The laboratory evaluation of platelet dysfunction
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The laboratory evaluation of platelet function plays a central role in the diagnosis of hemostatic disorders and in the assessment of patients with arterial thrombosis. During the last decade enormous progress has been made in understanding platelet function at the biochemical and molecular level. Although traditional platelet function studies, as first described by Gustav Born in the 1960s [1], continue to serve as the mainstay of platelet function testing, the direct evaluation of platelet membrane glycoproteins (GP) by fluorescence activated cell sorting (FACS) and the examination of platelet function under high shear conditions provide additional diagnostic information. Furthermore, the ability to define genetic defects by molecular methods in families with inherited thrombocytopathies has had a major impact on carrier detection, prenatal diagnosis, and genetic counseling.

Overview of platelet structure and function

Platelets constitute the smallest formed elements of the blood. Their structure and function continues to be the focus of intense investigation. An update of current concepts may be found in several textbooks of hemostasis and thrombosis [2–4]. Key features are summarized below.

Platelets are derived from bone marrow megakaryocytes and, unlike many other cells, possess no nucleus. They do contain a variety of intracellular organelles, however, including prominent granules. The most numerous of these, the platelet alpha granules, contain a variety of adhesive proteins and coagulation factors and inhibitors, and are responsible for the characteristic staining properties of platelets on Wright’s-stained peripheral
blood smears. Platelets also possess a lesser number of electron dense granules or dense bodies, which contain a variety of amines and nucleotides including ADP and ATP. The secretion of alpha and dense granules following platelet activation and aggregation is important for recruiting additional platelets to sites of vascular injury.

Both platelet quantity and quality are important for normal hemostasis. Platelets circulate in the bloodstream for 8 to 10 days at a count of 150,000 to 400,000 per microliter. Conditions associated with decreased circulating platelets (thrombocytopenia) are more common than those associated with increased circulating platelets (thrombocytosis). Whereas thrombocytopenia is frequently associated with mucocutaneous bleeding, thrombocytosis may be associated with increased thrombotic risk, particularly in the arterial circulation.

In the circulation, platelets are subject to varying shear stresses, depending in large part on the diameter of the blood vessel. For example, the shear stress in large veins is between 20 and 200 sec\(^{-1}\). In comparison, the shear rate in stenotic arteries can reach 800 to 10,000 sec\(^{-1}\) [5]. Platelets are activated by high shear [6], and this contributes significantly to their participation in hemostasis and thrombosis.

During normal hemostasis, platelets adhere to damaged blood vessel walls, secrete granule contents, aggregate with one another, and participate in clot formation. Defects in any of these functions may result in clinically significant bleeding (Table 1). Interestingly, the accumulation of platelets at sites of arterial stenosis occurs by similar biochemical and molecular pathways and can be regarded as hemostasis gone awry.

### Platelet adhesion

Platelets adhere to a variety of extracellular matrix proteins present beneath the blood vessel endothelial cell lining and in the blood vessel wall

<table>
<thead>
<tr>
<th>Functional defect</th>
<th>Inherited disorder</th>
<th>Acquired disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion</td>
<td>vWD</td>
<td>Acquired vWD, ITP, multiple myeloma,</td>
</tr>
<tr>
<td></td>
<td>Bernard-Soulier syndrome</td>
<td></td>
</tr>
<tr>
<td>Aggregation</td>
<td>Glanzmann’s thrombasthenia</td>
<td>DIC, fibrinolytic therapy, therapeutic drugs</td>
</tr>
<tr>
<td></td>
<td>Afibrinogenemia</td>
<td></td>
</tr>
<tr>
<td>Secretion</td>
<td>Familial disorders</td>
<td>Exhausted platelets</td>
</tr>
<tr>
<td>Dense granules</td>
<td>Gray platelet syndrome</td>
<td>Exhausted platelets</td>
</tr>
<tr>
<td>Alpha granules</td>
<td>Enzyme, cytoskeletal deficiencies</td>
<td>Aspirin, NSAID, ethanol</td>
</tr>
<tr>
<td>Defective secretion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** DIC, disseminated intravascular coagulation; ITP, idiopathic-immune thrombocytopenic purpura; NSAID, nonsteroidal anti-inflammatory drugs; vWD, von Willebrand’s disease.
The most important proteins in terms of hemostasis are collagen and von Willebrand’s factor (vWF). Other adhesive GPs, including fibronectin, thrombospondin, laminin, vitronectin, and fibrinogen, may play a role also. Their corresponding platelet membrane adhesion receptors, for the most part, are constitutively expressed and platelet-activation independent. Platelets recognize collagen predominantly by the integrin $\alpha_2\beta_1$ (GPIa-IIa). GPs IV and VI, however, play a role also, particularly in platelet activation and signal transduction [8]. GPIb is the primary platelet membrane receptor for vWF [9].

