

# **Genetic approach to the laboratory efficacy of anti-platelet treatment in patients after coronary stent implantation**

**Ph.D. thesis**

**by**

**Orsolya Rideg M.S.**

**Supervisors:**

**Attila Miseta M.D.DSc., Ph.D.**

**and**

**András Komócsi M.D., Ph.D.**

**Head of the Ph.D. program**

**Attila Miseta M.D.DSc., Ph.D.**

**University of Pécs, Faculty of Medicine**

**Department of Laboratory Medicine**

**2011**

## **Introduction**

Coronary artery disease (CAD) is known to be a very heterogeneous disease of multifactorial origin affecting millions world-wide and being the top leading cause of death among the middle-aged population.

The development of percutaneous coronary intervention (PCI) has revolutionized the treatment of ischaemic heart disease. However, despite the efforts some short and longer term complication has been realized; acute stent thrombosis (ST) and in-stent restenosis (ISR), both resulting in target-vessel failure after PCI, still occur.

Significant interindividual differences in response to anti-platelet therapy have recently been recognized with supposed environmental, clinical, pharmacokinetic and genetic background. While lack of standardization in platelet function assays and paucity of well defined cut off values represent further difficulties of the subject. The circumstances that individuals may have different needs for anti-platelet action as well as different risk for bleeding, point toward the need for an individualized therapeutic regime.

## **Adjuvant therapy of coronary stent implantation**

Platelets are relevant in the process of atherothrombosis and arterosclerosis, hence inhibition of thrombocyte activation and aggregation is one of the major pharmacological goals in our therapeutic regimen. Currently, three main groups of drugs are used after and before coronary stent implantation: GP-IIb/IIIa receptor antagonists, the cyclooxygenase-1 (COX-1) inhibitor Aspirin and ADP-receptor antagonists.

Since the ADP receptor, P2Y<sub>12</sub> transmitted effect plays a pivotal role in the amplification of platelet aggregation leading to a stable occlusive thrombus, inhibition of the receptor was an early focus in anti-platelet drug development.

The most frequently used ADP antagonist, clopidogrel, is an inactive pro-drug requiring metabolic activation of the hepatic cytochrome P450 (CYP450) pathway to produce active metabolites that irreversibly and covalently bind to the P2Y<sub>12</sub> receptor on platelets membrane and block the receptor-induced reduction in intracellular cAMP, resulting in reduced aggregation of the platelets.

Significant clinical benefit of using ADP antagonist + aspirine dual antiplatelet therapy (DAPT) instead of aspirine monotherapy after coronary stent implantation (PCI) have been confirmed in multiple clinical studies and became the “first-line” therapeutic regime in patients after PCI.

### **Interindividual differences in response to anti-platelet therapy**

In spite of the promising results through aspirin-clopidogrel DAPT, thrombotic events still occur and the term “clopidogrel resistance” became used, referring for patients with inappropriate response to DAPT.

Early studies had demonstrated that patients responses’ to DAPT are not uniform, large interindividual differences to fix-dose clopidogrel therapy have been recognized. Multifactorial background as patients’ compliance, clinical conditions and genetic variants affecting drug absorption and metabolism are important determinants of interindividual variability in platelet reactivity.

Relevant polymorphisms of the multidrug resistance 1 gene (mdr 1, ABCB1) as C3435T and G2677T/A, affecting drug absorption and transport, as well as functional polymorphisms of the CYP2C19 metabolizing enzyme as the CYP2C19\*2,\*3 loss-of-function (LOF) and CYP2C19\*17 gain-of-function allelic variants are highly relevant in therapy outcome. Recently another enzyme the paraoxonase-1 (PON1) gene’s Q192R functional polymorphism as well became in the focus of interest. PON1 is known to be a factor in the second step of the biotransformation of clopidogrel, forming active thiol derivative.

However despite of the growing number of genetic analysis and clinical studies still many questions are open.

## **Aims**

According to the recent guidelines of the European Society of Cardiology, the American College of Cardiology and the American Heart Association, for the prevention of recurrent thrombotic events after percutaneous coronary intervention (PCI), dual anti-platelet therapy (DAPT) with aspirin and clopidogrel is recommended. Considering the great interindividual differences in response to fix-dose clopidogrel therapy, as well as the absence of well defined cut off and prognostic values of high platelet reactivity, in our thesis we aimed to perform systematic review over the available literature highlighting on the methodical heterogeneity, comparing results and predictive value of different anti-platelet function assays on defining platelet reactivity and clinical outcome. Further, considering the heterogeneity and the lack of standardized platelet function assays we aimed to compare utility and reliability of light transmission aggregometry (LTA) with vasodilator-stimulated phosphoprotein phosphorylation (VASP-PRI) assay. We investigated the most optimal and predictive platelet aggregation parameter ( $Agg_{max}$ ,  $Agg_{late}$ , AUC and disAggregation) measuring platelet reactivity and predicting clinical outcome; and compared LTA estimates in determining the potency of P2Y<sub>12</sub> receptor inhibition to VASP-PRI assay; in low-risk, stable angina patients after elective PCI.

