Monitoring and optimizing antiplatelet therapy in patients undergoing percutaneous coronary intervention

PhD thesis

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1. INTRODUCTION

In the past decades, revascularization by percutaneous coronary intervention (PCI) became one of the most important therapeutic possibilities in cardiovascular disease and many procedural and pharmaceutical improvements have been carried out, to maximize its clinical benefit. Introduction of dual antiplatelet therapy with the COX_1 enzyme inhibitor aspirin and the $P2Y_{12}$ receptor inhibitor clopidogrel proved to be essential to prevent adverse thrombotic events. High residual platelet reactivity besides antiplatelet therapy measured by different laboratory methods proved to be an independent predictor of adverse thrombotic events, and recently correlation between low platelet reactivity and bleeding events was also described. However, the different tests have fairly variable predictive power regarding thrombotic and bleeding consequences. As the platelet function testing proven efficacy of clopidogrel showed wide variability, novel, more potent antiplatelet agents (prasugrel, ticagrelor and cangrelor) have been developed and approved. Also, the clinical utility of platelet function testing and tailored antiplatelet therapy became an extensively researched field in cardiology. The so far conducted large randomized controlled trials proved no clinical benefit of monitored and adjusted antiplatelet therapy in low and intermediate risk cardiac patients undergoing PCI. However, some recent smaller studies indicated better outcomes in acute coronary syndrome patients receiving platelet function testing guided intensified antiplatelet therapy.

2. AIMS

We aimed to investigate the contribution of platelet function testing to treatment optimization and risk stratification of patients undergoing percutaneous coronary intervention focusing on patients with acute coronary syndromes, who are

at high thrombotic risk. Also, there is a lack of knowledge regarding the clinical benefit of platelet testing based tailored antiplatelet therapy regarding these patients. Therefore, first we aimed to investigate the incidence of high on-treatment platelet reactivity, the effect of intensified antiplatelet therapy and long term follow-up of platelet function in patients with myocardial infarction and stable coronary artery disease. Secondly, we analyzed predictors of high on-clopidogrel platelet reactivity measured by a clinically recently available point-of-care method in a high thrombotic risk cohort represented by acute coronary syndrome patients. Detailed objectives of our studies were the followings:

1. To assess platelet function values and the rate of high on-clopidogrel platelet reactivity (HCPR) measured with light transmission aggregometry among patients with myocardial infarction (MI) and patients with stable coronary artery disease (SCAD).

2. To assess the influencing clinical and demographical factors of platelet reactivity measured by LTA.

3. To optimize antiplatelet therapy in patients with HCPR until laboratory certified platelet inhibition is achieved in patients with MI and SCAD.

4. To conduct a long-term follow up of individually tailored antiplatelet therapy with repeated platelet function testing and registration of clinical outcome data in patients with MI and SCAD.

5. To identify predictors of HCPR measured with the point-of-care multiple electrode aggregometry in the acute coronary syndrome patient cohort with high thrombotic risk.

6. To develop a HCPR risk prediction model and perform HCPR risk stratification of patients with ACS.

3. METHODS

To answer the above questions we conducted two prospective studies on separate patient cohorts.

3.1. Monitoring and optimizing antiplatelet therapy in patients with myocardial infarction and stable coronary artery disease

3.1.1. Patient population and study design

We enrolled 200 patients into our study: 133 patients with myocardial infarction (MI) and 67 patients with stable coronary artery disease (SCAD). All patients underwent PCI and intracoronary stenting.

A loading dose of 600 mg clopidogrel was given to all patients in the MI group and to clopidogrel naive patients in the SCAD group. MI patients were given 75 or 150 mg clopidogrel maintenance dose based on the physician's decision. Higher doses of clopidogrel were applied in the first 30 days after PCI, then standard 75 mg clopidogrel for the whole study period. In the SCAD group, clopidogrel maintenance dose was 75 mg daily. All patients were given ≥100 mg aspirin daily. Patients were re-interviewed and repeated LTA was performed at 6 and 12 month.

3.1.2. Aggregometry measurements

Platelet function was measured with LTA (Carat TX4, Budapest, Hungary) 72 hours after PCI in the MI group and 24 hours after PCI in the SCAD group. Platelet agonists were 10 μM epinephrine (EPI), 1 μg/ml collagen (COLL) and 0,5 μg/ml arachidonic acid (AA) to assess overall platelet reactivity and 1,25 μM; 5 μM; 10 μM ADP to assess

clopidogrel's effectiveness specifically. Control LTA was performed 5 days after therapeutic adjustments.

