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Acetylsalicylic acid resistance in long-term follow-up in subjects after myocardial infarction

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ABSTRACT:
Acetylsalicylic acid (ASA) is widely used as an antiplatelet therapeutic drug in secondary prevention. Despite its recognized benefits in treatment of subjects with cardiovascular (CV) diseases, last years brought many reports on ASA resistance or high-on treatment platelet reactivity (HTPR) despite aspirin treatment. The aim of this study is an evaluation of ASA resistance in long-term control basing on the result of arachidonic acid-induced platelet aggregation in reference to the results obtained during first 9 months of observation. This study is a prospective analysis with 30 subjects evaluated during control visit on average of 6,3 years after hospitalization from myocardial infarction. The examined population was divided into two subgroups according to the response to ASA. In order to estimate the function of blood platelets and their responsiveness to acetylsalicylic acid therapy, ASPI-test was used. The measurements were performed by the method of whole blood impedance aggregometry. During long-term visit significantly higher percentage of high platelet reactivity was observed, compared with previous visits ($p=0.00001$). Considering clinical endpoints of the research that were connected with coronary disease, no differences were obtained. The frequency of acetylsalicylic acid resistance in this study was higher than data reported in literature among subjects with CV diseases. In long-term observation the highest percentage of ASA non-responders was reported (58.6%), yet presence of ASA resistance did not affect the presence of the clinical endpoints for the study connected with coronary disease.

Keywords:
Acetylsalicylic acid, Aspirin, Aspirin resistance, High-on treatment platelet reactivity, Myocardial infarction

INTRODUCTION:
Cardiovascular (CV) diseases inducted by atherosclerosis based changes are currently major death cause in all over the world. This is confirmed by the European Heart Network Report from 2012, which indicates that they are the cause of 47% of all deaths in Europe [1]. Platelets play an important role in the pathophysiology of thrombotic atherosclerosis complications and therefore antiplatelet therapy, the essential element of which is acetylsalicylic acid (ASA), is at the heart of the use in secondary prevention at the optimal dose [2,3,4,5]. The benefits of using ASA in cardiology are widely known, but reports of resistance to acetylsalicylic acid have been reported for many years [6].

Acetylsalicylic acid resistance is defined by Patrono as an ontogenic limitation of ability to prolongate the bleeding time, inhibition of thromboxane (TXA) biosynthesis and ability of platelet function inhibition concluding with deficit of efficacy for preventing cardiovascular incidents [7]. Currently, aspirin resistance is also called high-on treatment platelet reactivity (HTPR) despite aspirin treatment. Despite multiple studies, criteria of ASA resistance have not been clearly defined, and the pathogenesis of this phenomenon is still a subject of debate. Numerous methods are available for laboratory evaluation of platelet function but this methods evaluate only specific aspects of platelet function [8]. Frequency of that phenomenon in a group of subjects after myocardial infarction and coronary disease is estimated from 5 to 15%, and even 29%, depending on the application of a testing method [9].

The main aim of the study is to assess the occurrence of aspirin resistance phenomenon during follow-up visits (average 6,3 years) after hospitalization due to myocardial infarction based on the result of platelet aggregation dependent on arachidonic acid in relation to the results obtained during the first 9 months of observation.

MATERIALS AND METHODS:
Study population and design: This study was designed by Stolarek et al. as post-hoc prospective research analysis and comprised of patients hospitalized in Department of Cardiology and Internal Medicine Cardiology due to acute coronary syndrome [10]. The study included 194 patients – 144 men and 50 women. Study was divided into two stages: clinical and ambulatory. Second stage of the study included control visits in 3rd, 6th and 9th month after hospitalization. Currently we followed the methods of Stolarek et al. 2015. Prospective follow up evaluation held averagely 6,3 years from primary hospitalization due to the myocardial infarction for various reasons, for example: death of the patient, old age or movement limitations, covered only 30 subjects from the original group (21 males and 9 females). Study was performed according to the Declaration of Helsinki together with the agreement of Bioethics Commission. Clinical and demographical characterization of studied population during hospitalization period (2010/2011 years) and during control visit (2016/2017 years) are shown in table 1. The study population was taking aspirin in enteric coated form at a dose of 75-100 mg.
Table 1. CLINICAL AND DEMOGRAPHIC CHARACTERISTICS IN STUDY POPULATION IN 2010/2011 YEARS AND IN 2016/2017 YEARS [MEDIAN (LOWER QUARTILE-UPPER QUARTILE) OR NUMBER (PERCENT)].

