

Personalized antiplatelet therapy with P2Y₁₂ receptor inhibitors: benefits and pitfalls

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Abstract

Antiplatelet therapy with P2Y₁₂ receptor inhibitors has become the cornerstone of medical treatment in patients with acute coronary syndrome, after percutaneous coronary intervention and in secondary prevention of atherothrombotic events. Clopidogrel used to be the most broadly prescribed P2Y₁₂ receptor inhibitor with undisputable benefits especially in combination with aspirin, but a considerable number of clopidogrel-treated patients experience adverse thrombotic events in whom insufficient P2Y₁₂-inhibition and a consequential high on-treatment platelet reactivity is a common finding. This clinically relevant limitation of clopidogrel has driven the increased use of new antiplatelet agents. Prasugrel (a third generation thienopyridine) and ticagrelor (a cyclopentyl-triazolo-pyrimidine) feature more potent and predictable P2Y₁₂-inhibition compared to clopidogrel, which translates into improved ischemic outcomes. However, excessive platelet inhibition and consequential low on-treatment platelet reactivity comes at the price of increased risk of major bleeding. The majority of randomized clinical trials failed to demonstrate improved clinical outcomes with platelet function testing and tailored antiplatelet therapy, but results of all recent trials of potent antiplatelets and prolonged antiplatelet durations point towards a need for individualized antiplatelet approach in order to decrease thrombotic events without increasing bleeding. This review focuses on potential strategies for personalizing antiplatelet treatment.

Key words: antiplatelet therapy, P2Y₁₂ receptor inhibitors, acute coronary syndromes, platelet reactivity.

Atherothrombosis

Atherosclerotic plaque rupture or erosion is thought to be the initial step in the development of acute coronary syndrome (ACS). At the site of vascular injury (due to plaque rupture) exposed subendothelial matrix recruits and activates platelets [1]. Platelets adhere to exposed collagen and von Willebrand factor (vWF). Via the platelet glycoprotein (GP)-VI receptor and integrin $\alpha 2\beta 1$, collagen can directly bind to and activate platelets, which leads to release of contents from the dense granules to the extracellular surrounding. Dense granules mostly consist of platelet agonists such as adenosine diphosphate (ADP), epinephrine, serotonin, thrombin, thromboxane A₂, which in turn promote aggregation, recruitment, and further activation of circulating platelets. The α -granules contain fibrinogen, factor V and P-selectin. ADP binds to platelet P2Y₁₂ and P2Y₁ receptors and by that amplifies the effect of other agonists such as thrombin [1–3]. Activation induces changes in platelet shape, increase of surface by pseu-

dopodia and secretion of further storage products. In the final step, GP IIb/IIIa is converted into its active form, which binds fibrinogen and vWF, leading to stable platelet aggregates and subsequent thrombus formation [4]. Additionally, the vascular injury exposes tissue factor which initiates the extrinsic clotting cascade and leads to generation of more thrombin and the propagation of the fibrin clot [5].

P2Y₁₂ receptor

The P2Y₁₂ receptor is a member of the P2Y purinergic G protein-coupled receptors (GPCR) family, which is activated by ADP, thromboxane A₂ and the PAR-1 receptor agonists [6, 7]. Activation of the P2Y₁ receptor by ADP initiates a weak and transient phase of platelet aggregation whereas binding of ADP to the P2Y₁₂ amplifies dense granule secretion, expression of P-selectin and platelet aggregation [8]. Further stimulation of the P2Y₁₂ receptor sustains the activation of the GP IIb/IIIa and GP Ia/IIa receptors and stabilization of platelet aggregates [9, 10].

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P2Y₁₂ receptor antagonism

Combination of aspirin with P2Y₁₂ receptor antagonists has been proven in a multitude of trials to have a favourable synergistic effect in patients after coronary stent implantation [11]. To date, ticlopidine, clopidogrel, prasugrel, ticagrelor and an intravenous compound, canagrelor, have been approved by the Food and Drug Administration (FDA) [1, 12, 13].

Ticlopidine

Ticlopidine, a first-generation thienopyridine, was the first FDA-approved P2Y₁₂ receptor inhibitor in clinical use [14]. It was the first drug that showed a decrease in major cardiovascular events in patients after stroke compared to aspirin or placebo, and in patients after percutaneous coronary intervention (PCI) compared to warfarin-based regimens [15]. Nevertheless, severe side effects like aplastic anaemia and agranulocytosis and slow onset of action limit the use of the compound and have led to the development of clopidogrel [16].

Clopidogrel

Clopidogrel, a second-generation thienopyridine-type irreversible inhibitor of the P2Y₁₂ receptor, has a more favourable safety profile compared to the ticlopidine. It is a pro-drug, requiring enteric and hepatic transformation by the cytochrome P450 (CYP) system to exert its antiplatelet effect. After absorption, up to 85% of clopidogrel is hydrolyzed by carboxyesterase-1 to an inactive metabolite, SR26334. The remaining approx. 15% of clopidogrel are metabolized to the active compound, R-130964, in a two-step process *via* formation of 2-oxo-clopidogrel. CYP 2C19

seems to have the most prominent role in this process, with less involvement of CYP2B6, CYP1A2, CYP3A/A5, and CYP2C9 [17, 18] (Figure 1). After administration of a 600 mg clopidogrel loading dose, the maximum achievable inhibition of ADP-induced platelet aggregation of 40–60% is achieved within 2 to 6 h [19].

Next generation P2Y₁₂ inhibitors

Despite the proven benefits of aspirin and clopidogrel, a non-negligible proportion of patients continue to experience recurrent ischemic events. These clinical failures have been attributed to response variability and to a relatively slow onset of action with clopidogrel and have prompted the development of new oral P2Y₁₂ inhibitors. Additionally, it has been shown that a moderate platelet inhibition by clopidogrel is insufficient to suppress an increase in ADP-induced platelet aggregation in the midmorning, in the period when myocardial infarction (MI), stroke and sudden cardiac death occur the most frequently [20–23]. Both prasugrel and ticagrelor have shown to have a more consistent, rapid and potent P2Y₁₂ receptor inhibition than clopidogrel, which translated into reduction in the ischemic events at the costs of bleeding events [12, 24–29].

Prasugrel

Prasugrel is a third generation thienopyridine, which acts as an irreversible inhibitor of the P2Y₁₂ receptor. Like clopidogrel, prasugrel is a pro-drug and requires hepatic bioactivation. The active metabolite is formed in a single-step oxidation via various CYP isoenzymes (CYP3A4/5, CYP2B6, CYP2C19, CYP2C9) [30] (Figure 1).

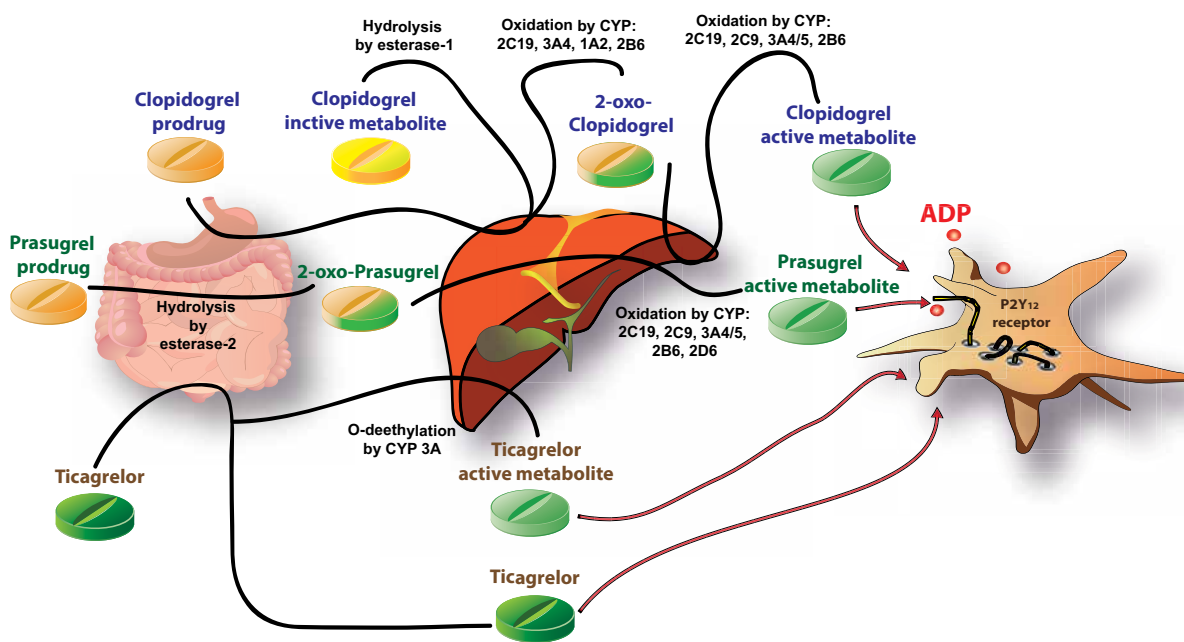


Figure 1. Metabolism of P2Y₁₂ receptor inhibitors

ADP – adenosine diphosphate, CYP – cytochrome 450.

It's worth noting that the known functional genetic CYP variants do not significantly affect formation of the active metabolite of prasugrel, that is faster and more efficient resulting in greater *in vivo* antiplatelet potency compared to clopidogrel [31, 32].

Ticagrelor

Ticagrelor, a cyclopentyl-triazolo-pyrimidine, is an oral antagonist of the P2Y₁₂ receptor, and unlike clopidogrel and prasugrel it is an active, noncompetitive antagonist of the P2Y₁₂ receptor. As an active drug ticagrelor does not require hepatic bioactivation, but has a metabolite (AR-C124910XX) formed by metabolism via CYP3A4, with also anti-aggregatory effects [33] (Figure 1). Genetic factors including *CYP2C19* and *ABCB1* polymorphisms do not influence the clinical outcome of ticagrelor-treated patients [34]. Ticagrelor is active immediately after oral administration, which results in a more rapid onset of action and a more pronounced platelet inhibition compared to clopidogrel [35].

The unprecedented mortality benefits observed in the PLATO trial, despite only a moderate decrease in the occurrence of MI, led to a hypothesis that ticagrelor therapy was associated with off-target effects [36]. Since P2Y₁₂ receptors were identified on vascular smooth muscle cells (VSMCs), we and others have earlier demonstrated in animal and human models that ticagrelor, but not clopidogrel and prasugrel, prevents ADP-induced VSMC contraction [37]. Additionally, other groups have demonstrated that ticagrelor inhibited the uptake of adenosine by human erythrocytes [38] and also induced the release of adenosine triphosphate from human erythrocytes, that is, followed by its degradation to adenosine [39]. The former mechanism was proposed to explain the enhancement of adenosine-induced increase in coronary blood flow observed in a canine model by ticagrelor [38].

High on-treatment platelet reactivity

In clinical practice, antiplatelet drugs are administered to patients at standard doses, without monitoring their pharmacological response as it is done in case of warfarin therapy guided by INR-control [40]. This fixed-dose or better "one size fits all" approach with clopidogrel therapy is a remnant of clinical trials and does not take the inter-individual pharmacodynamic variability of ADP-pathway inhibitors into account [41]. Starting in 2003, studies suggested that the level of platelet inhibition, especially by clopidogrel, considerably varies between patients [41, 42].

Dependent on the assay used and the population studied, up to 25–50% of clopidogrel-treated patients fail to show adequate pharmacological response to clopidogrel and are not adequately protected from major adverse cardiac events (MACE) [43–45]. There is robust data showing an association between clopidogrel non-

responsiveness or high on-treatment platelet reactivity (HPR) and adverse ischemic events, with the strongest association for short-term thrombotic events, like acute and subacute stent thrombosis, in patients after PCI [42, 46–55]. Nevertheless, the routine measurement of platelet reactivity has not been widely implemented and recommended in the guidelines. At least 40 studies demonstrated that ADP-induced platelet function testing is the best predictor of ischemic events in clopidogrel non-responders [54, 56–58]. Likewise, measurement of platelet function by light transmission aggregometry (LTA) in patients undergoing coronary stenting might predict adverse events [59–62]. Other tests, including the new generation impedance aggregometry test (Multiplate, MEA), VerifyNow™ and the vasodilator stimulated phosphoprotein (VASP) phosphorylation assay, have confirmed the association between poor clopidogrel responsiveness and increased risk of cardiac ischemic events during short- and long- term follow-up [46, 48, 63–69]. Nevertheless, lack of consensus concerning optimal method to quantify HPR and the best cut-off value associated with clinical risk has hindered the consideration of platelet function testing (PFT) in clinical guidelines. However, a recent analysis involving more than 20,000 patients after PCI tested uniformly-defined cutoff values for three relatively well-standardized assays (MEA, VerifyNow and VASP) and identified sharp cut points for HPR that were highly significant predictors of stent thrombosis and cardiovascular mortality [70].

Although new platelet aggregation inhibitors were invented to overcome HPR, it has been shown that this phenomenon is not exclusively true for clopidogrel treatment. In the acute phase of ST-elevation myocardial infarction (STEMI) 37% of the patients treated with prasugrel and 46% treated with ticagrelor exhibited HPR [69, 71–76]. Interestingly, a recent study has indicated that 12% of prasugrel-treated patients presented with the HPR phenotype [77], which might be explained by the fact that 43% of included patients displayed HPR under clopidogrel treatment and were switched to prasugrel [77]. Similarly, the TAILOR study as well as randomized trials in haemodialysis patients have shown that up to 20% of patients continued to exhibit HPR despite switch from clopidogrel to prasugrel [73, 74, 78]. Noteworthy, in the MADONNA study, direct switch to prasugrel from clopidogrel was associated with a satisfactory level of platelet inhibition by prasugrel in all patients [79]. Not surprisingly, however, the platelet inhibitory effect in patients treated with therapeutic hypothermia after cardiac arrest was reduced in prasugrel and ticagrelor treated patients reaching an incidence of HPR of 32% and 30%, respectively [80]. Interestingly, some studies indicated that in ACS patients with HPR while on clopidogrel ticagrelor produced stronger platelet inhibition compared with prasugrel [81]. Noteworthy, switching from ticagrelor main-

tenance dose to prasugrel maintenance dose was associated with a lower level of platelet inhibition as compared to continued ticagrelor therapy [82]. Nevertheless, at day 7 there was no difference in HPR frequency between the prasugrel and ticagrelor groups [82]. In ACS patients one month after the event ticagrelor was more effective than prasugrel in a pharmacodynamic study [83]. Interestingly, the antiplatelet activity of ticagrelor's active metabolite was more potent compared to ticagrelor or prasugrel's active metabolite in both humans and nonhuman primates [84].