3.2. Determining factors of high on-treatment platelet reactivity in patients with acute coronary syndrome

3.2.1. Patient population and study design

In this study, we enrolled 463 consecutive ACS patients referred for urgent coronary angiography (334 cases with ST segment elevation myocardial infarction (STEMI), 110 patients with non-ST segment elevation myocardial infarction (NSTEMI) and 19 cases with unstable angina (UAP)). 95.9% of the population underwent percutaneous coronary intervention and 93.9% had coronary stent implantation. Acetylsalicylic acid loading and maintenance treatment was applied according to current guidelines. A single loading dose of 600 mg clopidogrel was given to all patients prior to or at the time of angiography/PCI.

Use of glycoprotein IIb/IIIa receptor (GPIIb/IIIa) inhibitor (eptifibatide) was left to operator's discretion. Unfractionated heparin was administered for both diagnostics and percutaneous coronary intervention

3.2.2. Platelet function testing

Platelet function was measured in whole blood with multiple electrode aggregometry (Multiplate analyzer, Roche, Basel, Switzerland, agonist was adenosine-diphosphate (ADP) at 6.4 μM final concentration) 12 to 36 hours after 600 mg clopidogrel loading. When the GPIIb/IIIa inhibitor eptifibatide was applied during the PCI, based on the pharmacokinetic features of the drug, platelet function test was postponed to ensure a 24-hour time lag from cessation of eptifibatide administration. The definition of high on-clopidogrel-treatment platelet reactivity (HPR) was based on the consensus paper of the Working Group on On-Treatment Platelet Reactivity, using >46 U as the cut-off value. Below this

threshold, platelet inhibition was considered to be efficient (no HPR). Besides platelet function testing, other laboratory measurements were also performed (e.g. hematology testing, blood glucose and high sensitive C reactive protein (hs-CRP) and troponin I level measurements).

3.3. Statistical methods used in the studies

Categorical variables in 2×2 contingency tables were assessed using Fisher's exact test. Categorical data in 2×k contingency tables were analyzed using the unordered chi squared test or, to detect linear trend, the Cochran Armitage test (chi-squared test for trend). Continuous parameters were examined for normality with the Shapiro-Wilks W and the D'Agostino Pearson test. As none of the investigated continuous variables showed normal distribution, the Mann–Whitney test was applied for inter-group comparisons. The Wilcoxon signed-rank test or repeated measures ANOVA test was used for repeated measures. To adjust for differences in demographic data between patients with stable coronary artery disease and myocardial infarction, ANCOVA analysis was used.

For the risk prediction model construction, logistic regression analysis was used. Univariate logistic regression analysis was performed to identify parameters with a p value less than 0.2. These variables (along with clinical parameters that were previously found to be associated with HPR, irrespective of the univariate p value) were entered into a backward multivariate logistic regression model. In multivariate logistic regression, parameters with a p value above 0.1 on likelihood ratio testing were then sequentially removed. The likelihood ratio and the Hosmer-Lemeshow tests were used to assess model fit, whereas predictive power was evaluated by receiver operating characteristic (ROC) curve analysis. Since the performance of a prediction model in the derivation dataset may overestimate the true performance, we conducted internal validation using 10,000 bootstrap samples to assess optimism. Risk of HPR was stratified as low, intermediate or high based

on sensitivity, specificity and cumulative frequency distribution analyses of the predicted probability. For each of the risk classes, interval likelihood ratios were calculated.
A two-tailed p value less than 0.05 was considered statistically significant. All analyses were carried out with MedCalc 15.2 (MedCalc Software, Ostend, Belgium) except for internal validation, which were performed with R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria, available at http://www.R-project.org) using the 'rms' package 4.2-1 (authored by Frank E Harrell Jr, available at http://biostat.mc.vanderbilt.edu/rms).
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4. RESULTS

4.1. Monitoring and optimizing antiplatelet therapy in patients with myocardial infarction and stable coronary artery disease

Our first prospective study was conducted in patients with myocardial infarction (N=133) and stable coronary artery disease (N=67) undergoing percutaneous coronary intervention at our institute.

4.1.1. Baseline platelet aggregations in the MI and SCAD patients
Baseline platelet reactivity with respect to most agonists was significantly higher in patients receiving 75 mg clopidogrel in the MI group than in the SCAD group (COLL p=0.7, 1.25ADP p<0.001, 5ADP p=0.02, 10ADP p<0.01, EPI p<0.01, AA p=0.01). After adjustment to demographic parameters, the difference diminished in case of EPI and AA induced aggregations, however, it remained significant with all concentrations of the ADP agonist (1.25ADP: p=0.005; 5ADP: p=0.046; 10ADP: p=0.023).

