ACTA MEDICA LITUANICA. 2014. Vol. 21. No. 4. P. 182–194

Platelet function and indices in Lithuanian men with dyslipidemia: associations with inflammatory biomarkers

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Introduction. Platelet function, platelet volume indices, traditional markers of lipid metabolism, inflammatory markers and a novel biomarker, cyclophilin A (CyPA), are related as far as they have implications in pathogenesis of atherosclerosis. The purpose of this study was to evaluate interrelations among these factors, correlation between platelet function and inflammatory factors, also a function of CyPA.

Materials and methods. 160 male patients with high risk of atherosclerosis and metabolic syndrome were included in the study. Inclusion criteria were as follows: disturbances in lipids profile, increased weight, smoking, acute or chronic stress, no previous or current acute cardiovascular disease (CVD), high risk and presence of stress confirmed by physician. CRP, vWF, fibrinogen, CyPA levels were measured. Platelet function was assessed by aggregation and flow cytometry.

Results. Increasing number of risk factors gave statistically significant elevation in fibrinogen, thrombin receptors activating peptide, CRP, glucose, mean platelet volume (MPV), platelet large cell ratio, lipids and their ratios with extremely significant linearity. MPV correlated positively with CD42a ($r = 0.605$; $p < 0.001$) and negatively with CD42a/CD14 ($r = –0.327$; $p < 0.001$). Lipid ratios were found to be prognostic for metabolic syndrome biochemical markers. Platelet function parameters showed relation with lipid ratios, lipid-CRP ratios, MPV and vWF. CyPA correlated with CD42a/CD14 ($r = 0.202$, $p = 0.010$).

Conclusions. Platelets in men with dyslipidemia and other investigated risk factors have a tendency to be hyperreactive. It might be considered as a separate risk factor for CVD. Inflammatory status does not correlate with the platelet function. CyPA reflects inflammatory processes but not the platelet function.

Key words: atherosclerosis, dyslipidemia, platelet indices, cyclophilin A, flow cytometry

Abbreviations: AI – atherogenic index; CD14 – cluster of differentiation 14 (monocyte differentiation antigen); CD42a – cluster of differentiation 42a (glycoprotein IX); CD63 – cluster of differentiation 63 (type III lysosomal glycoprotein, expressed on activated platelets, monocytes and macrophages); CyPA – cyclophilin A; HDL-C – high density lipoprotein cholesterol; hs-CRP – C-reactive protein high sensitivity assay; CVD – cardiovascular diseases; LDL-C – low density lipoprotein cholesterol; MPV – mean platelet volume; MS – metabolic syndrome; PAC-1 – antibodies against an epitope on the glycoprotein IIb/IIIa (gpIIb/IIIa, αIIbβ3) complex of activated platelets; Pct – plateletcrit; PDW – platelet distribution width; PLT – platelet count; P-LCR – platelet large cell ratio; TC – total cholesterol; G – triglycerides; TRAP – thrombin receptors activating peptide; vWF – von Willebrand factor

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INTRODUCTION

Atherosclerosis is a chronic inflammatory disease that plagues humankind for millennia. Carl von Rokitansky and Rudolf Virchow controversially explained the concept of inflammatory causes in the middle of the 19th century (1). Since then importance of inflammation in the development of cardiovascular diseases (CVD) has been widely studied. At present, it is clear that inflammation plays a key role in pathogenesis of atherosclerosis and it is not anymore just a cholesterol storage disease characterized by the collection of cholesterol and thrombotic debris in the artery wall (2). It involves both innate and adaptive immune mechanisms. Direct factors affecting the process include lipid deposition and oxidation, accumulation of cell-derived microparticles and various non-lipid lipoprotein associated factors. Indirectly inflammation at non-vascular sites can also contribute to progression of atherosclerosis (i. e. autoimmune diseases, smoking, respiratory infections and pollution exposure) (3).

The well-known atherosclerotic cardiovascular disease risk factors (decreased high-density lipoprotein cholesterol (HDL-C) and increased low-density lipoprotein cholesterol (LDL-C) concentrations in blood) are not enough for complete risk evaluation (4) and creation of treatment strategies in clinical practice (5). For these purposes, other multiple markers have been established as risk factors. For example, C-reactive protein (CRP) is greatly used as an independent predictor of cardiovascular diseases due to discovery of high-sensitivity techniques for its determination, stable concentrations in blood and relatively low testing costs (6–8). Similarly, the von Willebrand factor (vWF) (9–11) and fibrinogen (12), given their key roles in arterial thrombus formation, as biomarkers attract considerable interest in predicting CVD. Platelets, previously known only for their clear role in hemostasis, now increasingly gain interest as inflammatory cells contributing to pathophysiology of CVD (13–15). One of the contribution theories states platelet hyperreactivity, which manifests as increased aggregability, alterations in platelet volume indices (increased mean platelet volume (MPV), increased platelet distribution width (PDW) and increased platelet large cell ratio (P-LCR) independently of combination of changes) (16–20), increased expression of P-selectin (21) and other molecules, also active counteraction between monocytes and other leucocyte populations (22, 23).

One of the novel biomarkers of CVD is cyclophilin A (CyPA). Normally an intracellular protein, CyPA, is secreted from monocytes / macrophages, endothelial cells, vascular smooth muscle cells and platelets in response to reactive oxygen species. Linkage between CyPA and cardiovascular events has been described in several studies, where increased concentrations of the protein were regarded as a risk factor to acute coronary syndromes (24–27).

Platelet function, platelet volume indices, traditional markers of lipid metabolism (total cholesterol (TC), LDL-C, HDL-C, triglycerides (TG)), inflammatory markers (CRP, vWF and fibrinogen) and CyPA, a novel biomarker, are all related as having implications in the pathogenesis of atherosclerosis. The purpose of the study was to evaluate relations among these factors, a correlation between the platelet function and inflammatory factors, also the function of CyPA.

MATERIALS AND METHODS

160 male patients (age mean 47.79 ± 4.01 years) with high risk of atherosclerosis and metabolic syndrome were included in the study. Inclusion criteria were represented by the presence of cardiovascular risk factors: disturbances in lipids profile (high TC and / or high LDL-C and / or low HDL-C), increased weight, smoking, acute or chronic stress, no previous or current acute cardiovascular illness, and high risk of diseases and presence of stress confirmed by a physician. The study was approved by the Vilnius Regional Biomedical Research Ethics Committee and followed the Declaration of Helsinki. All participants provided a written informed consent before inclusion into the study.