**Platelet aggregation**

In contrast to platelet adhesion, platelet aggregation is mediated exclusively by the GPIIb-IIIa receptor, a member of the $\beta_3$ integrin family [2,7,10]. Under basal conditions, GPIIb-IIIa exhibits low affinity for GP ligands, such as fibrinogen and vWF, but undergoes a conformational transition to a high-affinity ligand binding state when platelets are suitably activated [11]. Platelets can be activated by a variety of agonists including ADP, epinephrine, serotonin, vasopressin, collagen, thrombin, arachidonic acid, platelet activating factor, and immune complexes [6]. In all cases, platelet activation correlates with fibrinogen or vWF binding to GPIIb-IIIa.

**Platelet secretion**

Platelet adhesion and aggregation lead to a variety of intracellular signaling pathways that culminate in the secretion of platelet granule contents. Secretion of platelet granule constituents, especially ADP and serotonin from dense granules and adhesive proteins from alpha granules, is important for recruiting additional platelets to sites of vascular injury. The release of arachidonic acid from negatively charged platelet membrane phospholipids, and its subsequent conversion to thromboxane A$_2$ by the sequential action of the enzymes cyclooxygenase and thromboxane synthetase [6], is required for normal secretion when platelets are activated by weak agonists, such as ADP or epinephrine. Thromboxane A$_2$ formation by activated platelets is inhibited by aspirin through inhibition of the enzyme cyclooxygenase [12]. Platelet secretion induced by strong agonists, such as thrombin or collagen, does not require thromboxane A$_2$.

**Platelet procoagulant activity**

One of the earliest recognized effects of platelets on blood coagulation is the shortening of the plasma clotting time by stimulated platelets. The relevant platelet activity is termed *platelet factor 3* (PF3), and results from the combined effects of surface membrane microvesiculation; expression of procoagulant phospholipids (phosphatidylserine, phosphatidylinositol); and receptor-mediated interactions between platelets and coagulation proteins [13,14]. Specific receptor-like membrane components for factor Va, factor
Xa, and prothrombin have been described. In addition, the specific binding of factors VIII, IX, and XI, and high molecular-weight kininogen has been observed.

Platelet exposure to thrombin, collagen, or both, and to high shear stress can initiate shedding of procoagulant-containing microparticles [15]. These microparticles were first described by Wolf [16], and were noted to contain most of the platelet-related coagulant activity in whole blood and serum. The contribution of microparticle shedding to in vivo coagulation is illustrated by a patient with Scott syndrome, who suffered from a significant bleeding diathesis. The patient’s platelets expressed markedly reduced procoagulant activity, linked to a defect in calcium-mediated vesiculation of the plasma membrane and decreased membrane exposure of phosphatidylserine [17].

**Platelet function tests**

A variety of congenital and acquired disorders of platelet function [12,18] can be classified based on results of laboratory studies examining broad categories of platelet function, such as adhesion, aggregation, and secretion (see Table 1). Patients with platelet function disorders have characteristic patterns of bleeding. Most individuals have mucocutaneous bleeding, characterized by epistaxis, gingival bleeding, easy bruising, menorrhagia, gastrointestinal bleeding, and petechiae [19].

