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Laboratory Monitoring of $P2Y_{12}$ Inhibitors: a Position Statement of the Platelet Physiology Scientific and Standardization Committee.

Running title: Monitoring of $P2Y_{12}$ Inhibitors

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ticagrelor

Abbreviations:

AU = aggregation units

ACS = acute coronary syndromes

BMS = bare metal stent

CABG = coronary artery bypass graft

CAM = clopidogrel active metabolite

DAPT = dual antiplatelet therapy

DES = drug-eluting stent

HPR = high on-treatment platelet reactivity

LPR = low on-treatment platelet reactivity

LTA = light transmission aggregometry

MACE = major adverse cardiovascular event

NSTE-ACS =Non-ST elevation acute coronary syndromes

NSTEMI = non-ST-elevation myocardial infarction

PCI = percutaneous coronary intervention

PFT = platelet function testing

PRI = platelet reactivity index

PRU = platelet reaction units

STEMI = ST-segment elevation myocardial infarction

SIHD = stable ischemic heart disease

TEG = thromboelastograph

VASP = vasodilator stimulated phosphoprotein

Dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ platelet adenosine diphosphate (ADP) receptor antagonist reduces ischemic events in acute coronary syndrome (ACS) patients undergoing percutaneous coronary intervention (PCI) but also increases bleeding [1-3]. Residual high on-treatment platelet reactivity (HPR) and low on-treatment platelet reactivity (LPR) in response to $P2Y_{12}$ receptor stimulation, as measured by different platelet function testing (PFT) methodologies, are associated with increased risk for ischemic and bleeding outcomes, respectively (see [4, 5] and the references contained therein for descriptions of PFT assays and definitions of HPR and LPR), suggesting that altering antiplatelet therapy based on PFT would reduce adverse events. Small randomized and non-randomized studies demonstrated a reduction in ischemic events when $P2Y_{12}$ inhibitor therapy was modified if PFT indicated HPR (guided therapy) [6, 7]. However, larger randomized controlled trials, using different PFT methods and different therapeutic strategies, demonstrated no improved outcome with *vs.* without guided therapy [8-11]. PFT has also been proposed as a means to determine when platelet function has recovered sufficiently to enable surgery with minimum bleeding risk following $P2Y_{12}$ inhibitor withdrawal [12] and more recently to guide de-escalation therapy in ACS patients treated with PCI [13]. Goals of this position statement are to provide expert opinion on the utility of laboratory monitoring of $P2Y_{12}$ inhibitors to reduce ischemic and bleeding events in patients on DAPT and to guide timing of surgery if needed in $P2Y_{12}$ inhibitor-treated patients.

Clopidogrel is a second generation (after ticlopidine) thienopyridine oral antiplatelet drug which inhibits ADP-induced platelet aggregation and decreases major adverse cardiovascular events (MACE) when combined with aspirin, compared to aspirin alone [1]. Clopidogrel requires conversion by cytochrome P450 (CYP) enzymes to an active metabolite (CAM) which irreversibly inhibits platelet $P2Y_{12}$ [14].

CYP gene variants influence production of CAM and the pharmacodynamic response to the drug [15]. Loss of function alleles leading to reduced generation of CAM (e.g. CYP2C19*2) have been associated with poor clinical outcomes [16, 17] leading the Food and Drug Administration to issue a boxed warning advising that clopidogrel's effectiveness may be diminished in CYP2C19*2 carriers. Although CYP2C19 variants account for more than 10% of the variability to clopidogrel, other factors may contribute significantly to the variation in clopidogrel responsiveness, including non-adherence, underdosing, poor absorption, co-medications (atorvastatin, proton pump inhibitors, calcium antagonists), accelerated platelet turnover, inflammation and underlying platelet hyperreactivity. Thus, demonstration of HPR, the net effect of all of these factors, potentially offers a better predictive marker than these individual factors in clopidogrel-treated patients [18].

Newer $P2Y_{12}$ inhibitors (e.g. prasugrel and ticagrelor) produce greater inhibition of ADP-dependent platelet function and decrease MACE to a greater extent than clopidogrel [2, 3]. Prasugrel, a third generation thienopyridine compound, is, like clopidogrel, a prodrug. However, prasugrel's metabolism to active drug is independent of CYP2C19 and the possibility of mutations in this metabolic pathway that could influence platelet inhibition have been previously addressed [19]. In contrast to clopidogrel and prasugrel, ticagrelor, a direct $P2Y_{12}$ antagonist, is inherently active and thus is unaffected by CYP polymorphisms [20]. Clopidogrel-treated patients with HPR show significantly greater inhibition when switched to prasugrel or ticagrelor [21, 22]. Nevertheless, even with these antiplatelet agents on-treatment platelet reactivity is variable, albeit less than with clopidogrel [21, 22], thus their clinical benefit may be reduced in patients with HPR [5]. Moreover, unfortunately a significant fraction of the studies on the clinical efficacy of PFT-guided antiplatelet therapy have been performed by increasing clopidogrel dose and not by switching to prasugrel or ticagrelor [6-8, 10]. Thus, there is a need for such studies to be undertaken. Arguments against $P2Y_{12}$ monitoring include cost, variability in individual on-

treatment platelet responsiveness profile, the availability of $P2Y_{12}$ inhibitors with reduced variability, and the potential use of risk scores [23] to stratify patients.

