Platelet Function Monitoring

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Case Vignette

A 63-year-old farmer is admitted to the emergency department with an acute coronary syndrome. He takes aspirin (75 mg/day) and clopidogrel (75 mg/day) after he received a drug eluting stent in the proximal left coronary artery 8 months ago. The coronary angiography showed a near occlusion of this stent. In addition, there are subtotal occlusions in the distal right coronary artery and proximal circumflex artery, so the heart team decides for urgent coronary artery bypass graft surgery. The team questions whether the antiplatelet drugs were effective, and whether platelet function testing could provide sufficient information about the bleeding and thrombotic risk. Moreover, can platelet function analysis be used to estimate the bleeding risk, and valuable for changing therapy?

Why Is It Important?

Antiplatelet therapy is a cornerstone of primary and secondary prevention in cardiology. Particularly, patients with an acute coronary syndrome or a percutaneous intervention frequently receive dual antiplatelet therapy consisting of aspirin and a $P2Y_{12}$-receptor antagonist, like clopidogrel, prasugrel, or ticagrelor, to prevent progression of the disease and stent thrombosis. However, due to a variety of reasons this prophylaxis is not always effective, which renders a risk of a thrombotic event. In addition, treatment with antiplatelet drugs puts patients at risk for bleeding with 4–7 events per 100-person years. In case of an urgent operation this risk must be balanced against the risk of stent-thrombosis [1].

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Looking at cardiac surgery, bleeding still is one of the most important perioperative complications. It increases mortality independently with more than 10%, even if blood products are administered [2]. Some bleeding risk factors are known for a long time, such as the impact of surgical trauma, prolonged use of the extracorporeal circuit, and the use of high dose anticoagulants. Others gained more attention during the last decade, like the deliberate use and combination of antiplatelet and antithrombotic drugs before surgery. It is therefore of importance to understand the individual bleeding mechanism, followed by targeted therapy in order to correct coagulation abnormalities and reduce unnecessary transfusion of blood products. After surgery, most patients restart antiplatelet therapy to protect their grafts, and the timing of discontinuation and resuming of therapy is crucial in the perioperative management in cardiac surgery. The aim of this chapter is to describe the function of human platelets, the approach to test their function, and to translate this knowledge into clinical practice.

Platelet Function

With a diameter of 2–5 μm and counts between 150 and 350 × 10^9/L, platelets are the smallest and second most frequent particular components circulating in blood, with a life-span of about 10 days. Aside their involvement in inflammation, the host defense, angiogenesis, and atherosclerosis, initiation and augmentation of coagulation is the main function of platelets [3]. Platelet contribution to hemostasis was classically described as primary, corpuscular hemostasis separated from the secondary, plasmatic hemostasis. Nowadays it is thought that platelets activate and propagate coagulation in general.

In brief, due to their small size, platelets are pushed to the vessel walls in the blood stream. Here they keep a close contact to the endothelium where they adhere to injured, endothelium-denuded layers (e.g., after a vessel wall lesion) binding to von Willebrand factor (vWF), thrombospondin-1, and under low shear stress directly to collagen. This adhesion initiates the secretion of multiple mediators, like adenosine diphosphate (ADP), thrombin, and fibrinogen, which enhances further platelet activation. The process leads to contraction of the actin–myosin filaments and shape change of the platelets, turning them into an aggregation state. In the activated state, platelets express tissue factor on their membrane, which in concert with a complex of activated coagulation factor VII and tissue factor starts and amplifies the plasmatic coagulation [4]. Yet, the activation of platelets needs different receptors, which have specific agonists (Table 9.1).

The platelet receptor pathways are used as specific therapeutic targets and the assessment of platelet function. Like in other receptor–agonist conditions a high concentration of a weak agonist or a low concentration of a strong agonist is used in these tests to induce and maintain irreversible aggregation [4]. Figure 9.1 shows the different platelet function tests with their respective activators.

What is the Evidence from the Literature?

Continuing aspirin throughout cardiac surgery as a single therapy results in reduced cardiovascular morbidity and mortality, with an acceptable, mildly

Table 9.1 Platelet receptors and their activators

Activator	Receptor	Effect	Characteristics
ADP	P2Y1/P2Y$_{12}$	Enhancing of activation	Secreted from dense bodies Major platelet feedback agonist
Thromboxane A$_2$	Thromboxane receptor	Maintenance of activated state	Weak agonist
Thrombin	PAR 1/PAR 4	Induction of inside-out signaling	Strong agonist Induction of procoagulant surface
Collagen	GPIa/IIa, GPVI	Adhesion to subendothelial layer Secretion	Strong agonist Low shear rate Induction of procoagulant surface
vWF	GPIbalpha (GPIb/V/IX)	Adhesion to subendothelial layer Secretion	High shear rate
Thrombospondin	GPIbalpha	Adhesion to subendothelial layer Secretion	High shear rate
Fibrinogen	GPIIb/IIIa	Cross linking platelets Aggregation and activation (outside-in signaling)	–