Therefore, although the rate of HPR is lower with novel P2Y₁₂-inhibitors, it is not an exclusive feature of clopidogrel. While clinical, genetic and demographic variables associated with HPR in clopidogrel-treated subjects are well defined and the worse clinical course in such patients is unquestionable, such factors and clinical impacts should be investigated and clarified in future trials in case of prasugrel and ticagrelor.

Clinical factors associated with HPR

Drug-drug interactions, obesity, renal dysfunction, diabetes mellitus (DM), higher age, reduced left ventricular function, inflammation and the presence of an ACS are all associated with inadequate response to clopidogrel therapy and consequential HPR [85–90]. For further risk stratification, it has been suggested to use scoring systems that can integrate clinical risk factors and genetic variants identified by path models [91, 92].

Drug-drug interactions

It has been shown that certain proton pump inhibitors such as omeprazole, calcium channel blockers, ke-

toconazole and rifampicin may significantly influence clopidogrel metabolism [87, 93–102]. Interestingly, morphine co-medication in ACS is a strong predictor of HPR in patients treated with clopidogrel, prasugrel and ticagrelor. This effect might be partially explainable by the impaired or delayed absorption of those drugs induced by opioid-induced gastroparesis [76, 103, 104].

Diabetes mellitus

Among all clinical risk factors accounting for HPR, DM has a unique position. DM is a strong independent predictor of short-term and long-term recurrent ischemic events and mortality in the ACS setting [105]. The reported negative impact on mortality includes all ACS subtypes and especially the increased risk for short-term ischemic events suggests an important role of platelet activation-aggregation.

It is well evidenced that platelets of DM patients exhibit an increased reactivity, caused by dysregulation of several signalling pathways by hyperglycaemia, insulin resistance, metabolic conditions and inflammation [106].

Direct insulin effect on platelets

In general, insulin exhibits anti-aggregatory effects and this antithrombotic effects are diminished in diabetic patients [107]. Insulin can exhibit direct anti-aggregatory effect via attenuation of the thrombin-induced Ca²⁺ response and the release of ADP as well as inhibition of the P2Y₁₂ receptor [108, 109]. Furthermore, it enhances the platelet inhibitory effects of prostaglandin (PG) E1 and I2 (Figure 2) [110].

Hyperglycaemia

Hyperglycaemia has been shown to increase platelet reactivity in various ways (Figure 3). It induces P-selectin expression, alters membrane fluidity with subsequent platelet adhesion and activates protein C [106, 111]. In DM patients with ACS, glucose lowering therapy is proven beneficial independent of the treatment strategy [112, 113].

Insulin deficiency and resistance

Both insulin receptors and the insulin-like growth factor-1 (IGF-1) are expressed on thrombocytes. Binding of insulin to the platelets' insulin receptor increases surface expression of adenylate cyclase-linked prostacyclin receptor, but due to the low Insulin receptor expression, the effect is negligible. IGF-1 is stored in the α-granules of platelets, which may contribute to the amplification of platelet aggregation after alpha granule release [114]. Other mechanisms how insulin resistance affect platelet aggregation include increased intracellular calcium with chanced platelet degranulation, impaired response to prostacyclin and nitric oxide [115, 116].

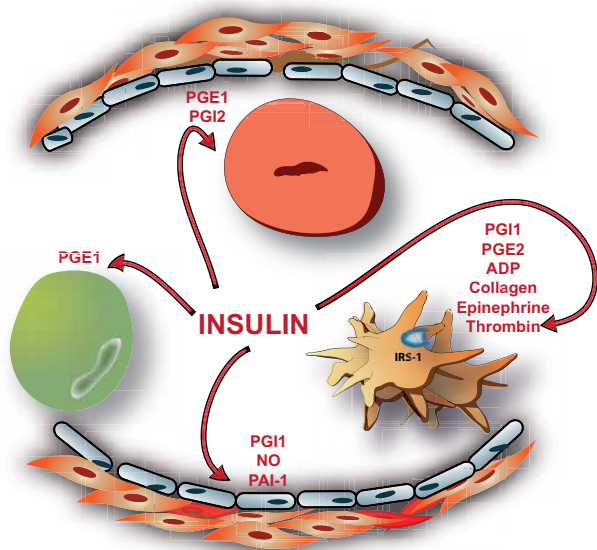


Figure 2. Effects of insulin on blood cells

PGE1 – prostaglandin E1, PGI2 – prostaglandin I2, NO – nitric oxide, ADP – adenosine diphosphate, PAI-1 – plasminogen activator inhibitor 1.

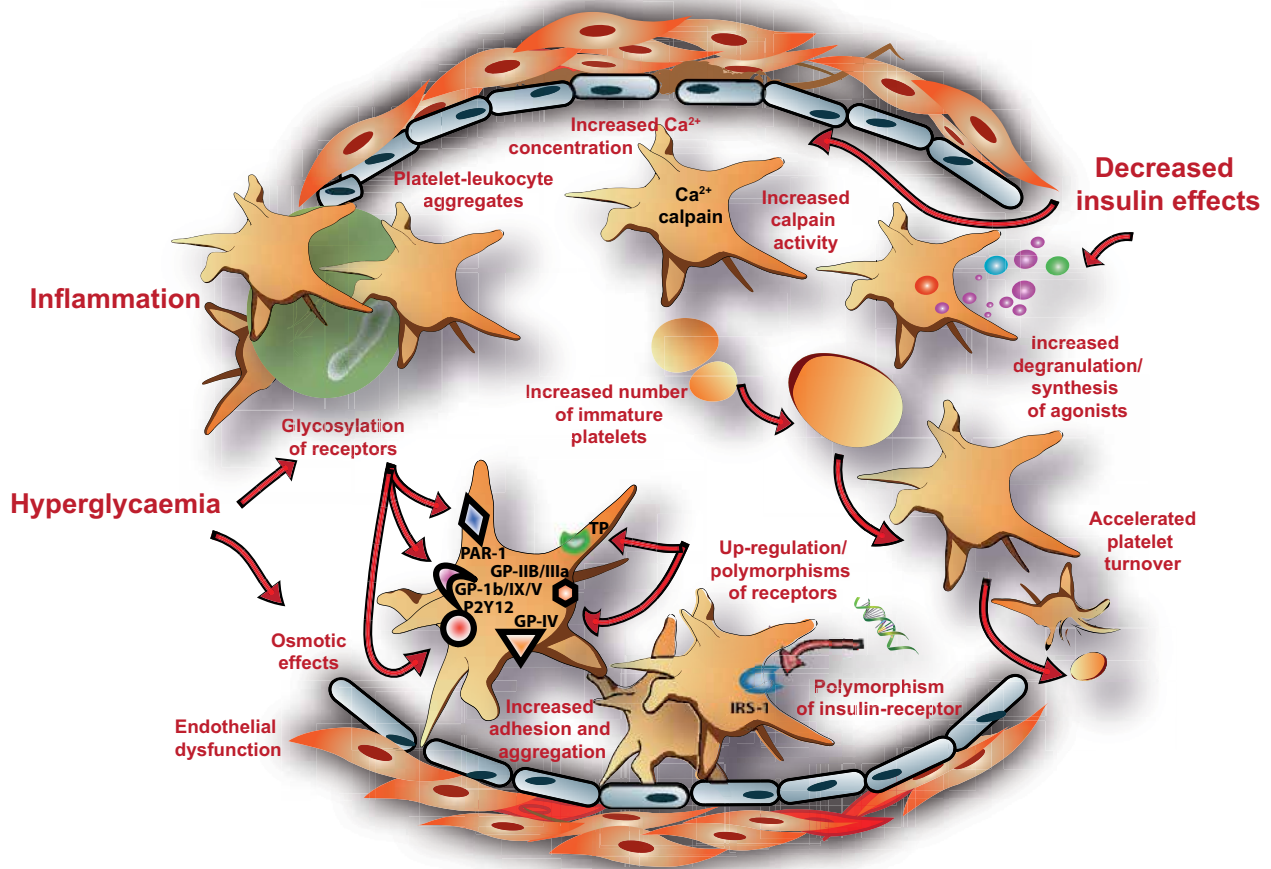


Figure 3. Effects of hyperglycaemia on platelets

Metabolic conditions

The DM is often accompanied by obesity, dyslipidemia, and enhanced systemic inflammation, every single one of which may contribute to the increased platelet reactivity. Obesity may enhance platelet aggregation via similar pathomechanisms as insulin resistance: higher mean platelet volume, high blood leptin, increased intracellular calcium concentration [106].

HPR in diabetic patients

A multitude of trials have proven the benefit of clopidogrel in combination with aspirin in post-ACS DM patients. Nevertheless, HPR under clopidogrel therapy is more prevalent in diabetic compared with non-diabetic patients, especially in those requiring insulin therapy [105, 107]. Similar factors leading to increased platelet reactivity in DM patients also cause HPR to clopidogrel. To date, only small *in vitro* and *ex vivo* studies have identified the following factors to cause HPR: lack of response to insulin in platelets, changes in calcium metabolism, P2Y₁₂ receptor signalling upregulation, increased exposure to ADP, and increased platelet turnover [106, 117, 118].

Another interesting mechanism for impaired P2Y₁₂ inhibition mediated by clopidogrel among DM patients has been linked to attenuation of clopidogrel's pharmacokinetics, which was characterized by lower plasma levels of clopidogrel active metabolite as compared with non-diabetic patients [119].

Low on-treatment platelet reactivity and the therapeutic window concept

Some studies postulated that there might be a therapeutic window for P2Y₁₂ receptor blockers, indicating that while HPR is associated with thrombotic events, low on-treatment platelet reactivity (LPR) may be related to bleeding events [61, 64, 65, 120–122]. The two sides of the coin regarding P2Y₁₂-inhibition, i.e. higher risk for thrombosis in HPR and higher risk for bleeding in LPR suggest that a sweet spot may exist for P2Y₁₂-inhibition. Validation of such therapeutic window with patients having optimal platelet reactivity was recently reported in a collaborative analysis including more than 20,000 patients [70]. According to the results, patients with LPR had an absolute 1.2% lower risk for stent thrombosis and

2.7% lower risk for bleeding, compared to HPR and LPR, respectively [70].

Test systems used for assessment of the effect of antiplatelet drugs

The effect of clopidogrel on platelet function can be measured by platelet function testing and corresponds to the phenotype of its response. There are several test systems available for monitoring the effect of antiplatelet drugs, all characterizing different pathways of platelet activation, unfortunately with no option to reflect the complexity of platelet biology.

Platelet aggregometry

Platelet aggregometry is based on the stimulation of platelet aggregation with different agents. There are two commercially available techniques: optical and impedance aggregometry.

Light transmission aggregometry

Light transmission aggregometry (LTA) used to be the most widespread platelet function test. P2Y₁₂ receptor inhibition is measured by adding ADP and the change in the light transmittance is recorded. The maximal aggregation and the final aggregation responses can be measured and expressed as percentage (Table I). The widespread use and the reported good correlation between the measured aggregation responses and adverse events are the most important advantages of optical aggregometry. Time-consuming centrifugation steps as well as the large sample volume needed and variable reproducibility make this test less favourable. The proposed cutoff for HPR is > 70% of the maximal ADP-induced aggregation, but as LTA is not standardized according to the concentration of agonist, centrifuging time and speed, this sharp cut point is not generalizable for different centers. LTA is able to predict ischemic events with a sensitivity between 60–79%, with a specificity of 59–82% and an area under the receiver operating curve (AUC) of 0.73–0.85, and an odds ratio (OR) for ischemic events in the range of 3–35 [52, 56, 59, 123–125]. Additionally, LTA has been shown to predict stent thrombosis and bleeding events [47, 49, 51, 52].

Impedance aggregometry: multiple electrode aggregometry

Multiple electrode aggregometry (MEA) is measuring whole blood platelet aggregation. ADP is used as agonist and, depending on the test, the antagonist PGE1 may be added (high sensitivity ADP test (ADP-HS)). Changes in the electrical impedance caused by adhesion and aggregation of platelets on two independent electrode-set surfaces is measured and expressed as U (units) [100, 126, 127]. The cut-off values to separate patients with HPR in prior studies were around 46–50 U (Table II) [100, 126, 127].

MEA can predict stent thrombosis quite effectively (OR: 9–37; AUC: 0.78–0.92; sensitivity: 70–90% and specificity: 84–100%) [46, 48, 63]. Similarly to LTA, MEA has been shown to predict major bleedings (AUC: 0.61–0.74; sensitivity: 72–77% and specificity: 62–66%) [64, 65, 128, 129].

