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REVIEW

High On-Treatment Platelet Reactivity in Peripheral Endovascular Procedures

Stavros Spiliopoulos · George Kassimis · Adam Hatzidakis · Miltiadis Krokidis

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Abstract The use of aspirin is considered the "gold standard" for the decrease of major adverse cardiovascular events in patients with atherosclerosis, including peripheral arterial disease (PAD), whereas a dual-antiplatelet regimen with aspirin and clopidogrel is usually indicated for such patients after angioplasty and stent deployment. However, a substantial number of subsequent adverse events still occur, even in patients who receive double-antiplatelet therapy. The "high on-treatment platelet reactivity" (HTPR) phenomenon has been lately recognized and plays a major role in the management of patients with PAD. Greater and more rapid inhibition of platelet aggregation has become the goal for new antiplatelet agents with the expectation of further improving outcomes for percutaneous intervention for PAD. The purpose of this review article is to highlight current evidence regarding the prevalence, aetiology, and clinical implications of HTPR in PAD as well as to discuss the possibilities of novel alternative antiplatelet regiments.

S. Spiliopoulos
Department of Radiology, Patras University Hospital, Rio, Greece

G. Kassimis
Oxford Heart Center, John Radcliffe Hospital, Oxford, UK

A. Hatzidakis
Department of Radiology, Medical School of Crete, Heraklion University Hospital, Heraklion, Greece

M. Krokidis (✉)
Department of Radiology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge CB2 0QQ, UK
e-mail: mkrokidis@hotmail.com

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Introduction

Peripheral endovascular procedures are usually performed for peripheral arterial disease (PAD), which is most commonly caused by atherosclerosis and is usually accompanied by the presence of diffuse atherosclerotic changes in rest of the arterial bed. Patients who undergo peripheral endovascular procedures are also at high risk for myocardial infarction (MI), stroke, or death from cardiovascular causes due to the impairment of arterial remodeling and the acceleration of atherosclerotic disease progression [1–4]. Patients affected by the combination of coronary artery disease (CAD) and intermittent claudication (IC) appear to have greater levels of inflammatory and prothrombotic biomarkers than patients with CAD alone [5]. Intensive risk-factor modification and prompt antiplatelet treatment is required for patients with PAD who undergo peripheral endovascular procedures to improve the outcome of the revascularization procedure itself; however, this is also to avoid major adverse cardiovascular events (MACE) [3, 6–8]. Aspirin monotherapy is required for decrease of MACE, whereas dual-antiplatelet regimen with aspirin and clopidogrel is indicated for patients who undergo stent deployment in the peripheral arteries [8, 9]. Nevertheless, despite the administered pharmacological regimen, a substantial number of adverse events still occur, including stent thrombosis (ST) [10–12].

High on-treatment platelet reactivity (HTPR)—also referred to in the literature as "low-responsiveness," "non-responsiveness," or "resistance" to aspirin or clopidogrel or

both—has been shown to be associated with increased risk of MACE and vessel occlusion in patients that undergo peripheral endovascular procedures [13–16]. The phenomenon is largely unrecognized by physicians who perform peripheral endovascular procedures; therefore, the exact prevalence of the phenomenon and the relative impact on clinical practice is not well established. The available data are mainly extrapolated from studies in patients with CAD [15, 17–26].

The purpose of this review is to highlight all current evidence regarding the prevalence, aetiology, and clinical implications of HTPR in patients undergoing peripheral endovascular procedures as well as to discuss the possibilities of novel alternative antiplatelet regiments.

Aspirin

The Role of Aspirin in the Prevention of Adverse Events

Aspirin is the most widely used antiplatelet agent in cardiovascular disease, and it has been shown to be effective for the prevention of MI, stroke, and death from cardiovascular causes [28]. In the acute management of MI, unstable angina, and ischemic stroke, as well as among high-risk vascular patients, the use of aspirin was associated with a mortality decrease of 34 % for MI, 25 % for stroke, and 18 % overall mortality decrease from cardiovascular causes [29–33]. However, the absolute risk of recurrent adverse cardiovascular events of aspirin remains relatively high, with an estimated range between 8 and 18 % for the first 2 years [33]. This suggests that the antiplatelet effectiveness of aspirin is not homogeneously distributed in all patients and that probably the effect of the single aspirin dose is not enough for such patients.