In view of the pivotal role of genetic variants on anti-platelet therapy we aimed to perform genetic analysis of the following genes' among low-risk, stable angina patients after elective PCI:

- CYP2C19\*2 and \*3 loss-of-function and CYP2C19\*17 gene-of-function allelic variants;

- multidrug resistance 1 gene (ABCB1, mdr1) as C3435T and G2677T/A polymorphisms;
- paraoxonase-1 (PON1) gene's Q192R polymorphism.

## **Methods**

### **Light Transmission Aggregometry (LTA)**

In our studies CARAT TX4 four-channel light transmission aggregometer (Carat Diagnostics, Hungary) was used for platelet function assessments. Calibration for aggregation measurements were established using light transmission percentage through platelet rich plasma (PRP) (0% transmission) and platelet poor plasma (PPP) (100% transmission). The assessment required 10ml sodium-citrate (3.8%) anti-coagulated blood from each patient. All samples were processed within two hours. After sample separation to PRP and PPP fractions, 5 $\mu$ M ADP was added into PRP to stimulate platelet aggregation. Platelet reactivity was described with the maximal platelet aggregation value (Agg<sub>max</sub>) of the registered optical curve, late aggregation (Agg<sub>late</sub>), steepness of slope, area under curve (AUC) and disAggregation (disAgg).

### **Flow cytometric assessment of vasodilator stimulated phosphoprotein phosphorylation assay (VASP-PRI)**

Vasodilator phosphoprotein (VASP) intracellular protein through binding to the ADP-specific P2Y<sub>12</sub> receptor, takes part in the cyclic adenosine monophosphate (cAMP) regulated cascade. VASP is important for regulation of the cytoskeleton and for conversion of glycoprotein IIb/IIIa to its active conformation, thus permitting platelets to aggregate. The cAMP cascade is inhibited by ADP through the P2Y<sub>12</sub> receptor; due to low intracellular level of cAMP VASP is dephosphorylated, in contrast when the P2Y<sub>12</sub> receptor is blocked cAMP level increases, VASP is in its phosphorylated state. Using immunofluorescence

based specific monoclonal antibodies (Biocytex Platelet VASP kit, Marseille, FR) phosphorylated and dephosphorylated level of VASP can be measured. Result of the P2Y<sub>12</sub> specific assay refers to the activated stage of the receptor, hence anti-platelet therapy efficiency.

### **Genetic analysis of the functionally relevant ABCB1, CYP2C19 and PON1 allelic variants; RT PCR**

For the genetic analysis of ABCB1, PON1 and CYP2C19 loci, genomic DNA was isolated from ethylenediaminetetraacetic acid (EDTA) -anticoagulated blood samples. All of the genotyping procedures were performed on LightCycler 2.0 Real-Time PCR, using fluorescent labeled sequence specific probes and primers. Results were evaluated by the Melting Curve Analyzer program; LightCycler software 4.05.

### **Study design and patient population**

During the systematic review and meta-analysis overview of observational studies, between 2003 January to 2010 February, presenting clinically relevant high platelet reactivity (HPR) with ADP-specific methods were performed. The primary clinical outcomes of interest, evaluated at the longest available follow-up (during that patients were on clopidogrel treatment) were (a) cardiovascular (CV) death, (b) definite/probable stent thrombosis (ST), (c) non-fatal myocardial infarction (MI) and (d) a composite endpoint of the reported ischemic events (CIE) that included CV death, MI, ischemic stroke, unplanned repeat revascularization or rehospitalization for acute coronary syndrome (ACS).

In 2008, our team set up a prospective, randomized, double-blind, placebo-controlled, monocentered clinical study, called: DOSER-trial (NCT006638326). The methodical and genetical studies are sub studies of this trial.

In the DOSER trial 200, clopidogrel-naïve stable angina patients, in whom elective percutaneous coronary interventions (PCI) were performed, had been recruited.

The day after the PCI and then in the 4<sup>th</sup> week after the intervention platelet reactivity was measured both with ADP-specific light transmission aggregometry (LTA) and with VASP-PRI assay. Results from both LTA and VASP-PRI measurements became available in case of 121 patients. The LTA-VASP methodical part processes these results.

During the genetical study, from the initial 200 patients, CYP2C19\*2, \*3, \*17 and PON1 genotyping were done in 189 cases, while ABCB1 genetic analysis were done in 181 cases.

## **Statistical analysis**

The review manager 5.0.22 freeware package and the SPSSv11.0/Graphpad Prism 5.0 softwares were used for statistical analysis to the systematic review and meta-analysis and to the clinical studies respectively.

