

Qualitative Platelet Disorders

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ABBREVIATIONS: ADP = adenosine diphosphate; BSS = Bernard-Soulier syndrome; GP = glycoprotein; GSA = guanidinosuccinic oxide MPDs = myeloproliferative disorders; NO = nitric oxide; NSAIDs = non-steroidal anti-inflammatory drugs; TXA₂ = thromboxane A₂; VWD = von Willebrand disease; VWF = von Willebrand factor.

INDEX TERMS: functional platelet disorders; platelets; thrombotic thrombocytopenic purpura.

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. recognize the clinical presentation of patients with dysfunctional platelets.
2. describe the defect in each of the following hereditary disorders: Glanzmann thrombasthenia and Bernard-Soulier syndrome (BSS).
3. distinguish among the following types of hereditary platelet disorders: membrane receptor abnormality, secretion disorder, and storage pool deficiency.

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4. for each of the inherited platelet disorders listed, name useful laboratory tests and recognize diagnostic results.
5. Discuss general mechanisms of action for antiplatelet drugs.
6. explain the effects of paraproteins on platelet function.
7. describe the effect of aspirin, clopidogrel, and α IIb/ β 3 inhibitors on platelet function.
8. discuss the mechanism of the platelet defects associated with myeloproliferative diseases, uremia, and liver disease.

Mucocutaneous bleeding in a patient whose platelet count is normal suggests a disorder of platelet function. Congenital and acquired disorders may cause abnormalities in each phase of platelet function: adhesion, aggregation, and secretion. The qualitative disorders are detected and monitored using platelet aggregometry.¹ Qualitative disorders are summarized in Table 1.

PLATELET MEMBRANE RECEPTOR DISORDERS

Bernard-Soulier (giant platelet) syndrome

Bernard-Soulier syndrome (BSS) usually manifests in infancy or childhood with mucocutaneous hemorrhage characteristic of defective platelet function: ecchymoses, epistaxis, and gingival bleeding. BSS is inherited as an autosomal recessive disorder in which the glycoprotein (GP) Ib/IX/V complex exhibits abnormal function. Heterozygotes with about 50% of normal levels of GP Ib, GP V, and GP IX have normal platelet function. Homozygotes have moderate to severe bleeding characterized by enlarged platelets, thrombocytopenia, and decreased platelet survival. Platelet counts range from 40,000/ μ L to near-normal.² Platelets typically are five to eight μ m in diameter although a few reach 20 μ m. Viewed by electron microscopy, BSS platelets contain a larger number of cytoplasmic vacuoles and membrane complexes.³⁻⁵

Four glycoproteins are required to form the GP Ib/IX/V complex: GP Ib α , GP Ib β , GP IX, and GP V. These are present in the ratio of 2:2:2:1. The gene for GP Ib α is located on chromosome 17, the gene for GP Ib β is located on chromosome 22, and the genes for GP IX and GP V are on chromosome 3. For surface expression of the complex, it seems that synthesis of three proteins, GP Ib α , GP Ib β , and GP IX, is required. GP V can be expressed alone in significant

quantities, but its expression seems to be enhanced if the rest of the complex is present. The most frequent forms of BSS involve defects in GP Ib α expression. The presence of GP Ib α is essential to normal function because it contains binding sites for von Willebrand factor (VWF) and thrombin. Defects in the GP Ib β and GP IX genes also are known to result in BSS.⁶⁻¹¹

BSS platelets have normal in vitro aggregation responses to the platelet activators (agonists) adenosine diphosphate (ADP), epinephrine, collagen, and arachidonic acid, but do not respond to ristocetin and have diminished response to thrombin.^{1,3,5} The lack of response to ristocetin is due to the lack of GP Ib/IX/V complexes and the inability of BSS platelets to bind von Willebrand factor (VWF). Platelets fail to adhere to exposed subendothelium resulting in bleeding. In many respects, this disorder resembles the defect seen in von Willebrand disease (VWD). In contrast to VWD, abnormality is not corrected by therapeutic administration of VWF-rich factor VIII concentrate.

Platelet transfusions are the only effective treatment, but patients invariably develop alloantibodies and become refractory. BSS patients tend to do better if apheresis platelets are used for transfusion because this limits the number of donors to which the patient is exposed, and the rate of alloimmunization is controlled.^{3,5}

Glanzmann thrombasthenia

Glanzmann thrombasthenia is an autosomal recessive disorder seen most frequently in consanguinity. Heterozygotes are clinically normal, whereas homozygotes have serious mucocutaneous bleeding from infancy. There are wide variations in symptoms but their severity seems to decrease with age.^{12,13}

The biochemical lesion is a deficiency or abnormality of the platelet membrane GP IIb/IIIa (α_{IIb}/β_3), a receptor that binds fibrinogen and VWF in normal in vivo and in vitro aggregation. The platelets of homozygous individuals lack surface-expressed α_{IIb}/β_3 , whereas the α_{IIb}/β_3 of platelets from heterozygotes is 50% to 60% of normal.¹⁴ Failure of binding results in defective hemostatic plug formation.¹⁵⁻¹⁷

More than 70 mutations give rise to Glanzmann thrombasthenia.^{19,20} The α_{IIb} and β_3 genes are present on chromosome 17, and genetic defects are distributed widely over the two genes. α_{IIb} is synthesized in megakaryocytes as pro- α_{IIb} which complexes β_3 in the endoplasmic reticulum. The complex is transported to the Golgi where α_{IIb} is cleaved to heavy and light chains to form the complete complex. Uncomplexed α_{IIb} and β_3 are not processed in the Golgi. Similar to the GP Ib/IX/V complex, both proteins of α_{IIb}/β_3 must be available for the complex to be expressed.