Laboratory analysis was performed in the Center of Laboratory Medicine of Vilnius University Hospital Santariškių Clinics. The list of performed routine and specific tests, methods (and / or used analyzers) and baseline characteristics of the studied population are summarized in Table 1. All laboratory tests were performed the same day when blood was drawn, except vWF and

Table 1. Baseline characteristics of the study population

Variable, units	Mean ± SD	Method details
CyPA, μg/mL	0.560 ± 0.779	ELISA (AMS Biotechnology (Europe) Ltd., UK) on GEMINI analyzer (Stratec Biomedical, Germany)
CyPA, s/co	1.041 ± 0.123	
Fibrinogen, g/L	3.78 ± 0.74	Claus method (STA Compact, Stago, France)
TRAP, U	123.41 ± 19.99	Whole blood aggregation with TRAP agonist on Multiplate analyzer (Roche Diagnostics, Germany)
vWF, %	123.57 ± 41.24	Immunoturbidimetric (STA Compact, Stago, France)
TC, mmol/L	6.45 ± 1.31	Enzymatic colorimetric (Architect ci8200, Abbott, USA)
TG, mmol/L	2.52 ± 1.87	Enzymatic colorimetric (Architect ci8200, Abbott, USA)
HDL-C, mmol/L	1.09 ± 0.25	Enzymatic colorimetric (Architect ci8200, Abbott, USA)
LDL-C, mmol/L	4.20 ± 1.13	Friedewald formula but if TG > 4.5 mmol/L – direct enzymatic colorimetric (Architect ci8200, Abbott, USA)
hs-CRP, mg/L	3.35 ± 3.90	High sensitivity immunoturbidimetric (Architect ci8200, Abbott, USA)
Glucose, mmol/L	5.67 ± 0.48	Hexokinase (Architect ci8200, Abbott, USA)
PLT, $\times 10^9$/L	218.84 ± 44.07	Part of full blood count by hematology analyzer SYSMEX XE-5000 (Sysmex Corporation, Japan)
MPV, fL	10.5 ± 0.82	
Pct, %	0.23 ± 0.04	
PDW, %	12.3 ± 1.8	
P-LCR, %	28.7 ± 6.7	
PAC-1/CD42a, %	0.156 ± 0.114	Flow cytometric analysis by BD FACSCanto (BD Biosciences, USA)
CD63/CD42a, %	0.211 ± 0.166	
CD42a/CD14, %	8.517 ± 2.611	

CyPA. Citrated plasma was kept frozen at –20 °C and vWF was analyzed in several batches. Serum was kept frozen at –70 °C and CyPA was performed in several batches. Due to zero CyPA μg/mL values (76 of 160) additional units for this parameter were calculated (signal to cut-off ratio, s/co).

Biochemical metabolic syndrome basis was confirmed as present (n = 23, 14.4 %) if all three biochemical criteria (TG ≥ 1.7 mmol/l, HDL-C <1.03 mmol/l and glucose >5.6 mmol/l) were met. Different lipid ratios (TG/HDL-C, atherogenic index – lg[TG/HDL-C], TC/HDL-C and LDL-C/HDL-C) and lipid-CRP ratios (LDL-C/CRP, CRP/LDL-C, TC/CRP, CRP/TC, HDL-C/CRP, CRP/HDL-C and lg[CRP/HDL-C]) were calculated. Lipid-CRP ratios were evaluated as absolute numbers without estimated units of measure.

In order to evaluate the prognostic value of lipid ratios or lipid-CRP ratios, we have used cut-offs of each ratio. Cut-offs were calculated in our another unpublished study where patients with acute myocardial infarction were involved. Cut-offs for lipid ratios and lipid-CRP ratios where CRP was used as a denominator were calculated as means of values obtained from individuals with risk factors and individuals with diagnosed CVD. Lipid-CRP ratios where CRP was used as a numerator cut-offs were calculated as means and one standard deviation of values obtained from individuals with risk factors.

Statistical analysis was performed using the SPSS software (PASW Statistics 18 version, SPSS Inc., USA). Continuous variables were expressed as means and standard deviations (SD). Data comparison was made by the Student t-test, ANOVA or Mann-Whitney U test where appropriate. Categorical variables were presented as frequencies and percentages and were compared by the chi-square test. The Spearman coefficient (r) was calculated to quantify the correlation between variables. Correlation was considered weak when the r value was below 0.3, moderate when the value was between 0.3 and 0.7, and strong when the value was above 0.7. Diagnostic characteristics of the markers were evaluated by ROC curves. The markers were considered as useful if the area under the ROC curve (AUC) was >0.5. Linear regression models were considered applicable if the coefficient of determination r^2 was ≥0.25. All

reported p values were two tailed and a p value of <0.05 was considered as statistically significant.

Results

Baseline characteristics of the study population (n = 160) are presented as means ±SD in Table 1. Parameters were evaluated according to the approved reference values at the Center of Laboratory Medicine. Frequencies of abnormal results, reference values and estimated abnormality are summarized in Table 2. CRP values were divided into four groups: only the results <1.0 mg/L were considered normal with low risk of CVD events. Each variable deviation from the reference range was considered as a separate risk factor, and analyzed according to the total number of risk factors (ranging from 2 to 7) registered among subjects. The ANOVA analysis revealed that an increasing number of deviations gives statistically significant elevations in fibrinogen (from 3.52 to 4.32 g/L; p < 0.001), TRAP (from 110.7 to 147.9 U; p < 0.001), hs-CRP (from 1.17 to 3.68 mg/L; p = 0.005), glucose (from 5.54 to 6.01 mmol/L; p = 0.026), MPV (from 9.95 to 10.83 fL; p = 0.033) and P-LCR (from 24.19 to 31.33 %; p = 0.036) with extremely significant linearity (p ≤ 0.004) (Fig. 1). The same tendency is seen with lipids and their ratios: TG (from 1.58 to 2.50 mmo/L; p = 0.044), TC (from 5.10 to 6.27 mmol/L; p < 0.001), LDL-C (from 3.29 to 4.16 mmol/L; p = 0.007), atherogenic index (from 0.14 to 0.38; p = 0.001), TC/HDL-C (from 4.87 to 6.60; p < 0.001), LDL-C/HDL-C (from 3.11 to 4.41; p < 0.001), CRP/LDL-C (from

