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RĪGAS STRADIŅA UNIVERSITĀTE

Inga Urtāne

THE ROLE OF GENETIC FACTORS ON THE EFFECT OF ADDITIONAL DOSES OF CLOPIDOGREL TO IMPROVE PERSONALIZED EFFECTIVENESS IN PATIENTS WITH HYPORESPONSIVENESS

Summary of the Doctoral Thesis
for obtaining the degree of a Doctor of Pharmacy
Speciality – Clinical pharmacy

Riga, 2014

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Doctoral thesis performed at: Latvian Centre of Cardiology, Pauls Stradins Clinical University Hospital and Latvian Research Institute of Cardiology, University of Latvia in collaboration with Latvian Biomedical Research and Study Centre and Cell Transplantation Centre, Pauls Stradins Clinical University Hospital

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Secretary of Promotion Council:
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CONTENT

ABBREVIATIONS

A2RB – angiotensin II receptor blockers

ABCB1 – ATP-binding cassette sub-family B member 1

ACEI – angiotensin converting enzyme inhibitors

ACS – acute coronary syndrome

ADP – adenosine diphosphate

ALT – alanine aminotransferase

APTT – activated partial thromboplastin time

AST – aspartate aminotransferase

BB – beta blockers

BMC – Biomedical Research and Study Centre

BMI – body mass index

CABG – coronary artery bypass grafting

CAD – coronary artery disease

CCB – calcium channel blocker

CHD – coronary heart disease

CHF – congestive heart failure

CI – cerebral infarction

CKD – chronic kidney disease

CK-MB – creatine kinase-MB mass

CRO – C-reactive protein

CV – cardiovascular

CYP – cytochrome

DAPT – dual antiplatelet therapy

DES – drug eluting stent

DM – diabetes mellitus

DNA – deoxyribonucleic acid

EDTA – ethylenediaminetetraacetic

FC – functional class

FITC – fluorecein isothiocyanate

FUP – follow up

GFR – glomerular filtration rate

GP – glycoprotein

HDL-C – high density lipoprotein cholesterol

INR – international normalized ratio

LD(s) – loading dose(s)

LDL-C – low density lipoprotein cholesterol

MD – maintenance dose

MDR1 – multi drug resistance protein 1

MFI – mean fluorescence intensity

MFIc – calculated mean fluorescence intensity

MI – myocardial infarction

NSAID(s) – nonsteroidal anti-inflammatory drug(s)

NSTEMI – non-ST-segment elevation myocardial infarction

NYHA – New York Heart Association

PCI – percutaneous coronary intervention

PCR – polymerase chain reaction

PE – fico-eritrin

PGE1 – prostaglandin E1

PPI – proton pump inhibitor

PRI – platelet reactivity index

SD – standart deviation

ST – stent thrombosis

STEMI – ST-segment elevation myocardial infarction

TC – total cholesterol

TG – triglycerides

TVR – target vessel revascularization

USA – United States of America

VASP – vasodilatorstimulated phosphoprotein

VASP-P – vasodilatorstimulated phosphoprotein phosphorylation

WBC – white blood cells

INTRODUCTION

Dual antiplatelet therapy (DAPT) as a combination of aspirin and $P2Y_{12}$ receptor antagonist clopidogrel reduces atherothrombotic complications in patients with acute coronary syndrome (ACS) and after percutaneous coronary intervention (PCI) with stent implantation [King et al., 2008, Wijns et al., 2010]. Lately, new generation $P2Y_{12}$ receptor antagonists (prasugrel and ticagrelor) are used in DAPT models [Bonello et al., 2010, Wijns et al., 2010]. The shortcomings of new generation $P2Y_{12}$ receptor antagonists are an increase of therapy costs and lack of evidence in patient after eletive PCI, which increase the importance of further research and understanding of clopidogrel [Brandt et al., 2007, Varenhorst et al., 2009].

Based on functional tests of platelet reactivity, reduced response to clopidogrel (hyporesponsiveness) is described in studies in up to 50% of cases [Gurbel et al., 2003, O'Donoghue et al., 2006, Angiolillo et al., 2007, Bonello et al., 2010], but the real resistance of clopidogrel is encountered more rarely – in 2 to 11% of cases [Papathanasiou et al., 2007, Kim et al., 2009]. In the conducted studies it was established that hyporesponsiveness of clopidogrel (platelet reactivity index (PRI) ≥50%) is related with the frequency of cardiovascular (CV) death, myocardial infarction (MI) and stent thrombosis (ST) in patients after PCI [Aradi et al., 2010, Brar et al., 2011]. As the incidence of ST was assessed very low (<1% during the first year after PCI with stent implantation) [Mallouk et al., 2012, Sudhir et al., 2013], the question arises whether PRI target <50% is not a too conservative aim and whether PRI <60% would be more eligible to select patients with clinically higher ST and CV risk [Motovska et al., 2009].

Cytochrome (*CYP*) *2C19* and ATP-binding cassette sub-family B member 1 (*ABCB1*) gene variations are related with the frequency of

hyporesponsiveness and CV events in patients with clopidogrel therapy [Simon et al., 2009, Wallentin et al., 2010, Mega et al., 2010, Bonello et al., 2012]. Decreased response to DAPT is observed more frequently in patients with diabetes mellitus (DM) [Gurbel et al., 2007, Ferreiro et al., 2010, Hall et al., 2011], increased body mass index (BMI) [Feher et al., 2007, Mallouk et al., 2012], and is reported also as the potential effect of concomitantly received drugs [Gilard et al., 2008, Kwok et al., 2012]. In literature there have been attemps to overcome the hyporesponsiveness by using additional loading doses (LDs) and higher maintenance doses (MDs) of clopidogrel [Price et al., 2011, Bonello et al., 2008, Bonello-Palot et al., 2009].

Till now there have been very few studies on the strategy of additional dosing regarding the effect of *CYP2C19* and *ABCB1* gene variation. It is not clear whether the efficiency of modified dosage of clopidogrel is related to genetic factors and whether the strategy of overcoming the hyporesponsiveness may differ according to the type of the risk allele.

HYPOTHESES OF THE STUDY

- genetic and phenotypic factors facilitating hyporesponsiveness of clopidigrel influence differently the efficiency of additional LDs and higher MD, so the optimal DAPT strategy may differ depending on the possible mechanism of hyporesponsiveness or resistance;
- PRI target below 60% for patients after PCI with DES is as safe and efficient as PRI target below 50%.

AIM OF THE STUDY

Analyse the effect of *CYP2C19*, *CYP2C9* and *ABCB1* genetic polymorphisms on additional clopidogrel LDs (600 mg) and higher MD (150 mg) to overcome hyporesponsiveness and to clarify the safety of higher PRI target (<60%) in long-term during 1 year.

OBJECTIVES OF THE STUDY

1. To analyse the differences of efficiency of initial LD and additional LDs of clopidogrel in following groups of genotype polymorphisms: *CYP2C19*, *CYP2C9*, *ABCB1*.
2. To analyse the general efficacy of the strategy of additional clopidogrel LDs.
3. To identify patients with clopidogrel resistance and clarify whether the new generation $P2Y_{12}$ receptor inhibitors can overcome hyporesponsiveness in these patients.
4. To analyse the effect of polymorphisms on efficacy of higher clopidogrel MD compared with standard MD.
5. To clarify the interaction of phenotypic factors with efficiency of clopidogrel additional LDs and higher MD.
6. To analyse the influence of other concomitant drug therapy on the efficacy of clopidogrel standard and modified doses.
7. To clarify the efficiency and safety of the strategy of PRI target <60% within 1 year after PCI with DES.

NOVELTY OF THE STUDY

Analysis of causes of clopidogrel hyporesponsiveness is of great scientific and practical importance. In the scientific field there is very little information on the therapeutical strategy for individual patient with decreased response to clopidogrel therapy. There is also lack of information about the efficiency of additional doses of clopidogrel according to genotype.

In the previous studies, efficacy of additional doses were verified for acute patients after receiving 600 mg LD of clopidogrel, but in this study the prevalence of clopidogrel hyporesponsivity was studied also in patients after elective PCI with 300 mg of clopidogrel LD. Individual LD and MD correction was developed for each patient. Different MD (150 mg and 75 mg) were tested for platelet reactivity for the same patients with established hyporesponsiveness of clopidogrel.

The influence of genetic factors was analysed for additional LDs and different MD of clopidogrel. The safety of PRI above 60% was also verified in order to select a group of patients with potentially higher CV risk.

1. MATERIALS AND METHODS

1.1. Place of the study

The research was performed in the Latvian Centre of Cardiology at Paula Stradina Clinical University Hospital and in the Latvian Research Institute of Cardiology in collaboration with Latvian Biomedical Research and Study centre (BMC) and Cell Transplantation Centre at Paula Stradina Clinical University Hospital.

