Clinical implications of monitoring the efficacy of antiplatelet therapy in patients after percutaneous coronary intervention

Ph. D. Thesis

by

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Introduction

Percutaneous coronary interventions (PCI) have revolutionized the treatment of ischemic heart disease. Following the pioneer interventions of Andreas Grüntzig in 1977, approximately 10.000 patients are treated with this technique every year in Hungary. Exploiting numerous advantages of a minimal invasive intervention, substantial technological and pharmacological advances were made to increase the feasibility and procedural success during the last decade. Consequently, balloon angioplasty, the initial method for dilating coronary stenoses has been replaced with coronary stent implantation due to the lower rates of acute (dissection, abrupt vessel closure) and chronic (restenosis) complications of the latter. (1, 2) Although stent implantation dramatically improved the overall success rate of PCI, limitations are also evident both in short- and long-term. The main shortcomings include stent thrombosis (ST) and instent restenosis (ISR) that both result in target-vessel failure after PCI.

Stent thrombosis

Stent thrombosis is an acutely occurring, quick and mostly total occlusion of a previously implanted coronary stent that usually manifests in high-mortality myocardial infarction (MI). (3) In parallel to de novo coronary artery thrombosis, stent thrombus is mainly formed by activated and aggregated platelets that are stabilized by fibrin clots. It is thought to be a multi-factorial event, in that both procedural (under-expansion, malposition and malapposition of stents, long stented segment, residual edge-dissection), clinical (low ejection fraction, acute myocardial infarction, impaired renal function, diabetes) and hemorrheologic abnormalities (premature discontinuation of antiplatelet therapy, aspirin/thienopyridine resistance) play substantial role. (4) Notably, the administration of dual antiplatelet therapy has decreased the occurrence of ST compared to aspirin monotherapy or aspirin plus anticoagulation in patients after PCI. (5-8) Thus, aggressive inhibition of platelet function seems essential to obviate these ischemic events. Although being a relatively rare event, the prevalence of ST varied largely between clinical trials and registries due to terminological heterogeneity. For obtaining universal definitions and in order to improve comparability and interpretation of results, the Academic Research Consortium (ARC) proposed consensus categories for ST, based on the event probability and level of evidence. Also known as the Glasgow-classification, these criteria define three categories of ST (ARC I: definite; II: probable; and III). Alternatively, ST can be classified based on the timing of occurrence. (Table 1) According to the ARC criteria, definite ST occurs in 0.5-1.5% after the first year of PCI, while possible ST has a prevalence of 2 to 4% (9, 10).

Instent restenosis

Instent restenosis (ISR) is a chronic process that progressively and irreversibly reduces the previously stented vessel lumen. Similarly to ST, ISR is also a multi-factorial event, in that procedural (under-expansion, malposition, malapposition of stents, long stented segment, smaller stent diameter), clinical (acute myocardial infarction, diabetes, inflammation and hypersensitivity) and hemorrheologic abnormalities (slow flow, not good outflow from

stented segment) might play significant part. (11, 12) Compared to normal neointimal formation, ISR is accompanied by overproliferation of the endothelial and smooth muscle cells around the stented segment that leads to a fibrous and thickened neointima. (13, 14) In most of the cases, ISR develops in the first year after coronary intervention and is usually manifested in recurrent stable angina. However, in a minority (2-3%) of the patients, ISR leads to myocardial infarction due to the critical ischemia. (15) Recently, the role of platelet activation and impaired response to antiplatelet therapy were also suggested in the development of ISR as activated platelets might trigger the proliferation of the neointimal tissue by releasing growth factors (PDGF) and recruiting leucocytes. (13)

With the implantation of bare metal or cobalt chromium stents, ISR occurred in 20 to 40% of patients after PCI. In the past years, drug-eluting stents (DES) proved to be extremely successful in preventing instent restenosis. (9, 10) The anti-inflammatory, anti-proliferative and/or cytostatic drugs that are released from stents to the vessel wall were successful to prevent neointimal hyperplasia and decreased the incidence of angiographic instent restenosis below 10%. On the other hand, the eluted antiproliferative drugs prevent healing and complete endothelization of such stents that prolongs the risk of stent thrombosis. Likewise, efficient and long-lasting antiplatelet therapy has a substantial importance in the DES era, as well. Despite being successful in decreasing the rate of target lesion revascularisations (TLR), the first-generation drug- (sirolimus and paclitaxel) eluting stents failed to reduce hard cardiovascular endpoints, such as cardiac death or MI compared to bare metal analogues after PCI. Moreover, higher prices also limit their availability. As the current penetration of DES does not exceed 20-25%, ISR remains a clinically relevant problem in Hungary.

Inter-individual differences in response to antiplatelet therapy

According to in vitro/ex vivo measurements, administration of aspirin does not inhibit platelet activation and aggregation in a proportion of patients; their platelet reactivity is comparable to aspirin-free subjects. Classified as ‘aspirin resistants’ or ‘non-responders’, these patients can be separated from good responders with laboratory assessments. (40) On the other hand, there are subjects who incur adverse thrombotic events despite being on aspirin therapy that is considered a ‘treatment failure’. Although treatment failure to aspirin and aspirin resistance are different the first is defined by clinical the latter by laboratory assessment aspirin resistant patients were shown to be at higher risk for ischemic vascular events. (41) Hence, aspirin resistance might be more than just a laboratory curiosity as high proportion of aspirin resistants ended up with a clinical treatment failure. (42) As a result of methodical and terminological heterogeneity, the prevalence of aspirin resistance varies a lot between authors; ranging from 5% to 65%, with a recent meta-analysis suggesting a mean prevalence of 28%. (43) These results highlight the fact that not all methods are equal in defining antiplatelet response to aspirin. (40, 44)

In patients after PCI, the administration of clopidogrel has dramatically reduced the rate of ischemic events as well as bleeding complications compared to the coumarin and aspirin combination. (5-8) However, thrombotic events still ocurred in spite of the dual antiplatelet therapy, thereby extensive research started to explore the individual response to clopidogrel. Early studies demonstrated large inter-individual differences in response to a fix-dose

clopidogrel, and similar to aspirin, the term “clopidogrel resistance” was created and widely applied to refer for patients with inappropriate response. (45-47)
Numerous observational studies pointed out the higher risk of patients with inappropriate response to clopidogrel to recurrent thrombotic events. (48-55) However, according to the 2005 ACC/AHA/SCAI guidelines on PCI, antiplatelet testing is only recommended in patients in whom ST would be of catastrophic consequences (18). This is due to the lack of a uniformly available, standardized, cheap, quick and reliable bedside assay that provides comparable and interpretable results of antiplatelet efficacy. Moreover, results of randomized, sufficiently powered trials are also awaited to clarify the clinically relevant cutoff values. Currently, light transmission aggregometry is considered the historical gold-standard in monitoring the efficacy of antiplatelet therapy. (56)

Aims

The main aims of our examinations were the following:

- to characterize the individual response and efficacy of both aspirin and thienopyridine therapy in patients admitted for percutaneous coronary intervention and compare the agreement between relevant methods in monitoring antiplatelet efficacy.

- to determine the possible clinical implications of the variability observed in the antiplatelet response among patients after PCI.

- to determine the significance of diabetes mellitus on the prevalence of low response to aspirin and clopidogrel.

- to determine alternative pharmacological approaches in order to overcome inadequate response to clopidogrel and to analyze possible predictors of the response to the alternative approach.

- to determine the prognostic significance of the used access site (femoral vs. radial) on both bleeding and ischemic complications after PCI in patients receiving dual/triple antiplatelet therapy.

Methods

Light Transmission Aggregometry

Carat TX4 four-channel light transmission aggregometer (LTA; CARAT Diagnostics, Budapest, Hungary) was used for ex vivo measurements to monitor the efficacy of antiplatelet therapy. LTA registers optical density of a suspension trans-illuminated with infrared light. The higher the optical density of the suspension, the less light reaches the sensor. LTA dynamically measures and displays infrared light intensity during assessment for 7 minutes. Having introduced in the late ’60-ies, LTA became the gold-standard of measuring platelet aggregation in subjects with platelet function disorders and in patients receiving antiplatelet therapy. The method is relatively cheap, broadly available and well accepted; enables

monitoring antiplatelet efficacy selectively with specific agonists. However, measurements are poorly standardized, time-consuming, non-automated, require trained personnel for sample preparation and measurement. As a conclusion, it is difficult to compare results and generalize consequences obtained with this method.
For assessments, 10 ml blood needs to be drawn into vacuum-tubes anticoagulated with 3.8% sodium-citrate from every patient. For getting platelet-rich plasma (PRP), blood is centrifuged at 2000 rpm for 4 minutes. Further centrifugation for 10 minutes at 4000 rpm results in platelet-poor plasma (PPP). At baseline, PPP is used to set 100%, while PRP 0% light transmission on the aggregometer. Then, platelet-specific agonists are added into PRP to stimulate platelet aggregation with a continuous magnetic stirring at 37 degree Celsius. In antiplatelet-free subjects, activation of resting platelets results in formation of platelet aggregates that decrease optical density of the plasma. Thus, light transmission increases steeply after the injection of the agonist forming a plateau thereafter. Platelet reactivity is usually expressed with the maximal platelet aggregation value (Aggmax) of the registered optical curve, while other parameters (late aggregation [Agglate], steepness of slope, area under curve [AUC]) and disaggregation [disAgg] may also be determined. (Figure 3, 4)
Efficient antiplatelet therapy prohibits platelet activation, limiting the formation of platelet aggregates and decreasing the peak value of the aggregation curve. Likewise, high Aggmax values are typical for untreated subjects and low responder patients, while low Aggmax reflects effective platelet inhibition. (Figure 3, 4)
As antiplatelet agents block a specific pathway of platelet activation, their efficacy can be measured with a specific agonist: ADP is used to test the efficacy of thienopyridine therapy, while adrenaline, collagen or arachidonic acid is suitable to measure efficacy of aspirin treatment. This means one of the most important advantages of the assay, i.e. to measure the efficacy of antiplatelet agents using specific agonist with high selectivity.

