Role of platelet reactivity in patients undergoing percutaneous coronary intervention

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Role of platelet reactivity in patients undergoing percutaneous coronary intervention

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Technische Universiteit Eindhoven, op gezag van de rector magnificus, prof.dr.ir. C.J. van Duijn, voor een commissie aangewezen door het College voor Promoties in het openbaar te verdedigen op woensdag 17 oktober 2012 om 14.00 uur

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Introduction
1.1 CORONARY ARTERY DISEASE AND PERCUTANEOUS CORONARY INTERVENTION

Coronary artery disease (CAD) is a major cause of death and disability in developed countries. Although CAD mortality rates have declined over the past four decades, CAD remains responsible for about one-third of all deaths in individuals over age 35. CAD results from the accumulation of atherosclerotic plaques within the coronary arteries. The latter are the arterial conduits through which the myocardium is supplied with oxygen and nutrients. Coronary atherosclerosis can determine diffuse or focal narrowing which in turn can result in a significant obstacle to blood flow. By impairing or obstructing normal blood flow, atherosclerotic plaques cause myocardial ischemia. Atherosclerotic plaques can also rupture triggering intravascular thrombosis with abrupt occlusion of the vessel, which manifests itself as acute coronary syndrome. In patients with stable CAD, the presence of myocardial ischemia is one of the most important prognostic factors, and should be used to guide treatment strategies. These include medical therapy, which consists of drugs capable of relieving symptoms of CAD and arresting atherosclerosis progression, and coronary revascularization by either coronary artery bypass surgery or percutaneous coronary intervention (PCI).

PCI was first developed in 1977 by Andreas Gruentzig. This technique consists of inflation of a balloon within the coronary artery (angioplasty), which in most of the cases is followed by the implantation of a stent. Other procedures that are done during a percutaneous coronary intervention include rotational atherectomy, a procedure that utilizes a high speed rotational "burr" coated with microscopic diamond particles which rotates at high speed (approximately 200,000 rpm), breaking up blockages into very small fragments (smaller than red blood cells) which can pass, harmlessly, into the circulation.

1.2 ROLE OF PLATELETS IN CORONARY ARTERY DISEASE

Platelets are produced by megakaryocytes as anucleate cells that lack genomic DNA, but contain megakaryocyte-derived messenger RNA (mRNA) and the translational machinery needed for protein synthesis. After leaving the bone marrow, platelets circulate for about 10 days. Their primary function is to stop hemorrhage after tissue trauma and vascular injury. However, they also participate in the process of forming and extending atherosclerotic plaques, and play a key role in the pathophysiology of thrombosis after plaque rupture. In fact, platelets that adhere to the vessel wall at sites of endothelial-cell activation contribute to the development of chronic atherosclerotic lesions, by releasing adhesive ligands that become expressed on the platelet membrane and mediate platelet–endothelium interactions. When atherosclerotic lesions rupture, spontaneously as in patients with acute coronary syndromes, or iatrogenically as in those undergoing PCI, platelets trigger the acute onset of arterial thrombosis through mechanisms dependent on collagen and thrombin.

1.3 ANTIPLATELET THERAPY IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION
In clinical practice, PCI is performed under anti-coagulation and anti-aggregation therapy. This is to avoid thrombotic complications related both to insertion of devices (catheters, balloons, stents) and coronary plaque disruption after balloon dilation and stent implantation, which is associated with platelet activation (1,2). Tromboxane-A2 (TXA2) and adenosine diphosphate (ADP) are released, leading to exposure and activation of glycoprotein IIb/IIIa receptor that, through the interaction with fibrinogen and von Willebrand factor, acts as the final common pathway of platelet aggregation and thrombus formation (1).

Randomized clinical trials support the assumption that the magnitude of platelet inhibition in patients undergoing percutaneous coronary intervention (PCI) and/or with acute coronary syndrome (ACS) correlates with adverse clinical outcomes (3-6). Yet, effectiveness of common anti-platelet therapy (e.g. aspirin, clopidogrel, etc.) in reducing the risk of cardiovascular events is limited by a large inter-individual variability in responsiveness to therapy. A significant number of patients (20-50%) present indeed high residual platelet reactivity, being therefore at increased risk for thrombotic complications. Interestingly, on the other side of the spectrum are those patients with extremely low platelet reactivity who have been recently reported at higher risk of bleeding events (7).

**Figure 1.** Theoretical relationship between platelet reactivity and risk of complications: Insufficient platelet inhibition can increase risk of ischemic complications (on the right), excessive platelet inhibition can increase risk of bleeding complications (on the left).

1.4 VARIABILITY IN PLATELET INHIBITION
Platelet activation is a complex process involving multiple pathways. Common therapeutic strategies have been proposed to target cyclo-oxygenase (COX-1) by aspirin, P2Y12 receptors by thienopyridine, and GP IIb/IIIa receptors by GP IIb/IIIa inhibitor. More recently, simultaneous targeting of these pathways has been pursued to achieve incremental inhibition of platelet activation and aggregation (8-10).

Figure 2. Platelet receptor pathways.

1.4.1 Variability in response to aspirin

Cyclooxygenase-1 (COX-1) is involved in the synthesis of prostaglandin and thromboxane (11), which is released by activated platelets to induce platelet aggregation. Aspirin irreversibly inhibits COX-1, therefore platelets are unable to produce new COX-1 for the whole lifetime of the cell (∼10 days). However, a near normal platelet aggregation might still be preserved in case of sub-maximal COX-1 inhibition (until 20% COX activity) (12,13). In addition, aspirin bears antioxidant effects and exerts beneficial effect by inhibiting the interaction between activated platelets, inflammatory cells and endothelium (14-16).

Aspirin administration in patients with cardiovascular disease, at doses in the range of 75-150 mg, is associated with reduction of adverse cardiovascular events (17,18). However, a significant proportion of patients demonstrate suboptimal platelet inhibition in response to aspirin, which has been described as “aspirin resistance” (19). Several factors might account for the heterogeneous response to aspirin.
treatment, including dosage, co-morbid conditions and drug interactions (19). In addition, irrespective of patient-related factors, intrinsic variability of the different tests used to assess platelet response to aspirin have questioned the value of testing for aspirin resistance (20). More recently, scarce compliance to therapy has appeared as the most important cause for the occurrence of aspirin resistance. Among 190 patients with previous myocardial infarction, after “witnessed” ingestion of single 325 mg dose of aspirin, only one patient failed to show adequate platelet inhibition (21). Aspirin non-response was found in up to 14% of the patients treated with PCI on chronic aspirin therapy, 30 days after the procedure. But after controlled administration of aspirin (75 mg), all but one of these supposedly non-responders showed adequate platelet inhibition and were therefore re-classified as non-compliant to therapy (22). Should the poor aspirin response be the consequence of “real” aspirin-resistance, then higher doses of aspirin (1000 mg) have been demonstrated more effective than current dosages (23).

1.4.2 Variability in response to clopidogrel

The rationale for the administration of additional antiplatelet drugs during and following PCI has come from clinical and laboratory investigations demonstrating thrombin generation and thrombus deposition at the site of arterial deep wall injury despite therapy with aspirin and unfractionated heparin (24-27). Due to its favorable safety profile, clopidogrel has replaced the first-generation thienopyridine ticlopidine (28), and its association with aspirin has represented the most common anti-platelet strategy in patients undergoing percutaneous coronary revascularization (29). Clopidogrel inhibits platelet aggregation, irreversibly binding ADP P2Y12 receptor on platelet surface (30). Being a precursor, clopidogrel needs to be oxidized by hepatic cytochrome P450 (CYP) to its active thiol metabolite. Like aspirin, individual response to clopidogrel is characterized by significant variability, which is depending upon several factors (31).

Mutations of the gene coding for CYP450 have been associated with important variability in clopidogrel response (32,33). In particular, the polymorphism CYP 2C19*2 is associated with loss of function of the CYP-450 enzyme and therefore reduced clopidogrel activation. This variant has been demonstrated to be a major determinant of clinical outcome in patients with coronary atherosclerotic disease receiving clopidogrel (34,35). Yet, increasing the doses of clopidogrel in carriers of such polymorphism might improve responsiveness and therefore antiplatelet effects of the drug (36,37).

Clinical factors associated with high platelet reactivity despite therapy with aspirin and clopidogrel are: diabetes mellitus (38,39), high body mass index (40), and unstable coronary syndrome (41). Degree of endothelial dysfunction has also been shown to be associated with impaired response to clopidogrel (42). In addition, a correlation has been described between severity of coronary atherosclerosis and clopidogrel response, suggesting reduced platelet inhibition with clopidogrel in patients with multivessel disease (43).

Drug interactions were also reported as potential cause of poor clopidogrel response. Atorvastatin administration has been associated with lower platelet inhibition to clopidogrel, due to the competitive metabolism through CYP (44). This interaction might be modulated by varying clopidogrel dose and treatment duration (45,46). Among proton pump inhibitors, omeprazole might also be associated with lower platelet inhibition to clopidogrel (47-49). A post-hoc analysis of the PRINCIPLE-TIMI 44 and TRITON-TIMI 38 has recently shown that the
administration of proton pump inhibitors in patients receiving concomitant dual anti-
platelet therapy does not seem to result in worse clinical outcome (50). Yet, the topic
is under active investigation and no definitive conclusion can be drawn.

To address whether sub-optimal response to clopidogrel may be overcome by
increased doses, several dose-finding studies have been conducted. A 300-mg loading
dose of clopidogrel (51), followed by 75 mg/day maintenance dose, in combination
with aspirin, improved clinical outcome in patients receiving coronary stenting, as
compared to aspirin alone (3, 4, 52). Subsequently, the ARMYDA-2 study showed that
higher loading dose (600 mg) of clopidogrel administered 4 to 8 hours before elective
PCI significantly reduced peri-procedural MI, as compared with the conventional
300-mg dose (53). More recently, the CURRENT/OASIS-7 trial compared 600 mg
loading dose, followed by 150 mg/day for seven days with 300 mg loading dose
followed by 75 mg/day in patients with unstable angina or acute MI (54). In patients
who underwent PCI, the high clopidogrel dose regimen was associated with a
significant reduction of the primary composite endpoint and stent thrombosis, without
any significant increase in bleedings. In most of the patients, clopidogrel loading
doses higher than 600 mg have not shown any additional benefit (55, 56), probably
due to limited drug absorption (55).

**Figure 3.** Normal distribution of P2Y12 reaction units (PRU), as measured by the
VerifyNow P2Y12 assay, in 1033 patients after 600 mg loading dose of clopidogrel
(F. Mangiacapra, unpublished data).

### 1.5 PLATELET FUNCTION TESTS

Several tests are available to assess the different pathways regulating platelet
activation and aggregation (57).

**1.5.1 Light transmittance aggregometry (LTA)**

LTA was first described in 1962 (58) and still represents the “gold standard”
to assess platelet aggregation. In LTA, platelet aggregation is measured by
turbidimetry in platelet-rich plasma, after exposure to an agonist (i.e., arachidonic
acid, ADP). Light transmission through the sample is proportional to the extent of
platelet aggregation, so the more light transmitted, the greater the degree of
aggregation. One of the major advantages of LTA is that it can be used to test multiple agonists thus providing valuable information on multiple signaling pathways. Nonetheless, LTA is time consuming, requires relatively large amounts of blood and expertise.

1.5.2 Whole blood aggregometry

Whole blood aggregometry (59), also known as multiple electrode platelet aggregation (MEA), measures the change in electrical impedance or resistance between two electrodes following stimulation with an agonist. The activated platelets adhere to the electrodes when aggregate causing an increase in the impedance between the two electrodes. The impedance is read over time and the change in impedance is converted to % aggregation. The major advantage of whole blood aggregometry is that it provides a more physiologically relevant environment, as compared to LTA. Although it does not require any processing of blood samples, it is still time-consuming and expensive. Recently, a point-of-care device using this platelet aggregation method has been developed (Multiplate Analyzer, Dynabyte, Munich, Germany).

1.5.3 VerifyNow

The VerifyNow (Accumetrics, San Diego, California) is a rapid, simple, fully automated, point-of-care device that measures the effects of aspirin, thienopyridines or glycoprotein IIb/IIIa receptor antagonists separately. This device exploits turbidimetric aggregation in whole blood samples, and is based on specific cartridges containing different agonists. In the aspirin cartridge the agonist of choice is arachidonic acid and results are expressed as aspirin reaction units (ARU). The cut off value for non-response to aspirin is any result ≥550 ARU. The P2Y12 assay contains both ADP and prostaglandin E1 (PGE1). The PGE1 is used to suppress the platelet activation contribution from ADP binding to the P2Y1 receptor, thereby making the assay more P2Y12 specific. For the P2Y12 the results are expressed as P2Y12 Reaction Units (PRU). Finally the IIb/IIIa assay contains thrombin receptor-activating peptide (TRAP) in the cartridge. The results for this cartridge are expressed as Platelet Aggregation Units (PAU).

1.5.4 Other tests

The Platelet Function Analyzer–100 (PFA-100; Siemens Healthcare Diagnostics, Inc., Deerfield, Illinois) evaluates platelet function by determining the time to sealing of an aperture within a membrane coated with platelet agonists as whole blood is aspirated under high-shear-rate conditions. This reaction takes place within disposable cartridges that are inserted into the device. PFA-100 is a rapid, whole blood assay that only requires a relatively small sample of blood and limited expertise. In addition, measuring platelet function under conditions of shear is more haemodynamically relevant than many other assays of platelet function. Nevertheless, the closure time is dependent upon the level of von Willebrand factor and on the haematocrit, and the PFA-100 device appears to be insensitive to clopidogrel (60).

Phosphorylation of vasodilator-stimulated phosphoprotein (VASP) is measured by whole blood flow cytometry (BioCytex, Marseilles, France) for the assessment of platelet inhibition. VASP is a marker protein for platelet response to thienopyridines, since its phosphorylation specifically correlates with inhibition of P2Y12 receptor (61). It requires relatively small amounts of whole blood, which
however need to be prepared and processed in a flow cytometer by experienced personnel.

Thromboelastography (TEG) was first developed in 1951 as a method to evaluate coagulation (62), but has recently been adapted to platelet function monitoring through the addition of platelet-specific agonists. It measures the contribution of platelet activation to clot formation (Haemoscope, Niles, Illinois). The main advantage is that it allows the evaluation of both coagulation and platelet function at once. Although it is a whole blood test, it requires pipetting and is therefore relatively time-consuming.

The Impact Cone and Plate(let) Analyzer (DiaMed, Cressier, Switzerland) tests platelet function under physiologically relevant arterial flow conditions. Small amounts of whole blood are used to measure activation induced by shear, which represents the major novelty of this assay compared to traditional methods, such as LTA or impedance aggregometry. In the two latter, platelets, albeit stirred, are not subjected to shear rates comparable to those relevant to in vivo conditions. However, the Impact analyzer requires blood pipetting, being thus not appropriate for bedside platelet evaluation.

The Plateletworks (Helena Laboratories, Beaumont, Texas) measures platelet aggregation and inhibition comparing cell count at baseline and in the presence of an agonist (ADP). It is a whole blood test requiring small sample preparation. However, it is highly time-dependent and samples need to be analyzed within 10 minutes from the collection. Longer waiting time may result in overestimation of platelet inhibition in patients receiving antiplatelet drugs. The latter represents the major disadvantage of the Plateletworks device and may limit its use in the routine clinical practice.

1.6 OUTLINE OF THE THESIS

As outlined in this introduction in patients with coronary artery disease undergoing PCI antiplatelet therapy is crucial in preventing thrombotic complications. However, a wide inter-individual variability exists in the response to antiplatelet agents and to clopidogrel in particular. This variability is related to genetic, cellular and clinical factors and determines either too high or too low residual platelet reactivity in a substantial proportion of patients.

The objective of the current thesis is to study the impact of platelet reactivity on clinical outcomes of patients with CAD undergoing PCI, and to understand the mechanisms leading to platelet activation in the setting of coronary intervention. Specifically, chapter 2 will illustrate the results of the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) Study. This is one of the first studies correlating platelet reactivity after clopidogrel with clinical outcomes after PCI and showing the usefulness of a point-of-care platelet function test in identifying patients in whom tailored antiplatelet strategies might be indicated. In chapter 3, the impact of high platelet reactivity on the incidence of PCI-related myonecrosis is presented. Next chapter 4 will address the modulator effect of diabetes mellitus on residual platelet reactivity after clopidogrel and on peri-procedural outcomes in patients undergoing PCI.

As outlined in the introduction, the ischemic benefit exerted by the antiplatelet therapy may be outweighed by the bleeding complications. Both are important determinants of future adverse events including mortality. Yet, identification of optimal platelet reactivity has not be determined. In chapter 5, we addressed this
unmet need and investigated whether assessment of platelet reactivity is predictive of both ischemic and bleeding events after elective PCI in stable CAD and whether a “therapeutic window” for platelet reactivity could be identified.

In the ensuing chapters 6 and 7, we went on to understand the mechanisms leading to higher platelet reactivity in the setting of PCI. We first evaluated the role of coronary atherosclerosis and its extent as underlying determinant of platelet reactivity and peri-procedural myocardial damage. Furthermore, we addressed whether PCI and its technical complexity may affect platelet reactivity. Importantly, biological and mechanical components underlying peri-procedural variations of platelet reactivity were dissected using detailed and well-characterized in vitro model of PCI.

Finally, we present a critical discussion of the gathered observations and outline future perspectives related to the role of platelet reactivity assessment in patients undergoing PCI.
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Point-of-Care Measurement of Clopidogrel Responsiveness Predicts Clinical Outcome in Patients Undergoing Percutaneous Coronary Intervention: Results of the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) Study

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ABSTRACT

Objectives. The aim of this study was to evaluate the correlation of point-of-care measurement of platelet inhibition with clinical outcome in patients undergoing percutaneous coronary intervention (PCI).

Background. Individual variability of clopidogrel response might influence results of PCI.

Methods. A total of 160 patients receiving clopidogrel before PCI were prospectively enrolled. Platelet reactivity was measured by the VerifyNow P2Y12 assay (Accumetrics Inc., San Diego, California). Primary end point was 30-day occurrence of major adverse cardiac events (MACE) according to quartile distribution of P2Y12 reaction units (PRU).

Results. Primary end point occurred more frequently in patients with pre-procedural PRU levels in the fourth quartile versus those in the lowest quartile (20% vs. 3%; p=0.034), and it was entirely due to periprocedural myocardial infarction (MI). Mean PRU absolute levels were higher in patients with periprocedural MI (258±53 vs. 219±69 in patients without; p=0.030). On multivariable analysis pre-PCI PRU levels in the fourth quartile were associated with 6-fold increased risk of 30-day MACE (odds ratio: 6.1; 95% confidence interval: 1.1 to 18.3, p=0.033). By receiver-operating characteristic curve analysis, the optimal cut-off for the primary end point was a pre-PCI PRU value ≥240 (area under the curve: 0.69; 95% confidence interval: 0.56 to 0.81, p=0.016).

Conclusions. This study indicates that high pre-PCI platelet reactivity might predict 30-day events. Use of a rapid point-of-care assay for monitoring residual platelet reactivity after clopidogrel administration might help identify patients in whom individualized antiplatelet strategies might be indicated with coronary intervention.
2.1 INTRODUCTION

Optimal platelet inhibition plays a key role in the prevention of early myocardial ischemic complications during percutaneous coronary intervention (PCI) (1,2); accordingly, the association of aspirin and clopidogrel, a non-reversible P2Y12 platelet receptor inhibitor, represents the established antiplatelet therapy in this setting. Because of inter-individual variability in clopidogrel responsiveness, platelet function monitoring assays are useful tools to identify patients with different drug responses, who may potentially be at increased risk of recurrent cardiac events or bleeding. Adenosine diphosphate (ADP)-induced light transmittance aggregometry (LTA) is the standard test for evaluating platelet reactivity in patients receiving clopidogrel, but this is non-specific and time-consuming (3); conversely, more rapid and specific tests measuring clopidogrel response in the individual patient might be widely applicable in clinical practice, thereby providing risk stratification and guide to antithrombotic therapy. Thus, the ARMYDA study group (1,4,5,6,7) has designed the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty - Platelet Reactivity Predicts Outcome) study to prospectively evaluate whether point-of-care measurements of platelet inhibition predict clinical outcome in patients undergoing PCI.
2.2 METHODS

2.2.1 Study Population and Design
ARMYDA-PRO is a prospective study including 160 patients receiving clopidogrel pre-PCI. Patients with a variety of coronary syndromes, including non ST-segment elevation acute coronary syndromes (ACS) were enrolled. Exclusion criteria were: primary PCI; platelet count <70x10^9/L; high bleeding risk; severe chronic renal failure (serum creatinine >2 mg/dL).

All patients received 600 mg clopidogrel load approximately 6 hours pre-intervention (N=120) or were on chronic clopidogrel therapy (75 mg/day for ≥5 days, N=40). Clopidogrel was continued for 1 month post-PCI and for 12 months in patients with ACS or receiving drug-eluting stents. All patients received aspirin.

Platelet reactivity was evaluated in the catheterization laboratory before PCI and at 8 and 24 hours after intervention by the VerifyNow™ P2Y12 assay (Accumetrics Inc., San Diego, CA), which is a rapid cartridge-based assay specifically measuring direct effects of clopidogrel on the platelet P2Y12 receptor. Technical details were already described elsewhere (8). Results are expressed as P2Y12 reaction units (PRU). The lower the PRU value, the greater the degree of P2Y12 receptor inhibition by clopidogrel and vice versa. Twenty randomly selected patients were analyzed to assess intra-assay variability, which was 2.1±1.3% (coefficient of variation: 6%).

Blood samples were also drawn before and at 8 and 24 hours in all patients for creatine kinase-MB (CK-MB, mass) and troponin-I (Tn-I, mass) levels; further measurements were obtained if symptoms suggested myocardial ischemia. Measurements were performed by Access 2 Immunochemiluminometric assay (Beckman Coulter); normal limits were ≤4 ng/mL for CK-MB and ≤0.08 ng/mL for Tn-I. One-month and 6-month clinical follow-up was obtained by office visit in all patients. Each patient gave informed consent to the study. The study was not supported by external sources of funding.

