The assessment of platelet function

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SUMMARY

Platelet activation is involved in physiological hemostasis and in a variety of thromboembolic events leading to stroke, myocardial ischemia, and other organ dysfunction. Apart from congenital platelet dysfunction, acquired platelet defects are increasingly common in patients due to the widespread use of antiplatelet agents. Newer antiplatelet agents targeting platelet glycoprotein IIb–IIIa and adenosine-5′-diphosphate receptors inhibit platelet activation, and therefore assessing platelet inhibition may be important to prevent major bleeding, especially before a surgical intervention. In the past decade, the knowledge from genotypic analyses and prospective outcome studies suggested inter-patient variability in the therapeutic response to antiplatelet therapies, which further stresses the need for individual platelet testing. In managing bleeding patients, platelet transfusion may be better guided by the use of platelet function tests because transfusion therapy is costly and potentially harmful if administered inappropriately. Basic understanding of the hemostatic roles of platelets, etiologies of platelet defects, and characteristics of current platelet assessment methods should help physicians who manage complex coagulation issues in critically ill patients.

INTRODUCTION

In the past century, there has been extensive research into both physiological and pathological roles that platelets play in hemostasis and thrombosis. Antiplatelet therapy with aspirin has become widely accepted for prevention of arterial thrombosis in patients with atherosclerotic cardiovascular disease. However, clinical assessment of platelet function has been limited to the workup of congenital bleeding disorder.

The importance of monitoring platelet function has drawn more attention recently for several reasons. First, more potent antiplatelet agents, namely inhibitors of glycoprotein IIb–IIIa (GPIIb–IIIa) receptor and adenosine-5′-diphosphate (ADP) receptor, were introduced. Second, clinicians realized that unrecognized underdosing or therapeutic resistance to antiplatelet therapy may compromise patients’ outcomes. Furthermore, antiplatelet agents are often used in combination with aspirin and/or anticoagulant drugs, therefore dosing of the respective agents may need to be adjusted to avoid major bleeding complications. Third, technological advances enabled the improvement of conventional platelet function tests as well as the development of novel assays.

Today, physicians are required to manage complex coagulation issues of critically ill patients, and therefore face various issues in coagulation monitoring and administering hemostatic agents and transfusion. The
The aim of this article is to provide a concise review of laboratory and point-of-care assessments of platelet function in relation to the current concept of hemostasis and clinical platelet dysfunction.

MECHANISM OF HEMOSTASIS

Platelets are involved in multiple key steps in normal hemostasis (Figure 1). Primary hemostatic plugs are formed by attachment of platelets to the subendothelial surface exposing collagen and tissue factor. Platelet glycoprotein Ib (GPIb) receptors and von Willebrand factors are required for this process. Subsequently, platelet activation mediated by platelet-derived thromboxane and ADP lead to accumulation and aggregation of platelets at the site of injury. Activated platelets undergo conformational changes of GPIIb/IIIa receptors which now bind to fibrinogen and fibrin. Furthermore, activated platelet surfaces support thrombin generation by binding to various coagulation factors including prothrombinase complex (factor Va-factor Xa). When prothrombin is converted to thrombin by this enzyme complex, fibrinogen is cleaved to form fibrin. Explosive thrombin generation follows this initial clot formation, and clot is stabilized by thrombin-catalyzed activation of platelets, factor XIII, and thrombin activatable fibrinolysis inhibitor. Alternatively, thrombin formation is controlled by endogenous anticoagulant system including antithrombin, protein C/protein S, and endothelial thrombomodulin.