Vasodilator Stimulated Phosphoprotein (VASP)

Platelets express two subtypes of ADP surface receptors: while P2Y1 is essential for initiation of platelet activation, stimulation of P2Y12 receptor causes a decrease in adenyl-cyclase resulting in lower levels of intracellular cyclic adenosine monophosphate (cAMP). Cyclic AMP is necessary for phosphorylation of a second messenger known as vasodilator-stimulated phosphoprotein (VASP). VASP is important for regulation of the cytoskeleton and for conversion of glycoprotein IIb/IIIa to its active conformation, thus permitting platelets to aggregate. VASP exists in both phosphorylated and dephosphorylated states. The phosphorylated form is characteristic for a resting platelet. Inhibition of cAMP-activity after activation of the P2Y12 receptor by ADP leads to an increase in VASP dephosphorylation, whereas blockade of P2Y12 receptor by the active metabolite of a thienopyridines inhibits dephosphorylation. Thus, the ratio of phosphorylated to dephosphorylated VASP reports the degree of the P2Y12 receptor blockade. (Figure 5)
Currently, flow cytometric measurement of VASP phosphorylation represents the most specific method for monitoring the efficacy of thienopyridine therapy. However, the kit is

expensive, the process is time-consuming, requires special instrumental and personal training giving very limited availability to the assay.
In our measurements, the phosphorylation status of VASP was analyzed with fluorescent antibody against VASP-P on a Beckmann Coulter flow cytometer using PLT VASP/P2Y12 kit (Biocytex, Marseille, France). For this assay, citrated whole blood was incubated with PGE1 with or without ADP. After fixation with paraformaldehyde, the cells were permeabilized and incubated with a primary mouse monoclonal antibody specific for phosphorylated VASP, followed by a secondary fluorescein isothiocyanate (FITC)-conjugated polyclonal goat-antimouse antibody. Samples were then analyzed by flow cytometry to measure the level of phosphorylated VASP. The efficacy of ADP-receptor inhibition is expressed with the platelet reactivity index (PRI), which is calculated from the corrected mean fluorescence intensity (MFI) of the PGE1 and the PGE1+ADP-incubated samples as follows: PRI= (MFIPGE1- MFIPGE1+ADP)/ MFIPGE1 x100. (Figure 5-7)

Soluble markers of platelet activation

We collected blood samples for examination of soluble markers of platelet activation using Multiplex Fluorescent Bead Immunoassay (Bender MedSystems GmbH, Vienna, Austria). The assay enabled the detection of soluble VCAM-1, soluble CD40L and soluble P-selectin in patients after coronary stent implantation. 5 ml blood was drawn with direct venipuncture, and centrifuged for separating plasma. Samples were immediately frozen to -20°C to avoid loss of bioactive markers. Prior to assay, the frozen plasma was brought to room temperature slowly, and added to the mixture of seven microbead clusters coated with different monoclonal antibody against the intent-to-measure activation markers (sVCAM-1, sP-seletctin and sCD40L). The activation markers present in the sample bind to the antibodies adsorbed to the fluorescent beads. Phycoerythrin (PE) conjugated second antibody mixture is added and the specific antibodies bind to the activation markers captured by the first antibodies. Microbead clusters were identified according to their intrinsic far red fluorescent activities (690nm). Rows of standard dilution of the intent-to-measure markers were created in parallel to sample analysis in order to obtain standard curves of PE fluorescent intensity (575nm) of the adequate bead cluster. Far red fluorescent intensity (indicate the bead cluster, the activation marker) and PE flurorescent intensity (indicate the concentration of the activation marker in the sample) of the microbeads was read on FACSCalibur flow cytometer. Plasma levels of activation markers were calculated using standard dilution curves of mean fluorescent intensity (MFI).

Experimental results

1. Monitoring P2Y12 receptor inhibition with light transmission aggregometry: a comparison with vasodilator stimulated phosphoprotein phosphorylation assay

Background

As LTA is poorly standardized, the optimal platelet aggregation parameter (Aggmax, Agglate, AUC or disaggregation) to monitor P2Y12 receptor inhibition is unclear. Most of the laboratories are using the maximum value (Aggmax) of the ADP-stimulated aggregation curve that is achieved early after agonist addition. However, LTA is not P2Y12-specific, as ADP also binds to P2Y1 receptor. (20) The latter initiates shape change with degranulation leading to an unstable, early-stage platelet aggregation that might result in disaggregation during assessment if the P2Y12 receptor is blocked. Disaggregation during ex vivo testing is typical when low ADP ($\leqslant$ 5 μM) concentrations are used. Based on these, some laboratories prefer to use late aggregation value believing that aggregation measured at 5-6 minutes after ADP stimulation might better represent P2Y12 receptor inhibition without the influence of P2Y1-activity. (50) However, the superiority of Agglate over other aggregometry parameters has not yet been proved by an independent, P2Y12-specific assay. Flow cytometric assessment of vasodilator stimulated phosphoprotein (VASP) phosphorylation is a recently developed, completely P2Y12-specific method that is considered the gold standard for measuring P2Y12-receptor inhibition.

Objective

We aimed to compare ADP-stimulated light transmission aggregometry to the P2Y12-specific VASP phosphorylation assay in order to analyze the agreement between them. Moreover, we sought to test the hypothesis that Agglate is superior to other estimates of the LTA measurement in monitoring P2Y12 receptor inhibition.

Methods

Eighty-nine clopidogrel-naïve stable angina patients were prospectively recruited in whom elective percutaneous coronary interventions were performed. Exclusion criteria were acute coronary syndrome, prior thienopyridine or oral anticoagulant therapy, known contraindication to aspirin or clopidogrel, stroke in the past 6 months, known bleeding disorders or low platelet count (<100 x109/L). All patients received a single loading dose of 600 mg clopidogrel and 300 mg enteric-coated aspirin after coronary angiography, just immediately before PCI, after giving written consent for participation in the study. Twelve to 18 hours after the 600-mg loading dose of clopidogrel, 20 ml blood was drawn from each patient from a peripheral vein and put into four 4.5-ml BD Vacuutainer tubes, with 3.8% sodium-citrate for anticoagulation for LTA and VASP assessment. All patients gave written consent for participation, and the study was approved by the local ethics committee. LTA and VASP measurements were performed as described previously. The efficacy of clopidogrel therapy was expressed using ADP 5 μM, while epinephrine 10 μM was used to refer for the efficacy of aspirin therapy. Maximal aggregation (Aggmax), 6-minute late aggregation (Agglate), disaggregation (disAGG) and the area under the aggregation curve (AUC) were calculated in every measurement.

Results

Correlation between LTA and VASP measurements

Eighty-nine VASP and LTA measurements were performed 19±2 hours after receiving a 600-mg loading dose of clopidogrel and 80 patients were sampled on maintenance-phase, at 25±2 days after PCI. Thereby, a total number of 169 measurements were analyzed. After the administration of a 600-mg loading dose of clopidogrel, all platelet function measures demonstrated high inter-individual variability in efficacy (Aggmax: 28.8±14.0; Agglate: 9.4±17.5; disAGG: 72.0±33.3; AUC: 66.8±52.2; VASP-PRI: 49.9±22.1) that also persisted in the maintenance period (Aggmax: 30.0±12.6; Agglate: 9.2±16.2; disAGG: 71.4±30.3; AUC: 68.6±49.5; VASP-PRI: 48.1±20.6). Based on the 169 LTA measurements, high correlation was found between the maximal and late aggregation values ($p<0.001$; Spearman's ρ: 0.91). When LTA values were compared to VASP-PRI, significant, moderate-strength correlations were registered with AUC showing the highest correlation coefficient to VASP-PRI. (Figure 1) Notably, the efficacy of aspirin therapy, measured by epinephrine 10 μM, did not correlate to VASP-PRI. ($p=0.75$). In a multivariable linear regression model with VASP-PRI as a dependent variable, AUC proved to be the independent predictor of VASP-PRI [0.50, 0.21(0.15-0.27)].

Bland-Altman plots were used to demonstrate intra-individual agreement among assays in measuring post-clopidogrel platelet reactivity. (Figure 2) In case of maximal aggregation, the plot showed that platelet reactivity is estimated quite similarly by both methods with significant disagreement in certain individuals (bias: 2.8; limits of agreement: -42.6 - 48.2, range: 90.8). When Agglate was plotted against VASP-PRI, the analysis showed that it underestimates platelet reactivity with similar intra-individual differences as Aggmax (bias: -9.4; limits of agreement: -53.6 - 34.9; range: 88.5). Disaggregation and AUC also underestimated VASP-defined platelet reactivity, with wider disagreement range of the former (bias: -19.8; limits of agreement: -75.0 - 35.5; range: 110.5 for disAGG and bias: -10.0; limits of agreement: -61.5 - 41.4, range: 102.9 for AUC). The plots also demonstrate that the underestimation of platelet reactivity by Agglate, disAGG and AUC is driven by differences in the low platelet reactivity range (markedly below 50%) where these measures of LTA give significantly lower values of platelet reactivity than VASP-PRI.