2.2.2 End-points
Primary end-point of ARMYDA-PRO was 30-day incidence of major adverse cardiac events (MACE): cardiac death, myocardial infarction (MI), target vessel revascularization, in relation to quartile distribution of platelet reactivity measured by PRU assay. MI was defined as post-procedural increase of cardiac biomarkers (Tn or CK-MB) >3 x 99th percentile of the upper reference limit (9). Target vessel revascularization included by-pass surgery or repeat PCI of the target vessel(s).

Secondary end-points were: 1) evaluation of mean PRU absolute values in patients with or without post-PCI elevation of markers of myocardial damage (CK-MB, Tn-I); 2) distribution in PRU quartiles of patients with any post-procedural increase of cardiac markers above normal limits; 3) correlation of vascular/bleeding complications to PRU quartiles; 4) clinical outcome at 6-month follow-up. Bleeding events were defined according to TIMI criteria (10).

2.2.3 Statistics
Based on patients’ enrollment criteria, we assumed 8% overall incidence of 30-day MACE (1,11). We tested the hypothesis that incidence of 30-day MACE differed by PRU quartiles. We assumed as effect size for the power analysis a 7-fold increased risk of 30-day MACE in patients of the 4th quartile (12); thus, a study population of at least 153 patients would be needed to verify this hypothesis with alpha of 0.05 (two-tailed) and beta of 0.08.
Continuous variables were compared by t-test for normally distributed values, otherwise the Mann-Whitney U-test was used. Proportions were compared by Fisher’s exact test when the expected frequency was <5, otherwise the chi squared test was applied. Event-free survival analysis was performed by Kaplan-Meier method with log-rank test group comparison. Odds ratios and 95% confidence intervals investigating the independent predictive role of PRU quartiles on the occurrence of the primary end-point were assessed by logistic regression. The following parameters were first evaluated in a univariate model: comparison of PRU quartiles (4th vs 1st quartile, 3rd vs 1st, 2nd vs 1st), each of the variables indicated in Table 1, and procedural variables, such as stent length, direct stenting, inflations duration. Only variables with P value <0.15 were then entered into the final model of multivariable logistic regression analysis. Ability of the assay to discriminate between patients with and without MACE at 30 days was evaluated by receiver-operating characteristic (ROC) curve analysis. The optimal cut-off value was calculated by determining the PRU value providing the greatest sum of sensitivity and specificity. Results are expressed as mean ± SD. P value <0.05 (two-tailed) was considered significant. Analysis was performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA) software.
2.3 RESULTS

Clinical and procedural characteristics according to pre-intervention PRU quartiles are indicated in Table 1. Procedural success was obtained in 99% of patients. No vessel or side branch (≥ 2 mm) closure occurred. No patient required emergency coronary artery by-pass graft.

Table 1. Main Clinical and Procedural Features.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cohort (N=160)</th>
<th>1st Quartile (N=40)</th>
<th>2nd Quartile (N=40)</th>
<th>3rd Quartile (N=40)</th>
<th>4th Quartile (N=40)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66±9</td>
<td>65±12</td>
<td>66±10</td>
<td>67±8</td>
<td>68±10</td>
<td>0.58</td>
</tr>
<tr>
<td>Male gender</td>
<td>129 (81)</td>
<td>30 (75)</td>
<td>32 (80)</td>
<td>29 (73)</td>
<td>38 (95)</td>
<td>0.07</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>55 (34)</td>
<td>15 (38)</td>
<td>11 (28)</td>
<td>15 (38)</td>
<td>14 (35)</td>
<td>1</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>119 (74)</td>
<td>32 (80)</td>
<td>26 (65)</td>
<td>32 (80)</td>
<td>29 (73)</td>
<td>0.48</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.8±3.1</td>
<td>25.6±3.2</td>
<td>26.2±3.4</td>
<td>26±3.1</td>
<td>25.7±3.2</td>
<td>0.83</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>45 (28)</td>
<td>7 (18)</td>
<td>12 (30)</td>
<td>13 (33)</td>
<td>13 (33)</td>
<td>0.52</td>
</tr>
<tr>
<td>ACS/Non STEMI</td>
<td>87 (54)</td>
<td>18 (45)</td>
<td>27 (67)</td>
<td>22 (55)</td>
<td>20 (50)</td>
<td>0.28</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>56±7</td>
<td>56±8</td>
<td>55±8</td>
<td>55±6</td>
<td>56±8</td>
<td>0.87</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.12±0.30</td>
<td>1.10±0.28</td>
<td>1.09±0.21</td>
<td>1.14±0.21</td>
<td>1.16±0.35</td>
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</tr>
<tr>
<td>Statin therapy</td>
<td>136 (85)</td>
<td>35 (88)</td>
<td>36 (90)</td>
<td>34 (85)</td>
<td>31 (78)</td>
<td>0.59</td>
</tr>
<tr>
<td>Vessel treated</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>93 (49)</td>
<td>21 (46)</td>
<td>24 (52)</td>
<td>26 (52)</td>
<td>22 (47)</td>
<td>0.93</td>
</tr>
<tr>
<td>Left circumflex</td>
<td>43 (23)</td>
<td>10 (22)</td>
<td>11(24)</td>
<td>11 (22)</td>
<td>11 (23)</td>
<td>1</td>
</tr>
<tr>
<td>Right coronary artery</td>
<td>52 (27.5)</td>
<td>15 (32)</td>
<td>11 (24)</td>
<td>12 (24)</td>
<td>14 (30)</td>
<td>0.98</td>
</tr>
<tr>
<td>Saphenous vein grafts</td>
<td>1 (0.5)</td>
<td>-</td>
<td>-</td>
<td>1 (2)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Restenotic lesions</td>
<td>6 (4)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>3 (8)</td>
<td>1 (3)</td>
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</tr>
<tr>
<td>Lesion type B2/C</td>
<td>112 (70)</td>
<td>26 (65)</td>
<td>28 (70)</td>
<td>31 (78)</td>
<td>27 (67)</td>
<td>0.88</td>
</tr>
<tr>
<td>Multivessel intervention</td>
<td>28 (18)</td>
<td>6 (15)</td>
<td>5 (13)</td>
<td>10 (25)</td>
<td>7 (18)</td>
<td>0.67</td>
</tr>
<tr>
<td>Use of stents</td>
<td>149 (93)</td>
<td>37 (92)</td>
<td>37 (92)</td>
<td>38 (95)</td>
<td>37 (92)</td>
<td>1</td>
</tr>
<tr>
<td>Use of drug-eluting stents</td>
<td>41 (26)</td>
<td>12 (30)</td>
<td>11 (28)</td>
<td>11 (28)</td>
<td>7 (18)</td>
<td>0.80</td>
</tr>
<tr>
<td>Use of IIb-IIa inhibitors</td>
<td>8 (5)</td>
<td>3 (8)</td>
<td>2 (5)</td>
<td>1 (3)</td>
<td>2 (5)</td>
<td>1</td>
</tr>
</tbody>
</table>
2.3.1 Primary End-point
Occurrence of composite primary end-point was significantly more frequent in patients with pre-procedural PRU levels in the 4th quartile vs those in the 1st quartile (20% vs 3%; P=0.034) (Figure 1); this was essentially due to peri-procedural MI. No further event occurred during 1-month follow-up.

![Figure 1](image)

**Figure 1.** Incidence of primary end-point (30-day MACE) according to quartile distribution of pre-intervention PRU values. MACE=Major adverse cardiac events; PRU=Platelet (P2Y12) Reaction Units

2.3.2 Secondary End-points
Higher PRU values prior to PCI (Figure 2, Panel A) predicted peri-procedural MI by CK-MB definition (258±53 PRU in patients with vs 219±69 PRU in those without MI; P=0.030), but not by Tn-I (Figure 2, Panel B).

Likewise, any post-procedural increase of CK-MB above upper normal limits was more frequently observed in patients in the 4th PRU quartile (48% vs 23% in the 1st quartile; P=0.035), but there was no difference in the proportion of patients with Tn-I elevation (65% in the 4th vs 45% in the 1st quartile; P=0.12). No relationship was found between MACE and PRU levels at 8 and 24 hours post-PCI (P≥0.30).

No patient had major bleeding or required transfusions; minor bleeding occurred in 9 patients (6%): 1 patient had urethral bleeding from a Foley catheter and 8 had small entry-site haematomas, independent of baseline PRU quartiles. There were no side-effects in either group requiring discontinuation of clopidogrel.
At 6-month follow-up, the cumulative incidence of MACE was 10% in the 1st, 13% in the 2nd, 17% in the 3rd, and 30% in the 4th quartile (P=0.05 1st vs 4th quartile).

Figure 2. Secondary end-points. Distribution of pre-intervention PRU levels according to post-PCI CK-MB (Panel A) and Tn-I (Panel B) values. CK-MB=Creatine kinase-MB; PRU=Platelet (P2Y12) Reaction Units; Tn-I=Troponin-I

2.3.3 Multivariable analysis

While comparison of 3rd and 2nd quartiles vs 1st was not different (P=0.68 and 0.71, respectively), multivariable analysis revealed that pre-PCI PRU levels in the 4th quartile were independent predictors of higher risk of adverse events at 30 days (OR 6.1, 95% CI 1.1-18.3; P=0.033). Age >70 years, left ventricular dysfunction and use of Glycoprotein IIb/IIIa inhibitors were also associated with increased risk, whereas statin therapy at the time of PCI had a protective effect (OR 0.21, 0.10-0.91; P=0.037) (4,7).

2.3.4 ROC curve analysis

ROC curve analysis showed that PRU levels significantly discriminate between patients with and without 30-day MACE with an area under the curve of 0.69 (95% CI 0.56-0.81; P=0.016). A PRU value ≥ 240 was identified as the optimal cut-off point to predict 30-day outcome, with sensitivity of 81% and specificity of 53%.
2.4 DISCUSSION

This prospective study indicates that high residual platelet reactivity after clopidogrel administration, measured by a point-of-care assay at the time of intervention, is associated with higher incidence of 30-day MACE after PCI; this outcome is mainly driven by increased risk of peri-procedural myocardial infarction.

Evaluation of individual clopidogrel responsiveness is an emerging issue in interventional cardiology. Wide variability is reported (13), ascribed in turn to differences in drug absorption, variations in biotransformation rate into active metabolite (due to drug-drug interactions at the site of cytochrome P or genetic CYP3A polymorphisms) (14), P2Y12 receptor polymorphisms affecting receptor number and activity (15), and even non-compliance (16). The prevalence of reduced clopidogrel responsiveness varies from 4% to 30% (2,13,17), depending on the assay used, definitions empirically applied and presence of potential confounders.

Several methods have been utilized to evaluate clopidogrel response in patients undergoing PCI, with the aim to identify those at higher risk of adverse events (18), with optical aggregometry after ADP stimulation being the most widely used in previous studies. A low platelet response to clopidogrel measured with LTA was associated with increased incidence of peri-procedural myocardial injury (19), cardiovascular events at short (12,20) and mid-term (2,21), and stent thrombosis (22). Nevertheless, this test has a number of limitations, such as need for highly-trained personnel, repeated centrifugations, large sample volume, length of assay time, sub-optimal reproducibility (3). Furthermore, it is non-specific, since it also measures aggregation due to binding of ADP to P2Y1 platelet receptor, which is not inhibited by clopidogrel.

Vasodilator-stimulated phosphoprotein phosphorylation by flow cytometry is more specific, as it directly measures effects of the drug on the target receptor; in observational studies, a lower platelet inhibition assessed by VASP was observed in patients with stent thrombosis (22). Cost and need for long sample preparation and skilled personnel limit a widespread use of this test (3).

Platelet reactivity by VerifyNow™ can be rapidly measured without sample preparation by moderately-experienced personnel utilizing low sample volumes; the test specifically reflects the extent of ADP-induced inhibition of adenylyl cyclase, which is mediated uniquely by action of clopidogrel on the P2Y12 receptor. The assay allows to detect absolute PRU values and percent P2Y12 receptor inhibition, which may be calculated as a percent change from baseline platelet aggregation (8,23). A close correlation was described between results of ADP-induced optical aggregometry and absolute PRU values by VerifyNow™ assay in patients undergoing PCI (24). This method was also tested and clinically validated in a multicenter, prospective study (VERITAS) (23).

In this study we demonstrate the correlation between clopidogrel response and clinical outcome after coronary intervention. Although multivariable analysis may be over-fitted given the number of end-point events included, the predictive value for adverse events of the 4th quartile of platelet reactivity was independent of possible confounding factors, with a 6-fold increased risk of peri-procedural MI vs the 1st quartile. ROC analysis indicated that this assay can predict outcome at 30 days, with optimal cut-off point to discriminate patients at higher risk of events of ≥ 240 PRU, and a positive predictive value of 81%. The threshold identified in our study coincides with that observed by Price et al. (25) in an observational study in which a cut-off ≥235 PRU correlated with 6-month events, including stent thrombosis, in patients
receiving sirolimus-eluting stents. In ARMYDA-PRO, the negative predictive value of PRU in the 1st quartile was 98%, with minimal probability of early adverse events with PRU levels <178. Interestingly, higher PRU values correlated with CK-MB elevation, but not with Tn-I elevation, suggesting that platelet-unrelated events, i.e. distal embolization, may be also responsible for Tn raise after PCI (12). No cut-off value for events was described in a recent study (26) on stable patients treated with drug-eluting stents after variable loading doses of clopidogrel (300 mg or 600 mg) which also utilized VerifyNow™.

We utilized quartile distribution since clopidogrel responsiveness is multifactorial and it should be considered as a continuum; accordingly, a progressively increasing incidence of events was observed across PRU quartiles (Figure 1), with a similar pattern of correlation between PRU values and peak levels of myocardial markers (Figure 2).

Unlike Price’s study (25), ARMYDA-PRO was not powered to evaluate relationship between degree of clopidogrel inhibition and occurrence of bleeding complications or late thrombotic events; larger prospective studies with longer follow-up are needed to address this issue.

Clopidogrel responsiveness variability, within the enhanced thrombotic milieu of PCI, requires careful evaluation. ARMYDA-PRO supports the use of a rapid point-of-care assay for monitoring residual platelet reactivity after clopidogrel administration: VerifyNow™ can be a useful and easily applicable method that may help establish a clinically-driven threshold of platelet reactivity that would identify patients at higher risk of peri-procedural events; in those, individualized antithrombotic strategies (i.e. restricted use of drug-eluting stents, more extensive utilization of Glycoprotein IIb/IIIa inhibitors, higher clopidogrel maintenance dose or use of newer P2Y12 receptor antagonists) may be indicated in order to improve clinical outcome after coronary intervention.
REFERENCES


Point-of-Care Assessment of Platelet Reactivity After Clopidogrel to Predict Myonecrosis in Patients Undergoing Percutaneous Coronary Intervention

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† Cardiovascular Center, OLV Hospital, Aalst, Belgium.

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ABSTRACT

Objectives. To evaluate the impact of platelet reactivity after clopidogrel, as assessed by the VerifyNow point-of-care assay, on myonecrosis in low-to-intermediate risk patients undergoing PCI.

Background. Inadequate platelet inhibition at the time of percutaneous coronary intervention is associated with a higher risk of recurrent ischemic events.

Methods. A total of 250 consecutive biomarker negative patients treated with clopidogrel and undergoing elective PCI were enrolled. Cardiac biomarkers (CK-MB and Troponin-I) were measured before and 8 and 24 hours after intervention. Platelet reactivity after clopidogrel was assessed immediately before PCI by the VerifyNow P2Y12 point-of-care assay. High platelet reactivity (HPR) after clopidogrel was defined as a platelet reaction unit (PRU) value $\geq 240$.

Results. Patients with HPR (31% of the overall population) showed more frequently myonecrosis, with statistical significance with regards to CK-MB elevation (35% vs. 20%; $p=0.011$), and by trend with regards to Troponin-I elevation (47% vs. 35%; $p=0.059$). Incidence of peri-procedural myocardial infarction was higher in patients with HPR, both by CK-MB (13% vs. 4%; $p=0.011$) and Troponin-I definition (32% vs. 19%; $p=0.019$). By multivariable analysis, HPR was an independent predictor of peri-procedural myocardial infarction.

Conclusions. HPR after clopidogrel, easily assessed by a point-of-care assay, is a frequent finding and is associated with increased risk of myonecrosis in low-to-intermediate risk patients undergoing planned PCI.
3.1 INTRODUCTION

Platelet inhibition in patients undergoing percutaneous coronary intervention (PCI) is of paramount importance to reduce the risk of recurrent ischemic events (1,2). Dual anti-platelet therapy with aspirin and clopidogrel is the strategy most commonly applied (3), although a large inter-individual variability in clopidogrel response (4) may result into an inadequate platelet inhibition at the time of PCI (5-7). The latter has been advocated to partly explain post-procedural myonecrosis, although recent studies (6,8-10) have yielded conflicting results, probably due to heterogeneous methods and definitions employed. Yet, even small post-procedural elevations of cardiac markers have been associated with an increased risk of long-term major cardiac adverse events (MACE), including death (11-15).

Aim of the present study was to evaluate the impact of platelet reactivity after clopidogrel, as assessed by the VerifyNow point-of-care assay, on myonecrosis in low-to-intermediate risk patients undergoing PCI.
3.2 METHODS

3.2.1 Patient Population
Consecutive patients with negative myocardial biomarkers undergoing elective PCI were recruited. Exclusion criteria were: acute coronary syndrome, administration of glycoprotein IIb/IIIa inhibitors, chronic total occlusion, lesions with extensive calcifications requiring rotational atherectomy, platelet count <70x10^9/l, high bleeding risk, coronary bypass surgery in the previous 3 months, severe chronic renal failure (serum creatinine >2 mg/dl). All patients were on chronic aspirin treatment. They received either 600 mg clopidogrel loading dose (at least 6 hours before intervention) or were on therapy with clopidogrel 75 mg/day for at least 5 days. Procedural anticoagulation was performed by administration of unfractionated heparin (100 U/kg) in all patients. Local ethical committee approved the study and all patients signed written informed consent.

3.2.2 Assessment of biomarkers and platelet reactivity
Blood samples were drawn in all patients before and 8 and 24 hours after intervention for cardiac biomarkers assessment (CK-MB, Tn-I), using the Access 2 Immunochemiluminometric assay (Beckman Coulter, Fullerton, California).

Platelet reactivity after clopidogrel was assessed immediately before PCI by the VerifyNow P2Y12 point-of-care assay. Blood was collected from the femoral artery immediately after sheath placement. Blood samples were collected in 2-mL tubes containing 3.2% sodium citrate. VerifyNow P2Y12 specifically evaluates clopidogrel effect on P2Y12 receptor by optical turbidimetry. Whole blood is challenged in dedicated cartridges with 20 μmol ADP, which activates platelets by binding P2Y1 and P2Y12 receptors, and 22 nmol prostaglandin E1 (PGE-1), which increases assay specificity by suppressing ADP-induced P2Y1-related intra-platelet signaling. Activated platelets agglutinate around fibrinogen-coated polystyrene beads, therefore increasing light transmittance through the sample. Results are reported as P2Y12 reaction units (PRU); the lower the PRU value, the higher the platelet aggregation inhibition by clopidogrel. High platelet reactivity (HPR) after clopidogrel was defined as a PRU value ≥240 (6).

3.2.3 Endpoints
Primary endpoint of the study was the correlation between occurrence of myonecrosis in patients undergoing elective PCI and presence of HPR. Myonecrosis was defined as any increase of cardiac biomarkers above the 99th percentile upper limit of normal (ULN). In addition, correlation between HPR and increases of biomarkers >3 x ULN and >5 x ULN were also evaluated.

Secondary endpoints were: incidence of HPR in patients with and without peri-procedural myocardial infarction (PMI), according to the universal definition (16); peak values of cardiac biomarkers according to the presence of HPR. In-hospital MACE (death, myocardial infarction, urgent target vessel revascularization) and bleedings were monitored.

3.2.4 Statistics
In the Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome (ARMYDA-PRO) study, mean PRU values were 219±69 in patients with normal post-PCI CK-MB, and 244±58 in those with post-procedural increase of CK-MB (6). Thus, assuming a CK-MB
increase in 30% of patients and expecting in these a mean PRU value 12% higher than
in those without myonecrosis, a total of 246 patients were needed to detect the
expected difference between the two groups with an estimated power of 80% at a 2-
sided alpha of 0.05.

Continuous variables are expressed as mean ± standard deviation, unless
otherwise specified. Categorical variables are reported as frequencies and
percentages. Comparisons between continuous variables were performed by Student’s
t test, or Mann-Whitney test if not normally distributed. Comparisons between
categorical variables were evaluated using two-tailed Fisher’s exact test or Pearson’s
$\chi^2$ test, as appropriate. Normal distribution of PRU levels in the study population
was confirmed by Kolmogorov-Smirnov test. All clinical and procedural features and
presence of HPR were evaluated in a univariate analysis for the association with PMI
using logistic regression. Only variables showing a p value <0.15 were then entered a
multivariable logistic regression model. Statistical analysis was performed using
SPSS 15.0 software (SPSS Inc., Chicago, Illinois) and p values <0.05 was considered
significant.
3.3 RESULTS

3.3.1 Study population
A total of 250 patients were recruited in the study. Mean PRU value in the general population was 207±65; 78 patients (31%) showed a PRU ≥240 and were assigned to the HPR group (Figure 1). Clinical and procedural features are listed in Tables 1 and 2, respectively. Diabetes mellitus was more frequent among patients with HPR (42% vs. 29% in patients with normal platelet reactivity; p=0.039), who also tended to have higher BMI and more commonly a multi-vessel disease. Procedural success was achieved in all patients and no bailout use of glycoprotein IIb/IIIa inhibitors was required.

Figure 1. Platelet reactivity. Distribution of platelet reactivity assessed by VerifyNow P2Y12 assay. PRU= P2Y12 reaction unit.
Table 1. Clinical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>HPR (n=78)</th>
<th>No HPR (n=172)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>66.5±9.6</td>
<td>65.4±9.8</td>
<td>0.405</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>66 (85)</td>
<td>134 (78)</td>
<td>0.219</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.6±3.1</td>
<td>25.9±3.2</td>
<td>0.103</td>
</tr>
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<td>Diabetes mellitus, n (%)</td>
<td>33 (42)</td>
<td>50 (29)</td>
<td>0.039</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>60 (77)</td>
<td>128 (74)</td>
<td>0.671</td>
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<td>60 (77)</td>
<td>124 (72)</td>
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<td>Current smoker, n (%)</td>
<td>13 (17)</td>
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<td>Previous myocardial infarction, n (%)</td>
<td>24 (31)</td>
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<td>Previous coronary intervention, n (%)</td>
<td>28 (36)</td>
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<td>Previous bypass surgery, n (%)</td>
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<td>Left ventricle ejection fraction, %</td>
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<td>56±8</td>
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<td>Creatinine, mg/dl</td>
<td>1.10±0.31</td>
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<td>0.810</td>
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<td>78 (100)</td>
<td>172 (100)</td>
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<td>Clopidogrel</td>
<td>9 (12)</td>
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<td>Clinical presentation, n (%)</td>
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<td>Stable angina</td>
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<td>64 (82)</td>
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<tr>
<td>PRU</td>
<td>281±34</td>
<td>173±44</td>
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HPR= high platelet reactivity; PRU= P2Y12 reaction unit.