Aspirin Resistance

Platelet aggregation, platelet activation, and bleeding time measurements have confirmed the variability of response to aspirin in the population [34–37]. The decreased responsiveness to aspirin therapy is associated with an increased risk of atherothrombotic events as shown by prospective clinical studies [38, 39]. These observations introduced the concept of "aspirin resistance," Although there are no defined diagnostic criteria, the term "aspirin resistance" generally describes aspirin's failure to produce the expected biological response (platelet inhibition) or to prevent adverse cardiovascular events. For the management of patients with peripheral vascular disease, the clinical impact of aspirin's resistance plays a major role. Although the prevalence of poor platelet response to aspirin is unclear, previous studies reported that it might affect between 5 and 45 % of the population. Therefore, identifying aspirin nonresponders and achieving appropriate levels of platelet inhibition with alternate therapy is of paramount importance for such patients.

Pharmacokinetic Properties and Mechanism of Action of Aspirin

Aspirin is absorbed rapidly by the gastrointestinal tract and reaches the plasma concentration peak within 40 min. The biological effect (blockage of thromboxane [TX] A2) is expected to begin in most individuals approximately 60 min after ingestion [40, 41]. The primary antithrombotic effect is achieved by the deactivation of cyclooxygenase (COX), a key enzyme in the arachidonate metabolism of the platelets [42, 43]. There are two COX isoforms; however, only COX-1 is constitutively expressed in mature platelets. Platelets have only minimal capacity for protein synthesis; the inactivation of COX-1 by aspirin is irreversible and lasts for the whole life of the platelet, which is 8–10 days. The second COX isoform (COX-2) is inducible in newly formed platelets (8–10 % of circulating platelets) and prostaglandin (PG) E2 is the main product [44]. COX-2 has been detected in a variety of cell types and tissue distributions, and its role in the inflammation process is widely recognized. The relatively weak anti-inflammatory effect of aspirin at low doses is in part explained by the fact that aspirin produces 170 times stronger inhibition of COX-1 than of COX-2 [40]. Aspirin may also influence haemostasis and cardiovascular disease by mechanisms independent of PG production (Table 1) [42, 43, 45, 46].

Table 1 PG-mediated and non-PG-mediated effects of aspirin

PG-mediated effects
Inactivation of COX-1 enzyme
Inactivation of COX-2 enzyme
Non-PG-mediated effects
Vitamin K antagonism
Decrease of the platelet production of thrombin
Acetylation of different clotting factors
Inhibition of neutrophil-mediated platelet activation
Protection of low-density lipoprotein from oxidative modification
Improvement of endothelial dysfunction in atherosclerotic patients
Anti-inflammatory response by acting as an antioxidant
Inhibition of inflammation-mediated endothelial dysfunction
Decrease of platelet release of interleukin-7
Decrease of the plasmatic levels of interleukin-7 and other cytokines

Measurement of Platelet Responsiveness to Aspirin

An optical aggregometer is traditionally used in platelet-rich plasma to test the platelet function. A platelet agonist is used (epinephrine, adenosine diphosphate (ADP), or collagen) to stimulate the aggregation, which is then graded on a scale of 0–100 % according to the degree of light transmission. Although this is a widely used technique, it requires appropriate expertise, and the results may vary [47]. Alternatively, whole-blood aggregometry (WBA) eliminates the need to prepare platelet-rich plasma and measures the platelet aggregation response using electrical impedance rather than optical density.

Another test is the (PFA)-100 (DadeBehring, Deerfield, IL), which uses whole blood and simulates haemostasis by flowing blood through a recipient coated with collagen and epinephrine or ADP [48]. The time required for platelet plug formation and the cessation of blood flow is used to measure platelet function. The PFA-100 system has been used to measure platelet response to aspirin therapy and shows reasonable correlation with optical aggregometry [48].

Another point-of-care test is the VerifyNow Aspirin Test (Accumetrics, San Diego, CA), which is an optical detection system that measures platelet-induced aggregation in citrated whole blood. The VerifyNow Aspirin Test uses arachidonic acid (AA) as the agonist to measure the antiplatelet effect of aspirin specifically along this pathway. Sample results are interpreted based on the extent of platelet aggregation reported in aspirin-reaction units (ARUs). Aspirin nonresponsiveness is defined as ARU $\geq$550 in a patient taking aspirin. Concomitant glycoprotein IIb/IIIa inhibitor, clopidogrel, dipyridamole, streptokinase, and nonsteroidal anti-inflammatory drug therapy affect the assay results. Recent studies have used this test to study the association between aspirin resistance and cardiovascular risk [49]. Urinary levels of 11-dehydro TX B2, a stable metabolite of TX A2, were used to study the extent of aspirin-mediated inhibition of TX generation [13]. Soluble CD 40 ligand and P-selectin have also shown promising results in the measurement of platelet activation [50].