## **Results**

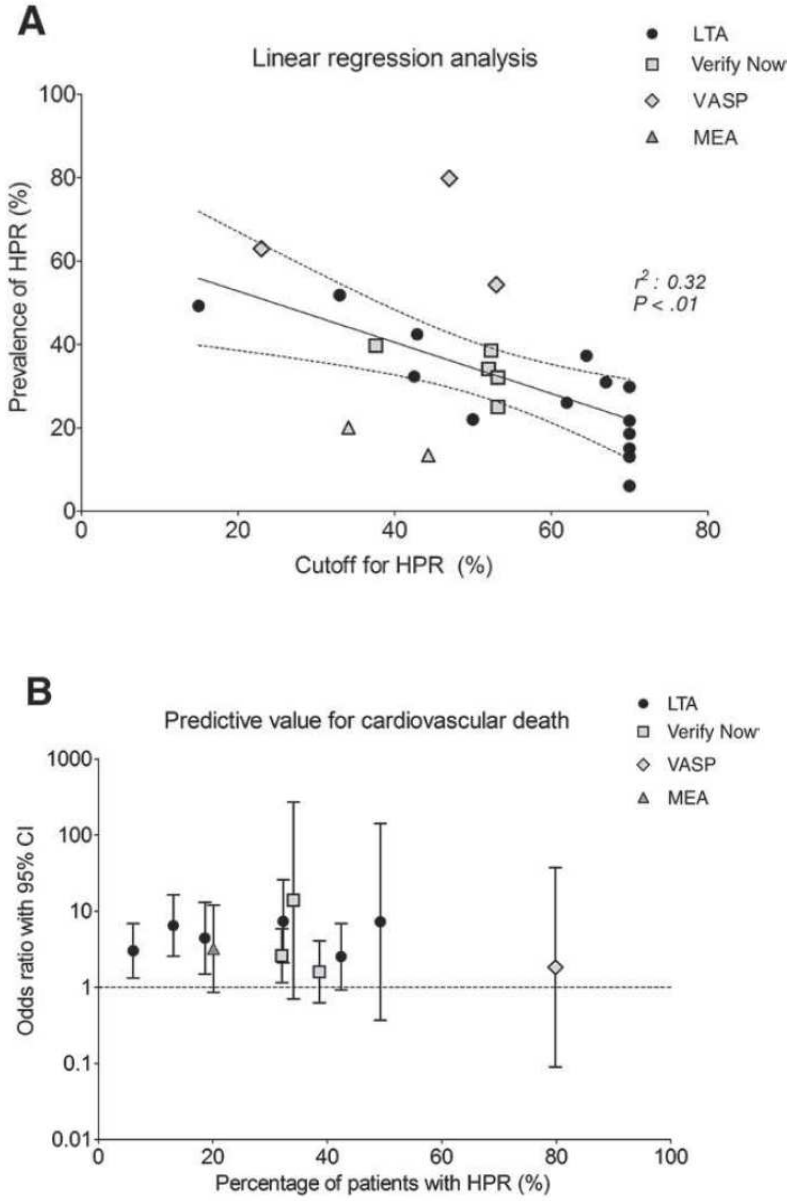
### *Prognostic significance of high on-clopidogrel platelet reactivity after percutaneous coronary intervention: Systematic review and meta-analysis*

Out of the 1801 citations, twenty articles, analyzing high platelet reactivity with ADP-specific assays, including 9187 patients, were selected for full text analysis and data extraction.

### **Prevalence of high on-clopidogrel platelet reactivity**

According to our analysis the rate of HPR showed large heterogeneity with a mean prevalence of 32.3% (95% CI for mean: 25.9–40.5; range: 6.06-79.86).

Among the recruited studies, the selected platelet reactivity cut off and the type of the platelet function device interacted significantly with the prevalence of HPR. The selected cut off was in strong, inverse correlation with the rate of HPR (Figure 1.).



**Figure 1. Impact of the methodological heterogeneity in platelet aggregation tests.**  
**A: Linear regression analysis between the selected cut off and the prevalence rate for high platelet reactivity (HPR).**  
**B, The impact of the prevalence rate of HPR on the relative risk of CV death.**



## Prognostic significance of HPR

Based on the pooled results, compared to HPR was associated with a significant, 3-fold increase in non-fatal MI (OR: 3.00; 95%CI: 2.26-3.99;  $p < 0.00001$ ), a 4-fold increase in definite/probable ST (OR: 4.14; 95%CI: 2.74-6.25;  $p < 0.0001$ ) and a 5-fold increase in the rate of composite ischemic events (OR: 4.95; 95%CI: 3.34-7.34;  $p < 0.00001$ ). When the subgroup of studies using receiver operating characteristic (ROC)-defined cut offs for HPR was analyzed separately, similar outputs were gained (CV death 2.34 [1.40-3.92], MI 2.89 [2.07-4.04], ST 4.75 [2.13-10.63], and CIE: 3.06 [2.07- 4.51];  $P < 0.001$  in all cases). Although there was large methodical heterogeneity among the platelet function assays as well as in the selected cutoffs for HPR, the predicted risk for CV death, non-fatal MI and ST were not heterogeneous between studies. On the contrary, there was significant heterogeneity in case of the less standardized, composite end point.