1.2. Study design and selection criteria

We selected patients among those who were referred to the Latvian Centre of Cardiology for elective or acute PCI with DES implantation. All patients received clopidogrel therapy for prevention of thrombosis after stent implantation and after ACS according to latest guidelines. The study was approved by Pauls Stradins Clinical Univeristy Hospital Ethical Committee for Clinical Research (statement No. 300610 – 2L, 30 June 2010).

Inclusion criteria:

- Patient underwent PCI with at least one DES implantation.
- Patient has received 300 mg or 600 mg LD of clopidogrel during last 24 hours before PCI.
- By signing the informed consent, patient agree to participate in the study, as wel for repeated withdrawl of blood samples, for repeated phone interviews and agree to arrive for follow-up (FUP) visits.

Exclusion criteria:

- The patient did not want to participate or noncompliance to therapy was expected.
- Congestive heart failure (CHF) NYHA functional class (FC) IV.
- Active bleeding or history of bleeding diathesis. Trombocytopathya. Platelet count $<100\times10^9$/l.
- Oral anticoagulant therapy.
- Severe liver disease (cirrhosis, hepatitis) or serum bilirubin level > 2 mg/dl (>34.2 μmol/l).
- Hemorrhagic stroke or any stroke of unspecified cause.

- Malignancy or other concurrent severe illness with expected survival <1 year.
- Allergy towards drugs used during the study.

1.3. Data Acquisition Methods

1.3.1. Phenotype data

After written consent was obtained, information on each patient involved in the study, was entered into a special study questionnaire with a special code. Information on patient's identity (encryption of code) and the questionnaires were kept only in Latvian Centre of Cardiology and were not transferred further. Phenotypic information was obtained about all patients. The analysis of laboratory and genetic polymorphism were also performed. Following conventional risk factors were registered for each patient: age, gender, smoking status (non-smoker, active or ex-smoker, if he/she has quit > 1 month, how long is he/she smoking (< or > 1 year)), arterial hypertension (AH) (stage). Special care was taken to summarize the data of risk factors for ST: DM and its type, congestive heart failure (CHF) and its class, malignancy, chronic liver diseases. Data on coronary heart disease (CHD) and the revascularization of coronary arteries in history were also collected: MI in history (amount, time since last MI), number of PCI, number and type of stents, treated artery, coronary artery bypass grafting (CABG) (number, type).

Data about prehospital and intra-hospital treatment were collected: clopidogrel (dose, length of application), aspirin (dose, length of application), other nonsteroidal anti-inflammatory drugs (NSAID), statins, angiotensin-converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (A2RB), beta blockers (BB), calcium channel blockers (CCB), nitrates, antiarrhythmic agents, proton pump inhibitors (PPI) (type, dose), antibacterial agent, agents for

DM therapy. The weight and height of patient was determined and BMI was calculated.

Other measurements included serum lipids (total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG)), glucose, liver functional markers: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, creatinine, C-reactive protein (CRP). In citrated plasma we determined the level of fibrinogen, activated partial thromboplastin time (APTT), international normalized ratio (INR), cardiologic markers: troponin I and fraction of creatine kinase-MB (CK-MB). In blood sample at presence of ethylenediaminetetra-acetate (EDTA) – haemoglobin, hematocrit and full blood count (erythrocytes, leucocytes, platelet), average erythrocyte and platelet volume and erythrocyte sedimentation rate.

On day 40 the patients were followed up and repeated VASP phosphorylation analysis was made determining PRI, and the registered data on hospitalization during the last five weeks (frequency and cause), observation of bleeding, pharmacotherapy was marked.

Interviews by phone were ensured in the following periods since the time of inclusion – 3 months, 6 months, 1 year.

1.3.2. Genetic analysis

After written informed consent was obtained, all patients were included in Latvian Genome Databse for that a permit from Ethics Commission (responsible person *Dr. biol.* J. Kloviņš) is received. Blood samples for genetic analysis (two tubes with EDTA (10ml, BD Vacutainer, Franklin Lakes, NJ, USA) and one SST tube (BD Vacutainer SST^{TM} Gel Separator Tube, 5ml, BD Vacutainer, Franklin Lakes, NJ, USA) were marked with patient's code and

delivered to Latvian BMC. Deoxyribonucleic acid (DNA) was extracted according to standard phenol-isopropanol method. Seven polymorphisms were determined: *CYP2C19**2, *CYP2C19**3, *CYP2C19**5, *CYP2C19**17, *CYP2C9**2, *CYP2C9**3, *ABCB1*. Genotyping was performed with *Applied BiosystemsTaqMan SNP Genotyping Assay*, by means of *Vii7 Real-Time PCR system (Life Technologies)*. Response of sample genotype signals were performed with AutoCaller 1.1 (Applied Biosystems) program.

1.3.3. Clopidogrel resistance analysis

Clopidogrel resistance (hyporesponsiveness) was defined as PRI ≥60% after the initial LD of clopidogrel 300 mg (elective PCI with DES) or 600 mg (primary or acute PCI with DES) was received. During the research PRI was determined by VASP test (PLT VASP/P2Y12) – in hospital (1-4 times), ambulatory – on day 10 (if reduced effectiveness of clopidogrel is established) and on day 40. VASP phosphorilation analysis, was made within 24 hours after LD of clopidogrel was received. If PRI was ≥60%, patient received additionally up to 3 additional LDs (3 x 600 mg) with the aim to get PRI <60%.

If this target could not be achieved with the first LD, but was later on with additional LDs, patients were determined as hyporesponders and received clopidogrel therapy 150 mg daily for the first month and continued the standard MD UD (75 mg daily) further on. For hyporesponders VASP phosphorylation analysis was performed also on day 10 while being on MD of 150 mg and on day 40 while on 75 mg of clopidogrel. If PRI target <60% was not reached with these 3 additional LDs, patients were determined as real nonresponders to clopidogrel therapy and received another antiplatelet (ticagrelor) therapy. For real nonresponders VASP phosphorylation analysis was performed also on day 40 while being on ticagrelor therapy.

If PRI target <60% was reached already after first LD, patient were determined as responders to clopidogrel therapy and received standard clopidogrel MD (75 mg daily) further on. For responders VASP phosphorylation analysis was performed also on day 40 while being on 75 mg of clopidogrel.

Analyses with flow cytometer

The VASP phosphorylation analysis was performed using platelet VASP kits (BIOCYTEX, France). Blood sample was incubated with prostaglandin E1 (PGE1) or with PGE1 and adenosine diphosphate (ADP). After cell permeabilization, the phosphorylated VASP was marked with indirect immunofluorescence method using a specific monoclonal antibody (16C2). Dual colour flow citometre analysis allows comparing the parameter in two testing circumstances and increasing ADP capacity of each sample inhibiting VASP phosphorylation.

During analyses with cytometer (Beckman Coulter FC Flow Cytometer Series, Beckman Coulter, Miami, Florida, USA) after radiating the samples with argon laser rays, radiation was obtained from the antibodies marked with flourochrome fluorescein isothiocyanate (FITC) and fico-eritrin (PE) respectively in range of 495–521nm and 565–585nm frequency. Intensity of FITC fluorochrome radiation correlates with VASP concentration in the sample. PRI was calculated, using the mean fluorescence intensity (MFI). To obtain accurate data, corrected MFI (MFI_c – mean fluorescence intensity corrected) is used. PRI was calculated according to the following formula: $PRI = (MFIc_{PGE1} - MFIc\ (_{PGE1 + ADP}) / MFI_{c\ PGE1}) \times 100$.

1.3.4. Statistical methods

All statistical calculations were performed with SPSS software (IBM SPSS Statistics Version 17, SPSS inc., USA). Statistically reliable data were regarded to be those with p value being <0.05. Quantitative variables were described with arithmetical mean and standard deviation (SD). In cases when division sharply differed from the normal one, the median value and standard error or interquartile interval was calculated. Categorical or qualitative variables were characterized as number and percentage. The variables the dispersion of which sharply differed from the normal one, were transformed, approaching the dispersion to the normal and only transformed values were used in further calculations. Comparison of normally divided quantitative variables was made with Student's t-test between two groups or with ANOVA method among three and more groups. Relation between two quantitative variables, and among the genetic variation groups and quantitative variable was analysed by means of linear regression method. Categorical variables were compared with Pearson χ^2 test or Fisher exact test in accordance with test usage instructions. Multivariate analyses were performed for genetic factors influence analysis. Multiple linear regression and ANCOVA method was used in quantitative variables analysis. Categorical variables were analysed with logistic regression analysis.

Conformity of observed genotypes to Hardy-Weinberg equilibrium was verified with χ^2 conformity test (*Chi-square Goodness of Fit test*), comparing the observed and anticipated genotype frequency. The anticipated genotype frequency was calculated from the observed frequency of alleles and their combination probability by means of formula: $(p \times q)^2 = p^2 + 2pq + q^2$, where

p – frequency of most widespread allele occurrence, q – frequency of most rare allele occurrence, pp, pq, qq – combination of both alleles that form the respective genotypes.