Prevalence and Clinical Relevance of High On-Aspirin Platelet Reactivity

The concept of therapeutic resistance originated in response to the fact that the immediate biological effects of aspirin are not uniformly distributed among all patients. Mehta et al. [51] showed that a single 650-mg dose of aspirin produced minimal platelet inhibition in 30 % of patients with CAD. Variability in aspirin-mediated platelet inhibition has subsequently been documented among normal subjects, in patients with cerebrovascular disease, in patients with stable CAD, and in patients presenting for coronary artery bypass surgery [52–69]. Wang et al. [49] showed that nearly one quarter of the patients (23.4 %) who were taking aspirin by self-report were found to be aspirin nonresponsive. Despite the apparent consistency of these observations, the exact prevalence of aspirin resistance remains uncertain.

Clinical observations have suggested that the relationship between aspirin resistance and cardiovascular risk is in fact causal. Grundmann et al. [55] reported that among patients with previous ischemic attack or stroke, the incidence of aspirin resistance was significantly greater (34 %) compared with a panel of asymptomatic patients with known cerebrovascular disease (0 %). In another study, investigators reported that among a population of high-risk patients taking daily therapy with aspirin, the incidence of aspirin resistance was 23.4 % and that individuals with a history of CAD had nearly twice the odds of being resistant [49].

Evidence of High On-Aspirin Platelet Reactivity in PAD

Only a few studies have investigated the responsiveness of patients undergoing peripheral endovascular procedures to aspirin antiplatelet therapy. Linnemann et al. [25] evaluated platelet responsiveness to aspirin over time in 98 patients with stable PAD using native platelet-rich plasma with the Behring Coagulation Timer and the PFA-100 analyser. The investigators reported a 4.1 % of aspirin nonresponsiveness according to light transmission aggregometry (LTA) (maximum aggregation $\geq$78 %) and 12.2 % according to the PFA-100 (coagulation time $<$192 s). After a second evaluation with the PFA-100 system, nonresponsiveness to aspirin was persistent over time in 5.3 %, whereas 26.3 and 7.0 % of patients had changes in response status during a 17 months mean follow-up period when platelet function was assessed by PFA-100 and the LTA respectively. The investigators concluded that nonresponsiveness to aspirin is not stable over time in a number of patients and that this phenomenon could be attributed to methodological inconsistency, noncompliance with therapy, inadequate dose response, or interactions with other drugs. Moreover, the investigators speculated that the status of platelets might change over time due to changing disease activity or related differences in platelet activation pathways [25]. Nevertheless, it is not known whether these results reflect a true change of response status or intraobserver variability owing to test errors. A slightly superior incidence of low responsiveness was observed by Madsen et al. [15] using PFA-100 testing (17 %) and LTA ($\leq$8.1 %), whereas, again, 23 % of the patients had changed responsiveness status over time when tested with the PFA-100.

In another recent study from Saunders et al. [24] the prevalence of poor response to aspirin in 80 patients with PAD was investigated using three different assays: optical aggregation with the use of AA, optical aggregation with the use of ADP, and PFA-100 testing with the use of collagen/epinephrine (Epi). Poor response to aspirin was defined as AA aggregation >30 %, ADP aggregation >70 %, or PFA-100 Epi <164 s. Once again, the prevalence of persistent poor response varied between the three assays because only 5 % of subjects showed persistent low response by AA aggregation compared with 9.9 % detected by the PFA-100 point-of-care testing and 27.5 % by ADP aggregation. Regarding the agreement of the assays, only AA aggregation and PFA-100 Epi agreed significantly [24]. Elsayed et al. [27] published the only study that investigated high on-aspirin platelet reactivity with the VerifyNow P2Y12 point-of-care assay. In this small series of 15 patients with critical limb ischemia (CLI) and poor response to aspirin, defined as ARU >550, was detected in 28.5 % of patients. Even fewer studies have investigated the clinical significance of this phenomenon in patients undergoing peripheral endovascular procedures. In particular, among patients with IC who presented for a peripheral vascular angioplasty procedure, Mueller et al. [16] reported a 40 % incidence of aspirin resistance measured with corrected WBA. After 18 months of follow-up, aspirin resistance was associated with an 87 % increase in the risk of arterial reocclusion. Recently, Van der Loo et al. [26] reported the results of a prospective study with 109 symptomatic patients after endovascular interventions. The platelet-function tests—including ADP-, collagen- and Epi-induced aggregation using LTA—were performed before and at multiple time points after the procedure and at ≤1 year during the follow-up period. The investigators reported that according to LTA, results varied considerably over time and did not correlate either with the 1-year restenosis/reocclusion end point or the composite end point of cardiovascular death, stroke, or MI during ≤8-year follow-up. However, only 25.6 % of patients were sampled at 1 year, and only 52 % of patients were measured at more than thee time points [26].