Study feature	Property value (n=30)
2010/2011	
Age [years]	61.0 (53.0-66.0)
Height [cm]	170.0 (164.0-176.0)
Body mass [kg]	80.5 (73.0-90.0)
BMI [kg/m^2]	28.7 (24.5-32.2)
Girth [cm]	95.0 (90.0-104.0)
Sex [men/women]	21/9
Ischemic disease recognized before admittance	9 (30.0%)
Infarction passed before admittance	4 (13.3%)
PCI passed before admittance	4 (13.3%)
CABG passed before admittance	0 (0.0%)
Risk factors for ischemic heart disease:	
Hyperlipidemia in interview	20 (66.7%)
Arterial hypertension	15 (50.0%)
Diabetes	6 (20.0%)
Current smokers	18 (60.0%)
Past smokers	4 (13.3%)
Treatment:	
PCI with DES implantation	30 (100%)
Qualification for further treatment:	
Conservative	24 (80.0%)
PCI	5 (16.7%)
CABG	1 (3.33%)
Final diagnosis:	
NSTEMI/UA	3 (10.0%)
STEMI including:	27 (90.0%)
Anterior myocard. inf.	9 (30.0%)
Inferior myocard. inf.	12 (40.0%)
Other localization	6 (20.0%)
LVEF [%]	44.0 (40.0-50.0)
2016/2017	
Height [cm]	169.5 (164.0-176.0)
Body mass [kg]	83.0 (73.0-87.0)
BMI [kg/m^2]	28.0 (24.2-32.3)
Girth [cm]	96.5 (93.0-100.0)
Alcohol:	
No	27 (90.0%)
Yes	0 (0.0%)
Past	3 (10.0%)
Smokers:	
No	7 (23.3%)
Current smokers	7 (23.3%)
Past smokers	14 (46.7%)
Occasionally	2 (6.7%)
NYHA class	
I	27 (90.0%)
II	3 (10.0%)
CCS class I	30 (100.0%)
Concomitant treatment	
Acetylsalicylic acid (dose:75-100 mg)	29 (96.7%)
Beta- blocker	30 (100.0%)
Angiotensin converting enzyme inhibitor	30 (100.0%)
Statin	28 (93.3%)
Proton pump inhibitor	5 (16.7%)
Clopidogrel	4 (13.3%)

BMI, Body Mass Index; CABG, Coronary Artery By-pass Grafting; CCS, Canadian Cardiovascular Society; DES, Drug Eluting Stent; LVEF, Left Ventricular Ejection Fraction; NSTEMI, Non ST- elevation Myocardial Infarction; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; STEMI, ST Elevation Myocardial Infarction; UA, Unstable Angina

Assessment of platelet reactivity: All aggregation measurements were performed with impedance aggregometry method in whole blood, using Multiplate® (Dynabyte, Munich, Germany) – platelet activity analyzer –according to the user's manual provided by producer. In turn for assay of platelet function and their response for ASA therapy ASPI-test was performed during all visits (Dynabyte, Munich, Germany). All platelet aggregation measurements were performed 2 hours after ASA intake. It comprises of arachidonic acid that is a substrate for cyclooxygenase to synthetize strong platelet agonist, thromboxane A_2 (TXA_2). When cyclooxygenase-1 (COX-1) is being blocked by ASA, thromboxane synthesis inhibition occurs that results in decreased activation of platelets. Based on the manufacturer's data, it was accepted that the value of 30U was indicated as a cutting point above which resting platelet activity is recognized despite treatment with a acetylsalicylic acid [11]. Recently, test producer differentiates two cutting points: value ≤40 U as COX-1 inhibited by acetylsalicylic acid and value ≤30 U as COX-1 strongly inhibited by acetylsalicylic acid [12]. In 2010 studied population was divided into aspirin resistant (ASA non-responders) and aspirin sensitive (ASA responders), according to then operating cutting point set to 30 U. Due to that fact, studied population was also divided according to the value 30 U during control after 6,3 years.