Agreement between assays in determining normal and high platelet reactivity

The predictive value of LTA variables in determining high platelet reactivity (HPR), defined as a VASP-PRI value greater than 50% was evaluated with receiver-operator characteristic (ROC) curve analysis. According to the analysis, the predictive value of the LTA estimates were similar. (Figure 3)

The optimal cutoff with the highest sensitivity and specificity for predicting HPR was also determined for each variable: AGGmax: 32.9%; AGGlate: 12.8%; DISagg: 63.6%; AUC: 96.5 %*min.

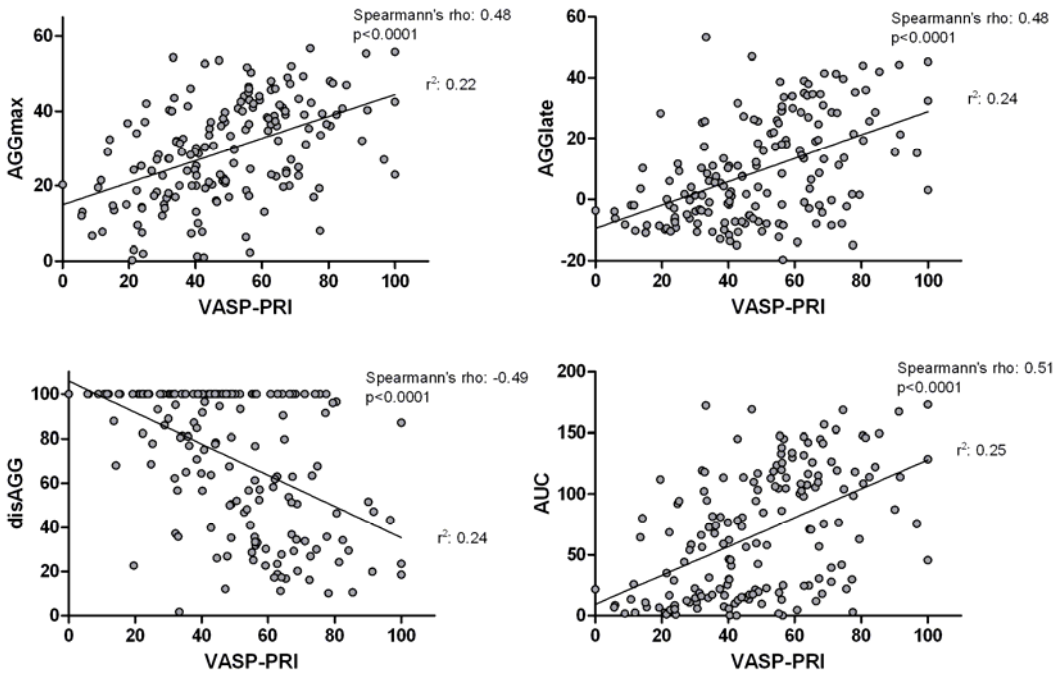

Figure 1. Scatterplots comparing vasodilator stimulated phosphoprotein phosphorylation index (VASP-PRI) and different measures of the light transmission curve. AGGmax: maximal aggregation; Agg_{late}: 6-minute late aggregation; disAGG: disaggregation; AUC: area under the light transmission curve.

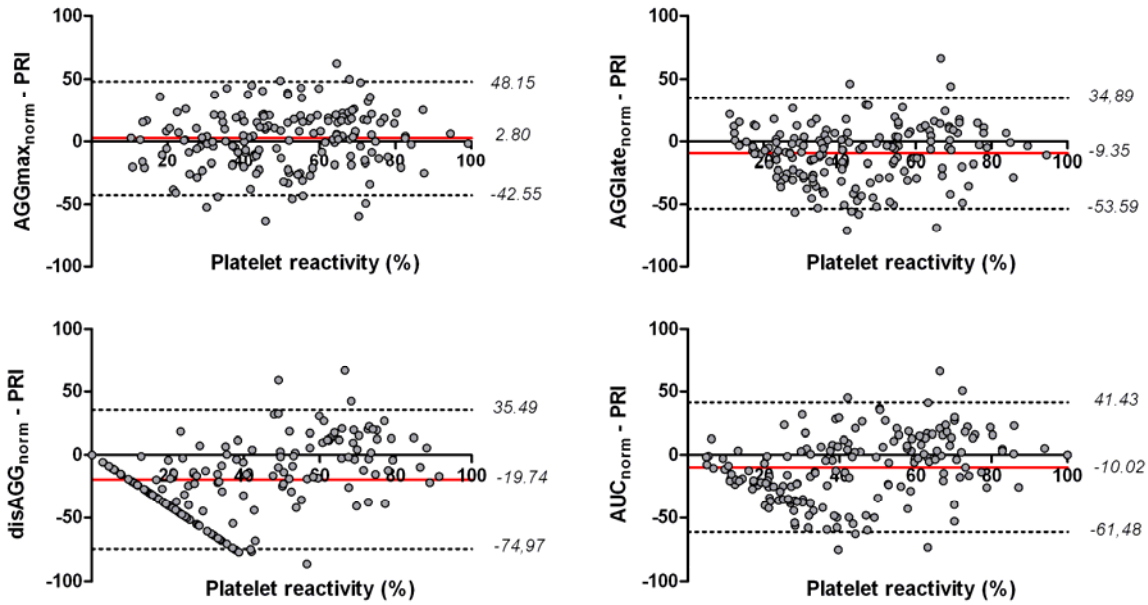

Figure 2. Bland–Altman plots are demonstrating intra-individual agreement between estimates of LTA and VASP-PRI in measuring platelet reactivity. Bias (red line) is a measure of a systematic error leading to over- or underestimation of a known value (VASP-PRI) by alternative parameters of the light transmission assessment. As a major principle of the Bland-Altman analysis is that both measurements evaluate a parameter on a same scale (platelet reactivity, %), all the light transmission parameters were normalized to the scale of VASP-PRI (from 0% to 100%). $Aggmax_{norm}$: normalized maximal aggregation, $Agglate_{norm}$: normalized 6-minute late aggregation; $disAGG_{norm}$: normalized disaggregation, AUC_{norm}: normalized area under the light transmission curve.

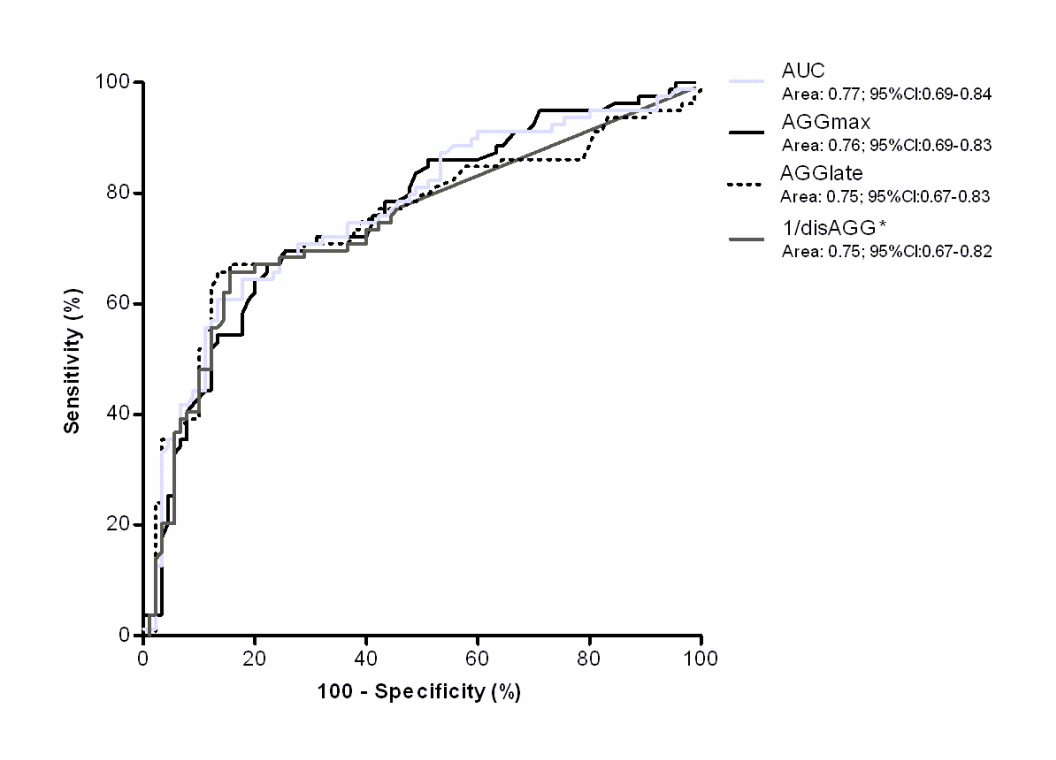

Figure 3. Receiver-operator characteristic (ROC) curve analysis of the ability of light transmission aggregation values to predict high platelet reactivity defined by VASP-PRI > 50%. The area under the ROC curve is shown with the 95% confidence intervals. AUC: area under the light transmission curve; Aggmax: maximal aggregation; Agglate: 6-minute late aggregation; disAGG: disaggregation.
*1/disAGG: as disaggregation is in inverse correlation to VASP-PRI, its reciprocal was used for better comparability with other parameters.