All of the above-mentioned studies reported considerable test intraobserver variability whenever LTA or PFA-100 was used, thus highlighting the fact that a patient identified as a responder to aspirin could be classified as a nonresponder over time and vice versa. Again, it should be outlined that this variability could also be attributed to methodological inconsistency or to other inherent testing deficiencies.

Ongoing Trials

Several on-going trials are investigating aspirin resistance in patients with PAD. The French cardiovascular events at 1 year of patients hospitalized for critical limb ischemia and aspirin resistant using the VerifyNow aspirin resistance and prognosis of patients with CLI trial, is currently recruiting hospitalized CLI patients who will be tested for aspirin resistance using the bedside point-of-care VerifyNow test. The study aims to recruit approximately 150 patients, and the primary end point will be the correlation between aspirin resistance and the 1-year composite end point of death, fatal and nonfatal acute coronary syndromes (ACS), cardiac insufficiency, stroke, and major amputation (www.clinicaltrials.gov; NCT01104441). Finally, the Italian Tailored Strategy for Residual Platelet Activity In Advanced Peripheral Artery Disease: New Optimal Management trial will recruit 410 patients to evaluate high residual platelet activity in patients with IC. Secondary end points will include target vessels thrombosis and major adverse events rates as well as comparison of the diagnostic efficiency of various platelet aggregation tests (www.clinicaltrials.gov; NCT01627431).

Clopidogrel

High On-Clopidogrel Platelet Reactivity

ADP binding to its P2Y12 membrane receptor mediates platelet activation and aggregation. The ADP-receptor antagonist clopidogrel is an inactive precursor requiring metabolic biotransformation by a two-step enzymatic conversion by the hepatic cytochrome P450 (CYP450) into a biologically active thiol-metabolite, which binds the ADP P2Y12 receptor expressed on the platelet surface, thus causing its irreversible blockade. The standard daily dose of clopidogrel, 75 mg, requires 3–7 days to achieve maximum platelet inhibition [9, 70].

Clopidogrel response variability (CRV) has been well established [70] in CAD patients, with insufficient platelet inhibition in approximately 21 % of patients [71], whereas high on-clopidogrel treatment platelet reactivity has been associated with MACEs [24], particularly ST [72]. A number of genetic and nongenetic variables have been identified as causative mechanisms of HTPR [73] (Table 2).

Nongenetic Variables for High On-Clopidogrel Platelet Reactivity

High on-clopidogrel reactivity nongenetic variables are either cellular or clinical factors that influence the overall lack of platelet response to clopidogrel. Cellular factors, accelerated platelet turnover, increased exposure to ADP, and upregulation of P2Y12 have been implicated [74]. Regarding clinical factors, lack of platelet responsiveness may be noted in ases

Table 2 Mechanisms causing high on-clopidogrel treatment platelet reactivity

Nongenetic variables	Genetic variables
Clinical factors	Gene variants
Noncompliance	CYP2C19*2, *3, *4, *5, *6, *7, *8
Poor absorption	CYP2C19*17
Underdosing	CYP2B6*5
Diabetes mellitus	CYP3A4 gene variants
Renal insufficiency	CYP3A5 gene variants
Increased body mass index	CYP1A2 gene variants
Female sex	ABCB1 gene variants
Older age	P2Y12 gene variants
Acute coronary syndromes	ITGB3 gene variants
Cardiogenic shock	PON-1 gene variants
Decreased ejection fraction	
Systemic inflammation	
Cellular factors	
Accelerated platelet turnover	
Exposure to adenosine diphosphate	
Decreased CYP3A metabolic activity	
Upregulation of P2Y12 pathways	
Drug–drug interactions	
PPIs	
CCBs	
Statins	
Phenprocoumon	

of poor absorption, diabetes mellitus, renal insufficiency, female sex, increased body mass index, age, acute coronary syndrome, cardiogenic shock, or systemic inflammation as showed by a variety of studies [75–84]. Tobacco abuse is also correlated with HTPR [85–89].

The interaction of clopidogrel with other medication that uses the same metabolic pathways is another cause of non-genetic HTPR. In particular, when clopidogrel is administered together with proton-pump inhibitors (PPIs), the bioactivation of clopidogrel is influenced due to the common involvement of the hepatic cytochrome P450 [90, 91]. Other blocking medications include calcium-channel blockers (CCBs), statins, and derivatives of coumarin [92–96].