**Platelet count**

A platelet count should accompany any evaluation of platelet function. Not only are quantitative disorders of platelets function considerably more common than qualitative disorders, but interpretation of platelet function studies may be influenced by the platelet count. For example, patients with thrombocytopenia (platelet count <100,000/μL) may demonstrate abnormal platelet function both in screening assays and in tests of platelet aggregation and secretion. For this reason, platelet function studies are often nondiagnostic in patients with thrombocytopenia. Moreover, an evaluation of platelet morphology by review of the peripheral blood smear, and an examination of the mean platelet volume obtained by automated analysis may provide further important diagnostic insights. Certain inherited thrombocytopenias are associated with small (eg, Wiskott-Aldrich syndrome) or large (eg, Bernard-Soulier syndrome) platelets, and alpha granule storage pool disease is characterized by platelets that do not stain with Wright’s-Giemsa stain (gray platelets).

**Platelet function screening**

Laboratory studies to evaluate platelet function often include screening tests, such as the bleeding time, and more recently platelet function analysis
under high shear conditions using the PFA-100 analyzer (Dade-Behring, Miami, FL) [20–22].

**Bleeding time**

The bleeding time measures *in vivo* platelet function. It involves making a standardized incision (1 × 9 mm) on the volar surface of a patient’s forearm and assessing how long bleeding takes to stop. To ensure standardized back pressure, a blood pressure cuff is placed on the arm before the incision is made and is inflated to 40 mm Hg. The bleeding time is difficult to standardize, and thus reproducibility can be a problem. In addition, the sensitivity of the bleeding time to mild platelet function disorders, particularly to von Willebrand’s disease, is poor [23], as is its predictive value for surgical bleeding risk [24].

**PFA-100 platelet function screen**

The PFA-100 simulates the process of primary hemostasis in vitro in an artificial vessel under standardized flow conditions (shear rate 5000–6000 sec⁻¹) [21]. The system measures the ability of platelets in a whole blood sample, anticoagulated with sodium citrate, to occlude a 150 µm aperture in a membrane treated with combinations of collagen and epinephrine or collagen and ADP. Results are reported in closure times (CT). Like the bleeding time, the CT is affected by platelet count and platelet function. The CT is more sensitive to von Willebrand’s disease than the bleeding time [23], and is considerably more reproducible. Because the test is performed under flow conditions, it is also sensitive to the patient hematocrit [21], and may complement platelet function studies by aggregometry.

**Platelet aggregation and secretion**

To perform in vitro platelet aggregation studies [25], blood is anticoagulated with sodium citrate and gently centrifuged to sediment the red cells and leukocytes, leaving platelets suspended in the supernatant plasma. This platelet-rich plasma (PRP) is then placed in a cuvette and subjected to the addition of discrete platelet agonists under constant stirring conditions in an aggregometer. The aggregometer quantifies changes in light transmission through a cuvette of PRP, with increased light transmission correlating with platelet aggregation (Fig. 1). Thus, the rate and extent of platelet aggregation can be evaluated. Some agonists like ADP and epinephrine produce two identifiable, successive waves of platelet aggregation. The first is due to GPIIb-IIIa activation produced by the initial agonist, and the second represents additional GPIIb-IIIa activation in response to synthesized platelet thromboxane A₂ and secreted dense granule ADP. The release of platelet dense granule ADP can be monitored during aggregation by assaying secreted ATP using the firefly luminescence assay (luciferin/luciferase) [25]. Abnormal platelet secretion constitutes one of the most common acquired platelet function defects [12]. Typical platelet responses to a variety of
Fig. 1. Typical aggregation responses of normal platelets to a variety of agonists, assessed using an aggregometer. Platelet aggregation is demonstrated by increasing light transmission (speckled arrows) as a function of platelet cohesion. The solid arrows indicate agonist addition to platelet samples. (A) 10 μm ADP. (B) 4 μm ADP. (C) 2 μm ADP. (D) 100 μm epinephrine. (E) Collagen (100 μg/mL). (F) Arachidonic acid (500 μg/mL). (G) Ristocetin (1.5 mg/mL). (H) Ristocetin (0.6 mg/mL). (I) Platelet aggregation and ATP secretion measured using chemiluminescence in a lumi aggregometer (Chronolog Corp., Haverton, PA).
agonists are summarized in Table 2. Artifacts affecting platelet aggregation include contamination of PRP by RBC, lipemia, pH, temperature, aggregometer stir speed, and platelet count.