ADP adenosine diphosphate, *vWF* von Willebrand Factor

increased bleeding risk [5]. Only in high bleeding risk situations, aspirin could be paused preoperatively. This is different for dual antiplatelet therapy. Although dual antiplatelet therapy reduces the cardiovascular risk, it also increases the burden of bleeding, which is associated with higher morbidity and mortality. For this reason, discontinuation of the P2Y$_{12}$ blocker in a timely manner is advised in most cases. Due to the urgent or emergency character of cardiac surgery, there is frequently no time to discontinue dual antiplatelet therapy on time, which requires intensified hemostatic monitoring. Moreover, some patients are insensitive to platelet inhibitors, and these patients are at an increased risk for postoperative thrombosis, which also justifies hemostatic and platelet function assessment [5].

Platelet Function Tests

Available platelet function tests can be divided into tests directly assessing platelets and assays which detect surrogate parameters of platelet activation. In this section, we focus on the direct tests, which are mainly point-of-care (POC) tests. In general, there are whole blood tests and assays using platelet rich plasma to assess platelet function. Moreover, global evaluations can be separated from (very) specific tests, such as flow cytometry.

PFA-100/200	ADP/EPI/ADP+PGE1
Light Transmission Aggregometry	All possible, ADP/AA/TRAP/Collagen
VerifyNow	ADP/AA/PGE1
PlateletWorks	ADP/AA/Collagen
WBA	ADP/TRAP/Collagen/Ristocetin
TEG-platelet mapping	ADP/AA
ROTEM-MEA	ADP/AA/TRAP

Fig. 9.1 Different platelet function analyzers with their respective activators. *PFA* platelet function analyzer, *WBA* whole blood impedance platelet aggregometry, *TEG* thromboelastography, *ROTEM* rotational thromboelastometry, *MEA* multiple electrode aggregometry, *ADP* adenosine diphosphate, *EPI* epinephrine, *PGE1* prostaglandin E1, *TRAP* thrombin receptor activating peptide, *AA* arachidonic acid

The gold standard for platelet function analysis is light transmission aggregometry (LTA). For this test a sample of citrated whole blood needs to be spun off two times and separated to retain platelet rich plasma and platelet poor plasma. In the next step the material will be mixed with platelet activators, and the change of turbidity is measured by change of light absorption to compare platelet rich and poor plasma. The platelet poor plasma is generally clear and serves as calibrator. An advantage of this method is the freedom to use different activators in various concentrations, and the possibility to detect even mild abnormalities. Unfortunately, this test is work-intensive and time consuming, and not considered as POC. Besides, it seems quite non-physiological, because it excludes all other corpuscular components of the blood. In addition, the test is not properly standardized, which makes it difficult to compare results between different laboratories [4, 6].

VerifyNow™

The VerifyNow is a system based on the light transmission aggregometry principle. The device is more frequently used in cardiology for quantification of antiplatelet drug effects [1]. It works fully automated with cartridges prefilled with fibrin-coated polystyrene beads combined with a platelet activator. According to the pathway to be assessed there are three different cartridges containing arachidonic acid (AA), ADP, or prostaglandin E1 as an activator. After adding a sample of citrated whole blood,

the system measures the change of light transmission over time. The main advantage of the technique is the use of whole blood and the complete automatization.

Whole Blood Impedance Aggregometry

Whole blood impedance aggregometry, like multiple electrode aggregometry, Multiplate™ or ROTEM-platelet™, is a frequently used method to assess platelet function. A blood sample is first anticoagulated, usually with hirudin, and pipetted into small cups. A predefined concentration of an activator, depending on the manufacturer (e.g., ADP, AA, collagen, thrombin activating peptide [11], or ristocetin) is added. For the Multiplate™, the reagents enable the diagnosis of von Willebrand disease with ristocetin, or the effect of antiplatelet drugs by ADP and AA. The ROTEM-platelet™ focuses on detecting an antiplatelet agent effect by ADP, AA, and the thrombin activating peptide (TRAP) test reflecting $P2Y_{12}$ inhibition, aspirin, or GPIIb/IIIa inhibition, respectively.

In the measurement cup, two pairs of electrical wires are built. Between each of them a current is running, while a magnetic stirrer keeps the diluted whole blood mixed. After activation, the platelets will adhere to fibrinogen, which is first condensed to the wires. This causes a change of electrical impedance, which is measured over time. Although initiation of this test needs some expertise, it is easy to perform and does not require much training or time. The results of the impedance change are displayed while the test is running. After 6 min, the test stops automatically and can be read out [4, 12].