VASP phosphorylation

Measurement of VASP phosphorylation, that is a second messenger in one of the intracellular signalling pathways downstream of the P2Y₁₂ receptor, forms the basis of this assay (BioCytex, Marseille, France) [130, 131]. Serine 239-phosphorylated VASP is labelled with a monoclonal antibody followed by a secondary fluorescein isothiocyanate (FITC)-conjugated polyclonal goat-anti-mouse antibody and then measured using a flow cytometer. Platelet reactivity is expressed as platelet reactivity index (PRI%). Due to this unique technique, the VASP assay is highly reproducible even after 24 h of sample storage [90]. The VASP assay is the most specific assay for P2Y₁₂ signalling, because it evaluates the extent of P2Y₁₂ receptor inhibition without influencing the P2Y₁ receptor with agonist. The cut-off for VASP to separate patients with HPR is 50% (70) PRI [93]. A positive VASP test result corresponded to an OR = 1–23 [124, 131] to develop a stent thrombosis or MACE (AUC: 0.55–0.79) with high sensitivity (70–100%) [48, 132] but low specificity (25–37%) (Table III).

VerifyNow™

The VerifyNow™ assay (Accumetrics, San Diego, USA) measures the agonist-induced activation of platelets and their binding to fibrinogen-coated polystyrene beads. Once the platelets have bound to the beads, the platelet-bead complexes fall out of the solution and infrared-light transmittance increases. The assay uses ADP as agonist and PGE1 as antagonist and results are reported as P2Y₁₂ reaction units (PRU) [133]. Beside the higher costs, the VerifyNow™ test shares the same advantages as the MEA, such as whole blood test condition, fast preparation time and small blood volume requirement. Although prior studies suggested 235 PRU to separate patients with HPR, data from the largest meta-analysis [70] and a sub-analysis of the GRAVITAS study suggest the benefit of a lower cutoff, 208 PRU (Table IV). VerifyNow™ has been shown to predict MACE (OR = 1–6.5; AUC: 0.56–0.87, sensitivity: 60–80% and specificity: 63–92%; Table IV) and major bleeding events (OR = 0.94; AUC = 0.84, sensitivity: 81% and specificity: 80%) [120, 134, 135] (Table IV).

Platelet Function Analyzer (PFA-100™)

The PFA-100™ (Dade Behring, Marburg, Germany) measures the time required for occlusion of a capillary tube by platelet aggregates (closure time – CT) under high shear rates (5000–6000 s⁻¹). To measure the effect of ADP antagonists, the membrane is coated with col-

Table 1. Studies investigating the association of ischemic or bleeding events and clopidogrel response with use of light transmission aggregometry (LTA)

Study author/acronym	Method:agonist	N	Population	Follow-up	Outcome	OR/HR	Cut-off value (%)	Prevalence of HPR/LPR (%)	AUC	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Thrombosis													
Matetzky <i>et al.</i> [42]	LTA:ADP	60	PCI + STEMI	6 months	MACE	6.00	103	25					
Gurbel <i>et al.</i> [56]	LTA:ADP	297	Elective PCI	2 years	MACE	3.90	46	30	0.77			63	82
CREST [53]	LTA:ADP	100	History of ST vs. no ST				42	60					
PREPARE-POST STENTING [123]	LTA:ADP	192	Elective PCI	6 months	MACE	2.70	67	25				37	79
CLEAR PLATELETS [62]	LTA:ADP	120	Elective PCI	In hospital	Periprocedural MI		50						
CLEAR PLATELETS-2 [58]	LTA:ADP	200	Elective PCI	In hospital	Periprocedural MI		40						
Frere <i>et al.</i> [124]	LTA:ADP	195	NSTE-ACS + PCI	1 month	MACE	8.00	70	27	0.74	21	98	79	76
Cuisset <i>et al.</i> [52]	LTA:ADP	598	NSTE-ACS + PCI	1 month	ST	5.80	67		0.70	4	99	70	68
Cuisset <i>et al.</i> [187]	LTA:ADP	106	NSTE-ACS + PCI	1 month	MACE	22.40	70	25					
Cuisset <i>et al.</i> [54]	LTA:ADP	190	NSTEMI + PCI	In hospital	Periprocedural MI	1.80	70	22					
POPULAR [59]	LTA:ADP	1049	Elective PCI	1 year	MACE	2.09	43	42	0.73	12	94	60	59
POPULAR [188]	LTA:ADP	921	Elective PCI	1 year	MACE	2.65	43	15					
Bliden <i>et al.</i> [125]	LTA:ADP	100	Elective PCI	1 year	MACE	34.60	50	22	0.86	73	91		
Lev <i>et al.</i> [57]	LTA:ADP	150	Elective PCI	In hospital	Myonecrosis	1.87	70	24					
Gori <i>et al.</i> [51]	LTA:ADP	746	PCI + DES	6 months	ST	3.15	70	12					
Geisler <i>et al.</i> [189]	LTA:ADP	379	PCI	3 months	MACE, death	4.90	70	6					
Geisler <i>et al.</i> [47]	LTA:ADP	1019	PCI	3 months	ST	2.21	42.5	33					
Geisler <i>et al.</i> [86]	LTA:ADP	1092	PCI	1 month	MACE	1.71	47	33					
EXCELSIOR [60]	LTA:ADP	802	Elective PCI	1 month	MACE	6.70	32	25					
EXCELSIOR [190]	LTA:ADP	797	Elective PCI	1 year	MACE	3.0	14	27					
Buonamici <i>et al.</i> [49]	LTA:ADP	804	PCI + DES	6 months	ST	3.08	70	13					
Migliorini <i>et al.</i> [50]	LTA:ADP	215	PCI	3 years	CD, ST	3.82	70	19					
Wang <i>et al.</i> [191]	LTA:ADP	386	Elective PCI + DES	1 year	MACE	2.44	10 difference	17					
Wenaweser <i>et al.</i> [192]	LTA:ADP	82	History of ST vs. no ST	Case/control			10 difference						
RECLOSE 2-ACS [61]	LTA:ADP	1789	ACS + PCI	2 years	MACE	1.49	70	16		15	91		
Bleeding													
Parodi <i>et al.</i> [61]	LTA:ADP	298	PCI + prasugrel	6 months	TIMI major bleeding	0.91	40	32					
Chen <i>et al.</i> [193]	LTA:ADP	45	Surgery under clopidogrel		Blood transfusion		40						

ADP – adenosine diphosphate, AUC – area under the curve (of the receiver operating curve – c-index), PPV – positive predictive value, NPV – negative predictive value, HPR – high platelet reactivity (prevalence is given for studies investigating thrombotic events), LPR – low platelet reactivity (prevalence is given for studies investigating bleeding events), PCI – percutaneous coronary intervention, NSTE-ACS – non ST-elevation acute coronary syndrome, STEMI – ST-elevation myocardial infarction, MACE – major adverse cardiac events, MI – myocardial infarction, ST – stent thrombosis, DES – drug eluting stent, CD – cardiac death, TIMI – thrombolysis in myocardial infarction, NS – not significant.

Table II. Studies investigating the association of ischemic or bleeding events and clopidogrel response with use of Multiplate Electrode Aggregometry (MEA)

Study author/ acronym	Method:agonist	N	Population	Follow-up	Outcome	OR/HR	Cut-off value	Prevalence of HPR/LPR (%)	AUC	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Thrombosis													
Sibbing <i>et al.</i> [46]	MEA:ADP	1608	Elective PCI	1 month	ST	9.40	468AU*min = 47 U	20	0.78			70	84
Sibbing <i>et al.</i> [64, 65]	MEA:ADP	2533	Elective PCI	1 month	ST	0.40	468AU*min = 47 U	17					
Eshteherdi <i>et al.</i> [66]	MEA:ADP	219	PCI	1 month	MACE		309AU*min = 31 U	15					
Müller-Schunk <i>et al.</i> [194]	MEA:ADP	50	Neurointerventional stent		ST + TIA/ stroke		52 U	28					
Siller-Matula <i>et al.</i> [195]	MEA:ADP	403	PCI	1 year	MACE	1.75	48 U	19	0.60				
PEGASUS-PCI [63]	MEA:ADP	416	PCI	1 year	ST, MACE		46 U	38	0.78	7	100	70	67
Dineva <i>et al.</i> [67]	MEA:ADP	603	PCI	1 month	ST	24.3	46 U	18	0.86			84	78
Siller-Matula <i>et al.</i> [48]	MEA:ADP + PGE1	416	PCI	6 months	ST		54 U	14	0.92	5	100	86	100
PEGASUS-PCI [63]	MEA:ADP + PGE1	416	PCI	1 year	ST, MACE	36.9	48 U	19	0.90	13	100	90	83
Bleeding													
Rahe-Meyer <i>et al.</i> [128]	MEA:ADP	60	Cardiac surgery	In hospital	Blood transfusion		13 U	33	0.74			77	63
Sibbing <i>et al.</i> [64, 65]	MEA:ADP	2533	PCI	In hospital	TIMI major bleeding	3.50	188AU*min = 19 U	38	0.61	2	99	62	62
Ranucci <i>et al.</i> [129]	MEA:ADP	87	Thienopyridine treatment	In hospital	Postoperative bleeding		31 U	40	0.71	29	92	72	66
PEGASUS-PCI [63]	MEA:ADP + PG	416	PCI	1 year	TIMI major bleeding	Ns	20 U						

ADP – adenosine diphosphate, PGE1 – prostaglandin E1, AUC – area under the curve (of the receiver operating curve – c-index), PPV – positive predictive value, NPV – negative predictive value, HPR – high platelet reactivity (prevalence is given for studies investigating thrombotic events), LPR – low platelet reactivity (prevalence is given for studies investigating bleeding events), PCI – percutaneous coronary intervention, MACE – major adverse cardiac events, ST – stent thrombosis, TIA – transient ischemic attack, TIMI – thrombolysis in myocardial infarction, NS – not significant.

Table III. Studies investigating the association of ischemic or bleeding events and clopidogrel response with use of vasodilator activated phosphoprotein assay (VASP)

Study author/acronym	Method:agonist	N	Population	Follow-up	Outcome	OR/HR	Cut-off value (%)	Prevalence of HPR/LPR (%)	AUC	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Thrombolysis													
Bonello <i>et al.</i> [132]	VASP assay	144	PCI	6 months	MACE		50	20	0.55	100	100	100	25
Bonello <i>et al.</i> [69]	VASP assay	301	ACS + PCI + prasugrel	1 month	MACE	23.0	53.5	25	0.86	92	100	88	77
Sillier-Matula <i>et al.</i> [48]	VASP assay	416	PCI	6 months	ST	NS	42	63	0.60	1	100	100	37
PEGASUS-PCI [63]	VASP assay	416	PCI	1 year	ST, MACE	NS	42	62	0.62	3	98	70	38
Blindt <i>et al.</i> [131]	VASP assay	99	PCI at high ST risk	6 months	ST	1.16	48		0.79			80	73
Frere <i>et al.</i> [124]	VASP assay	195	NSTE-ACS + PCI	1 month	MACE	11.18	53	54	0.73	12	99	93	50
Barragan <i>et al.</i> [196]	VASP assay	46	History of ST vs. no ST	1 month	ST		50						
Cuisset <i>et al.</i> [52]	VASP assay	598	NSTE-ACS + PCI	1 month	ST	NS			0.61				
WILMAA [68]	VASP assay	300	PCI	6 months	MACE	1.04	60	62	0.68	8	99	94	37
Bleeding													
Cuisset <i>et al.</i> [197]	VASP	597	NSTEMI + PCI	1 month	TIMI bleeding non-CABG related		40	25					
Mokhtar <i>et al.</i> [121]	VASP	346	PCI	In hospital	TIMI major bleeding non-CABG	0.96							
Michelson <i>et al.</i> [71]	VASP	125	ACS + PCI	> 3 days after PCI	Serious bleedings	0.97	50						

AUC – area under the curve (of the receiver operating curve – c-index), PPV – positive predictive value, NPV – negative predictive value, PCI – percutaneous coronary intervention, NSTE-ACS – non ST-elevation acute coronary syndrome, MACE – major adverse cardiac events, ST – stent thrombolysis, TIMI – thrombolysis in myocardial infarction, CABG – coronary artery bypass graft, HPR – high platelet reactivity (prevalence is given for studies investigating thrombotic events), LPR – low platelet reactivity (prevalence is given for studies investigating bleeding events), NS – not significant.

Table IV. Studies investigating the association of ischemic or bleeding events and clopidogrel response with use of VerifyNow assay

Study author/acronym	Method:agonist	N	Population	Follow-up	Outcome	OR/HR	Cut-off value	Prevalence of HPR/LPR (%)	AUC	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Thrombosis													
Price <i>et al.</i> [134]	VerifyNow	380	PCI	6 months	MACE	6.50	235 PRU	32	0.71	99	99	78	68
Campo <i>et al.</i> [120]	VerifyNow	300	PCI	1 year	MACE	1.02	239 PRU	13	0.87	43	98	81	92
Park <i>et al.</i> [198]	VerifyNow	2849	PCI + DES	2.2 years	MACE	NS	235 PRU	58					
Marcucci <i>et al.</i> [135]	VerifyNow	683	ACS + PCI	1 year	MACE	2.52	240 PRU	32	0.66	12	96	61	70
POPULAR [59]	VerifyNow	1055	Elective PCI	1 year	MACE	2.53	236 PRU	38	0.62	13	94	60	63
POPULAR [188]	VerifyNow	422	Elective PCI	1 year	MACE	2.5	236 PRU	25					
Cuisset <i>et al.</i> [199]	VerifyNow	120	Elective PCI	In hospital	Periprocedural MI	4.60	< 15% inhibition	25					
ARMYDA PRO [200]	VerifyNow	160	PCI	1 month	MACE	6.10	240 PRU	25	0.56			81	53
TRILOGY ACS Platelet Function Substudy [201]	VerifyNow	2564	ACS treated conservatively	30 months	MACE	NS	208 and 230 PRU	Clopidogrel: 45–55% Prasugrel: 10–15%	0.54 (for 178 PRU)			47	59
Bleeding													
Campo <i>et al.</i> [120]	VerifyNow	300	PCI	1 year	TIMI major bleeding	0.94	85 PRU	25	0.84	21	98	81	80

AUC – area under the curve (of the receiver operating curve – c-index), PPV – positive predictive value, NPV – negative predictive value, HPR – high platelet reactivity (prevalence is given for studies investigating thrombotic events), LPR – low platelet reactivity (prevalence is given for studies investigating bleeding events), PCI – percutaneous coronary intervention, ACS – acute coronary syndrome, MACE – major adverse cardiac events, MI – myocardial infarction, ST – stent thrombosis, DES – drug eluting stent, TIMI – thrombolysis in myocardial infarction, NS – not significant.

lagen/adenosine diphosphate (CADP) or collagen/ADP/PGE1. To date, there is conflicting data concerning the reproducibility of the test [136–138]. The normal value for CADP-CT in treatment of naive patients is 65–120 s [136]. Only small studies revealed the usefulness of the device for prediction of MACE (OR = 3–33) in clopidogrel users [139–142], but other studies found no association with clinical outcomes [59]. A closure time ≤ 72s has a sensitivity of 86%, and specificity of 76% [142] to detect ischemic events (Table V).