In order to provide a complete evaluation of platelet function, platelet aggregation is performed with several agonists including high-dose ADP (10–20 μM), high-dose epinephrine (100 μM), collagen (20–100 μg/ml), and arachidonic acid (500 μg/ml). In addition, it may be useful to examine platelet aggregation and secretion in response to low doses of ADP (<10 μM). These doses can be titred to elicit a secondary wave, or fixed ADP concentrations may be chosen (eg, 2 and 4 μM), especially if ATP secretion is measured simultaneously. To rule out dense granule storage pool disease in individuals with absent secondary wave aggregation in response to epinephrine
or low-dose ADP, platelet secretion in response to a high concentration of thrombin (1 U/ml) may be measured. High doses of thrombin will overcome inhibition of platelet granule secretion due to impaired thromboxane A\(_2\) synthesis. Since alpha thrombin will clot PRP, it will be impossible to examine the platelet aggregation response, but ATP secretion can be quantified (Fig. 1). The aggregation of platelets by thrombin can be examined, however, using \(\gamma\)-thrombin or peptide agonists of the two protease activated thrombin receptors on human platelets, designated PAR 1 and PAR 4 [26]. Although the GPIb-IX-V complex is also involved in thrombin binding to platelets [9], there are, as yet, no convenient clinical assays to evaluate this function.

The platelet response to collagen is also mediated by diverse membrane receptors [8,27]. The role of GPIa-IIa and GPVI have been investigated intensively. The relative contribution of these glycoproteins to platelet function may be dissected using low doses of collagen to screen for potential defects, followed by an evaluation of platelet adhesion to immobilized collagen to assess GPIa-IIa function, and platelet aggregation studies using specific synthetic peptides (CRP) or the snake venom convulxin [27] to activate platelets via GPVI. These studies may be indicated in individuals with abnormal platelet function screening studies, but apparently normal platelet responses to high doses of ADP, epinephrine, and collagen. FACS analysis to quantify the expression of GPIa-IIa and GP VI on the platelet membrane may also be useful (see below).

Table 2
Expected platelet aggregation results in normal individuals

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Expected platelet response</th>
</tr>
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<tbody>
<tr>
<td>Epinephrine</td>
<td>Primary and secondary wave aggregation.</td>
</tr>
<tr>
<td>Low-dose ADP</td>
<td>Primary and secondary wave aggregation at doses between 2 and 4 (\mu)m. Aggregation is reversible at doses insufficient to elicit a secondary wave aggregation.</td>
</tr>
<tr>
<td>High-dose ADP</td>
<td>Fused primary and secondary wave aggregation (10–20 (\mu)m ADP).</td>
</tr>
<tr>
<td>Collagen</td>
<td>Strong platelet aggregation following a short lag at high doses of collagen (20–100 (\mu)g/mL).</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>Strong aggregation inhibited at aspirin or nonsteroidal anti-inflammatory agents.</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>Strong aggregation at high concentrations (&gt;1.2 mg/mL) with diminished to absent response to low doses less than 0.8 mg/mL.</td>
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</tbody>
</table>

Platelet aggregation in response to Ristocetin is often performed as part of a work-up for von Willebrand’s disease (see below), but, in our hands, is more sensitive for identifying individuals with the Bernard Soulier Syndrome who have diminished surface expression of the platelet GPIb-IX-V complex. Ristocetin induced platelet aggregation most often involves an
analysis of platelet aggregation in response to high (~1.5 mg/ml) and low doses (~0.6 mg/ml) of Ristocetin. Platelet responses to high dose Ristocetin reflect vWF interaction with GPIb with subsequent platelet agglutination, followed by platelet activation and secretion. Normal platelets do not respond to low doses of Ristocetin. A response to Ristocetin concentrations below 0.6–0.8 mg/ml may suggest von Willebrand’s disease type IIB [28,29].

**Clot retraction**

The ability of platelets to retract a fibrin clot in whole blood results in the extrusion of a large volume of serum, and is an important component of thrombus consolidation and wound healing. Clot retraction may also play a role in resistance to fibrinolysis. Although the precise biophysical mechanism of clot retraction is not yet fully understood, platelet interactions with fibrinogen, in part via GPIIb-IIIa, are intimately involved. Also essential are activation and assembly of the platelet cytoskeleton for generation of contractile force via actin–myosin interactions. Platelets from patients with thrombasthenia demonstrate diminished clot retraction often in direct correlation with the level of GPIIb-IIIa expression [18]. Clot retraction can be performed simply by allowing whole blood to clot in a glass test tube at 37°C and observing the extrusion of serum from the clot over several hours [25].