Interestingly, patients receiving 150 mg clopidogrel compared to those taking the standard dose within the MI group, showed only a tendency of lower platelet reactivity, without reaching statistical significance. Moreover, after adjusting to demographic parameters, this tendency completely diminished regarding all agonists.

4.1.2. Definition and ratio of high on-clopidogrel platelet reactivity based on LTA results in MI and SCAD patients

5 μM ADP induced maximal aggregation (AGGRmax 5ADP) was considered as the indicator of the efficacy of clopidogrel therapy with the cut-off value of AGGRmax 5ADP>50 %. Above this value high on-clopidogrel platelet reactivity (HCPR), below this value no high on-clopidogrel platelet reactivity (no HCPR) was identified, independently of the applied clopidogrel maintenance dose.

The number of patients with high platelet reactivity tended to be higher in the MI than in the SCAD group (MI: 19.5% vs SCAD: 11.9%; p=0.232). Similarly to the baseline platelet aggregation data, proportion of HCPR patients within the MI group tended to be higher among patients on 75 mg maintenance dose compared to those being on 150 mg (26.8% vs. 16.3%, p=0.164).

4.1.3. Definition, ratio and management of clopidogrel pseudo and real non-responders

In HPR patients, antiplatelet therapy was adjusted as follows: in case of 75 mg clopidogrel, the drug dose was doubled. In patients with HPR already on 150 mg clopidogrel, 2x250 mg ticlopidine was induced, as the study was conducted before the prasugrel/ticagrelor era.

Patients with high initial platelet reactivity on 75 mg but reaching normal platelet reactivity on 150 mg clopidogrel were defined as clopidogrel pseudo non-responders (PsNR). In

contrast, patients with persisting high platelet reactivity even on 150 mg clopidogrel were defined as clopidogrel real non-responders (RNR).

The ratio of real non-responders was significantly higher in the MI group compared to SA group (MI: 18/133=13.5% vs SCAD: 2/67=2.9%; p=0.023). The ratio of pseudo non-responders also tended to be higher in the MI group but did not reach statistical significance (MI: 8/41=19.5% vs SA: 6/67=8.9%, p=0.143).

4.1.4. Functional results of antiplatelet therapy modification

In pseudo non-responders clopidogrel dose doubling resulted in effective platelet inhibition. On the other hand, switch of therapy to ticlopidine also resulted in normal platelet reactivity in all patients in the SCAD group (2/2) and in the majority of real non-responders in the MI group (16/18). Patients undergoing therapy intensification remained on modified therapy for the whole study period.

4.1.5. Long term follow-up of platelet function in MI and SCAD patients

In the MI group most of the patients (n=84) were already on standard 75 mg clopidogrel therapy by 6 month, however 8 patients received 150 mg clopidogrel and 10 patients received ticlopidine as a result of therapy intensification. At 12 month, 60 patients were on standard, 5 patients were on high dose clopidogrel and 10 patients were on ticlopidine. Platelet reactivity remained on the same level throughout 12 months follow-up in the MI group.

In the SCAD group 44 patients were on standard, 2 patients were on high dose clopidogrel and 2 patients were on ticlopidine at 6 month. At 12 month, 38 patients on 75 mg, 1 patient on 150 mg clopidogrel and 1 patient on ticlopidine

were available for retesting. In SCAD patients, platelet reactivity showed slight oscillation: at 6 months platelet aggregation was significantly higher compared to the 12 month values (5ADP6month vs. 5ADP12month p=0.005).
Platelet reactivity did not differ between the MI and SCAD group at 6 and 12 month and was also irrespective of the 75 mg or 150 mg clopidogrel maintenance therapy at 6 and at 12 months.

4.1.6. Incidence of new HCPR during 12 month follow-up in MI and SCAD patients

Despite the fact, that out of our 200 patients, 198 individuals were on effective antiplatelet therapy after modifications at baseline, there was a remarkable incidence of new HCPR at 6 month in both patient groups.

In the MI group new HCPR occurred in 12 patients receiving standard and in 2 patients receiving high dose clopidogrel therapy (13.7%). Interestingly, in the SCAD group incidence of new HCPR was unexpectedly high; 14 patients were on 75 mg and 1 patient was on 150 mg clopidogrel.