PLATELET DYSFUNCTION

Congenital platelet defects

Congenital platelet dysfunction can be categorized as: (i) adhesion defect: Bernard–Soulier syndrome, pseudo-von Willebrand disease; (ii) aggregation defect: Glanzmann’s thrombasthenia, P2Y1 deficiency; (iii) secretion defect: storage pool disease (SPD), Hermansky–Pudlak syndrome, α-SPD, δ-SPD; (iv) signaling defect: platelet polymorphism; and (v) procoagulant defect: Scott syndrome, factor V ‘Quebec’. Relevant diseases are briefly mentioned below with respect to platelet monitoring, and readers should refer to detailed reviews elsewhere.1,2

Acquired platelet defects

Platelet dysfunction is associated with multiple disorders. Underlying conditions include uremia, hepatic failure, the use of cardiopulmonary bypass (CPB), myeloproliferative disorders, dysproteinemia and numerous drugs.3

Antiplatelet therapies

Most acquired platelet dysfunctions are attributed to antiplatelet therapies. The procoagulant activity of platelets has been associated with pathological occlusion of vital vessels; therefore, antiplatelet drugs are widely prescribed for patients with cardiovascular diseases. The major classes of antiplatelet drugs are sum-

Figure 1. In vivo arterial hemostasis. A. Platelet adhesion and aggregation at injury site. B. Thrombin formation on activated platelet surface. C. Clot formation (thrombin catalyzed conversion of fibrinogen to fibrin). D. Inhibition of platelet accumulation and reduced thrombin formation at injury site. ADP, adenosine diphosphate; TxA2, thromboxane A2.
Aspirin is the prototypical inhibitor of cyclooxygenase (COX). Thromboxane A₂ formation is irreversibly inhibited by irreversible acetylation of COX-1. Aspirin has been the mainstay antiplatelet agent for prevention of myocardial infarction, ischemic stroke, and peripheral vascular disease. Aspirin is also available as a combination tablet with dipyridamole (Aggrenox®, Boehringer Ingelheim, Biberach, Germany), which is an inhibitor of adenosine metabolism. Adenosine stimulates adenylate cyclase and increases intraplatelet cyclic AMP (cAMP) levels. Aggrenox® is indicated for prophylaxis of transient ischemic attacks and stroke.4

**GPIIb/IIIa inhibitors.** Abciximab (ReoPro®, Eli Lilly, Indianapolis, IN, USA), eptifibatide (Integrilin®, Millenium Pharmaceuticals, Cambridge, MA, USA) and tirofiban (Aggrastat®, Merck, Whitehouse Station, NJ, USA) are the three intravenous agents that are currently available in North America. The interactions of platelet–fibrin via GPIIb/IIIa receptors play pivotal roles in normal hemostasis and pathological thrombosis. Implementation of GPIIb/IIIa antagonists with heparin anticoagulation has improved outcomes of coronary intervention. Pharmacokinetic and pharmacodynamic profiles vary among the three drugs (Table 1). Major bleeding may be observed within 12 hours of abciximab therapy, especially after cardiac surgery, but eptifibatide and tirofiban are less likely to result in excessive bleeding.5 Acute thrombocytopenia may be observed in 0.1–1% of patients receiving GPIIb/IIIa inhibitors, most commonly with abciximab.

**Thienopyridines.** Ticlopidine (Ticlid®, Roche Laboratories, Nutley, NJ, USA) and clopidogrel (Plavix®, Sanofi-Aventis, Bridgewater, NJ, USA) are thienopyridine-derived antiplatelet drugs that block platelet ADP receptors. The latter has replaced ticlopidine in North America because it is associated with fewer side effects (e.g. thrombocytopenia or neutropenia), and it has become a mainstay therapy in the coronary interventions and treatment of ischemic cardiovascular disease. Preoperative use of clopidogrel, especially in conjunction with aspirin, is associated with an increased risk of bleeding after cardiac surgery with CPB.

**Phosphodiesterase (PDE) inhibitor.** Cilostazol (Pletal®, Otsuka Pharmaceuticals, Tokyo, Japan) is a novel oral PDE type III inhibitor that is indicated for symptomatic relief of intermittent claudication. Inhibition of PDE leads to increased intraplatelet cAMP and causes disaggregation of platelets.6 Another type of oral PDE type III inhibitor, anagrelide (Agrylin®, Shire, Wayne, PA, USA), is specifically indicated for thrombocytosis due to myeloproliferative disorders. Anagrelide inhibits maturation of megakaryocytes.

