulin (β -TG) (Borsey et al., 1980; Kaplan and Owen, 1981). Moreover, within the atherosclerotic plaque platelets could remain activated for a long time providing for proinflammatory cytokines production, e.g. IL-6 (Nording et al., 2015). So far many biomarkers of inflammation have been utilized as a markers of ACS. Most of the studies concerned C-reactive protein, fibrinogen, and IL-6. These inflammatory biomarkers have high sensitivity and thus are indicated as a good prognostic and evolution-monitoring markers (Gilardi et al., 2014).

Elevated concentrations of cardiac troponins as a specific and sensitive biomarkers of myocardial-cell necrosis are noted in about 1/3 of ACS cases (Heeschen et al., 2003, 2005). However, cardiac troponins are not actively engaged in the pathophysiology of ACS (Heeschen et al., 2003). It is suggested that the main role in the development of ACS play both the inflammatory as well as thrombotic processes. According to Hansson G.K. “most cases of infarction are due to the formation of an occluding thrombus on the surface of the plaque” (Hansson, 2005). Inflammation plays the pivotal role in platelet activation and thrombotic complications in ACS (Tanindi et al., 2011; Zhang et al., 2007). In respect to the above mentioned issues, markers of inflammation together with platelet activation parameters may be useful indicators of disease activity, by the time myocardial-cell necrosis occurs. Therefore the aim of the current study was the evaluation of inflammation markers (white blood cells count/WBC, C-reactive protein/CRP, interleukin-6/IL-6) concentrations, chosen platelet morphological parameters (platelet count/PLT, mean platelet volume/MPV, large platelet/LPLT) as well as beta-thromboglobulin/ β -TG activity in the group of ACS patients (STEMI, NSTEMI, and UA) as compared to the control group without coronary artery disease. In the next step of the study we try to establish the diagnostic significance of parameters tested.

2. Material and methods

2.1. Study group and control group

The study group included 93 patients (34F/59 M, mean age 63, ranged 29–93 years) with ACS, diagnosed and hospitalized at the Department of Cardiology Intensive Care with the Hemodynamic Unit of the Provincial Hospital in Bialystok. Table 1

Table 1 Clinical characteristics of the total group of ACS patients and depending on the severity of signs and symptoms.

	ACS N = 93	STEMI N = 33	NSTEMI N = 30	UA N = 30	P-value STEMI vs. NSTEMI vs. UA
Age (years)	63 (29–93)	62 (35–87)	62 (29–85)	66 (48–93)	
Sex: F/M	34/59	13/20	11/19	11/19	
Systolic BP [mmHg]	140 (120–160)	130 (116–160)	150 (130–170)	130 (120–160)	NS
Diastolic BP [mmHg]	80 (70–90)	80 (70–90)	87 (80–96)	80 (75–85)	NS
TCH [mg/dL]	183 (159–211)	179 (162–205)	194 (157–224)	183 (156–212)	NS
LDL [mg/dL]	113 (80–135)	108 (89–134)	120 (83–139)	99 (73–131)	NS
HDL [mg/dL]	49 (41–59)	50 (42–57)	43 (38–56)	49 (43–61)	NS
TG [mg/dL]	105 (68–148)	99 (57–131)	106 (76–148)	111 (71–198)	NS
Glucose [mg/dL]	102 (93–112)	104 (94–129)	105 (101–126)	93 (86–101)	0.0012*
Sodium [mmol/L]	139 (137–140)	138 (135–140)	139 (136–140)	140 (139–141)	0.0034*
Potassium [mmol/L]	4.26 (3.96–4.50)	4.25 (3.88–4.42)	4.25 (3.96–4.47)	4.28 (4.13–4.50)	NS
Creatinine [mg/dL]	0.88 (0.71–0.99)	0.88 (0.71–1.01)	0.82 (0.71–0.95)	0.88 (0.78–0.97)	NS
eGFR [mL/min/1.73m ²]	91 (76–104)	92 (77–108)	93 (81–104)	88 (70–101)	NS
Urea [mg/dL]	38 (31–43)	40 (33–43)	33 (26–40)	39 (31–47)	NS
AST [U/I]	29 (20–79)	53 (28–116)	44 (19–79)	20 (16–22)	0.0013*
ALT [U/I]	22 (15–35)	27 (20–37)	25 (14–41)	20 (13–26)	NS
RBC [x10 ⁶ /μL]	4.61 (4.26–4.95)	4.53 (4.10–5.11)	4.69 (4.46–5.93)	4.56 (4.32–4.95)	NS
HGB [g/dL]	14.2 (13.2–15.0)	14.0 (12.0–14.9)	14.2 (13.5–15.3)	14.3 (13.1–15.4)	NS
HCT [%]	41.4 (38.1–43.7)	40.8 (36.2–43.8)	41.0 (39.3–43.5)	42.2 (38.0–44.2)	NS
Troponin I [ng/mL]	9.28 (0.22–25.00)	25.00 (25.00–25.00)	9.28 (6.06–23.4)	0.10 (0.10–0.14)	0.0000*
1-vessel disease [N]	40 (43%)	17 (43%)	11 (27%)	12 (30%)	
2-vessels disease [N]	24 (26%)	10 (42%)	9 (37.5%)	5 (20.5%)	
3-vessels disease [N]	19 (21%)	5 (26%)	8 (42%)	6 (32%)	

