Association of serum levels of lipoprotein A-I and lipoprotein A-I/A-II with high on-treatment platelet reactivity in patients with ST-segment elevation myocardial infarction

Aleksander Siniarski[1,#], Rafał Grzybczak[1,2,#], Paweł Rostoff[1], Jarosław Zalewski[1], Urszula Czubek[1], Jadwiga Nessler[1], Grzegorz Gajos[1]

[1]Department of Coronary Disease and Heart Failure, Faculty of Medicine, Jagiellonian University Medical College, John Paul II Hospital; Krakow-*Poland*
[2]Department of Cardiac Rehabilitation, John Paul II Hospital; Krakow-*Poland*

Abstract

Objective: High-density lipoproteins (HDLs) are a very heterogeneous group of particles. Little is known about the impact of their subfractions including lipoprotein A-I (LpA-I) and lipoprotein A-I/A-II (LpA-I/A-II) on platelet function and high on-treatment platelet reactivity (HPR), particularly in the acute phase of ST-segment elevation myocardial infarction (STEMI). The aim of the study was to evaluate the relationship between serum levels of LpA-I and LpA-I/A-II and HPR in STEMI patients.

Methods: Fifty-two consecutive STEMI patients (26.9% women, mean age 60.6±9.1 years) were enrolled into this study. Clinical and demographic data were collected and HDL subfractions were measured by rocket immunoelectrophoresis. Platelet reactivity was assessed using light transmission aggregometry and quantitative flow cytometry.

Results: We found a positive correlation between platelet aggregation after both ADP-5 and ADP-20 stimulation and serum level of LpA-I. Compared with subjects with satisfactory platelet response to clopidogrel, patients with HPR had 32.44% higher serum level of LpA-I (p=0.021). On the other hand, patients with HPR assessed by ADP-5 stimulation had 22.13% lower serum level of LpA-I/A-II (p=0.040). Regression analysis showed that LpA-I [odds ratio (OR) 1.03; 95% confidence interval (CI) 1-1.07; p=0.049] and current smoking (OR 0.18; 95% CI 0.04-0.81; p=0.025) were independent predictors of HPR. With receiver operating characteristic (ROC) curve analysis, we designated the cut-off point at serum level of 57.52 mg/dL for LpA-I for predicting HPR (AUC=0.71, p=0.010).

Conclusion: This study showed that higher serum level of LpA-I measured in the acute phase of STEMI is an independent risk factor for HPR. Our study is the first to demonstrate an important and distinct activity of LpA-I and LpA-I/A-II that can prove pleiotropic and different functions of HDL subfractions in acute STEMI. *(Anatol J Cardiol 2018; 19: 374-81)*

Keywords: high-density lipoproteins, lipoprotein A-I, platelets, high platelet reactivity, ST-elevation, myocardial infarction

Introduction

Platelets are involved in both atherothrombosis and inflammation, becoming a key factor in the pathophysiology of acute coronary syndrome (ACS), which includes ST-segment elevation myocardial infarction (STEMI) (1). Adequate platelet inhibition with dual antiplatelet therapy (DAPT) using aspirin and $P2Y_{12}$ antagonist (e.g., clopidogrel, prasugrel, ticagrelor, cangrelor) is an essential therapeutic target in STEMI patients and is strongly recommended by current guidelines (1, 2).

$P2Y_{12}$ antagonists selectively inhibit the adenosine diphosphate (ADP) pathway of platelet activation by antagonizing one of the two platelet ADP receptors irreversibly (thienopyridines) or reversibly (ticagrelor) (3). The incidence of high on-treatment platelet reactivity (HPR) can be as high as one-third of individuals treated with clopidogrel, and HPR is present even in those receiving the more potent $P2Y_{12}$ antagonists (4).

Inter-individual variability in the concentration of active metabolite and the level of platelet inhibition achieved following the administration of the recommended doses of clopidogrel is multi-factorial and is contributed to by non-compliance, drug

[#]A.S. and R.G. contributed equally to this work.

Address for correspondence: Grzegorz Gajos, MD, PhD, Department of Coronary Disease and Heart Failure, Faculty of Medicine, Jagiellonian University Medical College, 80 Pradnicka St., 31-202 Krakow-*Poland*
Phone: +48 12 614 22 18 Fax: +48 12 433 43 76 E-mail: grzegorz.gajos@uj.edu.pl

Accepted Date: 06.04.2018 **Available Online Date:** 22.05.2018

DOI:10.14744/AnatolJCardiol.2018.63549

absorption, drug interactions, intrinsic high platelet reactivity, and genetic polymorphisms (5). Numerous clinical studies have demonstrated that suboptimal responses to clopidogrel and HPR are significantly related to increased cardiovascular mortality and thrombotic events in STEMI patients or in those undergoing percutaneous coronary intervention (PCI) (5). It was also demonstrated that HPR was a major risk factor for post-PCI stent thrombosis (ST) and subsequent ACS (6). The relation between HPR and post-PCI ischemic events depended on the overall disease risk, post-PCI time interval (early vs. late), and ethnicity (6). HPR was proven to correlate with greater coronary atherosclerosis and calcification (7).