Statistical analysis: Statistic software Statistica 12.0 in polish version (StatSoft, Tulsa, United States) was used to calculate statistical parameters. Shapiro-Wilk test assay revealed that distribution of random variables did not comprise criteria of normal distribution. According to that quantitative variables were shown as medians and quartile ranges. To compare between medians of independent variables Mann-Whitney test, Kruskal-Wallis test and multiple comparison test were performed. To compare between medians of dependent variables ANOVA Friedmana test and Wilcoxon test were performed. Qualitative variables were presented as number of patients with particular feature and as a percent of analyzed group. Comparison between qualitative variables was performed using $\chi 2$, $\chi 2$ test with Yeats's correction or exact Fisher test, depending on group's abundance. Values $p < 0,05$ were treated as statistically significant (in tables numeric value in bold). Values p 0,05-0,10 were treated as a trend towards statistical significance. Values $p \geq 0,10$ as not significant were replaced with shortcut ns (not significant). Study was designed as post-hoc prospective research analysis and as we have no influence on the number of patients in long term follow-up power analysis was not performed.

RESULTS:

Studied population was divided in two groups, depending on aspirin responsiveness during control visit averagely 6,3 years after hospitalization due to myocardial infarction. HTPR was evaluated basing on the ASPI-test in accordance to the 30 U cutting point. Among 30 studied subjects evaluated during control visit, one subject did not treat aspirin due to stomach ulcer disease (chronic intake of clopidogrel). It was the reason that ASPI-test results from 29 subjects were included for final analysis. Eventually, groups of 12 ASA responders (ASPI-test ≤30 U) and 17 ASA non-responders (ASPI-test >30 U) were isolated.

Clinical and demographical characterization of studied population and results of selected laboratory tests in 2010/2011 years and in 2016/2017 years depending on the correlation of ASA sensitiveness during control after 6,3 years, are shown in table 2. Compared groups in 2010/2011 years differed statistically significant in anthropometric parameters (BMI- Body Mass Index, body mass, waist size). The trend in statistical significance was delivered in the parameters of height and age, whereas studied subgroups did not differ statistically significant among gender. In other values, i.e. coronary disease interview, risk factors and results of selected laboratory tests the differences were not statistically significant.

Compared groups in 2016/2017 years differed statistically significant in anthropometric parameters (body mass, waist size) and MCV (mean corpuscular volume) value. The trend in statistical significance was delivered in the parameter of RBC (red blood cells) value. In other values, i.e. pharmacotherapy and the rest of laboratory tests' results, the differences were not statistically significant.

Table 3 shows comparison of selected parameters both during hospitalization and control visit among studied population. Statistically significant differences were stated in respective components of lipid profile (excluding triglycerides) and blood morphology (excluding RBC value), respectively, together with BNP (Brain Natriuretic Peptide) and hsCRP (high sensitivity C- reactive protein) values. In anthropometric parameters the differences were not statistically significant.

Comparison of clinical endpoints of the study, comprising of unplanned revascularization and ACS (Acute Coronary Syndromes), is presented in table 4. For endpoints related to the coronary disease no statistically significant differences were obtained.

Table 5 shows comparison of results of arachidonic acid-induced platelet aggregation (ASPI-test) during both hospitalization and control visits in studied population. Statistically significant differences were stated comparing all time points with each other together with results obtained during visit after 6,3 years with parallel time point during observation.
Table 5 shows presence of ASA sensitiveness during both hospitalization and control visits in studied population basing on the result of arachidonic acid-induced platelet aggregation (ASPI-test). Comparing number of aspirin resistant obtained in relevant time point, a statistically significant differences were demonstrated.