ACS – acute coronary syndrome, F – female, M – male, BP – blood pressure, TCH – total cholesterol, LDL – low-density lipoprotein cholesterol, HDL – high-density lipoprotein cholesterol, TG – triglycerides, eGFR – estimate glomerular filtration rate, AST – aspartate transaminase, ALT – alanine transaminase, RBC – red blood cell count, HGB – hemoglobin, HCT – hematocrit, STEMI – ST-segment elevation myocardial infarction, NSTEMI – non-ST-segment elevation myocardial infarction, UA – unstable angina, NS – not statistically significant. Conversion factors to SI units are as follows: for glucose – 0.0555, for TCH – 0.0259, for LDL – 0.0259, for HDL – 0.0259, for TG – 0.0113, for creatinine – 88.4, for urea – 59.48, for RBC – 1.0, for HGB – 10.0, for HCT – 0.01.

* The analysis of differences between the ACS subgroups showed that significant variations were found, therefore in the next step the post hoc Dwass-Steale-Critchlow-Fligner test was conducted. Obtained P-value results are as follows: Glucose: STEMI vs. NSTEMI = NS, STEMI vs. UA = 0.0321, NSTEMI vs. UA = 0.0011; Sodium: STEMI vs. NSTEMI = NS, STEMI vs. UA = 0.0030, NSTEMI vs. UA = NS; AST: STEMI vs. NSTEMI = NS, STEMI vs. UA = 0.0008, NSTEMI vs. UA = NS.

presents the clinical characteristic of the study group. The criteria of ACS diagnosis included the detection of rise and/or fall of cardiac troponin I (cTnI) with at least one value above 99th percentile URL, and at least one of the following: clinical symptoms of ischemia, new or presumed new significant ST-segment-T wave changes or new Left Bundle Branch Block (LBBB), development of pathological Q waves, imaging evidence of new loss of viable myocardium, a new regional wall motion abnormality. The exclusion criteria were: active inflammation, cancer, chronic circulatory insufficiency, severe renal failure, acute coronary syndrome in medical history, antiplatelet drugs (acetylsalicylic acid, clopidogrel, ticagrelor), and anticoagulant treatment (acenocoumarol, warfarin, dabigatran, rivaroxaban).

The study group was divided according to the clinical and biochemical criteria of the European Society of Cardiology into three sub-groups:

1. patients with **STEMI**: 33 patients (13 F/20 M, mean age 62, ranged 35–87 years)
2. patients with **NSTEMI**: 30 patients (11F/19 M, mean age 62, ranged 29–85 years)
3. patients with **UA**: 30 patients (11F/19 M, mean age 66, ranged 48–93 years)

The control group included 30 healthy volunteers (15F/15 M, mean age 66, ranged 45–77 years), undergoing their periodical checkups at the Occupational Health Clinic of the Medical University Hospital in Bialystok. Their medical history did not reveal any cardiovascular problems, hypertension, neoplasia or diabetes. Moreover, routinely performed echocardiogram (ECG) was normal and the concentration of cTnI was within allowed range.

The study protocol was accepted by the Local Bioethics Committee (R-I-002/122/2013). All subjects included to the study gave their written informed consent.