Discussion

Our results confirm that all LTA estimates are in significant, moderate-strength relationship with VASP-PRI without the superiority of Agglate over Aggmax, AUC or disAGG in monitoring the degree of P2Y12-receptor inhibition. According to the highest correlation coefficient and the result of the multivariable linear regression analysis, not Agglate, but AUC seems to be the best linear predictor of VASP-PRI. The significant correlation also validates LTA assessment for monitoring the efficacy of thienopyridine therapy and is in line with prior studies that had compared VASP with LTA. (20)

Bland-Altman plots were useful to confirm that in spite of the significant correlation there might be substantial differences in certain individuals between VASP and LTA-defined platelet reactivity. Based on these, Agglate, disAGG and AUC are systematically underestimating VASP-defined platelet reactivity in the low platelet reactivity range (markedly below 50% platelet reactivity). The observed magnitude of differences between VASP-PRI and LTA estimates emphasize that two methods are closely related, but are not able to substitute for each other.

2. The efficacy of thienopyridine therapy influences late outcome after coronary stent implantation

Background

At present, antiplatelet agents are used in uniform, fixed-dose manner despite the growing body of evidence supporting large inter-individual variability in response to both aspirin and clopidogrel. (20, 45) Inter-individual differences indicate that a substantial number of patients after coronary intervention persist with high platelet reactivity despite aspirin and thienopyridine treatment. It is still debated whether low response to therapy and consequential high platelet reactivity is associated with adverse thrombotic events. Moreover, no consensus exists on defining the threshold for low response to therapy; however, this threshold should be clinically adjusted.

Objective

We designed a prospective study to evaluate the clinical impact of inter-individual differences in response to antiplatelet therapy. We hypothesized that low platelet reactivity measured with light transmission aggregometry after PCI was associated with a better 10-month clinical outcome.

Methods

Study Population

A total number of 134 eligible patients after elective PCI were recruited. Patients were considered eligible for enrolment into the study if they had clinically proven stable or unstable angina, had de novo lesion in one of the main coronary arteries causing a diameter stenosis over 50%, were feasible to direct stent implantation, were at least 18 years, and provided informed consent before enrolment. Exclusion criteria included contraindications to any antiplatelet/antithrombotic agents, haematological disorders and coagulopathies, 2 or 3 vessel disease with an indication for coronary artery bypass grafting, recent myocardial infarction (within 72 hours), poor left ventricle function (ejection fraction below 25%), history of stroke in the past 6 months and any other known disorder that may significantly influence survival (malignancy, serious liver disease, immunosuppressed conditions).
Antiplatelet agents were given in an oral bolus at the time of the procedure, 300 mg clopidogrel or 500 mg ticlopidine with 300 mg aspirin at the discretion of the operator. No platelet glycoprotein IIb/IIIa receptor blocker was used. After PCI, 75 mg clopidogrel or 2x250 mg ticlopidine were administered with 100 mg enteric-coated aspirin therapy until 12 months.

Platelet Function Analysis

Platelet aggregation studies were performed 30 ±5 days after the intervention with LTA. Efficacy of thienopyridine therapy was measured with ADP 5 and 10 μM, while efficacy of aspirin treatment was evaluated with collagen 2 μg/mL and adrenaline 10 μM. At the same time, 5 ml blood was drawn for assessment of plasma concentration of soluble markers of platelet activation including soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble

CD40 ligand (sCD40L) and soluble P-selectin (sP-selectin) using Human Cardiovascular Multiplex Fluorescent Bead Immunoassay (Bender MedSystems GmbH, Vienna, Austria).

Results

Between May 2003 and January 2005, 134 patients were enrolled. After the intended follow-up period of 10 months, 33 major adverse cardiac events (MACE) were traced (Table 1). Adverse events developed 151 ± 111 days after the index intervention, with 2 end points occurring within the first one month. These patients had subacute stent thrombosis on day 7 and 13. No cardiac or non-cardiac deaths were recorded, and 33 revascularizations were performed, including 10 CABG and 23 repeated PCIs. We registered 3 cases of MIs that were reperfused with primary PCI. Two of them were due to subacute stent thrombosis, whereas one de novo lesion triggered an athero-thrombotic event. On the basis of the control coronary angiographies, we found 27 cases of significant instent restenosis (ISR).
Based on the LTA measurements, there were large inter-individual differences in platelet reactivity in case of all agonist-stimulation. The distribution of ADP-induced maximal aggregation values shown a Gaussian pattern, whereas adrenaline-induced reactivity showed a detached group of nonresponders delineated. (Figure 4)

Table 1. Major adverse cardiac events (MACE) at 10 months in 134 patients after PCI

Death	0 (0%)
Myocardial Infarction	3 (2.2%)
Revascularisation	33 (24.6%)
Revascularisation with PCI	23 (17.1%)
Revascularisation with CABG	10 (7.5%)
Death or Myocardial infarction or revascularisation (1^{o} endpoint)	33 (24.6%)
Instent Restenosis	27 (20.1%)
Stent Thrombosis	2 (1.5%)
De Novo Lesion	5 (3.7%)

Maximal platelet aggregation values to ADP 5 and 10 μM were in significant relation with MACE in the univariate Cox regression model ($p<0.01$ in both cases), indicating better survival in case of more effective inhibition. In Kaplan-Meier analysis, cumulative event-free survival of patients in the lower 50 percentile of ADP-induced platelet aggregation was significantly better than that of patients in the upper 50 percentile. (Figure 5A) Overall, patients in the higher 50% of ADP-aggregation had a 6.84-fold HR for MACE (95%CI: 2.64-17.72, $p<0.001$). In case of collagen 2 μg/mL and adrenaline 10 μM, no association was detected with primary outcome ($p=0.84$ and $p=0.76$, respectively).
The plasma level of soluble P-selectin was also associated with MACE ($p<0.05$) in the univariate COX-regression model. This benefit was also demonstrated in the Kaplan-Meier log-rank test as well. (Figure 5B). Neither sVCAM-1 nor sCD40L had association with MACE in the Cox regression analysis.

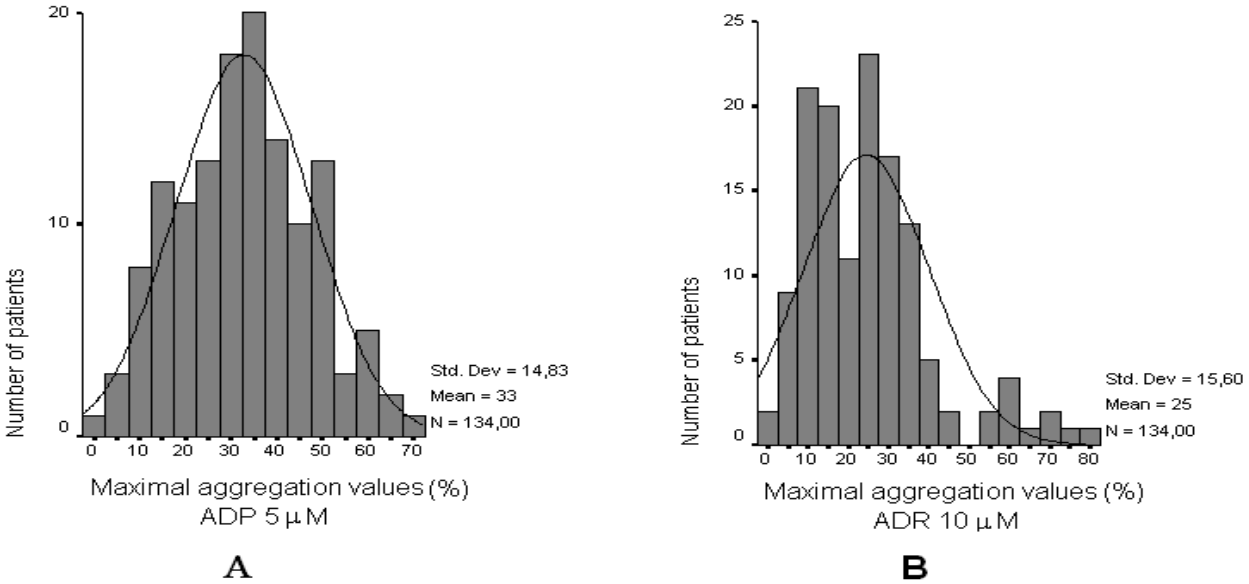

Figure 4. Distribution of maximal platelet aggregation (Aggmax) values using adenosine diphosphate 5 µM (ADP; Panel A) and adrenaline 10 µM (ADR; Panel B) reflecting the efficacy of thienopyridine and aspirin therapy, respectively. On-clopidogrel ADP-reactivity shows a normal distribution pattern (Panel A), while in case of aspirin patients with decreased efficacy constitute a segregated group above 50% of Aggmax values.