2. RESULTS

2.1. Phenotype characterization of the study sample

Between September 2010 and December 2012, we selected 94 patients according to the inclusion and exclusion criteria. Summary of the phenotype data (demographic data, risk factors, laboratory evaluation and pre-hospital treatment) is given in Table 2.1. Majority of the study patients (n=75, 79.8%) underwent elective PCI and only 19 patients (20.2%) underwent emergent or urgent PCI with DES due to ACS.

Table 2.1.

Non-genetic characteristics of the study population (n=94)

Characteristic	
Age (years), mean±SD	63.0±9.7
Men, n (%)	50 (53.2)
BMI (kg/m^2), mean±SD	29.7±4.6
Obesity (BMI>30kg/m^2), n (%)	40 (42.6)
Abdominal circumference (cm), mean±SD	103.5±11.0
Active smoker, n (%)	11 (11.7)
DM, n (%)	28 (29.8)
AH, n (%)	84 (89.4)
I stage	14 (18.2)
II stage	58 (75.3)
III stage	5 (6.5)
CHF, n (%)	59 (64.1)
NYHA I	18 (30.5)
NYHA II	38 (64.4)
NYHA III	3 (5.1)
History of CABG, n (%)	2 (2.1)
History of PCI, n (%)	30 (31.9)

Characteristic	
PCI indication, n (%)	
Stable CAD	75 (79.8)
Unstable angina	9 (9.6)
STEMI	8 (8.5)
NSTEMI	2 (2.1)
Pre-hospital therapy, n (%)	
Aspirin	71 (75.5)
Aspirin dose:	
50 mg/per day	1 (1.5)
75 mg/per day	15 (22.4)
100 mg/per day	45 (67.1)
150 mg/per day	6 (9.0)
Clopidogrel	6 (6.4)
PPI type:	
Pantoprazole	6 (50.0)
Omeprazole	6 (50.0)
Statins	68 (75.6)
BB	65 (72.2)
ACEI un A2RB	64 (71.1)
CCB	35 (38.9)
Nitrates	17 (18.9)
Antiarrhythmic drugs	1 (1.1)
Benzodiazepines	3 (3.4)
Omega-3	16 (18.0)
Laboratory evaluation, mean ± SD	
WBC, 10^9/l	7.7±2.2
Hemoglobin, g/l	1.4±0.2
Platelets, 10^3/l	224.7±52.4
Fibrinogen, g/l	3.4±1.3
Creatinine, μmol/l	83.8±23.7
GFR, ml/min	63.2±23.9
TC, mmol/l	4.6±1.4
HDL-C, mmol/l	1.2±0.4
LDL-C, mmol/l	2.7±1.1
TG, mmol/l	1.4±0.9

BMI – body mass index, CABG – coronary artery bypass grafting, STEMI – ST-segment elevation myocardial infarction, NSTEMI – non-ST- segment elevation myocardial infarction, DM – diabetes mellitus, MI – myocardial infarction, PCI – percutaneous coronary intervention, CHF – congestive heart failure, CAD – coronary artery disease, AH – arterial hypertension, PPI – proton pump inhibitor, BB – beta blockers, CCB – calcium channel blockers, ACEI – angiotensin converting enzyme inhibitors, A2RB – angiotensin II receptor blockers, WBC – white blood cells, GFR – glomerular filtration rate, TC – total cholesterol, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, TG – triglycerides, SD – standart deviation.

2.2. PRI variability after the initial LD of clopidogrel

Patients undergoing PCI with DES received LD of clopidogrel according to the guidelines, namely, 300 mg (89.4%) or 600 mg (10.6%) for patients with elective or acute PCI, respectively. The mean PRI after the initial LD of clopidogrel (PRI1) was 68.4±18.8%. The mean PRI for elective cases who received 300 mg LD of clopidogrel was not significantly lower compared to acute patients who received 600mg LD of clopidogrel (67.7±19.6 vs. 74.0±8.0; p=0.321). We observed large inter-individual variability in clopidogrel responsiveness, with PRI ranging from 8% to 94%. The majority of patients (n=68, 72.3%) were hyporesponsive (PRI1 ≥60%) to initial clopidogrel LD (Figure 2.1). All patients with initial LD of 600 mg had PRI1 ≥60%.

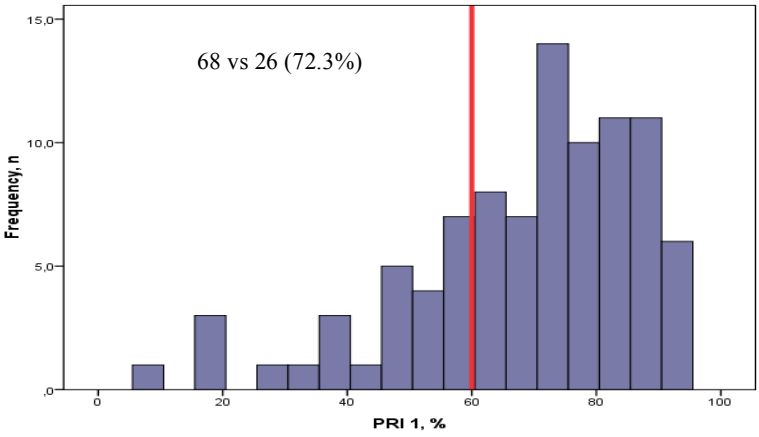

Fig. 2.1. Baseline PRI after the initial clopidogrel LD
PRI1 – the first VASP PRI analysis, PRI – platelet reactivity index

2.3. Effect of additional LDs in patients with PRI1 ≥60%

Figure 2.2. summarizes the effect of each clopidogrel additional LD on PRI in hyporesponders. The target PRI was attained with one, two and three additional LDs in 44 (46.8%), 13 (13.8%) and 9 (9.6%) patients, respectively. Two patients (2.1%) were identified as resistant (real nonresponders) to clopidogrel as the three additional LDs were unable to achieve PRI below 60%. Both participants responded, however, to ticagrelor 180 mg / per day, which reduced PRI statistically significantly compared to PRI4 (12.0±1.4 vs. 73.0±4.2; p=0.042) (Figure 2.4.).

Effect of the two MDs (150 mg and 75 mg) on PRI was investigated during follow-up on day 10 and 40, respectively, in hyporesponders, and on day 40 (75 mg MD) in responders (Figure 2.3). Only 8 (12.1%) hyporesponders had PRI ≥60% while on 150 mg MD on day 10 compared to 33 (50.0%) patients while on 75mg MD on day 40 ($p<0.001$). On day 40, fewer patients in responders group had PRI ≥60% (n=2, 7.7%) compared to hyporesponders (n=33, 50.0%) while on 75 mg MD ($p<0.001$).

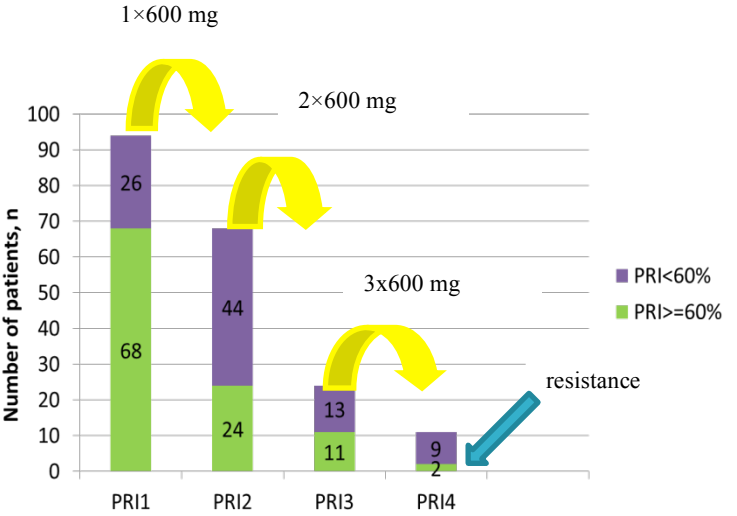

Fig. 2.2. Effect of each additional LD (600 mg) in hyporesponders (n=94)
PRI1 – the first VASP PRI analysis after the initial LD of clopidogrel, PRI2, PRI3, PRI4 – PRI after the first, second and third additional LD of clopidogrel