When the predictive value of each assay was analyzed separately, only LTA-defined HPR was significantly associated with CV death, MI, and ST (death: 4.18 [2.70-6.46], MI: 2.93 [1.97-4.35], ST: 3.66 [2.32-5.78];  $P < 0.0001$  in all cases). The VerifyNowP2Y12 predicted CV death and MI (death: 2.28 [1.23-4.25],  $P = 0.009$ ; MI: 2.98 [1.94-4.58],  $P < 0.00001$ ), but only a trend was observed regarding ST (4.17 [0.81-21.63],  $P = 0.09$ ). MEA<sub>ADP</sub> significantly predicted MI and ST (MI: 4.03 [1.16-14.00],  $P = 0.03$ ; ST: 13.89 [2.63-73.45],  $P = 0.002$ ), but only a trend was observed regarding CV death (3.21 [0.86-12.00],  $P = 0.08$ ). Based on the results of 2 small studies, VASP-defined HPR was predictive neither for CV death (1.84 [0.09-37.07],  $P = 0.69$ ) nor for ST (1.48 [0.28-7.77],  $P = 0.64$ ).

## *Comparison of conventional aggregometry with VASP for monitoring P2Y12-specific platelet inhibition.*

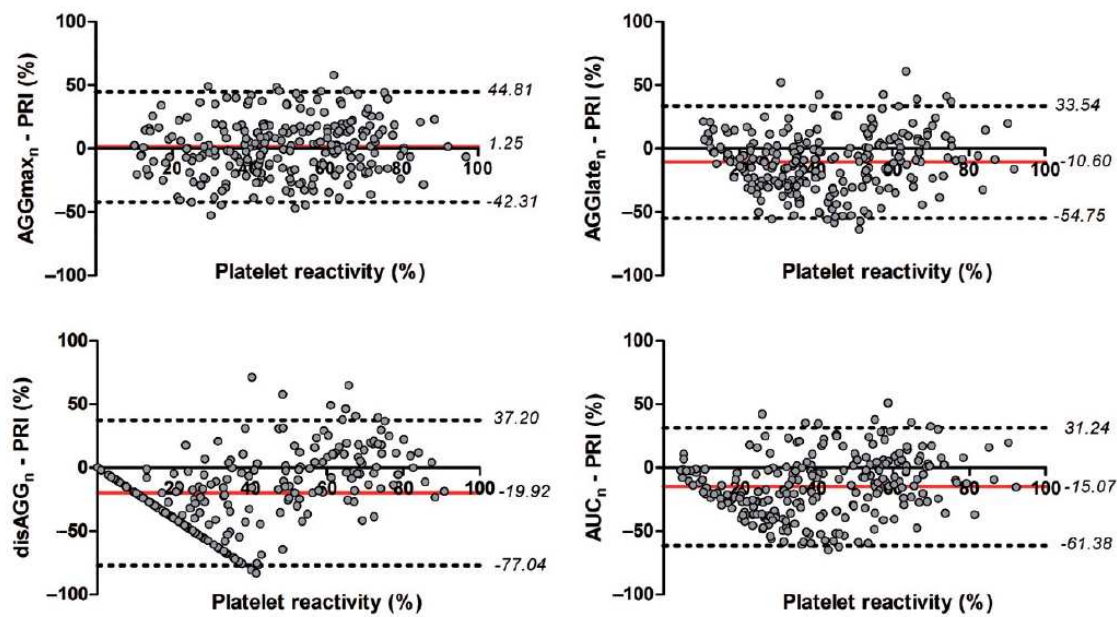
### **Correlation between LTA estimates and VASP-PRI**

One hundred twenty-one patients were enrolled in the study.

During the VASP-PRI and LTA measurements all platelets function parameters demonstrated high interindividual variability, that appeared both after the loading dose as well during the maintenance period (loading dose:  $\text{Agg}_{\text{max}}$ :  $29.1 \pm 14.4$ ;  $\text{Agg}_{\text{late}}$ :  $9.4 \pm 18.7$ ;  $\text{disAgg}$ :  $71.5 \pm 32.4$ ; AUC:  $67.6 \pm 55.0$ ; VASP-PRI:  $48.3 \pm 21.3$ ; maintenance period:  $\text{Agg}_{\text{max}}$ :  $29.6 \pm 12.7$ ;  $\text{Agg}_{\text{late}}$ :  $8.7 \pm 16.6$ ;  $\text{disAgg}$ :  $72.2 \pm 30.9$ ; AUC:  $67.7 \pm 49.3$ ; VASP-PRI:  $47.9 \pm 19.6$ ). Based on the LTA measurements, high correlation was found between the maximal and late aggregation values ( $p < 0.001$ ; Spearman's  $\rho$ : 0.91). When LTA values were compared to VASP-PRI, significant, moderate-strength correlations were registered without marked difference among the parameters ( $\text{Agg}_{\text{max}}$ :  $\rho = 0.47$ ;  $\text{Agg}_{\text{late}}$ :  $\rho = 0.45$ ;  $\text{disAgg}$ :  $\rho = -0.44$ ; AUC:  $\rho = 0.50$ ). Notably, the efficacy of aspirin therapy, measured by epinephrine 10  $\mu\text{M}$ , did not correlate to VASP-PRI. ( $p = 0.75$ ;  $\rho = -0.24$ ).

