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Emergence of the Concept of Platelet Reactivity Monitoring of Response to Thienopyridines

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BRIEF SUMMARY

Clinical trials have demonstrated the beneficial impact of clopidogrel in preventing major adverse cardiovascular events (MACE), particularly in patients undergoing percutaneous coronary intervention (PCI). The concept of biological clopidogrel resistance emerged with the finding of persistent platelet activation despite clopidogrel therapy in some patients. Further, a link between biological clopidogrel resistance and thrombotic recurrence following PCI was observed and a threshold of platelet reactivity (PR) for thrombotic events was suggested.

Consistently, in recent trials, enhanced PR inhibition translated into a reduction in the rate of MACE post-PCI. This review aims to present the emergence of the concept of PR monitoring in patients undergoing PCI following recent advances in this field.

INTRODUCTION

Historical perspectives

The advent of angioplasty has revolutionized coronary revascularization, thereby greatly improving the prognosis of coronary artery disease (CAD) and, in particular, of acute coronary syndromes (ACS). Stent implantation has improved the safety and efficacy of percutaneous coronary interventions (PCI) by decreasing the risk of acute vessel closure and restenosis.[1] In its early days, however, stenting was associated with a high risk of acute and subacute stent thrombosis due to platelet activation.[1] Ticlopidine pre-treatment followed by sustained administration after PCI was associated with a dramatic reduction in the rate of stent thrombosis, thus allowing stenting to become the gold standard of percutaneous coronary revascularization.[2] However, a high frequency of serious hematological side effects with ticlopidine have led to the introduction of a second generation thienopyridine, clopidogrel, with a similar antiplatelet efficacy and improved safety.[3]

Clinical trials have demonstrated the beneficial impact of clopidogrel in preventing major adverse cardiovascular events (MACE) in CAD patients and particularly for those undergoing PCI.[4] Despite clopidogrel use, however, thrombotic events after PCI, including acute and subacute stent thrombosis, have not been eliminated and may be considered as therapeutic failure of the drug. Further, biological assays have demonstrated persistent platelet activation despite clopidogrel therapy in some patients, which has led to the concept of biological clopidogrel resistance or clopidogrel low response. This review aims to summarize present data on the relationship between therapeutic failure and low response to clopidogrel and discuss the emergence of the concept of platelet reactivity (PR) monitoring in patients undergoing PCI.

CLOPIDOGREL

Mechanism of action

Clopidogrel is an inactive pro-drug rapidly absorbed by the intestine. Because 85% of the pro-drug is hydrolyzed by esterases in the blood to an inactive carboxyl acid derivative, only 15% of clopidogrel is converted to its active thiol metabolites by hepatic cytochrome P450 isoenzymes (CYP3A4, CYP3A5, and CYP2C19). These active metabolites selectively bind to the P2Y₁₂ adenosine diphosphate (ADP)-receptor by a covalent bisulfide bond, which is irreversible and permanent, thus inhibiting the receptor for the platelet's lifespan. This blockade acts early in the formation of platelet thrombus by inhibiting the transformation of the glycoprotein (GP) IIb/IIIa receptor into an active form that binds fibrinogen and link platelets, thus preventing the amplification ADP-mediated process of platelet aggregation and stabilization of platelet aggregates.[5]

Common methods to evaluate platelet reactivity

Several assays have been proposed to assess PR. Since there are numerous ways by which platelets can be activated and participate in coagulation and aggregation, no single test can encompass the complexity of platelet biology and function to accurately describe the global platelet activation state. Further, PR monitoring has been limited by the lack of consensus on an optimal test to assess platelet response to clopidogrel because of the lack of standardization. The most commonly used tests in the research field are described below. [Table 1]

Platelet Aggregometry

Aggregometry using ADP as an agonist is the most commonly used test to assess PR in response to clopidogrel. It is based on the stimulation of platelet-platelet aggregation in platelet-

rich plasma followed by light-transmission or impedance measurement. Aggregometry which is considered the gold standard of PR measurement presents several disadvantages that impair its ability to be used for platelet monitoring. It is not standardized and therefore has several methodological pitfalls, including agonist dosage, nature of the anticoagulant used for blood sampling, and whether peak or late aggregation value is used.[6] Moreover aggregometry is influenced by other anti platelet therapy including GP IIb/IIIa inhibitors and aspirin.[7]