Table 2. Procedural characteristics.

<table>
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<th>No HPR (n=172)</th>
<th>p value</th>
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<td>Treated vessel, n (%)</td>
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</tr>
<tr>
<td>Left anterior descending</td>
<td>46 (50)</td>
<td>103 (48)</td>
<td></td>
</tr>
<tr>
<td>Left circumflex</td>
<td>20 (22)</td>
<td>51 (24)</td>
<td></td>
</tr>
<tr>
<td>Right coronary artery</td>
<td>26 (28)</td>
<td>56 (26)</td>
<td></td>
</tr>
<tr>
<td>Saphenous vein graft</td>
<td>-</td>
<td>4 (2)</td>
<td></td>
</tr>
<tr>
<td>Restenotic lesion, n (%)</td>
<td>9 (11)</td>
<td>22 (13)</td>
<td>0.781</td>
</tr>
<tr>
<td>Lesion type B2/C, n (%)</td>
<td>62 (67)</td>
<td>132 (62)</td>
<td>0.342</td>
</tr>
<tr>
<td>Bifurcation lesions, n (%)</td>
<td>46 (27)</td>
<td>25 (32)</td>
<td>0.389</td>
</tr>
<tr>
<td>Multivessel intervention, n (%)</td>
<td>14 (18)</td>
<td>38 (22)</td>
<td>0.454</td>
</tr>
<tr>
<td>Use of stent, n (%)</td>
<td>77 (99)</td>
<td>169 (98)</td>
<td>0.787</td>
</tr>
<tr>
<td>Use of drug eluting stent, n (%)</td>
<td>20 (26)</td>
<td>41 (24)</td>
<td>0.758</td>
</tr>
<tr>
<td>Direct stenting, (%)</td>
<td>38 (49)</td>
<td>89 (52)</td>
<td>0.657</td>
</tr>
<tr>
<td>Stents implanted, n (%)</td>
<td>1.4±0.6</td>
<td>1.5±0.7</td>
<td>0.249</td>
</tr>
<tr>
<td>Total stent length, mm</td>
<td>28±17</td>
<td>29±16</td>
<td>0.662</td>
</tr>
<tr>
<td>Postdilatation, (%)</td>
<td>29 (37)</td>
<td>67 (39)</td>
<td>0.789</td>
</tr>
<tr>
<td>Maximal inflation pressure, atm</td>
<td>15.0±3.8</td>
<td>15.1±4</td>
<td>0.850</td>
</tr>
<tr>
<td>Side branch occlusion, n (%)</td>
<td>2 (3)</td>
<td>3 (2)</td>
<td>0.668</td>
</tr>
</tbody>
</table>

HPR= high platelet reactivity.
3.3.2 Primary endpoint

Myonecrosis was significantly more frequent in HPR group (Figure 2). CK-MB increase was more frequent in patients with HPR with respect to any elevation (35% vs. 20%; OR 2.15, 95%CI 1.18-3.91; p=0.011), more than 3 times ULN (13% vs. 4%; OR 3.47, 95%CI 1.27-9.48; p=0.011), and more than 5 times ULN (6% vs. 1%; OR 5.82, 95%CI 1.10-30-70; p=0.020). While there was no significant difference between the two groups with respect to any elevation of Tn-I (47% vs. 35%; OR 1.69, 95%CI 0.98-2.90; p=0.059), patients with HPR showed significantly more frequent increases in Tn-I when the value was more than 3 times ULN (32% vs. 19%; OR 2.06, 95%CI 1.12-3.80; p=0.019) and also when the value was more than 5 times ULN (21% vs. 6%; OR 4.18, 95%CI 1.80-9.71; p<0.001). Mean PRU value was significantly higher in patients with CK-MB increase, as compared to patients without (227±71 vs. 200±62; p=0.006).

![Figure 2. Platelet reactivity and myonecrosis. Incidence of myonecrosis in patients with (dark bars) and without (light bars) high platelet reactivity.](image)

3.3.3 Secondary endpoints

A total of 57 (23%) patients met the criteria for PMI (16). HPR was more frequent in patients with PMI (44% vs. 28% of patients without PMI; OR 2.06, 95%CI 1.12-3.80, p=0.019; Figure 3). The median peak value of Tn-I was significantly higher in HPR group (0.07, interquartile range [IQR] 0.03-0.30 vs. 0.04, IQR 0.01-0.16; p=0.002), while the median peak value of CK-MB was not significantly different between the two groups (2.4, IQR 1.1-5.0 vs. 1.9, IQR 1.1-3.4; p=0.098). No significant correlation was found between PRU values and peak levels CK-MB or Tn-I. No major adverse cardiac events, other than PMI, or major bleeding occurred during hospitalization. Nine minor bleedings were recorded, irrespective of HPR.
**Figure 3. Platelet reactivity and peri-procedural myocardial infarction.** Incidence of high platelet reactivity according to the presence of peri-procedural myocardial infarction. HPR= high platelet reactivity; PMI= peri-procedural myocardial infarction.

### 3.3.4 Multivariable analysis

At univariate analysis, multivessel intervention, lesion type B2/C, multivessel disease, diabetes and HPR were significantly associated with PMI by CK-MB definition, while total stent length, left ventricle ejection fraction <40%, multivessel disease and HPR were significantly associated with PMI by Tn-I definition. Multivariable analysis (Figure 4) showed that HPR was an independent predictor of increased risk of PMI either by CK-MB definition (OR 3.21, 95%CI 1.11-9.32; p=0.032; Figure 4, Panel A) or Tn-I definition (OR 2.25 95%CI 1.24-4.13; p=0.019; Figure 4, Panel B).
Figure 4. Multivariable analysis. Odds ratios (presented on log axis) for peri-procedural myocardial infarction by CK-MB (Panel A) and troponin (Panel B) definition. HPR= high platelet reactivity; LVEF= left ventricle ejection fraction.
3.4 DISCUSSION

In the present study, high residual platelet reactivity after clopidogrel was detected in almost one third of the patients and showed to be an independent predictor of PMI. Our results confirm the predictive role of post-clopidogrel platelet reactivity on myocardial injury during coronary intervention, as previously shown (6,10). Yet, Lev et al. (8) found only a trend towards increased myonecrosis in clopidogrel resistant patients and Buch et al. (9) did not observe any significant correlation between PRU values and cardiac biomarkers levels. Lack of cut-off values for impaired response to clopidogrel in these studies might have accounted for discrepancies. Conversely, we divided the study population based on the presence of HPR after clopidogrel, defined as a PRU value ≥ 240 by VerifyNow assay. In the Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome (ARMYDA-PRO) study (6), a PRU value ≥ 240 was found as optimal cut-off to discriminate patients at higher risk of 30-day major adverse cardiovascular events (MACE). More recently, Marcucci et al. (7) showed the same PRU threshold value to predict higher risk of 12-month cardiovascular death and non-fatal MI in 683 patients undergoing PCI for acute coronary syndrome. Furthermore, a similar cut-off value (PRU ≥ 235) was able to significantly predict stent thrombosis after drug eluting stent implantation (5). Beyond clinical significance, our definition of HPR is also supported by previous evidence that a PRU value ≥ 239 was the optimal cut-off for predicting high post-treatment platelet reactivity, defined as a 5 µM ADP-induced platelet aggregation >50% by light transmittance aggregometry (LTA) in 300 PCI patients (17).

In our study, HPR was significantly associated with peri-procedural myonecrosis, consistently over different degrees of post-procedural cardiac biomarkers elevations. Although the prognostic value of procedure-related myonecrosis is still under debate (18,19), growing evidence are supporting that even small increase of CK-MB (11-13) and Troponin (14,15) are significantly associated with long-term clinical events, including mortality. Selvanayagam et al. (20) provided important insights on the mechanism linking peri-procedural myonecrosis and long-term-outcome: an irreversible myocardial injury detected by delayed-enhancement MRI was observed in patients with post-procedural Troponin elevation. In addition, the risk of adverse events increases proportionally with cardiac markers elevation (11, 21), translating into an even higher risk for patients with PMI (16).

3.4.1 Clinical implications

Assessment of platelet reactivity by a rapid and user-friendly point-of-care assay, in combination with the use of a validated threshold value, can be a useful tool for discriminating patients with HPR despite clopidogrel therapy, therefore at increased risk of myonecrosis. In such patients a more aggressive antiplatelet treatment may be indicated to prevent further ischemic events. Recent reports have shown, in fact, a particularly beneficial effect of glycoprotein IIb/IIIa inhibitors in patients with impaired response to clopidogrel (22-24). A substudy of the Brief Infusion of Intravenous Eptifibatide Following Successful Percutaneous Coronary Intervention (BRIEF-PCI) trial, demonstrated that incidence of post-procedural myonecrosis was not affected by response to clopidogrel in patients receiving eptifibatide (22). Furthermore, in a relatively small study on 149 clopidogrel non-responders undergoing PCI, administration of abciximab significantly reduced rates of 30-day cardiovascular events, which were mainly driven by peri-procedural myonecrosis.
(23). Similarly, Valgimigli et al. have recently shown that in patients with poor responsiveness to clopidogrel, tirofiban administration can significantly decrease incidence of PMI, both by CK-MB and troponin definition (24).

Novel thienopyridines represent another therapeutic option in patients with impaired response to clopidogrel in order to prevent myonecrosis. In a subanalysis of the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38), treatment with prasugrel was superior to clopidogrel in reducing the risk of recurrent myocardial infarction, including those related to the procedure, in patients with acute coronary syndrome undergoing PCI (25).

3.4.2 Study limitations
By study protocol, only patients with low-to-intermediate risk PCI and baseline negative cardiac biomarkers were enrolled, therefore we cannot extend our results to more complex patients or acute coronary syndrome. However, low-to-intermediate risk patients are precisely those patients highly exposed to myonecrosis should platelet inhibition play a critical role, while coronary manipulation and side branch occlusion might indeed be more important determinants in more complex cases. We only monitored patients during in-hospital period, thus no data about the impact of platelet reactivity on long-term outcome is available.

3.4.3 Conclusions
HPR after clopidogrel, easily assessed by a point-of-care assay, is a frequent finding and is associated with increased risk of myonecrosis in low-to-intermediate risk patients undergoing PCI. In patients with HPR, tailored antiplatelet therapy may be required to reduce procedural complications.
REFERENCES


Comparison of Platelet Reactivity and Peri-Procedural Outcomes in Patients With versus Without Diabetes Mellitus and Treated with Clopidogrel and Percutaneous Coronary Intervention

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ABSTRACT

The effect of peri-procedural platelet reactivity and clinical outcomes in diabetic patients taking clopidogrel and undergoing percutaneous coronary intervention (PCI) is unclear. Aim of the present study was to prospectively evaluate the influence of diabetes mellitus (DM) on platelet reactivity measured by the VerifyNow P2Y12 assay, and on peri-procedural outcomes in patients receiving clopidogrel and undergoing PCI. A total of 285 consecutive clopidogrel treated patients undergoing elective PCI were included. Platelet function analysis was performed using the VerifyNow P2Y12 assay. High platelet reactivity (HPR) after clopidogrel was defined as a platelet reaction unit (PRU) value ≥240. Cardiac biomarkers were measured before, 8 and 24 hours after intervention. Patients with DM had significantly higher pre-PCI platelet reactivity compared to non-diabetics (214±83 PRU vs. 193±68 PRU; P=0.02). HPR was more frequently observed in diabetics (36% vs. 22%; P=0.01) pre-PCI. Patients with DM had an increased incidence of peri-procedural MI (11% vs. 4%; P=0.04). When the entire population was divided on the basis of the presence or absence of DM and HPR, patients with both DM and HPR presented the highest incidence of peri-procedural MI (P for trend=0.0008). HPR was an independent predictor of peri-procedural MI (OR 8.34, 95% CI 2.60-26.76; P=0.0003). In conclusion, patients with DM undergoing PCI have higher platelet reactivity at the time of PCI despite adequate clopidogrel pretreatment, and subsequently worse peri-procedural outcomes. Point-of-care platelet function testing may help to identify patients at higher risk of peri-procedural MI.
4.1 INTRODUCTION

The aim of this study was to prospectively evaluate the influence of DM on platelet reactivity measured by VerifyNow P2Y12 assay, and on peri-procedural outcomes in patients receiving clopidogrel and undergoing PCI.
4.2 METHODS

A total of 285 consecutive clopidogrel-treated patients undergoing elective PCI for stable angina or non ST-elevation acute coronary syndromes were recruited. All patients either received a 600-mg clopidogrel loading dose at least 6 hours before intervention or were pretreated with 75-mg/day clopidogrel for at least 5 days. Exclusion criteria were: ST-elevation myocardial infarction or upstream use of glycoprotein IIb/IIIa inhibitors or platelet count <70x10^9/L or high bleeding risk or coronary artery by-pass surgery in the previous 3 months or severe renal failure (serum creatinine >2 mg/dL). After PCI, patients were treated with clopidogrel (75 mg/day) for at least 4 weeks after bare metal stent implantation and for 12 months after an acute coronary syndrome or drug-eluting stent implantation. All patients received aspirin prior to intervention and continued aspirin (100 mg/day) indefinitely. Procedural anticoagulation consisted of unfractionated heparin (100 U/kg) for all patients.

Platelet function analysis was performed in the cardiac catheterization laboratory immediately before PCI using the VerifyNow P2Y12 assay (Accumetrics Inc., San Diego, California). This is a rapid cartridge-based turbidimetric whole blood aggregation assay that measures the response to thienopyridines. Blood samples were collected in 2 ml vacutainer tubes containing 3.2% sodium citrate. To avoid unwanted platelet activation the first 5 ml of blood was not used for platelet function testing. Technical details on the assay are already described elsewhere. Platelet reactivity results are expressed in P2Y12 reaction units (PRU). In general, the higher the PRU value, the lower the degree of platelet inhibition conferred by clopidogrel and vice versa.

Blood samples were also drawn in all patients before PCI, at 8 and 24 hours after intervention for the measurement of creatine kinase-MB (CK-MB) and troponin-I levels. Additional samples were obtained to measure markers of myocardial necrosis if patients developed symptoms after PCI that were suggestive of myocardial ischemia. Measurements were performed using the Access 2 Immunochemiluminometric assay (Beckman Coulter, Fullerton, California). Informed consent was obtained from all patients. No external source of funding supported this study.

Study end-points were: a) evaluation of influence of DM on peri-procedural platelet reactivity; b) comparison of peri-procedural outcomes in patients with and without DM, according to platelet reactivity levels. Diabetes mellitus was defined according to the World Health Organization Report criteria. High platelet reactivity (HPR) was defined as a PRU value ≥240 based on previous results from ARMYDA-PRO. Peri-procedural MI was defined as a post-procedural increase in CK-MB greater than 3 x 99th percentile of the upper reference limit for patients with baseline negative myocardial necrosis markers, according to the Joint ESC/ACCF/AHA/WHF Task Force consensus statement on the Redefinition of Myocardial Infarction for clinical trials on coronary intervention. In patients with raised baseline levels of creatine kinase-MB, a subsequent elevation ≥50% the baseline value fulfilled the criteria for peri-procedural MI.

Continuous variables are expressed as mean ± SD, while categorical variables are reported as frequencies and percentages. Normality was tested by the Kolmogorov-Smirnov test. Continuous variables were compared by t-test for normally distributed values; otherwise the Mann-Whitney U-test was used. Comparisons between categorical variables were made using the Fisher’s exact test.
when the expected frequency was <5, otherwise the chi-squared test was applied. Odds ratios and 95% confidence intervals investigating the independent association of clinical and procedural variables with peri-procedural MI were assessed by logistic regression. All variables indicated in Table 1 and 2 showing a significant univariate association with peri-procedural MI (P<0.05) and HPR were entered in a multivariable logistic regression model. A P value <0.05 was considered significant. Statistical analysis was performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).
4.3 RESULTS

A total of 104 (36%) diabetic and 181 non-diabetic patients were enrolled. The main clinical and procedural features are indicated in Tables 1 and 2. Patients with DM had significantly higher pre-PCI platelet reactivity compared to non-diabetics (214±83 PRU vs. 193±68 PRU; P=0.02). HPR was more frequently observed in diabetics vs. non-diabetics (36% vs. 22%; P=0.01; Figure 1) pre-PCI. This association remained significant after adjustment for the factors potentially related to HPR (i.e., age, gender, BMI, smoke, statin treatment, proton pump inhibitor treatment) (OR 1.895, 95%CI 1.11-3.21; P=0.02). There was no significant difference in the incidence of HPR in diabetic patients regardless of whether they were treated with insulin therapy or oral hypoglycaemic drugs (P>0.10). In the quartile with the highest pre-PCI platelet reactivity, DM was more prevalent compared to the 3 lowest quartiles (47% vs. 33%; P=0.04).

Patients with DM had an increased incidence of peri-procedural MI compared to non-diabetics (11% vs. 4%; P=0.04) (Figure 2). When the entire population was divided into four groups on the basis of the presence or absence of DM and HPR, we found that the combination of DM and HPR resulted in the highest incidence of peri-procedural MI (P for trend=0.0008; Figure 3). Univariate analysis showed that multi-vessel disease, DM and HPR were positively associated with peri-procedural MI, whereas statin therapy was inversely related to peri-procedural MI. Multivariate logistic regression analysis revealed that HPR was an independent predictor of peri-procedural MI (OR 8.34, 95% CI 2.60-26.76; P=0.0003; Figure 4).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Diabetes Mellitus</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (N=104)</td>
<td>No (N=181)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67 ± 8</td>
<td>66 ± 9</td>
</tr>
<tr>
<td>Men</td>
<td>83 (80%)</td>
<td>141 (78%)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.1±6.1</td>
<td>26.8±5.7</td>
</tr>
<tr>
<td>Systemic hypertension*</td>
<td>81 (78%)</td>
<td>145 (80%)</td>
</tr>
<tr>
<td>Hypercholesterolemia**</td>
<td>81 (78%)</td>
<td>134 (74%)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>17 (16%)</td>
<td>37 (20%)</td>
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<tr>
<td>Previous myocardial infarction</td>
<td>30 (29%)</td>
<td>58 (32%)</td>
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<tr>
<td>Previous coronary intervention</td>
<td>37 (36%)</td>
<td>73 (40%)</td>
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<td>Previous by-pass surgery</td>
<td>8 (8%)</td>
<td>8 (4%)</td>
</tr>
<tr>
<td>Non-ST elevation ACS</td>
<td>56 (54%)</td>
<td>98 (54%)</td>
</tr>
<tr>
<td>Left ventricle ejection fraction (%)</td>
<td>56±8</td>
<td>55±7</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.11 ± 0.3</td>
<td>1.09 ± 0.2</td>
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<tr>
<td>Baseline therapy</td>
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</tr>
<tr>
<td>Insulin</td>
<td>29 (28%)</td>
<td>-</td>
</tr>
<tr>
<td>Clopidogrel (pretreatment)</td>
<td>28 (27%)</td>
<td>43 (24%)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>104 (100%)</td>
<td>181 (100%)</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>31 (30%)</td>
<td>55 (33%)</td>
</tr>
<tr>
<td>Statins</td>
<td>93 (89%)</td>
<td>156 (86%)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>29 (28%)</td>
<td>63 (35%)</td>
</tr>
<tr>
<td>Ace-inhibitors</td>
<td>87 (84%)</td>
<td>138 (76%)</td>
</tr>
</tbody>
</table>

ACS = acute coronary syndrome.
* Defined as systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg or current antihypertensive treatment.
** Defined as total cholesterol >200 mg/dl or statin therapy.
Table 2. Procedural characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diabetes Mellitus</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (N=104) No (N=181)</td>
<td>0.96</td>
</tr>
<tr>
<td>Coronary treated</td>
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<td></td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>56 (54%) 94 (52%)</td>
<td></td>
</tr>
<tr>
<td>Left circumflex</td>
<td>22 (21%) 40 (22%)</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>25 (24%) 46 (25%)</td>
<td></td>
</tr>
<tr>
<td>Saphenous vein graft</td>
<td>1 (1%) 1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Lesion type B2/C</td>
<td>72 (69%) 116 (64%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Multi-vessel intervention</td>
<td>15 (14%) 29 (16%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Type of intervention</td>
<td></td>
<td>0.77</td>
</tr>
<tr>
<td>Balloon only</td>
<td>6 (6%) 12 (7%)</td>
<td></td>
</tr>
<tr>
<td>Stent</td>
<td>98 (94%) 169 (93%)</td>
<td></td>
</tr>
<tr>
<td>No. of stents/patient</td>
<td>1.3 ± 0.8 1.4 ± 0.9</td>
<td>0.33</td>
</tr>
<tr>
<td>Stent diameter (mm)</td>
<td>3.1 ± 0.9 3.0 ± 0.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Total stent length (mm)</td>
<td>16 ± 10 14 ± 10</td>
<td>0.11</td>
</tr>
<tr>
<td>Use of drug eluting stents</td>
<td>39 (38%) 59 (33%)</td>
<td>0.40</td>
</tr>
<tr>
<td>Stent deployment pressure (atm)</td>
<td>13 ± 6 14 ± 6</td>
<td>0.68</td>
</tr>
<tr>
<td>Bailout use of glycoprotein IIb/IIIa inhibitors</td>
<td>7 (7%) 11 (6%)</td>
<td>0.83</td>
</tr>
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</table>
Figure 1. Incidence of high platelet reactivity in patients with and without diabetes mellitus. HPR = high platelet reactivity; DM = diabetes mellitus.

Figure 2. Incidence of peri-procedural myocardial infarction according to the presence of diabetes mellitus. MI = myocardial infarction; DM = diabetes mellitus.
**Figure 3.** Incidence of peri-procedural myocardial infarction according to the presence of high platelet reactivity and diabetes mellitus. MI = myocardial infarction; HPR = high platelet reactivity; DM = diabetes mellitus.