2.2. Samples handling and storage

Blood samples from all subjects included to the study were drawn immediately after the diagnosis of ACS. Samples collected into EDTA-K₂ tubes were analyzed within 2 h of venipuncture. Tubes with the blood collected without anticoagulant were allowed to clot for 30 min before centrifugation (15 min at 1000×g); obtained serum was stored at –75 °C until further analysis. Blood collected into tubes containing CTAD (citrate, theophylline, adenosine and dipyridamole) anticoagulant was allowed in the ice bath for at least 15 min, and after that tubes were centrifuged (20 min at 2500×g, at 2–8 °C) within one hour from the blood collection. After the first cen-

trifugation one-third volume of the obtained plasma was removed from the middle region of the supernatant and centrifuged a second time (20 min at $2500\times g$, at $2-8^{\circ}\text{C}$). Once again one-third volume of the obtained plasma was removed from the middle region of the supernatant, aliquoted into several portions of $200\ \mu\text{L}$, and frozen at -20°C until assayed.

2.3. The evaluation of inflammation and platelet biomarkers

The platelet count (PLT), mean platelet volume (MPV), the percentage of large platelet (LPLT), and white blood cells count (WBC) were determined in the whole blood collected into EDTA- K_2 tubes with the use of SYSMEX XT 4000i hematological analyzer (Sysmex America, Mundelein, IL, USA) according to the manufacturer's instruction.

The C-reactive protein (CRP) and cTnI concentrations in the serum were determined with use of enzymatic-spectrophotometric method on the Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, USA).

Serum levels of IL-6 were measured using ELISA Quantikine® Human IL-6 Immunoassay kit (Catalog number: D60504; R&D Systems Europe Ltd., Abingdon, England) according to the manufacturer's instruction. Samples were not diluted before analysis. The manufacturer of assay kits referred to the intra-assay coefficient of variation (CV%) as 4.2% at IL-6 mean concentration of $16.8\ \text{pg/mL}$, $\text{SD} = 0.7\ \text{pg/mL}$.

Plasma β -TG activity was measured using commercially available ELISAs (Catalog number: 00950; ASSERACHROM® β -TG, Diagnostica Stago S.A.S., France) according to the manufacturer's instruction. Samples were not diluted before analysis. The manufacturer of assay kits referred to the intra-assay coefficient of variation (CV%) as 6.6% at β -TG mean activity of $28.7\ \text{IU/mL}$, $\text{SD} = 1.9\ \text{IU/mL}$.

2.4. Coronary angiography

Coronary angiography and angioplasty were performed in the course of ACS. During coronary angiography 6F catheter was applied. Percutaneous coronary interventions (PCIs) were routinely performed via right radial artery. Standard angiographic projections were used. A significant coronary lesion was defined as lumen stenosis of more than 70%. According to the coronary angiography results patients were distinguished into one-, two-, and three-vessel disease subgroups as well as patients without angiographically significant coronary lesion.

2.5. Statistical analysis

The obtained results were statistically analyzed with the use of the STATISTICA 12.0 PL software (StatSoft Inc., Tulsa, USA). The concentrations of protein tested did not follow the normal distribution in the preliminary statistical analysis (χ^2 -test), thus nonparametric statistical analysis was employed. The Mann-Whitney test and Kolmogorov-Smirnov test were used in order to compare two independent samples and ANOVA rank Kruskal-Wallis test was used for the comparison of three samples. The post hoc Dwass-Steele-Critchlow-Fligner test was conducted to assess which groups were different, if significant differences were found. The values for each given measured variable are given in medians and interquartile ranges. Differences were considered statistically

significant for $P < 0.05$. Correlation coefficients were obtained by applying Spearman's rank method.

The relation between the sensitivity and specificity was obtained using a receiver operator characteristic (ROC) curve. The ROC curve is a line graph that plots the probability of true positive results – or the sensitivity of the test – against the probability of false positive results for a range of different cut-off points. ROC curves to calculate the areas under the ROC curves (AUCs), positive predictive values (PPV), negative predictive values (NPV), diagnostic accuracy (ACC), as well as standard errors (SE) were generated with the use of the STATISTICA 12.0 PL software (StatSoft Inc., Tulsa, USA).

3. Results

3.1. Inflammation biomarkers

In the total group of ACS patients median (Me) WBC, CRP as well as IL-6 were significantly higher as compared to controls (Table 2).

Analyzing the inflammation biomarkers depending on the sensitivity of signs and symptoms in comparison to control group studies revealed that statistically significant differences were found for WBC, CRP and IL-6 (except between UA subgroup vs. controls for IL-6) (Table 3).

The analysis of differences between the ACS subgroups showed that significant variations were found for CRP and IL-6 concentrations (Table 4), therefore in the next step the post hoc Dwass-Steele-Critchlow-Fligner test was conducted. Studies revealed that among the ACS subgroups statistically significant differences were found between STEMI vs. UA subgroup ($P = 0.0230$) for CRP concentrations and between STEMI vs. UA subgroup ($P = 0.0000$) as well as between NSTEMI vs. UA subgroup ($P = 0.0000$) for IL-6 concentrations (Table 4). Statistically significant differences were also found for CRP and IL-6 concentrations in the STEMI + NSTEMI subgroup as compared to the UA subjects (Table 5).