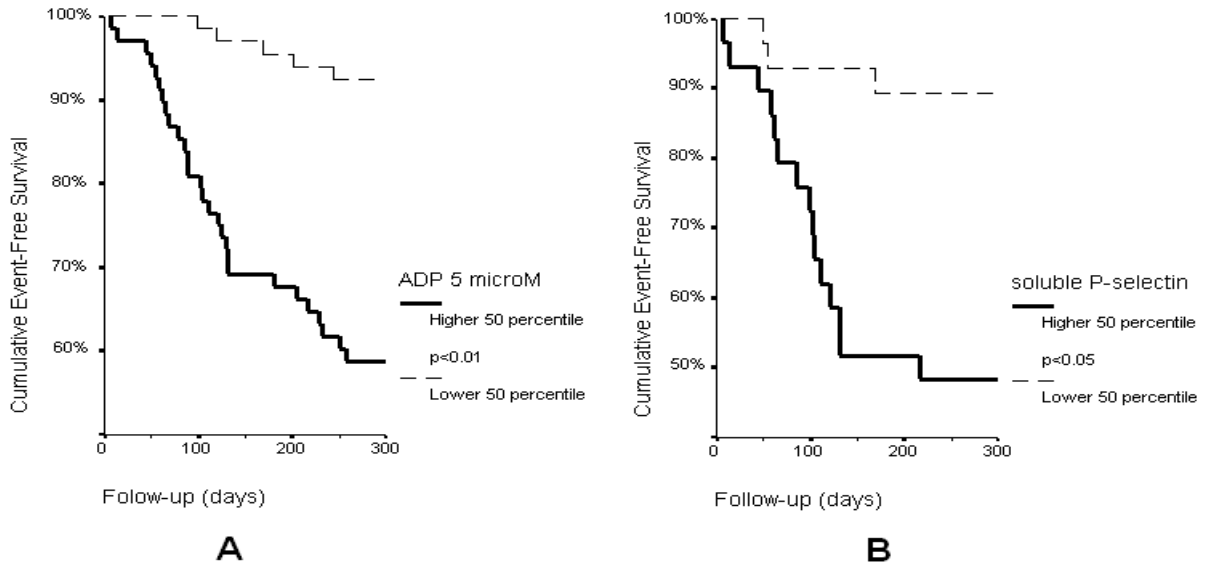

Figure 5. Kaplan-Meier diagrams of the cumulative event-free survival of patients after coronary stent implantation. Panel A: lower and higher 50 percentiles of maximal platelet aggregation values using ADP 5µM (log-rank test: $p<0.01$). Panel B: lower and higher 50 percentiles of plasma level of soluble P-selectin (log-rank test: $p<0.05$).

Discussion

The major finding of this study is that LTA-monitored on-clopidogrel ADP-reactivity is in significant relation with the occurrence of major adverse cardiac events after PCI, showing clinical benefit in patients with more effective ADP-receptor inhibition. As sP-selectin is also a sensitive marker of platelet activation, the relation between plasma levels and MACE

supports independently that the degree of platelet activation may interfere with clinical outcome. Notably, the efficacy of aspirin therapy assessed with collagen and adrenalin had no association with the primary endpoint. Notably, the composite end point of death, MI or repeat revascularization was dominantly triggered by instent restenosis (ISR) rather than thrombotic events. Thereby, somewhat link between platelet reactivity and ISR should be hypothesized. These results suggest that the more effective the thienopyridine therapy, the less frequent the need for repeat revascularization. As neither the design, nor the sample size of the study was powered to evaluate this hypothesis, further research is needed to clarify the link between high platelet reactivity and ISR.

3. The impact of diabetes mellitus on the efficacy of combined antiplatelet therapy after coronary stent implantation

Background

Aspirin plus thienopyridine therapy is one of the foot-stones of clinical success after coronary stent implantation. (18, 19) However, impaired response to aspirin and/or thienopyridines has been demonstrated and may contribute to the development of major adverse cardiac events. Several factors might contribute to the inappropriate response to clopidogrel: genetic polymorphism of the metabolizing enzymes (CYP2C19); clinical conditions (acute coronary syndrome) and low patient compliance. (60) Many reports have suggested an impaired antiplatelet efficacy in diabetic patients that might contribute to the higher risk of this subset.

Objectives

We sought to evaluate the impact of type two diabetes mellitus (DM) on the efficacy of antiplatelet therapy in patients after coronary stent implantation.

Methods

With a retrospective search in our LTA database in patients after PCI, we collected diabetic (DM) and matching, (according to age, gender, risk profile, stent type and antiplatelet regimen) non-diabetic (ND) control subjects in order to compare their efficacy of antiplatelet therapy. Among diabetics, we differentiated oral antidiabetic- (OAD) and insulin-treated (INS) groups in whom fasting plasma glucose was also measured after stent implantation.
Blood was drawn for LTA assessment >6 hours of PCI, after receiving a 600-mg loading dose of clopidogrel and 300 mg aspirin. 1x75 mg clopidogrel and 1x100 mg aspirin was administered in the maintenance phase of treatment. ADP 5 and 10 μM-induced maximal aggregation values (Aggmax) were used to refer for efficacy of thienopyridine therapy, while collagen 2 μg/ml and adrenaline 10 μM stimuli reflected the potency of aspirin treatment. The primary endpoint of the study was the difference in Aggmax values between ND and DM groups. Secondary analyses focused on Aggmax values in diabetic subgroups and correlation of actual fasting glucose to Aggmax.

Results

We found 79 type II diabetic patients for evaluation in the database and matched them with 81 control subjects. Clinical characteristics between diabetic and non-diabetic groups were similar. Among the 79 DM patients, 56 (71%) were on oral glucose-lowering therapy and 23 (29%) received insulin treatment. On the basis of the LTA measurements, ADP-induced Aggmax values did not differ significantly between the DM and ND group (ADP 5 μM: P=0.32; ADP 10 μM: P=0.47). In diabetic patients, Aggmax values of the OAD group were lower than that of the INS group, however, the difference did not reach the level of significance (ADP 5 μM: P=0.07; ADP 10 μM: P=0.06). Meanwhile, efficacy of thienopyridine therapy showed significant difference between INS and ND patients (ADP 5 μM and ADP 10 μM: $p<0.05$). (Figure 6A, C and E) Using our prevous results for the definition of high platelet reactivity (HPR: Aggmax>33%), insulin-treated patients had an OR of 2.46 (95%CI: 0.95-6.36, $p<0.05$) for HPR compared to non-diabetics. (59)
We found no relevant differences in efficacy of aspirin therapy in group comparisons according to adrenalin and collagen-stimulated measurements. (Figure 6B, D and F) Notably, the level of fasting glucose did not correlate with Aggmax values (Spearman r=0.13, p=0.32).

Discussion

In this study, we demonstrated that patients with insulin-treated DM have impaired response to clopidogrel. Insulin has specific effects on platelets, as insulin receptor complex is expressed on the platelet surface. Insulin mediates inhibition of platelet activation through inactivating the Gi_{α}-protein that leads to an increase in cAMP formation in the platelet. Rise in cyclic AMP levels results in phosphorilation of intracellular proteins that prohibit platelet activation. In diabetic subjects, insulin-resistance has a major impact in the development of multi-organ damage. Ferreira et. al demonstrated that antiplatelet effects of insulin are diminished in diabetic patients that leads to higher platelet reactivity. (62) Moreover, endothelial dysfunction with lower prostacyclin production might also contribute to HPR in diabetics. As both insulin and ADP-receptor converge to Gi_{α}-protein, diminishment of Gi inhibition in diabetics might explain the excess activity of ADP-receptor, with consequently lower response to clopidogrel. (63)