**Figure 4.** Odds ratios for peri-procedural myocardial infarction. Odds ratios presented on log axis. DM = diabetes mellitus; HPR = high platelet reactivity; PCI = percutaneous coronary intervention.
In the present study, we evaluated platelet function using the VerifyNow P2Y12 assay and found that diabetic patients have higher pre-PCI platelet reactivity despite adequate clopidogrel pretreatment, as compared to non-diabetics, in agreement with previous work. However, these data show for the first time that diabetic patients taking clopidogrel and aspirin have significantly higher rates of peri-procedural MI compared to non-diabetics. Furthermore the combination of diabetes and high platelet reactivity despite clopidogrel therapy results in a 4-fold increase in peri-procedural MI, as compared to diabetic patients with normal platelet reactivity.

Detailed studies have found that platelets from diabetics are generally more reactive and less responsive to antiplatelet therapy. Several factors may explain why diabetics more commonly have an impaired response to thienopyridines compared to non-diabetics. These include insulin resistance, poor glycaemic control and increased inflammatory status. Platelets from diabetic patients are poorly responsive to insulin, show an increased response to ADP and have heightened activity on contact with collagen. Moreover, diabetic patients with poor glycaemic control have increased platelet reactivity despite dual antiplatelet therapy. Ang et al. have recently shown that elevated plasma fibrinogen is significantly associated with a lower response to clopidogrel in patients with DM, possibly due to a direct interaction of fibrinogen with the Gp IIb/IIIa receptor. Furthermore, in diabetic patients enhanced production of platelet agonists, such as epinephrine and thrombin receptor agonist peptide (TRAP), may explain the higher levels of platelet activation through different signaling pathways besides those depending on the P2Y12 receptor. Thus, in patients with DM a global hyper-reactive platelet status is present, which may explain low responsiveness even after higher maintenance doses of antiplatelet drugs. In line with previous evidence, the Verify Now P2Y12 assay identified in the present study higher platelet reactivity in DM, with a 1.5-fold increase in occurrence of HPR. Moreover, almost 50% of the patients in the highest quartile of pre-PCI platelet reactivity were diabetic.

Although controversy surrounds the issue as to whether procedure-related myonecrosis is prognostically significant, there is considerable evidence to suggest that even a small increase in CK-MB is associated with increased long-term adverse events, including mortality. In the present study, DM was associated with worse peri-procedural outcomes. Although DM is a known determinant of procedural success and complications in patients undergoing PCI, this is the first evidence of an increased incidence of peri-procedural MI in diabetic patients. However the higher risk of peri-procedural MI in diabetic patients was only observed in patients with the combination of DM and HPR. A unifying hypothesis possibly explaining such a high incidence of peri-procedural MI in diabetics is that in the presence of HPR emboli that shower the microcirculation are more likely to be more sizeable because they consist of both plaque debris and platelet aggregates as opposed to plaque debris alone. This in turn may increase the ischaemic burden within the coronary microcirculation as a result of a higher degree of microcirculatory occlusion. This, in combination with microcirculatory dysfunction, frequently seen in diabetics, may explain the higher rates of peri-procedural myonecrosis in our diabetic population.

Our study suggests that the use of a simple, rapid point-of-care platelet function test may help to identify patients with high platelet reactivity despite
adequate clopidogrel pretreatment. Strikingly these patients experience much higher rates of peri-procedural myocardial injury, particularly when DM is combined with HPR, advocating for the need of a more aggressive antiplatelet regimen in patients with DM undergoing PCI. Greater platelet inhibition may be achieved by tailoring doses of clopidogrel, which in turn may reduce the incidence of events after PCI.\textsuperscript{25} Drugs that enhance the efficacy clopidogrel, such as cilostazol,\textsuperscript{26} or newer thienopyridines, such as prasugrel, might be more beneficial in diabetics. Indeed, a recent subanalysis of the TRITON-TIMI 38, comparing the efficacy of prasugrel to clopidogrel in patients with acute coronary syndrome undergoing PCI showed that only diabetics had a significant net clinical benefit (including ischemic events and major bleeding) at long-term follow-up.\textsuperscript{27} Moreover, the addition of GP IIb/IIIa should possibly be considered in patients that are poorly responsive to clopidogrel in order to reduce ischemic events after PCI.\textsuperscript{28,29}

Our study was not designed to evaluate differences in the incidence of bleeding complications and cardiovascular events, including stent thrombosis, during follow-up. Furthermore we did not explore the potential mechanisms for the association between high platelet reactivity and peri-procedural MI in diabetic patients.
REFERENCES


A therapeutic window for platelet reactivity for patients undergoing elective percutaneous coronary intervention: results of the ARMYDA-PROVE (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity for Outcome Validation Effort) study

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ABSTRACT

Objectives. To validate the ability of the VerifyNow P2Y12 assay in predicting both ischemic and bleeding events after elective percutaneous coronary intervention (PCI).

Background. High and low levels of platelet reactivity are associated with ischemic and bleeding events after PCI, respectively.

Methods. A total of 732 patients on dual antiplatelet therapy undergoing elective PCI were recruited. Platelet reactivity was measured prior to PCI. Primary end point was the 30-day incidence of net adverse clinical events (NACE), defined as the occurrence of ischemic or bleeding events, in relation to PRU distribution.

Results. At ROC curve analysis, PRU values could significantly discriminate between patients with and without bleeding events (area under the curve [AUC]: 0.72; 95% confidence interval [CI], 0.65 to 0.80; p<0.0001) and those with and without ischemic events (AUC 0.68; 95% CI 0.61-0.76; p<0.0001). The optimal cutoffs for bleeding (PRU ≤178) and ischemic events (PRU ≥239) were used to define 3 groups: low platelet reactivity (LPR = PRU ≤178), normal platelet reactivity (NPR = PRU 179 to 238) and high platelet reactivity (HPR = PRU ≥239). The incidence of NACE was 14.1% in the LPR group, 7.8% in the NPR group (p=0.025 vs. LPR group), and 15.4% in the HPR group (p=0.005 vs. NPR group). At multivariate analysis, PRU values in the NPR group were an independent predictor of reduced risk of 30-day NACE (odds ratio 0.47, 95% confidence interval 0.27-0.81).

Conclusions. A therapeutic window for platelet reactivity measured with the VerifyNow P2Y12 assay can be identified using specific thresholds that define a group of patients at lower risk for both ischemic and bleeding events. Adjunctive measures may be beneficial in patients with higher or lower platelet reactivity in order to improve clinical outcomes after PCI.
5.1 INTRODUCTION

Dual antiplatelet therapy with aspirin and clopidogrel is a cornerstone of the pharmacological treatment of patients with coronary artery disease (CAD) undergoing elective percutaneous coronary intervention (PCI). However, a wide inter-individual variability in response to clopidogrel therapy has been described (1-4), implying that a substantial proportion of patients have inadequate platelet inhibition (either too high or too low) at the time of PCI. In patients with a decreased response to clopidogrel, high platelet reactivity (HPR) may result in an increased risk of thrombotic complications after PCI (5-11). On the other hand, in patients with an increased response to clopidogrel, low platelet reactivity (LPR) may result in an increased risk of bleeding complications (12,13).

Various attempts have been made to identify patients with inadequate platelet reactivity through the use of different platelet function tests (14,15). Using the VerifyNow P2Y12 assay, several studies have shown that platelet reactivity above a threshold of ≈240 P2Y12 reaction units (PRU) is associated with an increase in both short- and long-term ischemic adverse events (8,9,11,16,17), while PRU values ≤189 are associated with a higher risk of early major bleeding or entry-site complications after PCI (13). Overall, these data suggest the possibility to identify a therapeutic window for platelet reactivity, measured with the VerifyNow P2Y12 assay, associated with the lowest risk for both thrombotic and bleeding complications.

Therefore, we aimed to validate the ability of the VerifyNow P2Y12 assay in predicting both ischemic and bleeding events after elective PCI in patients on dual antiplatelet therapy.
5.2 METHODS

5.2.1 Patient population and study design

ARMYDA-PROVE (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity for Outcome Validation Effort) is a prospective study enrolling consecutive patients with stable angina and established CAD undergoing elective PCI from April 2010 to February 2011 at Department of Cardiovascular Sciences, Campus Bio-Medico University, Rome, Italy, and at Cardiovascular Center Aalst, Aalst, Belgium. All patients received clopidogrel, either a 600-mg loading dose ≥6 hours before intervention or a maintenance dose of 75 mg/day for at least 5 days. Patients on chronic treatment did not receive any further loading dose of clopidogrel. Technicalities of the procedure, including use of radial approach, drug eluting stents and glycoprotein IIb/IIIa inhibitors, were left to the operator’s discretion. Procedural anticoagulation consisted of unfractionated heparin administrated to achieve an activated clotting time of 250 to 300 sec. Procedural success was defined as a reduction in % diameter stenosis to below 30% in the presence of Thrombolysis In Myocardial Infarction (TIMI) flow grade 3 in the main vessel and all side branches ≥2 mm in diameter. After PCI, patients receiving bare metal stents received clopidogrel 75 mg/day for at least 4 weeks whereas those receiving drug eluting stents received clopidogrel for 12 months. All patients were on aspirin treatment before intervention and continued aspirin (80-100 mg/day) indefinitely. Exclusion criteria were upstream use of glycoprotein IIb/IIIa inhibitors, treatment with oral anticoagulant drugs, platelet count <70x10^9/L, high bleeding risk (active internal bleeding, history of hemorrhagic stroke, intracranial neoplasm, arteriovenous malformation or aneurysm, ischemic stroke in the previous 3 months), coronary artery bypass surgery in the previous 3 months, and severe renal failure (serum creatinine>2 mg/dl). After PCI, patients receiving bare metal stents received clopidogrel 75 mg/day for at least 4 weeks, whereas those receiving drug eluting stents received clopidogrel for at least 12 months. Clinical follow-up data were obtained at 30 days following an office visit, a telephone interview or after chart review. All events were classified and adjudicated by a physician not involved in the follow-up process. This study complied with the Declaration of Helsinki and was approved by the local ethics committees, with all patients giving written informed consent.

5.2.2 Blood sampling and platelet function analysis

Platelet reactivity was measured in the catheterization laboratory using the VerifyNow P2Y12 assay (Accumetrics, San Diego, California) immediately before PCI. Blood was drawn from the femoral or radial artery immediately after sheath insertion. After discarding the first 5 ml of blood, a further sample was collected into a 2-ml tube containing 3.2% sodium citrate. The VerifyNow P2Y12 assay is a validated optical turbidimetric point-of-care assay specifically assessing the effects of P2Y12 receptor blockers (18,19). Results are expressed as P2Y12 reaction units (PRU): the lower the PRU value, the higher the platelet aggregation inhibition, and vice versa. In all cases, the operators were blinded to the results of the platelet function analysis.

Blood samples were drawn before, at 8 and 24 hours after intervention for the assessment of creatine kinase-myocardial band (CK-MB), and thereafter if clinically indicated. Measurements of CK-MB levels were performed by Access 2 Immunochemiluminometric assay (Beckman Coulter, Fullerton, California) and the
upper normal limits was <4 ng/ml.

5.2.3 End points and definitions

Primary end point of this study was the 30-day incidence of net adverse clinical events (NACE), defined as the occurrence of ischemic events or bleeding events, in relation to the PRU distribution. To evaluate the impact of pre-PCI platelet aggregation on the primary outcome measure, we stratified the study population according to their platelet aggregation value using cutoff points derived from receiver-operator characteristic (ROC) curve analysis. Patients with PRU values below the optimal cutoff for bleeding events were classified as having LPR; patients with PRU values above the optimal cutoff for ischemic events were classified as having HPR; the remaining patients with PRU values between the aforementioned cutoff point were classified as having normal platelet reactivity (NPR).

Secondary end points were (1) the occurrence of the single components of the primary end point in relation to the PRU distribution; (2) platelet reactivity according to the occurrence of outcome measures.

Ischemic events were defined as death, myocardial infarction (MI), or target vessel revascularization (TVR). MI included both periprocedural and spontaneous events. Periprocedural MI was defined as a post-procedural increase in CK-MB ≥3 times the 99th percentile of the upper reference limit for patients with baseline negative myocardial necrosis markers, according to the Joint European Society of Cardiology (ESC) / American College of Cardiology Foundation (ACCF) / American Heart Association (AHA) / World Heart Federation (WHF) task force consensus statement on the redefinition of MI for clinical trials on coronary intervention (20). In patients with increased baseline levels of CK-MB, a subsequent increase ≥50% the baseline value fulfilled the criteria for periprocedural MI (21). Occurrence of spontaneous MI, defined as the presence of symptoms compatible with recurrent ischemia associated with electrocardiographic changes indicative of new ischemia (new ST-T changes or new left bundle branch block) (20) was also registered. Definite stent thrombosis (ST) was defined according to the Academic Research Consortium definition (22). TVR was clinically driven and included by-pass surgery or repeat PCI of the target vessel(s). Bleeding events were defined as major bleeding according to the Thrombolysis In Myocardial Infarction criteria (23), or large entry-site hematoma (>10 cm in diameter) (13). Entry-site hematomas were repeatedly monitored throughout the hospitalization and the largest size detected was used for the analysis.

5.2.4 Statistics

In the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) study (9) the 30-day incidence of ischemic events in patients with PRU values ≥240 was 15.0%, while in the ARMYDA-BLEEDS (Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty - Bleeding Study) study (13) the 30-day incidence of bleeding events was 11.6% in patients with pre-PCI PRU values ≤189. Assuming that patients falling in the NPR group represent one third of the entire population, and expecting in these patients a 50% reduction in the occurrence of NACE, compared to patients in the LPR and HPR group, a total of at least 700 patients were needed to detect the expected difference with an estimated power of 80% at a 2-sided alpha of 0.05.

Continuous variables are expressed as mean ± SD or as median [interquartile
range], as appropriate, while categorical variables are reported as frequencies and percentages. Comparisons between continuous variables were performed using the Student t test or Mann-Whitney test. Comparisons between categorical variables were evaluated using the Fisher exact test or the Pearson chi-square test, where appropriate. Normal distribution of PRU levels in the study population was confirmed by Kolmogorov-Smirnov test. A receiver-operating characteristic (ROC) curve analysis was used to test the ability of PRU values to discriminate between patients with and without ischemic events, and with and without bleeding events at 30 days. The optimal cutoff point was calculated by determining the value that provided the greatest sum of sensitivity and specificity. All clinical and procedural features, as well as pre-PCI platelet reactivity groups, were evaluated in a univariate analysis for the association with 30-day NACE using logistic regression. Only variables with p value <0.15 were then entered into the final multivariable logistic regression model providing odds ratios (OR) and 95% confidence intervals (95%CI). Statistical analysis was performed using STATA/IC 10 (STATA Corp., College Station, Texas) and p values <0.05 (two tailed) were considered significant.
5.3 RESULTS

5.3.1 Study population

A total of 732 patients were recruited in the study. The distribution of platelet reactivity is shown in Figure 1. Clinical and procedural features are listed in Tables 1 and 2, respectively. Diabetes mellitus was more frequent among patients with HPR, who also had more frequently multivessel disease. Procedural success was achieved in all patients. In 23 patients a flow-limiting vessel dissection occurred after stent implantation and was successfully treated with the implantation of an additional stent in all cases. A total of 9 patients had no-reflow phenomenon (TIMI flow grade <2 not attributable to dissection, occlusive thrombosis, or epicardial spasm), which significantly improved in all cases after administration of intracoronary nitrates, adenosine, and glycoprotein IIb/IIIa inhibitors. No evident vessel or side branch (≥2 mm) closure occurred. Bailout use of glycoprotein IIb/IIIa inhibitors was low, as well as the use of radial access.

![Figure 1. Distribution of platelet reactivity. PRU = P2Y12 reaction units.](image_url)
Table 1. Clinical characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall population (n=732)</th>
<th>LPR (n=248)</th>
<th>NPR (n=244)</th>
<th>HPR (n=240)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>66±10</td>
<td>66±10</td>
<td>66±9</td>
<td>67±10</td>
<td>0.201</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>536 (73)</td>
<td>176 (71)</td>
<td>178 (73)</td>
<td>182 (76)</td>
<td>0.476</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.9±3.2</td>
<td>25.6±3.2</td>
<td>26.0±3.0</td>
<td>26.1±3.3</td>
<td>0.181</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>216 (30)</td>
<td>55 (22)</td>
<td>70 (29)</td>
<td>91 (38)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>570 (78)</td>
<td>192 (77)</td>
<td>190 (78)</td>
<td>188 (78)</td>
<td>0.971</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>552 (75)</td>
<td>180 (73)</td>
<td>185 (76)</td>
<td>187 (78)</td>
<td>0.386</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>145 (20)</td>
<td>57 (23)</td>
<td>44 (18)</td>
<td>44 (18)</td>
<td>0.303</td>
</tr>
<tr>
<td>Previous myocardial infarction, n (%)</td>
<td>220 (30)</td>
<td>81 (33)</td>
<td>68 (29)</td>
<td>71 (30)</td>
<td>0.501</td>
</tr>
<tr>
<td>Previous coronary intervention, n (%)</td>
<td>244 (33)</td>
<td>84 (34)</td>
<td>90 (37)</td>
<td>70 (29)</td>
<td>0.193</td>
</tr>
<tr>
<td>Previous bypass surgery, n (%)</td>
<td>36 (5)</td>
<td>10 (4)</td>
<td>12 (5)</td>
<td>14 (6)</td>
<td>0.743</td>
</tr>
<tr>
<td>Left ventricle ejection fraction (%)</td>
<td>56±7</td>
<td>56±8</td>
<td>56±7</td>
<td>55±7</td>
<td>0.804</td>
</tr>
<tr>
<td>Left ventricle ejection fraction &lt;40%, n (%)</td>
<td>56 (8)</td>
<td>25 (10)</td>
<td>15 (6)</td>
<td>16 (7)</td>
<td>0.106</td>
</tr>
<tr>
<td>White blood cells, x1000/mm³</td>
<td>8.1±3.5</td>
<td>8.3±3.5</td>
<td>7.9±3.6</td>
<td>8.2±3.1</td>
<td>0.407</td>
</tr>
<tr>
<td>C reactive protein, mg/l</td>
<td>2.5</td>
<td>2.3</td>
<td>2.5</td>
<td>2.5 [1.3-6]</td>
<td>0.217</td>
</tr>
<tr>
<td>[1.2–7.1]</td>
<td>[1.0-5.9]</td>
<td>[1.1-6.8]</td>
<td>[7.3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>170±49</td>
<td>169±52</td>
<td>175±57</td>
<td>168±47</td>
<td>0.315</td>
</tr>
<tr>
<td>Low density lipoprotein cholesterol, mg/dl</td>
<td>97±31</td>
<td>97±29</td>
<td>95±32</td>
<td>101±33</td>
<td>0.103</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>1.08±0.27</td>
<td>1.08±0.26</td>
<td>1.08±0.24</td>
<td>1.10±0.29</td>
<td>0.530</td>
</tr>
<tr>
<td>Chronic renal failure, n (%)</td>
<td>99 (14)</td>
<td>27 (11)</td>
<td>37 (15)</td>
<td>35 (15)</td>
<td>0.321</td>
</tr>
<tr>
<td>Multivessel disease, n (%)</td>
<td>312 (43)</td>
<td>93 (38)</td>
<td>102 (42)</td>
<td>117 (49)</td>
<td>0.041</td>
</tr>
<tr>
<td>Clopidogrel regimen, (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.922</td>
</tr>
<tr>
<td>Loading dose (600 mg)</td>
<td>644 (88)</td>
<td>219 (88)</td>
<td>213 (87)</td>
<td>212 (88)</td>
<td></td>
</tr>
<tr>
<td>Maintenance dose (75 mg &gt;5 days)</td>
<td>88 (12)</td>
<td>29 (12)</td>
<td>31 (13)</td>
<td>28 (12)</td>
<td></td>
</tr>
<tr>
<td>Baseline medications, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>732 (100)</td>
<td>248 (100)</td>
<td>244 (100)</td>
<td>240 (100)</td>
<td>1.000</td>
</tr>
<tr>
<td>Statin</td>
<td>651 (89)</td>
<td>218 (89)</td>
<td>215 (88)</td>
<td>218 (91)</td>
<td>0.518</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>168 (23)</td>
<td>53 (21)</td>
<td>58 (24)</td>
<td>57 (24)</td>
<td>0.767</td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>249 (34)</td>
<td>87 (35)</td>
<td>84 (34)</td>
<td>78 (33)</td>
<td>0.823</td>
</tr>
<tr>
<td>PRU</td>
<td>206±72</td>
<td>127±35</td>
<td>206±17</td>
<td>287±38</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Continues variables are expressed as mean±SD, or median [interquartile range]. LPR = low platelet reactivity; NPR = normal platelet reactivity; HPR = high platelet reactivity; PRU = P2Y12 reaction units.
Table 2. Procedural characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall population (n=732)</th>
<th>LPR (n=248)</th>
<th>NPR (n=244)</th>
<th>HPR (n=240)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated vessel, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left main</td>
<td>3 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0.189</td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>407 (55)</td>
<td>123 (50)</td>
<td>143 (58)</td>
<td>141 (59)</td>
<td></td>
</tr>
<tr>
<td>Left circumflex</td>
<td>136 (18)</td>
<td>56 (22)</td>
<td>45 (18)</td>
<td>35 (14)</td>
<td></td>
</tr>
<tr>
<td>Right coronary artery</td>
<td>182 (25)</td>
<td>67 (26)</td>
<td>55 (22)</td>
<td>60 (25)</td>
<td></td>
</tr>
<tr>
<td>Saphenous vein graft</td>
<td>3 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Lesion type B2/C, n (%)</td>
<td>425 (58)</td>
<td>146 (59)</td>
<td>137 (56)</td>
<td>142 (59)</td>
<td>0.758</td>
</tr>
<tr>
<td>Multivessel intervention, n (%)</td>
<td>106 (14)</td>
<td>30 (12)</td>
<td>33 (14)</td>
<td>43 (18)</td>
<td>0.165</td>
</tr>
<tr>
<td>Use of stent, n (%)</td>
<td>706 (96)</td>
<td>239 (96)</td>
<td>234 (96)</td>
<td>233 (97)</td>
<td>0.779</td>
</tr>
<tr>
<td>Use of drug eluting stent, n (%)</td>
<td>201 (27)</td>
<td>78 (31)</td>
<td>67 (27)</td>
<td>56 (23)</td>
<td>0.133</td>
</tr>
<tr>
<td>Stents implanted, n</td>
<td>1.41±0.86</td>
<td>1.34±0.83</td>
<td>1.42±0.80</td>
<td>1.47±0.98</td>
<td>0.252</td>
</tr>
<tr>
<td>Maximal inflation pressure, atm</td>
<td>15±2</td>
<td>15±2</td>
<td>15±3</td>
<td>15±2</td>
<td>0.893</td>
</tr>
<tr>
<td>Side branch occlusion, n (%)</td>
<td>11 (2)</td>
<td>3 (1)</td>
<td>4 (2)</td>
<td>4 (2)</td>
<td>0.897</td>
</tr>
<tr>
<td>Radial access, n (%)</td>
<td>29 (4)</td>
<td>12 (5)</td>
<td>8 (3)</td>
<td>9 (4)</td>
<td>0.661</td>
</tr>
<tr>
<td>Sheath size, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.799</td>
</tr>
<tr>
<td>6 French</td>
<td>681 (93)</td>
<td>231 (93)</td>
<td>225 (92)</td>
<td>225 (94)</td>
<td></td>
</tr>
<tr>
<td>7 French</td>
<td>51 (7)</td>
<td>17 (7)</td>
<td>19 (8)</td>
<td>15 (6)</td>
<td></td>
</tr>
<tr>
<td>Bailout use of glycoprotein IIb/IIIa inhibitors, n (%)</td>
<td>44 (6)</td>
<td>19 (7)</td>
<td>11 (5)</td>
<td>14 (6)</td>
<td>0.335</td>
</tr>
</tbody>
</table>

Continuous variables are expressed as mean±SD, or median [interquartile range]. LPR = low platelet reactivity; NPR = normal platelet reactivity; HPR = high platelet reactivity; PRU = P2Y12 reaction units.