Thromboelastography Platelet Mapping

The thromboelastography (TEG) platelet mapping uses citrated whole blood, which is processed by pipetting it into the test-cup. Blood is subsequently spiked with ADP or AA as platelet receptor activator. The results of these two tests are compared to the results of the conventional TEG-traces, which allows conclusions about platelet contribution to clot strength. Particularly, the newer version of the TEG devices, the TEG 6S, is semi-automated and uses cartridges which only need to be filled by pipetting.

PlateletWorks™

The PlateletWorks™ approach uses a standard impedance cell counter and two tubes of blood. The platelet function activator is added to one of these tubes, and the proportional difference in platelet count is given as a surrogate marker for platelet activation. This method correlates well with results from LTA studies [10]. The main advantage is the use of whole blood and of common counter systems, which are widely available. However, the system relies on end-point platelet aggregation, which means that it is sensitive to incomplete aggregation. More importantly, the method is sensitive to all kind of situations, where a cell counting device struggles with (e.g. giant cells,

pseudothrombocytopenia, cell fragments). According to the literature, one EDTA tube is needed for basic counting, while the second tube spiked with an activator should contain citrated blood. This induces a dilution mismatch, resulting in lower platelet counts in the citrated sample even if no platelet activation took place.

Platelet Function Analyzer

The platelet function analyzer (PFA-100/200™) uses citrated whole blood. Blood is aspirated through an activator-coated capillary which mimics shear stress. The time that is required to close the capillary is quantified and correlates with platelet function. The system does not need any preparation of the sample, which eases its use. The application of shear stress allows screening for moderate to severe forms of von Willebrand diseases and Glanzmann's thrombasthenia. There are three cartridges available (collagen/ADP, collagen/epinephrine, and collagen/prostaglandin E1) for the detection of aspirin and $P2Y_{12}$ blocker effects. The main disadvantage is the low sensitivity and specificity of the system [6].

VASP test

Aside to the described tests there are some more which are not very well studied in cardiac surgery. These are mainly the one using surrogate parameters like the Vasodilator-stimulated phosphoprotein phosphorylation assay (VASP), which is more used in cardiology. Principally the VASP test uses PRP in which the phosphorylation state is assessed rather by flow cytometry or by ELISA based assays.

Limits of Platelet Function Tests

Apart from the light transmission aggregometry, all systems have moderate to low sensitivity to mild platelet dysfunction, which could play a role as an ancillary factor when the coagulation system is generally disturbed. This means that a combination of low coagulation factor concentration, low hemoglobin, and mildly decreased platelet dysfunction could result in clinical bleeding, while all laboratory parameters are still at the lower edge of normality.

Moreover, whole blood impedance aggregometry is influenced by temperature, protamine, and tranexamic acid, but not by anesthetic drugs like midazolam, propofol, lidocaine, and magnesium [13]. The literature regarding drug interactions with other platelet function tests is however limited. To a certain degree, all systems are sensitive for changes in hemoglobin, but also for thrombocytopenia. In general, a platelet count below 150×10^9/L will impair platelet function readings, but the critical platelet count threshold depends on the activation pathway. For the Multiplate™ and PFA-100™, a hematocrit below 30% is critical with respect to the reliability of the test results [14]. This could be a major disadvantage in cardiac surgery when

postoperative findings are compared with preoperative data. In particular, the impairment of the $P2Y_{12}$ and collagen receptor due to exposure of blood to the extracorporeal circuit may be detected by these devices [15].

Most systems use whole blood, which is an advantage in terms of user-friendliness and the physiological value of test results. However, discrepancies in the composition of blood impede clear conclusions regarding one corpuscular compartment only, which are the platelets. Except for thromboelastography, which compares the intrinsic activity of fibrinogen, all methods using fibrinogen as an anchor for platelets are sensitive to fibrinogen levels, but specific literature for this phenomenon is lacking. Finally, the human factor still plays a major role, and less automated systems are more subject to measurement errors by the operator than automated systems with cartridges.