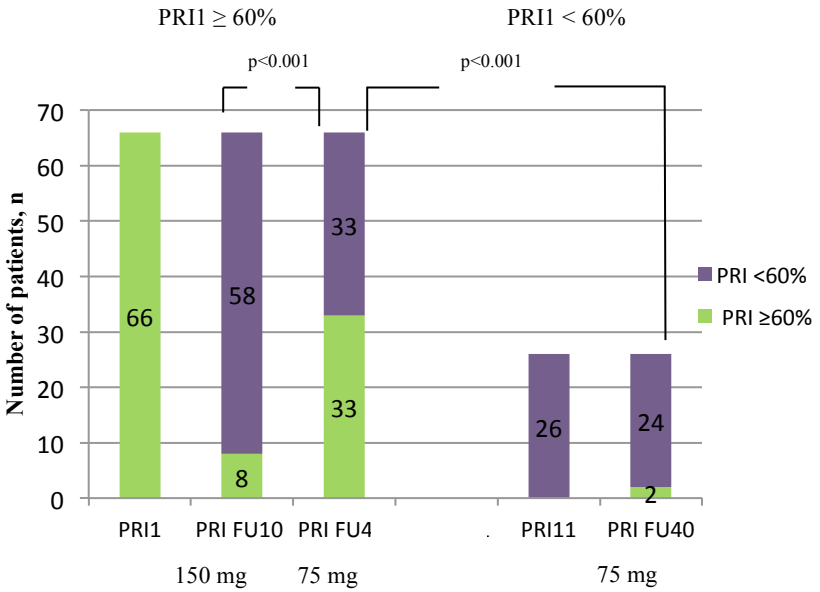

Fig. 2.3. VASP PRI in patients with PRI1 ≥60% and PRI1 <60% and follow-up (n=66 vs 26)*

*Two resistant patients switched to ticagrelor excluded. *Chi-square* test. PRI – the first VASP PRI analysis, PRI FU10 – VASP PRI analysis on day 10, PRI FU40 – VASP PRI analysis on day 40

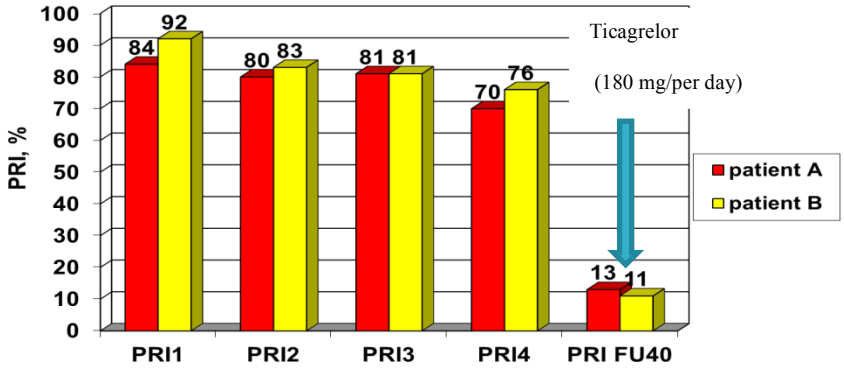

Fig. 2.4. PRI in clopidogrel resistant patients

PRI1 – the first VASP PRI analysis after the initial LD of clopidogrel, PRI2, PRI3, PRI4 – PRI after the first, second and third additional LD of clopidogrel, PRI FU40 – VASP PRI on day 40

2.4. Characteristics of clopidogrel resistant patients

Patient A

Female, 69 years, undergoing elective PCI due to stable CAD, received 300 mg LD of clopidogrel. BMI 40.0 kg/m^2, non-smoker. Duration of AH – 40 years, CHF II functional class (NYHA). Without history of previous MI, DM, CI, CKD, without history of PCI and CABG. **Pre-hospital therapy:** aspirin, statin, BB, ACEI/A2RB, CCB; without previous clopidogrel therapy. Laboratory evaluations in hospital: TC 7.2 mmol/l, HDL-C 1.17 mmol/l, LDL-C 4.8 mmol/l, TG 2.6 mmol/l. **Genotypes** – *CYP2C9**3 wt/wt, *CYP2C9**2 wt/wt, *CYP2C19**2 wt/*2, *CYP2C19**3 wt/wt, *CYP2C19**5 wt/wt, *CYP2C19**17 wt/*17, *ABCB1* C/T. **PRI results:** PRI = 84%, 80%, 81%, 70%; after initiation of ticagrelor PRI FU40 = 13%.

Patient B

Female, 48 years, undergoing elective PCI due to stable CAD, received 300 mg LD of clopidogrel. BMI 33.0 kg/m2, ex-smoker (less than 1 year). AH, II stage, duration – 10 years, CHF I functional class (NYHA), non-insulin dependent DM for 10 years.Without history of previous MI, CI, CKD, without history of PCI and CABG. **Pre-hospital therapy:** aspirin, statin, BB, ACEI/A2RB; without previous clopidogrel therapy. Laboratory evaluations in hospital: TC 3.7 mmol/l, HDL-C 1.01 mmol/l, LDL-C2.0 mmol/l, TG 1.4 mmol/l, glucose 7.9 mmol/l. **Genotypes** – *CYP2C9**3 wt/wt, *CYP2C9**2 wt/wt, *CYP2C19**2 wt/*2, *CYP2C19**3 wt/wt, *CYP2C19**5 wt/wt, *CYP2C19**17 wt/wt, *ABCB1* T/T. **PRI results:** PRI = 92%, 83%, 81%, 76%; after initiation of ticagrelor PRI FU40 = 11%.

2.5. Genetic characteristics of the study population

Genotype distribution and allele frequencies of the genetic variations studied are presented in Table 2.2. No deviations from the Hardy-Weinberg equilibrium were detected.

Table 2.2.

Genetic distributions and allele frequencies of all investigated genetic variations

Polymorphism	Genotype	Patients, n (%)	p	Allele	Allele frequency
CYP2C19					
*CYP2C19**2 (*G681A/ rs4244285*) ‡	GG (wt/wt) AG (wt/*2) AA (*2/*2)	71 (76.3) 21 (22.6) 1 (1.1)	0.686	G A	0.876 (163) 0.124 (23)
*CYP2C19**3 (*G636A/ rs4986893*)	GG (wt/wt) AG (wt/*3)	92 (98.9) 1 (1.1)	0.958	G A	0.995 (185) 0.005 (1)
*CYP2C19**5 (*C1297T/ rs56337013*)	CC (wt/wt) CT (wt/*5)	90 (100.0)	NA	C	1.000 (90)
*CYP2C19**17 (*C806T/ rs12248560*)	CC (wt/wt) CT (wt/*17) TT (*17/*17)	36 (38.7) 46 (49.5) 11 (11.8)	0.523	C T	0.634 (118) 0.366 (68)
CYP2C9					
*CYP2C9**2 (*C430T/ rs1799853*)	CC (wt/wt) CT (wt/*2)	86 (92.5) 7 (7.5)	0.706	C T	0.962 (179) 0.038 (7)
*CYP2C9**3 (*A1075C/ rs1057910*)	AA (wt/wt) CA (wt/*3)	79 (84.9) 14 (15.1)	0.433	A C	0.925 (172) 0.075 (14)
ABCB1					
ABCB1 (*C3435T/ rs1045642*)	CC CT TT	17 (18.2) 50 (53.8) 26 (28.0)	0.410	C T	0.452 (84) 0.548 (102)

‡ According to *National Center for Biotechnology Information;* SNP -*single-nucleotide polymorphism.* Hardy-Weinberg equilibrium; NA - non-applicable; wt - wild-type.

2.6. Relationship of genotypes with PRI

2.6.1. PRI1 results according to *CYP2C19*, *CYP2C9* and *ABCB1* genotypes

Table 2.3. summarizes platelet reactivity after the initial LD (PRI1) by genotypes. Carriers of the *CYP2C19**2 allele (wt/*2 and *2/*2) had significantly higher PRI1 compared with patients homozygous for *CYP2C19* wild-type genotype (78.2±13.1 vs 65.3±19.5; p=0.005) (Figure 2.5). Patients with *CYP2C19**17 allele (wt/*17 and *17/*17) had lower PRI1 compared with homozygous for *CYP2C19* wild-type genotype 64.7±20.7; 68.7±20.8 and 73.0±15.0; p=0.140, respectively. No other polymorphism in recessive or dominant model had significant association with PRI1.

Prevalence of carriers of the *CYP2C19**2 allele (wt/*2 and *2/*2) after the initial LD of clopidogrel remained higher in compared with homozygotes of *CYP2C19* wild-type genotype, 86.4% (19/22) and 67.6% (48/71); p=0.087, respectively. Patients with *CYP2C19**17 unchanged function alleles (wt/wt) were mostly in group with PRI ≥60% that in group of patients with PRI <60%, respectively 80.6% and 19.4% (p=0.146).

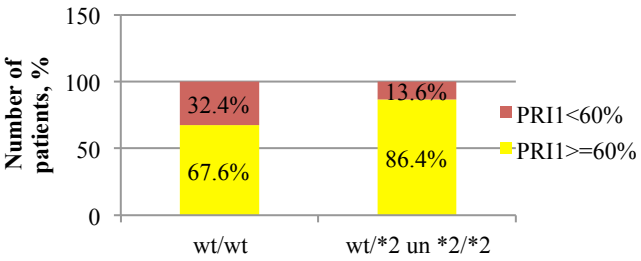

Fig. 2.5 Repartition of genetic polymorphism of *CYP2C192 according to response of the initial dose of clopidogrel (p=0.087)**

Chi-square test. PRI1 – the first VASP PRI analysis after the initial LD of clopidogrel. The data are presented as percentage of the total number of patients with this genotype

Table 2.3.