5.3.2 Primary end point

No patients were lost at 30-day follow-up. A total of 57 (7.8%) ischemic events and 36 (4.8%) bleeding events occurred. Ischemic events included 3 deaths, 51 MI (45 periopercular and 6 spontaneous, of which 4 due to ST), and 6 TVR. The sources of major bleeding were genitourinary in 4 patients, gastrointestinal in 3 patients, and cerebral in 1 patient. Twenty-seven patients had entry site hematoma >10 cm. In 2 patients both an ischemic (MI) and a bleeding event (entry site hematoma >10 cm) occurred. A total of 91 (12.3%) patients experienced at least one NACE.

ROC curve analysis demonstrated that pre-PCI PRU values could significantly
discriminate between patients with and without ischemic events (area under the curve [AUC]: 0.68; 95% confidence interval [CI], 0.61 to 0.76; p<0.0001). A PRU value ≥239 was the optimal cutoff point to predict ischemic events with a sensitivity of 63%, specificity of 70%, a negative predictive value (NPV) of 96% and a positive predictive value (PPV) of 15% (Figure 2, Panel A). Similarly, pre-PCI PRU values could significantly discriminate between patients with and without bleeding events (AUC 0.72; 95% CI 0.65-0.80; p<0.0001). A PRU value ≤178 was the optimal cutoff point to predict bleeding events with a sensitivity of 78%, specificity of 63%, a negative predictive value (NPV) of 98% and a positive predictive value (PPV) of 10% (Figure 2, Panel B). Based on these results, we divided our study population in 3 groups based on the distribution of PRU values: LPR (PRU ≤178; n=248 [33.9%]), NPR (PRU between 179 and 238; n=244 [33.3%]) and HPR (PRU ≥239; n=240 [32.8%]).

The incidence of NACE was 14.1% in the LPR group, 7.8% in the NPR group (p=0.025 vs. LPR group), and 15.4% in the HPR group (p=0.005 vs. NPR group) (Figure 3). Considering patients with LPR and HPR together, the incidence of NACE was 14.8% (p=0.007 vs. NPR group). PRU values in the LPR or HPR group could predict the occurrence of NACE with a sensitivity of 79%, a specificity of 35%, a NPV of 92%, a PPV of 15%, and an overall prognostic accuracy of 41%.

![Ischemic events](image1)

![Bleeding events](image2)
Figure 2. ROC curves for PRU Values. (A) Receiver-operator characteristic (ROC) analysis for ischemic events. (B) ROC analysis for bleeding events. PRU values are in the opposite direction for the two curves. AUC = area under the curve; NPV = negative predictive value; PPV = positive predictive value; PRU = P2Y12 reactivity unit.

Figure 3. Kaplan-Meier curves for 30-day NACE-free survival. NACE = net adverse clinical event; LPR = low platelet reactivity; NPR = normal platelet reactivity; HPR = high platelet reactivity.

5.3.3 Secondary end points

Patients in the LPR, NPR and HPR groups presented an incidence of ischemic events of 4.0%, 4.9% and 15.0%, respectively (p for trend <0.0001; Figure 4, Panel A), and an incidence of bleeding events of 10.5%, 2.9% and 1.3%, respectively (p for trend <0.0001; Figure 4, Panel B). The incidence of the single components of the primary end point according to PRU groups is reported in Figure 4. The incidence of death was overall low and similar in the three study groups (0.4%). Patients in the HPR group showed the highest incidence of MI (p for trend <0.001) and TVR (p for trend = 0.041). Periprocedural MI occurred in 3.2%, 4.5% and 13.3% of patients with LPR, NPR and HPR, respectively (p for trend <0.001). Spontaneous MI occurred in 0.4%, 0.0% and 2.1% of patients with LPR, NPR and HPR, respectively (p for trend = 0.041). ST occurred in 4 patients (1.7%) in the HPR group and in none of LPR or NPR groups (p for trend = 0.013). Patients in the LPR group showed the highest incidence of major bleeding (p for trend = 0.003) and hematoma >10 cm (p for trend <0.001).

The average pre-PCI platelet reactivity was 207±73 PRU in the overall population, and 206±72 in patients without adverse events at 30-day follow-up. Patients experiencing bleeding events had the lowest PRU values (154±59, p<0.0001 vs. patients without events), while patients experiencing ischemic events had the highest PRU values (247±67, p<0.0001 vs. patients without events) (Figure 5).
Figure 4. Single components of the primary end point and platelet reactivity. Ischemic events (A) and bleeding events (B) according to P2Y12 reaction unit groups. MI = myocardial infarction; ACS = acute coronary syndrome; ST = stent thrombosis; TVR = target vessel revascularization; LPR = low platelet reactivity; NPR = normal platelet reactivity; HPR = high platelet reactivity.

Figure 5. Platelet reactivity and adverse events. PRU = P2Y12 reaction units. Boxes extend from the 25th to the 75th percentile, with a line at the 50th percentile (median). Whiskers show the highest and the lowest value.
5.3.4 Multivariate analysis

At univariate analysis, diabetes mellitus, multivessel disease, chronic renal failure, total stent length, use of IIb/IIIa glycoprotein inhibitors and PRU values in the NPR range were significantly associated with the occurrence of NACE. Multivariate analysis (Table 3) showed that PRU values in the NPR range were an independent predictor of decreased risk of NACE (OR 0.47, 95%CI 0.27-0.81).

Table 3. Univariate and multivariate predictors of net adverse clinical events at 30 days.

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th></th>
<th></th>
<th>Multivariate</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>OR [95%CI]</td>
<td>p value</td>
<td>OR [95%CI]</td>
<td>p value</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal platelet reactivity</td>
<td>0.49 [0.29-0.83]</td>
<td>0.008</td>
<td>0.47 [0.27-0.81]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.52 [0.91-1.98]</td>
<td>0.072</td>
<td>1.32 [0.83-2.10]</td>
<td>0.218</td>
<td></td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>1.39 [0.89-2.18]</td>
<td>0.148</td>
<td>1.20 [0.73-1.98]</td>
<td>0.332</td>
<td></td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>1.68 [1.02-3.36]</td>
<td>0.048</td>
<td>1.46 [0.80-2.67]</td>
<td>0.297</td>
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</tr>
<tr>
<td>Total stent length</td>
<td>1.02 [1.01-1.04]</td>
<td>0.004</td>
<td>1.50 [0.91-2.47]</td>
<td>0.181</td>
<td></td>
</tr>
<tr>
<td>Glycoprotein IIb/IIIa inhibitors</td>
<td>2.20 [1.05-4.63]</td>
<td>0.037</td>
<td>2.12 [0.99-4.54]</td>
<td>0.085</td>
<td></td>
</tr>
</tbody>
</table>

OR = odds ratio; 95%CI = 95% confidence interval.
5.4 DISCUSSION

In the present study we have identified a therapeutic window for platelet reactivity, as measured by the VerifyNow P2Y12 assay, in patients with stable angina undergoing elective PCI. Those patients with PRU values within this therapeutic window accounted for one third of our patients and had the lowest risk for the combined end point of ischemic and bleeding complications, 30 days after elective PCI. Of note, PRU values between 179 and 238 resulted in almost a 50% risk reduction for net adverse clinical events compared to patients with either low or high platelet reactivity.

Given the wide inter-individual variability in response to clopidogrel (4), platelet reactivity is highly heterogeneous in the overall population of patients on dual antiplatelet therapy, and follows a normal Gaussian distribution (Figure 1). Therefore, it ranges from low levels on one extreme of its bell-shaped distribution to high levels on the other extreme. Several studies have shown that patients who have high platelet reactivity prior to PCI are at an increased risk of ischemic events despite taking dual antiplatelet therapy (5-11). Several attempts have been made to identify optimal thresholds of platelet reactivity in order to stratify those patients at risk of ischemic events following PCI (14,15). Amongst the many commercially available platelet function tests, the VerifyNow P2Y12 assay has proven to be particularly useful. Not only is it an effective tool for identifying high platelet reactivity in patients taking dual antiplatelet therapy using a threshold of \( \approx 240 \) PRU, but knowing this information helps to predict which patients will experience an ischemic event (8,9,11,16,17,24). Consistent with these studies, we also found a very similar optimal cutoff value to predict ischemic events in our population of patients undergoing elective PCI with a PRU value of \( \geq 239 \). Our data also corroborate the hypothesis that a threshold effect exists for ischemic events (15,25). In fact, the incidence of ischemic events was similar in patients with low and normal platelet reactivity, suggesting that below the safety threshold of PRU ischemic events are not further significantly reduced.

In addition to that of recurrent ischemic events, the prognostic importance of bleeding complications following PCI has also been established. Ndrepepa et al. (26) have shown that patients with bleeding events within 30 days after PCI have a 3-fold higher risk for 1-year mortality compared to patients without bleeding. Moreover, in a study of 6,995 patients undergoing PCI, periprocedural bleeding complications were significantly associated with increased risk for mortality and adverse cardiac events at follow-up (27). Recently, two studies have sought to define thresholds of platelet reactivity to identify patients at higher risk for bleeding events after PCI. In one of these studies enrolling patients undergoing PCI after a 600-mg clopidogrel loading, increased platelet inhibition (<188 aggregation units) measured with multiple electrode aggregometry, resulted in a 3.5-fold higher in-hospital incidence of major bleeding (12). Similarly, the ARMYDA-BLEEDS study (13) showed that low residual platelet reactivity after clopidogrel, as measured with the VerifyNow P2Y12 assay prior to PCI, is associated with a significantly higher incidence of 30-day major bleeding or entry-site complications, also suggesting a threshold of PRU \( \leq 189 \) as the optimal cutoff to predict bleeding events. In the present study, we confirm the predictive value of platelet reactivity, as measured with the VerifyNow P2Y12 assay, on the occurrence of bleeding events after PCI, and we found that the optimal cutoff to predict such events was a PRU value \( \leq 178 \). Similar to ischemic events, a threshold effect seems to be present for bleeding, as no significant difference in the incidence of bleeding events was found between patients with normal and high platelet reactivity.
Using the two thresholds for ischemic and bleeding event, we have found a therapeutic window for platelet reactivity, ranging from 179 to 238 PRU, which was associated with the lowest incidence of net adverse events. Despite being relatively small, this range accounts for one third of the overall population, while the remainder of patients was equally distributed between the low and high platelet reactivity group (Figure 1). While the optimal cutoff for ischemic events (PRU >239) was very close to the one described in ARMYDA-PRO (9) and other studies (8,10,11), we found that the optimal cutoff to predict bleeding (PRU ≤178) was lower than the one previously described in ARMYDA-BLEEDS (PRU ≤189) (13). As expected, the positive predictive value for both the ischemic and bleeding thresholds was very low (15% and 10%, respectively). As predictive values of a test depend on the prevalence of the tested condition, this could be partly explained with the relatively low occurrence of adverse events in a low-risk population of stable patients undergoing elective PCI.

Sibbing et al. (28) have also found a therapeutic window for pre-PCI platelet reactivity, as measured with multiple electrode aggregometry, identifying the group of patients with aggregation values in the range of 189 to 467 aggregation units as having the lowest risk for the occurrence of both bleeding and ischemic events. However, in this analysis only in-hospital bleeding and 30-day ST were considered. Recently, Campo et al. (25) have also found a therapeutic window for platelet reactivity measured with the VerifyNow P2Y12 assay (between 86 and 238 PRU). However, in this study platelet reactivity was measured 30 days after PCI, and the adverse events occurred within the first month were excluded from the analysis. Moreover, a different definition for bleeding events, including both TIMI and BleedScore classifications, was used compared to the present study.

According to our results, pre-PCI evaluation of platelet reactivity carries important prognostic information, and may guide the therapeutic approach to those patients that do not fall within the described therapeutic window. In particular, in patients with a low response to clopidogrel and higher ischemic risk, more aggressive antiplatelet strategies might be useful in obtaining platelet reactivity values that fall within the desired range. These include higher clopidogrel doses, the use of inducers of clopidogrel metabolism (i.e. cilostazol), or newer, more potent P2Y12 receptor blockers. Although one recent study that did tailor antiplatelet therapy by doubling the clopidogrel dose in patients with low response using VerifyNow P2Y12 results did not show any significant benefit (29), further studies are necessary to clarify this issue (30). On the other hand, in patients with increased response to clopidogrel and higher bleeding risk, besides deferring PCI until platelet reactivity falls within the desired range, individualized preventive measures could also be indicated (i.e., restricted use of glycoprotein IIb/IIIa inhibitors and drug eluting stents, more extensive use of the radial approach, bivalirudin, and gastroprotective agents).

By study design, we aimed at identifying a group of patients with intermediate platelet reactivity presenting the lowest incidence of adverse clinical events among our total population of patients undergoing PCI. This approach implies that the set of patients used to test the hypothesis corresponds to the validation set. The absence of an independent validation set represents a limitation of this study.

In conclusion, this study using the VerifyNow P2Y12 assay identified a therapeutic window for platelet reactivity that defines a group of patients at lower risk for both ischemic and bleeding events. Adjunctive measures may be beneficial in patients with higher or lower platelet reactivity in order to improve clinical outcomes after PCI. Larger studies, possibly with longer follow-up, are warranted to test this hypothesis.
REFERENCES


High residual platelet reactivity after clopidogrel: extent of coronary atherosclerosis and peri-procedural myocardial infarction in patients with stable angina undergoing percutaneous coronary intervention

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ABSTRACT

Objectives. We tested the hypothesis that residual platelet reactivity after clopidogrel correlates with the extent and severity of coronary atherosclerosis in patients undergoing elective percutaneous coronary intervention (PCI).

Background. Platelets are actively involved in vascular atherosclerosis.

Methods. We prospectively enrolled 338 patients undergoing PCI for stable angina, loaded with 600 mg clopidogrel. Platelet reactivity was assessed 12 hours later by measuring platelet reactivity unit (PRU) with VerifyNow P2Y12 assay. High platelet reactivity (HPR) was defined as PRU value ≥ 240. Presence of multi-vessel disease (MVD) and total stent length (TSL) were used as surrogate markers of atherosclerosis severity and extension.

Results. Patients with MVD showed higher PRU compared to single vessel disease (SVD) patients (222±85 vs. 191±73; p<0.001). PRU increased with the number of stenotic coronaries (1-VD: 191±73; 2-VD: 220±88; 3-VD: 226±80; p=0.002). PRU was higher in the 3rd tertile compared with 1st tertile TSL (217±83 vs. 191±73; p=0.015). HPR was most frequently observed among MVD patients (40.5% vs. 21.6% in patients with SVD, respectively; p<0.001) and those in 3rd tertile TSL (35.8% vs. 22.2% 1st tertile; p=0.028). Higher incidence of peri-procedural myocardial infarction was observed in patients with HPR (41.2% vs. 26.7% in patients without HPR; p=0.008) and in those in the 3rd tertile TSL (37.7% vs. 23.1% 1st tertile; p=0.020). By multivariate analysis, HPR was the only independent predictor of peri-procedural myocardial infarction (p=0.034).

Conclusions. Platelet reactivity after clopidogrel significantly correlates with the extent of coronary atherosclerosis and is a strong predictor of peri-procedural myocardial infarction.
6.1 INTRODUCTION

Platelets are actively involved in the inflammatory cascade leading to vascular atherosclerosis (1,2). Platelet aggregability has been directly related with systemic atherosclerotic disease (3). In addition, the most detrimental manifestations of coronary atherosclerotic disease, e.g. myocardial infarction and acute coronary syndromes, are mediated by platelet activation (4). On this basis, numerous therapeutic options, targeting platelet aggregability, have been proposed.

Dual therapy with aspirin and clopidogrel is the most commonly used anti-platelet strategy in patients undergoing percutaneous coronary intervention (PCI) (5). In these patients a residual high platelet reactivity (HPR) after clopidogrel has been associated with increased cardiovascular events both peri-procedural (6) and at long-term follow-up (7,8). Several mechanisms have been described for the suboptimal platelet response to clopidogrel including genetic, cellular and clinical factors (9). In addition, baseline platelet reactivity is a strong predictor of platelet response to clopidogrel (10). Provided higher baseline platelet reactivity in patients with more diffuse vascular atherosclerosis, the potential relationship between residual platelet reactivity after clopidogrel and extent and severity of coronary atherosclerosis has not yet been investigated.

In the present study, we tested the hypothesis that residual platelet reactivity after clopidogrel correlates with the extent and severity of coronary atherosclerosis in stable angina patients at the occasion of PCI. In addition, we evaluated whether higher residual platelet reactivity after clopidogrel might partly account for an unfavorable peri-procedural outcome in patients with more extensive coronary atherosclerosis.
6.2 METHODS

6.2.1 Patient population and study protocol

We prospectively enrolled 338 patients undergoing PCI for stable angina or a positive functional test and presence of an angiographic significant stenosis (diameter stenosis >50%) in at least one native coronary artery. Patients were excluded in the presence of an acute coronary syndrome in the previous month, elevated myocardial necrosis markers before the procedure, thrombocytopenia (platelet count <100,000/l), left ventricular ejection fraction <30%, high bleeding risk, allergy to thienopyridines, PCI for chronic total occlusions, and lesions with extensive calcifications requiring rotational atherectomy. All patients received standardized anti-platelet therapy with clopidogrel 600 mg and aspirin 500 mg loading doses at least 12 hours before PCI, irrespectively of the ongoing antiplatelet therapy. Of note, there was no significant difference in chronic thienopyridine therapy among patients with single and multivessel disease (24% vs. 26%, p=0.617). Local ethical committee approved the study and signed written informed consent was obtained from all the included patients.

Technicalities of the procedure, including use of drug eluting stents and glycoprotein IIb/IIIa inhibitors, were left to the operator’s discretion. Heparin was administrated to achieve an activated clotting time (ACT) of 250-300 seconds. Procedural success was defined as a reduction of stenosis to <30% residual narrowing.

Platelet reactivity after clopidogrel was assessed in the catheterization laboratory by the VerifyNow P2Y12 assay immediately before PCI (and where appropriate, before the administration of glycoprotein IIbIIIa inhibitors). Blood was collected from the femoral artery immediately after sheath placement. The first 5-ml of blood were discarded, then samples were collected in 2-mL tubes containing 3.2% sodium citrate. VerifyNow P2Y12 is a validated point-of-care assay specifically assessing clopidogrel effects on P2Y12 receptor by optical turbidometry.(11, 12) Specific cartridges contain 20 µmol ADP, which activates platelets by binding P2Y1 and P2Y12 receptors, and 22 nmol prostaglandin E1 (PGE-1), which increases assay specificity by suppressing P2Y1-induced intra-platelet signaling. Activated platelets agglutinate around fibrinogen-coated polystyrene beads, therefore increasing light transmittance through the sample. Results are reported as P2Y12 reaction units (PRU); the lower the PRU value, the higher the platelet aggregation inhibition by clopidogrel.

6.2.2 End-points

Primary end-point of the study was the correlation between extent of atherosclerotic coronary artery disease and residual platelet reactivity after clopidogrel. Presence of multi-vessel disease (defined as coronary artery stenosis > 50%, as assessed by quantitative coronary angiography, in at least two major epicardial coronary arteries) and total length of the implanted stents were considered as surrogate markers of atherosclerosis extension and severity, as previously described (13).

Secondary end-point was to assess whether HPR, as defined by a PRU value ≥240, in the context of a more extensive atherosclerosis, might partly account for the peri-procedural myocardial infarction. Peri-procedural myocardial infarction was defined as a post-procedural Troponin-T (Tn-T) increase more than three times the 99th percentile of the upper reference limit (14).
6.2.3 Statistics

Statistical analysis was performed using SPSS 15.0 software (SPSS Inc., Chicago, Illinois). Continuous variables are expressed as mean ± SD. Categorical variables are reported as frequencies and percentages. Normality of PRU distribution among the whole population was confirmed by Kolmogorov-Smirnov test. Student’s t test was used to compare continuous variables. PRU values within total stent length (TSL) tertiles and patients with 1-, 2- or 3-vessel disease were compared using one-way ANOVA. Comparisons between categorical variables were evaluated using two-tailed Fisher’s exact test or Pearson’s χ² test, as appropriate. All clinical and procedural variables that showed a significant univariate association to peri-procedural MI (p<0.05) were entered in a multivariable logistic regression model. A receiver-operating characteristic (ROC) curve analysis was used to test the ability of TSL to discriminate between patients with and without HPR. The optimal cutoff point was calculated by determining the TLS value that provided the greatest sum of sensitivity and specificity. Statistically significance was defined as a p value <0.05.
6.3 RESULTS

A total of 338 patients were enrolled in the study, 185 (55%) with single-vessel disease (SVD) and 153 with multi-vessel disease (MVD). Among patients with MVD, 97 (63%) presented 2-vessel disease and 56 (37%) had 3-vessel disease. Main clinical and procedural features are shown in Table 1. Left ventricle ejection fraction (LVEF) was lower and previous myocardial infarction more common in patients with MVD. C reactive protein (CRP) was higher in patients with MVD as compared with patients with SVD. No significant differences between the 2 groups were observed with respect to other risk factors and ongoing medical therapy.