Table 2. CLINICAL, DEMOGRAPHIC CHARACTERISTICS AND THE RESULTS OF SELECTED LABORATORY TESTS IN STUDY POPULATION IN 2010/2011 YEARS AND IN 2016/2017 YEARS IN DEPENDENCE ON ASA RESPONSIVENESS DURING THE CONTROL VISIT AFTER 6.3 YEARS [MEDIAN (LOWER QUARTILE-UPPER QUARTILE) OR NUMBER (PERCENT)].

Study feature	ASA resistant (ASPI>30) (n=17)	ASA responsive (ASPI≤30) (n=12)	*p*
2010/2011			
Age [years]	63.0 (59.0-67.0)	53.0 (52,0-63,0)	0,0802
Height [cm]	165.0 (164.0-174.0)	171.5 (168,5-176,0)	0,0655
BMI [kg/m^2]	28.3 (23.5-31.2)	30.6 (27,1-35,2)	**0,0476**
Body mass [kg]	76.0 (69.0-81.0)	90.0 (82,0-100,5)	**0,0031**
Girth [cm]	92.0 (90.0-95.0)	100.5 (99,0-106,5)	**0,0123**
Sex [men/women]	11 (64.7%)/ 6 (35.3%)	9 (75.0%)/ 3 (25.0%)	
Infarction passed before admittance	1 (5.9%)	3 (25.0%)	ns
PCI passed before admittance	1 (5.9%)	3 (25.0%)	ns
CABG passed before admittance	0 (0.0 %)	0 (0.0 %)	-
Final diagnosis:			
NSTEMI/UA	2 (11.8%)	1 (8.3%)	ns
STEMI	15 (88.2%)	11 (91.7%)	ns
LVEF [%]	49.0 (42.0-50.0)	43.0 (40.0-47.0)	ns
Risk factors for ischemic heart disease:			
Hyperlipidemia in interview	9 (52.9%)	10 (83.3%)	ns
Arterial hypertension	8 (47.1%)	6 (50.0%)	ns
Diabetes	2 (11.8%)	4 (33.3%)	ns
Current smokers	11 (64.7%)	6 (50.0%)	ns
Past smokers	3 (17.7%)	1 (8.3%)	ns
Lipid profile			
Total cholesterol [mg/dL]	215.0 (182.0-262.0)	198.0 (176.0-249.5)	ns
LDL cholesterol [mg/dL]	136.0 (104.0-164.0)	130.5 (119.0-153.0)	ns
HDL cholesterol [mg/dL]	42.0 (34.0-48.0)	37.5 (31.0-43.0)	ns
Triglycerides [mg/dL]	93.0 (76.0-198.0)	137.5 (93.5-165.0)	ns
WBC [10^3/uL]	8.41 (7.45-10.38)	8.19 (6.14-10.09)	ns
RBC [10^6/uL]	4.5 (4.1-4.7)	4.6 (4.3-4.9)	ns
HGB [g/dL]	13.7 (13.1-14.3)	13.7 (13.5-14.2)	ns
HCT [%]	39.7 (37.8-42.4)	40.3 (38.3-42.6)	ns
MCV	89.8 (87.9-93.3)	87.2 (86.0-90.0)	ns
PLT [10^3/uL]	210.0 (179.0-267.0)	198.0 (162.5-237.5)	ns
MPV [fL]	10.6 (10.2-11.1)	10.8 (10.0-11.5)	ns
Others			ns
BNP [mg/dL]	97.4 (53.0-155.5)	128.5 (57.6-282.3)	ns
hsCRP [pg/mL]	8.7 (4.2-32.4)	6.8 (3.2-25.7)	ns
HbA1C [mg/dL]	5.9 (5.7-6.5)	6.1 (5.7-6.3)	ns
Arachidonic acid-dependent aggregation [U]	17.0 (5.0-24.0)	10.5 (5.0-21.0)	ns