The VerifyNow P2Y₁₂ is a point-of-care assay which negates some of these limitations. It is a user-friendly assay which doesn't necessitate any sample processing, thus limiting loss of time and potential platelet activation. Some of the previous limitations of aggregometry such as GP IIb/IIIa influence, however, couldn't be overcome. Of interest is that this point of care assay which correlates with LTA and vasodilator-stimulated phosphoprotein (VASP) assays could be available in the catheterization laboratory and therefore for clinical use.[8]

Vasodilator-stimulated Phosphoprotein Phosphorylation

VASP assay is a P2Y₁₂ ADP-receptor specific assay based on intra-cellular signal measurement using flow cytometry. The ratio of phosphorylated/dephosphorylated VASP-protein within platelets is correlated with the inhibition of the binding of fibrinogen and glycoprotein IIb/IIIa and thus of the inhibition of PR induced by clopidogrel through P2Y₁₂ ADP-receptor blockade. The advantages of this technique are the stability of blood sample for 24 hours and that it is not influenced by other antiplatelet medications.[9]

	Advantages	Disadvantages
Aggregometry	Gold standard	Variable reproducibility, time consuming, requires an experienced operator, sample preparation, not standardized, not specific of the P2Y12 ADP receptor Influenced by aspirin and GP IIb/IIIa inhibitors
VerifyNow P2Y12	Small blood volume, whole blood assay, rapid, bedside	Influenced by GP IIb/IIIa
VASP	Small blood volume, whole blood assay, specific of the P2Y12 ADP receptor	Sample preparation, requires flow-cytometer, experienced operator

Table 1: Main advantages and disadvantages of the most commonly used platelet assays

BIOLOGICAL CLOPIDOGREL RESISTANCE / CLOPIDOGREL LOW RESPONSE

Jaremo et al. were the first to describe a large, inter-individual variability in PR after a 300-mg clopidogrel pretreatment.[10] Since then, several studies using various platelet assays have confirmed these findings and have underlined the fact that in some patients PR was not- or was only slightly influenced by clopidogrel intake.[11] The large, inter-individual variability shown with a 300-mg loading dose (LD) remains when a 600-mg dose is used.[12]

Interestingly, biological resistance to the LD also predicts poor drug effect at 1 month.[11] Initially, biological clopidogrel resistance was based on the inhibition of platelet aggregation assessed by LTA and empirically defined as a <10% decrease in PR after the LD compared to baseline.[11, 12] Later, post-treatment PR, which does not require baseline measurement of PR, was shown to more reliably reflect thrombotic risk.[13] Because of the numerous assays currently available, to the methodological variability within each technique, and to the absence of a common endpoint to define a cut-off value, a consensual definition of biological clopidogrel resistance is still lacking. We will therefore refer to the term clopidogrel low response instead of biological clopidogrel resistance in the following.

Mechanisms of inter-individual variability in response to clopidogrel (14)

The mechanisms of inter-individual variability in response to clopidogrel are numerous and include clinical factors, baseline individual variability, and genetic polymorphism. The clinical factors that contribute to variability include poor compliance, inadequate doses, and high body mass index. Of importance patients suffering of an ACS have an increased baseline PR and therefore more often exhibit clopidogrel low-response¹⁵.

Individual differences or drug-drug interaction in intestinal absorption of the drug or in cytochromes P450 isoenzyme activity may also account for variability in the response to the drug. Internal factors of inter-individual variability include genetic polymorphisms of platelet receptors, including GP IIb/IIIa receptor, liver cytochromes P450 isoenzyme, and up-regulation of platelet activation pathways.

Finally, as summarized in Table 2, the mechanisms of inter-individual variability in response to clopidogrel are numerous and could participate alone or combined.

Clinical factors and biodisponibility

Poor compliance
 Inadequate dose
 High BMI
 Timing of loading
 Poor absorption
 Acute coronary syndrome
 Diabetes/insulin resistance
 Smoking
 Drug-drug interaction involving CYP3A4

Baseline individual variability

Increased baseline platelet reactivity
 Up-regulation of the P2Y12 pathway
 Up-regulation of the P2Y1 pathway
 Up-regulation of the P2Y independent pathway
 Increased platelet production by the bone-marrow
 Incomplete suppression of the ADP signal (P2Y1)
 Increased platelet sensitivity to collagen/ADP

Genetic variation

Polymorphism of the cytochrome P 450

Polymorphism of the P2Y12 gene

Other platelet receptor polymorphism

Table 2: Main suspected mechanisms of clopidogrel low response

The multiple determinants of clopidogrel metabolism, the impossibility to clinically predict responsiveness to the drug, and the large inter-individual variability in response to the drug support the need for evaluation of individual response using platelet monitoring.