Table 2 Inflammation and platelet biomarkers in the total group of ACS patients (STEMI, NSTEMI, and UA) compared to the controls. Results are presented as medians and interquartile ranges.

	ACS <i>N</i> = 93	C <i>N</i> = 30	<i>P</i>
WBC [$\times 10^3/\mu\text{L}$]	8.20 (6.70–10.1)	6.31 (5.38–7.35)	0.0000
CRP [mg/L]	2.1 (0.9–4.2)	0.75 (0.5–1.1)	0.0000
IL-6 [pg/mL]	12.0 (5.5–17.6)	7.6 (6.9–8.1)	0.0009
PLT [$\times 10^3/\mu\text{L}$]	217 (179–243)	233 (207–250)	NS
MPV [fL]	11.0 (10.1–11.3)	9.4 (9.2–9.8)	0.0000
β -TG [IU/mL]	81 (53–101)	29 (23–33)	0.0000

WBC – white blood cells count, CRP – C-reactive protein, IL-6 – interleukin-6, PLT – platelet count, MPV – mean platelet volume, L-PLT – large platelet, β -TG = β -thromboglobulin, STEMI – ST-segment elevation myocardial infarction, NSTEMI – non-ST-segment elevation myocardial infarction, UA – unstable angina.

Conversion factors to SI units are as follows: for WBC – 1.0, for PLT – 1.0.

Table 3 Inflammation and platelet biomarkers in the subgroups of ACS patients depending on the sensitivity of signs and symptoms (STEMI, NSTEMI, and UA) compared to controls. Results are presented as medians and interquartile ranges.

	STEMI N = 33	NSTEMI N = 30	UA N = 30	C N = 30	P
WBC [$\times 10^3/\mu\text{L}$]	8.70 (7.50–10.00)	8.05 (7.50–10.00)	7.65 (5.80–9.50)	6.31 (5.38–7.35)	0.0000 0.0000 0.0030
CRP [mg/L]	3.5 (1.4–5.0)	2.0 (0.9–3.9)	1.3 (0.9–2.9)	0.75 (0.5–1.1)	0.0000 0.0002 0.0018
IL-6 [pg/mL]	12.8 (9.9–17.4)	18.8 (12.2–24.1)	5.4 (2.2–11.0)	7.6 (6.9–8.1)	0.0001 0.0000 NS
PLT [$\times 10^3/\mu\text{L}$]	222 (188–273)	206 (168–254)	210 (175–223)	233 (207–250)	NS NS 0.0062
MPV [fL]	10.9 (10.0–11.5)	10.9 (10.2–11.2)	11.1 (10.4–11.3)	9.4 (9.2–9.8)	0.000 0.000 0.000
β -TG [IU/mL]	69 (53–118)	71 (53–128)	70 (53–95)	29 (23–33)	0.000 0.000 0.000

WBC – white blood cells count, CRP – C-reactive protein, IL-6 – interleukin-6, PLT – platelet count, MPV – mean platelet volume, L-PLT – large platelet, β -TG – β -thromboglobulin, STEMI – ST-segment elevation myocardial infarction, NSTEMI – non-ST-segment elevation myocardial infarction, UA – unstable angina.

Conversion factors to SI units are as follows: for WBC – 1.0, for PLT – 1.0.

Table 4 Inflammation and platelet biomarkers in the subgroups of ACS patients depending on the sensitivity of signs and symptoms (STEMI, NSTEMI, and UA). Results are presented as medians and interquartile ranges.

	STEMI N = 33	NSTEMI N = 30	UA N = 30	P-value
WBC [$\times 10^3/\mu\text{L}$]	8.70 (7.50–10.00)	8.05 (7.50–10.00)	7.65 (5.80–9.50)	NS
CRP [mg/L]	3.5 (1.4–5.0)	2.0 (0.9–3.9)	1.3 (0.9–2.9)	0.0251*
IL-6 [pg/mL]	12.8 (9.9–17.4)	18.8 (12.2–24.1)	5.4 (2.2–11.0)	0.0000*
PLT [$\times 10^3/\mu\text{L}$]	222 (188–273)	206 (168–254)	210 (175–223)	NS
MPV [fL]	10.9 (10.0–11.5)	10.9 (10.2–11.2)	11.1 (10.4–11.3)	NS
LPLT [%]	30.9 (25.6–34.2)	31.4 (28.0–35.1)	32.4 (27.9–34.0)	NS
β -TG [IU/mL]	69 (53–118)	71 (53–128)	70 (53–95)	NS

WBC – white blood cells count, CRP – C-reactive protein, IL-6 – interleukin-6, PLT – platelet count, MPV – mean platelet volume, L-PLT – large platelet, β -TG – β -thromboglobulin, STEMI – ST-segment elevation myocardial infarction, NSTEMI – non-ST-segment elevation myocardial infarction, UA – unstable angina.