In these patients, further therapeutic interventions were not performed. Interestingly, the majority of patients with HCPR at 6 month, returned with normal platelet reactivity at 12 month (MI group: 8 patients on 75 mg and 1 patient on 150 mg clopidogrel; SCAD group: 11 patients on 75 mg and 1 patient on 150 mg clopidogrel). Nevertheless, HCPR persisted from 6 to 12 month in 4 patients being on 75 mg and 1 patient being on 150 mg clopidogrel in the MI group. Persisting HCPR from 6 to 12 month was also observed in 3 SCAD patients receiving 75 mg clopidogrel.

By 12 month, new HCPR was observed in 8 MI patients being on standard clopidogrel therapy and no further incidence of HCPR was observed on high dose clopidogrel

therapy. The ratio of new HCPR at 12 month was substantially lower in the SCAD group: only 1 patient on 75 mg clopidogrel returned with new HCPR and no further incidence of HCPR was observed on high dose clopidogrel therapy.

Interestingly, all clopidogrel real non-responders switched over to ticlopidine remained effectively inhibited during the whole follow-up period.

4.1.7. Clinical end points during 12 month follow up

The 12 month cumulative event rate was 8.3% in the MI and 1.5% in the SCAD group. During the 12 month follow-up no TIMI major or minor bleeding events were documented.

4.2. Determining factors of high on-treatment platelet reactivity in patients with acute coronary syndrome

In a second prospective cohort study, we investigated the predictors of HCPR measured by the point-of-care multiple electrode aggregometry.

4.2.1. Distribution of platelet aggregation values measured by MEA

The ADP induced-platelet aggregation units (U) of the overall population showed a right skewed, unimodal distribution with a median (M) of 29.0 U (IQR 21.0 to 39.0 U, Figure 10). Based on the >46 U cut off value, the proportion of HCPR was 74/463=16.0%. The median platelet aggregation values in the HCPR and no HCPR groups were 56.5 U (IQR 50.0 to 62.0 U) and 27.0 U (IQR 20.0 to 34.0 U), respectively.

4.2.2. Factors related to HCPR, model construction

Univariate logistic regression analysis was performed to a wide scale of parameters. Variables univariately associated

with HCPR with a p value less than 0.2 were as follows: PLT count (per G/L, p=0.0006), white blood cell count (WBC, per G/L, p=0.06), CRP level (per mg/L, p=0.03), troponin I level >50 ng/mL (p=0.07) upon admission, female gender (p=0.06) and non-smoking status (p=0.07). These factors and clinical parameters that were previously found to be associated with HCPR, such as diabetes mellitus, BMI and renal function were entered into the backward multivariate logistic regression model. Based on the analysis, PLT count (per G/l, odds ratio [OR]: 1.0073, 95% confidence interval [95% CI]: 1.0035 to 1.0112, p=0.0002), CRP level (per mg/L, OR, [95% CI]: 1.0077 [1.0016 to 1.0137], p=0.01) upon admission, and current smoking (OR [95% CI]: 0.51 [0.29 to 0.89], p=0.02) proved to be predictors of HCPR.

The likelihood ratio and Hosmer-Lemeshow tests showed good model fit ($p<0.0001$ and p=0.43, respectively).

The area under the ROC curve analysis revealed moderate predictive power (AUC=0.665).

4.2.3. Internal validation

Since the performance of a model in the development dataset may overestimate the true performance, we conducted internal validation using 10,000 bootstrap samples. Predictive accuracy was characterized by the AUC value while calibration was assessed by means of the intercept and slope of the calibration line. Moreover, we evaluated calibration graphically by applying a LOWESS smoother on scatterplots of predicted versus observed probabilities. Considering accuracy, optimism proved to be 0.013 resulting in an optimism-corrected AUC value of 0.653. Both the optimism adjusted intercept and slope were on average correct (-0.102 and 0.930, respectively), not necessitating recalibration of the apparent model.

4.2.4. Risk stratification

Based on sensitivity, specificity and cumulative frequency distribution analyses of the predicted probability, three risk classes (low, intermediate, and high risk) were defined. Using this classification, low- and high risk patients, who represent some 60% of the population, may be precisely identified. There is more than a fourfold increase in post-test probability of HCPR between patients of the low- and high risk strata (8.7% versus 35.7%). On the contrary, pre- and post-test probabilities are almost identical (16.0% versus 18.1%) in the intermediate risk group corresponding to some 40% of the cases.

Using risk stratification, low, intermediate and high risk patients were successfully separated. 95% confidence intervals of the interval likelihood ratios do not overlap each other suggesting clearly different levels of risk in the three strata.