There have been reports in which over 50% of patients with ACS had abnormal HDL-C levels, which were the most common forms of lipid abnormalities in this group of patients (8, 9). STEMI patients with low and very low levels of HDL-C have significantly increased in-hospital mortality compared with those with higher levels of HDL-C (9). Furthermore, the analysis of MIRACL trial showed that HDL-C level, but not LDL-C levels, is a better short-term prognostic factor in predicting adverse cardiovascular events in patients after ACS (8). Several studies have confirmed the anti-inflammatory, anti-atherogenic, and anticoagulant properties of HDL-C are in part associated with the stimulation of production of platelet activation inhibitors such as nitric oxide and prostacyclin (10-12). In addition, high level of HDL-C is related to low platelet activation in ACS patients treated with PCI (13, 14).

On the other hand, there have also been reports on HDL-C's procoagulant properties (15, 16). Human plasma HDL is a very heterogeneous group of lipoproteins with uncertain roles in cardiovascular risk (17-19). Apolipoprotein A-I (Apo A-I) and apolipoprotein A-II (Apo A-II) are found in two subfractions of HDL particles.

Apo A-I is a predominant protein of HDL particles. Gotto et al. (20) showed that Apo A-I level is a better predictor of the first cardiovascular event than any other lipid parameter. However, its oxidation by myeloperoxidase could change its properties and was associated with impairment on potential atheroprotective features of HDL particles (21). Apo A-II is the second major component of HDLs. It has been hypothesized that higher level of Apo A-II in HDL particles promotes procoagulant lipid redistribution and atherosclerosis progression, but these results remain unclear (22, 23). On the other hand, in the EPIC-Norfolk project, a negative association was found between the level of Apo A-II and development of CAD and its complications in potentially healthy population (24).

HDL particles containing only Apo A-I are called lipoprotein A-I (LpA-I) and consist of 25% of plasma Apo A-I; the rest of HDL particles contain both Apo A-I and Apo A-II and are called lipoprotein A-I/A-II (LpA-I/A-II) (25).

To our knowledge, there is no information regarding the impact of serum levels of LpA-I and LpA-I/A-II on platelet function, particularly in the acute phase of STEMI. Thus, the aim of this study was to evaluate the relationship between serum levels of LpA-I and LpA-I/A-II, and HPR in STEMI patients.

Methods

Study population

Fifty-two consecutive patients with acute STEMI within 12 h of the onset of symptoms were included in this study. Inclusion criteria were ≥45 years of age and first-ever clinical manifestation of CAD. STEMI diagnosis was established according to the Third Universal Definition of Myocardial Infarction by European Society of Cardiology. The study was performed according to the Helsinki Declaration with the approval of the local ethics committee. Each study participant provided written informed consent.

Exclusion criteria were as follows: previous treatment with hypolipidemic agents, acetylsalicylic acid (ASA), clopidogrel, proton pump inhibitor, or non-steroid anti-inflammatory drugs 12 months prior to hospitalization; use of GP IIb/IIIa receptor inhibitor during PCI; and concomitant severe systemic disease including cancer.

When choosing a power of 90% and a two-sided alpha level of 0.05, at least 46 patients in the group were required to find the significance between LpA-I and ADP-5.

Lipid analysis

HDL-C subfractions and Apo A-I were determined by taking 10 mL blood samples into tubes without any anticoagulant. After 30–60 min at room temperature, blood samples were centrifuged for 10 min (3000 r/min). After clot separation, centrifugation was repeated. Serum was drained and portioned into parts and then stored in eppendorf tubes at –80°C until assayed. Basic laboratory results and all lipid parameters were measured during hospital admission up to 12 h from symptom onset of chest pain.

LpA-I measurement

To measure LpA-I levels, we used rocket immunoelectrophoresis. In serum agarose gel electrophoresis, excess concentration of anti-Apo A-I blocked the migration of particles containing Apo A-II (LpA-I/A-II). The height of generated characteristic rockets was proportional to the concentration of LpA-I particles. The concentration of the HDL-C-related Apo A-I in serum was measured by manual immunonephelometric assay. For further calculations, we used a previously defined formula:

Apo A-I (serum)=Apo A-I in (LpA-I) + Apo A-I in (LpA-I/A-II).

Other lipid analysis such as LDL-C, HDL-C, TG, and TC was performed using standard enzymatic assay.

Light transmission aggregometry

Blood-citrate vacutainer tubes were centrifuged for 10 min at 120 g and 10 min at 850 g to recover platelet-rich plasma (PRP) and platelet-poor plasma (PPP), respectively. Prior to the analysis, platelets were stimulated with 0.5 mmol/L solution of arachidonic acid (AA) and 5 and 20 μmol/L solution of adenosine diphosphate (ADP-5 and ADP-20, respectively). Measurements were performed 30-60 min after blood collection. Light transmission was adjusted using PRP to represent 0% and PPP

Anatol J Cardiol 2018; 19: 374-81
DOI:10.14744/AnatolJCardiol.2018.63549

to represent 100% transmission for each measurement. Curves were recorded for 8 min or until the plateau phase was obtained. Platelet aggregation was expressed as the maximal percentage of change in light transmission from baseline using PPP as a reference. Aggregation was measured with the two-channel Chronology Aggregometer (Chrono-Log 490; Chrono-Log Corp., Havertown, Pennsylvania, USA).