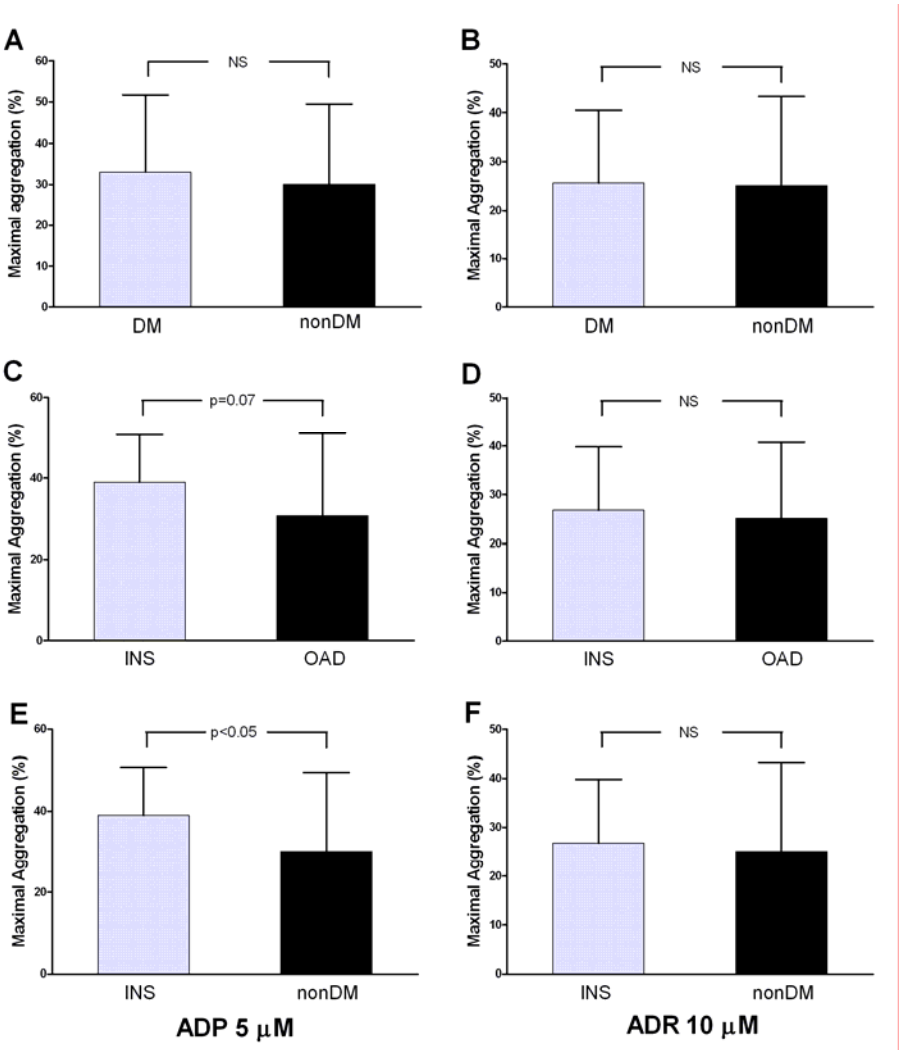

Figure 6. Inter-group comparisons in maximal aggregation values reflecting the efficacy of clopidogrel (Panel A, C, E using ADP 5 μM) and aspirin therapy (Panel B, D, F using adrenalin 10 μM). DM: type II. diabetes mellitus, nonDM: non-diabetic patients, INS: insulin-treated diabetes mellitus, OAD: oral glucose-lowering therapy.

4. Low platelet disaggregation predicts poor response to 150 mg clopidogrel in patients with elevated platelet reactivity

Introduction

The response to a fixed-dose clopidogrel is not uniform between patients. Growing body of evidence suggests that low response to clopidogrel and consequential high platelet reactivity (HPPR) is associated with adverse thrombo-ischemic events. In a prospective cohort of 134 patients we found that patients with Aggmax values >33% had a 6.84-fold HR for MACE (95CI: 2.64-17.72, p<0.001) compared to those with ⩽33%. (59) Recently, numerous antiplatelet protocols were tested in order to overcome elevated platelet reactivity. (64-69) Doubling the maintenance dose of clopidogrel after PCI is a promising option and has been recommended in patients in whom less than 50% of platelet inhibition is demonstrated. (18)

Importantly, all of the studies that evaluated the biological effect of 150 mg clopidogrel described large inter-individual differences in the extent of the benefit, indicating that there are patients who profited from dose-shift while others persisted with EPR. As the latters might remain at higher risk for adverse outcome, identification of variables that predict the response to 150 mg clopidogrel is awaited.

Objective

The main aim of this study was to investigate the pharmacological benefits of administering a high maintenance dose (150 mg) of clopidogrel as compared to standard therapy (75 mg) in patients with verified HPPR after 600-mg loading dose. Furthermore, we sought to analyze possible determinants of response to 150 mg clopidogrel.

Methods

Patients and study design

We aimed to recruit three patient populations to compare their efficacy of clopidogrel treatment at two separate time points after PCI: a group of good responders receiving 75 mg clopidogrel (no HPPR), a group of patients with HPPR taking 75 mg (HPPR+75 mg) and a group of HPPR subjects with increased maintenance dose of clopidogrel (HPPR+150 mg. Clopidogrel-naïve patients who underwent PCI with stent implantation after the administration of 600 mg loading dose (LD) of clopidogrel were eligible. Both stable angina and acute coronary syndrome patients were enrolled. Exclusion criteria included recent (<7 days) treatment with thienopyridines, administration of GPIIbIIIa inhibitors, concomitant anticoagulant therapy, recent (<6 months) hemorrhagic stroke, intolerance to clopidogrel/aspirin and low platelet count (<100 x109/L).

Results

Platelet function assessment

The study cohort comprised 287 patients after PCI. 85 patients with verified baseline HPPR received 150 mg maintenance dose until 30 days of PCI (HPPR + 150 mg), 85 patients with HPPR were administered the standard 75 mg clopidogrel (HPPR + 75 mg) and 117 good responder ones (no HPPR) received 75 mg clopidogrel daily after coronary stent implantation. At baseline, after the loading dose of 600 mg clopidogrel there were no difference in Aggmax between the groups of HPPR. (Figure 7) The administration of 150 mg clopidogrel resulted in significantly lower Aggmax at time point 2 compared to 75 mg in patients with high post-treatment platelet reactivity supporting the enhanced P2Y12 receptor inhibition due to higher dose of clopidogrel. In parallel, the relative percentage of HPPR patients decreased significantly with the high maintenance dose. Despite administering 150 mg clopidogrel, neither Aggmax nor the prevalence of HPPR patients reached that of good responders.

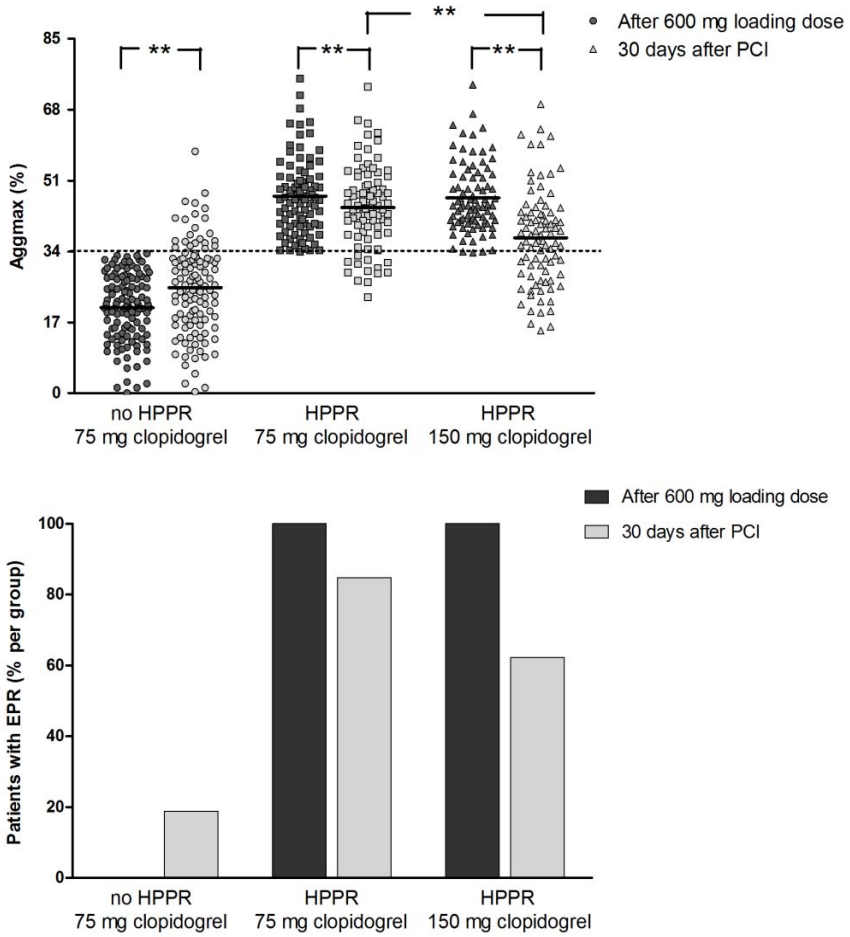

Figure 7. Comparison of platelet function profiles at baseline and on maintenance phase assessed with light transmission aggregometer in patients with good response to clopidogrel (no HPPR), in patients with high post-clopidogrel platelet reactivity receiving 75 mg maintenance dose (HPPR + 75 mg) and in patients with high post-clopidogrel platelet reactivity taking 150 mg clopidogrel (HPPR + 150 mg). Panel A shows group comparison according to Agg_{max} where dots represent individual data along with the median in each group. The dotted line indicates the cutoff limit for HPPR. (*: $p<0.01$; **: $p<0.001$; LD: loading dose). Panel B presents the relative percentage of patients with high residual platelet reactivity (HPPR) in study groups between time points.