Genetic Variables for High On-Clopidogrel Platelet Reactivity

The enzymatic systems involved in the absorption and metabolism of clopidogrel are also responsible for the high on-clopidogrel platelet reactivity phenomenon [97–99]. In particular, a gene called "ABCB1" regulates clopidogrel absorption. In case of gene polymorphism, absorption is significantly decreased [100]. In terms of metabolism, only 15 % of clopidogrel follows the bioactivation pathway from the CYP2 enzymatic system, whereas the rest of it is hydrolyzed by esterases [101]. Several iso-enzymes contribute to the *CYP2* enzymatic pathway (mainly *CYP2C19, CYP3A4* or *CYP3A5, CYP2C9, CYP1A2*, and *CYP2B6*). When the metabolite is activated, it antagonizes irreversibly to the receptor of ADP, which is coded by the *P2RY12* gene.

Clopidogrel's biotransformation is mainly influenced by genetic variability of the isoenzyme *CYP2C19,* which affects approximately 30–55 % of the population [102]. The decreased function of the iso-enzyme is controlled by the *CYP2C19*2* genetic variant, which is carried by 95 % of the population [98]. The loss-of-function (LOF) allele follows an autosomal codominant inheritance. Therefore, individuals who are heterozygotes (or *1/*2) are expected to have an intermediate response between the *1/*1 and the *2/*2 genotypes [98]. Hence, they may be classified as extensive clopidogrel metabolizers (*1/*1), intermediate metabolizers (*1/*2), or poor metabolizers (*2/*2) according to the *CYP2C19* genotype they carry. The latter are expected to occur approximately 2–5 % in the white population and 15 % in the Asian population [73]. According to clinical data, the LOF *CYP2C19*2* allele is associated with increased HTPR in patients eho receive double-antiplatelet therapy after percutaneous coronary stent deployment [73, 98]. Due to the established decrease of the pharmacokinetic and pharmacodynamic response to clopidogrel, the LOF allele is also associated with an increased risk of major adverse cardiovascular events, in particular ST [103–107].

Measurement of Platelet Responsiveness to Clopidogrel

Platelet functional testing (PFT) to evaluate the antiplatelet effect of clopidogrel is currently performed using laboratory tests, such as (1) LTA, which requires platelet-rich plasma preparation; (2) Multiplate ADPtest HS multiple-electrode aggregometry method, which requires reagent preparation and vasodilator-stimulated phosphoprotein (VASP) phosphorylation; (3) flow cytometry analysis (BioCytex, Marseille, France), which requires platelet fixation and permeabilisation; (4) IMPACT-R Assay (Matis Medical, Inc., Beersel, Belgium), which requires extensive sample handling as well as true point-of-care testing, which entails only whole blood sampling, such as the VerifyNow P2Y12 assay (Accumetrics, San Diego, CA); (5) Plateletworks assay (Helena Laboratories, Beaumont, TX), (6) Innovance PFA P2Y (Siemens Health Care Diagnostic GMBH, Marburg, Germany); and (7) platelet-function analyzer PFA-100 system (DadeBehring, Deerfield, IL) [47–49, 108, 109]. All of the above-mentioned test are based on different principles and demonstrate diverse diagnostic performance measures, such as sensitivity and

specificity values. However, the most validated methods are the LTA, VerifyNow, VASP, and Multiplate tests. However, the best correlation between tests in the laboratory or clinically setting has been reported between the LTA, VerifyNow, VASP, and Multiplate tests. The best overall agreement is between the VerifyNow and VASP tests and the poorest with the LTA and Multiplate tests. In contrast, the "gold standard" of PFT has not yet been established because all of the available tests measure biological responses to the drug and do not include absolute analytic concentrations. [109] In the LTA and VerifyNow tests, both platelet activation and aggregation are assessed; in the VASP test, only the potential for platelet activation is evaluated; and the Multiplate test is based on the combination of platelet activation and adhesion to a heterologous surface. A substantial difference between the various measurements is the fact that the VerifyNow, VASP, and Multiplate assays use PGE1, which provides a more specific measurement of the effect of P2Y12 receptor inhibitors than LTA, which is influenced by the activity of both the P2Y12 and P2Y1 receptors [109]. A recent consensus opinion established the threshold values of HTPR to ADP as defined by receiver operator curve (ROC) analyses for the most commonly used PFT (LTA, VerifyNow, VASP, and Multiplate) tests to stratify patient risk for ischemic/thrombotic events after PCI as follows: (1) >46 % maximal 5 mol/l ADP-induced aggregation with LTA; (2) 235 to 240 P2Y12 reaction units (PRU) by VerifyNow P2Y12 assay; (3) platelet reactivity index (PRI) >50 % by VASP-P analysis; and (4) >468 arbitrary aggregation units/min in response to ADP by Multiplate (Table 3). Of note, in the same document it is reported that diabetic patients undergoing percutaneous coronary intervention (PCI) and patients with ACS managed with conservative medical treatment compared with those treated with PCI may demonstrate different cut-off values of HTPR [110].