Quantitative flow cytometry

Quantitative flow cytometry (FACSCalibur System, BD Bioscience, Warsaw, Poland) was used with labeled monoclonal antibodies against serine 239-phosphorylated vasodilator-stimulated phosphoprotein (VASP), followed by a secondary fluorescein isothiocyanate-conjugated polyclonal goat anti-mouse antibody. The mean fluorescence intensity (MFI) of the phosphorylated VASP levels after stimulation with prostaglandin E1 (PGE1) and PGE1+ADP was measured. In the analysis, we used $P2Y_{12}$ receptor activation-Platelet Reactivity Index (PRI) calculated according to the following formula:

$[(MFI_{PGE1})-(MFI_{PGE1+ADP})]/(MFI_{PGE1})\times100\%$.

HPR was defined as PRI of ≥50% and aggregation of >46% after ADP-5 stimulation or >59% for ADP-20 was considered as inadequate response to clopidogrel (26). All platelet reactivity measurements were performed 12-24 h after the loading dose of clopidogrel and ASA (300-600 mg and 150-300 mg, respectively).

Statistical analysis

All continuous variables were expressed as mean±SD, and categorical variables were expressed as percentages. Continuous data were tested for fit-to-normality by the D'Agostino-Pearson omnibus normality test. Normally distributed variables were presented as mean±SD and compared using independent samples t-test. Non-normally distributed variables were expressed in medians with interquartile range (IQR), and Mann-Whitney U test was used to determine the significant differences among the groups. Categorical variables were compared between the groups using the χ^2 test or Fisher's test when the expected population size was small. The linear relationship between the two quantitative variables was investigated using the Pearson correlation coefficient (normally distributed variables) or Spearman's rank correlation coefficient (non-normally distributed variables). To determine platelet activation during clopidogrel treatment, univariate and multiple stepwise logistics regression analysis was performed. The final model was constructed by stepwise logistic regression to determine the independent determinants of HPR. To assess the optimum cut-off point, we used receiver operating characteristic (ROC) curve. Discriminatory ability of the model was tested using the area under the ROC curve (AUC). The level of significance was set at $p<0.05$. Statistical analysis was performed using the R statistical package for Windows (version 2.15.1, The R Foundation for Statistical Computing, Vienna, Austria) and MedCalc for Windows (version 12.4, MedCalc Software, Mariakerke, Belgium) was used.

Results

The study included 52 consecutive STEMI patients including 14 women (26.9%) and 38 men (73.1%) aged 46-76 years (mean age of 60.6±9.14 years). The baseline demographic and clinical characteristics, laboratory results, evaluation of atherosclerotic lesions, culprit artery location, coronary angioplasty outcome, hospital course, and enzymatic assay with LV function parameter of the study population are presented in Table 1.

HDL-C subfractions and platelet reactivity

Lipids levels with HDL-C subfractions measurements, platelet reactivity, and numbers of patients diagnosed with HPR (ADP-5, ADP-20, and PRI, respectively) are listed in Table 1. We have found a positive correlation between platelet aggregation after

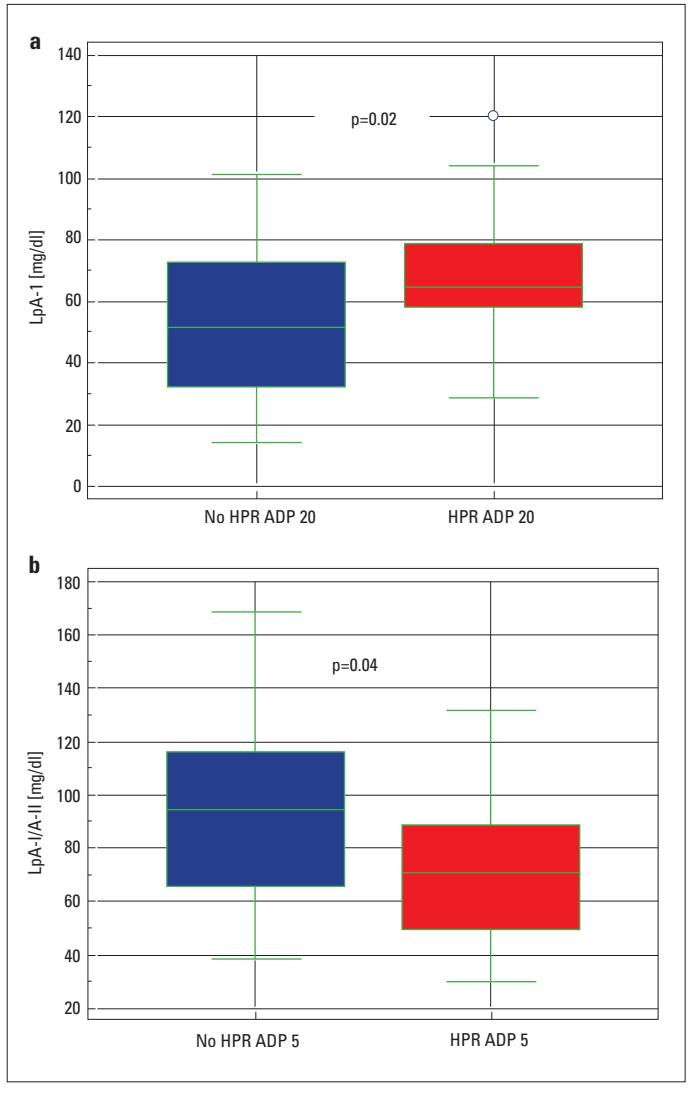

Figure 1. Concentration of HDL-C subfractions (LpA-I and LpA-I/A-II) in patients with and without HPR.
Data are presented as: median, interquartile range (IQR) and 1,5 IQR.
AA - aggregation after stimulation with arachidonic acid; ADP-5 (20) - aggregation after stimulation with 5 (20) μmol of adenosine diphosphate; PRI - platelet reactivity index; HPR - high platelet reactivity during treatment