Table 2. Frequencies of abnormal results according to laboratory reference ranges

Variable, units	Reference values	Estimated abnormality	Frequency of abnormality, %
Fibrinogen, g/L	2.0–4.0	>4.0	57 (35.6%)
TRAP, U	92–150	>150	14 (8.8%)
vWF, %	50–160	>160	55 (34.4%)
TC, mmol/L	<5.2	≥5.2	132 (82.5%)
TG, mmol/L	≤1.8	>1.8	100 (62.5%)
HDL-C, mmol/L	>0.91 (men only)	≤0.91	40 (25.0%)
LDL-C, mmol/L	2.6–3.5	>3.5	117 (73.1%)
hs-CRP, mg/L	≤5.0	1.0–3.0 (average risk)	68 (42.5%)
		3.0–5.0 (high risk)	38 (23.8%)
		>5.0 (very high risk)	28 (17.5%)
Glucose, mmol/L	4.2–6.1	>6.1	29 (18.1%)
MPV, fL	7.4–10.4	>10.4	78 (48.8%)

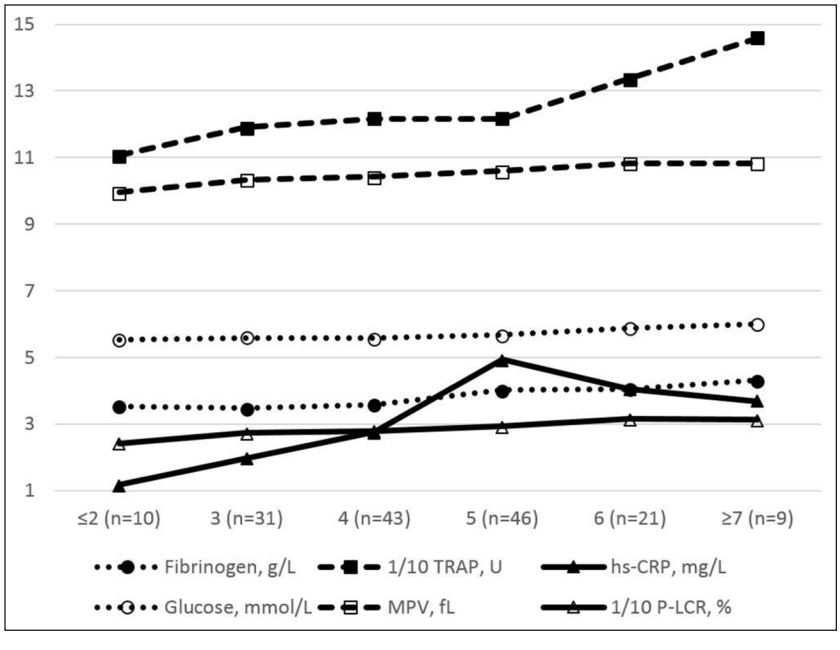

Fig. 1. Statistically significant, dependent on the total number of laboratory risk factors increase of fibrinogen, glucose, CRP, TRAP, MPV and P-LCR values

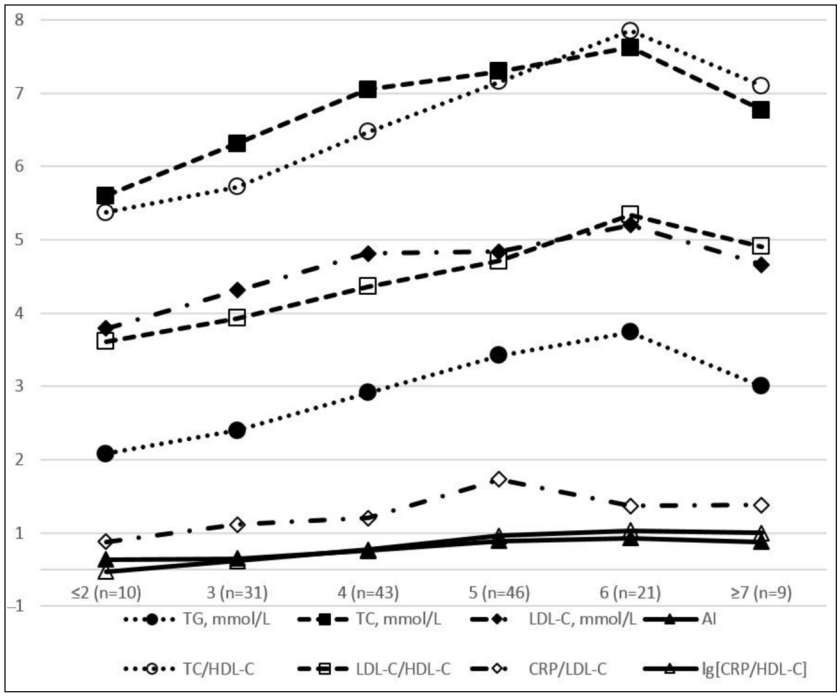

Fig. 2. Statistically significant, dependent on the total number of laboratory risk factors increase of lipids and their ratios values

0.38 to 0.88; p = 0.030) and lg[CRP/HDL-C] (from –0.03 to 0.50; p < 0.001) (Fig. 2). It should be noted that none of the platelet flow cytometry results depended on the number of risk factors (changes were not significant; p ≥ 0.05).

In the analysis of the whole investigated population (n = 160) correlations of lipids (TC, TG, HDL-C and LDL-C) were as expected: TC correlation with other lipids was moderate to strong and significance level was high (p ≤ 0.009); HDL-C significant negative correlation was noted with TG (r = –0.333; p < 0.001).

Statistically significant interrelatedness of inflammatory factors was noted: vWF correlated positively with fibrinogen (r = 0.229; p = 0.004) and CRP (r = 0.224; p = 0.004), whereas the CRP correlation with fibrinogen (r = 0.471; p < 0.001) was moderate and positive (Fig. 3).