High On-Clopidogrel Platelet Reactivity in Peripheral Endovascular Procedures

Until recently, there have been few data on the clinical implications of high on-clopidogrel platelet inhibition in patients undergoing interventions for PAD. The phenomenon was first reported in the MIRROR randomized controlled trial, which compared dual-antiplatelet therapy with aspirin and clopidogrel with aspirin monotherapy in 80 patients undergoing femoro-popliteal interventions due to IC or CLI. Patients randomized to the dual-antiplatelet therapy group received a loading dose of 300 mg of clopidogrel 6–12 h before the procedure. The incidence of HTPR was 30 % (12 of 40 patients) assessed with an ex vivo flow model (the Chandler-Loop vessel model). The investigators reported that the two patients who required a clinically driven revascularization procedure during the 6-month follow-up period were resistant to clopidogrel [110]. Two recent studies investigated the incidence and clinical significance of HTPR in patients undergoing peripheral endovascular procedures using the VerifyNow P2Y12 point-of-care assay. Pastromas et al., in an audit that included 113 patients returning for regular follow-up or clinical relapse after infrainguinal angioplasty or stenting (femoropopliteal or infrapopliteal or both), reported that clopidogrel resistance defined as PRU values $\geq$235 was identified as the only independent predictor of decreased target-limb revascularization survival in $\leq$7-year follow-up [Cox multivariable regression analysis hazard ratio (HR) 0.536, 95 % confidence interval (CI) 0.31–0.90; $p = 0.01$]. Of note, concomitant aspirin intake did not affect outcomes according the multivariable model. The PRU cut-off value of 235 was adopted by trials investigating HTPR after PCI because at the time no data regarding the optimal cut-off PRU value in peripheral endovascular procedures were available [111, 112]. The incidence of clopidogrel resistance was 53.9 % and was associated with diabetes mellitus, CLI, and renal disease (Fisher's exact test $p < 0.05$).

Motivated by the results of this pilot study, the same group designed a prospective trial (Platelet REsponsiveness to CLOpidogrel treatment after Peripheral endovascular procedures [PRECLOP]; NCT01744613) for determination of the optimal cut-off PRU value influencing clinical outcomes as well as the clinical significance of HTPR in peripheral endovascular procedures [113]. The study's clinical end point was the 1-year major adverse event rate (death, major stroke, major amputation, target vessel

Table 3 Most commonly used PFT and their relative cut-off values of HTPR in patients undergoing PCI

	Cut-off value	Comments
LTA	>46 % maximal 5 mol/l ADP-induced aggregation	Laboratory method; requires complex sampling and trained staff; time consuming; no standardization
VerifyNow	235–240 PRU and >550 ARU	True point-of-care test; rapid, standardized, and repeatable; easy to use.
VASP	PRI >50 %	Involves complex sample preparation; time consuming; requires flow cytometer
Multiplate analyzer	>468 ARUs	Involves pipetting and rapid sample processing