Table 1. General characteristics, lipids measurements, and platelets parameters of the study population (n=52)

Basic characteristics	
Age (years)	60.6±9.1
Weight (kg)	77.5±13.5
Waist (cm)	94±12
BMI, kg/m^2	26.6±3.9
Clinical data	
Hypertension	27 (51.9)
Diabetes mellitus	11 (21.1)
IFG or IGT	11 (21.1)
Positive family history	13 (25)
Current cigarette smoking	21 (40.4)
Ex-smokers	13 (25)
Metabolic syndrome	26 (50)
Basic laboratory results	
eGFR (MDRD) ml/kg/1.73 m^2	80.6±16.9
AST, U/L	204.3±211.4
ALT, U/L	54.1±42.6
HGB, g/dL	14.4±1.3
RBC, 10^6/µL	4.7±0.5
PLT, 10^3/µL	224.2±66.0
CRP, mg/L	11.2±19.9
WBC, 10^3/µL	10.7±3.0
Glucose, mmol/L	7.8±2.4
Evaluation of atherosclerotic lesions	
Gensini score, points	52±27.4
Culprit artery occlusion before procedure	35 (67.3)
IMT, mm	9.23±2.55
Hospital course	
Killip–Kimball classification I and II	49 (96.2)
Killip–Kimball classification III and IV	3 (3.8)
Recurrent ischemia	2 (3.8)
Necessity of urgent revascularization	1 (1.9)
AF/SVT	10 (19.2)
Complex ventricular arrhythmias	10 (19.2)
Atrioventricular block class II or III	7 (13.5)
Stroke or TIA	1 (1.9)
Cardiac arrest in the acute phase of MI	3 (3.8)
Deaths	0 (0)
Enzymatic assays and LV function parameter	
TnI, ng/mL	74.6±72.2
CK, U/L	1914±1715
LVEF, %	49.1±7.5

Table 1. Cont.

Lipids measurements	
HDL-C, mmol/L	1.3±0.3
LDL-C, mmol/L	3.5±0.9
TG, mmol/L	1.4±1.4
TC, mmol/L	5.6±1.2
Apo A I, mg/dL	140.9±30.4
LpA-I, mg/dL	59.3±23.8
LpA-I/A-II, mg/dL	80.0±30.9
LpA-I/HDL, %	42.3±15.3
[LpA-I/LpA-II]/HDL, %	57.7±15.3
Platelet reactivity	
AA	2.8±1.7
ADP-5	52.2±23.3
ADP-20	53.1±23.1
PRI	45.7±26.4
MPV, fl	10.5±1.1
HPR (ADP-5)	33 (63.4)
HPR (ADP-20)	23 (44.2)
HPR (PRI)	27 (51.9)

Data are given as mean±SD or number (percentage)
BMI - Body mass index; IFG - impaired fasting glucose; IGT - impaired glucose tolerance; eGFR - estimated glomerular filtration rate; AST - aspartate transaminase; ALT - alanine transaminase; HGB - hemoglobin; RBC - red blood cells; PLT - platelets; CRP - C-reactive protein; WBC - white blood count; IMT - intima-media thickness; AF - atrial fibrillation; SVT - supraventricular tachycardia; TIA - transient ischemic attack; MI - myocardial infarction; TnI - cardiac troponin I; CK - creatine kinase; LVEF - left ventricle ejection fraction; SD - standard deviation; HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol; TG - triglycerides; TC - total cholesterol; Apo A-I - apolipoprotein A I; LpA-I - HDL lipoprotein consisting only apolipoprotein A-I; LpA-I/A-II - HDL lipoprotein consisting both apolipoprotein A-I and A-II; AA - result of the optical aggregometry using arachidonic acid; ADP-5 (20) - result of the optical aggregometry using 5 (20) µmol/L of adenosine diphosphate; PRI - platelet reactivity index; MPV - mean platelet volume; HPR - high platelet reactivity during treatment; SD - standard deviation

both ADP-5 and ADP-20 stimulation and serum level of LpA-I (Table 2). Compared with subjects with satisfactory platelet response to clopidogrel, patients with HPR assessed by ADP-20 stimulation had 32.44% higher serum level of LpA-I (p=0.021). On the other hand, patients with HPR assessed by ADP-5 stimulation had 22.13% lower serum level of LpA-I/A-II (p=0.040) (Table 3 and Fig. 1).