**Anticoagulants.** Thrombin is the most potent platelet agonist, and protease-activated receptors (PAR 1 and PAR 4) are present on platelet surface, and their activation leads to granule release and platelet aggregation via GPIIb/IIIa receptors. Hence, anticoagulants which inhibit thrombin activity reduce platelet activation via PAR receptors. Recombinant hirudin (Refludan®, Berlex, Wayne, NJ, USA), argatroban (Slonnon®, Daiichi Pharmaceuticals, Tokyo, Japan), bivalirudin (Angiomax®, Medicines Company, Parsippany, NJ, USA) are three major intravenous direct thrombin inhibitors.

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**Table 1. Current antiplatelet drugs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Trade name</th>
<th>Route</th>
<th>Description</th>
<th>Half-life</th>
<th>Platelet function return</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abciximab</td>
<td>ReoPro</td>
<td>IV</td>
<td>Chimeric mAb</td>
<td>30 min</td>
<td>48 h</td>
</tr>
<tr>
<td>Eptifibatide</td>
<td>Integrilin</td>
<td>IV</td>
<td>Cyclic heptapeptide</td>
<td>2.5 h</td>
<td>4–8 h</td>
</tr>
<tr>
<td>Tirofiban</td>
<td>Aggrastat</td>
<td>IV</td>
<td>Non-peptide</td>
<td>1.6 h</td>
<td>4–8 h</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Plavix</td>
<td>PO</td>
<td>Thienopyridine</td>
<td>8 h</td>
<td>5–10 d</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>Ticlid</td>
<td>PO</td>
<td>Thienopyridine</td>
<td>12–96 h</td>
<td>2 wk</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Ecotrin</td>
<td>PO</td>
<td>Acetylsalicylic acid</td>
<td>2–20 h</td>
<td>5–10 d</td>
</tr>
<tr>
<td>Aspirin/dipyridamole</td>
<td>Aggrenox</td>
<td>PO</td>
<td>ASA+AC stimulator</td>
<td>15 h</td>
<td>5–10 d</td>
</tr>
<tr>
<td>Cilostazol</td>
<td>Pletal</td>
<td>PO</td>
<td>PDE III inhibitor</td>
<td>12 h</td>
<td>3 d</td>
</tr>
</tbody>
</table>

AC, adenylate cyclase; ASA, aspirin; d, day; h, hour; IV, intravenous; min, minute; PDE III, phosphodiesterase type III; PO, peroral; wk, week.
PLATELET MONITORING

Platelet count is a simple yet useful screening test. Platelet count below $20 \times 10^9/L$ is regarded as a trigger point for platelet transfusion in adult acute leukemia patients, and a platelet count below $100 \times 10^9/L$ is often associated with increased bleeding after CPB. A low platelet count has been shown to be a negative predictor of survival in critically ill patients. Screening patients for acute thrombocytopenia caused by GPIIb–IIIa inhibitors and subacute thrombocytopenia caused by thienopyridines may be important during antiplatelet therapy. A drop of the platelet count by 50% is one of the diagnostic criteria for heparin-induced thrombocytopenia. The mean platelet volume (MPV) and the platelet distribution width (PDW) are also provided with the platelet count. MPV is elevated when platelet production is increased, and is inversely related to the platelet count in normal subjects.