Cone and Platelet Analyzer

The Cone and Platelet Analyzer (DiaMed, Cressier, Switzerland) tests thrombocyte adhesion and aggregation under shear stress [143]. Adherent platelets are stained under flow conditions, the percentage of surface coverage (SC) and the average size (AS) of the objects are determined [144]. The variability is relatively low (< 5%). To date, no study could show that the CPA is sensitive enough to predict ischemic events in clopidogrel-treated patients (AUC: 0.53–0.62; Table V).

Plateletworks

The Plateletworks (Helena Laboratories, Beaumont, Texas) is based on counting platelets before and after ADP agonist incubation. The ratio between the aggregated platelets after stimulation and the platelet count in the reference tube is used as the degree of platelet aggregation [59]. One study investigated the predictive value of Plateletworks for ischemic events. In the POPULAR study, the Plateletworks assay predicted the composite of major ischemic events with a sensitivity of 63%, specificity of 59% and the AUC of 0.61 (Table V) [59].

Thrombelastography (TEG)

The TEG haemostasis analyser (Haemoscope Corp., Niles, Illinois) only measures platelet-fibrin clot strength and is therefore insensitive to P2Y₁₂ inhibition and aspirin effect. P2Y₁₂ receptor inhibitions can be measured only in modified protocols (Table V) [123, 125, 127, 145].

Limitations of platelet function testing

It is well known that technical factors, like type of anti-coagulant or agonist used, time delay and pipetting errors, can influence the results of platelet testing [146, 147].

Beside all technical obstacles related to the procedure itself we must not forget that all these *ex vivo* tests do not reproduce the complexity of thrombocyte activation *in vivo*. Moreover, those tests ignore other platelet activating factors during ACS that might influence outcome, such as cytokines or other paracrine factors [148]. Because of this fact, one cannot assume that an *in vitro* observed clopidogrel effect will show the same efficacy *in vivo*, but vice versa one can prove at least the pharmacological efficacy, because if a drug fails to block ADP-in-

Table V. Studies investigating the association of ischemic events and clopidogrel response with use of Cone and Platelet Analyzer (CPA), Plateletworks, Thromboelastography (TAG) or Platelet Function analyser 100 (PFA 100)

Study author/acronym	Method:agonist	N	Population	Follow-up	Outcome	OR/HR	Cut-off value	Prevalence of HPR (%)	AUC (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Thrombosis													
Matetzky <i>et al.</i> [42]	CPA	60	PCI + STEMI	6 months	MACE	6.00	9% difference	25					
POPULAR [59]	CPA	910	Elective PCI	1 year	MACE	NS	8.4%	47	0.56	7	90	56	53
POPULAR [59]	CPA:ADP	905	Elective PCI	1 year	MACE	NS	3%	54	0.53	8	91	44	54
PEGASUS-PCI [63]	CPA:ADP	416	PCI	1 year	ST, MACE	NS	4.6%	61	0.62	3	98	90	36
POPULAR [59]	Plateletworks	606	Elective PCI	1 year	MACE	2.22	80.5%	43	0.61	13	94	63	59
PREPARE-POST STENTING [123]	TEG:ADP	192	Elective PCI	6 months	MACE	22.60	67%	25				74	89
Bliden <i>et al.</i> [125]	TEG:ADP	100	Elective PCI	1 year	MACE	26.80	70%	22	0.88	67	94		
POPULAR [59]	PFA100:CADP	812	Elective PCI	1 year	MACE	NS	116 s	44	0.50	5	93	63	44
PEGASUS-PCI [63]	PFA100:CADP	416	PCI	1 year	ST, MACE	NS	105 s	38	0.66	4	98	70	61
Chiu <i>et al.</i> [141]	PFA100:CADP	144	PCI	2 years	MACE	5.3	95 s						
Campo <i>et al.</i> [142]	PFA100:CADP	135	STEMI + PCI	2 years	MACE	4.5	72 s		0.85			86	76
Gianetti <i>et al.</i> [139]	PFA100:CADP	175	ACS or CAD	6 months	MACE	22.9	82 s	25					
Fuchs <i>et al.</i> [140]	PFA100:CADP	208	ACS	28 months	MACE	3.2	73 s	25					
POPULAR [59]	PFA100: Imo-vance	588	Elective PCI	1 year	MACE	NS	299 s	30	0.56	5	90	61	29

ADP – adenosine diphosphate, CADP – collagen-adenosine diphosphate, HPR – high platelet reactivity, AUC – area under the curve (of the receiver operating curve – c-index), PPV – positive predictive value, NPV – negative predictive value, PCI – percutaneous coronary intervention, ACS – acute coronary syndrome, STEMI-ST – elevation myocardial infarction, MACE – major adverse cardiac events, ST – stent thrombosis, DES – drug eluting stent, CAD – coronary artery disease, NS – not significant.

duced aggregation *in vitro*, it will also fail *in vivo*. For that reason, platelet function assays cannot overcome the uncertainty of antithrombotic therapy efficacy in all patients.

It should be emphasized that the consensus regarding the optimal cut-off for HPR is necessary as well as standardization of methods before platelet function testing is introduced in clinical practice.

Genes associated with the response variability to clopidogrel

Cytochrome P450 genetic polymorphisms

Due to its complex metabolism, P2Y₁₂ inhibitors involve multiple genes in absorption, activation, and inhibition of the receptor. Those detected gene variants have been shown to be associated with both bleeding and ischemic events.

Although CYP2C9 has an integral role in clopidogrel metabolism, the sparse data do not support the genotyping for CYP2C9*3 for prediction of events [87, 149].

CYP2C19*2 (loss of function allele), the most common known allele with 30% of Caucasians and up to 50% Asian being carriers, is associated with a reduced antiplatelet effect of clopidogrel and increased risk for adverse cardiovascular events [87, 150–154]. Although the CYP2C19*2 allele accounts only for 5–12% of the variation in the response to clopidogrel, several studies have shown an influence of CYP2C19*2 on clinical outcome [91, 95, 154–156]. Platelet function studies have shown a gene-dose effect in carriers of this polymorphism, showing that increase of dosage led to a sufficient level of platelet inhibition in heterozygous patients, whereas most homozygous patients failed to respond despite daily doses of 300 mg clopidogrel (Table VI) [157].

Due to a relatively low allele frequency (< 1%), other identified CYP2C19 variants (*3–*8) have only a minor impact on HPR [149, 158, 159].

In contrast to CYP2C19*2, CYP2C19*17 is a gain of function mutation leading to intensified activation of clopidogrel and so-called ultra-metabolizers with exaggerated bioactivation of clopidogrel. Data on whether there is an association of CYP2C19*17 with haemorrhagic events is conflicting, and to date not convincing [120, 155, 156, 160, 161].

ABCB1

Thienopyridine absorption is mediated via the intestinal efflux transport pump P-glycoprotein encoded by the ABCB1 gene (*MDR1*). The influence of different ABCB1 alleles is unclear. Some studies have shown that patients harbouring genetic variants in ABCB1 (specifically homozygous for the C3435T variant), have lower levels of the active compound and higher rates of adverse clinical outcomes (Table VI). However, this finding could not be

confirmed in several subsequent studies. Further studies are needed to clarify the impact of this gene on the antiplatelet effect of clopidogrel [34, 162, 163].

PON1

PON1 QQ192, a genetic variant in the gene encoding for the paraoxonase 1 (PON1) enzyme was linked to lower clopidogrel active metabolite concentrations in one study [164], which however was not confirmed in the following studies [91, 150, 165–167] (Table VI).

ITGB3

ITGB3 that encodes the integrin β_3 of the GP IIb/IIIa receptor has been linked with response variability of clopidogrel treatment and the risk of stent thrombosis [87]. Again, these results are challenged by another study that could not confirm these observations [158].

P2Y₁₂

Genetic variations for the gene encoding the binding site for clopidogrel active metabolite on the P2Y₁₂ receptor have shown a reduced efficacy of clopidogrel, but the clinical importance is doubtful [87, 158, 168].

IRS-1

Polymorphism of the insulin receptor substrate (IRS)-1 have been shown to be associated with hyperactive platelets and increased risk for ischemic events in patients with type 2 DM and stable coronary artery disease [169].

Studies investigating personalized antiplatelet treatment

The last decades of clopidogrel use have raised concerns that the “one dose fits all” approach is questionable in P2Y₁₂-treated patients. There are numerous studies that linked HPR on clopidogrel to adverse ischemic events and gave credit to the need of platelet inhibition testing in case of clopidogrel. In multiple trials, it has been observed that ADP-antagonist induced platelet inhibition can be improved with increased clopidogrel loading and maintenance doses or simply by switching to novel compounds like prasugrel or ticagrelor. For example, increase to 150 mg maintenance dose of clopidogrel resulted in more intense inhibition of platelet aggregation than administration of the standard 75 mg dose in a subset of patients [170–172]. Nevertheless, it must be emphasized that increase in dosage is not sufficient in a number of patients, as it has been shown that even 900 mg loading doses of clopidogrel did not overcome HPR to clopidogrel in homozygous CYP2C19*2 allele carriers [157]. Adjusted loading doses of clopidogrel according to platelet monitoring were shown to achieve a reduction of MACE without an increase of bleeding complications,

Table VI. Studies investigating the genotype and its association with bleeding or ischemic events

Study author/acronym	Polymorphism	N	Population	Follow-up	Outcome	OR/HR	Prevalence (%) carriers	Prevalence (%) homozygote	Prevalence (%) heterozygote
Thrombolysis: CYP2C19*2, *3, *4, *5									
AFIII [152]	2C19*2	259	MI (< 45 years of age)	6 months	MACE	3.69	28	0	25
AFIII [150]	2C19*2	371	MI (< 45 years of age)	6 years	MACE	2.26	31	4	26
TRITON TIMI-38 [153]	2C19*2	1477	PCI + ACS	15 months	MACE	1.53	34		
Oh <i>et al.</i> [202]	2C19*2	2146	PCI + DES	1 year	MACE	2.62	47		
Shuldiner <i>et al.</i> [154]	2C19*2	227	Elective PCI	1 year	MACE	2.42	33	2	31
RECLOSE [151]	2C19*2	772	PCI	6 months	ST	3.43	32	3	29
Harmsze <i>et al.</i> [149]	2C19*2	176/420	PCI (ST case/control)	1 year	ST	1.7	40	5	35
Sibbing <i>et al.</i> [203]	2C19*2	2485	PCI	30 days	ST	3.81	27	2	25
Sibbing <i>et al.</i> [165]	2C19*2	127/1439	PCI (ST case/control)	30 days	ST	2.27	25	2	23
ONASSIST [87]	2C19*2	123/246	PCI (ST case/control)		ST	1.99	49	16	33
Harmsze <i>et al.</i> [96]	2C19*2	725	Elective PCI	1 year	MACE	NS	31	3	28
Campo <i>et al.</i> [120]	2C19*2	300	PCI	1 year	MACE	NS	27	2	25
CHARISMA [156]	2C19*2	4819	CAD or at high risk	2 years	MACE	NS	15		
Tiroch <i>et al.</i> [204]	2C19*2	928	MI	1 year	MACE	NS	27	2	25
Sawada <i>et al.</i> [205]	2C19*2	100	PCI + DES	8 months	MACE	NS	42		
Tello-Montoliu <i>et al.</i> [206]	2C19*2	428	NSTE-ACS	6 months	MACE	NS	28	3	25
Malek <i>et al.</i> [207]	2C19*2	261	ACS	1 year	Death	NS	21	2	19
PEGASUS-PCI [63]	2C19*2	416	PCI	1 year	ST	NS	20	2	18
Jeong <i>et al.</i> [159]	2C19*2 and *3	266	MI	1 year	MACE	2.81	45	8	37
FAST-MI [158]	2C19*2,3,*4,*5	2208	PCI + MI	1 year	MACE	1.98	28	2	26
Yamamoto <i>et al.</i> [208]	2C19*2 or *3	123	CAD	12 months	MACE		44	11	33
CURE and ACTIVE [209]	2C19*2 or *3	5059	ACS or AF	1 year	MACE	NS	20	2	18
PLATO [210]	2C19*2-*8	10285	ACS	30 days	MACE	1.37	20	2	18
Harmsze <i>et al.</i> [149]	2C9*3	176/420	PCI (ST case/control)	1 year	ST	2.4	16	1	15
ONASSIST [87]	2C9*3	123/246	PCI (ST case/control)		ST	NS	17	0	17
Harmsze <i>et al.</i> [149]	CYP3A4*1B	176/420	PCI (ST case/control)	1 year	ST	NS	9	2	7
Suh <i>et al.</i> [211]	CYP3A5*3	348	PCI	6 months	MACE	4.89	45		
Harmsze <i>et al.</i> [149]	CYP3A5*3	176/420	PCI (ST case/control)		ST	NS	13	0	13
FAST-MI [158]	CYP3A5*3	2208	PCI + MI	1 year	MACE	NS	17	1	16
Campo <i>et al.</i> [120]	CYP3A5*3	300	PCI	1 year	MACE	NS	13	1	12
ONASSIST [87]	CYP3A5*3	123/246	PCI (ST case/control)		ST	NS	20	4	16

Table VI. Cont.