Predicting of Perioperative Bleeding

Most evidence regarding the predictive value of platelet function tests for perioperative bleeding is derived from studies using multiple electrode aggregometry. A retrospective study showed that a patient blood management algorithm including preoperative platelet function analysis reduced blood transfusion in general, but the volume of transfused platelets increased [16]. This study underlines the concept of assessing platelet function before surgery, which might contribute to an estimation of remnant platelet function and help in timely ordering of platelet concentrates or the cessation of $P2Y_{12}$ inhibitors [17–19]. Others demonstrated that preoperative multiple electrode aggregometry in a cohort of patients under treatment of $P2Y_{12}$ blockers could identify patients at risk for bleeding [17]. Moreover, they identified preoperative cut-off values for ADP and TRAP tests that were associated with bleeding [17]. In a comparison of multiple electrode aggregometry and the rotational thromboelastometry (ROTEM™) platelet function test it was shown that only multiple electrode aggregometry has a predictive value for bleeding [20]. Both devices showed however a good correlation with perioperative blood loss [21].

Kong and co-workers proposed in 2015 an algorithm to test patients treated under $P2Y_{12}$ inhibition for MEA analysis. They recommend to use the MEA ADP assay and the TRAP assay. Others showed that the ADP test is an indicator for $P2Y_{12}$-related bleeding using multiple electrode aggregometry, while the TRAP test reflects platelet function in general [22]. A comparison of multiple electrode aggregometry and thromboelastography-platelet mapping before and after cardiopulmonary bypass showed that both postoperative tests did not correlate with blood loss during the first 12 h after surgery [23] and is associated with a reduction in blood transfusions [24]. This is in contrast to results from a Scandinavian group, showing that preoperative thromboelastography-platelet mapping was superior in the prediction of bleeding compared to multiple electrode aggregometry [25].

When the VerifyNow™ system was tested in a preoperative and retrospective setting as a predictor for platelet inhibition, it demonstrated a good indication of aspirin-induced platelet inhibition, but without an association with postoperative bleeding [26]. Others however showed that one should be cautious to use the

Table 9.2 Advantages and disadvantages of different platelet function analyzers

Test	Advantages	Disadvantages
PFA-100/200	WB, easy to perform, flow makes it suitable for vWF-disease	Depending on hematocrit and platelet count
LTA	Allows use of multiple activators in different concentrations	Needs preparation of PRP and PPP, poorly standardized
VerifyNow	WB, easy to perform, cartridges	Sensitive to all
PlateletWorks	WB, easy and simple, uses standard devices	Sensitive to all abnormalities interfering with cell count
WBA	WB, easy to perform	Sensitive to low platelet count
TEG-platelet mapping	WB, easy to perform, cartridges	Sensitive to low platelet count
ROTEM-MEA	WB, easy to perform	Sensitive to low platelet count

PFA platelet function analyzer, *LTA* light transmission aggregometry, *WBA* whole blood impedance platelet aggregometry, *TEG* thromboelastography, *ROTEM* rotational thromboelastometry, *MEA* multiple electrode aggregometry, *WB* whole blood, *vWF* von Willebrand, *PRP* platelet rich plasma, *PPP* platelet poor plasma

VerifyNow™ to decide when antiplatelet drugs should be discontinued [27]. In contrast, in a smaller, prospective study the VerifyNow™ had a good association with bleeding when used in the preoperative setting in a cohort of patients treated with dual antiplatelet therapy, including $P2Y_{12}$ inhibitors [28]. Yet, there are several studies deriving from the cardiology field looking at periprocedural events after percutaneous interventions that provide positive evidence for the use of this system in the confirmation of adequate platelet inhibition, which is associated with a decreased risk of stent thrombosis [1].

Implications for Daily Practice

Regarding estimation of thrombosis and bleeding risk around PCI procedures the VerifyNow™ system and the MEA device have been investigated. Additionally, thromboelastography-based platelet mapping and the VASP assay have been tested in this setting, and all systems show added value in the definition of the optimal dose–response relation for antiplatelet drugs, and the reduction of thrombotic and bleeding risks [29]. The evidence for platelet function testing in cardiac surgery is contrasting, and depends on the clinical settings and devices used (see Table 9.2). However, it seems reasonable to plan elective interventions guided by platelet function tests to determine when to stop $P2Y_{12}$ inhibition. The VerifyNow™ and multiple electrode aggregometry are well established in assessing the recovery of platelet function in patients who discontinued antiplatelet drugs. Also, in urgent situations, preoperative platelet function tests demonstrated to help estimating the bleeding risk. Furthermore, platelet function assessment could lead to timely ordering of platelet concentrates.

Intraoperative and postoperative platelet function testing should be interpreted with caution, since the test results are influenced by hemodilution and a reduced

number of corpuscular compartments. Likewise, the use of drugs and cardiopulmonary bypass influences platelet function to an uncertain degree, and it is not reliably to assess platelet function under these circumstances. One should remember that platelet function usually recovers within 12–24 h after surgery. Finally, with the assumption that platelet count and hemoglobin levels are not critical, platelet function testing could be helpful in timing restarting of antiplatelet therapy.

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