HDL-C and Apo A-I did not differ between groups with vs. without HPR (Table 3). We have designated the cut-off point at 57.52 mg/dL for LpA-I level in ROC curve for predicting HPR after stimulation with ADP-20 (Fig 2). Age, sex, weight, waist circumference, BMI, smoking history, current smoking, diabetes, impaired glucose tolerance, impaired fasting glucose, metabolic syndrome, mean platelet volume, and levels of HDL-C, LDL-C, triglycerides, total cholesterol, Apo A-I, LpA-I, LpA-I/A-II, glucose and C-reactive protein ($p<0.05$ in a univariate analysis) were included in the final regression model. Multiple stepwise

Anatol J Cardiol 2018; 19: 374-81
DOI:10.14744/AnatolJCardiol.2018.63549

Table 2. Correlations between following lipids and platelet reactivity measurements

Parameter	Concentration in the acute phase of myocardial infarction			
	HDL-C	Apo A I	LpA-I	LpA-I/II
AA	*P*=0.281	*P*=0.210	*P*=0.270	*P*=0.122
	r=0.12	r=0.16	r=0.16	r=-0.04
ADP-5	*P*=0.453	*P*=0.362	***P*=0.042**	*P*=0.443
	r=-0.20	r=0.13	**r=0.31**	r=-0.10
ADP-20	*P*=0.112	*P*=0.781	***P*=0.041**	*P*=0.790
	r=-0.17	r=0.07	**r=0.32**	r=-0.13
PRI	*P*=0.762	*P*=0.334	*P*=0.762	*P*=0.392
	r=0.01	r=0.05	r=0.04	r=0.02
MPV	*P*=0.810	*P*=0.240	*P*=0.550	*P*=0.671
	r=-0.008	r=0.04	r=0.13	r=0.03

Data are given as *P*-value and r-value
AA - result of the optical aggregometry using arachidonic acid; ADP-5 (20) - result of the optical aggregometry using 5 (20) µmol/L of adenosine diphosphate; PRI - platelet reactivity index; MPV - mean platelet volume; NS - not significant; HDL-C - high-density lipoprotein cholesterol; Apo A-I - apolipoprotein A-I; LpA-I - HDL lipoprotein consisting only apolipoprotein A I; LpA-I/A-II - HDL lipoprotein consisting both apolipoprotein A-I and A-II

Table 3. Concentration of high-density lipoprotein cholesterol subfractions and Apo A-I in acute phase of ST-segment elevation myocardial infarction in groups with and without high platelet reactivity

Variables		Concentration							
		HDL		Apo A I		LpA-I		LpA-I/A-II	
ADP-5	HPR (+)	1.13		136.1±28.6		63.0±23.2		71.8±26.5	
		0.96-1.39	*P*=0.241		*P*=0.092		*P*=0.321		***P*=0.040**
	HPR (-)	1.30							
		0.98-1.43		148.9±33.5		53.5±25		92.2±37.1	
ADP-20	HPR (+)	1.09		144.6±21		69±21.8		73.8±23.2	
		0.91-1.39	*P*=0.330		*P*=0.190		***P*=0.021**		*P*=0.542
	HPR (-)	1.28		137.7±36.78		52.1±23.5		83.5±37.3	
		0.98 -1.43							
PRI	HPR (+)	1.21		135.9±28.9		59.7±27.8		76.2±22.7	
		0.97-1.41	*P*=0.392		*P*=0.762		*P*=0.681		*P*=0.382
	HPR (-)	1.23		141±31.8		55.9±22.3		82.2±38.6	
		0.96-1.41							

Data are given as mean±SD or median (Q1-Q3).
NS - non-significant; AA - result of the optical aggregometry using arachidonic acid; ADP-5 (20) - result of the optical aggregometry using 5 (20) µmol/L of adenosine diphosphate; PRI - platelet reactivity index; HPR - high platelet reactivity during treatment; HDL-C - high-density lipoprotein cholesterol; Apo A-I - apolipoprotein A I; LpA-I - HDL lipoprotein consisting only apolipoprotein A I; LpA-I/A-II - HDL lipoprotein consisting both apolipoprotein A I and A II

logistics regression analysis showed that LpA-I level and current cigarette smoking were independent predictors of HPR (Table 4). With every 1 mg/dL of increasing LpA-I level, the risk of HPR grew by 3%. On the other hand, with cigarette smoking, the risk of HPR decreased more than five times. In the analyzed population of STEMI patients, HDL-C subfractions did not prove to be useful in predicting in-hospital adverse cardiovascular events.

Discussion

To our knowledge, this is the first study to show that serum levels of LpA-I and LpA-I/A-II in STEMI patients are associated with platelet reactivity in response to DAPT with aspirin and clopidogrel. We have found that both current smoking and LpA-I altered the prevalence of HPR. Our study extends prior findings