revascularization, bypass). In total, 100 consecutive patients programmed to undergo femoro-popliteal angioplasty or stenting were enrolled and stratified into four quartiles according to their PRU value (progressively increased PRU from 1st to 4th quartile). Blood sampling and platelet responsiveness testing with the VerifyNow assay was performed after at least 1 month of antiplatelet therapy. According to ROC analysis, the optimal cut-off value for the composite end point was PRU $\geq$234 (area under the curve 0.883; 95 % CI 0.811–0.954; $p < 0.0001$) achieving very satisfactory sensitivity (92.1 %), specificity (84.2 %), and positive (67.3 %) and negative (93.9 %) predictive values comparable with those reported from PCI trials [110] and was identical to that proposed for PCI patients according to a recently published international consensus document [112]. The 1-year composite end point showed a significant difference in the composite end point between successive quartiles because patients in the first two quartiles had significantly fewer adverse events than those in the last two quartiles. Cox multivariable regression analysis identified HTPR (PRU $\geq$234) as the only independent predictor for increased number of adverse events (HR 16.9; 95 % CI 5–55; $p < 0.0001$). Interestingly, patients with PRU lower than the cut-off value showed 1-year event-free survival of approximately 90 % regardless of lesion length or grade (stenosis or occlusion), stent use, or baseline clinical presentation (IC or CLI), all of which are considered to influence outcomes of endovascular procedures. In contrast, the 1-year event-free survival in the HTPR patient subgroup (PRU values above the cut-off value) was <40 %. The incidence of high on-clopidogrel platelet reactivity based on the estimated cut-off value (PRU $\geq$234) was 51 %. Again, following subgroup analysis, the investigators reported that CLI, diabetes mellitus, and chronic renal disease was associated with HTPR, as has been reported elsewhere [76–78, 114, 115]. Individual variability of platelet responsiveness to clopidogrel was not correlated with any complications requiring drug discontinuation or any additional treatment/hospitalization because no significant difference in bleeding events was detected between the study quartiles [113]. In both studies, the incidence of HTPR was superior to the nearly 30 % reported in the MIRROR study and in trials investigating patients undergoing PCI. However, these results were in line with previously reported data coming from studies investigating patients with advanced intracranial atherosclerotic disease [116, 117]. Moreover, Elsayed et al. recently reported in the 2012 ACC congress the results of a prospective study investigating HTPR assessed with the VerifyNow assay in a small series of 15 CLI patients who underwent endovascular procedures. HTPR to clopidogrel was found in 78.5 %, to aspirin in 28.5 %, and to both aspirin and clopidogrel in 14 % of patients [27]. Although the sample was too small, once again a trend versus greater incidence of high on-clopidogrel and aspirin platelet reactivity in patients with advanced PAD was noted. Finally, Kliger et al. [118] recently presented at the American College of Cardiology (ACC) 2012 a study investigating responsiveness to dual-antiplatelet therapy aspirin and/or clopidogrel in a population undergoing coronary percutaneous revascularization procedures with the VerifyNow aspirin and P2Y12 assay using the validated threshold for ARU <550 and PRU <230. In total, 58 patients underwent peripheral and 531 patients underwent coronary percutaneous revascularization procedures. Measurements were performed 6–12 h after the procedure. Again, a significantly greater incidence of aspirin and clopidogrel nonresponsiveness was noted in patients undergoing peripheral compared with coronary procedures.

The unexpectedly increased incidence of high on-clopidogrel platelet reactivity in patients with severe PAD undergoing endovascular treatment is currently of unknown etiology and remains to be confirmed by larger prospective multicenter trials. Nevertheless, if accurate, the investigators speculate that it could be attributed to factors, such as marked endothelial decrease and/or multiple drug intake due to various comorbidities, which are commonly encountered in patients with advanced atherosclerotic arterial disease causing severe lifestyle-limiting IC or CLI.

Novel Antiplatelet Agents

New, more potent P2Y12 inhibitors, such as prasugrel and ticagrelor, have been introduced in common clinical practice for the management of patients with coronary disease (Table 4). More specifically, according to the 2011 European Society of Cardiology Guidelines for patients with ACS, a P2Y12 inhibitor should be immediately added to aspirin therapy and maintained for 12 months unless contraindicated due to excessive risk of bleeding (level of evidence A) [30]. Both prasugrel and ticagrelor have shown superior clinical results compared with clopidogrel (composite end point of cardiovascular events: HR 0.81; 95 % CI 0.73–0.90; $p < 0.001$ for prasugrel versus clopidogrel [30, 119]. However, in the TRITON-TIMI multicentre randomized trial, which included 13,608 subjects with ACS, prasugrel also showed greater risk of fatal bleeding compared with clopidogrel (bleeding rate 4 % vs. 0.1 % respectively; $p = 0.002$) [119]. Although prasugrel was proven to provide superior clinical results with no additional bleeding risk versus clopidogrel in diabetic patients, it should be avoided in patients >75 years old and body weight <60 kg because no net clinical benefit was achieved in this subgroup [30]. Ticagrelor has also been associated with increased rates of minor bleeding and

Table 4 Aspirin and P2Y12 inhibitors

	Aspirin (acetylsalicylic acid)	Clopidogrel (thienopyridines)	Prasugrel (thienopyridines)	Ticagrelor (triazolopyrimidine)
Activation	Active drug	Pro-drug	Pro-drug	Active drug
Onset of effect	Within 1 h	2–4 h	30 min	30 min
Reversibility	No	No	No	Yes
Effect duration (days)	8–10	3–10	5–10	3–4
Discontinuation before major surgery (days)	7–10	5	7	5

noncoronary bypass graft–related major bleeding compared with clopidogrel. Specifically, in the PLATO trial, although no difference in the overall rates of fatal hemorrhage was detected between the two study groups (0.3 % in both groups), a greater rate of fatal intracranial hemorrhage was present in the ticagrelor group [120]. Therefore, in patients with ACS, the use of P2Y12 inhibitors should be accompanied by individual bleeding risk assessment according to baseline characteristics and duration of therapy [30].