Determinants of response to 150 mg maintenance dose

To determine variables that significantly correlate to Aggmax at maintenance phase, linear regression models were generated including estimates of the aggregation curve (Aggmax, Agglate, disAgg, Figure 3, 4) as well as clinical/procedural variables. In univariate linear regression models, baseline Aggmax, Agglate, disAgg, administration of beta-blockers and CYP3A4-metabolized statins as well as acute coronary syndrome correlated significantly with primary endpoint. Active smoking, drug-eluting stent implantation and proton pump inhibitors also showed a trend ($p<0.10$) towards. However, in multiple linear regression analysis, disaggregation and acute coronary syndrome prevailed as the independent predictors of maintenance-phase Aggmax values. To corroborate these findings, multivariate logistic regression models were used to calculate odds ratios for HPPR. Without adjustment for confounding clinical and procedural variables, disaggregation (per 1% increase) and acute

coronary syndrome (ACS) were associated with an OR for HPPR of 0.95 (95% CI: 0.92-0.98, p=0.002) and 5.82 (95% CI: 1.94-17.47, p=0.002), respectively. Adjustment for beta-blockers, CYP3A4-metabolized statins and baseline Aggmax resulted in an adjusted OR of 0.96 (95% CI: 0.93-0.99, p=0.009) and 4.83 (95% CI: 1.54-15.09, p=0.008) for disaggregation and ACS, respectively. Likewise, ACS was associated with a 1.7-fold absolute risk of remaining HPPR compared to stable angina following 150 mg clopidogrel. (Table 7)
As the results confirmed that platelet disaggregation predicts the response to 150 mg clopidogrel, the optimal cut-off for predicting normal platelet reactivity (Aggmax<34%) after 150 mg clopidogrel was determined with receiver-operator characteristic (ROC) curve analysis. An area under the curve of 0.724±0.055 (Asymptotic significance: 0.001; 95% Asymptotic Confidence Interval: 0.616-0.833) was obtained and an optimal cutoff value of 16.5% was suggested, with a sensitivity of 94% and a specificity of 43%. Based on these, in HPPR+150 mg group 25 (29.8%) patients were below and 59 (70.2%) above this threshold. Among 25 patients below this cut-off, only 2 patients (8%) achieved a Aggmax value lower than 34% after 150 mg clopidogrel, representing a 92% negative predictive value. Among 59 patients with a disaggregation value higher than 16.5%, 29 (49.2%) showed good response and turned to nomal platelet reactivity.

Discussion

Our results confirm that the administration of a high maintenance dose of clopidogrel enhances platelet inhibition and reduces high post-clopidogrel platelet reactivity (HPPR) in a consecutive cohort of patients after PCI. This impact was characterized by a statistically significant decrease in Aggmax and Agglate values with increased disaggregation levels among patients receiving 150 mg clopidogrel. These results support the pharmacological benefit of administering 150 mg maintenance dose of clopidogrel in patients with HPPR evaluated after a 600-mg LD. However, the antiplatelet efficacy in response to a 150 mg maintenance dose did not reach the level of patients with normal platelet reactivity. Moreover, less than 40% of the patients with HPPR shifted to normal platelet reactivity. This means that there are patients who benefit from dose-shift, while in others, HPPR persists despite 150 mg clopidogrel. These results suggest that dose elevation to 150 mg is not the right answer in many poor responders to clopidogrel. Likewise, elucidating predictors of response to 150 mg clopidogrel might be of great importance. We evaluated possible clinical, procedural and platelet aggregation variables in multivariable models and identified platelet disaggregation and acute coronary syndrome as independent predictors of elevated platelet reactivity after 150 mg clopidogrel. Patients with acute coronary syndrome had a 1.7-fold higher absolute risk to persist with EPR compared to those with stable angina. Likewise, patients with ACS might be candidates for more potent antiplatelet therapy with newer ADP-receptor inhibitors (prasugrel, ticagrelor) being introduced in the following years. Most importantly our study shows that low platelet disaggregation is the strongest independent predictor of poor response to a high maintenance dose of clopidogrel. Based on these results, patients with a low (16.5%) disaggregation do not show substantial pharmacological benefit from an increased maintenance dose of clopidogrel, while half of those with good disaggregation might shift to normal platelet reactivity.

5. Transradial versus transfemoral percutaneous coronary intervention in acute myocardial infarction. Systematic overview and meta-analysis

Introduction

Transradial coronary angioplasty (TRPCI) has gained widespread acceptance since its introduction by Kiemeneij and Laarman. (71) Radial access has been proven to be a highly safe and effective technique for both diagnostic- and therapeutic procedures. (72, 73) Advantages of the transradial approach over the transfemoral include safe and easy haemostasis due to compressibility of the artery, and consequent lack of need for postprocedural bed rest permitting immediate ambulation, greater comfort, and earlier discharge. These have been shown to reduce the costs of hospitalization and improve quality of life for patients. (74, 75) Although it is technically more challenging, transradial intervention is feasible in the setting of acute coronary syndromes. (76-82) The major advantage of the TRPCI is the near elimination of clinically significant access site complications, even in patients at high risk for bleeding (i.e. patients treated with GP IIb/IIIa inhibitors or shortly after systemic thrombolysis). Bleeding events, and the consequent need for transfusion, are independent determinants of survival in acute coronary syndromes. Their relation to short- and long-term mortality has been demonstrated in major randomized trials as well as through the evaluation of registries. (83-86) Thereby, low incidence of vascular access site bleeding complications suggests that the transradial approach may be a safe alternative to the femoral technique employed in acute myocardial infarction with ST segment elevation (STEMI), particularly when an aggressive anticoagulation- and antiplatelet regimen is applied. On the contrary, the possible greater occurrence of procedural failure and longer procedural times occasioned by difficulty in puncturing the radial artery, inability to cannulate the coronaries, or impossibility to perform the angioplasty, are factors that raise concerns as to whether radial access remains beneficial in the setting where timely reperfusion is critical, in STEMI for instance. The safety of transradial- and transfemoral PCI in AMI were compared in numerous trials; however, most of them included small patient groups. Despite consistent demonstration of lower bleeding rates, only inconclusive results are available regarding recurrent ischemic events; most of these studies were underpowered to evaluate this issue.

Objective

Our aim was to perform a systematic review of the literature comparing the safety and efficacy of the two vascular accesses in STEMI and to complete a meta-analysis in order to achieve greater statistical power and more precise effect estimates.

Methods

Search strategy

We performed a systematic review of the available literature according to the MOOSE guidelines for the conduct of meta-analyses of observational studies. (87) Relevant studies

published between January 1993 and August 2009 were identified from MEDLINE®, SCOPUS®, the Web of Science® with Conference Proceedings, and the Cochrane Central Register of Controlled trials (CENTRAL) using a search strategy that combined text word and MeSH heading. Search keywords included various combinations of the following terms: "transradial", "radial access", "myocardial", "infarct*", and "coronary". No language restrictions were imposed. Furthermore, we searched reference lists of relevant studies and reading reviews and editorials on this topic. In addition, relevant abstracts and presentations from the annual meetings of the American Heart Association, the American College of Cardiology, the European Society of Cardiology and Transcatheter Cardiovascular Therapeutics were identified.

Selection criteria

Inclusion criteria for retrieved studies were a) controlled comparison of the radial- versus femoral approach for coronary intervention b) acute myocardial infarction (either primary- or rescue PCI) and c) intention-to-treat analysis. Exclusion criteria were a lack of clear- and reproducible results and incomplete follow-up, and lack of clear distinction of the clinical setting of the patients included (i.e. separate data for the acute- and elective interventions included).

Results

Search results and study selection

Our search detected 213 citations. These included editorials, reviews, letters, or articles regarding other aspects of the radial approach. Twelve studies were included in the final analysis. These comprised 5 randomized trials involving 516 patients: 266 of the transradial- and 250 of the transfemoral approaches. (76; 91-94) Seven further reports using the registry approach of single- or dual center experiences of primary TRPCI were identified. These included 2808 cases, made up of 1212 transradial- and 1596 transfemoral interventions. (82; 95-100) All studies were published in peer-reviewed journals.

Clinical results

The radial approach reduced risk for major bleeding by 70% compared to TFPCI (0.77% vs 2.61%, OR: 0.30 [95% CI: 0.16, 0.55] P=0.0001). Reductions in the composite of death, myocardial infarction, and stroke were also significant (3.65% vs. 6.55%, OR: 0.56 [95% CI: 0.39, 0.79] P = 0.01). Pooling the 29 events (2.59%) of 1421 TRPCI and 55 (3.18%) of 1800 TFPCI demonstrated a significant mortality reduction in the case of TRPCI. (OR: 0.54 [95% CI: 0.33, 0.86] P=0.01). There were no differences in procedural time and in time to reperfusion between the two access routes. Fluoroscopic times were longer in case of TRPCI; however, there was significant heterogeneity among studies in these parameters. Access site crossover was less frequent in the case of the transfemoral approach while the total hospital charge, assessed in eight studies was lower in the case of the transradial.

Discussion

The current meta-analysis of the literature demonstrated that transradial coronary intervention improves clinical outcomes by reducing major bleeding and postprocedural ischemic complications in patients with acute myocardial infarction. Thereby, this access site might maximize the benefits and reduce the potential harm of the aggressive antiplatelet and anticoagulant therapy required among these patients.

Novel findings

Based on the results of the cited experiments and studies, our major novel findings can be summarized as follows:

- Results of our measurements with light transmission aggregometry confirmed that the response to a fixed-dose aspirin and thienopyridines are not uniform between patients. While the response to aspirin follows a bimodal, yes-or-no distribution, the efficacy of thienopyridine therapy is normally distributed. Likewise, it is rationale to use the term "aspirin resistance" based on laboratory assessments; however, the term "high-post treatment platelet reactivity" (HPPR) is more appropriate compared to "clopidogrel resistance" in case of thienopyridines as the measured effect (P2Y12 receptor inhibition and ADP-reactivity) seems dose-dependent.
- The significant correlation with VASP validates LTA for monitoring the efficacy of P2Y12 receptor inhibition; however, there might be clinically meaningful differences in the results in certain individuals. Indeed, 6-minute late aggregation is not superior to other estimates of LTA in monitoring the efficacy of P2Y12-receptor inhibition.
- Low response to clopidogrel and high post-clopidogrel platelet reactivity is associated with a higher risk of recurrent ischemic events in low-risk patients after percutaneous coronary interventions. Patients above the median value of maximal aggregation (>33%) had a 6.8-fold HR to MACE.
- Doubling the maintenance dose of clopidogrel to 150 mg intensifies the efficacy of clopidogrel therapy in patients with high platelet reactivity. However, less than 40% of the patients with HPPR might return to normal platelet reactivity. Low platelet disaggregation and acute coronary syndrome are independent predictors of poor response to 150 mg clopidogrel.
- In patients with AMI receiving dual or triple antiplatelet therapy, using radial artery as an access site to perform primary PCI not only reduces access site complications and major bleeding but also major adverse cardiac events and mortality.