Also, with increasing risk class, there is a monotonic rise in the observed rates of high on clopidogrel platelet reactivity (Cochran Armitage test, $p<0.0001$). Moreover, differences between the predicted and actual event rates were small across the risk strata.

5. CONCLUSIONS

5.1. We found an increased level of ADP induced platelet reactivity measured by light transmission aggregometry in patients with myocardial infarction compared to those with stable coronary artery disease, representing heightened atherothrombotic risk of the acute patient cohort.

5.2. In patients with myocardial infarction, initial, clinical risk profile but not platelet function test guided clopidogrel dose

doubling resulted in the same inhibitory potency as the standard dose clopidogrel in patients with expectedly lower risk for high on-treatment platelet reactivity.

5.3. The ratio of patients, who had high on-clopidogrel platelet reactivity even on high dose clopidogrel (real non-responders) was significantly higher in the myocardial infarction group, suggesting that this patient cohort with higher thrombotic risk might benefit from prospective platelet function testing, especially in view of reported prevalence of high on-treatment platelet reactivity even beside the novel antiplatelet agents.

5.4. Therapy modification resulted in effective platelet inhibition in majority of the patients, according to in vitro testing. The potent inhibitory effect of therapy conversion proved to be stable during 12 month follow-up.

5.5. Among patients on clopidogrel therapy, appearance of new high on-clopidogrel platelet reactivity was observed during 12 months, irrespective of applied clopidogrel dose or representing clinical syndrome. This may suggest the vulnerability of clopidogrel effect in the long term, and highlights the potential significance of repeated evaluation of antiplatelet therapy.

5.6. We analyzed the association of a wide range of clinical and laboratory parameters with high on-clopidogrel platelet reactivity measured by multiple electrode aggregometry in an acute coronary syndrome cohort and identified elevated platelet count and CRP level upon admission and a non-smoking status as predictors.

5.7. We developed and internally validated a risk score for high on-clopidogrel platelet reactivity, which enabled successful classification of the patients into low, intermediate and high risk strata. Patients with the highest risk had more than a fourfold increase in post-test probability of high on-clopidogrel platelet reactivity compared to those with low risk. Such a risk assessment of high on-treatment platelet reactivity might enable more targeted use of platelet function testing to identify patients who may benefit from therapy intensification.

5.8. With recent reclassification of on-treatment platelet reactivity into low, optimal and high categories, platelet function testing may be used to find a therapeutic window to optimize the balance of ischemic and bleeding consequences in patients on dual antiplatelet therapy.

6. OWN PUBLICATIONS

Publications related to the thesis:

Leé S, Vargová K, Hizoh I, Horváth Z, Gulácsi-Bárdos P, Sztupinszki Z, Apró A, Kovács A, Préda I, Tóth-Zsámboki E and others. High on clopidogrel treatment platelet reactivity is frequent in acute and rare in elective stenting and can be functionally overcome by switch of therapy. Thromb Res 2014;133(2):257-64. **IF: 2.447**

Lee S, Hizoh I, Kovacs A, Horvath Z, Kiss N, Toth-Zsamboki E, Kiss RG. Predictors of high on-clopidogrel platelet reactivity in patients with acute coronary syndrome. Platelets 2015:1-9.
IF: 2.982

Leé S, Kiss RG, Noori E, Kiss N. Vérlemezkegátlás a kardiológiában – Új horizontok. Antiplatelet therapy in cardiology – new horizons. Cardiologia Hungarica 2011;41:46-52. Article in Hungarian.

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Horvath Z, Csuka D, Vargova K, Kovacs A, Molnar AA, Gulacsi-Bardos P, Lee S, Varga L, Kiss RG, Preda I and others. Elevated C1rC1sC1inh levels independently predict atherosclerotic coronary heart disease. Mol Immunol 2012;54(1):8-13. **IF: 3.003**

Homorodi N, Kovacs EG, Lee S, Katona E, Shemirani AH, Haramura G, Balogh L, Bereczky Z, Szoke G, Peterfy H, Kiss RG, Edes I, Muszbek L. The lack of aspirin resistance in patients with coronary artery disease. J Transl Med 2016;14(1):74. **IF: 3.930**

Vargová K, Pállinger É, Horváth Zs, Kovács A, Leé S, Gulácsi-Bárdos P, Falus A, Kiss RG, Préda I. A mikrovezikulumok szerepe cardiovascularis kórképekben. The role of microvesicles in cardiovascular disease. Orvosképzés 2012;2:55-60. Article in Hungarian.