Repartition of genetic polymorphisms according to response to the initial LD of clopidogrel (n = 93)

Polymorphism	PRI1, % (SD)	p
*CYP2C19**2		
wt/wt	65.3 (19.5)	**0.016**
wt/*2	77.7 (13.2)	
*2/*2	89.0 (-)	
*CYP2C19**3		
wt/wt	68.2 (19.0)	0.472
wt/*3	82.0 (-)	
*CYP2C19**17		
wt/wt	73.0 (15.0)	0.140
wt/*17	64.7 (20.7)	
*17/*17	68.7 (20.8)	
*CYP2C9**2		
wt/wt	68.6(18.9)	0.687
wt/*2	65.6 (20.6)	
*CYP2C9**3		
wt/wt	69.1 (17.7)	0.385
wt/*3	64.3 (25.2)	
ABCB1		
CC	67.0 (22.5)	0.379
CT	66.5 (18.7)	
TT	72.8 (16.9)	

Independent sample t-test (for two groups) and one-way ANOVA analysis (for three groups). The data are presented as the mean±SD. PRI1 – the first VASP PRI analysis after the initial LD of clopidogrel.

2.6.2. PRI2 results according to *CYP2C19*, *CYP2C9* and *ABCB1* genotypes

Platelet activity remained higher in carriers of the *CYP2C19**2 allele (wt/*2 and *2/*2) after the first additional LD (PRI2) compared with homozygotes of *CYP2C19* wild-type genotype (57.0±19.1 vs 40.8±21.5, p=0.006) (Table 2.4). The absolute decrease of mean PRI following the first additional LD (PRI1-PRI2) was significantly higher in non-carriers vs carriers

of *2 (35.5±16.8 vs 25.2±15.6; p=0.025). Among other genotypes we did not observe such kind of tendencies.

Table 2.4.

Mean PRI2 and absolute decrease of mean PRI (ΔPRI1-2) according to genetic polymorphisms (n=67)*

Polymorphism	n	Mean PRI2, % (SD)	p	Absolute decrease of mean ΔPRI1-PRI2, %p (SD)	p
*CYP2C19**2					
wt/wt	48	40.8 (21.5)	0.006	35.5 (16.8)	**0.025**
wt/*2 un *2/*2	19	57.0 (19.1)		25.2 (15.6)	
*CYP2C19**17					
wt/wt	29	47.4 (20.9)	0.517	31.7 (16.2)	0.705
wt/*17un *17/*17	38	43.9 (22.9)		33.3 (17.8)	
*CYP2C19**3					
wt/wt	66	45.1 (22.0)	0.398	32.8 (17.1)	0.392
wt/*3	1	64.0 (-)		18.0 (-)	
*CYP2C9**2					
wt/wt	63	45.5 (21.9)	0.878	32.3 (17.1)	0.659
wt/*2	4	43.8 (27.3)		36.3 (17.4)	
*CYP2C9**3					
wt/wt	58	44.7 (22.5)	0.525	32.9 (17.5)	0.691
wt/*3	9	49.8 (19.2)		30.4 (14.3)	
ABCB1					
CC	11	49.6 (21.9)	0.504	32.9 (17.1)	0.719
CT/TT	56	44.6 (22.1)		30.8 (17.3)	

%p – percentage points, PRI2 – PRI after the first additional LD of clopidogrel, ΔPRI1-2 – absolute decrease in PRI after the first additional LD of clopidogrel.

2.6.3. PRI3 results according to *CYP2C19*, *CYP2C9* and *ABCB1* genotypes

24 patients in our study received the second additional LD of clopidogrel. Platelet reactivity remained higher in carriers of the *CYP2C19**2 allele (wt/*2 and *2/*2) compared with homozygotes of *CYP2C19* wild-type genotype (61.8±11.6 and 51.5±15.2; p=0.078).

Absolute decrease of PRI following the second additional LD of clopidogrel was significantly smaller in carriers vs. non-carriers of *2 (9.0±5.9 vs. 18.9±10.2; p=0.009). Carriers of *CYP2C9**2 more often were identified as hyporesponders. Patients with *CYP2C19**17 allele (wt/*17 and *17/*17) had lower PRI3 (53.3±15.3 vs 59.6±13.1; p=0.293) and higher absolute decrease of mean PRI after the second additional LD of clopidogrel compared with homozygous for *CYP2C19* wild-type genotype (17.2±10.8 vs 11.1±7.6; p=0.131). No other polymorphism in recessive or dominant model had significant association with PRI3.

2.6.4. PRI4 results according to *CYP2C19*, *CYP2C9* and *ABCB1* genotypes

The target PRI <60% still was not achieved in 11 patients who received third additional LD. Patients with *CYP2C19**2 allele (wt/*2 and *2/*2) did not have significantly smaller PRI4 compared with homozygous for *CYP2C19* wild-type genotype (57.2±14.4 vs 54.8±2.0; p=0.731), which was presumably due to smaller number of patients in this group. Also among other genotypes we did not observe PRI differencies. Platelet activity remainded higher in carriers of the *CYP2C19**2 allele after each clopidogrel additional LD compared with homozygotes of the wild-type allele (wt/wt) (Figure 2.6).

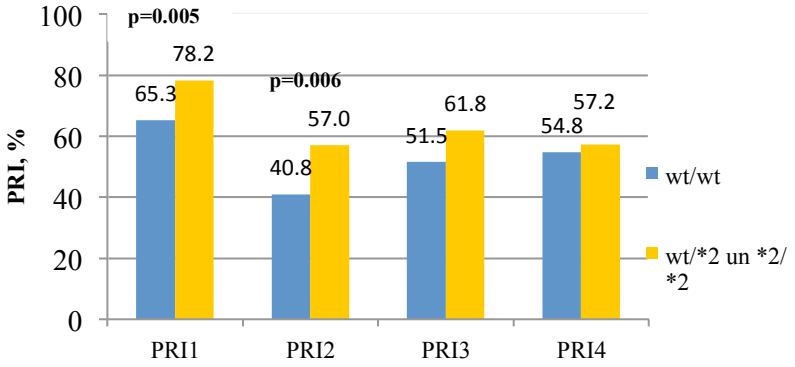

Fig. 2.6. PRI after each LD of clopidogrel depending on *CYP2C192 genetic polymorphism (n=93)**

Independent sample t-test. The data are presented as the mean±SD. PRI1 – the first VASP PRI analysis after the initial LD of clopidogrel. PRI2, PRI3, PRI4 – PRI after the first, second and third additional LD of clopidogrel

2.6.5. PRI on day 10 and on day 40 stratified by the *CYP2C19*, *CYP2C9* and *ABCB1* genotypes

Carriers of CYP2C19*2 (wt/*2 and *2/*2) had significantly higher PRI on day 10 while on the 150 mg MD (53.3±12.1 vs 40.3±13.5; p=0.001) and on day 40 while on the 75 mg MD of clopidogrel when this dose also was less effective in presence of *CYP2C19**2 compared with homozygous for *CYP2C19* wild type genotype (65.5±10.4 vs 56.3±14.5; p=0.020). Among other genotypes we did not observe statistically significant differencies.

In the whole study group carriers of *CYP2C19* *2 had significantly higher PRI with 75 mg MD (63.1±11.3 vs 50.3±17.1, p=0.002), but such kind of tendencies we did not observe in other genotypes.

Target PRI <60% both with increased and standard clopidogrel MD statistically reliably rarely was achieved in carriers of *CYP2C19**2 reduced function allele, when compared with carriers of wild-type genotype (wt/wt),

respectively with MD 150 mg daily 70.6% and 93.8%; p=0.024 and with MD 75 mg daily 23.5% and 58.5%; p=0.014.

2.6.6. Number of LD according to *CYP2C19*, *CYP2C9* and *ABCB1* genotypes

Patients with at least one *CYP2C19**2 reduced function allele (wt/*2 and *2/*2) more often needed several repeated additional LDs to reach the target PRI (<60%), when compared with carriers of unchanged function allele (wt/wt), respectively PRI1, PRI2, PRI3, PRI4: 18.2%, 36.4%, 22.7%, 22.7% and 36.6%, 46.5%, 9.9%, 7.0%; p=0.043. Patients with *CYP2C19**2 normal genotype (wt/wt) needed mostly only one clopidogrel additional LD (46.5%), when compared with patients with reduced function alleles of this genotype which often need the third and the fourth clopidogrel additional LD.

