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Review

# The use of platelet reactivity testing in patients on antiplatelet therapy for prediction of bleeding events after cardiac surgery

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ABSTRACT

Many patients are treated with platelet inhibitors such as aspirin and clopidogrel for prevention of thrombotic cardiovascular events. However, the inhibitory effect of antiplatelet therapy is variable between patients; in some, the platelets are hardly inhibited, while in others, the platelets are excessively inhibited. The newer and more potent platelet inhibitors, prasugrel and ticagrelor, often lead to low platelet reactivity, which potentially leads to bleeding events. Preoperative measurement of platelet reactivity in patients receiving platelet inhibitors who undergo cardiac surgery, could be useful to identify those with low platelet reactivity and thus have an increased risk of bleeding during or after surgery. In this review, we discuss the most commonly used platelet inhibitors and platelet function tests. Furthermore, we will provide an overview of the evidence for the prediction of post-operative bleeding at the operation site with preoperative platelet reactivity testing in patients undergoing cardiac surgery.



**Contents**



## 1. Introduction

Platelets are anucleated blood cells that play a key role in the maintenance of vascular integrity. They continuously monitor the vascular wall for breaches and are able to respond rapidly when defects are encountered. Upon exposure to the unique features of an injured vessel wall, they adhere to the site of injury despite the shear forces of the circulating blood, and immediately aggregate with other platelets. This results in the formation of a primary haemostatic plug that prevents blood loss and remains in place until it is reinforced with a strong fibrin network [1]. Failure to form an adequate platelet plug due to low platelet

*Abbreviations:* ADP, Adenosine diphosphate; CABG, Coronary artery bypass graft; CTO, Chest tube output; CVE, Cardiovascular event; LTA, Light transmittance aggregometry; PFA-100, Platelet function analyser- 100; MEA, Multiple electrode aggregometry; PR, Platelet reactivity; PRU, P- reactive units; TEG, Tromboelastography; TRAP, Thrombin receptor-activating peptide; VASP, Vasodilator- stimulated phosphoprotein.

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reactivity (LPR) or low platelet count will lead to bleeding complications, while high platelet reactivity (HPR) is associated with an increased risk of thrombosis, mainly in the arteries.

Although arterial thrombosis is a multifactorial disease with diverse genetic and acquired predisposing risk factors, the importance of platelets in this process is widely recognized. As a result, many people are treated with antiplatelet therapies for secondary prevention of thrombotic cardiovascular events (CVE), such as myocardial infarction, cerebrovascular accident and transient ischemic attack. Since the 1970s, the effect of aspirin as a platelet inhibitor has been studied extensively [2]. Aspirin has been very efficient in the prevention of secondary CVE; a meta- analysis of randomized studies of various antiplatelet drugs showed a 25% decrease in thrombotic vascular outcomes in patients with pre-existing conditions on aspirin [3]. With the addition of alternative and more potent $P2Y_{12}$ inhibitors such as clopidogrel, prasugrel and ticagrelor, the prevention of secondary CVEs has become even more effective [4,5].

The current recommended antiplatelet treatment for patients who undergo invasive cardiac surgery, such as coronary artery bypass graft (CABG), is aspirin monotherapy, with a recommended dose of 100-325 mg daily. Therapy should be initiated preoperatively, or at least within 6 hours post procedure and should be continued indefinitely to reduce the occurrence of vein graft closure and adverse cardiovascular events [6,7]. However, a recently published review showed that HPR despite aspirin treatment (HAPR) occurs in some patients [8]. The prevalence of aspirin resistance after CABG varies greatly though, with incidence rates ranging from 10% up to >90% in different studies, and seems to depend on both the assay used and the timing of measurements. [9] Possible causes of HAPR could be inadequate dosage, drug interactions, genetic polymorphisms of COX-1 and other genes involved in thromboxane biosynthesis, upregulation of non-platelet sources of thromboxane biosynthesis and increased platelet turnover [10,11].

To circumvent HAPR, alternative antiplatelet therapy as dipyridamole, warfarin or clopidogrel have been explored in multiple trials [12–15]. So far, no other treatment regimen has been proven to be superior to aspirin, but multiple studies, including trials with the newer agents prasugrel and ticagrelor, are still underway (POPular CABG [NCT02352402] and Prasugrel for Prevention of Early Saphenous Vein Graft Thrombosis [NCT01560780]).