In the univariate linear regression analyses, all variables of the LTA curve showed similar, significant relationship with VASP assessments. In the multivariate model, AUC proved to be the independent linear predictor of VASP-PRI.

Bland-Altman plots were used to demonstrate intraindividual agreement among assays in measuring on-clopidogrel platelet reactivity (Figure 2.). These plots demonstrated that  $\text{Agg}_{\text{late}}$ ,  $\text{disAgg}$  and AUC are underestimating VASP-PRI (bias: -10.6, -19.9 and -15.1, respectively) while platelet reactivity is estimated quite similarly by  $\text{Agg}_{\text{max}}$  (bias: 1.3) and VASP-PRI. The wide ranges of agreement in case of all LTA variables underscored that there are substantial intraindividual differences between LTA and VASP assessments (Figure 2.).



**Figure 2. Bland–Altman plots to demonstrate intraindividual agreement in platelet reactivity.**

The comparison was performed between vasodilator stimulated phosphoprotein phosphorylation index (VASP-PRI) and estimates of light transmission aggregometry. Bias (red line) is a measure of a systematic error leading to over- or underestimation of a known value (VASP-PRI) by the alternative parameters of the light transmission assessment. Dashed lines represent limits of 95% agreement that form a range within 95% of the measurements can be found. As the principle of Bland-Altman analysis is that both measurements evaluate a parameter on the same scale (platelet reactivity, %), all the light transmission parameters were normalized to the scale of VASP-PRI (from 0% to 100%).

### **Agreement between assays in determining normal and high platelet reactivity**

The predictive value of LTA variables in determining HPR as well as the optimal cut off values for the best agreement were evaluated with receiver-operator characteristic (ROC) curve analysis. LTA estimates were equal in predicting HPR with AUC showing the highest area under the ROC curve.

Based on the optimal cut off values, we registered significant relationships with moderate-strength agreements between VASP and LTA parameters in classifying patients to normal or HPR categories.

Cut-off	HPR <sup>#</sup>	Specificity	Sensitivity	Concordant	Discordant	$\kappa$
AGG <sub>max</sub> >34.5%	39.3%	79.4%	61.3%	71.1%	28.9%	0.4 <sup>*</sup>
AGG <sub>late</sub> >12%	37.2%	83.2%	62.2%	73.1%	26.9%	0.45 <sup>*</sup>
disAGG >63.5%	37.6%	80.2%	63.1%	72.7%	27.3%	0.44 <sup>*</sup>
AUC >82 × min	39.7%	86.7%	60.8%	72.3%	27.7%	0.44 <sup>*</sup>

**Figure 3. Diagnostic accuracy of ROC-defined cut off values in identifying patients as normal (VASP-PRI<50%) or high platelet reactivity (VASP-PRI ≥50%). Optimal cut off values for each aggregometric variable (Agg<sub>max</sub>, Agg<sub>late</sub>, disAgg, AUC) were determined with receiver-operating characteristic curve (ROC) analysis.**

*Determining the impact of genetic variants on post-clopidogrel platelet reactivity in patients after elective percutaneous coronary intervention*

## Genotype distribution

Out of the 200 patients, allelic variants of CYP2C19 and PON1 were determined in 189 cases, while genetic information was available on ABCB1 genotypes in 181 patients.