Interestingly, some mechanisms of this variability are modifiable and therefore could be overcome by targeted interventions.

Relationship between clopidogrel low-response and thrombotic events post-PCI

Numerous studies have aimed to investigate a potential association between biological assessment of response to clopidogrel and therapeutic failure as defined by acute/subacute stent thrombosis or thrombotic recurrence.[16-25] Barragan et al. were the first to report a link between persistence of a high PR assessed by the VASP index and stent thrombosis, which represents the perfect endpoint of clopidogrel failure since the drug's main objective is to prevent such dramatic events.[16] Subsequent studies have confirmed these preliminary data linking biological clopidogrel low response and acute and subacute stent thrombosis.[16-19](Table 3)

Endpoint	Study (ref)	Platelet assays used	n
Stent thrombosis	Barragan et al. (165)	VASP-P index	36
	Gurbel et al. (17)	VASP-P index, ADP induced aggregometry	120
	Buonamici et al. (18)	ADP-induced aggregometry	804
	Blindt et al. (19)	VASP-P index, ADP-induced aggregometry	99
Ischemic events			
CV death, MI, unstable angina, stroke	Gurbel et al. (21)	ADP-induced aggregometry	192
Death, MI, stent thrombosis, stroke, ischemia	Bliden et al. (22)	ADP-induced aggregometry	100
CV death, acute or subacute ST, ACS, ischemic stroke	Frere et al. (24)	ADP-induced aggregometry, VASP-P index	195
CV death, MI, urgent TVR	Bonello et al. (23)	VASP-P index	144
CV death, acute and subacute stent thrombosis, MI	Price et al. (20)	VerifyNow P2Y12	380

Table 3. Main studies investigating the relationship between biological clopidogrel resistance and thrombotic events post-PCI

Because of the low rate of acute and subacute stent thrombosis, several studies have used a broader definition of therapeutic failure to investigate the potential link between platelet reactivity and thrombotic events. [20-25](Table 3)

The Platelet Reactivity in Patients and Recurrent Events Post-Stenting (PREPARE POST-STENTING) study observed a link between PR measure using LTA and the occurrence of post-discharge thrombotic events, which included death secondary to cardiovascular cause, myocardial infarction, unstable angina, and stroke. Interestingly in this study, a cut-off value of 50% of post-treatment PR was associated with the occurrence of thrombotic events during 6 months' follow-up.[21] Consistently, with the concept of a threshold for thrombotic events, 2 additional studies using LTA observed an association between a cut-off value of PR and thrombotic events.[17, 22]

Consistently, we have demonstrated that a cut-off value of 50% of PR using VASP index predicted 6 months' MACE including cardiac death, myocardial infarction, and urgent revascularization with a very high negative value.[23](Figure 1) This threshold was already described by Barragan et al. in their prospective study and was later confirmed by other investigators.[16, 19, 24] In fact, in these studies, patients with a VASP index below 50% had a very low rate of thrombotic event after PCI. On the contrary, those with a VASP index above 50% had a high event rates after PCI. More recently, Price et al. similarly described a very high negative predictive value of post-treatment PR using the VerifyNow P2Y₁₂ assay in predicting thrombotic events including death of cardiac origin, myocardial infarction, and stent thrombosis for patients undergoing PCI.[20]

Together, these data suggest that post-treatment PR may reliably predict thrombotic events in patients undergoing PCI with stent implantation and that a threshold of PR inhibition may exist to prevent them.

HOW TO MANAGE PATIENTS WITH LOW RESPONSE TO CLOPIDOGREL

Clopidogrel dosage

The Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect (ISAR-CHOICE) trials has demonstrated that increasing the LD from 300 to 600 mg provided a greater inhibition of PR, thus reducing the frequency of clopidogrel low response.[25] Moreover, increasing drug doses from 300 to 600 mg reduces the time required to reach the steady-state from 6 to 2 hours. Interestingly, together with its efficacy on low response, the increased LD was shown to be beneficial on myocardial enzyme release and thrombotic events post-PCI.[26, 27] These data suggest that increasing clopidogrel LD