$P2Y_{12}$-monitoring can be potentially useful in three situations: 1) to assess risk of thrombosis or bleeding in patients treated with $P2Y_{12}$ inhibitors, 2) to guide antiplatelet therapy, 3) to determine the optimum timing of surgery following $P2Y_{12}$ inhibitor discontinuation.

There is general consensus that HPR has a negative prognostic value for MACE in $P2Y_{12}$ inhibitors-treated patients [5] and, although less certain, a predictive value for bleeding [4, 5]. Until 2018, no large randomized clinical trial had demonstrated improved clinical outcomes with PFT-guided antiplatelet therapy [8-11]. However, the recent CREATIVE trial [24] showed that intensification of antiplatelet therapy (addition of cilostazol) in clopidogrel plus aspirin-treated PCI patients with HPR, as measured by thromboelastography, significantly improved clinical outcomes (hazard ratio 0.55, 95% CI 0.35-0.87) without increasing bleeding. Thus, despite these encouraging results, until this recent finding is replicated, consistent with previous guidelines [25], we believe that this strategy cannot be recommended at this time (Table 1).

Given that the newer $P2Y_{12}$ inhibitors provide greater platelet inhibition and reduce ischemic outcomes compared to clopidogrel, using these agents in patients at high risk for ischemic events without $P2Y_{12}$-monitoring is reasonable and potentially more cost effective than repeated testing. However, improved efficacy is associated with enhanced bleeding and limitations exist to the use of prasugrel [2, 3]; moreover, HPR is still observed and is associated with increased risk of ischemic outcomes [5]. Recently, monitoring prasugrel-treated elderly patients undergoing PCI for ACS was not found to be superior to conventional treatment with respect to both ischemic and bleeding outcomes [11]. Several explanations have been put forth for the failure of large randomized controlled trials to demonstrate improved clinical outcomes with PFT-guided antiplatelet therapy. HPR might be a non-modifiable risk factor and/or PFT may not affect prognostic factors, such as adherence to treatment, procedure-related technical factors,

or coexisting conditions influencing platelet reactivity [10]. Nevertheless, given that platelets contribute to arterial thrombosis, greater inhibition of platelet function is predicted to result in reduction of MACE. Thus, it has been alternatively proposed [26] that some previous studies may have been flawed with respect to one or more of the following: a) study design (e.g. sample size, definition of clinical endpoints), b) patient selection (low vs. high risk), c) PFT issues (poor predictive value, incorrect cut-off, improper timing), d) inability of alternative therapy to overcome HPR. The CREATIVE trial may be an example of an appropriate combination of PFT and choice of intensified antiplatelet therapy leading to improved outcomes.[24] Whether optimizing additional parameters would result in improved clinical outcomes with $P2Y_{12}$-monitoring is unknown, but the results of the CREATIVE trial, registry studies [27] and model-based analyses [28] suggests this approach deserves additional testing.

Whether $P2Y_{12}$-monitoring can be of assistance in deciding on DAPT duration has not been assessed. The treatment algorithm for duration of $P2Y_{12}$ inhibitor therapy suggests that in NSTE-ACS or STEMI patients treated with medical therapy or PCI, $P2Y_{12}$ inhibitor should be maintained for up to 12 months, and thereafter it may be reasonable to continue it if risk of bleeding is not high [29]. Likewise, in patients with stable ischemic heart disease treated with stenting it is reasonable to continue $P2Y_{12}$ inhibitor beyond 1 or 6 months (for BMS or DES, respectively) if risk of bleeding is not high. Long duration ticagrelor 60mg or 90mg twice daily significantly reduced ischemic outcomes [30] and virtually eliminated HPR [31]. However, major bleeding was also increased in these patients, highlighting the need to consider the balance between increased risk of non-fatal bleeding associated with LPR relative to the reduced risk of fatal and non-fatal ischaemic events. While studies have shown a connection between risk of bleeding and LPR [4, 5], prospective evaluation in clinical trials is required to establish whether PFT may guide duration of DAPT. Most recently, a large randomized trial has shown that a strategy of early PFT-guided de-escalation to clopidogrel is non-inferior to standard treatment with prasugrel in patients with ACS managed with PCI [13], suggesting that PFT may be useful

in patients not suitable for prolonged therapy with potent $P2Y_{12}$ inhibitors.