Conversion factors to SI units are as follows: for WBC – 1.0, for PLT – 1.0

* The analysis of differences between the ACS subgroups showed that significant variations were found, therefore in the next step the post hoc Dwass-Steele-Critchlow-Fligner test was conducted. Obtained P-value results are as follows: CRP: STEMI vs. NSTEMI = NS, STEMI vs. UA = 0.0230, NSTEMI vs. UA = NS; IL-6: STEMI vs. NSTEMI = NS, STEMI vs. UA = 0.0000, NSTEMI vs. UA = 0.0000.

3.2. Platelet biomarkers

In the total group of ACS patients Me MPV and β -TG activity were significantly higher as compared to controls (Table 2).

Analyzing the platelet biomarkers depending on the sensitivity of signs and symptoms as compared to control group studies revealed that statistically significant differences were found for MPV and β -TG. Differences regarding PLT were found only in case of UA subgroup vs. control group (Table 3).

The analysis of differences between the ACS subgroups did not reveal any statistically significant variations (Table 4). Also

analyzing the STEMI + NSTEMI subgroup as compared to the UA subjects studies did not show any differences (Table 5).

3.3. Association between inflammation and platelet parameters and angiographic extent of the coronary artery disease in the total group of ACS patients

There were no significant differences between the advancement of coronary artery changes and inflammation as well as platelet parameters, except for CRP concentrations (Table 6). However to post hoc test conducted for CRP revealed that protein

Table 5 Inflammation and platelet biomarkers in the group of STEMI + NSTEMI subjects as compared to UA subjects. Results are presented as medians and interquartile ranges.

	STEMI + NSTEMI N = 63	UA N = 30	P
WBC [$\times 10^3/\mu\text{L}$]	8.40 (7.30–10.20)	7.65 (5.80–9.50)	NS
CRP [mg/L]	2.8 (0.9–4.6)	1.3 (0.9–2.9)	0.0357
IL-6 [pg/mL]	12.2 (4.1–18.8)	5.4 (2.2–11.0)	0.0000
PLT [$\times 10^3/\mu\text{L}$]	212 (183–257)	210 (175–223)	NS
MPV [fL]	10.9 (10.1–11.3)	11.1 (10.4–11.3)	NS
LPLT [%]	31.3 (2.57–35.1)	32.4 (27.9–34.0)	NS
β -TG [IU/mL]	69 (53–123)	70 (53–95)	NS

WBC – white blood cells count, CRP – C-reactive protein, IL-6 – interleukin-6, PLT – platelet count, MPV – mean platelet volume, L-PLT – large platelet, β -TG – β -thromboglobulin, STEMI – ST-segment elevation myocardial infarction, NSTEMI – non-ST-segment elevation myocardial infarction, UA – unstable angina.

Conversion factors to SI units are as follows: for WBC – 1.0, for PLT – 1.0.

tested was statistically lower only in the group of ACS patients with 1-vessel coronary artery disease as compared to subjects with 2-vessel coronary artery disease ($P = 0.0292$).

3.4. Diagnostic usefulness of inflammation and platelet biomarkers

The percentage of elevated results (diagnostic sensitivity) were higher for WBC (98%) and CRP (96%) than those of IL-6 (67%). However the highest specificity revealed IL-6 (90%). IL-6 also showed the highest PPV (94%) as well as diagnostic accuracy (76%). The highest NPV was found for WBC (67%) (Table 6). Among inflammation biomarkers the highest areas under the ROC curves (AUCs) were found for WBC (AUC = 0.787) and for CRP (AUC = 0.798). The percentage of elevated results were higher for MPV (93%) than those of β -TG (89%); however the highest specificity revealed β -TG (93%). β -TG also showed the highest PPV (90%), NPV (97%), and diagnostic accuracy (90%) (Table 7). Among platelet biomarkers the highest AUC was revealed for β -TG (AUC = 0.961) (Table 7).