Procedural characteristics were comparable among the 2 groups, except for a higher number of stents implanted and a longer total stent length (TLS) in the MVD group. In the latter group 59 (39%) patients underwent multi-vessel PCI.

Table 1. Clinical and Procedural Features

<table>
<thead>
<tr>
<th></th>
<th>Overall group (n=338)</th>
<th>SVD (n=185)</th>
<th>MVD (n=153)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Age, years</td>
<td>67±10</td>
<td>66±10</td>
<td>68±10</td>
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<tr>
<td>Male gender, n (%)</td>
<td>274 (81)</td>
<td>147 (79)</td>
<td>127 (83)</td>
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<td>Diabetes, n (%)</td>
<td>124 (37)</td>
<td>63 (34)</td>
<td>61 (40)</td>
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<td>Smoking, n (%)</td>
<td>65 (19)</td>
<td>34 (18)</td>
<td>31 (20)</td>
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<td>Hypertension, n (%)</td>
<td>242 (72)</td>
<td>132 (71)</td>
<td>110 (72)</td>
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<td>Dyslipidemia, n (%)</td>
<td>250 (74)</td>
<td>137 (74)</td>
<td>113 (74)</td>
<td>0.967</td>
</tr>
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<td>Body mass index, kg/m²</td>
<td>28.0±4.1</td>
<td>28.3±4.1</td>
<td>27.7±4.0</td>
<td>0.366</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>60±13</td>
<td>61±13</td>
<td>57±12</td>
<td>0.004</td>
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<td>Previous MI, n (%)</td>
<td>85 (25)</td>
<td>34 (18)</td>
<td>51 (33)</td>
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<td>Apirin, n (%)</td>
<td>338 (100)</td>
<td>185 (100)</td>
<td>153 (100)</td>
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<td>Beta-blockers, n (%)</td>
<td>109 (32)</td>
<td>54 (29)</td>
<td>55 (36)</td>
<td>0.186</td>
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<td>ACE-inhibitors/ARB, n (%)</td>
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<td>143 (77)</td>
<td>126 (82)</td>
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<td>Statins, n (%)</td>
<td>251 (74)</td>
<td>134 (72)</td>
<td>117 (76)</td>
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<td>Proton Pump Inhibitors, n (%)</td>
<td>88 (26)</td>
<td>50 (27)</td>
<td>38 (25)</td>
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<td>CRP, mg/dl</td>
<td>0.61±0.69</td>
<td>0.47±0.49</td>
<td>0.75±0.83</td>
<td>0.003</td>
</tr>
<tr>
<td>LAD</td>
<td>192 (48)</td>
<td>88 (48)</td>
<td>104 (48)</td>
<td>0.872</td>
</tr>
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<td>LCx</td>
<td>91 (23)</td>
<td>46 (25)</td>
<td>45 (21)</td>
<td>0.349</td>
</tr>
<tr>
<td>RCA</td>
<td>117 (29)</td>
<td>51 (28)</td>
<td>66 (31)</td>
<td>0.493</td>
</tr>
<tr>
<td>B2/C lesions, n (%)</td>
<td>178 (53)</td>
<td>92 (50)</td>
<td>86 (56)</td>
<td>0.235</td>
</tr>
<tr>
<td>Multivessel PCI, n (%)</td>
<td>59 (18)</td>
<td>-</td>
<td>59 (39)</td>
<td>-</td>
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</tbody>
</table>
Use of drug eluting stent, n (%)  144 (43)  82 (44)  62 (41)  0.482
Direct stenting, n (%)  125 (37)  67 (36)  58 (37)  0.748
Stents implanted per patient, n  1.62±0.86  1.49±0.84  1.79±0.85  0.002
Total stent length, mm (range)  28±17 (8-117)  26±17 (8-112)  32±17 (9-117)  0.002
Glycoprotein IIb/IIIa inhibitors, n (%)  3 (1)  1 (1)  3 (2)  0.229

LVEF= left ventricle ejection fraction; ACE= angiotensin converting enzyme; ARB= angiotensin receptor blockers; CRP= C reactive protein; LAD= left anterior descending; LCx= left circumflex; RCA= right coronary artery; PCI= percutaneous coronary intervention.

6.3.1 PRU and extent of atherosclerosis
Mean PRU value in the overall population was 205±80. Patients with MVD showed significantly higher mean value of PRU compared with subjects with SVD (222±85 vs. 191±73; p<0.001). PRU values progressively increased with number of diseased coronary arteries (1-vessel disease: 191±73; 2-vessel disease: 220±88; 3-vessel disease: 226±80; p=0.002; Fig. 1A). In addition, a significantly higher PRU was also detected in patients in the 3rd tertile of total stent length compared to those in the 1st tertile (217±83 vs. 191±73; p=0.015; Fig. 1B).

![Figure 1. Correlation between extent coronary atherosclerosis and platelet reactivity after clopidogrel.](image)

6.3.2 High platelet reactivity (HPR) and extent of atherosclerosis
Incidence of residual HPR in the entire population was 30% and it was most frequently observed among patients with MVD (40.5% vs 21.6% in patients with SVD, respectively; OR 2.47, 95% CI 1.53-3.98, p<0.001). Patients with 3-vessel disease presented the highest incidence of HPR (42.9% in 3-vessel disease, 39.2% in 2-vessel disease, and 21.6% in 1-vessel disease, respectively; p for trend<0.001; Fig. 2A). Likewise, individuals in the 3rd tertile of total stent length had significantly
higher incidence of HPR compared to those in the 1st tertile (35.8% vs. 22.2%, respectively; OR 1.96, 95% CI 1.07-3.57, p=0.028; Fig. 2B). Receiver operating characteristics (ROC) curve analysis showed that total stent length values could significantly discriminate between patients with and without HPR (area under the curve 0.59, 95% CI 0.52-0.66, p=0.010). A value ≥27 mm of total length of stents implanted was identified as the optimal cut-off to predict HPR (sensitivity=58%, specificity=61%, positive predictive value=39%, negative predictive value: 77%).

Figure 2 – Correlation between extent of coronary atherosclerosis and high residual platelet reactivity after clopidogrel. Incidence of high platelet reactivity (HPR, %) according to the number of diseased vessels (A) and to the tertiles of total stent length (TSL) (B).

6.3.3 High platelet reactivity (HPR) and peri-procedural myocardial infarction

Peri-procedural MI occurred in 105 patients (31% of the overall population). Patients with HPR experimented more frequently peri-procedural MI (41.2 vs. 26.7 in subjects without HPR, respectively; OR 1.92, 95% CI 1.18-3.13, p=0.008; Fig. 3A). Furthermore, peri-procedural MI was significantly more frequent in patients in the 3rd tertile TLS compared to those in the 1st tertile (37.7% vs. 23.1%, respectively; OR 2.01, 95% CI 1.11-3.65, p=0.020). By univariate analysis, presence of diabetes, HPR, MVD and TLS values in the 3rd tertile versus 1st tertile showed a significant correlation with peri-procedural MI. By the multivariate analysis (including only MVD as a marker of coronary atherosclerosis extent), HPR was the only independent predictor of peri-procedural MI (p=0.043; Table 2).
Figure 3 – Peri-procedural myocardial infarction. Incidence of peri-procedural myocardial infarction (MI) according to presence of high platelet reactivity (HPR) (A) and to the tertiles of total stent length (TSL) (B).

Table 2. Multivariate analysis: predictors of peri-procedural myocardial infarction.

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPR</td>
<td>1.684</td>
<td>1.016-2.789</td>
<td>0.043</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.525</td>
<td>0.943-2.466</td>
<td>0.085</td>
</tr>
<tr>
<td>MVD</td>
<td>1.507</td>
<td>0.934-2.432</td>
<td>0.093</td>
</tr>
</tbody>
</table>

HPR= high platelet reactivity; MVD= multi-vessel disease.
6.4 DISCUSSION

This prospective study conducted in patients with stable angina at the occasion of PCI showed a significant correlation between the extent of coronary atherosclerosis and residual platelet reactivity after clopidogrel administration. In addition, HPR was the strongest predictor of peri-procedural MI and was more common in patients with extensive coronary atherosclerosis.

6.4.1 Role of platelets in vascular atherosclerosis

Platelets are actively involved in vascular atherosclerosis (1,2,15) Even in the absence of endothelial damage, platelets adhere to vascular wall and promote the initial phases of atherosclerotic plaque formation (16). Activated platelets also participate to progression of atherosclerosis, by interacting with inflammatory and endothelial cells (17). Human studies have demonstrated an association between platelet activation and clinical manifestations of vascular atherosclerotic disease. In particular, platelet activation is increased in patients with peripheral artery disease compared with healthy controls (18). In addition, patients with subcritical limb ischemia showed even higher platelet reactivity as compared with those presenting with claudicatio intermittens (19). Platelet activation significantly correlated with morphological and quantitative parameters of atherosclerotic lesions in human carotid artery (20).

Patients with stable coronary artery disease have increased platelet reactivity and circulating monocyte-platelet aggregates (21), which have been also shown to be an early marker of acute myocardial infarction (22). In addition, platelet reactivity is progressively increased as a function of the number of vascular districts involved by atherosclerosis (cerebral, cardiac, peripheral) (3). Our findings extend these observations, showing higher residual platelet reactivity after clopidogrel in those patients who have more extensive and severe coronary artery disease. In particular, this association was found significant for both parameters used to evaluate coronary atherosclerotic burden (e.g., number of diseased vessel and total stent length).

6.4.2 Vascular atherosclerosis and variability in platelet response after clopidogrel

A large variability in platelet response to clopidogrel has been described, ranging from patients with high (so-called “low-responders”) to low residual platelet reactivity (9). Optimal platelet inhibition is crucial in patients undergoing PCI, as suggested by the fact that patients with low response to clopidogrel present higher risk of recurrent ischemic events (23). Different studies aimed at identifying low-responders to clopidogrel, supporting the use of a simple and readily available point-of-care assay VerifyNow P2Y12. Platelet aggregation as assessed by the VerifyNow P2Y12 has been demonstrated to significantly predict cardiovascular adverse events in patients undergoing PCI. In the Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome (ARMYDA-PRO) study (6) a PRU value ≥240 was proposed as optimal cut-off to discriminate patients undergoing PCI at higher risk of 30-day major adverse cardiovascular events (MACEs). Yet, the same PRU threshold value was found able to predict higher risk of 12-month cardiovascular death and non-fatal MI in 683 acute coronary syndrome patients undergoing PCI (8) Price et al., studying patients treated with drug eluting stent implantation, found that a similar cut-off value (PRU ≥235) was able to significantly predict stent thrombosis (7). In addition, the GRAVITAS trial, an international multicenter clinical trial, is currently aiming at enrolling 2800 patients
undergoing PCI, who are randomized on the basis of the presence of high residual platelet reactivity (as defined by PRU > 230) to clopidogrel 75 mg/day vs. 150 mg/day (24). In the present study, we defined patients with residual high platelet reactivity (HPR) after clopidogrel as those patients with PRU value ≥ 240 at the VerifyNow assay. Interestingly, we found a significant correlation between HPR and extension/severity of coronary atherosclerosis. In particular, a higher incidence of HPR was observed in those patients with multi-vessel disease or higher total stent length. Our results also propose a cut-off value of 27 mm total stent length as practical tool to discriminate patients at risk of HPR.

6.4.3 HPR and peri-procedural myocardial infarction
Coronary manipulation and side branch occlusion are known determinants of peri-procedural MI in patients undergoing elective PCI (25). In fact, several studies have clearly shown a direct relationship between the implantation of multiple stents, total length of stents implanted and peri-procedural outcome (26-28). In keeping with this study, we found a higher rate of peri-procedural MI in patients in the highest tertile of total stent length compared to those in the lowest tertile.

Recently, HPR was found to be significantly associated with peri-procedural MI (6, 29). We also found a higher incidence of peri-procedural MI in subjects with HPR. Interestingly, HPR was the only independent predictor of peri-procedural MI by multivariate analysis. This suggests that residual high platelet reactivity after clopidogrel plays an additive role over the procedural-related features. This finding is also in line with a recent post-hoc analysis of the Enhanced Suppression of the Platelet Glycoprotein IIb/IIIa Receptor with Integrisin Therapy (ESPRIT) trial showing a significant increase in peri-procedural MI across increasing stent length quartiles. The increased risk observed in the highest quartiles has been largely mitigated by eptifibatide treatment to the level observed in patients of the lowest quartile (28).

6.4.4 Study limitations
By study design, only patients with stable angina were enrolled, therefore our results are not applicable to patients with acute coronary syndromes. No clinical follow-up of the patients is available.

Coronary atherosclerosis severity and extension has been evaluated by the presence of coronary artery stenosis > 50% and/or total length of the implanted stents. This latter has been described as a valid surrogate of total lesion length (13). We cannot exclude that actual final stent length has been slightly overestimated by stent overlapping.

6.4.5 Clinical implications
This study underlines the importance of HPR in predisposing worse procedural outcome. Patients with more extensive coronary atherosclerosis have a higher rate of HPR, which might partly account for the higher risk of peri-procedural MI. Appropriate pre-procedural risk stratification, based on simple angiographic characteristics, should be easily applied to help identifying those patients in whom more aggressive anti-platelet therapy is warranted.
REFERENCES


Peri-Procedural Variations of Platelet Reactivity During Elective Percutaneous Coronary Intervention: insights from clinical observations and in vitro experiments

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ABSTRACT

Background. Percutaneous coronary intervention (PCI) may affect platelet reactivity. Objective of the current study was to assess whether peri-procedural variations of platelet reactivity are related to the type of coronary interventions and to peri-procedural myocardial infarction (PMI). Secondary objective was to investigate the mechanisms of these variations in an in vitro PCI model.

Methods and results. We enrolled a total of 65 patients (pts): 15 pts undergoing coronary angiography (CA group), 40 pts undergoing PCI (PCI group), and 10 pts undergoing rotational atherectomy (RA) plus PCI (RA group). All pts were on aspirin (80-100 mg/day) and received 600 mg clopidogrel at least 12 hours before the procedure. Prior to, immediately post and 24 hours after the procedure, platelet reactivity was measured by the Multiplate Analyzer using ADP, hs-ADP and TRAP test. E-selectin and ICAM-1 were assessed prior to and immediately post-procedure.

In the in vitro model, platelet reactivity was measured during pulsatile blood flow at baseline, after balloon inflation and after stent implantation in 6 porcine carotid arteries and 5 plastic tubes (sham group). Platelet reactivity tended to decline over time in the CA group, while in both PCI and RA groups it significantly increased immediately post-procedure, and decreased to baseline levels at 24 hours. Peri-procedural variations (post-PCI minus pre-PCI) of platelet reactivity were increasingly higher across the three study groups (ADP test: -2.7±7.4 aggregation units [AU] for CA vs. 6.0±12.4 AU for PCI vs. 18.6±7.7 AU for RA, p<0.0001). In the PCI group, peri-procedural variations of platelet reactivity were directly related to total inflation time (r=0.435, p=0.005) and total stent length (r=0.586, p<0.001). E-selectin peri-procedural variations significantly and inversely correlated with platelet reactivity variations (ADP test: r=-0.398, p=0.004; hs-ADP test: r=-0.480, p<0.001; TRAP test: r=-0.496, p=0.001). No correlation was found with sICAM-1. In pts with PMI, the periprocedural increase of platelet reactivity was significantly higher than in those without PMI (ADP test: 11.5±12.73 vs. 1.87±10.61, p=0.013). In the in vitro PCI model, platelet activation was observed only in the presence of an arterial conduit, while no significant variations in platelet reactivity were observed when the same procedures were performed on plastic tubes.

Conclusions. Despite dual antiplatelet therapy, coronary interventions by itself significantly affect platelet function, and these changes directly relate to the procedural complexity and to the extent of vascular damage. Patients experiencing larger increase of platelet reactivity are exposed to a higher risk of PMI.
7.1 INTRODUCTION

Optimal platelet inhibition is crucial to prevent procedural thrombotic complications and recurrent ischemic events in patients undergoing percutaneous coronary intervention (PCI).\textsuperscript{1,2} A 600-mg loading dose of clopidogrel has been established as pre-treatment strategy in stable angina patients undergoing PCI.\textsuperscript{3,4} However, a wide inter-individual variability in clopidogrel responsiveness hampers its protective effect, and contributes to higher thrombogenic risk in patients with high residual platelet reactivity.\textsuperscript{5} Genetic, cellular and clinical factors contribute to the suboptimal platelet response to clopidogrel.\textsuperscript{5} We have recently demonstrated that the extent and severity of coronary atherosclerosis is an additional patient-related factor that is significantly associated with high platelet reactivity and impaired response to clopidogrel.\textsuperscript{5}

Coronary interventional procedure by itself is another important determinant of platelet reactivity and responsiveness to clopidogrel. In fact, platelet inhibition to clopidogrel may significantly vary in the peri-procedural setting.\textsuperscript{7-14} Yet, it is unclear whether variations in platelet reactivity are related to the introduction of devices (i.e. catheters, balloons, stents, etc.) into the coronary circulation, rather than the consequence of the vascular damage induced by balloon inflation and stent implantation.

In this study, we addressed whether: (1) type of coronary interventions affects platelet reactivity after 600-mg clopidogrel loading dose; (2) related peri-procedural variations of platelet reactivity has an impact on myonecrosis after PCI; (3) using \textit{in vitro} PCI model of porcine carotid artery, we addressed underlying mechanisms of peri-procedural variations in platelet reactivity.
7.2 METHODS

7.2.1 Patient population
Between October 2009 and April 2010 consecutive thienopyridine-naive patients with suspected/established stable coronary artery disease (CAD) scheduled for diagnostic and/or interventional coronary procedures were recruited. Patients were divided in 3 groups: those undergoing coronary angiography (CA) alone (CA group), those undergoing PCI with stent implantation (PCI group), and those undergoing rotational atherectomy (RA) plus PCI (RA group). All patients received a 600-mg loading dose of clopidogrel at least 12 hours before the procedure and were on chronic treatment with aspirin (80-100 mg/day). Exclusion criteria were acute coronary syndrome, use of glycoprotein IIb/IIIa inhibitors, treatment with oral anticoagulant drugs, platelet count <70x10^9/L, high bleeding risk (active internal bleeding, history of hemorrhagic stroke, intracranial neoplasm, arteriovenous malformation or aneurysm, ischemic stroke in the previous 3 months), coronary artery bypass surgery in the previous 3 months, and severe renal failure (serum creatinine>2 mg/dl). The study complied with the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from all patients enrolled.

7.2.2 Catheterization laboratory
All interventional procedures in the catheterization laboratory were performed according to standard techniques. Procedural anticoagulation consisted of weight-adjusted unfractionated heparin (100 U/kg). PCI success was defined as a reduction in % diameter stenosis below 30% in the presence of Thrombolysis In Myocardial Infarction (TIMI) flow grade ≥2 in the main vessel and all side branches ≥2 mm in diameter.

7.2.3 Blood sampling
Blood samples were collected at 3 time points: (1) in the catheterization laboratory, after arterial sheath insertion and immediately before heparin administration; (2) immediately post-procedure; (3) 24 hours after the procedure. To assess the potential effect of weight-adjusted unfractionated heparin administrated at the beginning of the coronary intervention, in 10 patients an additional blood sample was measured also 5 minutes after heparin administration. Blood was collected into a 3-ml tube containing 200 U/ml hirudin (Dynabyte Medical, Munich, Germany) and samples were stored at room temperature for at least 30 minutes before testing.

7.2.4 Platelet reactivity
Platelet reactivity was measured using the Multiplate Analyzer (Dynabyte Medical, Munich, Germany), a whole blood platelet function test based on multiple electrode platelet aggregometry (MEA). After diluting 300 µl of whole blood with 300 µl of 0.9% NaCl solution and stirring for 3 minutes in the test cuvettes at 37°C, 6.4 µmol/l ADP (ADP test), or 6.4 µmol ADP + 9.4 nmol prostaglandin E1 (high sensitivity [hs]-ADP test), or 32 µM thrombin receptor activator peptide 6 (TRAP test) were added and the increase in electrical impedance was recorded over a period of 6 minutes. The mean values of two independent determinations are expressed as aggregation units (AU).

7.2.5 Adhesion molecules
Blood samples were also taken immediately before and post-procedure for the measurement of soluble E-selectin (sE-selectin) and soluble ICAM-1 (sICAM-1) using commercially available Quantikine Immunoassay kits (R&D systems, Minneapolis, MN).

7.2.6 Peri-procedural myocardial necrosis

High sensitivity Troponin T (hs-TnT; Roche Diagnostics, Mannheim, Germany) was determined in blood samples taken before, at 8 and 24 hours after intervention. Periprocedural myocardial infarction (PMI) was defined as a post-procedural increase in hs-TnT >3 times the 99th percentile of the upper reference limit (URL=14 ng/ml) for patients with baseline negative myocardial necrosis markers, according to the Joint ESC/ACCF/AHA/WHF task force consensus statement on the redefinition of myocardial infarction for clinical trials on coronary intervention. In patients with increased baseline levels of hs-TnT, a subsequent increase >50% the baseline value fulfilled the criteria for PMI.

7.2.7 Animal experimental set-up and procedures

Porcine carotid arteries and porcine blood were obtained at the local slaughterhouse. The blood was stored in plastic bottles and unfractionated heparin (1 IU/ml) was added immediately after collection. After removing connective/adipose tissue from the arterial samples we isolated segments of unstretched length of 40-60 mm and 2.5-3.5 mm in lumen diameter. Two polypropylene connectors were inserted at proximal and distal ends of each arterial segment and fixed with a tie-wrap. All experiments were performed within 4 hours from sample collection.