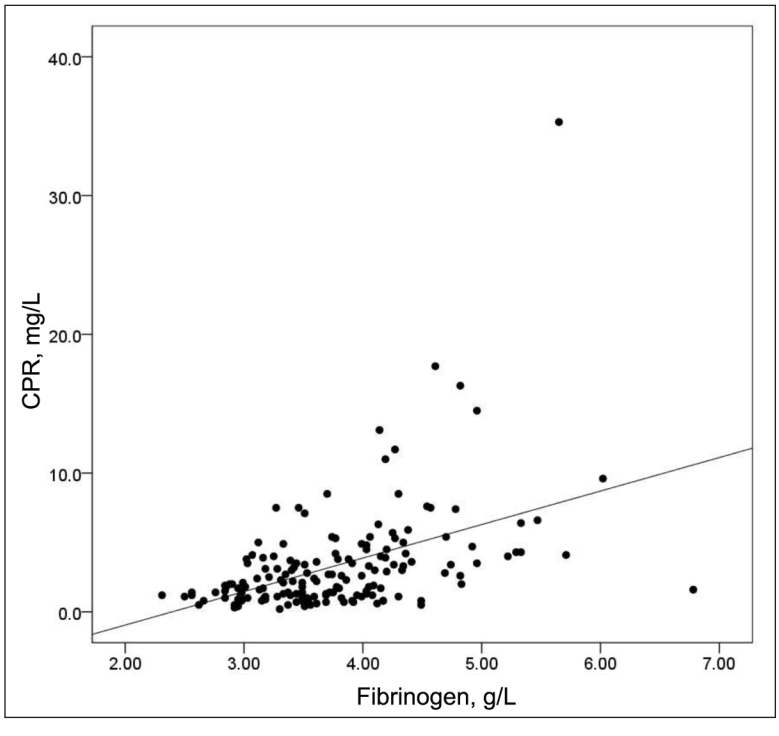

Fig. 3. Scatterplot of CRP and fibrinogen values (r^2 = 0.210, p < 0.001)

The following statistically significant correlations were noted for PLT: platelet numbers correlated positively with TRAP (r = 0.158; p = 0.045), negatively with MPV (r = –0.379; p < 0.001), PDW (r = –0.416; p < 0.001) and P-LCR (r = –0.387; p < 0.001). PLT also correlated with flow cytometry parameters: positively with CD42a/CD14 percentage (r = 0.479; p < 0.001), CD14 percentage (r = 0.185; p = 0.019) and negatively with CD42aPE events number (r = –0.287; p < 0.001) and CD63/CD42a percentage (r = –0.177; p = 0.025). PLT correlations with CD42a/CD14 and CD42a are depicted in Fig. 4.

The mean platelet volume correlated positively with TRAP (r = 0.173; p = 0.029), PDW (r = 0.964; p < 0.001) and P-LCR (r = 0.994; p < 0.001), also with CD42aPE events number (r = 0.605; p < 0.001), PAC-1 events number (r = 0.200; p = 0.011) and CD63/CD42a percentage (r = 0.195; p = 0.013).

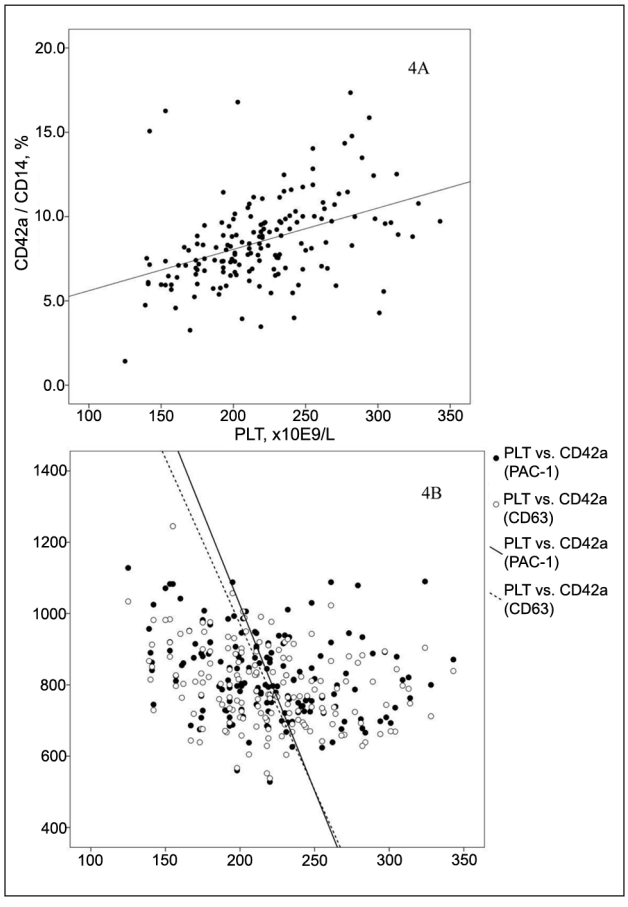

Fig. 4. Distribution of CD42a/CD14 (4A) and CD42a (4B) values according to PLT counts: PLT and CD42a/CD14 $r^2 = 0.171$; PLT and CD42a in combination with PAC-1 $r^2 = 0.064$, and in combination with CD63 $r^2 = 0.077$. All measures had $p \leq 0.001$

The negative correlation of MPV with CD42a/CD14 percentage (r = –0.281; p < 0.001), CD42a/CD14 events number (r = –0.327; p < 0.001) and CD14 number (r = –0.198; p < 0.012) was registered. Similar statistically significant correlations were seen for PDW and P-LCR, except their negative correlations with CD14 number (PDW r = –0.240, p = 0.002 and P-LCR r = –0.209, p = 0.008, respectively). MPV correlations with CD42a/CD14 and CD42a are depicted in Fig. 5.