4. Discussion

The aim of the current study was the evaluation of inflammation (WBC, CRP, IL-6) as well as platelet (PLT, MPV, LPLT, β -TG) biomarkers in the group of ACS patients (UA, STEMI, and NSTEMI) compared to the control group without coronary artery disease. According to the best of our knowledge studies evaluating in conjunction both groups of biomarkers engaged in the pathophysiology of CAD are limited. Moreover, which should be emphasized, this is the first study assessing the activity of β -TG in STEMI, NSTEMI, and UA subjects.

Studies revealed that patients with CAD have increased concentrations of proinflammatory biomarkers, such as TNF- α , CRP, and IL-6 (Lindahl et al., 2000; Sukhija et al., 2007; Williams et al., 2014; Zhang et al., 2007). Our study also showed statistically significant elevation in chosen inflammation biomarkers (WBC, CRP, IL-6) in the whole group of ACS patients in comparison to controls. Analyzing the inflammation biomarkers depending on the sensitivity of signs and symptoms as compared to control group studies revealed, that statistically significant differences were also found for all above parameters tested. The analysis of differences between the ACS subgroups showed that significant variations were found for CRP and IL-6 concentrations, but not for WBC.

Synthesis of CRP is regulated by IL-6, thus the strong correlation between these biomarkers was reported (Lai et al., 2011; Tan et al., 2008). It is indicated that CRP has also influence on atherosclerotic process via damage of endothelial cells, and formation, maturation, and final disruption of atheromatous plaque. Studies of Lai et al. designed to evaluate the relationship between the characteristics of coronary atherosclerotic plaques and inflammatory biomarkers, indicated that mean concentrations of high-sensitivity CRP (hs-CRP) and IL-6 were significantly higher in the three plaque groups in comparison to controls (Lai et al., 2011). Their findings support our results, which may indicate that inflammation is engaged in the occurrence and development of plaque in ACS. Moreover, findings of Lai et al. suggested that both hs-CRP as well as IL-6 concentrations may be recognized as one of the indexes to evaluate the degree of coronary heart disease (Lai et al., 2011). Interestingly our findings indicated that the highest percentage of negative results (specificity) revealed

Table 6 Association between inflammation and platelet parameters and angiographic extent of the coronary artery disease in the total group of ACS patients.

	No changes in coronary arteries N = 10	1-vessel disease N = 40	2-vessels disease N = 24	3-vessels disease N = 19	P-value
WBC [$\times 10^3/\mu\text{L}$]	7.75 (6.50–8.60)	8.75 (7.00–10.20)	8.75 (6.60–11.00)	7.70 (6.50–10.10)	NS
CRP [mg/L]	3.0 (1.6–3.9)	2.3 (0.8–2.8)	4.4 (1.3–5.7)	3.8 (0.9–4.7)	0.0419
IL-6 [pg/mL]	11.1 (10.1–12.0)	13.7 (5.0–18.9)	18.0 (9.1–32.8)	10.4 (3.8–14.7)	NS
PLT [$\times 10^3/\mu\text{L}$]	231 (198–286)	216 (173–248)	222 (190–254)	208 (176–216)	NS
MPV [fL]	11.1 (10.9–11.5)	10.9 (10.3–11.3)	10.8 (10.0–11.3)	10.5 (9.3–11.2)	NS
LPLT [%]	33.2 (30.1–37.0)	31.3 (27.6–33.8)	30.4 (24.9–34.5)	29.4 (24.5–35.1)	NS
β -TG [IU/mL]	58 (36–80)	89 (53–120)	75 (61–105)	55 (41–67)	NS

ACS – acute coronary syndrome, WBC – white blood cells count, CRP – C-reactive protein, IL-6 – interleukin-6, PLT – platelet count, MPV – mean platelet volume, L-PLT – large platelet, β -TG – β -thromboglobulin.

Conversion factors to SI units are as follows: for WBC – 1.0, for PLT – 1.0.

Table 7 Diagnostic usefulness of inflammation and platelet biomarkers in the total study group of ACS patients.