decreases the number of patients with low response and may translate into a reduction of the risk of thrombotic events. However using a 600 mg loading dose does not overcome the inter-individual variability in the response to clopidogrel and some patients are still considered as low responders. Of importance is that the use of single doses >600 mg was not associated with further PR inhibition because of failed increase in plasma concentration of the active metabolite, suggesting that intestinal absorption may be limited. Because of this limitation, recent studies have used repeated bolus of 600 mg of clopidogrel given at 24-hour intervals to further decrease PR. In two recent trials such strategy achieved a greater PR inhibition than a single bolus of 600 mg of clopidogrel thus overcoming the intestinal absorption limit.[28, 29] In addition we have recently demonstrated that giving an additional bolus of 600 mg of clopidogrel every 24 hours according to PR monitoring was particularly efficient to sensitize patients with low response to a first loading dose.[28](Figure 2) Of importance in this study, despite the use of up-to 2400 mg of clopidogrel, 15% of these patients did not reach a PR <50%, which was used to define a good response. These findings demonstrate that low response could be surmounted by dose adjustment through repeated dose of clopidogrel in the vast majority of cases and that PR monitoring could be used to guide the dose required to obtain a certain degree of PR inhibition in each patients. However, we have observed that some patients do not respond to the drug despite very high doses of clopidogrel which is consistent with a ceiling effect.

According to these data, patients could be divided into 3 groups depending on their response to clopidogrel: 'good responders' who achieve a high degree of PR inhibition (VASP <50 %) after a single 600-mg loading dose; 'low responders' who exhibit a low response (VASP > 50%) to the first 600-mg LD but reach a high level of PR inhibition (VASP <50 %)

with repeated LD; and 'resistant' who have little or no response (VASP >50 %) despite the use of up to 4 bolus of 600-mg of clopidogrel.

New P2Y₁₂ ADP receptor antagonists

New P2Y₁₂ ADP receptor antagonists, including AZD6140, cangrelor, and prasugrel, are in development in order to overcome the inter-individual variability in clopidogrel response.

Cangrelor and AZD6140 have both demonstrated a faster and greater PR inhibition and are currently being compared to standard clopidogrel therapy in clinical studies.[30, 31]

Prasugrel, a third generation thienopyridine, is a pro-drug which requires activation in the liver.

It is associated with a faster and greater PR inhibition without large inter-individual variability compared to clopidogrel.[32] In the Evaluation of prasugrel compared with clopidogrel in

patients with acute coronary syndromes: design and rationale for the TRial to assess

Improvement in Therapeutic Outcomes by optimizing platelet Inhibition with prasugrel

Thrombolysis In Myocardial Infarction 38 (TRITON-TIMI 38) trial investigating the safety and efficacy of prasugrel compared to the clopidogrel standard therapy in patients undergoing PCI

for an ACS, prasugrel was associated with less thrombotic events, including stent thrombosis.

However, this beneficial effect resulting from improved PR inhibition was counterbalanced by a

significant increase in major bleeding. Finally, the overall mortality did not differ between

treatment groups.[33] In conclusion, the TRITON-TIMI 38 trial demonstrated that increased PR

inhibition as achieved with prasugrel translates into with a decrease in thrombotic events but at

the cost of increased bleeding in patients undergoing PCI.

Platelet reactivity monitoring of individual response to thienopyridines

As mentioned previously, studies linking low response to clopidogrel and thrombotic events have observed a threshold of PR inhibition to prevent thrombotic events. These findings have given birth to the concept of individual PR monitoring of clopidogrel response. The hypothesis is such that by decreasing the PR under this level in patients with low response, an improved outcome should result. Accordingly, a multicenter, randomized study investigated the clinical impact of LD adjustment of clopidogrel according to PR monitoring in patients with clopidogrel low response after a 600-mg LD requiring PCI. A PR value $>50\%$ as assessed by the VASP index after the 600-mg LD of clopidogrel was considered as low response. All patients with low response to clopidogrel were included in the study. The hypothesis was that by decreasing PR below this cut-off value using an additional LD of clopidogrel and according to platelet monitoring, the rate of post-PCI thrombotic events would be decreased. Patients were therefore randomized to a control group or to a VASP-guided group. In the latter group, up to 3 additional 600-mg LDs of clopidogrel were used in order to obtain a VASP index $<50\%$ before PCI. The group of patients who received a tailored clopidogrel LD according to PR monitoring had a mean PR inhibition of 63% after adjustment which was associated with significantly fewer thrombotic events, including death of cardiovascular origin, myocardial infarction and urgent target vessel revascularization, than the control group during a 1-month follow-up. This improved outcome was mainly driven by a decrease in stent thrombosis. Interestingly, no increase in bleeding was observed in the VASP-guided group compared to the control group. This study suggested that PR monitoring could be use to individually tailor the dose of clopidogrel to be use in each individual in order to reduce the rate of post-PCI thrombotic events without increasing bleedings.[28]