2016/2017			
Height [cm]	168.0 (164.0-174.0)	171.5 (165.5-177.0)	ns
Body mass [kg]	76.0 (71.0-86.7)	85.0 (83.0-88.5)	**0,0476**
BMI [kg/m^2]	27.5 (23.8-31.0)	28.6 (27.3-32.3)	ns
Girth [cm]	94.0 (89.0-97.0)	99.0 (96.5-105.0)	**0,0161**
Smokers:			
No	3 (17.7%)	4 (33.3%)	
Current smokers	4 (23.5%)	2 (16.7%)	ns
Past smokers	8 (47.1%)	6 (50.0%)	
Occasionally	2 (11.8%)	0 (0.0%)	
Alcohol:			
No	16 (94.1%)	10 (83.3%)	ns
Past	1 (5.6%)	2 (16.7%)	
NYHA class			
I	14 (82.4%)	12 (100.0%)	ns
II	3 (17.7%)	0 (0.0%)	
CCS class			
I	17 (100.0%)	12 (100.0%)	-
Concomitant treatment			
Aspirin (dose 75-100 mg)	17 (100.0%)	12 (100.0%)	-
Beta blocker	17 (100.0%)	12 (100.0%)	-
Angiotensin converting enzyme inhibitor	17 (100.0%)	12 (100.0%)	-
Statin	16 (94.1%)	11 (91.7%)	ns
Proton pump inhibitor	1 (5.9%)	4 (33.3%)	ns
Clopidogrel	1 (5.9%)	3 (25.0%)	ns
Lipid profile			
Total cholesterol [mg/dL]	175.0 (161.0-214.0)	172.5 (146.5-203.5)	ns
LDL cholesterol [mg/dL]	96.0 (85.0-127.0)	101.0 (74.5-131.0)	ns
HDL cholesterol [mg/dL]	47.0 (41.0-55.0)	45.5 (39.5-51.0)	ns
Triglycerides [mg/dL]	147.0 (116.0-217.0)	130.0 (109.5-207.5)	ns
Morphology			
WBC [10^3/uL]	7.12 (6.05-7.79)	6.33 (5.44-7.16)	ns
RBC [10^6/uL]	4.6 (4.4-4.8)	4.7 (4.5-5.0)	0,0802
HGB [g/dL]	14.4 (13.5-15.0)	14.1 (14.0-14.9)	ns
HCT [%]	42.1 (40.2-43.9)	41.1 (40.8-43.7)	ns
MCV	91.2 (89.5-95.4)	89.3 (87.6-91.3)	**0,0469**
PLT [10^3/uL]	249.0 (199.0-279.0)	202.0 (186.0-245.0)	ns
MPV [fL]	10.2 (9.7-10.6)	10.2 (9.8-10.7)	ns
Others			
BNP [mg/dL]	48.0 (20.1-100.9)	44.1 (26.5-68.4)	ns
hsCRP [pg/mL]	2.3 (0.8-5.6)	1.0 (0.5-2.0)	ns
HbA1c [mg/dL]	6.5 (5.9-8.0	6.2 (5.9-6.4)	ns

BMI, Body Mass Index; BNP, Brain Natriuretic Peptide; CABG, Coronary Artery By-pass Grafting; CCS, Canadian Cardiovascular Society; HbA1c, glycated haemoglobin; Hct, hematocrit; HDL, High Density Lipoproteins; HGB, hemoglobin, hsCRP, high sensitivity C-reactive protein; LDL, Low Density Lipoproteins; LVEF, Left Ventricular Ejection Fraction; PCI, percutaneous coronary intervention; PLT, Platelets; MCV, Mean Corpuscular Volume; MPV, mean platelet volume; NSTEMI, Non ST-elevation Myocardial Infarction; NYHA, New York Heart Association; RBC, red blood cells; STEMI, ST Elevation Myocardial Infarction; UA, Unstable Angina; WBC, white blood cells

Table 3. COMPARISON OF SELECTED PARAMETERS IN STUDY POPULATION IN 2010/2011 AND 2016/2017 YEAR [MEDIAN (LOWER QUARTILE-UPPER QUARTILE) OR NUMBER (PERCENT)].