There are currently no data regarding the use of these new antiplatelet agents in patients with PAD, and their safety and efficacy is currently under investigation. On July 2012, AstraZeneca announced a global multicenter trial investigating ticagrelor, which is currently approved only for ACS, in 11,500 patients with PAD (www.reuters.com).

Future Considerations

Although high on-clopidogrel platelet reactivity has been shown to negatively affect clinical outcomes after PCI, international guidelines recommend that PFT may be considered in selected cases when clopidogrel is used because currently level A evidence is lacking [30]. One could argue that if new antiplatelet agents demonstrate more satisfactory platelet inhibition levels, why not administrate them to all patients undergoing percutaneous endovascular procedures? However, currently there are insufficient data to support a "one drug fits all" strategy because novel antiplatelet agents might be contraindicated in certain patients and have been also associated with increased bleeding risk [112]. As a result, individual risk assessment is essential and should be performed to avoid unnecessary adverse events that would increase the very satisfactory periprocedural morbidity and mortality rates of peripheral angioplasty and stenting. Moreover, due to the additional cost of these agents compared with plain aspirin or clopidogrel, their everyday clinical use should be validated by appropriate cost-effectiveness analysis.

After these initial studies reporting the influence of HTPR in patients undergoing peripheral endovascular procedures, point-of-care-guided individualized antiplatelet therapy is slowly being introduced into common clinical practice of patients with symptomatic PAD. Although recent data advocate the clinical utility of PFT in improving MACE-free survival, which is mainly driven by acute in-stent thrombotic events, by adjusting the antiplatelet therapy of nonresponders, their performance in specific population subgroups and the mid- to long-term restenosis rates after peripheral interventions remain to be determined [121]. In both studies investigating peripheral procedures and high on-clopidogrel platelet reactivity, the latter was not correlated with acute thrombotic events; however, these were clinically driven repeat procedures attributed to mid- or long-term restenosis or occlusion. In-stent stenosis for both bare and drug-eluting stents may be related to HTPR [122]. The development of progressive neointimal de novo (neo)-atherosclerosis has been described as a potential mechanism involved in both drug-eluting and bare metal in-stent restenosis [123]. In the aforementioned studies stents were used in approximately 70 % of the cases. Although hypothesis generated, knowing that antiplatelet therapy has a protective role in atherosclerotic disease, the investigators consider that in-stent progressive atherosclerotic disease might have influenced outcomes in patients with HTPR. However, the relation between antiplatelet therapy and restenosis remains unclear.

PFT has been widely investigated in various clinical trials and has also been used in phase I and II trials during new antiplatelet drug development to assess dosing and efficacy issues [109]. Despite lack of standardization in most PFT, a significant correlation of the various tests with the active metabolite concentration has been reported (maximum correlation for the VerifyNow and VASP and minimum for LTA and Multiplate) [124]. Moreover, the use of PFT has been recently introduced in clinical practice for the evaluation of significant P2Y12 inhibitor–remaining effect after discontinuation of clopidogrel before major surgery [125]. In a recent consensus document regarding HTPR to ADP in patients with coronary disease published in 2010, the absolute level of platelet reactivity during treatment was proposed to be a better measure of thrombotic risk after coronary stenting than responsiveness to clopidogrel [112]. Moreover, Dahlen et al. [109] reported that HTPR shows equivalent or greater net reclassification improvement compared with several other established risk assessment factors, such as HDL cholesterol, C-reactive

protein, carotid intima-media thickness, coronary artery calcification, and family history of premature CAD. As a result, published evidence advocates the significance of confirming the therapeutic effect of antiplatelet therapy using PFT. Nonetheless, proper use and correct data interpretation of PTF is imperative to attain accurate results regarding HTPR and maximize the clinical effect of individually adjusted antiplatelet therapy [109].

Conclusion

Current published data suggest that HTPR significantly influences outcomes in patients undergoing coronary, cerebro-vascular, and peripheral endovascular interventions. The incidence of the phenomenon in patients undergoing endovascular interventions due to symptomatic PAD is approximately 50 %. With the advent of novel stronger antiplatelet agents, point-of-care-guided individualized antiplatelet therapy might contribute to high-quality patient management and could improve outcomes of interventional radiology revascularization procedures. These initial findings certainly merit further investigation.

Conflict of interest None.

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