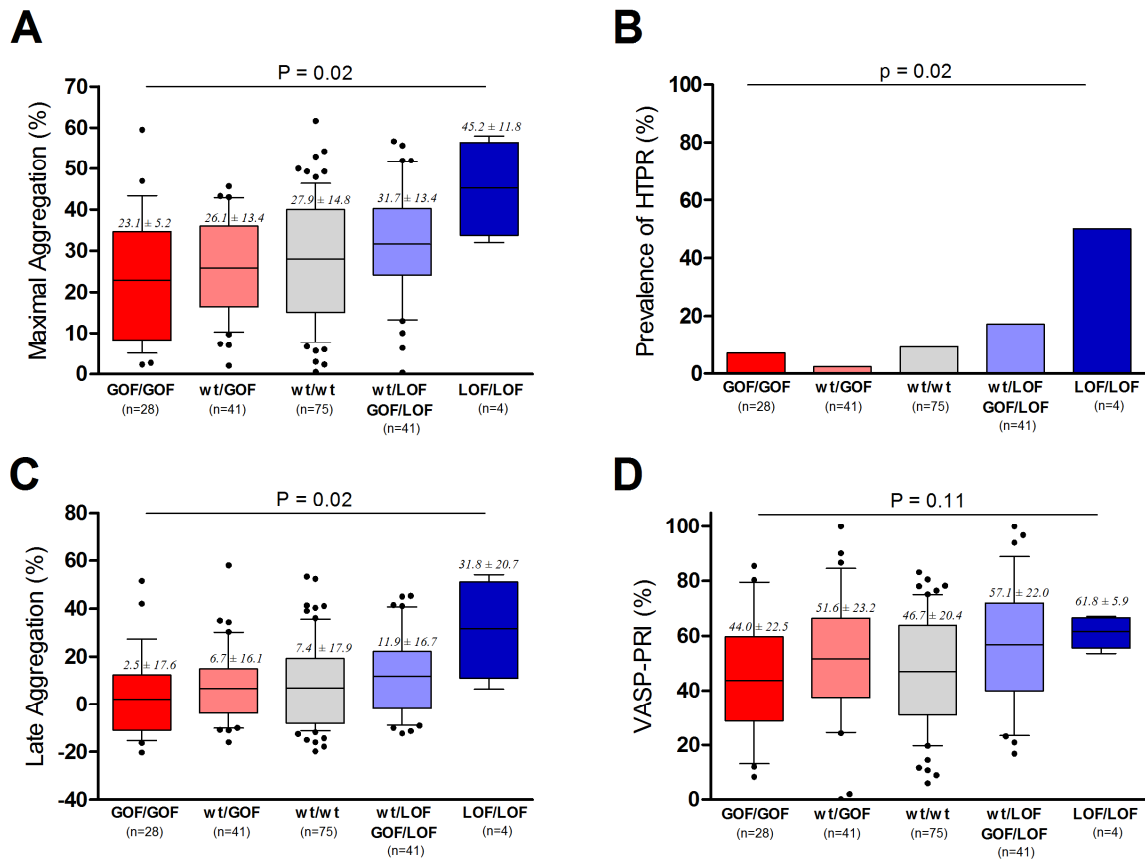
## Definition of normal and high platelet reactivity

In the current genetical trial, following the recommendation of the consensus paper, we defined HPR as an Agg<sub>max</sub> ≥46% value.

## CYP2C19 genotypes and platelet reactivity

According to the platelet function results in the case of CYP2C19 locus, patients harboring a LOF allele had significantly higher Agg<sub>max</sub> (32.9±13.6 vs. 26.4±14.5; P=0.01), 6-minute late aggregation (13.7±17.8 vs. 6.3±17.3, p<0.01) and VASP phosphorylation (57.6±20.8 vs. 47.6±.6; P=0.02) than those with

wild-type alleles. On the contrary, harboring at least one GOF allele only slightly decreased platelet reactivity ( $\text{Agg}_{\text{max}}$ :  $26.4 \pm 14.4$  vs.  $29.2 \pm 14.6$ ,  $P=0.19$ ;  $\text{Agg}_{\text{late}}$ :  $6.1 \pm 16.8$  vs.  $9.5 \pm 18.3$ ,  $P=0.19$ ). When patients were divided into groups according to different genotypes, a gene-dose effect appeared (Figure 4.).



**Figure 4. Comparison in platelet reactivity according to CYP2C19 genotypes.**

Platelet reactivity was compared with light transmission aggregometry (Panel A and C) and vasodilator stimulated phosphorylation (VASP) assay (Panel D) among patients with different CYP2C19 genotypes.

Platelet reactivity increased gradually through genotypes according to the following: GOF homozygotes, GOF/wt heterozygotes, wt homozygotes, wt/LOF or LOF/GOF carriers and LOF homozygotes (Figure 4.). Similarly, the proportion of patients with HPR increased across genotypes. Despite the increase in platelet reactivity through CYP2C19 genotypes, a wide variability in platelet function results in all genotype groups existed.

## PON1 genotypes and platelet reactivity

Based on the results of LTA and VASP assessments, we found lack of evidence of association between PON1 Q192R polymorphism and post-clopidogrel platelet reactivity.

## ABCB1 genotypes and platelet reactivity

There were no significant differences in  $Agg_{max}$ ,  $Agg_{late}$  and VASP-PRI values regarding ABCB1 3435 and 2677 genotypes. In fact, low expresser patients with T3435T genotype had numerically lower  $Agg_{max}$  values than the high expresser C3435C carriers ( $30.7 \pm 15.3$  vs.  $26.1 \pm 13.8$ ;  $P=0.11$ ). In parallel, C3435C genotypes were associated with a higher rate of HPR (9 [19.6%] vs. 9 [6.7%],  $P=0.02$ ); but the predictive value of C3435C genotype on HPR was poor (Table 1.).