The bleeding time was first introduced in the early 20th century, and modern bleeding time measurements were performed according to Duke’s earlobe method and Ivy’s modification with applied proximal pressure in the arm. Harker and Slichter’s study expanded its acceptance and clinical use by showing quantitative (platelet count) and qualitative (functional) relationships between platelets and bleeding time. Bleeding time is thought to reflect early hemostatic events of platelet adhesion and aggregation (Figure 1). It has been used for the screening of preoperative patients and the evaluation of patients with bleeding episodes or hereditary bleeding disorders. Its lack of sensitivity and specificity for predicting surgical bleeding and diagnosing von Willebrand disease, which was the subject of a controversy in the 1990s, is attributed to intra- and interobserver variability, day-to-day variation of von Willebrand factor/factor VIII levels, and other variables including skin temperature and hematocrit. Hence, bleeding time is no longer a part of routine preoperative workup and an alternative test is being adapted for screening of von Willebrand disease (see below). There is no question about the utility of bleeding time in research settings where it still provides insights on drug effects and hemostatic mechanisms.

The Platelet Function Analyzer (PFA-100, Dade-Behring) is a device which uses a disposable cup-capillary assembly (Figure 2) for the measurement of platelet function under the controlled shear (4000–5000/s). While being vacuumed through the capillary, the sample blood contacts the membrane disc with a central pore (150 µm) coated with collagen/epinephrine or collagen/ADP. In the presence of functional levels of GPIIb receptors and plasma von Willebrand factor, platelets attach to collagen and subsequently aggregate in response to epinephrine or ADP. The occlusion of the membrane
Pore with platelet plugs results in the cessation of blood flow and is detected as a ‘closure time’. For example, in vitro addition of anti-GPIb monoclonal antibody dose-dependently prolongs closure time (Figure 3). Its use for diagnoses of von Willebrand disease (type I) has been most extensively studied. PFA-100 has a greater sensitivity in detecting von Willebrand disease compared with the bleeding time. PFA-100 was also evaluated as a screening tool for platelet dysfunction and/or von Willebrand disease in 305 patients at a tertiary care center. Prolonged closure time was found in 37.3% of patients, which was mostly attributed to isolated prolongation of collagen/epinephrine closure time due to aspirin (69.3%). Prolonged collagen-ADP closure time was associated with qualitative platelet defect and/or decreased von Willebrand factor after confirmation with aggregometry or plasma von Willebrand factor levels. This study also showed that prolonged closure time was frequently encountered in anemic patients with sickle cell disease. Anemia in renal failure is also reported to prolong closure time. With regard to thrombocytopenia, closure time linearly prolongs with platelet counts below 100 × 10^9/L. In other clinical areas, conclusive evidence of the usefulness of PFA-100 is still missing; mixed results have been reported in antiplatelet therapies with aspirin or GPIIb–IIIa antagonists and in cardiac surgical patients after CPB.

Platelet aggregation is performed in platelet-rich plasma, washed platelets or whole blood using various types and doses of platelet agonists (collagen, ristocetin, arachidonic acid, ADP). Blood sample is collected in tubes with anticoagulants (citrate, heparin, PPACK) which prevent thrombin-mediated activation of platelets and fibrinogen cleavage (clotting). In optical aggregometry, changes in light transmittance after platelet stimulation are measured in platelet-rich plasma, and several variables (slope, peak, disaggregation slope) can be recorded. Although being suitable for laboratory diagnoses of platelet disorders such as Glanzmann’s thrombasthenia, Bernard–Soulier syndrome, or SPD, the utility of optical aggregometry is limited for preoperative screening and point-of-care evaluation of anti-platelet therapy. Disadvantages of optical methods include sample preparation time, artificial activation of platelets during centrifugation, interference from lipemia, and lack of other cellular components of blood. Whole-blood aggregometry circumvents some of the limitations by monitoring impedance changes with a pair of electrodes on which activated platelets accumulate upon activation. The Ultegra RPFA (Accumetrics, San Diego, CA, USA), another form of whole-blood aggregometry, has been developed as an automated platelet-function tests for monitoring platelet GPIIb–IIa inhibitors. The anticoagulated whole blood sample is mixed with fibrinogen-coated beads which agglutinate upon platelet activation, resulting in increased light transmission. The Ultegra calculates a numerical index, PAU (platelet aggregation unit) which is proportional to the number of functional GPIIb–IIa receptors. The Ultegra system has been evaluated extensively in conjunction with clinical studies of pharmacokinetics and pharmacodynamics of GPIIb–IIa inhibitors, abciximab, tirofiban and epifibatide. To monitor aspirin or clopidogrel, separate cartridges are available for use in the Ultegra system. Another commercial device is the Plateletworks system (Helena Laboratories, Beaumont, TX, USA) which is based on the method of platelet-particle counting. This method estimates the extent of platelet aggregation by counting platelets before and after adding a platelet agonist (e.g. ADP 20 μM). As aggregates of platelet form, each aggregate is still counted as one particle, hence the ADP-induced reduction of platelet count is used as a simple estimate of platelet aggregation (percent aggregation) based on the following formula: %aggregation = 100 × ([baseline platelet count] – [platelet count after adding ADP]) / [baseline platelet count]. Platelet function after clopidogrel therapy has been reported with this method. The baseline platelet count can be also useful in assessing acute thrombocytopenia which may occur during anti-GPIIb–IIa therapy.