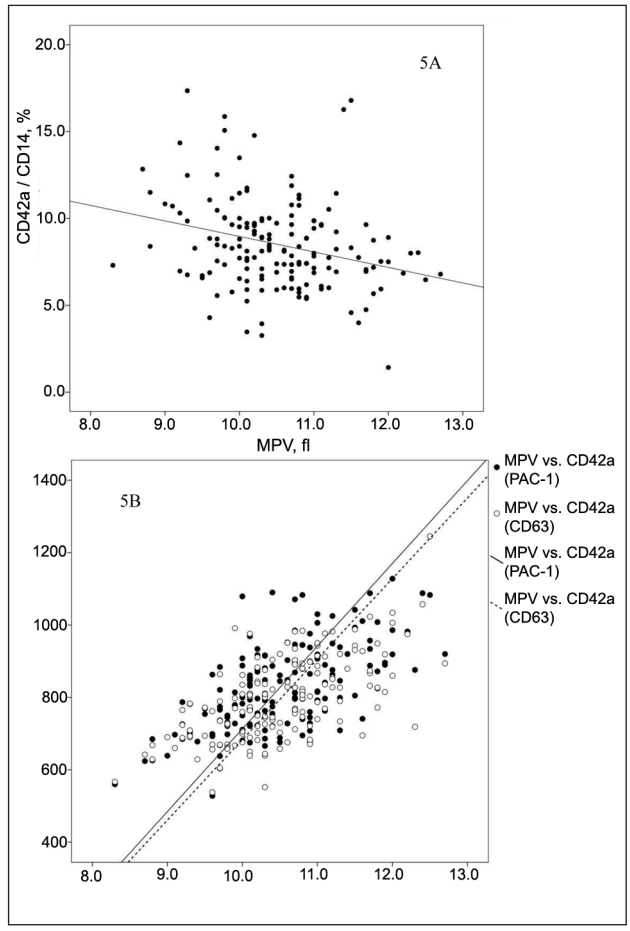

Fig. 5. Distribution of CD42a/CD14 (5A) and CD42a (5B) values according to MPV: MPV and CD42a/CD14 $r^2 = 0.078$; MPV and CD42a in combination with PAC-1 $r^2 = 0.388$, and in combination with CD63 $r^2 = 0.394$. All measures had $p < 0.001$

The following associations of inflammatory and platelet markers were noted: CRP weak positive correlation with TRAP (r = 0.166; p = 0.036) and weak negative correlation with CD42a/CD14 percentage (r = –0.191; p = 0.015) and CD42a/CD14 events number (r = –0.174; p = 0.028) existed; vWF correlated negatively with TRAP (r = –0.203;

p = 0.01), Pct (r = –0.156; p = 0.036) and CD42a/CD14 percentage (r = –0.208; p = 0.008); fibrinogen correlated with PLT (r = 0.192; p = 0.015).

Other correlations of biochemical parameters were the following: glucose correlated positively with HDL-C (r = 0.164; p = 0.038) but negatively with PLT (r = –0.181; p = 0.022), CD42a/CD14 percentage (r = –0.199; p = 0.012) and CD42a/CD14 events number (r = –0.193; p = 0.014); PLT correlated positively with LDL-C (r = 0.223; p = 0.005); TC and LDL-C correlation with CD42a/CD14 percentage was noted (r = 0.157, p = 0.047 and r = 0.165, p = 0.038, respectively).

CyPA correlations were as follows: CyPA μg/mL with CD42a/CD14 events number (r = 0.158, p = 0.046), CyPA s/co with CD42a/CD14 percentage (r = 0.175, p = 0.027) and CD42a/CD14 events number (r = 0.202, p = 0.010). After removing zero CyPA μg/mL values the correlation analysis of remaining 84 subjects resulted in more or less the same tendencies in associations of the analyzed parameters.

Dividing individuals according to the presence of biochemical criteria of metabolic syndrome resulted in four subgroups: individuals without biochemical criteria (MS[–] n = 19, 11.9%), individuals having one criterion (MS[+1] n = 42, 26.3%), individuals having two criteria (MS[+2] n = 76, 47.5%) and individuals having all three biochemical MS criteria (MS[+3] n = 23, 14.4%). Only absolute numbers of PAC-1 and CD42a/CD14% differences were seen among these subgroups. The PAC-1 highest numbers (50 ± 22) were seen in the MS[+2] subgroup and the lowest numbers (37 ± 13) were seen in the MS[–] subgroup (p = 0.019). The sequential decrease of CD42a/CD14 percentage was seen among the MS subgroups (from 10.06 ± 3.45 to 8.11 ± 1.55, p = 0.047, linearity p = 0.018). Using MS[+3] as a target of prognosis AI, TG/HDL-C ratio and TC/HDL-C ratio were found to be better markers (see Fig. 6). Other analytes and lipid-CRP ratios did not meet the AUC criterion of >0.5 and cannot be used as markers of MS[+3] prediction.

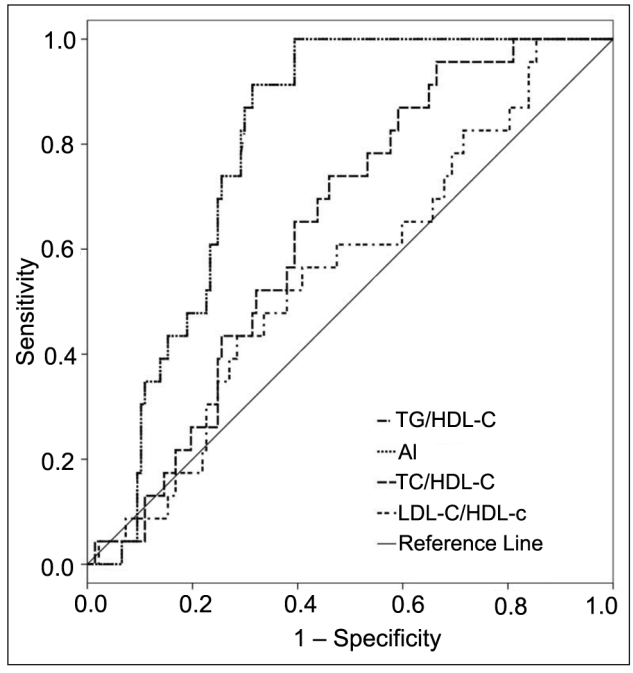

Fig. 6. ROC curves for MS[+3] prediction. TG/HDL-C ratio AUC = 0.797 (95% CI 0.728–0.865, p < 0.001), AI index AUC = 0.797 (95% CI 0.728–0.865, p < 0.001), TC/HDL-C ratio AUC = 0.640 (95% CI 0.540–0.741, p = 0.031)

According to significant correlations, linear regression models were adapted for each laboratory risk factor. Successful models of different independent markers are shown in Tables 3 and 4.