CONCLUSION

The recent literature suggests that when it comes to antiplatelet therapy for patients undergoing PCI and thienopyridines dose, 'one size does not fit all.' In fact, the effect of thienopyridines as measured by platelet assays predicts the outcomes of patients undergoing PCI. Interestingly, studies have suggested a threshold of PR to prevent thrombotic events post-PCI. Further, although enhanced PR inhibition has been associated with reduced thrombotic events, very high levels of PR inhibition result in more bleeding. It is therefore possible that a therapeutic window of PR inhibition for P2Y₁₂ ADP receptor antagonists in PCI exists to prevent thrombotic events without increasing bleeding. However, to date, there is no consensus on the optimal threshold of PR and of the platelet assay that should be used. If the hypothesis of a therapeutic window for P2Y₁₂ ADP receptor antagonist is confirmed, PR monitoring of thienopyridine response could guide the use of P2Y₁₂ ADP receptor antagonist and may offer the promise of an improved clinical outcome after PCI.

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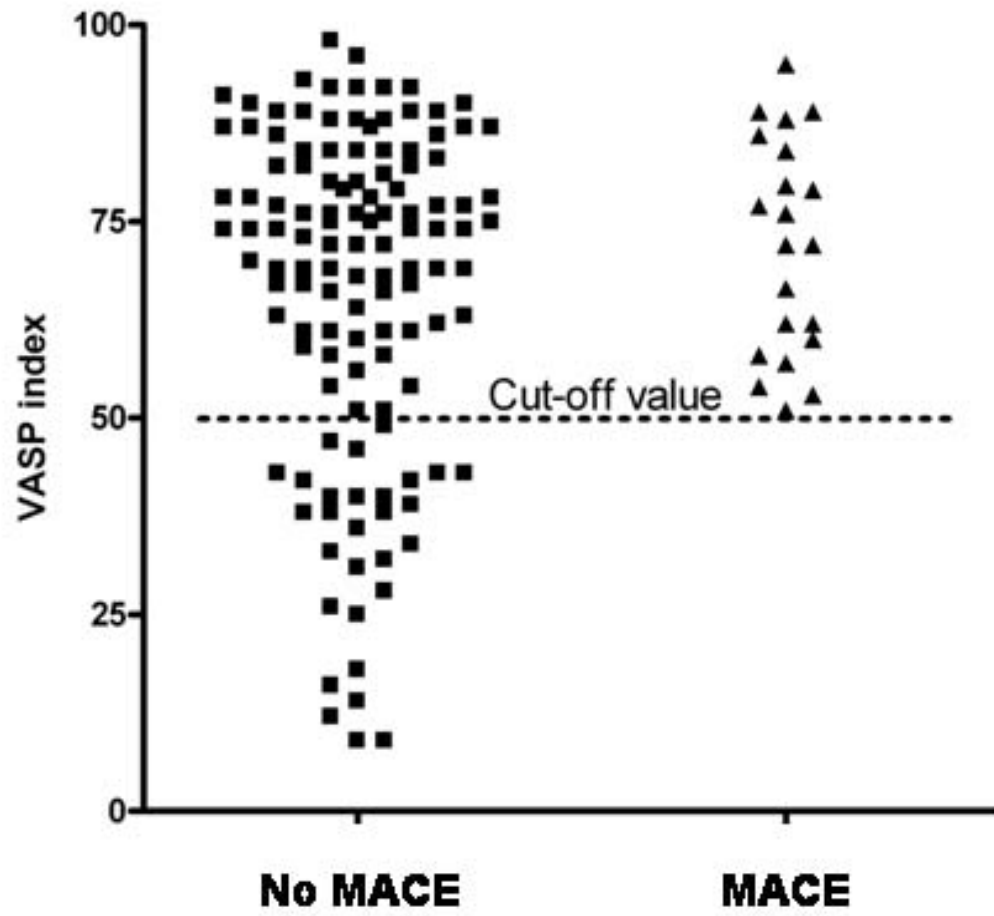
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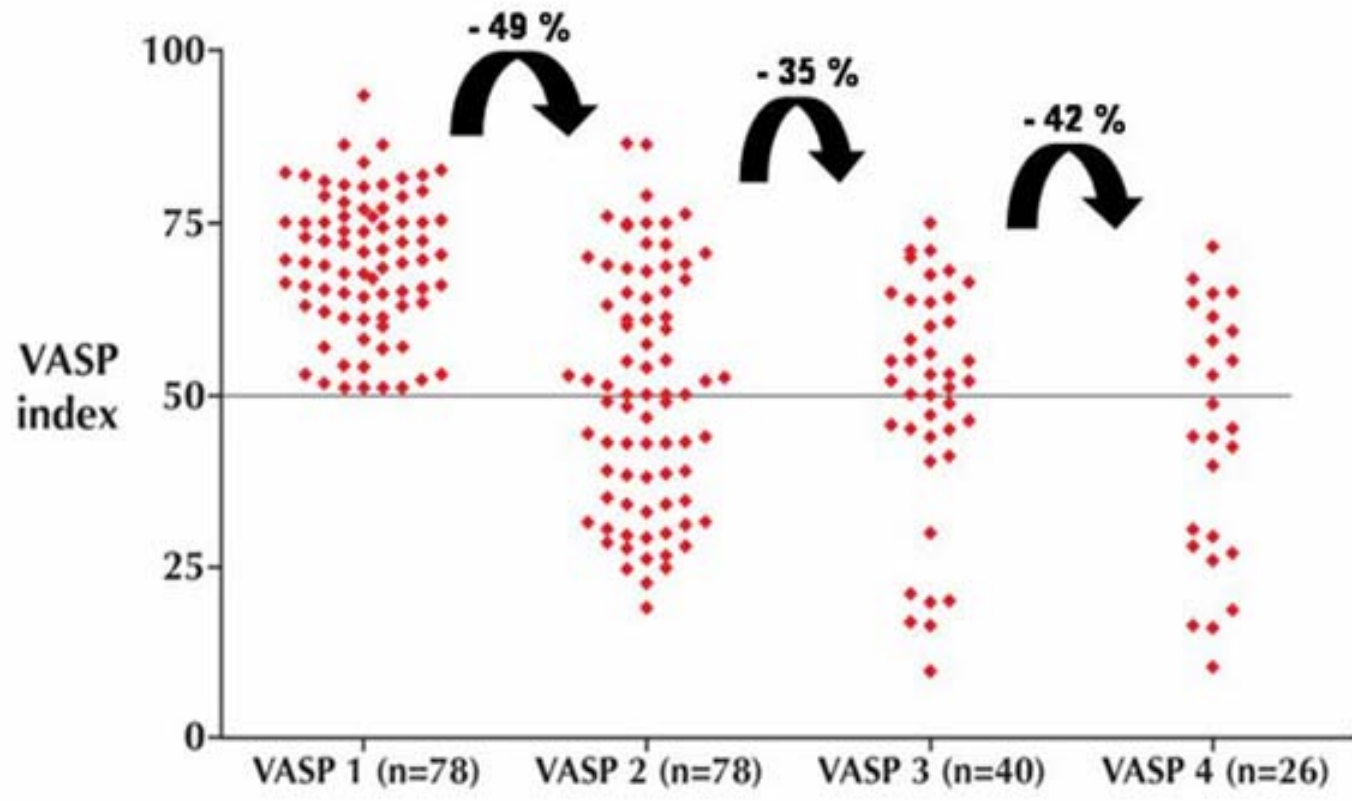
Figure legend

Figure 1: Evidence of a cut-off value of platelet reactivity to predict thrombotic events post-PCI.

All patients suffering a MACE during a 6-month follow-up after PCI had a VASP index $>50\%$ in this monocenter prospective study.[22]

Figure 2: Effect of loading dose repetition in patients with clopidogrel low response using the VASP index. This figure illustrates the impact of an additional 600-mg loading dose in patients with clopidogrel low response after a first 600-mg LD. Each additional bolus decreases the number of patients with low response of 35 to 49%. Finally this strategy was able to overcome low response in 86% of the patients. However, in 15 of the patients despite 2400 mg of clopidogrel, platelet reactivity remained $>50\%$. (from Bonello et al [27] with permission).





VASP: vasodilator-stimulated phosphoprotein