2.7. Influence of prehospital pharmacotherapy and concurrent diseases on PRI

When analysing the relation of prehospitally used medications with PRI, it was observed that patients who had used previously clopidogrel had lower result of mean PRI1±SD, when compared with patients who hadn't received previously clopidogrel therapy, respectively 49.8±16.0 and 69.7±18.4 (t=2.568; p=0.012). PRI1 result statistically significantly differed at different aspirin doses (F=4.668; p=0.005), but statistically reliably difference (F=4.365; p=0.063) wasn't observed at different PPI types. No statistically reliable difference was observed between other received prehospital drug therapy and PRI1 result.

The analysis of PRI1 mean results depending on patient's diseases in anamnesis was also made. The mean PRI1 result in patients with and without stroke differed statistically significantly (t=2.106; p=0.038). No statistically reliable difference was observed between other patients' diseases and PRI1 result.

2.8. Comparison of general and anamnesis data depending on PRI1

In patients with PRI1 ≥60% higher BMI was observed, respectively 30.7±4.8kg/m^2 and 27.1±2.8kg/m^2 (p=0.001), statistically reliably more often also adiposity was observed, respectively in 35 (51.5%) and 5 (19.2%) patients (p=0.005). Patient group with PRI1 <60% comprised mostly patients who already before PCI has used clopidogrel ambulatory, respectively 5 (19.2%) and 1 (1.5%) patient (p=0.006). In patient group with PRI1 ≥60% a statistically reliable trend for more frequent PSI usage was observed (p=0.046). Omega-3 fatty acids were used mostly by the patients from group with PRI1 <60% (n=8; 33.3%, when compared with patient group with PRI ≥60% (n=8; 12.3%) (p=0.022). No material difference was observed between other ambulatory used medications and PRI groups. Statistically reliable differences between platelet reactivity and other variables were not observed (see table 2.5.).

Table 2.5.

Baseline Demographic, Clinical, Angiographic and Biologic Characteristics according to PRI1 ≥60% and PRI1 <60% (n=94)

	PRI1 <60% (n=26)	**PRI1 ≥60% (n=68)**	**p**
Age (yrs), mean±SD	63.6±10.9	62.8±9.2	0.713
Men, n (%)	13 (50.0)	37 (54.4)	0.701
BMI (kg/m^2), mean±SD	27.1±2.8	30.7±.,8	**0.001**
Obesity (BMI>30kg/m^2), n (%)	5 (19.2)	35 (51.5)	**0.005**
Previous MI, n (%)	8 (30.8)	25 (36.8)	0.586
CHF, n (%)	15 (57.7)	44 (66.7)	0.473
NYHA I	7 (46.7)	11 (25.0)	
NYHA II	8 (53.3)	30 (68.2)	0.209
NYHA III	-	3 (6.8)	
History of CABG, n (%)	1 (3.8)	1 (1.5)	0.643
Cardiovascular risk factors, n (%)			
Current smoker	2 (7.7)	9 (13.4)	0.556
DM	9 (34.6)	19 (27.9)	0.527
AH	24 (92.3)	60 (89.6)	0.803
I stage	5 (23.8)	9 (16.1)	0.304
II stage	16 (76.2)	42 (75.0)	
III stage	-	5 (8.9)	
PCI indication, n (%)			
Stable CAD	23 (88.5)	52 (76.5)	0.258
Unstable angina	2 (7.7)	7 (10.3)	
STEMI	-	8 (11.8)	
NSTEMI	1 (3.8)	1 (1.5)	
Pre-hospital therapy, n (%)			
Aspirin	22 (84.6)	49 (72.1)	0.205
Aspirin dose:			
50 mg/per day	1 (5.0)	-	
75 mg/per day	3 (15.0)	12 (25.5)	0.098
100 mg/per day	16 (80.0)	29 (61.7)	
150 mg/per day	-	6 (12.8)	
Clopidogrel	5 (19.2)	1 (1.5)	**0.006**
PPI	3 (12.5)	9 (13.6)	0.888
PPI tipy:			
Pantoprazole	3 (100.0)	3 (33.3)	**0.046**
Omeprazole	-	6 (66.7)	
Statins	18 (75.0)	50 (75.8)	0.941

Continuation of the table 2.5.

	PRI1 <60% (n=26)	**PRI1 ≥60% (n=68)**	**p**
BB	17 (70.8)	48 (72.7)	0.859
ACEI un A2RBs	19 (79.2)	45 (68.2)	0.309
CCB	7 (29.2)	28 (42.4)	0.254
Nitrates	4 (16.7)	13 (19.7)	0.745
Antiarrhythmic drugs	-	1 (1.5)	0.544
Benzodiazepines	1 (4.2)	2 (3.1)	0.800
Omega-3	8 (33.3)	8 (12.3)	**0.022**
Intra-hospital therapy, n (%)			
PPI	18 (69.2)	51 (76.1)	0.496
PPI type: Pantoprazole	16 (88.9)	41 (80.4)	0.662
Omeprazole	2 (11.1)	9 (17.6)	
Statins	25 (96.2)	65 (97.0)	0.833
BB	23 (88.5)	59 (88.1)	0.957
ACEI un A2RBs	22 (84.6)	62 (92.5)	0.246
CCB	12 (46.2)	32 (47.8)	0.889
Nitrates	8 (30.8)	23 (34.3)	0.744
Antiarrhythmic drugs	1 (3.8)	3 (4.5)	0.893
Benzodiazepines	5 (19.2)	7 (10.4)	0.257
Omega-3	1 (9.1)	6 (20.7)	0.389
Laboratory evaluation, mean±SD			
WBC, 10^9/l	7.5±1.5	7.8±2.4	0.445
Hemoglobin, g/l	1.4±1.2	1.4±2.2	0.591
Platelets, 10^3/l	239.1±48.8	219.1±53.0	0.090
Fibrinogen, g/l	3.2±0.8	3.4±1.4	0.573
Creatinine, µmol/l	80.2±16.2	84.5±26.1	0.440
GFR, ml/min	55.8±15.3	66.0±26.0	0.063
TC, mmol/l	4.3±1.1	4.6±1.4	0.297
HDL-C, mmol/l	1.3±0.4	1.2±0.4	0.649
LDL-C, mmol/l	2.5±0.9	2.1±1.2	0.404
TG, mmol/l	1.2±0.7	1.5±0.9	0.179
Angiography and intervention			
Number of treated vessels, mean±SD	1.1±0.3	1.0±0.2	0.531
Number of stents per patient, mean±SD	1.4±0.6	1.4±0.6	0.987
Number of DES per patient, mean±SD	1.2±0.4	1.2±0.5	0.920

End of table 2.5.

	PRI1<60% (n=26)	PRI1≥60% (n=68)	p value
GP IIb/IIIa inhibitor use, n (%)	12 (46.2)	32 (47.8)	0.889

Independent sample t-test (for two groups), one-way ANOVA analysis (for three groups), Chi-square test. The data are presented as the mean±SD or as percentage of the total number of patients in group. BMI – body mass index, CABG – coronary artery bypass graft, CAD – coronary artery disease, STEMI – ST-segment elevation myocardial infarction, NSTEMI – non-ST-segment elevation myocardial infarction, DM – diabetes mellitus, CHF – congestive heart failure, AH – arterial hypertension, MI – myocardial infarction, PCI – percutaneous coronary intervention, PPI – proton pump inhibitor(s), BB – beta blocker(s), CCB – calcium channel blocker(s), ACEI – angiotensin converting enzyme inhibitor(s), A2RB – angiotensin II receptor blocker(s), TC – total cholesterol, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, TG – triglycerides, GP – glycoprotein, DES – drug-eluting stent(s), SD – standart deviation, WBC – white blood cells, GFR – glomerular filtration rate.

2.8.1. Influence of BMI on efficiency of additional LDs and higher MD of clopidogrel

After the initial LD of clopidogrel, higher BMI was observed for patients in group of PRI1 ≥60% compared with group of PRI1 <60%, respectively 32.8±3.9kg/m^2 and 29.6±4.9kg/m^2 (p=0.007). Patients after receipt of the third and second LD maintained this trend although it was not statistically reliable. Patients with PRI FU10 ≥60% and PRI FU40 ≥60% had higher BMI (33.1±4.9 and 30.2±4.6 (p=0.097)) when compared with the other group (PRI <60%) during these both follow-ups (30.9±4.3 and 29.0±4.7 (p=0.058).

Mean PRI decrease after the first additional LD (mean ΔPRI1-PRI2) was statistically significantly lower in group of patients with BMI ≥30kg/m^2, respectively 26.0±15.8%p and 39.6±15.3%p (p=0.001) (Fig. 2.7). Both increased and standard MD in this group was less efficient, respectively 62.5% and 51.4% patients when compared with group where BMI <30kg/m^2.