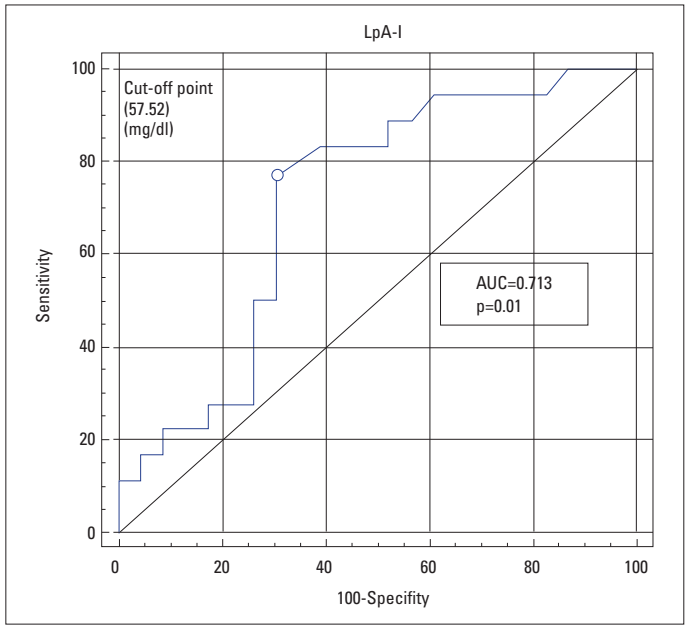

Figure 2. The ROC curve with designated cut-off point for LpA-I concentration in predicting HPR after stimulation with 20 µmol/L ADP.
LpA-I - HDL lipoprotein consisting only apolipoprotein A-I; LpA-I/A-II - HDL lipoprotein consisting both apolipoprotein A-I and A-II; HPR - high on-treatment platelet reactivity; ADP - adenosine diphosphate; AUC - area under the curve

Table 4. Independent determinants of high platelet reactivity after stimulation with 20 µM ADP in the acute phase of ST-segment elevation myocardial infarction

Parameter	OR	95% CI	*P*-value
sex, y	1.07	0.98-1.16	0.221
smoking (currently)	0.18	0.04-0.81	**0.025**
glucose, mmol/L	1.35	0.90-2.03	0.450
LpA-I, mg/dL	1.03	1-1.07	**0.049**

Data are given as: OR, 95% CI
HPR - high platelet reactivity during treatment; STEMI - ST-elevation myocardial infarction; OR -odds ratio; CI - confidence interval; *P* - p-value; LpA-I - HDL lipoprotein consisting only apolipoprotein A-I, NS - non-significant

concerning the influence of HDL particles on platelet activation, particularly during clopidogrel treatment.

Previous studies have shown that HDL particles inhibited coagulation processes and platelet activation mostly by the inhibition of the tissue factor expression on the endothelial surface (27). Furthermore, Apo A-II increased the activation of VIIc and Xc factors, which could indicate its prothrombotic properties (15). Nevertheless, Schäfer et al. (28) identified decreased HDL-C levels among factors determining HPR, both in patients with stable coronary artery disease and STEMI. Nofer et al. (29) showed that HDL particles led to the activation of protein kinase C, alkalization of platelets' intracellular matrix, and inactivation of phospholipase C, thus preventing platelet activation.

There are no well-known explanations of the interactions between LpA-I and LpA-I/A-II levels and platelet activity. Discovery of a scavenger receptor (SR-BI) gave us new insights into potential mechanisms involved in the inhibition of platelet activation. HDL particles binding to the SR-BI receptor resulted in the inhibition of thrombin-induced platelet aggregation, decrease in the binding of fibrinogen, decrease in the expression of P-selectin, and mobilization of intracellular calcium ions (11). It has also been shown that platelets loaded with cholesterol esters are more sensitive to ADP (30). Experimental studies in recent years have shown that the $P2Y_{12}$ receptor could present as an oligomer associated with membrane lipid rafts, which has been proven to split off from the rafts on clopidogrel treatment (31). Therefore, there is a possibility of significant influence of LpA-I and LpA-I/A-II levels on platelet reactivity and clinical responsiveness during clopidogrel treatment.

Assessment of platelet function during DAPT after PCI in STEMI patients is a useful diagnostic tool in predicting the incidence of both ischemic and hemorrhagic complications (32). In our study, the prevalence of HPR on DAPT with aspirin and clopidogrel varied from 44.2% to 63.4% (as assessed by ADP stimulation). HPR occurrence in the study by Schäfer et al. (28) reached 74%; hence, it was greater than that in our study (28). On the other hand, Cuisset et al. (33) showed a lower (44%) prevalence of HPR in patients with non-STEMI. These differences between studies may result from different populations studied and varied time-intervals between blood sample collection and clopidogrel administration.

The assessment of platelet reactivity in our study suggested that the "smokers' paradox" can also be present in STEMI patients. Over fivefold reduction in the risk of HPR in the group of smokers can be explained by the induction of hepatic CYP1A2, also involved in the metabolism of clopidogrel and other expression of the P_2Y_{12} receptor in smokers vs. non-smokers (34). It is still unclear whether obesity is associated with lower response to clopidogrel treatment. It was observed that obese individuals after PCI had lower short- and long-term mortality than do normal-weight patients. Our study did not confirm this "obesity paradox". There is a study that has found that despite the initial period of low platelet response to loading dose of clopidogrel, after 30 days of clopidogrel administration, platelet response was similar between obese and normal-weight patients (35).

Study limitations

Several limitations of the study must be acknowledged. First, there was no correlation between HDL-C levels and clinical outcomes, which probably resulted from the small population of the study. Second, there is a lack of uniform criteria for HPR diagnosis, which is different for each technique and change dynamically in time. Hence, it seems that the diagnostic criteria may be different for each clinical situation. Some authors have proposed a different cut-off point value of PRI for predicting i.e., in-stent thrombosis (69.7%) and recurrence ischemia after NSTEMI (53%) and STEMI (57%) (28, 36). Third, blood samples were collected only once during the hospitalization period; thus,

Anatol J Cardiol 2018; 19: 374-81
DOI:10.14744/AnatolJCardiol.2018.63549

we could not track possible changes in platelet reactivity. Finally, a small group of patients made accurate comparisons between subgroups (i.e., smokers vs. non-smokers or between sexes) impossible; therefore, drawing conclusions from them could only be hypothetical.