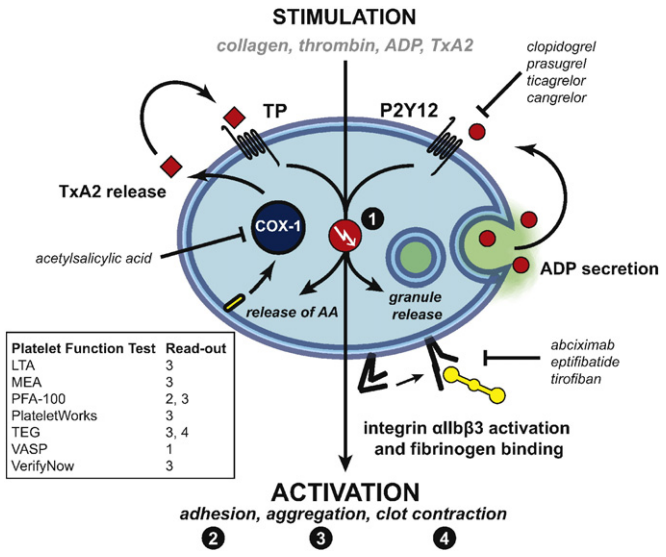
However, the newer and more potent $P2Y_{12}$ inhibitors have a potential downside: excessive inhibition of the platelets may cause LPR, potentially leading to bleeding events. Preoperative usage of clopidogrel on top of aspirin has already been associated with an increased risk of bleeding and mortality [16,17]. In general, postoperative bleeding is a common complication after invasive cardiac surgery. The need for administration of blood products after cardiac surgery due to major blood loss is associated with an 8.1-fold (95% CI, 3.9-17.0) increased risk of in-hospital mortality [18]. Moreover, blood transfusion is associated with an increased risk for postoperative adverse events such as mortality, renal failure, extended ventilatory support, major infection, cardiac complications and neurologic events. [19] Besides extensive inhibition of the platelets by platelet inhibitors, multiple other mechanisms may contribute to LPR during cardiac surgery, including dysfunction of alpha-granule release, decreased glycoprotein Ib expression on the platelet membrane, prolonged circulation of activated, "exhausted" platelets, and impaired platelet aggregation [20,21].

The association of HPR with increased risk of thrombotic events [22,23] and the relation of LPR with bleeding risk [24,25], suggest that there is a therapeutic window for platelet reactivity. If this is the case, assessment of PR in patients receiving antiplatelet therapy could identify those who are 'overtreated' and have an increased risk of bleeding during or after surgery. The timing of the operation can then be optimized in this group of patients and the risk of bleeding and transfusion requirement can be reduced. In this issue of vascular pharmacology, Polzin and colleagues have analysed the relationship between platelet reactivity and thrombotic or bleeding events in patients undergoing cardiac surgery. This review provides an overview of the evidence of preoperative platelet reactivity testing as predictor of post-operative bleeding complications in patients receiving aspirin or P2Y12 during invasive cardiac surgery. Since most of the available data are derived from studies involving CABG, we have used CABG as a model procedure.

## 2. Antiplatelet therapy regimens

Many antiplatelet regimens have been investigated, interfering with one or more stages in platelet activation. A brief overview of the antiplatelet regimens available and the activation pathways with which they interfere is provided below (Fig. 1).

**Aspirin, or acetylsalicylic acid** (ASA) is an irreversible cyclooxygenase (COX)-1 inhibitor. When platelets are activated, arachidonic acid (AA) is liberated from the internal plasma membrane of the platelet by phospholipase A2 and converted into prostaglandin $H_2$ by the enzyme COX-1. Prostaglandin H2 is subsequently converted into thromboxane A2, which will diffuse from the platelet interior and bind the thromboxane prostanoid receptor (TP). Signalling downstream from this G-protein coupled receptor leads to a rise in the intracellular $Ca^{2+}$ concentration, as well as cytoskeletal rearrangement, causing shape change and enhanced platelet activation. Because platelets are anucleate, de novo synthesis of COX-1 by platelets is absent. As a result the effect of aspirin lasts for the remainder of the platelet lifetime (8-10 days). Although some studies have shown that high-dose aspirin also inhibits thrombin generation [26] and erythrocyte-mediated platelet activation [27], aspirin is regarded as a weak platelet inhibitor. Because




| Platelet Function Test | Read-out |
|---|---|
| LTA | 3 |
| MEA | 3 |
| PFA-100 | 2, 3 |
| PlateletWorks | 3 |
| TEG | 3, 4 |
| VASP | 1 |
| VerifyNow | 3 |

**Fig. 1. Schematic representation of the major targets of antiplatelet therapy and the assays to measure their effects.** When platelets are activated, an intracellular signaling cascade is initiated. This will lead to activation of the fibrinogen receptor, the integrin αIIbβ3. Aside from activation of the fibrinogen receptor, stimulation of platelets will lead to the initiation of two major auxiliary activation processes. First, platelets will secrete the content of their intracellular granules. Platelet dense granules contain ADP, which stimulate the purinergic receptors P2Y1 and $P2Y_{12}$. Out of these two receptors, $P2Y_{12}$ contributes most to the propagation of the prothrombotic response. Several antiplatelet agents target this receptor, rendering platelets less susceptible to activation. Aside from granule release, platelet activation will lead to activation of phospholipase A2, which will liberate the fatty acid arachidonic acid (AA). AA will subsequently be converted by cyclooxygenase (COX)-1 into prostaglandin H2, which will then be converted into thromboxane A2 (TxA2). TxA2 will pass through the platelet membrane and bind to the prostanoid receptor (TP). This will further augment platelet activation. The conversion of AA to prostaglandin H2 by COX-1 is inhibited by acetylsalicylic acid. By far the most platelet reactivity tests measure the final stages of platelet activation, i.e. platelet aggregation, platelet adhesion or clot retraction. The vasodilator stimulated phosphoprotein (VASP) assay is the exception, as it measures changes in signaling molecules within the platelet. LTA light transmission aggregometry, MEA multiple electrode aggregometry, PFA-100 platelet function analyzer 100, TEG thromboelastography.