Conclusions and perspective

We started our examinations in an era when only limited evidence were available on the rationale and consequences of monitoring the efficacy of antiplatelet therapy in patients after

percutaneous coronary intervention. At that time, “aspirin resistance” was a popular and well-known term among clinicians; however, due to heterogenic definitions and non-standardized laboratory assessments, the range of aspirin resistance varied largely between authors. (43) One should be aware that the wide range of the prevalence of aspirin resistance cannot be explained by the different risk profile of the patient groups. It should be attributed to the arbitrary-used definitions and non-specific laboratory tests that might have lead to both over- and underestimation of the true incidence of aspirin resistance in many examinations.

As landmark studies demonstrated a significant clinical benefit of adding thienopyridines to aspirin monotherapy after coronary stent implantation, dual antiplatelet therapy became gold-standard in patients after PCI. (5-8) Knowing that clopidogrel has less haematological side-effects than ticlopidine, the former has become the thienopyridine of choice.

As thrombotic events still ocurred in spite of the dual antiplatelet therapy, extensive research started to explore the individual response to thienopyridines. Early studies demonstrated large inter-individual differences in response to a fix-dose clopidogrel, and similar to aspirin, the term “clopidogrel resistance” was created and widely applied to refer for patients with inappropriate response. (45-47) However, compared to aspirin, there are major differences in testing the response to clopidogrel therapy. Clopidogrel is a platelet surface receptor blocker while aspirin is an intracellular enzyme inhibitor. Using a specific agonist (ADP) for testing aggregation is reliable in reflecting the inhibition of the P2Y12 receptor. Though our results showed that ADP-stimulated LTA is not fully P2Y12-specific as it was also influenced by P2Y1-receptor activation, all aggregation values were in significant correlation with VASP-PRI. In contrast, the recently available assays to measure the efficacy of aspirin (urinary thromboxane and serum thromboxane levels, PFA-100 collagene-epinephrine closure time, LTA with arachidonic acid, adrenalin or collagen stimuli) are varying largely in determining the prevalence of aspirin resistance and correlating poorly amongst themselves making it difficult to compare and impossible to interpret their results. (40) These facts emphasize that not all methods are equal in estimating antiplatelet response and there are substantial differences in the reliability between assays in monitoring the efficacy of aspirin and clopidogrel treatment. (44) Based on our results, the CARAT TX4 aggregometer with ADP 5 μM stimuli shows fair correlation to VASP measurements in monitoring the efficacy of clopidogrel therapy. Without doubting its shortcomings, LTA-assessment is a validated and valuable tool for testing clopidogrel’s response.

By measuring the efficacy of antiplatelet therapy in patients after PCI, we found that the distribution of clopidogrel response follows a normal, Gaussian distribution pattern. This is in line with the findings of several other studies and it is not considered as a laboratory artefact. (42, 45) This distribution pattern is quite frequent in processes that are under both environmental and polygenetic control. Clopidogrel is a prodrug that needs to be metabolized into a thiol derivate to inhibit P2Y12 ADP receptor. (20) However, more than two thirds of the absorbed clopidogrel is converted into a pharmacologically inactive carboxyl metabolite by blood esterases, whereas only a small proportion might be metabolized into an active derivate through the cytochrome enzyme system (CYP2C19, 2C9, 3A4, 3A5, 1A2, 2B6). Importantly, the loss-of-function alleles (*2, *3, *4) of the CYP2C19 enzyme, that plays a pivotal role in the bioactivation process is very frequent in the Caucasian population (22) Moreover, the absorption of clopidogrel might also differ between patients according to the

genetic polymorphisms of the P-glycoprotein. (104) Clinical factors like diabetes, acute coronary syndrome and renal insufficiency might also impair the efficacy of clopidogrel therapy. (61) As a result, high post-clopidogrel platelet reactivity (HPPR) persists in a substantial proportion of patients despite receiving the recommended dose of clopidogrel. As the distribution of platelet aggregation values and VASP-PRI is normal, the term "elevated or high platelet reactivity" is more appropriate than "clopidogrel resistance" as the effect seems dose dependent. (105)

Growing body of evidence shows that high platelet reactivity is a risk marker for recurrent thrombo-ischemic events after PCI. (48-55) In our study recruiting 134 patients, ADP-stimulated Aggmax values were significantly associated with clinical outcome. Dividing patients into quartiles according their Aggmax values showed that those in the two higher quartiles had a 6.8-fold risk to MACE compared to those in the two lower ones. (59) The worse prognosis of patients in the two higher quartiles of residual platelet aggregation values was independently demonstrated by the EXCELSIOR study in 802 patients. They registered a 6.7-fold higher risk for death, MI or urgent TLR among patients above the median value of platelet aggregation. (50) A meta-analysis also confirmed the prognostic significance of high platelet reactivity is patients after PCI. (54) Despite these results, it is unclear whether high platelet reactivity is a marker for worse prognosis (risk marker) or a modifiable parameter (risk factor). It would be of enormous importance to know whether patients with HPPR might return to normal platelet reactivity with alternative antiplatelet strategies. Results of a recent work of Bonello et al. confirmed that in patients with high platelet reactivity, repeated loading doses of 600 mg clopidogrel were able to override low response to clopidogrel in the majority of patients. (58) However, findings of other studies suggest that returning to the standard dose of clopidogrel in these patients resumes high platelet reactivity. (68) In case of the maintenance-phase treatment, there are a few studies that have demonstrated the pharmacological benefit of doubling the maintenance dose of clopidogrel. (64-69) Our results supported that giving 150 mg clopidogrel for patients with HPPR enhanced platelet inhibition. Notably, the degree of platelet reactivity after 150 mg clopidogrel did not reach that of good responders and less than 40% of the patients returned to normal platelet reactivity with the elevated maintenance dose. Analyzing possible determinants of response to 150 mg clopidogrel revealed platelet disaggregation and acute coronary syndrome as independent predictors of poor response. Acute coronary syndrome was associated with a 1.7-fold, while low disaggregation with a 6.1-fold absolute risk for persisting with HPPR after 150 mg clopidogrel. Low disaggregation had a 92% negative predicting value emphasizing that these patients are not likely to benefit from dose shift. These results might carry important clinical implications as clinicians might be able to choose optimal candidates for a 150-mg maintenance dose based on the clinical status and the result of a LTA assessment.

Based on these findings, the potency of the platelet inhibition can be influenced by the administered dose of clopidogrel. However, many of the patients with HPPR would require such high loading and maintenance doses to achieve optimal platelet reactivity that are not tolerable and also not financed by our heath care system. (70) These individuals might be the candidates for alternative, more potent platelet inhibitors. One of the most important alternatives of clopidogrel is prasugrel that is recently under approval in Hungary. Based on the results of the TRITON-TIMI38 trial, the more potent antiplatelet therapy in ACS was

associated with significantly less myocardial infarctions and stent thromboses. (32) Ticagrelor, a reversible and also potent, direct-acting ADP-receptor antagonist has also demonstrated a 16% reduction in the composite of MI, cardiovascular death and stroke in the PLATO trial. (33)

Notably, the administration of prasugrel increased bleeding complications including spontaneous, fatal bleeds highlighting that the uniformly intensified antiplatelet therapy might do harm to some patients. As bleeding is an equally important predictor of morbidity and mortality as ischemia, preventing bleeding events is of essential importance. (82, 108) Patients admitted to primary PCI due to STEMI represent a high-risk group of both bleeding and ischemia. The double or triple antiplatelet therapies with excessively dosed anticoagulants are important causes of bleeding events among these patients. However, choosing the radial artery as the preferred access site dramatically lowers not only periprocedural bleeding complications but also major adverse cardiac events and mortality. (106) Many operators are fearing to puncture the radial artery for PCI in the setting of AMI, believing that it prolongs the intervention and increases the amount of contrast and radiation exposure. Our meta-analysis confirmed that the major cause of the longer procedural times and the higher radiation exposures is the learning-curve required to master the technique. This aspect was indicated by the significant heterogeneity between the included studies. Based on these results, the radial approach is also a way to maximize the benefits of an intensified antiplatelet therapy in the peri-interventional period of primary PCI.

Concluding these findings, there is a great need for an individualized antiplatelet therapy for patients in order to maximize the anti-ischemic benefits and minimize bleeding risk. Beyond clinical presentation and the genetic constellation, platelet function testing might help to find a therapeutic window for each antiplatelet agent. The novel, more potent antiplatelet agents will be essential tools to customize antiplatelet therapy to the patient's needs as suboptimal response to clopidogrel is common. The clinical relevance of an individualized antiplatelet regimen needs to be tested and supported in further clinical trials.

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