Study feature	Property value (n=29) 2010/2011 year	Property value (n=29) 2016/2017 year	*p*
Height [cm]	170.0 (164.0-176.0)	169.0 (164.0-176.0)	ns
Body mass [kg]	81.0 (76.0-90.0)	83.0 (74.5-87.0)	ns
BMI [kg/m^2]	28.1 (25.1-32.3)	29.0 (24.5-32.2)	ns
Girth [cm]	95.0 (91.0-104.0)	96.0 (93.0-100.0)	ns
Lipid profile			
Total cholesterol [mg/dL]	211.0 (182.0-261.0)	175.0 (155.0-210.0)	**0,0410**
LDL cholesterol [mg/dL]	134.0 (110.0-164.0)	100.0 (84.0-127.0)	**0,0133**
HDL cholesterol [mg/dL]	40.0 (33.0-44.0)	47.0 (40.0-54.0)	**0,0008**
Triglycerides [mg/dL]	114.0 (83.0-180.0)	135.0 (116.0-217.0)	ns
Morphology			
WBC [10^3/uL]	8.41 (6.94-10.38)	6.71 (5.83-7.75)	**0,0014**
RBC [10^6/uL]	4.5 (4.2-4.8)	4.6 (4.4-4.8)	ns
HGB [g/dL]	13.7 (13.1-14.3)	14.3 (13.9-15.0)	**0,0065**
HCT [%]	40.2 (38.0-42.5)	41.2 (40.6-43.9)	**0,0169**
MCV	89.5 (86.5-91.4)	90.0 (88.2-92.0)	**0,0214**
PLT [10^3/uL]	208.0 (178.0-248.0)	215.0 (190.0-271.0)	**0,0342**
MPV [fL]	10.6 (10.2-11.3)	10.2 (9.7-10.7)	**0,00003**
Others:			
BNP [mg/dL]	99.0 (53.0-226.8)	46.4 (26.4-99.0)	**0,0007**
hsCRP [pg/mL]	8.4 (3.4-29.0)	1.2 (0.7-3.3)	**0,00006**
HbA1C [mg/dL]	5.9 (5.7-6.4)	6.3 (5.9-6.4)	ns

BMI, Body Mass Index; BNP, Brain Natriuretic Peptide; HbA1c, glycated haemoglobin; Hct, hematocrit; HDL, High Density Lipoproteins; HGB, hemoglobin; hsCRP, high sensitivity C- reactive protein; LDL, Low Density Lipoproteins; PLT, Platelets; MCV, Mean Corpuscular Volume; MPV, mean platelet volume; RBC, red blood cells; WBC, white blood cells

Table 4. CLINICAL END-POINTS OF THE STUDY IN DEPENDENCE ON ASA RESPONSIVENESS DURING THE ALL OBSERVATION IN STUDY POPULATION BASED ON ARACHIDONIC ACID-DEPENDENT PLATELET AGGREGATION (ASPI-TEST) [NUMBER (PERCENT)].

Study feature	ASA resistant (ASPI >30) (n=17)	ASA responsive (ASPI≤30) (n=12)	p
ACS	3 (17.6%)	5 (41.7%)	ns
Unplanned revascularization (PCI, CABG)	3 (17.6%)	5 (41.7%)	ns
End-points (ACS, unplanned revascularization)	3 (17.6%)	5 (41.7%)	ns

ACS, acute coronary syndrome; ASA, acetylsalicylic acid; CABG, Coronary Artery By-pass Grafting; PCI, percutaneous coronary intervention

Table 5. ARACHIDONIC ACID-DEPENDENT PLATELET AGGREGATION (ASPI-TEST) AND ASA RESISTANCE COMPARISON DURING HOSPITALIZATION AND CONTROL VISITS IN IN STUDY POPULATION [MEDIAN (LOWER QUARTILE-UPPER QUARTILE) OR NUMBER (PERCENT)].