Study author/abbreviation	Polymorphism	N	Population	Follow-up	Outcome	OR/HR	Prevalence (%) carriers	Prevalence (%) homozygote	Prevalence (%) heterozygote
Bleeding: CYP2C19*17									
Sibbing <i>et al.</i> [160]	2C19*17	1524	PCI	30 days	TIMI major bleeding	1.8	41	5	36
Campo <i>et al.</i> [120]	2C19*17	300	PCI	1 year	TIMI major bleeding	2.3	34	6	28
Harmsze <i>et al.</i> [212]	2C19*17	820	Elective PCI	1 year	TIMI major bleeding	2.7			
Jeong <i>et al.</i> [159]	2C19*17	266	MI	1 year	TIMI major bleeding	NS	1	0	1
PLATO [210]	2C19*17	10285	ACS	1 year	Major bleeding	1.25	32	5	27
CURE and ACTIVE [209]	2C19*17	5059	ACS or AF	1 year	Major bleeding	NS	34		
CHARISMA [156]	2C19*17	4819	CAD or at high risk	2 years	GUSTO severe bleeding	NS	22		
PEGASUS-PCI [63]	2C19*17	416	PCI	1 year	TIMI major bleeding	NS	34	4	30
Thrombosis: PON1									
Bouman <i>et al.</i> [164]	PON1	1982	ACS	1 year	ST	12.80	54	13	41
EXCELISIOR [166]	PON1	760	Elective PCI	1 year	MACE	NS	50	10	40
Sibbing <i>et al.</i> [165]	PON1	127/1439	PCI (ST case/control)	30 days	ST	NS	47	8	39
Campo <i>et al.</i> [163]	PON1	300	PCI	1 month	MACE	NS	76	27	49
Simon <i>et al.</i> [167]	PON1	2210	MI	1 year	MACE	NS			
AFIII [150]	PON1	371	MI (< 45 years of age)	6 years	MACE	NS	55	15	40
Thrombosis: ACBC1									
TRITON TIMI 38 [213]	ACBC1	2932	ACS + PCI	15 months	MACE	1.72	73	23	50
FAST-MI [158]	ACBC1	2208	PCI + MI	1 year	MACE	1.72	74	26	48
ONASSIST [87]	ACBC1	123/246	PCI (ST case/control)		ST	2.16	76	32	44
Jaitner <i>et al.</i> [162]	ACBC1	66/1408	PCI (ST case/control)		ST	NS	78	29	49
Campo <i>et al.</i> [163]	ACBC1	300	PCI	1 year	MACE	NS	77	25	52
PLATO [210]	ACBC1	10285	ACS	1 year	MACE	NS	76	27	49
Harmsze <i>et al.</i> [149]	ACBC1	176/420	PCI (ST case/control)	1 year	ST	NS	68	14	54
Jeong <i>et al.</i> [159]	ACBC1	266	MI	1 year	MACE	NS	54	13	41
Spiewak <i>et al.</i> [214]	ACBC1	98	ACS + PCI	1.7 years	MACE	NS	72	21	51
Tiroch <i>et al.</i> [204]	ACBC1	928	MI	12 months	MACE	NS	82	29	49
Thrombosis: ITGB3, P2Y12, IRS-1									
ONASSIST [87]	ITGB3	123/246	PCI (ST case/control)		ST	0.52	16	0	16
FAST-MI [158]	ITGB3	2208	PCI + MI	1 year	MACE	NS	29	2	27
Ziegler <i>et al.</i> [215]	P2Y12	137	PAD	2 years	Neurological event	4.02	31	4	27
FAST-MI [158]	P2Y12	2208	PCI + MI	1 year	MACE	NS	25	3	25
ONASSIST [87]	P2Y12	123/246	PCI (ST case/control)		ST	NS	32	5	27
Angiolillo <i>et al.</i> [169]	IRS-1	187	DM + CAD	2 years	MACE	2.88	31	NN	NN

AF – atrial fibrillation, PCI – percutaneous coronary intervention, NSTE-ACS – non ST-elevation acute coronary syndrome, MACE – major adverse cardiac events, MI – myocardial infarction, ST – stent thrombosis, PAD – periphery artery disease, DES – drug eluting stent, CAD – coronary artery disease, TIMI – thrombolysis in myocardial infarction, DM – diabetes mellitus, NS – not significant.

however this strategy is not as sufficient as switch to prasugrel or ticagrelor [79, 173, 174]. Concordant with this, intensified platelet inhibition with GP IIb/IIIa antagonists could be used as a “bridging strategy” at the time point of PCI [175] and showed to lower the incidence of MACE without increased in-hospital bleeding rates in smaller studies [176, 177].

Most importantly, three randomized clinical trials (ARCTIC, $n = 2,440$; GRAVITAS, $n = 2,200$; and TRIGGER-PCI, $n = 423$) investigated if the outcome can be influenced using individualized antiplatelet strategy. In the GRAVITAS trial, clopidogrel treated patients with HPR received either standard dosing of clopidogrel or a second clopidogrel loading dose of 600 mg plus a maintenance dose of 150 mg. Within the 6-month follow-up, no significant differences in event rates could be shown in this patient population with a low-to-moderate thrombotic risk [178]. The TRIGGER-PCI trial compared prasugrel versus clopidogrel in patients with low thrombotic risk. The trial had to be stopped prematurely, because an interim analysis indicated a lower than expected incidence of the primary endpoint. Therefore, no meaningful conclusions may be drawn regarding clinical events from this study [179]. The ARCTIC trial included patients with low to moderate thrombotic risk with planned coronary stenting, that were randomised to bedside platelet function monitoring versus no monitoring. In the monitoring arm, antiplatelet therapy was intensified by increasing the dose of aspirin or an additional loading dose followed by an increased maintenance dose of clopidogrel, by additional treatment with a GP IIb/IIIa inhibitor or by switching to prasugrel. Adjustment of antiplatelet therapy based on platelet function monitoring did not lead to any improvement in the composite endpoint of coronary ischemic events [180].

There are several possible explanations why these trials failed to show improved clinical outcome. Firstly, the three trials (GRAVITAS, TRIGGER-PCI, ARCTIC) only included low-to-moderate risk patients, whereas STEMI patients with a much higher ischemic risk were excluded. Moreover, in ARCTIC and GRAVITAS trials, only a minority of patients included had a non-ST-elevation-ACS (NSTEMI-ACS), whereas the TRIGGER-PCI trial included only patients with elective drug-eluting stent implantation during PCI and without procedural complications [179]. It is likely that exclusion of high-risk patients may have accounted in part for the negative study results. Based on these findings one can argue that intensified antiplatelet treatment might not be beneficial in patients with a low-to-moderate risk for thrombotic events, but improve outcome in higher-risk patients or in those with a high risk for stent thrombosis [181]. In line with this assumption, Aradi *et al.* could prove in a meta-regression analysis that the net clinical benefit of intensified P2Y₁₂ inhibition depends on the baseline risk for stent thrombosis [182]. This meta-analysis including 10 randomized trials with more than 4000 patients also proved that the intensified antiplatelet treatment was

associated with a significant reduction in cardiovascular mortality, stent thrombosis and MI [182]. The net clinical benefit of a personalized antiplatelet treatment also has been shown in the MADONNA study [79, 183]. Similarly, individualisation of dual antiplatelet therapy minimised early thrombotic events in an all-comers PCI population without increasing bleeding in an IDEAL registry [184].

Individualised antiplatelet therapy – algorithm approach

Due to the lack of prospective double-blind randomised studies demonstrating an improvement in clinical outcome by personalised antiplatelet therapy, there is no recommendation regarding a routine approach of individualised antiplatelet therapy. To date, there is only a class IIb recommendation for platelet function testing to facilitate the choice of P2Y₁₂ inhibitor in selected patients on clopidogrel at high risk for thrombotic events [185].

The novel platelet inhibitors prasugrel and ticagrelor have been shown to be superior concerning platelet inhibition and reduction of thrombotic events and for that it is feasible to use those compounds in all ACS patients, especially those at high risk. Nevertheless the ACC/AHA guidelines recommend either clopidogrel or ticagrelor or prasugrel in interventional managed ACS (all of them received a class IIb recommendation) and because of that an individualized antiplatelet therapy is conceivable. For that purpose it might be useful to use an algorithm for personalised antiplatelet therapy in patients who are at high thrombotic risk. This global risk algorithm is based on clinical (PREDICT score), biological (platelet function) and genetic (*CYP2C19*2* carrier status) information [186]. Nevertheless, this algorithm has not been tested prospectively yet.

Conclusions

Although the tailored antiplatelet treatment monitored by platelet function testing seems to be feasible, the contradictory results of smaller registry studies and larger randomized trials with regards to outcome leave a big uncertainty. It is tempting to speculate that the different study populations, follow-ups, treatment strategies, study endpoints or time-points of blood sampling and therapy adjustment might disguise the real effect of tailored treatment [181]. Therefore, further research is needed to define:

- i) patient populations, which would benefit from the tailored antiplatelet strategy in terms of net clinical outcome,
- ii) which time points of platelet function testing are most predictive for outcome,
- iii) whether multiple testing is necessary,
- iv) whether genotyping adds useful information,
- v) how tailored antiplatelet strategy should be applied to patients with bleeding events,

- vi) whether algorithm based approach to tailored antiplatelet strategy is feasible and improves net clinical outcome.

Conflict of interest

The authors declare no conflict of interest.

References

- Franchi F, Angiolillo DJ. Novel antiplatelet agents in acute coronary syndrome. *Nat Rev Cardiol* 2015; 12: 30-47.
- Raju NC, Eikelboom JW, Hirsh J. Platelet ADP-receptor antagonists for cardiovascular disease: past, present and future. *Nat Clin Pract Cardiovasc Med* 2008; 5: 766-80.
- Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med* 2007; 357: 2482-94.
- Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol* 2008; 28: 403-12.
- Monroe DM, Hoffman M, Roberts HR. Transmission of a procoagulant signal from tissue factor-bearing cell to platelets. *Blood Coagul Fibrinolysis* 1996; 7: 459-64.
- Dorsam RT, Kunapuli SP. Central role of the P2Y12 receptor in platelet activation. *J Clin Invest* 2004; 113: 340-5.
- Zhang K, Zhang J, Gao ZG, et al. Structure of the human P2Y12 receptor in complex with an antithrombotic drug. *Nature* 2014; 509: 115-8.
- Rollini F, Franchi F, Angiolillo DJ. Switching P2Y-receptor inhibitors in patients with coronary artery disease. *Nat Rev Cardiol* 2015 Aug 18. doi: 10.1038/nrcardio.2015.113.
- Gachet C. ADP receptors of platelets and their inhibition. *Thromb Haemost* 2001; 86: 222-32.
- Ostrowska M, Adamski P, Kozinski M, et al. Off-target effects of glycoprotein IIb/IIIa receptor inhibitors. *Cardiol J* 2014; 21: 458-64.
- Siller-Matula JM, Delle-Karth G, Christ G, et al. Dual non-responsiveness to antiplatelet treatment is a stronger predictor of cardiac adverse events than isolated non-responsiveness to clopidogrel or aspirin. *Int J Cardiol* 2013; 167: 430-5.
- Siller-Matula JM, Krumpalhuber J, Jilma B. Pharmacokinetic, pharmacodynamic and clinical profile of novel antiplatelet drugs targeting vascular disease. *Br J Pharmacol* 2009; 159: 502-17.
- Kubica J, Kozinski M, Navarese EP, et al. Cangrelor: an emerging therapeutic option for patients with coronary artery disease. *Curr Med Res Opin* 2014; 30: 813-28.
- Angiolillo DJ. The evolution of antiplatelet therapy in the treatment of acute coronary syndromes: from aspirin to the present day. *Drugs* 2012; 72: 2087-116.
- Sharis PJ, Cannon CP, Loscalzo J. The antiplatelet effects of ticlopidine and clopidogrel. *Ann Intern Med* 1998; 129: 394-405.
- Ono K, Kurohara K, Yoshihara M, et al. Agranulocytosis caused by ticlopidine and its mechanism. *Am J Hematol* 1991; 37: 239-42.
- Huber K. Genetic variability in response to clopidogrel therapy: clinical implications. *Eur Heart J* 2010; 31: 2974-6.
- Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol* 2007; 49: 1505-16.
- Jakubowski JA, Matsushima N, Asai F, et al. A multiple dose study of prasugrel (CS-747), a novel thienopyridine P2Y12 inhibitor, compared with clopidogrel in healthy humans. *Br J Clin Pharmacol* 2007; 63: 421-30.
- Kozinski M, Bielis L, Wisniewska-Szmyt J, et al. Diurnal variation in platelet inhibition by clopidogrel. *Platelets* 2011; 22: 579-87.
- Kasprzak M, Kozinski M, Bielis L, et al. Pantoprazole may enhance antiplatelet effect of enteric-coated aspirin in patients with acute coronary syndrome. *Cardiol J* 2009; 16: 535-44.
- Navarese EP, Buffon A, Kozinski M, et al. A critical overview on ticagrelor in acute coronary syndromes. *QJM* 2013; 106: 105-15.
- Kubica A, Kasprzak M, Siller-Matula J, et al. Time-related changes in determinants of antiplatelet effect of clopidogrel in patients after myocardial infarction. *Eur J Pharmacol* 2014; 742: 47-54.
- Wallentin L, Becker RC, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2009; 361: 1045-57.
- Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007; 357: 2001-15.
- Jakubowski JA, Riesmeyer JS, Close SL, et al. TRITON and beyond: new insights into the profile of prasugrel. *Cardiovasc Ther* 2012; 30: e174-82.
- Navarese EP, Verdoia M, Schaffer A, et al. Ischaemic and bleeding complications with new, compared to standard, ADP-antagonist regimens in acute coronary syndromes: a meta-analysis of randomized trials. *QJM* 2011; 104: 561-9.
- Siller-Matula JM, Petre A, Delle-Karth G, et al. Impact of preoperative use of P2Y12 receptor inhibitors on clinical outcomes in cardiac and non-cardiac surgery: a systematic review and meta-analysis. *Eur Heart J Acute Cardiovasc Care* 2015 May 5; doi: 10.1177/2048872615585516.
- Gouya G, Arrich J, Wolzt M, et al. Antiplatelet treatment for prevention of cerebrovascular events in patients with vascular diseases: a systematic review and meta-analysis. *Stroke* 2014; 45: 492-503.
- Schrör K, Siller-Matula JM, Huber K. Pharmacokinetic basis of the antiplatelet action of prasugrel. *Fundam Clin Pharmacol* 2012; 26: 39-46.
- Sugidachi A, Ogawa T, Kurihara A, et al. The greater in vivo antiplatelet effects of prasugrel as compared to clopidogrel reflect more efficient generation of its active metabolite with similar antiplatelet activity to that of clopidogrel's active metabolite. *J Thromb Haemost* 2007; 5: 1545-51.
- Kozinski M, Obonska K, Stankowska K, et al. Prasugrel overcomes high on-clopidogrel platelet reactivity in the acute phase of acute coronary syndrome and maintains its antiplatelet potency at 30-day follow-up. *Cardiol J* 2014; 21: 547-56.
- Oprea AD, Popescu WM. P2Y12 receptor inhibitors in acute coronary syndromes: what is new on the horizon? *Cardiol Res Pract* 2013; 2013: 195456.
- Wallentin L, James S, Storey RF, et al. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. *Lancet* 2010; 376: 1320-8.
- Kowalczyk M, Banach M, Mikhailidis DP, et al. Ticagrelor: a new platelet aggregation inhibitor in patients with acute coronary syndromes. An improvement of other inhibitors? *Med Sci Monitor* 2009; 15: MS24-30.
- Adamski P, Kozinski M, Ostrowska M, et al. Overview of pleiotropic effects of platelet P2Y12 receptor inhibitors. *Thromb Haemost* 2014; 112: 224-42.
- Grzesk G, Kozinski M, Navarese EP, et al. Ticagrelor, but not clopidogrel and prasugrel, prevents ADP-induced vascular smooth