Platelets contain many substances in their storage granules. The alpha granules contain platelet factor 4 and beta-thromboglobulin, both of which can be quantitated by ELISA as markers of platelet activation and release. P-selectin (CD62P, GMP-140, PADGEM) is translocated to the platelet surface from the alpha-granule membrane on activation, which can be quantitated by flow cytometry (see below). Platelet secretion studies are useful in the diagnosis of SPD and release defect. ATP release from platelet dense granules can be measured by adding luciferase in an aggregometry setup. Other assays of platelet dense granule secretion include the serotonin-release assay. Radioabeled serotonin (14C serotonin, 2 mCi/mL) is loaded in platelets,
and released $^{14}$C is determined in plasma after agonist stimulation and centrifugation. Thromboxane A$_2$ is also released from activated platelets, and its stable metabolite thromboxane B$_2$ can be quantitated for evaluation of aspirin resistance by ELISA. However, non-platelet source of TxA$_2$ should also be considered (e.g. endothelial cells).

Thrombelastography (TEG$^*$, Haemoscope Corp., Niles, IL, USA) and Thromboelastometry (ROTEM$^*$, Pentapharm, Munich, Germany) are the point-of-care coagulation analyzers that measure the viscoelastic property of clotting blood. Both TEG$^*$ and ROTEM$^*$ use the similar principles of evaluating blood clotting in relation to viscoelasticity between a cup (cuvette) and a pin connected to a detector system. A torsion wire is used in TEG$^*$ to transduce the viscoelastic force exerted by fibrin fibers, an optical detector is used in ROTEM$^*$. Various activators and type of samples can be used for TEG$^*$ and ROTEM$^*$. Commonly measured variables of TEG$^*$ are shown in Figure 4. The reaction time (R time) is the duration from the start of measurement until the amplitude of 2 mm is reached. Two subsequent variables, $\alpha$ angle and K time, reflect the rapidity of fibrin build-up and cross-linking. The maximum amplitude (MA) reflects the peak tensile strength exerted by fibrin-platelet bonds, which is most commonly used to determine hypocoagulable (e.g. thrombocytopenia) and hypercoagulable (e.g. hyperfibrinogenemia) state. For ROTEM$, corresponding variables are clotting time (CT) for reaction time, clot formation time (CFT) for K time, and maximum clot firmness (MCF) for MA. The blockade of fibrinogen receptors (GPIIb–IIIa) on platelets with abciximab (ReoPro$^*$) reduces TEG$^*$-MA in a dose-dependent manner. The abciximab-modified TEG$^*$ may be used to ablate the platelet contribution to the clot strength, which allows estimation of plasma fibrinogen levels and detection of low-grade fibrinolysis. Similar analyses can be also achieved in ROTEM$^*$ using cytochalasin D (FIBTEM test). The gradual decrease of amplitude after MA occurs due to platelet-mediated clot retraction and/or fibrinolysis. For the latter, a decrease in amplitude greater than 15% at 60 minutes after MA indicates the threshold for intervention. Systemic fibrinolysis may be encountered during hepatic transplantation, and antifibrinolytic therapy may be administered based on TEG$^*$ variables.