Time point	ASPI-test result [U] (n=29)	p*	p#
Hospitalization	12.0 (5.0-22.0)	0.00001	0.000154
3rd month visit	12.0 (7.0-21.0)		0.000017
6th month visit	11.0 (6.0-16.0)		0.000154
9th month visit	11.0 (6.0-17.5)		0.000321
After 6.3 - year visit	32 (17.0-54.0)		-
Time point	**ASA resistant (ASPI>30), (n=29)**	**p***	**p#**
Hospitalization	2 (6.9%)	< 0.00001	0.00001
3rd month visit	4 (13.8%)		0.00026
6th month visit	3 (11.1%)		0.00012
9th month visit	3 (10.7%)		0.00008
After 6.3- year visit	17 (58.6%)		-

** - p for comparing all time points*

 # - p for comparing results from a given time point with the result of the 6.3-year visit

DISCUSSION:

ASA is a basic antiplatelet therapeutic drug applied in prevention for secondary coronary disease. Its effectiveness was confirmed in many studies with randomization [2]. Studied group was evaluated basing mainly on the presence of aspirin resistance during control visit averagely 6,3 years after hospitalization from myocardial infarction. For various reasons covered 30 subjects from the original group- it should be emphasized that probably the obtained results were influenced by the size of the study group.

In aspect of total observation it is important to notice statistically significant differences of arachidonic acid-induced platelet aggregation (ASPI-test) during both hospitalization and control visits. Median of ASPI-test values during control visits up to 9 months after myocardial infarction in subjects equaled ≤30 U, while during control visit after 6,3 years this value reached >30 U (Table 5). Those aggregation results reflect the occurrence of ASA sensitiveness among subjects during total observation basing on ASPI-test (Table 5). The least percentage of aspirin resistant subjects was stated during hospitalization (6,9%), while the utmost percentage was observed during visit after 6,3 years (58,6%). Comparison of ASA resistant subjects value obtained in relevant time point indicated statistically significant differences.

There are several factors possibly responsible for presented results, all of which are confirmed in existing studies. Time passed from hospitalization with simultaneous complaint deficit and cardiovascular complications might lead to disruption of antiplatelet treatment (although all subjects declared regular drug intake). It is considered to be most frequent trigger of deceptive ASA resistance [13].

Similar conclusions were stated in the study of Schwartz et al. and Tantra et al., where it was proven that disobedience of doctor's recommendations was causing resistance for ASA [14,15]. The irregularity of drug intake is estimated as 40% among subjects with cardiovascular system diseases [7, 14, 16].

Another reason indicates usage of non-steroid anti-inflammatory drugs, especially ibuprofen, which competes for binding with COX-1 active center [17, 18]. These drugs are not recommended for subjects with cardiovascular diseases, although such drugs are widely used in pharmacotherapy of many diseases and commonly available. Other drug interactions may include i.e. intensified hydrolysis of aspirin caused by esterases after proton pump inhibitors intake. These drugs are commonly used during gastroprotection [19, 20].

More reasons confirming obtained results data might be both decreased bioavailability of aspirin, connected with impaired absorption, and insufficient aspirin dosage [13].

Another reason confirming obtained results may be therapy-span-related drug tolerance that is confirmed by the research from Pulcinelli et al. [21]. The study group was over 6 years after myocardial infarction.

Although the utmost percentage aspirin resistant was observed during visit after 6,3 years- 58,6% no statistically significant differences were stated between the groups studied in terms of endpoints that were linked with coronary disease. In previous studies including two meta-analysis covering 15 to 20 test conducted on circa 3 thousand subjects with CV disease the results indicated that aspirin resistance is linked with almost 4-fold increase in the risk of ACS, 6-fold increase in mortality, and nearly 4-fold increase in the risk of any CV event among patients with ASA resistance compared to those sensitive to ASA [6, 22]. Undoubtedly, laboratory resistance to ASA has been demonstrated in the study population, which did not affect cardiovascular events, although the cohort studied is too small to draw further conclusions.

Another evaluation applied among testes subjects was comparison of both ASA non-responders and ASA responders in the field of chosen parameters during primary hospitalization and control visit.

During 2010/11 years hospitalization caused by myocardial infarction statistically significant differences between both groups were noticed in waist size, mass and BMI. Similar results (excluding BMI) were obtained during control visit 6,3 years after myocardial infarction, whereby higher values in abovementioned were noticed in ASA responders. Obtained results are at variance with those from literature data, for in previous studies obesity was indicated as a risk factor for aspirin resistance [23, 14, 16].