Test Result Variables	AUC	95% CI	P
LOF+GOF+ABCB1	0.697	0.558 - 0.837	0.006
LOF+GOF	0.670	0.538 - 0.802	0.018
LOF	0.639	0.495 - 0.783	0.053
ABCB1	0.630	0.484 - 0.776	0.070
PON-1	0.583	0.448 - 0.719	0.247
GOF	0.559	0.422 - 0.696	0.415

GOF: gain-of-function, HPR: high on-treatment platelet reactivity; LOF: loss-of-function; PON-1: paraoxonase-1.

Classification: GOF: \*17-carriers vs. non-carriers; LOF: \*2 or \*3 carriers vs. non-carriers; ABCB1: 3435 CC carriers vs. CT and TT; PON-1: 192 RR and QR vs. QQ carriers; LOF+GOF: \*17\*17 or \*1\*17 vs. \*1\*1 vs. \*1\*2 or \*1\*3 or \*2\*17 or \*2\*2; LOF+GOF+ABCB1: CYP2C19 \*17 carriers and 3435 CC non-carriers vs. CYP2C19 \*1\*1 and 3435 CC non-carriers vs. CYP2C19 \*1\*1 and 3435 CC carriers vs. CYP2C19 \*2 or \*3 carriers and 3435 CC non-carriers vs. CYP2C19 \*2 or \*3 carriers and 3435 CC carriers.

**Table 1. Predictive values of different genotypes on HPR.**

In the multivariate binary logistic regression model, including ABCB1, PON1 and CYP2C19 genotypes, the carriage of CYP2C19 LOF alleles proved to be the independent determinant of HPR. This was also confirmed by the ROC analysis, in that CYP2C19 LOF allele carriage had the highest predictive value in forecasting HPR (Table 1.). Combining the genotype information from LOF and GOF alleles increased the ability to predict HPR; however, the highest area under the curve value was obtained when ABCB1 C3435C carrier status was added to CYP2C19 genetic information (Table 1.).

### **Clinical outcome**

When patients with any LOF alleles were compared to non-LOF carriers, there were no significant differences in the rate CV death, MI or TVR, during the one year follow up (Kaplan-Meier estimate: 13.8% vs. 11.1%, HR: 1.24 95%CI: 0.44-3.47, P=0.69). However, when patients were grouped according to the number of the LOF alleles, it appeared that those with one LOF allele had similar risk for the primary composite outcome as non-LOF carriers (HR: 0.80 95%CI: 0.23-2.79, P=0.72), while poor metabolizer patients with two LOF alleles had a more than 7-fold unadjusted relative risk to CV death, MI or unplanned TVR (HR: 7.22, 95%CI: 1.61-32.65, P=0.01). When we adjusted for possible confounders between patients, including the presence of a GOF allele, this excess risk remained significant (HR: 9.44, 95%CI: 1.96-45.38, p<0.01).

There were no significant differences in the evaluated composite outcome between patients with different ABCB1 C3435T and G2677T/A genotypes. Patients with the C3435C genotype had numerically higher risk to CV death, MI or TVR compared to those with C/T or T/T genotype (Kaplan Meier estimate: 14.8% vs. 10.6%, HR: 1.61, 95%CI: 0.60-4.35, P=0.35).

There were no differences in clinical outcomes according to PON1 Q192R SNP.

## Discussion

High platelet reactivity level, after receiving percutaneous coronary intervention (PCI), detected by an ADP-specific laboratory assay, refers to higher risk for CV death, non-fatal MI, stent thrombosis and recurrent ischemic events. In our meta-analysis, although there were large differences in the methodology, patient selection and cut off definition between studies, the predicted risk of CV death, MI and ST, according to HPR were not heterogeneous.

Therefore, the 3-fold higher risk for non-fatal MI, the 3.4-fold increase in CV death and the 4-fold higher rate for definite / probable ST were demonstrated in almost 9,200 patients.

In the meta-analysis, we observed large inter-study and intra-assay heterogeneity in the prevalence of HPR that resulted in a range of 6 to 80%. This was mostly due to the differences in the methodologies and in the diverse definitions of platelet reactivity cut offs.

We evaluated the utility and reliability of different parameters of conventional aggregometry and VASP-PRI on monitoring platelet reactivity and predicting clinical outcome, and found that all involved estimates of the LTA assessment are equal in monitoring specific P2Y<sub>12</sub> receptor inhibition or in predicting VASP-defined high platelet reactivity. We found according to the results of ROC analysis, LTA measures were equivalent in predicting HPR.

Following these, by defining the optimal cut off values of LTA parameters normal and HPR patients were separated. When these optimal thresholds were adopted, Agg<sub>late</sub> showed the highest categorical agreement with VASP-defined NPR and HPR.

Our results also affirmed that, the most widely used LTA parameter the Agg<sub>max</sub>, predicts clinical outcomes after PCI, equally precise as the Agg<sub>late</sub>, hence superiority of one over the other has not been evidenced.