Table 3. Linear regression analysis of LDL-C, TG and HDL-C

Dependent variable	Independent variables	B	t	p	VIF	r^2
LDL-C, mmol/L	(constant)	1.139	3.636	<0.001		0.453
	TC/HDL-C	0.666	10.955	<0.001	2.302	
	AI	–3.507	–10.299	<0.001	2.302	
TG, mmol/L	(constant)	–1.416	–3.106	0.002		0.426
	TC/HDL-C	0.732	10.606	<0.001	1.021	
	LDL-C/CRP	–0.119	–2.795	0.006	1.237	
	CRP, mg/L	–0.076	–2.343	0.020	1.249	
HDL-C, mmol/L	(constant)	1.270	53.394	<0.001		0.416
	lg[CRP/HDL-C]	–0.091	–2.308	0.022	1.042	
	AI	–0.512	–9.653	<0.001	1.042	

Table 4. Linear regression analysis of fibrinogen, CRP, glucose and MPV

Dependent variable	Independent variables	B	t	p	VIF	r^2
Fibrinogen, g/L	(constant)	2.199	6.630	<0.001		0.267
	vWF, %	0.003	2.712	0.007	1.026	
	CRP/HDL-C	0.064	5.676	<0.001	1.018	
	Pct, %	4.192	3.493	0.001	1.022	
	(constant)	2.149	6.960	<0.001		0.281
	vWF, %	0.003	2.358	0.020	1.034	
	CRP/TC	0.456	5.880	<0.001	1.024	
	PLT, $\times 10^9$/L	0.003	4.112	<0.001	1.010	
CRP, mg/L	(constant)	0.234	2.625	0.010		0.951
	CRP/TC	5.809	55.155	<0.001	1.000	
	(constant)	0.623	5.955	<0.001		0.923
	CRP/HDL-C	0.821	43.495	<0.001	1.000	
	(constant)	0.856	3.483	0.001		0.620
	lg[CRP/HDL-C]	7.677	16.070	<0.001	1.000	
Glucose, mmol/L	CRP, mg/L	0.729	11.020	<0.001	1.000	0.433
	CRP, mg/L	0.064	1.953	0.053	1.739	0.921
	TC/HDL-C	0.820	31.182	<0.001	1.739	
	CRP, mg/L	0.144	4.290	<0.001	1.586	0.908
	CD42a/CD14, %	0.544	28.581	<0.001	1.586	
	CRP, mg/L	0.087	2.382	0.018	1.720	0.899
	CD42a (in combination with CD14)	0.002	27.027	<0.001	1.720	
MPV, fL	(constant)	7.815	17.158	<0.001		0.478
	CD42a (in combination with PAC-1)	0.002	2.074	0.040	4.039	
	CD42a (in combination with CD63)	0.003	3.350	0.001	3.978	
	CD42a/CD14, %	–0.064	–3.431	0.001	1.048	
	CD14, %	–0.057	–2.161	0.032	1.020	

Fibrinogen concentration depended on the combination of three variables, i. e. vWF, PLT and Pct (one of those but not together), and CRP or one of lipid-CRP ratios (when CRP is positioned in a fraction as a numerator). Two examples of the latter are shown in Table 4. Despite statistically significant TRAP correlations with vWF, CRP, PLT, Pct, MPV, P-LCR, CD42a (in two different combinations) and lipid-CRP ratios, there were no successful linear regression models for TRAP. The univariate regression analysis revealed that the prognostic marker for vWF is fibrinogen but not CRP. The CRP linear regression model with fibrinogen shows reliable dependency of these analytes (F 41.880, constant –5.782, $p < 0.001$), although $r^2 = 0.210$ is somewhat lower than the established criterion (Fig. 1). Relations of glucose with CRP, CD42a/CD14%, CD42a (in combination with CD14) and TC/HDL-C ratio were reliable only when the constant factor was not used. There were no successful models of MPV with TRAP, PDW and P-LCR, but a significant link existed with flow cytometry parameters (Table 4).

In the univariate regression analysis we have found that flow cytometry parameters of platelets can be predicted by established lipid ratios and lipid-CRP ratios cut-offs. Among other analytes high values of vWF and MPV were predictors of platelet activity (absolute numbers of CD42a, PAC-1 and CD63) and formation of platelet monocyte complexes (absolute numbers of CD42a/CD14). Results of the platelet function univariate analysis are presented in Tables 5 and 6. The former table depicts prediction capabilities for platelet function parameters from the point of view of increased CVD risk (individuals having 5–7 laboratory risk factors), while the latter one from the point of view of increased MS risk (individuals with having 3 MS biochemical risk factors). Only absolute numbers of PAC-1/CD42a and CD42a (in combination with CD14) were

Table 5. Univariate regression analysis of flow cytometry parameters of the platelet function when presence of 5–7 laboratory CVD risk factors is set as a fixed factor. CI – confidence interval

Dependent variable	Independent variables	B	95% CI		t	p
			Min	Max		
PAC-1/CD42a, %	AI > 0.26	0.054	0.018	0.090	2.9	0.004
	LDL-C/CRP > 2.32	0.192	0.065	0.318	3.0	0.003
	TC/CRP > 3.53	–0.180	–0.305	–0.055	–2.8	0.005
PAC-1/CD42a*	AI > 0.26	15.6	5.1	26.1	2.9	0.004
	LDL-C/CRP > 2.32	55.5	18.6	92.4	3.0	0.003
	TC/CRP > 3.53	–52.4	–89.0	–15.9	–2.8	0.005
CD42a (in combination with PAC-1)	vWF > 130 %	–43.7	–76.7	–10.7	–2.6	0.010
	MPV > 10.4 fL	99.0	66.7	131.3	6.0	<0.001
	CRP/LDL-C > 1.83	60.0	7.4	112.6	2.3	0.026
PAC-1	LDL-C/CRP > 2.32	15.4	1.8	29.0	2.2	0.026
	HDL-C/CRP > 0.63	–17.2	–31.1	–3.3	–2.4	0.016
CD63/CD42a, %	MPV > 10.4 fL	0.058	0.005	0.111	2.1	0.034
	lg[CRP/HDL-C] > 0.73	0.080	0.004	0.155	2.1	0.039
CD63/CD42a	MPV > 10.4 fL	16.6	1.2	32.0	2.1	0.034
	lg[CRP/HDL-C] > 0.73	22.0	0.06	44.0	2.0	0.049
CD42a (in combination with CD63)	MPV > 10.4 fL	113.7	82.1	145.4	7.1	<0.001
CD63	vWF > 130%	18.1	3.7	32.4	2.5	0.014
	CRP/LDL-C > 1.83	31.7	8.9	54.7	2.7	0.007
CD42a/CD14, %	vWF > 130%	–0.99	–1.8	–0.2	–2.3	0.021
CD42a/CD14	MPV > 10.4 fL	–128.2	–203.2	–53.1	–3.4	0.001
CD42a (in combination with CD14)*	TC/HDL-C > 6.0	–439.1	–730.9	–147.2	–3.0	0.003

* Presence of 5–7 laboratory CVD risk factors, $p \leq 0.045$.