Conclusion

This study demonstrated that higher serum levels of LpA-I measured during the acute phase of STEMI is an independent risk factor for HPR, which is a novel observation. There is a significant negative correlation between serum level of LpA-I/A-II and HPR. Our study is the first to demonstrate an important and distinct activity of LpA-I and LpA-I/A-II, which can prove pleiotropic and different functions of HDL subfractions in acute STEMI. The results of the present study are an interesting hypothesis and should be explored further in a larger study on a greater number of patients.

Acknowledgments: The study was supported by research grant: 2011/03/B/NZ5/0576 from the National Science Centre, Poland (to G.G.).

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – R.G., J.N.; Design – R.G., U.C., J.N.; Supervision – R.G., U.C., J.N., G.G.; Fundings – G.G.; Materials – G.G., J.Z.; Data collection &/or processing – A.S., J.Z.; Analysis &/or interpretation – A.S., P.R.; Literature search – A.S., P.R., J.Z.; Writing – A.S., R.G.; Critical review – A.S., P.R., J.Z., U.C., J.N., G.G.

References

1. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2018; 39: 119-77.
2. Valgimigli M, Bueno H, Byrne RA, Collet JP, Costa F, Jeppsson A, et al. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS. Eur J Cardiothorac Surg 2018; 53: 34-78. [CrossRef]
3. Cattaneo M. High on-treatment platelet reactivity--definition and measurement. Thromb Haemost 2013; 109: 792-8. [CrossRef]
4. Zhou Y, Wang Y, Wu Y, Huang C, Yan H, Zhu W, et al. Individualized dual antiplatelet therapy based on platelet function testing in patients undergoing percutaneous coronary intervention: a meta-analysis of randomized controlled trials. BMC Cardiovasc Disord 2017; 17: 157. [CrossRef]
5. Johnston LR, Larsen PD, La Flamme AC, Michel JM, Simmonds MB, Harding SA. Suboptimal response to clopidogrel and the effect of prasugrel in acute coronary syndromes. Int J Cardiol 2013; 167: 995-9.
6. Tantry US, Bonello L, Aradi D, Price MJ, Jeong YH, Angiolillo DJ, et al.; Working Group on On-Treatment Platelet Reactivity. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. J Am Coll Cardiol 2013; 62: 2261-73. [CrossRef]
7. Chirumamilla AP, Maehara A, Mintz GS, Mehran R, Kanwal S, Weisz G, et al. High platelet reactivity on clopidogrel therapy correlates with increased coronary atherosclerosis and calcification: a volumetric intravascular ultrasound study. JACC Cardiovasc Imaging 2012; 5: 540-9. [CrossRef]
8. Olsson AG, Schwartz GG, Szarek M, Sasiela WJ, Ezekowitz MD, Ganz P, et al. High-density lipoprotein, but not low-density lipoprotein cholesterol levels influence short-term prognosis after acute coronary syndrome: results from the MIRACL trial. Eur Heart J 2005; 26: 890-6. [CrossRef]
9. Ji MS, Jeong MH, Ahn YK, Kim YJ, Chae SC, Hong TJ, et al.; Korea Acute Myocardial Infarction Registry Investigators. Impact of low level of high-density lipoprotein-cholesterol sampled in overnight fasting state on the clinical outcomes in patients with acute myocardial infarction (difference between ST-segment and non-ST-segment-elevation myocardial infarction). J Cardiol 2015; 65: 63-70.
10. Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res 1995; 36: 211-28.
11. Brodde MF, Korporaal SJ, Herminghaus G, Fobker M, Van Berkel TJ, Tietge UJ, et al. Native high-density lipoproteins inhibit platelet activation via scavenger receptor BI: role of negatively charged phospholipids. Atherosclerosis 2011; 215: 374-82. [CrossRef]
12. Dzięgielewska-Gęsiak S, Bielawska L, Zowczak-Drabarczyk M, Hoffmann K, Cymerys M, Muc-Wierzgoń M, et al. The impact of high-density lipoprotein on oxidant-antioxidant balance in healthy elderly people. Pol Arch Med Wewn 2016; 126: 731-8.
13. Caughey GE, Cleland LG, Penglis PS, Gamble JR, James MJ. Roles of cyclooxygenase (COX)-1 and COX-2 in prostanoid production by human endothelial cells: selective up-regulation of prostacyclin synthesis by COX-2. J Immunol 2001; 167: 2831-8. [CrossRef]
14. Tselepis AD, Tsoumani ME, Kalantzi KI, Dimitriou AA, Tellis CC, Goudevenos IA. Influence of high–density lipoprotein and paraoxonase–1 on platelet reactivity in patients with acute coronary syndromes receiving clopidogrel therapy. J Thromb Haemost 2011; 9: 2371-8. [CrossRef]
15. Atta M, Crook D, Shafique F, Johnston DG, Godsland IF. Procoagulant activities of plasma factor VIIc and factor Xc are positively and independently associated with concentrations of the high-density lipoprotein apolipoprotein, apoA-II. Thromb Haemost 2008; 100: 391-6. [CrossRef]
16. Rosenson RS. Functional assessment of HDL: moving beyond static measures for risk assessment. Cardiovasc Drugs Ther 2010; 24: 71-5. [CrossRef]
17. Pająk A, Szafraniec K, Polak M, Polakowska M, Kozela M, Piotrowski W, et al.; WOBASZ Investigators. Changes in the prevalence, treatment, and control of hypercholesterolemia and other dyslipidemias over 10 years in Poland: The WOBASZ study. Pol Arch Med Wewn 2016; 126: 642-52.
18. Zdrojewski T, Solnica B, Cybulska B, Bandosz P, Rutkowski M, Stokwiszewski J, et al. Prevalence of lipid abnormalities in Poland. The NATPOL 2011 survey. Kardiol Pol 2016; 74: 213-23. [CrossRef]
19. Piotrowski W, Waśkiewicz A, Cicha-Mikołajczyk A. Global cardiovascular mortality risk in the adult Polish population: Prospective assessment of the cohorts studied in multicentre national WOBASZ and WOBASZ Senior studies. Kardiol Pol 2016; 74: 262-73. [CrossRef]