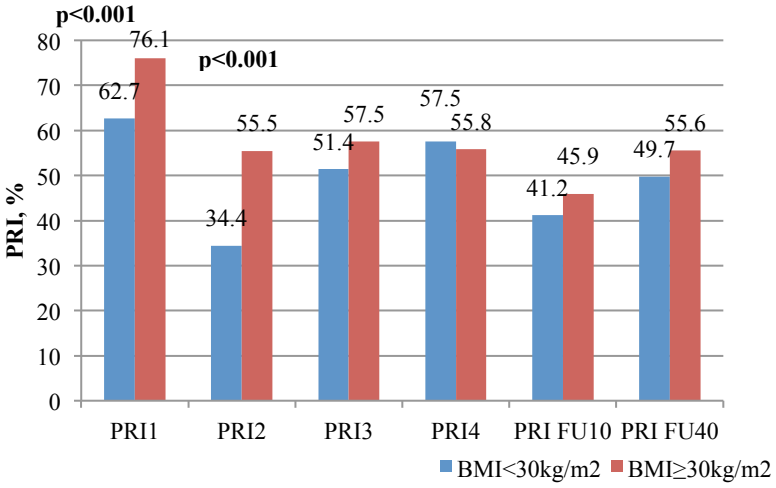

Fig. 2.7. The effect of obesity on dosing strategy of clopidogrel

Indepentent samples t-test, the data are presented as the mean results. PRI – platelet reactivity index. PRI1 – the first VASP PRI analysis after the initial LD of clopidogrel. PRI2, PRI3, PRI4 – PRI after the first, second and third additional LD of clopidogrel, PRI FU10 – VASP PRI analysis on day 10, PRI FU40 – VASP PRI analysis on day 40

2.8.2. Influence of PPIs on efficiency of additional LDs and higher MD of clopidogrel

Concomitantly with DAPT 69 (73.4%) patients during intra-hospital period received therapy of PPI of which 1 (1.5%) patient received esomeprazole, 11 (15.9) patients – omeprazole and 57 (86.2%) patients – treatment with pantoprazole. After the initial LD of clopidogrel patients with PPI therapy were mostly in group of hyporesponders compared with patients without PPIs (16 (69.6%) vs 7 (30.4%); p=0.381). Patients with PPI were more frequently hyporesponders of clopidogrel in time of both FUP visits (87.5% and 76.5%). PRI remained higher in presence of omeprazole and esomeprazole

compared with pantoprazole group during both FUPs in patients with concomitantly use of PPI (table 2.6).

Table 2.6.

Influence of PPI type on the mean PRI in patients after additional LDs and modified MD of clopidogrel

Mean PRI, % (SD)	n	Omeprazole and esomeprazole group	n	Pantoprazole group	p
PRI2	10	52.3 (41.3)	41	41.3 (19,8)	0.124
PRI3	4	63.0 (4.1)	12	50.8 (11.0)	0.052
PRI4	4	50.5 (9.1)	2	58.0 (0.0)	0.366
PRI FU10	10	51.1 (16.8)	41	42.2 (12.0)	0.060
PRI FU40	12	55.5 (17.2)	57	52.0 (16.9)	0.534

2.9. Results of long term FUP for study patients

Long-term FUP was conducted of all 94 (100%) patients with a mean FUP of 20.9±8.2 months. One cardiac death was observed after 9 months in patient from group of respnders (PRI1 <60%), which can be considered as a possible ST (1.1%). MI were detected in 2 (2.2%) patients, both of them were from group with PRI1 ≥60%. One (1.1%) patient from group of hyporesponders underwent CABG, 22 (23.7%) patients – PCI of which two were target vessel revascularization (TVR), 1 (1.1%) – TLR, 21 (22.8%) patients underwent PCI on other vesssel.

DISCUSSION

Results obtained during the study state to important negative influence of *CYP2C19**2 allele on the efficiency of additional LDs and higher MD of clopidogrel. For the carriers of this allele PRI decreases less after the first additional LD of clopidogrel, and higher MD is also rarely efficient compared with patients without this polymorphism. Trend to smaller PRI decrease maintains also during the administration of further additional LDs, but not enough to obtain statistical signifficance due to small groups of patients. According to the information at our disposal, in internationally quotable literature there are no studies showing the influence of aforementioned genetic polymorphisms in the same patients with both the additional LDs and different MD of clopidogrel. Since most of the patients were admitted to hospital to perform elective PCI with DES therewith data of our study testify on the influence of these polymorphisms also on initial LD of clopidogrel (300 mg) in group of these patients according to the guidelines.

Results of the study state that patients with at least one reduced function *CYP2C19**2 allele have statistically significantly higher PRI after initial LD of clopidogrel, when compared with patients without this polymorphism. This finding matches the literature data [Bonello et al., 2010, Bonello et al., 2012], where higher PRI was observed in *CYP2C19**2 heterozygotes after receipt of LD of clopidogrel when compared with group of patients without reduced function allele. But in patients with at least one *CYP2C19**17 increased function allele we observed a trend to better clopidogrel efficiency during the research that is approved also in the scientific literature where lower PRI values are observed in carriers of this allele after LD of clopidogrel is received [Sibbing et al., 2010].

In our research we did not observe the influence of other analysed *CYP2C19* and *CYP2C9* genotypes on PRI after the initial LD of clopidogrel. It must be noted that reduced function alleles in these genotypes were rarely encountered which can prevent findings of similar association as in case of *CYP2C19**2.

According to results of our research, mean PRI after the initial loading dose of clopidogrel was statistically significantly (p=0.005) higher in carriers of *CYP2C19**2 allele. Also additional LD strategy in carriers of *CYP2C19* reduced function allele was less efficient, respectively less PRI decrease, larger number of required LDs was observed when compared with patients without the reduced function allele. However, most patients irrespective of presence of *CYP2C19**2 reduced function allele managed to achieve the PRI target of our study. Also for *CYP2C19**2 homozygote in our study this dose strategy was successful. It must be remarked that patients with real resistance had only one *CYP2C19**2 reduced function allele that states to potential influence of other factors on hyporesponsiveness predisposition. In case of other *CYP2C19* and *CYP2C9* genotypes, LD effects were similar in polymorphism groups. Influence of *CYP2C19**2 genotype is widely studied and described also in the scientific literature [Simon et al., 2009, Trenk et al., 2008, Wallentin et al., 2010], where this reduced function allele is connected with the increased risk of ischemia and ST. Although in the studies VASP analysis is more often made with patients before PCI, linking hyporesponsiveness with the risk to develop early ST [Blindt et al., 2007, Gurbel et al., 2005]. Also in the study of *Bonello et al*., in 88% of patients with at least one *CYP2C19**2 reduced function allele 3 additional LDs of clopidogrel attested efficiency achieving PRI<50% [Bonello et al., 2010]. It can be concluded that in *CYP2C19**2 heterozygotes and homozygotes a sufficient level of clopidogrel active metabolite can be reached

using high doses of clopidogrel. Since the number of patients with both *CYP2C19**2 reduced function alleles is small, wider research of this groups is required.

Results of our research do not approve the influence of *ABCB1* C3435T genotype on efficiency of clopidogrel therapy. On the base of the data we have obtained, we can speak of the trend to higher PRI1 values in patients with TT genotype after initial LD of clopidogrel although statistically significant difference was not obtained. Although in the lately published meta-analysis, *ABCB1* genotype T allele was associated with CV risk for the patients who had received 300 mg LD of clopidogrel, unlike the patients after 600 mg LD [Su et al., 2012], results of other publications on the role of this genotype are conflicting. For example, in PLATO study it is reported on correlation of *ABCB1* 3435CC genotype with increased CV death, MI and cerebral infarction (CI) [Wallentin et al., 2010], but the result of TRITON-TIMI 38 study testify on higher abovementioned CV risk in patients with contrary (3535TT) *ABCB1* genotype [Mega et al., 2010].

After the first VASP analysis that was made after standard LD of clopidogrel was received, unsatisfactory activity of clopidogrel in the group of patients during our study were observed in most of the patients. Irrespective of increased PRI limit value, prevalence of hyporesponsiveness among the patients after initial LD was higher (72.3%) as expected. It's unlikely that the number of patients with reduced response to clopidogrel in Latvian is on average higher than in other published studies [Bonello et al., 2010, Gurbel et al., 2003, Angiolillo et al., 2007, O'Donoghue et al., 2006], where hyporesponsiveness is mostly established in 50–60% of cases. If we would apply the hyporesponsiveness definition with PRI ≥50% that is more frequently used in researches, a bit more patients with reduced response to clopidogrel –

86.2% were in our study. Perhaps higher PRI value in group of study patients could be indirectly provoked by application of 300 mg LD of clopidogrel although lower PRI was observed in patients after elective PCI after 600 mg LD that is indicated in case of ACS. Reason for such researched could be that higher platelet reactivity is observed in ACS patients due to higher platelet activation. These patients tend to have also other uncorrected risk factors while CAD in the patients with elective PCI is determined already sooner and medication for secondary prophylaxis are already used in most of the cases. Perhaps more reliable explanation for high PRI values could be that the inclusion criterion was selection of DES stents that is chosen more frequently by the invasive cardiologist for patients with DM and more complicates blood vessel damage. Also the fact that VASP was performed after PCI could increase PCI although this is not proven.