Table 6. Univariate regression analysis of flow cytometry parameters of the platelet function when presence of three MS biomarkers is set as a fixed factor. CI – confidence interval

Dependent variable	Independent variables	B	95% CI		t	p
			Min	Max		
PAC-1/CD42a, %	AI > 0.26	0.047	0.010	0.084	2.5	0.013
	LDL-C/CRP > 2.32	0.214	0.087	0.340	3.3	0.001
	TC/CRP > 3.53	–0.195	–0.321	–0.069	–3.1	0.003
PAC-1/CD42a	AI > 0.26	13.5	2.6	49.4	2.5	0.015
	LDL-C/CRP > 2.32	62.1	25.3	98.9	3.3	0.001
	TC/CRP > 3.53	–57.0	–93.8	–20.2	–3.1	0.003
CD42a (in combination with PAC-1)	vWF > 130%	–41.5	–75.1	–7.8	–2.4	0.016
	MPV > 10.4 fL	104.7	72.7	136.6	6.5	<0.001
PAC-1	vWF > 130%	6.2	0.02	12.5	2.0	0.049
	LDL-C/CRP > 2.32	16.0	2.7	29.4	2.4	0.019
	HDL-C/CRP > 0.63	–17.3	–30.7	–3.8	–2.5	0.012
CD42a (in combination with CD63)	vWF > 130%	–36.5	–67.9	–5.1	–2.3	0.023
	MPV > 10.4 fL	115.4	85.5	145.2	7.6	<0.001
	CRP/LDL-C > 1.83	62.4	12.6	112.3	2.5	0.014
CD63	vWF > 130%	19.0	4.2	33.7	2.5	0.012
CD42a/CD14, %	vWF > 130%	–1.1	–1.9	–0.3	–2.6	0.011
	MPV > 10.4 fL	–1.2	–2.0	–0.4	–2.9	0.004
CD42a/CD14	MPV > 10.4 fL	–135.6	–208.5	–62.7	–3.7	<0.001
CD42a (in combination with CD14)	TC > 5.2 mmol/l	395.4	5.5	785.2	2.0	0.047
	TC/HDL-C > 6.0	–416.4	–718.0	–114.9	–2.7	0.007
CD14	MPV > 10.4 fL	–626.6	–1185	–68.6	–2.2	0.028
CD14, %	MPV > 10.4 fL	–0.6	–1.2	–0.05	–2.1	0.033

statistically significant predictors of increased CVD risk and none of flow cytometry parameters could reach the level of significance for MS[+3] prediction.

According to the established cut-offs in Table 2 MS[+3] presence could be prognosticated by decreased HDL-C (cut-off < 0.91 mmol/L, odds ratio 3.41, 95% CI 1.37–8.53, Chi square 7.464, p = 0.009) and increased glucose concentrations (cut-off > 6.1 mmol/L, odds ratio 2.95, 95% CI 1.11–7.82, Chi square 5.023, p = 0.030), but not by deviations of fibrinogen, vWF, TRAP, TG, TC, LDL-C, CRP or MPV. The presence of 5–7 laboratory CVD risk factors could be prognosticated by the most analysed factors (Table 7). Considering that CRP values below 1.0 mg/L are associated with low CVD risk, CRP values corresponding to high CVD risk may influence increment of MPV values (>10.4 fL) with an odds ratio of 2.46 (95% CI 1.00–6.05, Chi square 4.017, p = 0.050) and vice versa – high MPV values are associated with increased CRP values. Higher concentrations of fibrinogen (>4 g/L) may be determined by increased TRAP aggregability (>150 U) (odds ratio 5.27, 95% CI 1.57–17.67, Chi square 8.576, p = 0.007). Likewise higher TG concentrations (>1.8 mmol/l) may be detected 5.12 times more often when the acute stress fact is concerned (95% CI 1.37–19.14, Chi square 6.731, p = 0.015).

DISCUSSION

The purpose of the study was to evaluate three aspects of the investigated markers' associations in men having increased risk of CVD (especially with alterations in lipids profile): first, behaviour of platelets (possibility of hyperreactivity), second, activity of inflammatory markers, and third, association of inflammation with platelet function.

Platelets role in the pathogenesis of atherosclerosis can be described by platelet increased reactivity markers: number, size, tendency to form aggregates and concentrations of released substances from granules (28). We have studied actual numbers of platelets, their indices reflecting size variations (especially MPV and P-LCR). Platelet function was analysed by flow cytometry (expression of CD42a for Glycoprotein IX; expression of CD63 for an integral protein of platelet dense granule and lysosomal membranes; PAC-1 binding capacity for GP IIb/IIIa in activated platelets; and expression of CD14 for monocytes). Additionally, aggregability was assessed with TRAP agonist and concentrations of vWF and fibrinogen were measured. Platelet count and MPV may change independently, and our results show an inverse correlation, which is the most common combination of results (16). Despite this, PLT and MPV can be separately associated with increased platelet reactivity and also with increased risk of coronary syndromes

Table 7. Likelihood of having 5–7 laboratory CVD risk factors according to established cut-offs of separate markers. OR – odds ratio. CI – confidence interval. NS – not significant

Variable	Cut-off	OR	95% CI	Chi square	p
Fibrinogen, g/L	4.0	4.979	2.455–10.097	21.191	<0.001
TRAP, U	151	4.569	1.223–17.065	5.940	0.024
vWF, %	130	1.231	0.641–2.367	0.391	NS
TC, mmol/L	5.2	7.200	2.367–21.904	15.014	0.001
TG, mmol/L	1.8	6.156	2.948–12.854	25.691	<0.001
HDL-C, mmol/L	0.91*	3.009	1.414–6.404	8.555	0.004
LDL-C, mmol/l	3.5	4.271	1.926–9.469	13.860	<0.001
CRP, mg/L	3.0**	2.360	1.223–4.515	6.486	0.009
Glucose, mmol/L	6.1	4.481	1.788–11.233	11.425	0.001
MPV, fL	10.4	2.771	1.460–5.259	9.931	0.002
Acute stress	–	1.138	0.542–2.391	0.116	NS
3 MS markers	–	1.527	0.627–3.719	0.877	NS
CRP and MPV	–***	2.778	1.170–6.595	5.640	0.021

* Cut-off for men only.