**Platelet flow cytometry**

The evaluation of platelets by flow cytometry is becoming increasingly popular for the quantitation of constitutively expressed platelet membrane proteins in patients with congenital deficiencies, and to assess activation dependent expression of platelet membrane proteins, as indicators of either in vivo or in vitro platelet activation [30]. For the evaluation of in vivo platelet activation, care must be taken to prevent ex vivo platelet stimulation, for example, during phlebotomy or sample processing. Often, blood is collected into a suitable anticoagulant with platelet inhibitors, such as the CTAD blood collection tube from Becton Dickinson (Franklin Lakes, NJ), which contains theophylline, adenosine, and dipyridamole in addition to the sodium citrate anticoagulant.

Antibodies directed against a variety of platelet activation markers exist and allow an assessment of platelet secretion and GPIIb-IIIa activation. For example, platelet alpha granule (CD62) or lysosomal granule membrane (CD63) proteins are convenient markers of platelet granule secretion. Similarly the interaction of fibrinogen with GPIIb-IIIa can be used as a specific marker of platelet activation, even in the absence of granule secretion. Monoclonal antibodies that react with a conformation of fibrinogen that is assumed only after fibrinogen binding to platelet GPIIb-IIIa are commercially available, as are a variety of antibodies that recognize conformational changes in GPIIb-IIIa following fibrinogen binding. FACS analysis,
therefore, lends itself to detecting in vivo platelet activation, as well as in vitro GPIIb-IIIa function, or GPIIb-IIIa blockade in patients treated with GPIIb-IIIa antagonists, such as abciximab or peptide analogs. The small sample requirement, and the observation that platelets can be studied in whole blood has made this technique very attractive as a potential alternative to platelet aggregation particularly in pediatric populations.

When analysis is done in whole blood, two monoclonal antibodies, labeled with different fluorophores, are typically chosen. One antibody reacts with a constitutively expressed platelet surface protein such as GPIb, IIb or IIIa and serves as a platelet marker, whereas the second antibody is directed against the antigen of interest. Background binding of monoclonal antibodies can be assessed using isotype species-specific immunoglobulins.

**Molecular studies**

Diagnosis of congenital platelet dysfunctions, particularly of well-characterized membrane glycoproteins can be enhanced by evaluation of genomic DNA or platelet mRNA by polymerase chain reaction and cDNA sequencing [18]. Results of such studies are useful for genetic counseling and prenatal diagnosis. Moreover, the identification of mutations has played an important role in understanding platelet membrane glycoprotein structure and function.

In addition to mutations, several platelet membrane glycoprotein polymorphisms have been identified. These play important roles in alloimmune thrombocytopenia, transfusion refractoriness, and post transfusion purpura [32]. More recently, molecular polymorphisms in platelet membrane glycoproteins, particularly in GPIIb-IIIa, GPIb, and GPIa-IIa have been implicated in some studies as potential risk factors for arterial thrombosis [31].

**vWF studies**

von Willebrand’s disease constitutes the most common platelet function abnormality [28]. The defect, however, resides not in platelets, but in the plasma protein vWF. Diagnostic tests for von Willebrand’s disease include a platelet function screen, preferably performed by PFA-100 analysis, as this test has demonstrated superior sensitivity for von Willebrand’s disease compared to the bleeding time [23], and measurements of factor VIII activity, vWF antigen, and vWF activity [25]. vWF antigen studies measure total vWF protein, whereas vWF activity assays measure vWF function using the Ristocetin cofactor assay. This assay is performed using normal platelets (usually obtained as a fixed lyophilized preparation from commercial sources) and dilutions of patient plasma. Platelet agglutination in response to high dose Ristocetin is measured, comparing the ristocetin cofactor activity of patient plasma to that of a plasma standard.