- muscle cell contraction: a placebo-controlled study in rats. *Thromb Res* 2012; 130: 65-9.
38. van Giezen JJ, Sidaway J, Glaves P, Kirk I, Bjorkman JA. Ticagrelor inhibits adenosine uptake in vitro and enhances adenosine-mediated hyperemia responses in a canine model. *J Cardiovasc Pharmacol Ther* 2012; 17: 164-72.
 39. Ohman J, Kudira R, Albinsson S, Olde B, Erlinge D. Ticagrelor induces adenosine triphosphate release from human red blood cells. *Biochem Biophys Res Commun* 2012; 418: 754-8.
 40. Cattaneo M. High on-treatment platelet reactivity: definition and measurement. *Thromb Haemost* 2013; 109: 792-8.
 41. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003; 107: 2908-13.
 42. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 2004; 109: 3171-5.
 43. Tantry US, Bonello L, Aradi D, et al. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. *J Am Coll Cardiol* 2013; 62: 2261-73.
 44. Siller-Matula JM, Trenk D, Schrorr K, et al. Response variability to P2Y₁₂ receptor inhibitors: expectations and reality. *JACC Cardiovasc Interv* 2013; 6: 1111-28.
 45. Komosa A, Siller-Matula JM, Kowal J, et al. Comparison of the antiplatelet effect of two clopidogrel bisulfate formulations: plavix and generic-Egitromb. *Platelets* 2015; 26: 43-7.
 46. Sibbing D, Braun S, Morath T, et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. *J Am Coll Cardiol* 2009; 53: 849-56.
 47. Geisler T, Zurn C, Simonenko R, et al. Early but not late stent thrombosis is influenced by residual platelet aggregation in patients undergoing coronary interventions. *Eur Heart J* 2010; 31: 59-66.
 48. Siller-Matula JM, Christ G, Lang IM, et al. Multiple electrode aggregometry predicts stent thrombosis better than the VASP assay. *J Thromb Haemost* 2010; 8: 351-9.
 49. Buonamici P, Marcucci R, Migliorini A, et al. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. *J Am Coll Cardiol* 2007; 49: 2312-7.
 50. Migliorini A, Valenti R, Marcucci R, et al. High residual platelet reactivity after clopidogrel loading and long-term clinical outcome after drug-eluting stenting for unprotected left main coronary disease. *Circulation* 2009; 120: 2214-21.
 51. Gori AM, Marcucci R, Migliorini A, et al. Incidence and clinical impact of dual nonresponsiveness to aspirin and clopidogrel in patients with drug-eluting stents. *J Am Coll Cardiol* 2008; 52: 734-9.
 52. Cuisset T, Frere C, Quilici J, et al. Predictive values of post-treatment adenosine diphosphate-induced aggregation and vasodilator-stimulated phosphoprotein index for stent thrombosis after acute coronary syndrome in clopidogrel-treated patients. *Am J Cardiol* 2009; 104: 1078-82.
 53. Gurbel PA, Bliden KP, Samara W, et al. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. *J Am Coll Cardiol* 2005; 46: 1827-32.
 54. Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity is associated with a high incidence of myonecrosis after stenting for non-ST elevation acute coronary syndromes. *Thromb Haemost* 2007; 97: 282-7.
 55. Geisler T, Elke Schaeffeler E, Gawaz M, Schwab M. Genetic variation of platelet function and pharmacology: an update of current knowledge. *Thromb Haemost* 2013; 110: 876-87.
 56. Gurbel PA, Antonino MJ, Bliden KP, et al. Platelet reactivity to adenosine diphosphate and long-term ischemic event occurrence following percutaneous coronary intervention: a potential antiplatelet therapeutic target. *Platelets* 2008; 19: 595-604.
 57. Lev EI, Patel RT, Maresch KJ, et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. *J Am Coll Cardiol* 2006; 47: 27-33.
 58. Gurbel PA, Bliden KP, Saucedo JF, et al. Bivalirudin and clopidogrel with and without eptifibatid for elective stenting: effects on platelet function, thrombelastographic indexes, and their relation to periprocedural infarction results of the CLEAR PLATELETS-2 (Clopidogrel with Eptifibatid to Arrest the Reactivity of Platelets) study. *J Am Coll Cardiol* 2009; 53: 648-57.
 59. Breet NJ, van Werkum JW, Bouman HJ, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. *JAMA* 2010; 303: 754-62.
 60. Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol* 2006; 48: 1742-50.
 61. Parodi G, Bellandi B, Venditti F, et al. Residual platelet reactivity, bleedings, and adherence to treatment in patients having coronary stent implantation treated with prasugrel. *Am J Cardiol* 2012; 109: 214-8.
 62. Gurbel PA, Bliden KP, Zaman KA, et al. Clopidogrel loading with eptifibatid to arrest the reactivity of platelets: results of the Clopidogrel Loading With Eptifibatid to Arrest the Reactivity of Platelets (CLEAR PLATELETS) study. *Circulation* 2005; 111: 1153-9.
 63. Siller-Matula JM, Delle-Karth G, Lang IM, et al. Phenotyping versus genotyping for prediction of clopidogrel efficacy and safety: the PEGASUS-PCI study. *J Thromb Haemost* 2012; 10: 529-42.
 64. Sibbing D, Steinhubl SR, Schulz S, et al. Platelet aggregation and its association with stent thrombosis and bleeding in clopidogrel-treated patients: initial evidence of a therapeutic window. *J Am Coll Cardiol* 2010; 56: 317-8.
 65. Sibbing D, Schulz S, Braun S, et al. Antiplatelet effects of clopidogrel and bleeding in patients undergoing coronary stent placement. *J Thromb Haemost* 2010; 8: 250-6.
 66. Eshthardi P, Windecker S, Cook S, et al. Dual low response to acetylsalicylic acid and clopidogrel is associated with myonecrosis and stent thrombosis after coronary stent implantation. *Am Heart J* 2010; 159: 891-8 e1.
 67. Dineva D, Paskaleva I, Gotcheva N, et al. Assessment of platelet response with Multiplate impedance aggregometry in patients with coronary stents on Clopidogrel and aspirin treatment. *Bulgarska Kardiologia* 2011; 17: 16-25.
 68. Freynhofer MK, Brozovic I, Bruno V, et al. Multiple electrode aggregometry and vasodilator stimulated phosphoprotein-phosphorylation assay in clinical routine for prediction of postprocedural major adverse cardiovascular events. *Thromb Haemost* 2011; 106: 230-9.
 69. Bonello L, Pansieri M, Mancini J, et al. High on-treatment platelet reactivity after prasugrel loading dose and cardiovascular events after percutaneous coronary intervention in acute coronary syndromes. *J Am Coll Cardiol* 2011; 58: 467-73.
 70. Aradi D, Kirtane A, Bonello L, et al. Bleeding and stent thrombosis on P2Y₁₂-inhibitors: collaborative analysis on the role of

- platelet reactivity for risk stratification after percutaneous coronary intervention. *Eur Heart J* 2015; 36: 1762-71.
71. Michelson AD, Frelinger AL 3rd, Braunwald E, et al. Pharmacodynamic assessment of platelet inhibition by prasugrel vs. clopidogrel in the TRITON-TIMI 38 trial. *Eur Heart J* 2009; 30: 1753-63.
 72. Bonello L, Mancini J, Pansieri M, et al. Relationship between post-treatment platelet reactivity and ischemic and bleeding events at one year follow-up in patients receiving prasugrel. *J Thromb Haemost* 2012; 10: 1999-2005.
 73. Alexopoulos D, Xanthopoulou I, Davlouros P, et al. Prasugrel overcomes high on-clopidogrel platelet reactivity in chronic coronary artery disease patients more effectively than high dose (150 mg) clopidogrel. *Am Heart J* 2011; 162: 733-9.
 74. Alexopoulos D, Panagiotou A, Xanthopoulou I, et al. Antiplatelet effects of prasugrel vs double clopidogrel in patients on hemodialysis and high on-treatment platelet reactivity. *J Thromb Haemost* 2011; 9: 2379-85.
 75. Alexopoulos D, Xanthopoulou I, Gkizas V, et al. Randomized assessment of ticagrelor versus prasugrel antiplatelet effects in patients with ST-segment-elevation myocardial infarction. *Circ Cardiovasc Interv* 2012; 5: 797-804.
 76. Parodi G, Valenti R, Bellandi B, et al. Comparison of prasugrel and ticagrelor loading doses in ST-segment elevation myocardial infarction patients: RAPID (Rapid Activity of Platelet Inhibitor Drugs) primary PCI study. *J Am Coll Cardiol* 2013; 61: 1601-6.
 77. Mayer K, Orban M, Bernlochner I, et al. Predictors of antiplatelet response to prasugrel during maintenance treatment. *Platelets* 2015; 26: 53-8.
 78. Dridi NP, Johansson PI, Clemmensen P, et al. Prasugrel or double-dose clopidogrel to overcome clopidogrel low-response – The TAILOR (Thrombocytes And Individualization of ORal antiplatelet therapy in percutaneous coronary intervention) randomized trial. *Platelets* 2014; 25: 506-12.
 79. Siller-Matula JM, Francesconi M, Dechant C, et al. Personalized antiplatelet treatment after percutaneous coronary intervention: the MADONNA study. *Int J Cardiol* 2013; 167: 2018-23.
 80. Ibrahim K, Christoph M, Schmeinck S, et al. High rates of prasugrel and ticagrelor non-responder in patients treated with therapeutic hypothermia after cardiac arrest. *Resuscitation* 2014; 85: 649-56.
 81. Alexopoulos D, Galati A, Xanthopoulou I, et al. Ticagrelor versus prasugrel in acute coronary syndrome patients with high on-clopidogrel platelet reactivity following percutaneous coronary intervention: a pharmacodynamic study. *J Am Coll Cardiol* 2012; 60: 193-9.
 82. Angiolillo DJ, Curzen N, Gurbel P, et al. Pharmacodynamic evaluation of switching from ticagrelor to prasugrel in subjects with stable coronary artery disease: results of the SWAP-2 Study. *J Am Coll Cardiol* 2014; 63: 1500-9.
 83. Deharo P, Bassez C, Bonnet G, et al. Prasugrel versus ticagrelor in acute coronary syndrome: a randomized comparison. *Int J Cardiol* 2013; 170: e21-2.
 84. Tomizawa A, Ohno K, Jakubowski JA, et al. Comparison of antiplatelet effects of prasugrel and ticagrelor in cynomolgus monkeys by an ELISA-based VASP phosphorylation assay and platelet aggregation. *Thromb Haemost* 2013; 110: 769-76.
 85. Angiolillo DJ. Antiplatelet therapy in diabetes: efficacy and limitations of current treatment strategies and future directions. *Diabetes Care* 2009; 32: 531-40.
 86. Geisler T, Grass D, Bigalke B, et al. The residual platelet aggregation after deployment of intracoronary stent (PREDICT) score. *J Thromb Haemost* 2008; 6: 54-61.
 87. Cayla G, Hulot JS, O'Connor SA, et al. Clinical, angiographic, and genetic factors associated with early coronary stent thrombosis. *JAMA* 2011; 306: 1765-74.
 88. Neubauer H, Kaiser AF, Endres HG, et al. Tailored antiplatelet therapy can overcome clopidogrel and aspirin resistance: the BOchum CLopidogrel and Aspirin Plan (BOCLA-Plan) to improve antiplatelet therapy. *BMC Med* 2011; 9: 3.
 89. Htun P, Fateh-Moghadam S, Bischofs C, et al. Low responsiveness to clopidogrel increases risk among CKD patients undergoing coronary intervention. *J Am Soc Nephrol* 2011; 22: 627-33.
 90. Muller K, Aichele S, Herkommer M, et al. Impact of inflammatory markers on platelet inhibition and cardiovascular outcome including stent thrombosis in patients with symptomatic coronary artery disease. *Atherosclerosis* 2010; 213: 256-62.
 91. Siller-Matula JM, Lang IM, Neunteufl T, et al. Interplay between genetic and clinical variables affecting platelet reactivity and cardiac adverse events in patients undergoing percutaneous coronary intervention. *PLoS One* 2014; 9: e102701.
 92. Droppa M, Tschernow D, Muller KA, et al. Evaluation of clinical risk factors to predict high on-treatment platelet reactivity and outcome in patients with stable coronary artery disease (PREDICT-STABLE). *PLoS One* 2015; 10: e0121620.
 93. Siller-Matula JM, Lang I, Christ G, Jilma B. Calcium-channel blockers reduce the antiplatelet effect of clopidogrel. *J Am Coll Cardiol* 2008; 52: 1557-63.
 94. Harmsze AM, Robijns K, van Werkum JW, et al. The use of amlodipine, but not of P-glycoprotein inhibiting calcium channel blockers is associated with clopidogrel poor-response. *Thromb Haemost* 2010; 103: 920-5.
 95. Hochholzer W, Trenk D, Fromm MF, et al. Impact of cytochrome P450 2C19 loss-of-function polymorphism and of major demographic characteristics on residual platelet function after loading and maintenance treatment with clopidogrel in patients undergoing elective coronary stent placement. *J Am Coll Cardiol* 2010; 55: 2427-34.
 96. Harmsze AM, van Werkum JW, Souverein PC, et al. Combined influence of proton-pump inhibitors, calcium-channel blockers and CYP2C19*2 on on-treatment platelet reactivity and on the occurrence of atherothrombotic events after percutaneous coronary intervention. *J Thromb Haemost* 2011; 9: 1892-901.
 97. Gilard M, Arnaud B, Cornily JC, et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol* 2008; 51: 256-60.
 98. Cuisset T, Frere C, Quilici J, et al. Comparison of omeprazole and pantoprazole influence on a high 150-mg clopidogrel maintenance dose the PACA (Proton Pump Inhibitors And Clopidogrel Association) prospective randomized study. *J Am Coll Cardiol* 2009; 54: 1149-53.
 99. Siller-Matula JM, Jilma B, Schror K, et al. Effect of proton pump inhibitors on clinical outcome in patients treated with clopidogrel: a systematic review and meta-analysis. *J Thromb Haemost* 2010; 8: 2624-41.
 100. Siller-Matula JM, Spiel AO, Lang IM, et al. Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. *Am Heart J* 2009; 157: 148.e1-5.
 101. Teng R. Pharmacokinetic, pharmacodynamic and pharmacogenetic profile of the oral antiplatelet agent ticagrelor. *Clin Pharmacokinet* 2012; 51: 305-18.