	Cut-off	AUC	SE	Sensitivity [%]	Specificity [%]	PPV [%]	NPV [%]	ACC [%]
WBC[x10 ³ /μL]	4.7	0.787	0.043	98	13	78	67	77
CRP [mg/L]	0.4	0.798	0.040	96	20	79	60	77
IL-6 [pg/mL]	9.1	0.707	0.052	67	90	94	55	76
MPV [fL]	9.6	0.904	0.026	93	57	87	71	84
β-TG [IU/mL]	36.3	0.961	0.016	89	93	90	97	90

ACS – acute coronary syndrome, WBC – white blood cells count, CRP – C-reactive protein, IL-6 – interleukin-6, PLT – platelet count, MPV – mean platelet volume, L-PLT – large platelet, β-TG – β-thromboglobulin, AUC – area under the ROC curve, SE – Standard Error, PPV – positive predictive value, NPV – negative predictive value, ACC – diagnostic accuracy.

Conversion factors to SI units are as follows: for WBC – 1.0, for PLT – 1.0.

IL-6. The AUCs for all inflammation parameters tested were similar, however the highest AUCs showed WBC and CRP.

Literature data regarding the evaluation of CRP and IL-6 concentrations depending on the sensitivity of signs and symptoms as compared to control individuals are limited. Tan et al. indicated higher IL-6 concentrations in patients with STEMI compared with controls (Tan et al., 2008), which is in agreement with our findings. Zhang et al. reported increased concentrations of CRP and IL-6 in NSTEMI patients in comparison to stable angina pectoris subjects and healthy individuals (Zhang et al., 2007), which is also in line with our research. In the current study significant differences for IL-6 were not found only between UA vs. controls. These results may indicate that IL-6 can be recognized as an inflammatory biomarker to identify individuals at high risk of myocardial infarction.

The analysis of differences between the ACS subgroups showed that statistically significant variations were found between STEMI vs. UA subgroup for CRP and between STEMI vs. UA subgroup as well as between NSTEMI vs. UA subgroup for IL-6 concentrations. Moreover, our study showed that CRP revealed a trend toward to be lower in NSTEMI patients as compared to STEMI subjects, while IL-6 showed inverse tendency; however these differences were not significant (Table 4). These observations are in opposite to results obtained by Di Stefano et al. and Habib et al., which indicated differences between STEMI vs. NSTEMI concerning CRP and IL-6 (Habib et al., 2011). Additionally Di Stefano and colleagues revealed higher median value of WBC in STEMI subjects as compared to NSTEMI (Di Stefano et al., 2009). These findings altogether suggest a differential inflammatory pattern regarding CRP and IL-6 in ACS patients depending on the severity of signs and symptoms of CAD. However further studies are needed to elucidate this aspect.

In the study group of patients with ACS except for CRP concentrations we did not reveal significant differences between the advancement of coronary artery changes and inflammation as well as platelet parameters. Statistically lower CRP concentrations were found in the group of ACS patients with 1-vessel coronary artery disease as compared to subjects with 2-vessel coronary artery disease. Studies of other authors revealed a correlation between lower fibrous cap thickness with elevated inflammation biomarkers and accumulation of inflammatory cells within atherosclerotic plaque (Raffel et al., 2007). It is hypothesized that increased inflammation conduct to increased platelet activation in ACS subjects and thus to the formation of atherosclerotic plaque (Ueba et al., 2014; Williams et al., 2014).

It is well established that platelets play significant role in the development of atherosclerotic plaque and thrombus formation after coronary plaque destabilization (Pal et al., 2014; Zhang et al., 2007). The rupture of an unstable plaque contributes to the local thrombin generation and fibrin deposition, which lead to the platelet activation, adhesion and aggregation, and thus the formation of intra-coronary thrombus (Parguñña et al., 2010). It is reported that individuals with CAD have circulating activated platelets, platelet-derived microparticles, monocyte-platelet aggregates, and increased reactivity of the platelet (Brambilla et al., 2008; Zhang et al., 2007). Activated platelets and platelet-leukocyte aggregates were found to be statistically elevated in subjects with unstable angina as compared to patients with stable angina (Brambilla et al., 2008).