Worldwide over three million patients undergo PCI each year, >90% with stenting, and it is estimated that ≥5% will need non-cardiac surgery within the first year. Current guidelines state that in patients who require non-emergency major non-cardiac surgery, postponing surgery for at least 5 days after cessation of ticagrelor or clopidogrel, and 7 days for prasugrel should be considered unless the patient is at high risk of ischemic events [32]. Nevertheless, shortening the delay to surgery is often highly desirable. PFT demonstrated variation between individuals in the time required to recover platelet function following $P2Y_{12}$ inhibitor discontinuation, and prospective studies showed that a strategy based on preoperative PFT reduced post-operative bleeding and blood consumption and/or shortened waiting time [33, 34]. These results suggest it may be reasonable to decide about surgical timing based on PFT.

Conclusions and recommendations

Recommendations were based on a multistep consensus process (Figure 1).

PFT cannot at present be recommended to guide $P2Y_{12}$ inhibitor choice or select patients most likely to benefit of prolonged antiplatelet treatment but may be considered in deciding an early de-escalation from prasugrel to clopidogrel in patients considered not suitable for prolonged prasugrel therapy (Table 1).

It is reasonable, in patients requiring surgery, to consider the results of $P2Y_{12}$ inhibitor monitoring to determine the timing of surgery (Table 1). New, larger, prospective studies are however warranted to confirm PFT usefulness.

While present evidence does not support PFT-guided antiplatelet therapy, limitations of the studies performed, differences in cost between generic clopidogrel and newer $P2Y_{12}$ antagonists, and the enhanced bleeding risk of the latter continue to motivate clinical research on this subject. Critical issues in future studies include study design (particularly sample size

and control groups), choice of high risk populations, appropriate selection of monitoring test and cut off, appropriate timing of (and possibly repeated) testing and switching to alternative therapy (i.e. within days rather than weeks of stent placement), and clearly defined clinical efficacy outcomes.

A critical issue remains the most appropriate PFT method: limitations of currently used techniques urge further research on new methods.

Addendum

A.L. Frelinger, C. Gachet, P. Harrison, and P. Gresele conceived the project, A.L. Frelinger wrote the manuscript, P. Gresele, C. Gachet, A.D. Mumford, P. Noris, D. Mezzano, and P. Harrison, provided critical comments and revisions, and all authors have approved the final version.

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Table 1. Position Statement of the Platelet Physiology Scientific and Standardization Committee on the Laboratory Monitoring of $P2Y_{12}$ Inhibitors

Recommendation	Class[a]	Level[b]	Refs.
$P2Y_{12}$ inhibitor monitoring to assess risk for bleeding or thrombosis during prolonged DAPT			
HPR and LPR determined by $P2Y_{12}$-monitoring as described in [4, 5] are associated with risk for ischemic and hemorrhagic events (respectively) and therefore may be considered in the overall management of patients. Optimal timing and frequency of this monitoring is unclear.	IIa	A	[4, 5]
$P2Y_{12}$ inhibitor monitoring to adjust $P2Y_{12}$ inhibitor dose or adjust $P2Y_{12}$ inhibitor selection			
Monitoring $P2Y_{12}$ inhibition for the purpose of guiding the intensity of antiplatelet therapy is not recommended. Monitoring $P2Y_{12}$ inhibition for the purpose of guiding the duration of DAPT is not recommended.	IIb	B	[8-10, 24]
$P2Y_{12}$ inhibitor monitoring for early de-escalation from prasugrel to clopidogrel in patients considered not suitable for prolonged prasugrel therapy			
Monitoring $P2Y_{12}$ inhibition may be considered for early de-escalation from prasugrel to clopidogrel in patients considered not suitable for prolonged prasugrel therapy	IIb	B	[13]
$P2Y_{12}$ inhibitor monitoring to shorten the time window to surgery following $P2Y_{12}$ inhibitor discontinuation			
It is reasonable, in balancing the risk of thrombosis during a delay to surgery with the risk of surgical bleeding, to consider the results of $P2Y_{12}$ inhibitor monitoring to determine the timing of surgery. • A cut-off of TEG MA_{ADP} >50 is recommended if this test is available. • A cut-off of PFA-100®P2Y CT <106 seconds is recommended if this test is available.	IIa	B	[33, 34]
• For other $P2Y_{12}$ inhibitor monitoring tests, cut-offs with respect to CABG bleeding have not been established. However, it may be reasonable to consider proceeding to surgery if platelet reactivity is >80% that seen in $P2Y_{12}$ inhibitor-free patients.	IIb	C	[33, 34]

[a]Class of recommendation: IIa, weight of evidence/opinion is in favor of usefulness/efficacy; IIb, usefulness/efficacy is less well established by evidence/opinion; III, evidence or general agreement that the given treatment or procedure is not useful/effective, and in some cases may be harmful

[b]Level of evidence: A, data derived from multiple randomized clinical trials or meta-analyses; B, data derived from a single randomized clinical trial or large non-randomized studies; C, consensus of opinion of the experts and/or small studies, retrospective studies, registries.

Abbreviations: CABG, coronary artery bypass graft; CAD, coronary artery disease; DAPT, dual antiplatelet therapy; HPR, high on-treatment platelet reactivity; LPR, low on-treatment platelet reactivity; PFA, platelet function analyzer; TEG, thromboelastograph

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