The arterial segments were placed into an in-vitro setup (Figure 1) and mounted between two plastic tubes immersed in a bath filled with phosphate buffered saline (Sigma-Aldrich, St. Louis, Missouri). The arterial segments were stretched to obtain a 20% increase in length. The plastic tubes were connected to silicone rubber tubes using polypropylene connectors. On one side of the bath, the rubber tube was connected with a pressure pump; on the other side of the bath it was connected with a 200-ml blood reservoir. Using another silicone rubber tube, the blood was transported from the reservoir back to the pump, making up a closed circuit. A pressure transducer (P10EZ, Becton-Dickinson, St. Niklaas, Belgium) was positioned between the pump and the bath at the proximal site in order to measure absolute pressure waveforms during the experiment. Through a Y-connector, angioplasty materials (guidewires, balloon and stent catheters) could be advanced into the lumen of the arterial segments in the setup.

Figure 2 shows the experimental design. All experiments were performed at room temperature. After placing the arterial segment into the setup, a balloon catheter sized 3.0 x 18 mm (Apex, Boston Scientific, Nanterre Cedex, France) over a metallic guidewire (Hi-Torque Balance Middle Weight Universal, Abbott Vascular, Diegem, Belgium) was positioned into the middle of the artery. An ultrasound scanner featuring a linear array probe (Esaote Europe, Maastricht, the Netherlands) was used to visualize the position of the balloon in the artery. Pulsatile blood flow with a frequency of 60 beats per minute was then activated through the circuit. After 10 minutes, a 1-ml blood sample was drawn from the outflow (baseline sample) of the experimental setup. The balloon catheter was then inflated until complete occlusion of the arterial lumen. Five consecutive inflations of 10 seconds each were carried out and the balloon catheter was then removed from the setup. A 1-ml blood sample was taken immediately after the balloon inflations (T1 sample) and 10 minutes later (T2
sample). Next, a 3.0x18 mm stent (Pro-Kinetic, Biotronik, Berlin, Germany) was advanced into the artery and deployed with 10-second balloon inflation. After implantation, the stent was post-dilated with 4 additional balloon inflations each lasting 10 seconds. One-ml blood samples were taken immediately after stent post-dilatation (T3 sample) and 10 minutes later (T4 sample). ADP-induced (6.4 μmol/l ADP) platelet reactivity was measured on blood samples (within 3 minutes from collection) drawn at each time point by the means of the Multiplate analyzer (Dynabyte Medical, Munich, Germany). A set of sham experiments was performed with the same experimental design using collected blood and a silicone rubber tube instead of the porcine carotid artery (control group). We performed a total of 6 experiments using collected blood and 6 carotid arteries from 6 pigs (artery group), and a total of 5 sham experiments using collected blood from 5 of the 6 pigs (control group). Figure 4. Schematic representation of the experimental setup of the animal in-vitro experiments.

Figure 4. Schematic representation of the experimental setup of the animal in-vitro experiments.
7.2.8 Statistics

Assuming that platelet reactivity measured with ADP test in patients pretreated with 600 mg clopidogrel was 50 AU, and expecting a 15% increase in platelet reactivity after PCI with a standard deviation (SD) of 30% at both time points (baseline and post-PCI), at least 36 patients were needed to detect such difference with a power of 80% at a 2-sided alpha of 0.05. Therefore, we aimed at recruiting a total of 40 patients in the PCI group. Continuous variables are expressed as mean ± SD or as median [interquartile range], as appropriate. Categorical variables are reported as frequencies and percentages. Normal distribution was tested with the Kolmogorov-Smirnov test. Comparisons between continuous variables were performed using the Student t test or Mann-Whitney test. One-way analysis of variance (ANOVA) was used to compare platelet reactivity in different groups. The Kruskall-Wallis test was used to compare non-normally distributed variables in different groups. These tests were corrected for repeated measures where appropriate.
A 2-way ANOVA for repeated measures followed by pairwise comparisons was used to detect changes in platelet reactivity levels over time in different study groups. The Pearson test was used to assess correlations between normally distributed variables, while the Spearman test was used to assess correlations between non-normally distributed variables. Comparisons between categorical variables were evaluated using the Fisher exact test or the Pearson chi-square test, as appropriate. Statistical analysis was performed using STATA/IC 10 (STATA Corp., College Station, Texas) and p values <0.05 (two tailed) were considered significant.
7.3 RESULTS

7.3.1 Patient population

A total of 65 patients were enrolled in the study. Of these, 15 underwent coronary angiography alone (CA group), 40 underwent PCI (PCI group) and 10 underwent PCI plus rotational atherectomy (RA group). The clinical and procedural characteristics are listed in Tables 1 and 2, respectively. No significant differences in demographics, previous clinical history and baseline medications were noticed between the 3 groups. RA group presented more frequently with lesions type B2/C and larger diameter of implanted stent as compared with the PCI group. Procedure duration was progressively longer across the 3 groups.

Table 1. Clinical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Angio n=15</th>
<th>PCI n=40</th>
<th>Rota n=10</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>69±11</td>
<td>66±12</td>
<td>72±9</td>
<td>0.280</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>10 (67)</td>
<td>28 (70)</td>
<td>7 (70)</td>
<td>0.970</td>
</tr>
<tr>
<td>Body mass index</td>
<td>24.9±2.8</td>
<td>27.9±4.3</td>
<td>27.5±5.3</td>
<td>0.064</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>2 (13)</td>
<td>17 (43)</td>
<td>4 (40)</td>
<td>0.116</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>6 (40)</td>
<td>24 (60)</td>
<td>5 (50)</td>
<td>0.381</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>9 (60)</td>
<td>33 (83)</td>
<td>7 (70)</td>
<td>0.205</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>3 (20)</td>
<td>4 (10)</td>
<td>4 (40)</td>
<td>0.072</td>
</tr>
<tr>
<td>Previous myocardial infarction, n (%)</td>
<td>2 (13)</td>
<td>13 (33)</td>
<td>0 (0)</td>
<td>0.057</td>
</tr>
<tr>
<td>Previous coronary intervention, n (%)</td>
<td>8 (53)</td>
<td>26 (65)</td>
<td>3 (30)</td>
<td>0.145</td>
</tr>
<tr>
<td>Previous bypass surgery, n (%)</td>
<td>4 (27)</td>
<td>4 (10)</td>
<td>1 (10)</td>
<td>0.340</td>
</tr>
<tr>
<td>Baseline medications, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>12 (80)</td>
<td>32 (80)</td>
<td>6 (60)</td>
<td>0.385</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>1 (7)</td>
<td>4 (10)</td>
<td>2 (20)</td>
<td>0.556</td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>4 (27)</td>
<td>10 (24)</td>
<td>3 (30)</td>
<td>0.948</td>
</tr>
</tbody>
</table>

Table 2. Procedural characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Angio n=15</th>
<th>PCI n=40</th>
<th>Rota n=10</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseased vessels, n</td>
<td>1.6±1.5</td>
<td>1.6±0.7</td>
<td>1.8±0.9</td>
<td>0.783</td>
</tr>
<tr>
<td>Treated vessel, n</td>
<td></td>
<td>1.1±0.3</td>
<td>1.1±0.3</td>
<td>0.975</td>
</tr>
<tr>
<td>Multivessel intervention, n (%)</td>
<td>4 (10)</td>
<td>1 (10)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Target vessel, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.828</td>
</tr>
<tr>
<td>Left anterior descending</td>
<td></td>
<td>18 (41)</td>
<td>4 (36)</td>
<td></td>
</tr>
<tr>
<td>Left circumflex</td>
<td></td>
<td>5 (11)</td>
<td>2 (18)</td>
<td></td>
</tr>
<tr>
<td>Right coronary artery</td>
<td></td>
<td>21 (48)</td>
<td>5 (45)</td>
<td></td>
</tr>
<tr>
<td>Lesion type B2/C, n (%)</td>
<td>-</td>
<td>21 (47)</td>
<td>11 (100)</td>
<td>0.001</td>
</tr>
<tr>
<td>Bifurcation lesions, n (%)</td>
<td>-</td>
<td>7 (16)</td>
<td>2 (18)</td>
<td>1.000</td>
</tr>
<tr>
<td>Chronic total occlusions, n (%)</td>
<td>9 (20)</td>
<td>1 (9)</td>
<td></td>
<td>0.667</td>
</tr>
<tr>
<td>Predilatation, n (%)</td>
<td></td>
<td>32 (73)</td>
<td>11 (100)</td>
<td>0.184</td>
</tr>
<tr>
<td>Use of stent, n (%)</td>
<td></td>
<td>44 (100)</td>
<td>11 (100)</td>
<td>1.000</td>
</tr>
<tr>
<td>Use of drug eluting stent, n (%)</td>
<td>-</td>
<td>33 (74)</td>
<td>11 (100)</td>
<td>0.096</td>
</tr>
<tr>
<td>Stents implanted, n</td>
<td></td>
<td>1.5±0.9</td>
<td>1.8±1.0</td>
<td>0.407</td>
</tr>
<tr>
<td>Stent diameter, mm</td>
<td></td>
<td>3.1±0.4</td>
<td>3.5±0.6</td>
<td>0.018</td>
</tr>
</tbody>
</table>
Total stent length, mm | - | 23 [12-132] | 35 [12-68] | 0.543
Postdilatation, n (%) | - | 25 (57) | 9 (82) | 0.174
Total inflation time, sec | - | 1.6±1.2 | 2.5±2.2 | 0.097
Maximal inflation pressure, atm | - | 15.8±3.2 | 16.2±2.2 | 0.700
Procedure duration, min | 32.7±10.3 | 66.0±38.8 | 97.6±51.8 | <0.001

7.3.2 Platelet reactivity
The results of the ADP, hs-ADP and TRAP tests are shown in Table 3. At baseline, platelet reactivity was similar between the 3 study groups. Platelet reactivity tended to decline over time in the CA group. In both PCI and RA groups, platelet reactivity increased immediately post-procedure, and decreased towards baseline levels at 24 hours. In those 10 patients within PCI group, in whom platelet reactivity was also measured 5 minutes after heparin, no significant difference in platelet reactivity was observed between pre and post-heparin administration (ADP test: 39±24 vs. 41±22 AU, p=0.543; hs-ADP test: 26±19 vs. 25±18 AU, p=0.866; TRAP test: 85±15 vs. 84±17 AU, p=0.881).

Table 3. Platelet reactivity.

<table>
<thead>
<tr>
<th></th>
<th>CA (n=15)</th>
<th>PCI (n=40)</th>
<th>RA (n=10)</th>
<th>Between group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP test (AU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>41±26</td>
<td>47±25</td>
<td>43±26</td>
<td>0.618</td>
</tr>
<tr>
<td>Post</td>
<td>38±23</td>
<td>53±24</td>
<td>61±29</td>
<td>0.048</td>
</tr>
<tr>
<td>24 h</td>
<td>34±19</td>
<td>40±21</td>
<td>52±23</td>
<td>0.108</td>
</tr>
<tr>
<td>Within group p value</td>
<td>0.138</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>hs-ADP test (AU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>25±17</td>
<td>29±19</td>
<td>28±19</td>
<td>0.746</td>
</tr>
<tr>
<td>Post</td>
<td>24±16</td>
<td>35±22</td>
<td>45±27</td>
<td>0.065</td>
</tr>
<tr>
<td>24 h</td>
<td>18±15</td>
<td>26±16</td>
<td>34±18</td>
<td>0.045</td>
</tr>
<tr>
<td>Within group p value</td>
<td>0.006</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>TRAP test (AU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>82±41</td>
<td>90±33</td>
<td>83±24</td>
<td>0.693</td>
</tr>
<tr>
<td>Post</td>
<td>69±37</td>
<td>94±30</td>
<td>108±25</td>
<td>0.006</td>
</tr>
<tr>
<td>24 h</td>
<td>65±36</td>
<td>82±33</td>
<td>82±16</td>
<td>0.189</td>
</tr>
<tr>
<td>Within group p value</td>
<td>0.003</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

7.3.3 Peri-procedural variations of platelet reactivity
Peri-procedural variations (defined as the difference between post-procedure and pre-procedure) in platelet reactivity were increased in all study groups (Figure 3). With ADP test, peri-procedural variations of platelet reactivity were -2.7±7.4 AU in the CA group, 6.0±12.4 AU in the PCI group, and 18.6±7.7 AU in the RA group, respectively (ANOVA p<0.0001). With hs-ADP test, peri-procedural variations of platelet reactivity were -1.0±5.5 AU in the CA group, 6.4±10.1 AU in the PCI group, and 16.7±10.4 AU in the RA group, respectively (ANOVA p<0.0001). With TRAP
test, peri-procedural variations of platelet reactivity were -13.9±16.3 AU in the CA group, 4.1±21.6 AU in the PCI group, and 24.9±11.2 AU in the RA group, respectively (ANOVA p<0.0001).

Figure 3. Peri-procedural variations of platelet reactivity. Difference in platelet reactivity between the pre-procedural and the post-procedural time points. CA = coronary angiography group; PCI = PCI group; RA = PCI plus rotational atherectomy group.

7.3.4 Procedural parameters and variations of platelet reactivity

In the PCI group, peri-procedural variations of platelet reactivity showed a significant correlation with total inflation time (Figure 4, Panel A) and total stent length (Figure 4, Panel B). Peri-procedural variations of platelet reactivity also moderately correlated with total procedure time (ADP test: r=0.326, p=0.040; hs-ADP: r=0.322, p=0.045; TRAP test: r=0.575, p<0.001). No significant correlation between peri-procedural variations of platelet reactivity and any other procedural parameters was found.

Figure 4. PCI features and variations of platelet reactivity. Relationship of peri-procedural variations of platelet reactivity with total inflation time (Panel A) and total stent length (Panel B) in the PCI group.
7.3.5 Adhesion molecules

Levels of sE-selectin and sICAM-1 are reported in Table 4. Peri-procedural variations of sE-selectin levels were significantly different across the three study groups (ANOVA p<0.0001). In the PCI group, peri-procedural variations of sE-selectin levels significantly and inversely correlated with platelet reactivity variations (ADP test: r=-0.398, p=0.004; hs-ADP test: r=-0.480, p<0.001; TRAP test: r=-0.496, p<0.001). Moreover, peri-procedural variations of sE-selectin levels significantly and inversely correlated with total inflation time (r=-0.469, p=0.002) and total stent length (r=-0.403, p=0.009). Peri-procedural variations of sICAM-1 levels were not significantly different across the three study groups (ANOVA p=0.196). Neither correlation was found between peri-procedural variations of sICAM-1 levels with platelet reactivity variations, nor with procedural parameters.

Table 4. Adhesion molecules.

<table>
<thead>
<tr>
<th></th>
<th>CA (n=15)</th>
<th>PCI (n=40)</th>
<th>RA (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sE-selectin (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>24.6±8.7</td>
<td>29.0±12.7</td>
<td>39.1±15.9</td>
</tr>
<tr>
<td>Post</td>
<td>24.2±8.5</td>
<td>25.0±11.6</td>
<td>31.1±12.3</td>
</tr>
<tr>
<td>Within group p value</td>
<td>0.160</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>209.8±49.0</td>
<td>206.0±58.2</td>
<td>206.6±45.9</td>
</tr>
<tr>
<td>Post</td>
<td>187.2±48.8</td>
<td>174.4±49.3</td>
<td>168.2±44.8</td>
</tr>
<tr>
<td>Within group p value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.006</td>
</tr>
</tbody>
</table>

7.3.6 Peri-procedural myocardial necrosis

Baseline levels of hs-TNT were 8.96 [3.00-10.96] ng/ml in the CA group, 8.90 [4.04-24.88] ng/ml in the PCI group and 6.58 [3-13.83] ng/ml in the RA group, respectively (Kruskall-Wallis p=0.282). The peri-procedural increase in hs-TnT (defined as the difference between post-procedure and pre-procedure) levels was 1.16 [0.00-1.82] ng/ml in the CA group, 14.63 [4.53-42.27] ng/ml in the PCI group, and 33.52 [23.97-130.07] ng/ml in the RA group, respectively (Kruskall-Wallis p<0.0001). PMI occurred in no patients in the CA group, 17 patients (43%) in the PCI group, and 5 patients (50%) in the RA group, respectively. In the PCI group, a significant correlation was found between peri-procedural increase in hs-TnT and peri-procedural variations of platelet reactivity (ADP test: rho=0.462, p=0.003; hs-ADP: rho=0.631, p<0.001; TRAP test: rho=0.386, p=0.014). Moreover, in patients with PMI the periprocedural increase in platelet reactivity was significantly higher than in those without PMI (ADP test: 11.5±12.73 vs. 1.87±10.61, p=0.013; hs-ADP test: 12.0±11.6 vs. 6.3±2.3, p=0.002: TRAP test: 14.8±25.6 vs. -1.7±14.4, p=0.013; Figure 5).
Figure 5. Platelet reactivity and periprocedural myocardial injury. Periprocedural variations of platelet reactivity in patients with and without periprocedural myocardial infarction in the PCI group. PMI = periprocedural myocardial infarction.

7.3.7 Animal experiments

At baseline, platelet reactivity measured with the ADP test was 34±16 in the artery group and 48±42 in the control group (p=0.450; Figure 6, Panel A). In the artery group, platelet reactivity significantly varied over time (ANOVA p=0.033), with a progressive increase after balloon dilatation (T1) and stent implantation (T3). No significant variations in platelet reactivity were observed in the control group over time (ANOVA p=0.452). The 2-way ANOVA showed a significant interaction between experimental groups and timings (p=0.012). Percent variations in platelet reactivity compared to the baseline in the two groups are shown in Figure 6, Panel B.

Figure 6. Results of the in-vitro studies. Absolute levels of platelet reactivity at the different time points (A) and percent variations in platelet reactivity compared to the baseline (B) in the two study groups.
7.4 DISCUSSION

In the present study we demonstrated that (1) platelet reactivity increases with more extensive and aggressive coronary interventions (i.e. with multiple stenting or rotational atherectomy), predisposing stable angina patients to a suboptimal platelet inhibition despite currently recommended loading dose of clopidogrel; (2) peri-procedural variations of platelet reactivity are specifically linked to the degree of vascular damage and endothelial dysfunction induced by coronary interventions; (3) procedure-related platelet activation is associated with an increased risk of myonecrosis.

7.4.1 Platelet reactivity and coronary procedures

Platelet reactivity, despite ongoing dual antiplatelet therapy, is increased early after the procedure as compared to 24 hours later in patients undergoing elective PCI with stent implantation. Yet, the non-availability of platelet reactivity at baseline in these studies does not allow establishing the relative contribution of the coronary intervention. We extended these findings demonstrating that, unlike coronary angiograms, both PCI and PCI plus RA result into a significant increase of platelet reactivity immediately after the procedure compared to baseline; at 24 hours platelet reactivity tends to decrease towards baseline levels. In addition, our data provide mechanistic explanation to these peri-procedural variations in platelet reactivity. Degree of PCI-related platelet activation, in fact, is proportional to the complexity of the procedure. Platelet reactivity was higher after rotational atherectomy and complex PCI procedures as related to procedural factors like total inflation time, total stent length and procedure time.

7.4.2 Platelet activation and vascular damage

Platelet reactivity after complex PCI procedures has been previously linked to the increased shear stress induced by the use of coronary devices such as rotational atherectomy. Whether the vascular damage occurring during PCI might also play a role in platelet activation remained unclear. In an in vitro model of angioplasty, we demonstrated that platelet reactivity increased only when angioplasty and stent implantation was performed in the presence of an arterial conduit (i.e. porcine carotid artery). At the variance, when the same procedures were performed on plastic tubes, no significant variations in platelet reactivity were observed. These findings suggest that flow turbulence and shear stress induced by the procedures are not sufficient to induce platelet activation, and underscore the importance of the interaction between the damaged vessel and circulating platelets. These in-vitro findings are corroborated by the significant decrease in the levels of endothelial biomarkers, sE-selectin and sICAM-1, observed after PCI, supporting the concept that increased platelet reactivity is mediated by the damaged vascular endothelium. In particular, the more aggressive the procedures (i.e. with RA), the more pronounced this biomarkers reduction, suggesting a status endothelial dysfunction potentially occurring post-PCI, in line with previous studies. In the context of coronary atherosclerosis, a close relationship between platelets and endothelium has been described: intact endothelium normally prevents platelet adhesion, while in the presence of endothelial damage and activation, selectins mediate the first loose contact between circulating platelets and vascular endothelium. This interaction represents the first step of vascular thrombosis, it is predominantly mediated by endothelial cells, and does not require platelets to be already activated. Our study suggests that similar events might also occur during
coronary interventions. In fact, the occurrence of coronary endothelial injury post-PCI (as suggested by a significant reduction of sE-selectin) was proportional to the extent of coronary manipulation. This was paralleled by an increase in platelet reactivity, despite pretreatment with aspirin and 600-mg loading dose of clopidogrel. Unlike for sE-selectin, sICAM-1 reduction did not correlate with platelet activation, probably because sICAM-1 primarily mediates the interaction between endothelial cells and leukocytes rather than platelets, and it is mostly involved in the inflammation rather than thrombosis.24

7.4.4 Clinical implications of peri-procedural platelet activation
The impact of baseline platelet reactivity on peri-procedural myonecrosis has been well established.25 We extended this notion by demonstrating for the first time that platelet activation induced by PCI can play a role that is even more important in determining post-procedural myocardial injury. In fact, the higher the increase in platelet reactivity post-PCI, the higher the increase in hs-TnT.

Our finding of a transient platelet activation immediately after the procedure has another important clinical implication, especially when trying to link residual platelet reactivity assessed post-PCI with future clinical outcomes. This finding might help explaining the negative results of the “Gauging Responsiveness with A VerifyNow assay - Impact on Thrombosis And Safety” (GRAVITAS) trial.26 This study aimed at demonstrating among patients with high platelet reactivity after PCI that the use of high-dose clopidogrel compared with standard-dose could improve clinical outcomes at follow-up. The fact that platelet reactivity was measured 12 to 24 hours post-PCI might have led to an incorrect patients selection. In fact, at least some of the patients classified as “non-responders” (i.e. with high on-treatment platelet reactivity) could have been reclassified had the platelet function been assessed pre-PCI or at least 24 hours later.

Finally, the notion of an increase in peri-procedural platelet reactivity proportionally with the complexity of PCI, and with subsequent increased risk of myocardial injury, supports the use of more effective platelet inhibition strategies in complex interventional procedures. This is corroborated by a recent analysis of the Enhanced Suppression of the Platelet Glycoprotein IIb/IIIa Receptor with Integrilin Therapy (ESPRIT) trial,27 which demonstrated an adjunctive beneficial effect of IIbIIIa inhibitors (in terms of reduction of early and late risk of complications after PCI) in patients undergoing more complex procedures (i.e. higher number of stents implanted, longer total stent length).