20. Gotto AM Jr, Whitney E, Stein EA, Shapiro DR, Clearfield M, Weis S, et al. Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). Circulation 2000; 101: 477-84. [CrossRef]
21. Zheng L, Nukuna B, Brennan M-L, Sun M, Goormastic M, Settle M, et al. Apolipoprotein AI is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. J Clin Invest 2004; 114: 529-41. [CrossRef]
22. Blanco-Vaca F, Escolà-Gil JC, Martín-Campos JM, Julve J. Role of apoA-II in lipid metabolism and atherosclerosis: advances in the study of an enigmatic protein. J Lipid Res 2001; 42: 1727-39.
23. Tailleux A, Duriez P, Fruchart JC, Clavey V. Apolipoprotein A-II, HDL metabolism and atherosclerosis. Atherosclerosis 2002; 164: 1-13.
24. van der Steeg WA, Holme I, Boekholdt SM, Larsen ML, Lindahl C, Stroes ES, et al. High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein AI: significance for cardiovascular risk: the IDEAL and EPIC-Norfolk studies. J Am Coll Cardiol 2008; 51: 634-42. [CrossRef]
25. Phillips MC. New insights into the determination of HDL structure by apolipoproteins Thematic Review Series: High Density Lipoprotein Structure, Function, and Metabolism. J Lipid Res 2013; 54: 2034-48. [CrossRef]
26. Bonello L, Tantry US, Marcucci R, Blindt R, Angiolillo DJ, Becker R, et al.; Working Group on High On-Treatment Platelet Reactivity. Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. J Am Coll Cardiol 2010; 56: 919-33. [CrossRef]
27. Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. Circ Res 2006; 98: 1352-64. [CrossRef]
28. Schäfer A, Flierl U, Kössler J, Seydelmann N, Kobsar A, Störk S, et al. Early determination of clopidogrel responsiveness by platelet reactivity indexidentifies patients at risk for cardiovascular events after myocardial infarction. Thromb Haemost 2011; 106: 141-8. [CrossRef]
29. Nofer JR, Tepel M, Kehrel B, Walter M, Seedorf U, Assmann G, et al. High density lipoproteins enhance the Na+/H+ antiport in human platelets. Thromb Haemost 1996; 75: 635-41.
30. Shattil SJ, Anaya-Galindo R, Bennett J, Colman RW, Cooper RA. Platelet hypersensitivity induced by cholesterol incorporation. J Clin Invest 1975; 55: 636-43. [CrossRef]
31. Savi P, Zachayus JL, Delesque-Touchard N, Labouret C, Hervé C, Uzabiaga MF, et al. The active metabolite of Clopidogrel disrupts P2Y12 receptor oligomers and partitions them out of lipid rafts. Proc Natl Acad Sci U S A 2006; 103: 11069-74. [CrossRef]
32. Matetzky S, Shenkman B, Guetta V, Shechter M, Beinart R, Goldenberg I, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 2004; 109: 3171-5. [CrossRef]
33. Cuisset T, Quilici J, Loosveld M, Gaborit B, Grosdidier C, Fourcade L, et al. Comparison between initial and chronic response to clopidogrel therapy after coronary stenting for acute coronary syndrome and influence on clinical outcomes. Am Heart J 2012; 164: 327-33.
34. Warner TD, Paul-Clark M, Mitchell JA. Smoking, atherothrombosis and clopidogrel. Heart 2012; 98: 963-4. [CrossRef]
35. Haberka M, Mizia-Stec K, Lasota B, Kyrcz-Krzemień S, Gąsior Z. Obesity and antiplatelet effects of acetylsalicylic acid and clopidogrel in patients with stable angina pectoris after percutaneous coronary intervention. Pol Arch Med Wewn 2015; 125: 620-30. [CrossRef]
36. Frere C, Cuisset T, Quilici J, Camoin L, Carvajal J, Morange PE, et al. ADP-induced platelet aggregation and platelet reactivity index VASP are good predictive markers for clinical outcomes in non-ST elevation acute coronary syndrome. Thromb Haemost 2007; 98: 838-43. [CrossRef]