The utility of TEG$^*/$ROTEM$^*$ in guiding hemostatic component therapies has been demonstrated in a high-risk cardiac surgical population where thrombocytopenia and platelet dysfunction are commonly seen due to the use of CPB. Morgan et al. stratified 115 patients undergoing coronary bypass surgery with TEG$^*$ before administering desmopressin acetate (DDAVP) or placebo. In patients who had a postoperative TEG$^*$-MA less than 50 mm and did not receive DDAVP, chest tube drainage (i.e. blood loss) was greater than in those who received DDAVP or who had a TEG$^*$-MA greater than 50 mm. When compared with the standard laboratory coagulation tests, a TEG$^*$-based algorithm was found to be effective in reducing transfusion of hemostatic components (fresh frozen plasma, platelet concentrate) without increasing postoperative blood loss. Nuttall et al. also reported that the implementation of a transfusion algorithm for hemostatic components using point-of-care PT/PTT and TEG$^*$ effectively reduced transfusion requirements and postoperative blood loss in patients who developed microvascular bleeding after cardiac surgery.

More recently, modified versions of TEG became available, which allow specific platelet-function analysis (Platelet Mapping$, Haemoscope, Niles, IL, USA). For this assay, a heparinized blood sample is used to suppress thrombin-mediated activation of fibrinogen and platelets. Alternatively, fibrinogen conversion to fibrin is achieved with heparin-insensitive reptilase (Batroxobin) premixed with activated factor XIII. Platelets are separately activated with arachidonic acid for aspirin assay, or with ADP for clopidogrel assay. These modifications may be useful in stratifying the bleeding risk in preoperative patients, and await additional clinical validation studies.

The Sonoclot$^*$ analyzer (Sienco, Inc., Morrison, CO, USA) is also a viscoelastic monitor which measures...
impedance changes of blood clots. A disposable hollow probe is mounted on a transducer which oscillates vertically for a distance of 1 µm at 200 Hz. Fibrin strands exert a viscoelastic force on the probe, causing impedance changes in the vibration of the probe. The CFT of Sonoclot® signature (Figure 5) corresponds to activated CT when celite or kaolin are used as a activator. The onset of clot formation on Sonoclot® is shorter than on TEG®, suggesting that the former detects impedance changes due to forming fibrin monomers. In the presence of heparin (2.5 U/mL), TEG® reaction time increases over 40 minutes, whereas Sonoclot® onset is within 15 minutes. The initial rise in the signature reflects thrombin-mediated fibrinogen conversion and fibrin polymerization. In the presence of functional platelets, Sonoclot® signature shows a second rise in the impedance followed by a decrease (deflection point) (Figure 5). This is due to platelet-mediated clot retraction that disrupts the linkage of fibrin fibers between the cuvette wall and the probe. After addition of GPIIb–IIIa antagonists, clot retraction disappears.