** Cut-off for differentiation of high CVD risk individuals.

*** The same cut-offs as above.

(19). Our results show that increasing PLT correlates with increasing TRAP aggregability and fibrinogen concentrations; MPV increase correlates with higher expression of GP IX (CD42a); platelets express higher levels of procoagulant surface protein GP IIb/IIIa (high numbers of PAC-1 events); there is higher percentage of CD63/CD42a showing that release of platelet granules constituents is more active.

We next focused on inflammatory markers: CRP, fibrinogen and vWF. Each of these is separately known as a risk factor for CVD (6–12). A meta-analysis of published data from 18 studies, involving about 4 000 CVD cases, indicated a relative risk of 1.8 (95% CI 1.6–2.0) per 1 g/L increase in plasma fibrinogen level (12). For vWF data is ambiguous: not all authors succeeded in showing a positive relationship between vWF levels and CVD events (29, 30). It has been demonstrated that high CRP levels can predict risk of myocardial infarction, stroke or cardiac death in apparently healthy subjects. At the same time, presence of metabolic syndrome correlates with high levels of CRP. The most important aspect of this biomarker is that it has an independent predictive potential if high-sensitivity assay (hs-CRP) is used. Thus, cardiovascular risk may be categorized depending on the level of CRP (31). In our results, we have shown interrelatedness of CRP, fibrinogen and vWF values. In most cases, vWF was seen as a predictor of platelet functions. CRB was associated with platelets only indirectly by using lipid-CRP ratios.

Additionally, a statistically significant positive correlation was observed for CyPA in different risk categories according to CRP levels. Although linearity was not perfect, CyPA results correspond to the theory of protein's involvement in inflammatory processes (24, 26). A relationship between CyPA and CD42a/CD14 expression was found, which might indicate that monocytes and/or platelet-monocyte complexes, but not platelets, are responsible for increased CyPA levels in blood. Furthermore, CD42a/CD14 expression related to TC and LDL-C might indicate that monocytes and / or platelet-monocyte complexes play an important role in the development of atherosclerosis.

CONCLUSIONS

The present study suggests that platelets in men with dyslipidemia and other risk factors have a tendency to be hyperreactive and this fact might be considered as a separate risk factor for CVD. Inflammatory status of individuals having risk factors of CVD does not correlate with the platelet function. According to our results CyPA reflects inflammatory processes but not the platelet function.

ACKNOWLEDGEMENTS

We would like to gratefully acknowledge the participants who took part in this research study. We would like to thank the laboratory professionals at the Center of Laboratory Medicine of Vilnius University Hospital Santariškių Clinics. Also we are grateful to Toma Tamulevičienė (JSC Interlux, Lithuania) for her needful help with the CyPA ELISA protocol. All authors did not use any source of funding for this study.

Received 22 December 2014
Accepted 13 February 2015

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LIETUVOS VYRŲ SU DISLIPIDEMIJA TROMBOCITŲ FUNKCIJOS IR JŲ INDEKSŲ SĄSAJOS SU UŽDEGIMO ŽYMENIMIS

Santrauka

Įvadas. Trombocitų funkcija, jų tūrio indeksai, tradiciniai lipidų apykaitos žymenys, uždegimo žymenys ir naujasis žymuo ciklofilinas A (CyPA) yra tarpusavyje susiję, nes dalyvauja aterosklerozės patogenezėje. Šio tyrimo tikslas – įvertinti galimus minėtų veiksnių ryšius, nustatyti sąsajas tarp trombocitų funkcijų ir uždegiminių žymenų, taip pat CyPA funkciją.

Tiriamieji ir metodika. Į tyrimą įtraukta 160 vyriškos lyties asmenų, turinčių išreikštų aterosklerozės ir metabolinio sindromo (MS) rizikos veiksnių. Kriterijai: lipidų apykaitos žymenų pakitimai, antsvoris, rūkymas, ūminis arba lėtinis stresas, ūminės širdies ir kraujagyslių ligos nebuvimas ir kiti rizikos veiksniai, patvirtinti gydytojo. Atlikti C-reaktyviojo baltymo (CRB), Vilebrando faktoriaus (vWF), fibrinogeno, CyPA tyrimai. Trombocitų funkcija įvertinta agregacijos ir tėkmės citometrijos metodais.

Rezultatai. Didėjant rizikos veiksnių skaičiui statistiškai patikimai didėjo fibrinogeno, agregacijos su trombino receptorius aktyvinančiu peptidu (TRAP), triacilglicerolių, bendrojo cholesterolio, mažo tankio lipoproteinų cholesterolio, CRB, gliukozės, vidutinio trombocitų tūrio (MPV), trombocitų didelių ląstelių santykio (P-LCR) ir kai kurių lipidų santykių vertės, o tiesiškumo reikšmingumo lygmuo buvo labai didelis ($p \leq 0{,}004$). MPV koreliacija su CD42a buvo teigiama ($r = 0{,}605$; $p < 0{,}001$), o su CD42a/CD14 – neigiama ($r = -0{,}327$; $p < 0{,}001$). Nustatyta, jog lipidų santykiai – biocheminių MS žymenų prognostiniai požymiai. Trombocitų funkcijų parametrai buvo susiję su lipidų santykiais, lipidų-CRB santykiais, MPV ir vWF. Nustatyta CyPA koreliacija su CD42a/CD14 ($r = 0{,}202$, $p = 0{,}010$).

Išvados. Vyrų su dislipidemija ir kitais rizikos veiksniais trombocitai turi polinkį į padidėjusį reaktyvumą. Tai gali būti vertinama kaip atskiras širdies ir kraujagyslių ligų rizikos veiksnys. Uždegiminė būklė nekoreliuoja su trombocitų funkcija. CyPA atspindi uždegiminius procesus, o ne trombocitų funkciją.

Raktažodžiai: aterosklerozė, dislipidemija, trombocitų indeksai, ciklofilinas A, tėkmės citometrija