102. Siller-Matula JM, Trenk D, Krahenbuhl S, et al. Clinical implications of drug-drug interactions with P2Y₁₂ receptor inhibitors. *J Thromb Haemost* 2014; 12: 2-13.
103. Hobl EL, Stimpfl T, Ebner J, et al. Morphine decreases clopidogrel concentrations and effects: a randomized, double-blind, placebo-controlled trial. *J Am Coll Cardiol* 2014; 63: 630-5.
104. Kubica J, Adamski P, Ostrowska M, et al. Morphine delays and attenuates ticagrelor exposure and action in patients with myocardial infarction: the randomized, double-blind, placebo-controlled IMPRESSION trial. *Eur Heart J* 2015 Oct 21; doi: 10.1093/eurheartj/ehv547
105. Roffi M, Chew DP, Mukherjee D, et al. Platelet glycoprotein IIb/IIIa inhibitors reduce mortality in diabetic patients with non-ST-segment-elevation acute coronary syndromes. *Circulation* 2001; 104: 2767-71.
106. Ferreiro JL, Angiolillo DJ. Diabetes and antiplatelet therapy in acute coronary syndrome. *Circulation* 2011; 123: 798-813.
107. Angiolillo DJ, Bernardo E, Ramirez C, et al. Insulin therapy is associated with platelet dysfunction in patients with type 2 diabetes mellitus on dual oral antiplatelet treatment. *J Am Coll Cardiol* 2006; 48: 298-304.
108. Fleming I, Schulz C, Fichtlscherer B, et al. AMP-activated protein kinase (AMPK) regulates the insulin-induced activation of the nitric oxide synthase in human platelets. *Thromb Haemost* 2003; 90: 863-71.
109. Ferreira IA, Mocking AI, Feijge MA, et al. Platelet inhibition by insulin is absent in type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2006; 26: 417-22.
110. Vinik AI, Erbas T, Park TS, et al. Platelet dysfunction in type 2 diabetes. *Diabetes Care* 2001; 24: 1476-85.
111. Assert R, Scherk G, Bumbure A, et al. Regulation of protein kinase C by short term hyperglycaemia in human platelets in vivo and in vitro. *Diabetologia* 2001; 44: 188-95.
112. Malmberg K. Prospective randomised study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus. DIGAMI (Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction) Study Group. *BMJ* 1997; 314: 1512-5.
113. Malmberg K, Ryden L, Wedel H, et al. Intense metabolic control by means of insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2): effects on mortality and morbidity. *Eur Heart J* 2005; 26: 650-61.
114. Hers I. Insulin-like growth factor-1 potentiates platelet activation via the IRS/PI3K pathway. *Blood* 2007; 110: 4243-52.
115. Ishida M, Ishida T, Ono N, et al. Effects of insulin on calcium metabolism and platelet aggregation. *Hypertension* 1996; 28: 209-12.
116. Betteridge DJ, El Tahir KE, Reckless JP, Williams KI. Platelets from diabetic subjects show diminished sensitivity to prostacyclin. *Eur J Clin Invest* 1982; 12: 395-8.
117. Li Y, Woo V, Bose R. Platelet hyperactivity and abnormal Ca²⁺ homeostasis in diabetes mellitus. *Am J Physiol Heart Circ Physiol* 2001; 280: H1480-9.
118. Michno A, Bielarczyk H, Pawelczyk T, et al. Alterations of adenosine nucleotide metabolism and function of blood platelets in patients with diabetes. *Diabetes* 2007; 56: 462-7.
119. Angiolillo DJ, Jakubowski JA, Ferreiro JL, et al. Impaired responsiveness to the platelet P2Y₁₂ receptor antagonist clopidogrel in patients with type 2 diabetes and coronary artery disease. *J Am Coll Cardiol* 2014; 64: 1005-14.
120. Campo G, Parrinello G, Ferraresi P, et al. Prospective evaluation of on-clopidogrel platelet reactivity over time in patients treated with percutaneous coronary intervention relationship with gene polymorphisms and clinical outcome. *J Am Coll Cardiol* 2011; 57: 2474-83.
121. Mokhtar OA, Lemesle G, Armero S, et al. Relationship between platelet reactivity inhibition and non-CABG related major bleeding in patients undergoing percutaneous coronary intervention. *Thromb Res* 2010; 126: e147-9.
122. Cuisset T, Grosdidier C, Loundou AD, et al. Clinical implications of very low on-treatment platelet reactivity in patients treated with thienopyridine: the POBA study (predictor of bleedings with antiplatelet drugs). *JACC Cardiovasc Interv* 2013; 6: 854-63.
123. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. *J Am Coll Cardiol* 2005; 46: 1820-6.
124. Frere C, Cuisset T, Quilici J, et al. ADP-induced platelet aggregation and platelet reactivity index VASP are good predictive markers for clinical outcomes in non-ST elevation acute coronary syndrome. *Thromb Haemost* 2007; 98: 838-43.
125. Bliden KP, DiChiara J, Tantry US, et al. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *J Am Coll Cardiol* 2007; 49: 657-66.
126. Sibbing D, Braun S, Jawansky S, et al. Assessment of ADP-induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. *Thromb Haemost* 2008; 99: 121-6.
127. Scharbert G, Auer A, Kozek-Langenecker S. Evaluation of the platelet mapping assay on rotational thromboelastometry ROTEM. *Platelets* 2009; 20: 125-30.
128. Rahe-Meyer N, Winterhalter M, Boden A, et al. Platelet concentrates transfusion in cardiac surgery and platelet function assessment by multiple electrode aggregometry. *Acta Anaesthesiol Scand* 2009; 53: 168-75.
129. Ranucci M, Baryshnikova E, Soro G, et al. Multiple electrode whole-blood aggregometry and bleeding in cardiac surgery patients receiving thienopyridines. *Ann Thorac Surg* 2011; 91: 123-9.
130. Siller-Matula J, Schror K, Wojta J, Huber K. Thienopyridines in cardiovascular disease: focus on clopidogrel resistance. *Thromb Haemost* 2007; 97: 385-93.
131. Blindt R, Stellbrink K, de Taae A, et al. The significance of vasodilator-stimulated phosphoprotein for risk stratification of stent thrombosis. *Thromb Haemost* 2007; 98: 1329-34.
132. Bonello L, Paganelli F, Arpin-Bornet M, et al. Vasodilator-stimulated phosphoprotein phosphorylation analysis prior to percutaneous coronary intervention for exclusion of postprocedural major adverse cardiovascular events. *J Thromb Haemost* 2007; 5: 1630-6.
133. Jakubowski JA, Payne CD, Li YG, et al. The use of the VerifyNow P2Y₁₂ point-of-care device to monitor platelet function across a range of P2Y₁₂ inhibition levels following prasugrel and clopidogrel administration. *Thromb Haemost* 2008; 99: 409-15.
134. Price MJ, Endemann S, Gollapudi RR, et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. *Eur Heart J* 2008; 29: 992-1000.
135. Marcucci R, Gori AM, Paniccia R, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome pa-