Endogenous thrombin potential can be measured using chromogenic thrombin substrate in subsamples obtained from clotting whole blood or plasma, which is useful for evaluating hypocoagulable state and responses to anticoagulants. The method for automated estimation of endogenous thrombin generation (Thrombogram®, Synapsee bv, Maastricht, the Netherlands) has been developed and described in detail by Hemker and Beguin. Briefly, for the thrombin generation experiments, 80 µL of platelet-rich (or poor) plasma and 20 µL of trigger (tissue factor) are added to wells of 96-well microtitre plate followed by 20 µL of substrate/calcium chloride buffer. Thrombin generation is continuously (every 20 seconds) monitored with a microplate fluorometer set at 390 nm (excitation wavelength) and 460 nm (emission wavelength) for fluorescence generated by thrombin-catalyzed fluorogenic substrate (Z-Gly-Gly-Arg-AMC). In contrast to other clot-based coagulation assays, there are several unique characteristics about the Thrombogram®. Prothrombin time and activated partial thromboplastin time only reflect the early thrombin formation that is sufficient (5–10 pM) to clot fibrin, whereas the peak level and total amount (area under the curve) are measured with the Thrombogram®. Furthermore, when the endogenous thrombin potential is measured in platelet-rich plasma with low-dose tissue factor, it may be possible to evaluate the contribution of platelets to thrombin formation and the inhibitory effects of antiplatelet agents. Thrombin-generation parameters include lag time, peak thrombin and endogenous thrombin potential (area under the curve) (Figure 6). There is an increasing interest in this technology for both research and clinical applications.

The flow cytometric assay has become a useful laboratory tool in assessing platelet function. Platelets are identified in whole blood by their forward and orthogonal light scattering properties and by a fluorescent platelet-specific monoclonal antibody. Flow cytometry can be used to obtain platelet counts in small amount of blood (5 µL), or applied to diagnose a GPIb disorder (Bernard–Soulier Syndrome), Glanzmann’s thromboasthenia and SPD.

Multiple platelet-activation markers can be labeled for flow cytometric assessment using monoclonal antibodies. Activation-dependent conformational changes
in GPIIb–IIIa receptors (integrin αIIbβ3, CD41/CD61) can be detected with PAC-1 monoclonal antibody. For thrombin receptor on the platelet surface, mouse IgG1 antibodies, WEDE15 and SPAN12, are commercially available. SPAN12 recognizes only uncleaved PAR-1 receptors, whereas WEDE15 binds to both cleaved and uncleaved PAR-1 receptors. The exposure of granule membrane proteins on activated (degranulated) platelets can be measured with antibodies against P-selectin or CD40L (TRAP1). Furthermore, leukocyte-platelet aggregates can be identified by flow cytometry. Activated platelets express P-selectin and rapidly binds to monocytes and neutrophils via the leukocyte surface P-selectin glycoprotein-1 (PSGL-1). Michelson et al. measured platelet–neutrophil aggregates in baboons and clinical patients with coronary diseases, and found that platelet–monocyte aggregates circulate in blood with an approximate half-life of 30 minutes. They concluded that platelet–monocyte aggregates may be superior, as platelet-activation markers, to platelet–neutrophil aggregates or platelet surface P-selectin, both of which are rapidly cleared in vivo. Although platelet assessments with flow cytometry are mainly performed at specialized laboratories due to time-consuming and complex sample processing, technological advancement and cost reduction may make this more routine assay.

CONCLUSIONS

Diverse functions of platelets in blood have been elucidated in laboratory and clinical investigations. Platelets are not only hemostatic plugs but also play key roles in inflammation and wound healing. In this post-
genomic era, large amounts of information on polymorphisms are being obtained and novel diagnostic modalities are being brought into clinical testing. At present, there is no single device that measures platelet function at the different stages of hemostasis. The platelet function tests that are performed with anticoagulants may allow assessment of platelet adhesion and aggregation, while other tests in recalcified or non-anticoagulated samples are more reflective of thrombin formation and subsequent clotting (Figure 7). Physicians who manage complex coagulation issues are advised to choose test methods based on their patient population (e.g. congenital syndromes), the underlying pathological mechanisms (e.g. types of antiplatelet therapy) and the acuity of disease (point-of-care vs. bench-top devices). It is important to remember that blood component transfusions, including platelet concentrate transfusions, may cause serious adverse events. The routine use of platelet testing may improve the outcomes of patients undergoing antiplatelet therapy, quality and cost-effectiveness of transfusion therapy, and overall safety of patients with platelet dysfunction.

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