7.4.5 Limitations
In our study, only patients with stable CAD were enrolled and therefore we cannot extend these observations to patients with acute coronary syndrome. Similarly, all study patients received a 600-mg loading dose of clopidogrel; therefore these results might not be applicable to patients on chronic clopidogrel treatment or taking different P2Y12 receptor inhibitors.

7.4.6 Conclusions
Coronary interventions significantly affect platelet function: the more complex the procedure, the larger the transient changes in platelet response to clopidogrel therapy. Damage of the vascular wall and endothelium activation seem to be the underlying mechanisms. Furthermore, patients experiencing larger peri-procedural increase of platelet reactivity are also exposed to higher risk of myocardial necrosis. This study
supports the need for more aggressive antiplatelet strategies when complex coronary procedures are planned.
REFERENCES


GENERAL DISCUSSION
8.1 Clinical impact of residual platelet reactivity in patients undergoing PCI

Platelets participate in the genesis and progression of coronary atherosclerosis, and play a key role in the triggering of arterial thrombosis. Antiplatelet therapy is therefore a cornerstone of the pharmacological treatment of patients with CAD, and especially of those treated with PCI. However, individual response to antiplatelet treatment is variable and particular attention should be paid to those patients exhibiting either too high or too low residual platelet reactivity.

8.1.1 High platelet reactivity

A large body of evidence has accumulated over the last years on the relationship between high residual platelet reactivity and clinical outcomes (Table 1). The detrimental effect of high platelet reactivity has been established in the whole spectrum of clinical syndromes of CAD. Both patients with acute coronary syndrome and stable CAD have been shown to have higher risk of adverse clinical events in the presence of high platelet reactivity. In a study of patients with ST-elevation myocardial infarction treated with primary PCI, higher post-clopidogrel platelet reactivity (assessed by LTA, Verify-Now) was associated with higher incidence of ischemic events at the follow-up (1). Similarly, in patients with NSTE-ACS treated with PCI, higher platelet reactivity (assessed with LTA, Verify-Now) has been shown to be associated with higher risk of peri-procedural myonecrosis and adverse events at follow-up, including death (3-6). In patients undergoing elective PCI, suboptimal response to clopidogrel (assessed by LTA, VASP, Verify-Now) has been shown to predict the occurrence of ischemic complications in the peri-procedural setting and up to 2 years after the procedure (7-18). Finally, several studies have reported, regardless of the test used for the assessment (LTA, VASP, MEA, Verify-Now), a strong association between high levels of platelet reactivity on clopidogrel and occurrence of stent thrombosis (2,19-21).

To translate these findings into clinically practical tools, a number of studies, including those outlined in this thesis (chapters 2 to 6) have explored the existence of reliable threshold values able to predict worse clinical outcome, especially using the Verify-Now point-of-care system. In general, a PRU cut-off ≈235-240 was found to predict both periprocedural and mid term events (2,18,22-25,27,28). Taken together, this collective evidence implies that patients showing high platelet reactivity could benefit from more aggressive antiplatelet strategies (see paragraph 8.2 Future Perspectives).

Table 1. Studies assessing the impact of high platelet reactivity on clinical outcomes after PCI.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Study design</th>
<th>Platelet function test</th>
<th>Definition of HPR</th>
<th>Clopidogrel dose</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matetzky et al. (1)</td>
<td>Primary PCI for STEMI (n=60)</td>
<td>Prospective cohort</td>
<td>LTA</td>
<td>First quartile of platelet inhibition</td>
<td>300 mg/75 mg</td>
<td>6-month MACE</td>
</tr>
<tr>
<td>Gurbel et al. (7)</td>
<td>Elective PCI (n=120)</td>
<td>Randomized controlled study</td>
<td>LTA</td>
<td>NA</td>
<td>300-600 mg/75 mg</td>
<td>Peri-procedural myonecrosis</td>
</tr>
<tr>
<td>Lev et al. (8)</td>
<td>Elective PCI (n=150)</td>
<td>Prospective cohort</td>
<td>LTA</td>
<td>Fourth quartile of ADP aggregation</td>
<td>300 mg/75 mg</td>
<td>Peri-procedural myonecrosis</td>
</tr>
<tr>
<td>Hochholzer et al. (11)</td>
<td>Elective PCI</td>
<td>Prospective cohort</td>
<td>LTA</td>
<td>NA</td>
<td>600 mg/75 mg</td>
<td>1-month MACE</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Sample</td>
<td>Method</td>
<td>Platelet aggregation cut-off</td>
<td>Platelet inhibition cut-off</td>
<td>Duration</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>------------------------------</td>
<td>----------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Cuisset et al. (3)</td>
<td>Randomized controlled study</td>
<td>LTA in NSTE-ACS (n=292)</td>
<td>ADP aggregation &gt;70%</td>
<td></td>
<td>300-600 mg/75 mg</td>
<td>1-month MACE</td>
</tr>
<tr>
<td>Geiser et al. (4)</td>
<td>Prospective cohort</td>
<td>LTA in NSTE-ACS (n=363)</td>
<td>ADP aggregation &gt;70%</td>
<td></td>
<td>600 mg/75 mg</td>
<td>3-month MACE</td>
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<td>Cuisset et al. (5)</td>
<td>Prospective cohort</td>
<td>LTA in NSTE-ACS (n=106)</td>
<td>Fourth quartile ADP aggregation</td>
<td></td>
<td>300 mg/75 mg</td>
<td>1-month MACE</td>
</tr>
<tr>
<td>Bleden et al. (13)</td>
<td>Prospective cohort</td>
<td>LTA/TEG in PCI (n=100)</td>
<td>ADP aggregation (LTA) ≥50%</td>
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<td>75 mg &gt;1 month before PCI</td>
<td>1-year MACE</td>
</tr>
<tr>
<td>Cuisset et al. (17)</td>
<td>Prospective cohort</td>
<td>LTA in NSTE-ACS (n=190)</td>
<td>ADP aggregation &gt;70%</td>
<td></td>
<td>600 mg</td>
<td>Peri-procedural myonecrosis</td>
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<td>Buonamici et al. (19)</td>
<td>Prospective cohort</td>
<td>LTA in NSTE-ACS (n=804)</td>
<td>ADP aggregation &gt;70%</td>
<td></td>
<td>600 mg/75 mg</td>
<td>6-month definite/probable stent thrombosis</td>
</tr>
<tr>
<td>Gurbel et al. (14)</td>
<td>Prospective cohort</td>
<td>LTA in PCI (n=297)</td>
<td>ADP aggregation &gt;59%</td>
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<td>601 mg/75 mg</td>
<td>2-year MACE</td>
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<td>Wang et al. (15)</td>
<td>Prospective cohort</td>
<td>LTA in PCI (n=386)</td>
<td>Platelet inhibition ≤10%</td>
<td></td>
<td>300 mg/75 mg</td>
<td>1-year MACE</td>
</tr>
<tr>
<td>Gurbel et al. (10)</td>
<td>Prospective cohort</td>
<td>LTA/TEG in PCI (n=192)</td>
<td>Fourth quartile of ADP aggregation/fourth quartile of clot strength</td>
<td></td>
<td>300-600 mg/75 mg</td>
<td>6-month MACE</td>
</tr>
<tr>
<td>Gurbel et al. (9)</td>
<td>Randomized controlled study</td>
<td>LTA/TEG in PCI (n=200)</td>
<td></td>
<td>NA</td>
<td>75-600 mg/75 mg</td>
<td>Peri-procedural myonecrosis</td>
</tr>
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<td>Gurbel et al. (20)</td>
<td>Case-control</td>
<td>LTA/TEG in PCI (n=120)</td>
<td>Fourth quartile of ADP aggregation</td>
<td></td>
<td>300 mg/75 mg</td>
<td>Sub-acute stent thrombosis</td>
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<tr>
<td>Frere et al. (6)</td>
<td>Prospective cohort</td>
<td>VASP in NSTE-ACS (n=195)</td>
<td>Platelet aggregation</td>
<td></td>
<td>600 mg/75 mg</td>
<td>1-month MACE</td>
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<td>Sibbing et al. (21)</td>
<td>Prospective cohort</td>
<td>MEA in PCI (n=1608)</td>
<td>Fifth quintile of platelet aggregation</td>
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<td>600 mg/75 mg</td>
<td>1-month definite thrombosis</td>
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<td>Bonello et al. (16)</td>
<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=144)</td>
<td>PRU ≥235</td>
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<td>6-month MACE</td>
</tr>
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<td>Buch et al. (26)</td>
<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=330)</td>
<td>PRU &gt;235</td>
<td></td>
<td>600 mg/75 mg</td>
<td>6-month MACE</td>
</tr>
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<td>Price et al. (27)</td>
<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=380)</td>
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<td>600 mg/75 mg</td>
<td>Peri-procedural myonecrosis</td>
</tr>
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<td>Cuisset et al. (17)</td>
<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=122)</td>
<td>Fourth quartile of PRU values</td>
<td></td>
<td>600 mg/75 mg</td>
<td>1-month MACE</td>
</tr>
<tr>
<td>Patti et al. (18)</td>
<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=160)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marcucc et al. (22)</td>
<td>Prospective cohort</td>
<td>VerifyNow in ACS (n=683)</td>
<td>PRU ≥240</td>
<td></td>
<td>600 mg/75 mg</td>
<td>1-year cardiovascular death/non-fatal MI</td>
</tr>
<tr>
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<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=338)</td>
<td>PRU ≥240</td>
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<td>Peri-procedural myonecrosis</td>
</tr>
<tr>
<td>Mangiacapra et al. (23)</td>
<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=250)</td>
<td>PRU ≥240</td>
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<td>75-600 mg/75 mg</td>
<td>Peri-procedural myonecrosis</td>
</tr>
<tr>
<td>Breet et al. (28)</td>
<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=1069)</td>
<td>PRU ≥236</td>
<td></td>
<td>75-300-600 mg/75 mg</td>
<td>1-year MACE</td>
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<tr>
<td>Mangiacapra et al. (25)</td>
<td>Prospective cohort</td>
<td>VerifyNow in PCI (n=732)</td>
<td>PRU ≥240</td>
<td></td>
<td>75-600 mg/75 mg</td>
<td>30-day MACE</td>
</tr>
</tbody>
</table>
8.1.2 Low platelet reactivity

The risk of inadequate response to antiplatelet drugs does not only reside in high residual platelet reactivity, but also in opposite of it, low platelet reactivity. In fact, following a Gaussian distribution, residual platelet reactivity after clopidogrel might be too low in a substantial proportion of patients falling under the left-hand side of the bell-shaped curve (see Figure 3 in Chapter 1). Recent evidence suggests that low platelet reactivity might be associated with higher risk of bleeding events after PCI (29,30). Several analyses from randomized studies and registries have highlighted the clinical importance of haemorrhagic complications, also demonstrating an association between bleeding and mortality (31,32). Thus, being able to identify those patients with low residual platelet reactivity and therefore at higher risk of bleeding events might be of clinical importance from the safety perspective. Using the VerifyNow P2Y12 assay, the ARMYDA-BLEEDS study has shown that an enhanced response to clopidogrel is indeed associated with higher risk for early major bleeding or entry-site complications in patients who undergo PCI, suggesting that a point-of-care monitoring of platelet reactivity after clopidogrel administration may help identify patients in whom individualized strategies are indicated to limit bleeding complications after coronary intervention (30).

8.1.3 Therapeutic window for platelet reactivity

Taken together, the aforementioned considerations suggest the possibility to identify a “therapeutic window”, representing the range of optimal levels of platelet reactivity associated with the lowest risk for adverse events. An initial evidence for such optimal range was provided by Sibbing et al. who measured platelet reactivity with the Multiplate Analyzed in a large cohort of patients (n=2533) undergoing coronary stenting after pre-treatment with clopidogrel 600 mg. They found that patients falling within the “sweet spot” of aggregation values between 189 and 467 AU*min showed remarkably low risk for the occurrence of both major bleeding and stent thrombosis (33). In ARMYDA-PROVE, we confirmed these results using the point-of-care system VerifyNow P2Y12 Assay (chapter 5) (25). In particular, PRU values between 179 and 238 resulted in almost a 50% risk reduction for net adverse clinical events (including both ischemic and bleeding events) compared to patients with either low or high platelet reactivity. Provided the lower risk for patients falling in this therapeutic window, adjunctive measures may be of benefit for those with higher or lower platelet reactivity.

8.1.4 Peri-Procedural Variations of Platelet Reactivity in PCI

Coronary intervention is capable by itself to induce platelet activation and aggregation. In chapter 7, we demonstrated the novel finding that PCI-induced platelet activation is related to the type of intervention and proportional to the extent and complexity of the coronary intervention. The experimental part of this study has shown that vessel wall is critical component in this process. On one hand, the platelet reactivity is related to the extent of atherosclerotic remodelling within the coronary tree (see chapter 3). On the other hand, mechanism of action induced by mechanical dilation at time of intervention and associated activation of vascular wall is critical trigger in platelet activation leading to transient increase in platelet reactivity. The degree of activation is strongly correlated to peri-procedural myocardial damage. These observations have several clinical implications:

• In addition to baseline platelet reactivity, its peri-procedural variations are
important determinants of PCI outcomes;

- as peri-procedural variations of platelet reactivity correlate with the complexity of PCI, more intensive antiplatelet strategies may be warranted in patients in whom more aggressive coronary procedures are planned;
- transient variations of platelet reactivity in the peri-procedural period should be taken into account when deciding the timing for platelet function testing.
8.2 Future perspectives

Both low and high platelet reactivity are associated with adverse events in patients undergoing PCI. A selective assessment of residual platelet reactivity based upon reliable predictors of suboptimal platelet inhibition might be a reasonable strategy. In patients taking clopidogrel with suboptimal platelet reactivity, either too high or too low, several alternative antiplatelet strategies are available. They consist of more potent P2Y12 receptor inhibitors in case of high platelet reactivity, or preventive measures to reduce bleeding in case of low platelet reactivity.

The potential advantage of tailoring clinical treatment on the basis of platelet reactivity is yet to be established. The GRAVITAS (Gauging Responsiveness With a VerifyNow Assay–Impact on Thrombosis and Safety) trial has failed to show that patients with high platelet reactivity benefit from a double dose of clopidogrel after PCI (34). Other on-going studies will help clarify this issue. The ARCTIC (Double Randomization of a Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation, and Interruption Versus Continuation of Double Antiplatelet Therapy) trial (http://www.clinicaltrials.gov, NCT00827411) will enrol 2500 patients randomized to conventional arm or to monitoring arm. In the latter, both aspirin and clopidogrel responsiveness will be measured by the VerifyNow device and, if necessary, antiplatelet therapy will be appropriately modified. Primary endpoint is the composite of death, myocardial infarction, stroke, urgent coronary revascularization and stent thrombosis at 1-year follow-up. The ADAPT-DES (Assessment of Dual Antiplatelet Therapy With Drug Eluting Stents) registry (http://www.clinicaltrials.gov, NCT00638794) has enrolled a large number (11000) of patients treated with drug eluting stent implantation in which platelet reactivity levels will be correlated to the incidence of stent thrombosis over 2 year of follow-up. Finally, in the DANTE (Dual Antiplatelet Tailored Therapy Based on the Extent of Platelet Inhibition trial (http://www.clinicaltrials.gov, NCT00774475), nearly 450 patients with NSTE-ACS and high platelet reactivity undergoing PCI will be recruited. Patients will be randomized to either a double clopidogrel maintenance dose (150 mg) or a standard dose (75 mg) and monitored for cardiovascular events over 1 year of follow-up.

Another potential strategy for patients with high platelet reactivity is the use of newer, more potent P2Y12 inhibitors. Third-generation thienopyridine prasugrel has been recently tested in patients undergoing PCI. Similar to clopidogrel, prasugrel is a prodrug that needs to be transformed by hepatic CYP in an active form that irreversibly blocks platelet P2Y12 receptor. The antiplatelet effect of prasugrel has been clearly shown to be superior to that of clopidogrel, achieving a more intense and less variable platelet inhibition (35-36). Among 13,608 moderate-to-high risk ACS patients with scheduled PCI enrolled in the TRITON-TIMI 38 trial, treatment with prasugrel (60 mg load, 10 mg daily), as compared with clopidogrel (300 mg load, 75 mg daily), resulted in a significant reduction in ischemic events (cardiovascular death, nonfatal myocardial infarction or nonfatal stroke) at follow-up (37). However, more intense platelet inhibition achieved by prasugrel also resulted in a significant increase in life-threatening and fatal bleeding events (37). Based on this evidence, according to the latest guidelines (38), the use of prasugrel is contraindicated in patients with high bleeding risk, such as elderly patients (~75 years of age), those with history of cerebrovascular accidents and those with body weight <65 kg.

The use of prasugrel as an alternative to clopidogrel in patients treated with PCI showing high residual platelet reactivity has been investigated in the Testing
Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel (TRIGGER-PCI) trial (39). However, in this study switching from clopidogrel to prasugrel, despite affording effective platelet inhibition, did not show any clinical utility, probably due to the low rate of adverse ischemic events after PCI in stable CAD. Whether the selective use of prasugrel could be of benefit in higher risk patients with high platelet reactivity, in order to reach a balance between protection from ischemic events and prevention of bleeding, should be investigated in clinical studies focused on this specific population.

Ticagrelor (AZD6140), a cyto-pentyl-triazolo-pyrimidine, is an oral, direct and, in contrast to thienopyridines, reversible inhibitor of the P2Y12 receptor, and does not require CYP biotransformation. The latter, is a factor significantly reducing interindividual variability in the response to the drug, which is a major limitation of thienopyridine clopidogrel. The antiplatelet effect of ticagrelor has been demonstrated to be more potent and rapid, as compared with clopidogrel (40). In the Platelet Inhibition and Patient Outcomes (PLATO) trial (41), 18,624 ACS patients were randomly assigned to receive either ticagrelor (180 mg loading dose, 90 mg twice-daily thereafter) or clopidogrel (300-600 mg loading dose, 75 mg daily thereafter). Treatment with ticagrelor resulted in a significant reduction in the primary end point of cardiovascular death, myocardial infarction and stroke. While no significant excess in overall major bleedings were associated with ticagrelor use, this resulted in a significant increase in rates of major bleeding not related to bypass surgery. Similar clinical benefit from ticagrelor was found in the cohort of patients treated with PCI (42). The use of ticagrelor in patients with high platelet reactivity has not been yet tested and should be investigated in future large clinical trials.

Another clinically tested platelet inhibitor is cangrelor. This drug belongs to a family of analogs of ATP displaying high affinity for the P2Y12 receptor. Cangrelor is a potent inhibitor of platelet aggregation, does not require conversion to an active metabolite, and is immediately active after intravenous infusion, with a half-life of 3 to 6 minutes. A study that directly compared the effects of clopidogrel and cangrelor administration in patients with ischemic heart disease showed that cangrelor (2 and 4 µg/ml/min) almost completely inhibited 10 µmol/L ADP–induced platelet aggregation, whereas 4 to 7 days of clopidogrel treatment resulted in only 60% inhibition (43). In the Clinical Trial Comparing Cangrelor to Clopidogrel in Subjects Who Require Percutaneous Coronary Intervention (CHAMPION PCI) (44) the efficacy of cangrelor was compared to that of clopidogrel in subjects requiring PCI. The primary objective of the Clinical Trial Comparing Treatment With Cangrelor (in Combination With Usual Care) to Usual Care, in Subjects Who Require Percutaneous Coronary Intervention (CHAMPION PLATFORM) (45) was to demonstrate that the efficacy of cangrelor (combined with usual care) is superior to that of usual care alone in subjects requiring PCI as measured by a composite of all-cause death, myocardial infarction, and ischemia-driven revascularization. Both studies were terminated prematurely because of insufficient evidence of the clinical effectiveness of cangrelor.
8.3 Conclusions

The main findings from this thesis can be summarized as follows:

• high pre-PCI platelet reactivity measured with a rapid point-of-care assay may predict 30-day event rates;
• high platelet reactivity is associated with increased risk of myonecrosis in low-to-intermediate risk patients undergoing planned PCI;
• patients with diabetes mellitus undergoing PCI have higher platelet reactivity at the time of PCI despite adequate clopidogrel pretreatment, and subsequently worse peri-procedural outcomes;
• a therapeutic window for platelet reactivity measured with the VerifyNow P2Y12 assay can be identified using specific thresholds that define a group of patients at lower risk for both ischemic and bleeding events;
• platelet reactivity after clopidogrel significantly correlates with the extent of coronary atherosclerosis;
• PCI by itself affects platelet function, determining variations that are directly related to the procedural complexity and to the extent of vascular damage; patients experiencing larger increase of platelet reactivity are also exposed to a higher risk of myocardial damage.
REFERENCES


CURRICULUM VITAE AND LIST OF PUBLICATIONS
Curriculum vitae

The author of this thesis was born on 08-04-1981 in Naples, Italy. After finishing high school in 1999 at Liceo Classico “D. Cirillo” in Aversa, Italy, he studied medicine at campus Bio-Medico University in Rome, Italy. In 2006 he graduated and in 2009 he completed his fellowship in Cardiology at Campus Bio-Medico University in Rome, Italy. In 2011 he completed his fellowship in Interventional Cardiology at Cardiovascular Center in Aalst, Belgium. In 2010 he started a PhD project at Technische Universiteit Eindhoven, the Netherlands, the results of which are presented in this dissertation. Since 2011 he is employed at the Department of Cardiovascular Sciences, Campus Bio-Medico University of Rome as a clinical and interventional cardiologist.

Main fields of interest of his research activity are antiplatelet therapy for patients undergoing coronary interventions, management of patients with acute coronary syndromes, physiology of coronary and renal circulation. Since 2010 he has contributed more than 30 publications in peer-reviewed journals.

In 2010 he received the Cardiology Graduation Thesis Award and in 2011 the Young Investigator Award, both from the Italian Society of Cardiology. In 2012 he has received the Young Author Achievement Award for JACC Cardiovascular Interventions and is the winner of the Young Investigator Award in Coronary Pathophysiology and Microcirculation at the European Society of Cardiology annual meeting.
List of publications


Crede quod habes et habes