Our studies showed that among chosen platelet morphological parameters only statistically increased median MPV in the total group of ACS patients as compared to controls was found. Current study did not reveal significant differences concerning PLT, which is in line with results of other researchers (Bychowski, 2002; Mathur et al., 2001; Turk et al., 2013). Interestingly, increased MPV was indicated as a predictor of death in ACS patients, but the cut-off point of MPV related to poor prognosis has not been settled (Maden et al., 2009; Slavka et al., 2011). However studies of Klovaite et al. indicated that subjects with MPV < 7.4 fL vs. > 7.4 fL had increased risk of myocardial infarction (Klovaite et al., 2011). Platelet size reflects platelet function (Sharpe and Trinick, 1993). Larger platelets are more reactive, contributing to the formation of free arachidonic acid, which can be converted into prostaglandins (e.g. thromboxane A₂ – the most potent platelet-aggregating factor) and leukotrienes (which are able to enhance the inflammatory response) (Pal et al., 2014; Sharpe and Trinick, 1993). Larger and more active platelets may accelerate the formation and extension of intracoronary thrombus, which lead to the acute thrombotic events in ACS subjects (Pal et al., 2014). In the whole group of ACS patients current study showed very strong positive correlation between MPV and LPLT (which are platelets with MPV > 12 fL) (data not present). Similar correlations were also found in the study group depending on the severity of signs and symptoms (data not present). Martin et al. indicated that in myocardial infarction cases large platelet size was recognized as an independent risk factor and may be a predictive parameter of recurrent myocardial infarction (Martin et al., 1991). Kiliçli-Camur et al. found increased MPV in patients with myocardial infarction in comparison to subjects with stable angina and healthy individuals (Kiliçli-Camur et al., 2005).

Also findings of Pal et al. indicated higher MPV in ACS patients than in healthy individuals (Pal et al., 2014). Likewise our results are able to support the hypothesis that increased MPV may be a potentially useful predictive biomarker in CAD. Moreover, analyzing MPV depending on the sensitivity of signs and symptoms as compared to control group, studies revealed that statistically significant differences were also found. Yilmaz et al. indicated that elevated MPV in NSTEMI determined both an increased risk of myocardial infarction as well as ischemic complications (Yilmaz et al., 2008). These data may indicate that platelet activation can play a significant role in the pathogenesis and clinical outcome of ACS, and thus might reflect the plaque instability and accompanying inflammation and vascular thrombosis (Zhang et al., 2007).

Activated platelets realize from α -granules markers of their activation, such as β -TG and soluble form of P-selectin (sP-selectin) (Kaplan and Owen, 1981; Michelson et al., 2001). In the total group of ACS patients median β -TG activity was significantly higher as compared to controls. Analyzing the β -TG activity depending on the sensitivity of signs and symptoms as compared to control group studies revealed that likewise differences were found. β -TG also revealed the best diagnostic usefulness among all parameters tested in the study. It should be emphasized that investigations evaluating the β -TG in STEMI, NSTEMI, and UA subjects are very limited and imprecise. The evaluation of β -TG, however regarding the concentration of protein tested, so far has been conducted only by Smitherman et al. (1981). According to authors the β -TG levels obtained in subjects with acute myocardial infarction were increased in general within the first 3–4 days of hospitalization (Smitherman et al., 1981). Current study indicated that the evaluation of β -TG activity may be a suitable parameter to grade the platelet activity in ACS patients. It should be emphasized, that in the available literature there are no data regarding the evaluation of β -TG activity in ACS, therefore the discussion concerning this aspect is unsatisfactory. However studies of Zhang et al. revealed that sP-selectin, which is also recognized as biomarker of platelet activation in vivo, was increased in NSTEMI and UA subjects as compared to controls (Zhang et al., 2007).

5. Study limitations

Current study was designed to evaluate the diagnostic value of chosen inflammation and platelet activation parameters in ACS patients depending on the severity of signs and symptoms. However, the study had some limitations. Firstly, small number of subjects included in the study could cause a selection bias. Hence, such studies should be performed on a larger study group. Secondly, it would be also valuable to perform the analysis of above-mentioned parameters in patients with stable angina and compare these findings with results obtained in ACS (similar study is in progress).

6. Conclusion

Our study revealed that WBC, CRP, and IL-6 as an inflammation parameters and MPV and β -TG as platelet biomarkers may be useful indicators of the presence of CAD. The AUCs for all above-mentioned parameters were significantly higher than AUC = 0.500, which may indicate their potential clinical

significance in ACS. Additionally current findings indicated a different inflammatory pattern regarding CRP and IL-6 concentrations in ACS patients depending on the severity of signs and symptoms; however further studies are needed to elucidate this aspect.

Author Contributions

JK: concept and design of the study, statistical analysis and interpretation of data, drafting the article, final approval of the submitted version of article.

OMK: the concept and design of the study, statistical analysis and interpretation of data, drafting the article, final approval of the submitted version of article.

ESK: acquisition of data, revising article critically, final approval of the submitted version of article.

JMK: analysis and interpretation of data, revising article critically, final approval of the submitted version of article.

JB: analysis and interpretation of data, revising article critically, final approval of the submitted version of article.

HK: the concept and design of the study, revising article critically, final approval of the submitted version of article.

Disclosures

The authors declare no conflict of interests.

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