Clinically, it is important to differentiate between at least three subtypes of von Willebrand’s disease. In patients with type I disease (approximately 80%
of cases), both vWF antigen and activity are similarly decreased. This is a quantitative disorder of vWF production. In type III von Willebrand’s disease, vWF levels are extremely low or undetectable. This subtype is extremely rare. In contrast, type II von Willebrand’s disease is due to a qualitative and, most often, also a quantitative defect in vWF. Laboratory studies will demonstrate discordance in vWF antigen and activity levels, with the antigen levels being significantly higher than the activity measurements. Approximately 20% of patients with von Willebrand’s disease will have type II disease. vWF multimer analysis [25] is useful to identify these patients, as high and intermediate size vWF multimers are characteristically absent from plasma.

Type II von Willebrand’s disease can be further subdivided into types IIA and IIB [28]. Ristocetin-induced platelet aggregation is useful for distinguishing between these subtypes. In type IIB von Willebrand’s disease, vWF has an abnormal affinity for its platelet receptor, and thus the largest multimers with the greatest biologic activity, will have been removed from the plasma by circulating platelets. Platelets demonstrate an enhanced responsiveness to low doses of Ristocetin, compared to patients with type IIA or type I disease. Patients with type IIA disease do not synthesize sufficiently large vWF multimers to adequately support platelet function. It is important to identify patients with type IIB von Willebrand’s disease because they may suffer thrombotic complications following administration of 1-deamino 8-D arginine vasopressin (DDAVP), used to release endogenous vWF from vascular endothelial cells. Patients with type IIB von Willebrand’s disease may also exhibit a low to normal platelet count.

In rare patients with pseudo-von Willebrand’s disease, a GPIb defect is responsible for high affinity binding of circulating vWF. Platelets aggregate spontaneously when presented with high molecular weight vWF multimers such as are present in cryoprecipitate [28]. These patients have a true platelet defect, and are treated with platelet concentrates rather than DDAVP or vWF containing plasma products [28].

Platelet function abnormalities in selected thrombocytopathies

A summary of major platelet function defects in a variety of inherited and acquired platelet abnormalities is provided in Table 1.

Acquired platelet dysfunctions

Aspirin

The ingestion of aspirin or other nonsteroidal anti-inflammatory agents is a common acquired cause of platelet dysfunction. The bleeding time is variably prolonged. Test results of platelet function screening with the PFA-100 analyzer typically reveal abnormal results with the collagen-epinephrine cartridge, whereas platelet function in the collagen-ADP
cartridge is normal. Platelet aggregation studies demonstrate absent secondary aggregation in response to epinephrine and ADP, and a delayed onset of aggregation in response to collagen. In addition, platelets fail to respond to arachidonic acid. Platelet secretion in response to high doses of thrombin or thrombin receptor activation peptides is normal, and helps to distinguish this acquired defect from dense granule storage pool disease.

**Thienopyridines**

Plavix and Clopidogrel are widely used as antithrombotic agents in arterial diseases [12]. Both agents are prodrugs that depend on metabolites for their effects. Effects on platelet aggregation may be seen within 24–48 hours of the first dose but are not maximal for 4–6 days. Moreover, inhibitory effects may last for 4–10 days after the drugs have been discontinued. These agents inhibit platelet responses to many agonists, but particularly to ADP, via antagonism of the recently described platelet purinergic ADP receptor, P2Y12 [33].

**GPIIb-IIIa receptor antagonists**

Inhibitors of GPIIb-IIIa, either antibodies or synthetic peptide derivatives that block adhesive protein binding, have been designed for use as antithrombotic agents in the setting of ischemic coronary artery disease [34]. These agents lead to marked inhibition of platelet aggregation in response to all agonists, except high doses of Ristocetin. Using platelet aggregation studies to monitor GPIIb-IIIa blockade or platelet inhibition by other drugs (eg, Plavix, Clopidogrel) may not be efficient in acute settings such as in interventional cardiology or cardiopulmonary bypass. Rapid, whole blood platelet function analyzers are becoming available (eg, Platelet Works Point of Care, Helena Laboratories, Beaumont, TX), and offer the potential for rapid, quantitative platelet aggregation/function assessment.