In our study the strategy of additional LDs in patients with hyporesponsiveness could be assessed as quite successful. Most of the patients managed to achieve PRI target with additional LDs of clopidogrel, except 2 patients whose PRI maintained above 60%. The convincing efficiency of applied additional LD strategy testifies on the real resistance of clopidogrel to be very rare. That indirectly indicates on selection of higher PRI target in patients with reduced response to clopidogrel to select the group of patients with higher risk of ST and CV event. Also the data of other authors confirm the positive effect of this additional LDs to overcome the hyporesponsivity of clopidogrel [Bonello et al., 2009, Bonello et al., 2010].

It must be noted that for 2 patients with real clopidogrel resistance the change of antiaggregant therapy to new generation medication ticagrelor was convincingly successful. During the follow-up of 40 days a rapid fall was observed in the decrease of PRI obtained numeric value, by 57%p and 65%p

respectively. Ticagrelor as the new generation $P2Y_{12}$ receptor inhibitor shows also in other studies greater degree of platelet inhibition both during the usage of LD and MD when compared with clopidogrel [Wallentin et al., 2009, James et al., 2012]. Therewith in case of real clopidogrel resistance it is a good alternative to medications if not contraindicated.

VASP analysis for determination of platelet reactivity was used in our study for the first time in Latvia. We chose this method on the base of advantages of this test the most important of which are that the analysis is not affected by other concurrently received medications (for example, aspirin, GP IIb/IIIa receptor inhibitors) and that small amount of blood (4ml) is required for the analysis that can be stored at room temperature up to 48 hours till the analysis is made [Bonello et al., 2010]. The main disadvantage of this method is the labour-consuming and time-consuming sample preparation and analysis process.

Considering the fact that there is still no unified approach on PRI target value in case of VASP analysis that would define the activity of decreased clopidogrel and influence the clinical result to decrease CV risk. Our selected PRI target was <60%. During the analysis of long-term safety and efficiency of this target (<60%), In our study ST was established in 1 (1.1%) patient during the first year with according clopidogrel activity – PRI1 was 52%. Perhaps PRI target <50% would be safer for this patient although during the 40 day follow-up PRI of this patient was even below this norm – 46%. Most frequently PRI limit values used in other scientific researches variate from 50–70% [Blindt et al., 2007, Aradi et al., 2012, Bonello et al., 2012].

In our study we observed higher PRI values in patients with *CYP2C19**2 reduced function allele during the usage of both MDs (75 mg and 150 mg). It must be noted that increased MD (150 mg daily) was more efficient for carriers

of this allele when compared with standard MD (75 mg daily). In case of other genotypes analysed in our study, statistically reliable differences in efficiency of MD of clopidogrel were not observed. In literature data is available that for *CYP2C19**2 heterozygotes a tripled MD (225 mg daily) achieves the same effect as standard MD of clopidogrel in patients without reduced function allele [Mega et al., 2011]. Such correlation on efficiency of higher MD of clopidogrel in carriers of *CYP2C19**2 allele was observed also in other previously published reports [Price et al., 2011, Price et al., 2012, Aleil et al., 2008], although there is still no data on decrease of CV events after application of such therapeutic strategy.

Results of our study reflect association of body mass and abdominal circumference with PRI both during LD and MD of clopidogrel that shows the statistic reliability after the initial and first additional LD. It can be observed very clearly in patients with obesity (BMI $\geq$30kg/m^2). In case of other (CV) risk factors (DM, smoking) statistical differences on the strategy of research therapy were not observed. Also in other studies, statistically reliable association of BMI with PRI after LD of clopidogrel was observed that is related with insufficient dose of clopidogrel to these patients [Bonello-Palot et al., 2009].

Considering the concurrently used medication during the clopidogrel therapy, results of our study point to a trend of higher PRI values during the whole research strategy, concurrently using omeprazole or esomeprazole. Both additional LDs and higher MD of clopidogrel were less efficient in the group of patients using omeprazole or esomeprazole, when compared with the group using pantoprazole. These PRI group differences didn't reach the statistical reliability due to small amount of patients who used omeprazole or esomeprazole ambulatory. Also in the hospital pantoprazole therapy was

appointed more frequently. Influence of other concurrently received medications on clopidogrel therapy wasn't observed during the study. The results of clinical researches on PPI and clopidogrel interaction are still conflicting [D'Ugo et al., 2013, Fernando et al., 2011]. Although there is no convincing data on interaction of clopidogrel and PPI, it is advised to avoid concurrent usage of these medications with increased affinity to *CYP2C19* enzyme.

Main differences from other similar researches, for example *Bonello et al.* research [Bonello et al., 2012] are than the number of patients in our study was smaller, most of the patients were refered to hospital for elective PCI receiving 300 mg LD of clopidogrel according to guidelines [Wijns et al., 2010], also PRI target was higher. It must be noted that PRI was determined in patients after PCI.

As the advantage of our research we can name that we used directly VASP method to determine PRI the result of which cannot be affected by concurrent usage of aspirin and GP IIb/IIIa inhibitors unlike such frequently used methods as Verify Now tests and PlateletWorks. Main shortage of the research was the relatively small number of patients, groups of inhomogeneous patients (both elective and acute patients were included).

PRACTICAL APPLICATION

Results of our study may facilitate the choice of successful therapeutic strategy for patients with reduced response to clopidogrel therapy. Determination of concurrent platelet reactivity ad *CYP2C19**2 allele can be superior than using the platelet functional tests individually to reach optimum $P2Y_{12}$ inhibition stage in each patient. According to the data of our study,

determination of *CYP2C19**2 reduced function allele would allow forecasting that initial LD and MD will be less efficient, especially in patients with other hyporesponsiveness provoking factors – increased BMI, and concurrent PPI, especially omeprazole or esomeprazole therapy.

CONCLUSIONS

1. The ability of initial LD of clopidogrel in patients with at least one *CYP2C19**2 reduced function allele to inhibit the platelet reactivity is lower. Carriers of *CYP2C19**17 increased function allele have a trend to better clopidogrel activity. Presence of *CYP2C19**2 allele in patients with hyporesponsiveness of clopidogrel is associated with decreased efficiency of additional LDs.
2. Almost two thirds of patients with hyporesponsiveness of clopidogrel managed to achieve the target PRI <60% already after the first additional LD that testified of efficiency of additional LDs of clopidogrel.
3. Real clopidogrel resistance is observed rarely, 2% of patients who cannot achieve the target PRI <60% even with three additional LDs. Ticagrelor is convincingly efficient in case of clopidogrel resistance.
4. Corresponding platelet inhibition is not observed in patients who are carriers of *CYP2C19**2 allele with standard MD (75 mg) in ¾ of cases. Higher 150 mg MD of clopidogrel is more efficient for these patients, but it is still insufficient in 29% of cases.
5. Adiposity is an important phenotypic factor that worsens the efficiency of additional LDs and higher MD in patients with clopidogrel hyporesponsivity.

6. Users of PPI have a trend to decreased efficiency of clopidogrel both after additional LDs and during usage of both MDs. Efficiency of clopidogrel in users of omeprazole or esomeprazole is lower during the whole clopidogrel dose strategy period when compared with users of pantoprazole.
7. During a year only one possible ST was established that makes us to think of safety of LD strategy in long-term in accordance with PRI target <60%. However, it is necessary to confirm PRI target <60% in larger prospective studies.

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APROBATION OF THE STUDY – PUBLICATIONS AND THESIS

Doctoral thesis is based on following SCI publication:

Urtane I., Aitullina A., Pukite K. Clopidogrel and the possibility of drug-drug interaction in the primary health care. Journal of Young Pharmacists, 2013; 5:18–21.

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Latkovskis G., **Urtane I**., Knipse A., Peculis R., Cakstina I., Klovins J., Erglis A. Role of Genetic Factors on the Effect of Additional Loading Doses and Two Maintenance Doses Used to Overcome Clopidogrel Hyporesponsiveness.

Non SCI publications:

Šaļapina A., Štokmane A.S., **Urtāne I**. Klopidogrela un citu zāļu vienlaicīgas lietošanas prevalence ambulatorā praksē farmaceita skatījumā. Rīgas Stradiņa universitātes Zinātniskie raksti, 2012; 157.–162. lpp.

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Latkovskis G., **Urtane I**., Knipse A., Puceta L., Peculis R., Klovins J., Erglis A. Role of genetic factors on the effect of additional loading doses and two maintenance doses used to overcome clopidogrel hyporesponsiveness. 42th ESCP Synposium on Clinical Pharmacy, Prague, Czech Republic, October 16–18, 2013, Programme and abstract book, P.77.

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