In multivariable linear regression analysis, although the differences were minimal between  $Agg_{late}$ ,  $Agg_{max}$ ,  $disAgg$  and AUC, not  $Agg_{late}$ , but AUC proved to be the independent predictor of VASP-PRI. In spite of the significant correlation, there were considerable intraindividual differences between LTA and VASP assessment.

As genetic variations may account for the interindividual differences in the achieved anti-platelet efficacy, the impact of CYP2C19, ABCB1 and PON1 genes' allelic variants on Platelet Reactivity in Patients after PCI were investigated. Results of the study showed that CYP2C19 allelic variants exert a gene-dose effect on post-clopidogrel platelet reactivity: those harboring two gain-of-function alleles (\*17) have the lowest average platelet reactivity values, while platelet reactivity increased gradually through ultrarapid - rapid - extensive - intermediate - poor/rapid - poor metabolizer phenotypes reaching the highest degree among carriers of two LOF alleles. Despite the clear effect of CYP2C19 alleles on platelet function, there was large variability in platelet reactivity according to genotypes and the carriage of a LOF allele explained only 3.6% of this variability.

Although our study was not empowered to demonstrate clinical differences between genotypes, we found that in parallel to platelet function results, patients carrying two LOF alleles had significantly higher risk to ischemic events after elective PCI.

Based on results of previous studies, effects of ABCB1 genetic variants are still confusing. The recent analysis showed that common polymorphisms of ABCB1 (C3435T; G2677T/A) did not significantly influence platelet function results. Though according to our findings numerically higher risk for HPR and adverse outcome among C3435C carriers was observed; but these differences remained non-significant probably due to the small sample size. Although the G2677T/A genotype is in linkage disequilibrium with C3435T genotype, our findings

excluded any interaction of this SNP with clinical outcome in clopidogrel-treated patients.

Due to the results of two independent platelet-function assays, the main allelic variant of PON1 gene (Q192R) neither did significantly influence platelet function results, nor associated with clinical outcome.

## **Novel findings of the thesis**

High on-clopidogrel platelet reactivity (HPR), measured by an ADP-specific platelet function assay is a strong predictor of cardiovascular death, myocardial infarction and stent thrombosis in patients after percutaneous coronary intervention. Although there were large differences in the methodology, patient selection and cut off definition between studies, the predicted risk of cardiovascular death, myocardial infarction and stent thrombosis were not heterogeneous.

The moderate significant correlation with VASP validates LTA for monitoring the efficacy of P2Y<sub>12</sub> receptor inhibition. LTA parameters were also equivalent in predicting HPR or in classifying patients to VASP-defined categories; however, there might be clinically meaningful differences in the results in certain individuals. Indeed, 6-minute late aggregation (Agg<sub>late</sub>) is not superior to other estimates of LTA in monitoring the efficacy of P2Y<sub>12</sub>-receptor inhibition.

Genetic variants in CYP2C19 have a gene-dose effect on platelet reactivity (HPR), with homozygote LOF carriers having the highest risk for HPR and for adverse ischemic events. Neither ABCB1, nor PON1 genotypes influenced significantly platelet reactivity or outcome.

## **Conclusions and perspectives**

Great hope has been expressed towards the development of personalized medical care strategies in terms of appropriate diagnosis, treatment, and CVD prevention. The issue of validated point-of-care testing and their ability to predict clinical outcomes remains unresolved for anti-platelet drugs. Recent research findings highlight the role of genetic variation as an important variable for optimizing the response to anti-platelet drugs such as clopidogrel. The goal of personalized medicine is to utilize in part the person's genetic makeup as guiding information in clinical decision making. In addition, this approach should also include the impact of important non-genetic factors, such as the clinical status of the patient, the environmental factors including diet, and drug–drug interactions. These together with concurrent diseases and clinical presentation defined risk for recurrent ischemic events and for bleeding should optimally be considered in selecting the most appropriate drugs and doses.

## **Acknowledgement**

I am very grateful to Prof. Dr. Attila Miseta and Prof. Dr. Gábor L. Kovács for giving me the chance to work at the University of Pécs, Institute of Laboratory Medicine in a very pleasant environment, for helping me in my scientific progress and I do appreciate their exceptional enthusiasm, productive and inspiring suggestions that advanced my scientific thinking and carrier.

I would also gratefully appreciate the support and assistance of my tutor, Dr. András Komócsi, who enabled to progress and tackle difficulties during the whole scientific period.

I would like to express my special thanks to Dr. Dániel Aradi for his help and support.

I am also thankful to the late Dr. Tamás Magyarlaki, Dr. Margit Tőkés-Füzesi and Sarolta Bácskai, for their great enthusiasm in helping to accomplish my work. They are not only my colleagues, but very good friends of mine.

Finally, I would like to thank to my family and friends for standing beside me every time I really needed them.