- tients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. *Circulation* 2009; 119: 237-42.
136. Jilma B. Platelet function analyzer (PFA-100): a tool to quantify congenital or acquired platelet dysfunction. *J Lab Clin Med* 2001; 138: 152-63.
 137. Madsen EH, Schmidt EB, Maurer-Spurej E, Kristensen SR. Effects of aspirin and clopidogrel in healthy men measured by platelet aggregation and PFA-100. *Platelets* 2008; 19: 335-41.
 138. Grove EL, Hvas AM, Johnsen HL, *et al.* A comparison of platelet function tests and thromboxane metabolites to evaluate aspirin response in healthy individuals and patients with coronary artery disease. *Thromb Haemost* 2010; 103: 1245-53.
 139. Gianetti J, Parri MS, Sbrana S, *et al.* Platelet activation predicts recurrent ischemic events after percutaneous coronary angioplasty: a 6 months prospective study. *Thromb Res* 2006; 118: 487-93.
 140. Fuchs I, Frossard M, Spiel A, *et al.* Platelet function in patients with acute coronary syndrome (ACS) predicts recurrent ACS. *J Thromb Haemost* 2006; 4: 2547-52.
 141. Chiu FC, Wang TD, Lee JK, *et al.* Residual platelet reactivity after aspirin and clopidogrel treatment predicts 2-year major cardiovascular events in patients undergoing percutaneous coronary intervention. *Eur J Intern Med* 2011; 22: 471-7.
 142. Campo G, Valgimigli M, Frangione A, *et al.* Prognostic value of serial platelet reactivity measurements on long-term clinical outcome in patients with ST-elevation myocardial infarction undergoing primary PCI. *J Thromb Haemost* 2008; 6: 1824-6.
 143. Panzer S, Eichelberger B, Koren D, *et al.* Monitoring survival and function of transfused platelets in Bernard-Soulier syndrome by flow cytometry and a cone and plate(let) analyzer (Impact-R). *Transfusion* 2007; 47: 103-6.
 144. Varon D, Lider O, Dardik R, *et al.* Inhibition of integrin-mediated platelet aggregation, fibrinogen-binding, and interactions with extracellular matrix by nonpeptidic mimetics of Arg-Gly-Asp. *Thromb Haemost* 1993; 70: 1030-6.
 145. Craft RM, Chavez JJ, Bresee SJ, *et al.* A novel modification of the Thrombelastograph assay, isolating platelet function, correlates with optical platelet aggregation. *J Lab Clin Med* 2004; 143: 301-9.
 146. Cattaneo M. Resistance to antiplatelet drugs: molecular mechanisms and laboratory detection. *J Thromb Haemost* 2007; 5 Suppl. 1: 230-7.
 147. Sumaya W, Daly RL, Mehra S, *et al.* Hirudin anticoagulation allows more rapid determination of P2Y₁₂ inhibition by the VerifyNow P2Y₁₂ assay. *Thromb Haemost* 2013; 109: 550-5.
 148. Schror K, Huber K, Hohlfeld T. Functional testing methods for the antiplatelet effects of aspirin. *Biomark Med* 2011; 5: 31-42.
 149. Harmsze AM, van Werkum JW, Ten Berg JM, *et al.* CYP2C19*2 and CYP2C9*3 alleles are associated with stent thrombosis: a case-control study. *Eur Heart J* 2010; 31: 3046-53.
 150. Hulot JS, Collet JP, Cayla G, *et al.* CYP2C19 but not PON1 genetic variants influence clopidogrel pharmacokinetics, pharmacodynamics, and clinical efficacy in post-myocardial infarction patients. *Circ Cardiovasc Interv* 2011; 4: 422-8.
 151. Giusti B, Gori AM, Marcucci R, *et al.* Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. *Am J Cardiol* 2009; 103: 806-11.
 152. Collet JP, Hulot JS, Pena A, *et al.* Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 2009; 373: 309-17.
 153. Mega JL, Close SL, Wiviott SD, *et al.* Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; 360: 354-62.
 154. Shuldiner AR, O'Connell JR, Bliden KP, *et al.* Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA* 2009; 302: 849-57.
 155. Siller-Matula JM, Delle-Karh G, Lang IM, *et al.* Phenotyping vs. genotyping for prediction of clopidogrel efficacy and safety: the PEGASUS-PCI study. *J Thromb Haemost* 2012; 10: 529-42.
 156. Bhatt DL, Pare G, Eikelboom JW, *et al.* The relationship between CYP2C19 polymorphisms and ischaemic and bleeding outcomes in stable outpatients: the CHARISMA genetics study. *Eur Heart J* 2012; 33: 2143-50.
 157. Mega JL, Hochholzer W, Frelinger AL 3rd, *et al.* Dosing clopidogrel based on CYP2C19 genotype and the effect on platelet reactivity in patients with stable cardiovascular disease. *JAMA* 2011; 306: 2221-8.
 158. Simon T, Verstuyft C, Mary-Krause M, *et al.* Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009; 360: 363-75.
 159. Jeong YH, Tantry US, Kim IS, *et al.* Effect of CYP2C19*2 and *3 loss-of-function alleles on platelet reactivity and adverse clinical events in East Asian acute myocardial infarction survivors treated with clopidogrel and aspirin. *Circ Cardiovasc Interv* 2011; 4: 585-94.
 160. Sibbing D, Koch W, Gebhard D, *et al.* Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation* 2010; 121: 512-8.
 161. Frere C, Cuisset T, Gaborit B, *et al.* The CYP2C19*17 allele is associated with better platelet response to clopidogrel in patients admitted for non-ST acute coronary syndrome. *J Thromb Haemost* 2009; 7: 1409-11.
 162. Jaitner J, Morath T, Byrne RA, *et al.* No association of ABCB1 C3435T genotype with clopidogrel response or risk of stent thrombosis in patients undergoing coronary stenting. *Circ Cardiovasc Interv* 2012; 5: 82-8, S1-2.
 163. Campo G, Ferraresi P, Marchesini J, *et al.* Relationship between paraoxonase Q192R gene polymorphism and on-clopidogrel platelet reactivity over time in patients treated with percutaneous coronary intervention. *J Thromb Haemost* 2011; 9: 2106-8.
 164. Bouman HJ, Schomig E, van Werkum JW, *et al.* Paraoxonase-1 is a major determinant of clopidogrel efficacy. *Nat Med* 2011; 17: 110-6.
 165. Sibbing D, Koch W, Massberg S, *et al.* No association of paraoxonase-1 Q192R genotypes with platelet response to clopidogrel and risk of stent thrombosis after coronary stenting. *Eur Heart J* 2011; 32: 1605-13.
 166. Trenk D, Hochholzer W, Fromm MF, *et al.* Paraoxonase-1 Q192R polymorphism and antiplatelet effects of clopidogrel in patients undergoing elective coronary stent placement. *Circ Cardiovasc Genet* 2011; 4: 429-36.
 167. Simon T, Steg PG, Becquemont L, *et al.* Effect of paraoxonase-1 polymorphism on clinical outcomes in patients treated with clopidogrel after an acute myocardial infarction. *Clin Pharmacol Ther* 2011; 90: 561-7.
 168. Fontana P, Dupont A, Gandrille S, *et al.* Adenosine diphosphate-induced platelet aggregation is associated with P2Y₁₂ gene sequence variations in healthy subjects. *Circulation* 2003; 108: 989-95.

169. Angiolillo DJ, Bernardo E, Zononi M, et al. Impact of insulin receptor substrate-1 genotypes on platelet reactivity and cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol* 2011; 58: 30-9.
170. von Beckerath N, Kastrati A, Wiecek A, et al. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. *Eur Heart J* 2007; 28: 1814-9.
171. Angiolillo DJ, Bernardo E, Sabate M, et al. Impact of platelet reactivity on cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol* 2007; 50: 1541-7.
172. Angiolillo DJ, Bernardo E, Palazuelos J, et al. Functional impact of high clopidogrel maintenance dosing in patients undergoing elective percutaneous coronary interventions. Results of a randomized study. *Thromb Haemost* 2008; 99: 161-8.
173. Bonello L, Camoin-Jau L, Armero S, et al. Tailored clopidogrel loading dose according to platelet reactivity monitoring to prevent acute and subacute stent thrombosis. *Am J Cardiol* 2009; 103: 5-10.
174. Bonello L, Camoin-Jau L, Arques S, et al. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. *J Am Coll Cardiol* 2008; 51: 1404-11.
175. Christ G, Hafner T, Siller-Matula JM, et al. Platelet inhibition by abciximab bolus-only administration and oral ADP receptor antagonist loading in acute coronary syndrome patients: the blocking and bridging strategy. *Thromb Res* 2013; 132: e36-41.
176. Valgimigli M, Campo G, de Cesare N, et al. Intensifying platelet inhibition with tirofiban in poor responders to aspirin, clopidogrel, or both agents undergoing elective coronary intervention: results from the double-blind, prospective, randomized Tailoring Treatment with Tirofiban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel study. *Circulation* 2009; 119: 3215-22.
177. Cuisset T, Frere C, Quilici J, et al. Glycoprotein IIb/IIIa inhibitors improve outcome after coronary stenting in clopidogrel non-responders: a prospective, randomized study. *JACC Cardiovasc Interv* 2008; 1: 649-53.
178. Price MJ, Berger PB, Teirstein PS, et al. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. *JAMA* 2011; 305: 1097-105.
179. Trenk D, Stone GW, Gawaz M, et al. A randomized trial of prasugrel versus clopidogrel in patients with high platelet reactivity on clopidogrel after elective percutaneous coronary intervention with implantation of drug-eluting stents: results of the TRIGGER-PCI (Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel) study. *J Am Coll Cardiol* 2012; 59: 2159-64.
180. Collet JP, Cuisset T, Range G, et al. Bedside monitoring to adjust antiplatelet therapy for coronary stenting. *N Engl J Med* 2012; 367: 2100-9.
181. Siller-Matula JM, Jilma B. Why have studies of tailored antiplatelet therapy failed so far? *Thromb Haemost* 2013; 110: 628-31.
182. Aradi D, Komocsi A, Price MJ, et al. Efficacy and safety of intensified antiplatelet therapy on the basis of platelet reactivity testing in patients after percutaneous coronary intervention: systematic review and meta-analysis. *Int J Cardiol* 2013; 167: 2140-8.
183. Siller-Matula JM, Gruber C, Francesconi M, et al. The net clinical benefit of personalized antiplatelet therapy in patients undergoing percutaneous coronary intervention. *Clin Sci (Lond)* 2015; 128: 121-30.
184. Christ G, Siller-Matula JM, Francesconi M, et al. Individualising dual antiplatelet therapy after percutaneous coronary intervention: the IDEAL-PCI registry. *BMJ Open* 2014; 4: e005781.
185. Jneid H, Anderson JL, Wright RS, et al. 2012 ACCF/AHA focused update of the guideline for the management of patients with unstable angina/non-ST-elevation myocardial infarction (updating the 2007 guideline and replacing the 2011 focused update): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2012; 60: 645-81.
186. Siller-Matula JM, Trenk D, Schror K, et al. How to improve the concept of individualised antiplatelet therapy with P2Y₁₂ receptor inhibitor – is an algorithm the answer? *Thromb Haemost* 2015; 113: 37-52.
187. Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. *J Thromb Haemost* 2006; 4: 542-9.
188. Breet NJ, van Werkum JW, Bouman HJ, et al. High on-treatment platelet reactivity to both aspirin and clopidogrel is associated with the highest risk of adverse events following percutaneous coronary intervention. *Heart* 2011; 97: 983-90.
189. Geisler T, Langer H, Wydymus M, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. *Eur Heart J* 2006; 27: 2420-5.
190. Trenk D, Hochholzer W, Fromm MF, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol* 2008; 51: 1925-34.
191. Wang ZJ, Zhou YJ, Liu YY, et al. Impact of clopidogrel resistance on thrombotic events after percutaneous coronary intervention with drug-eluting stent. *Thromb Res* 2009; 124: 46-51.
192. Wenaweser P, Dorffler-Melly J, Imboden K, et al. Stent thrombosis is associated with an impaired response to antiplatelet therapy. *J Am Coll Cardiol* 2005; 45: 1748-52.
193. Chen L, Bracey AW, Radovancevic R, et al. Clopidogrel and bleeding in patients undergoing elective coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 2004; 128: 425-31.
194. Müller-Schunk S, Linn J, Peters N, et al. Monitoring of clopidogrel-related platelet inhibition: correlation of nonresponse with clinical outcome in supra-aortic stenting. *AJNR Am J Neuroradiol* 2008; 29: 786-91.
195. Siller-Matula JM, Delle-Karth G, Christ G, et al. Dual non-responsiveness to antiplatelet treatment is a stronger predictor of cardiac adverse events than isolated non-responsiveness to clopidogrel or aspirin. *Int J Cardiol* 2013; 167: 430-5.
196. Barragan P, Bouvier JL, Roquebert PO, et al. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. *Catheter Cardiovasc Interv* 2003; 59: 295-302.
197. Cuisset T, Cayla G, Frere C, et al. Predictive value of post-treatment platelet reactivity for occurrence of post-discharge bleed-

- ing after non-ST elevation acute coronary syndrome. Shifting from antiplatelet resistance to bleeding risk assessment? *Euro-Intervention* 2009; 5: 325-9.
198. Park DW, Lee SW, Yun SC, et al. A point-of-care platelet function assay and C-reactive protein for prediction of major cardiovascular events after drug-eluting stent implantation. *J Am Coll Cardiol* 2011; 58: 2630-9.
199. Cuisset T, Hamilos M, Sarma J, et al. Relation of low response to clopidogrel assessed with point-of-care assay to periprocedural myonecrosis in patients undergoing elective coronary stenting for stable angina pectoris. *Am J Cardiol* 2008; 101: 1700-3.
200. Patti G, Nusca A, Mangiacapra F, et al. 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. *J Am Coll Cardiol* 2008; 52: 1128-33.
201. Gurbel PA, Erlinge D, Ohman EM, et al. Platelet function during extended prasugrel and clopidogrel therapy for patients with ACS treated without revascularization: the TRILOGY ACS Platelet Function Substudy. *JAMA* 2012; 308: 1785-94.
202. Oh IY, Park KW, Kang SH, et al. Association of cytochrome P450 2C19*2 polymorphism with clopidogrel response variability and cardiovascular events in Koreans treated with drug-eluting stents. *Heart* 2011; 98: 139-44.
203. Sibbing D, Stegherr J, Latz W, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J* 2009; 30: 916-22.
204. Tiroch KA, Sibbing D, Koch W, et al. Protective effect of the CYP2C19 *17 polymorphism with increased activation of clopidogrel on cardiovascular events. *Am Heart J* 2010; 160: 506-12.
205. Sawada T, Shinke T, Shite J, et al. Impact of cytochrome P450 2C19*2 polymorphism on intra-stent thrombus after drug-eluting stent implantation in Japanese patients receiving clopidogrel. *Circ J* 2011; 75: 99-105.
206. Tello-Montoliu A, Jover E, Marin F, et al. Influence of CYP2C19 polymorphisms in platelet reactivity and prognosis in an unselected population of non ST elevation acute coronary syndrome. *Rev Esp Cardiol* 2012; 65: 219-26.
207. Malek LA, Przulski J, Spiewak M, et al. Cytochrome P450 2C19 polymorphism, suboptimal reperfusion and all-cause mortality in patients with acute myocardial infarction. *Cardiology* 2010; 117: 81-7.
208. Yamamoto K, Hokimoto S, Chitose T, et al. Impact of CYP2C19 polymorphism on residual platelet reactivity in patients with coronary heart disease during antiplatelet therapy. *J Cardiol* 2011; 57: 194-201.
209. Pare G, Mehta SR, Yusuf S, et al. Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. *N Engl J Med* 2010; 363: 1704-14.
210. Wallentin L, James S, Storey RF, et al. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. *Lancet* 2010; 376: 1320-8.
211. Suh JW, Koo BK, Zhang SY, et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. *CMAJ* 2006; 174: 1715-22.
212. Harmsze AM, van Werkum JW, Hackeng CM, et al. The influence of CYP2C19*2 and *17 on on-treatment platelet reactivity and bleeding events in patients undergoing elective coronary stenting. *Pharmacogenet Genomics* 2012; 22: 169-75.
213. Mega JL, Close SL, Wiviott SD, et al. Genetic variants in ABCB1 and CYP2C19 and cardiovascular outcomes after treatment with clopidogrel and prasugrel in the TRITON-TIMI 38 trial: a pharmacogenetic analysis. *Lancet* 2010; 376: 1312-9.
214. Spiewak M, Malek LA, Kostrzewa G, et al. Influence of C3435T multidrug resistance gene-1 (MDR-1) polymorphism on platelet reactivity and prognosis in patients with acute coronary syndromes. *Kardiol Pol* 2009; 67: 827-34.
215. Ziegler S, Schillinger M, Funk M, et al. Association of a functional polymorphism in the clopidogrel target receptor gene, P2Y₁₂, and the risk for ischemic cerebrovascular events in patients with peripheral artery disease. *Stroke* 2005; 36: 1394-9.