**Congenital platelet dysfunctions**

**Bernard-Soulier syndrome**

The hallmark of this syndrome is diminished or absent GPI-XI-V complex expression on the surface of platelets. The molecular basis for this disorder has been determined in a growing number of patients. Molecular defects have been detected in GPIb alpha or beta, or GPIX [18]. So far, no defect in GPV has been reported. Laboratory features of Bernard-Soulier Syndrome include thrombocytopenia, giant platelets, and failure of platelets to aggregate in response to Ristocetin and von Willebrand factor. Responses to other agonists, with the possible exception of thrombin are normal. GPIb-IX-V may serve as a possible high affinity thrombin receptor [35].

**Glanzmann’s thrombasthenia**

Glanzmann’s thrombasthenia is characterized by absent or markedly abnormal platelet aggregation in response to all agonists requiring GPIIb-
IIIa activation and fibrinogen binding. Platelet responses to Ristocetin and von Willebrand factor are normal. Platelet clot retraction is absent or diminished. Patients have normal platelet counts, but abnormal screening platelet function test results. Genetic mutations affecting either GPIIb or GPIIIa have been described and lead to defective glycoprotein expression and, less frequently, abnormal glycoprotein function [18].

**Dense granule storage pool deficiency**

Dense granule storage pool deficiency is often associated with a multi-system disorder such as Hermansky-Pudlak syndrome, Chediak Higashi syndrome, or Wiskott-Aldrich syndrome [18]. In Hermansky-Pudlak syndrome there may be a total failure of dense granule formation, as judged by electron microscopy of platelets and megakaryocytes. The disorder is particularly common in northeastern Puerto Rico. In other forms of dense granule storage pool disease, dense granule membranes are formed but are not properly filled.

The results of screening platelet function studies are variable in these disorders. Platelet aggregation abnormalities are characterized by lack of secondary wave aggregation in response to epinephrine or low doses of ADP. Normal aggregation in response to high doses of thrombin and collagen is expected, but dense granule secretion, as quantified by ATP secretion using chemiluminescence, is markedly abnormal. The absence of dense granules can be confirmed by electron microscopy.

**Gray platelet syndrome**

The gray platelet syndrome is characterized by the absence of platelet alpha granules, and lack of characteristic platelet staining on Wright-stained peripheral blood films. Platelet aggregation abnormalities are quite variable. Often ADP or epinephrine induced aggregation is normal, but thrombin and collagen induced platelet aggregation may be variably abnormal. In addition, platelets fail to express the alpha granule membrane protein P-selectin (CD62) following stimulation with high doses of thrombin or thrombin receptor activation peptides. Similarly, ELISA assays for platelet specific alpha granule constituents, PF4 and β-thromboglobulin [20], are abnormal. Thrombocytopenia is common in patients with alpha granule storage pool deficiency.

**Defective platelet procoagulant activity**

Only a few patients with abnormal platelet procoagulant activity have been described [13,14]. The bleeding manifestations in these patients are not typically mucocutaneous [18]. The most widely studied and extensively characterized patient, named Scott, gives the name to this syndrome [17]. Scott syndrome is characterized by failure of platelets to undergo microvesiculation in response to different stimuli. Screening platelet function studies, and
Platelet aggregation studies are normal; however, the serum prothrombin time, which is a measure of the completeness of clotting of whole blood as reflected by prothrombin consumption, is a convenient screening test [19]. Shedding of platelet microparticles may be examined further by flow cytometry.

**Summary**

An algorithm for platelet function testing is presented in Fig. 2. The proposed algorithm takes into account the importance of a thorough clinical bleeding history and provides an integration of screening tests with more specific diagnostic assays. As our understanding of the biochemical and molecular aspects of platelet function becomes increasingly refined, it is becoming clear that current clinical testing strategies may not be adequate to identify all significant platelet function defects. The repertoire of distinct platelet agonists is increasing to allow a more discrete look at the function of isolated
platelet membrane agonist receptors, especially those involved in platelet activation by ADP, thrombin, and collagen. In addition, FACS analysis of platelets, with emphasis on surface membrane glycoprotein expression, may offer a more specific and quantitative view of platelet membrane constituents and their function. Furthermore, screening tests evaluating platelet function under physiologic and pathologic flow conditions are becoming important adjuncts to in vitro analyses, and may accentuate platelet function abnormalities, not easily discerned by platelet aggregation studies. It remains to be seen whether such screening tests will better predict clinical bleeding or thrombotic risk.

References