Another applied comparison among studied group involving chosen parameters during both hospitalization and control visit indicates statistically significant differences primarily in the field of laboratory tests. Statistically significant differences were stated in respective components of lipid profile (excluding triglycerides) – during control visit lower values of total cholesterol (p<0,0410) and LDL (Low Density Lipoproteins) (p<0,0133) comparing to those from hospitalization period were collected. It is important to notice that LDL concentration (median 100 mg/dl) does not reach a target value (<70 mg/dl) recommended by ESC (European Society of Cardiology) in secondary prevention despite that most of the subjects declared regular statin intake [24].

Inflammatory state parameters analysis also indicated statistically significant differences between compared subject groups in leucocytes level (p=0,0014) and hs-CRP (p=0,00006) in comparison to the results from hospitalization and control visit after 6,3 years. Median of the results obtained during control was lower what might be connected with stable clinical state. Inflammation and associated oxidative stress play a major role in the development of ASA resistance. Cells participating in the inflammatory response show increased expression of COX-2, leading to increased generation of TXA_2 and its precursors, prostaglandins H2 and G2 which also may serve as ligands for platelet TXA_2 receptors or be used as a substrate for TXA_2 synthesis by platelet COX-1. In addition, inflammation is usually accompanied by a prothrombotic state [25]. Higher results during myocardial infarction might have been connected with the occurrence of vulnerable plaques that correlates with inflammatory markers. Activation of inflammatory trails is strictly connected with pathogenesis of arteriosclerosis. This confirms a relation between high-risk plaques and indigenous inflammation process, where inflammatory mediators both increase platelets activity and activate coagulation cascade [26- 28]. Collected data indicates high influence of inflammation process on aspirin-resistance and the development of arteriosclerosis taking part in formation of unstable arteriosclerosis plaque.

In the range of BNP values, statistical analysis indicates statistically significant difference between compared subject groups in comparing results from both hospitalization and control visit after 6,3 years (p=0,0007). BNP is one of the markers used in risk stratification of i.e. acute coronary syndromes. Although it must be remembered that temporal increase of BNP concentration might occur shortly after percutaneous coronary intervention [29]. Median of BNP values during hospitalization was 99 mg/dl (according to ESC diagnostic algorithm – heart failure is then less probable) [30]. Results obtained during control were lower what might be connected with stable clinical state.

Comparing respective components of peripheral blood morphology (HGB- hemoglobin, HCT- hematocrit, MCV, PLT- platelets, MPV- mean platelet volume) excluding RCB statistically significant differences between compared subject groups were stated. Median from results obtained during control was higher what might be connected with stable clinical state. Higher concentration of thrombocytes might be connected with increased distribution of platelets leading to augmented release of their young forms that are able to produce COX-1-independent TXA_2 [31,32]. Obtained result might be consecutive explanation for stated highest percentage of aspirin resistant (58,6%) during control visit after 6,3 years.

Currently routine test for resistance as well as monitoring of anti-platelet treatment effects or treatment modification is currently not recommended as a standard progressing in daily clinical practice [33].

Limitations:

Our study had some important limitations. First, small count of the study group. Second, compliance regarding aspirin treatment was evaluated based on patient self-reports. It is difficult to verify to what extent the demonstrated aspirin resistance may be associated with reduced bioavailability. An optimal approach would involve directly observed administration of an aspirin dose, followed by testing performed after a specified uniform time in all participants. Third, we did not analyze genetic polymorphisms involved in biotransformation of aspirin, nor did we assess alternative platelet activation or TXA_2 synthesis pathways. Fourth, although the ASPI test assay remains a very well validated method of platelet function monitoring, we did not assess platelet activation by another method. Fifth, the evaluated clinical endpoints of the study were associated only with coronary artery disease. Sixth, this study was designed as post-hoc prospective research analysis and as we have no influence on the number of patients in long term follow-up power analysis was not performed.

CONCLUSIONS:

In conclusion it must be stated that HTPR is a well-documented phenomenon. Available literature indicates that scale of the phenomenon in subjects with cardiovascular diseases is wide but depends greatly on applied laboratory method, actual clinical state of a subject, concurrent diseases, drug interaction etc. In specific situations, the assessment of aspirin resistance should be considered, although this is not a standard practice.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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