The laboratory features of Glanzmann thrombasthenia include a normal platelet count and morphology, but no ag-

Table 1. Qualitative platelet abnormalities

DISORDERS OF PLATELET ADHESION

Congenital adhesion disorders

- Bernard Soulier syndrome: GP Ib/IX/V deficiency
- Von Willebrand disease

Acquired defects of platelet adhesion

- myeloproliferative disorders
- lymphoproliferative disorders, multiple myeloma and dysproteinemias
- antiplatelet antibodies
- cardiopulmonary bypass
- chronic liver disease
- drug-induced membrane modification

DISORDERS OF PLATELET AGGREGATION

Congenital aggregation abnormalities

- Glanzmann thrombasthenia: α_{IIb}/β_3 deficiency
- hereditary afibrinogenemia

Storage pool disorders: dense granule deficiencies

- Hermansky-Pudlak syndrome
- Wiskott-Aldrich syndrome
- thrombocytopenia with absent radii syndrome
- Chédiak-Higashi syndrome

DISORDERS OF PLATELET SECRETION (RELEASE)

Hereditary thromboxane pathway disorders: aspirin-like defects

- cyclooxygenase deficiency
- thromboxane synthase deficiency

Drug inhibition of the prostaglandin pathways

- drug inhibition of platelet phosphodiesterase activity
- uremia

gregation response to ADP, collagen, thrombin, or epinephrine.^{15,17} A strong agonist such as thrombin induces platelet secretion in the absence of aggregation. Ristocetin-induced platelet agglutination is normal.

Thrombasthenia is one of the few platelet disorders in which hemorrhage is severe and disabling. Bleeding of all types is reported, including epistaxis, ecchymoses, hemarthroses, subcutaneous hematomas, menorrhagia, and gastrointestinal and urinary tract hemorrhage. Treatment of bleeding episodes requires platelet concentrate. The defective Glanzmann platelets may interfere with normal platelets, and it may be necessary to infuse more donor platelets than expected to control bleeding.²¹ As in BSS or any situation in which repeated transfusions are required, patients with Glanzmann thrombasthenia may become alloimmunized.²² Strategies to reduce alloimmunization include use of single-donor platelet apheresis products, human leukocyte antigen-matched donor platelets, or ABO-matched donor platelets.

A variety of treatments have been used successfully to control or prevent bleeding alone or in combination with platelet transfusion. To a large extent, the site of hemorrhage determines these therapeutic approaches. Hormonal therapy (norethindrone acetate) has been used to control menorrhagia. Oral contraceptives reduce excessive bleeding. Menorrhagia at the onset of menses is uniformly severe and can be life-threatening, leading some to suggest that birth control pills be started before menarche. Also, antifibrinolytic (aminocaproic acid or tranexamic acid) therapy can be used to control gingival hemorrhage or excessive bleeding after tooth extraction.²² Recombinant factor VIIa has proven useful to treat severe bleeding in patients with isoantibodies to α_{IIb}/β_3 and for patients undergoing invasive procedures.²³ Recombinant factor VIIa is thought to enhance thrombus formation at the site of a lesion by stimulating tissue factor-independent thrombus generation and fibrin formation.²⁴

STORAGE POOL DISORDERS: DENSE GRANULE DEFICIENCIES

A variety of genetic abnormalities lead to dense granule (dense body, δ -granule) deficiency, most associated with albinism, where the number of dense granules is reduced. In non-albino or acquired dense granule deficiency, as in myelodysplastic syndromes, dense granule membranes are present in near-normal numbers, suggesting inability to package dense granule contents.²⁵⁻³⁰

Bleeding is limited to mild easy bruisability. Dense granules are intracellular storage sites for ADP, ATP, calcium, pyrophosphate, and serotonin, all expressed during platelet secretion (release). Secreted ADP recruits and activates platelets, promoting aggregation and propagation of the hemostatic plug. In lumiaggregometry, agonists (even thrombin) fail to induce platelet secretion, although the aggregation response is normal or moderately suppressed.

Hermansky-Pudlak syndrome is an autosomal recessive disorder characterized by tyrosinase-positive oculocutaneous albinism, defective lysosomal function in a variety of cell types, cereoid-like deposition in the cells of the reticuloendothelial system, and profound platelet dense granule deficiency.³¹⁻³⁴ Mutations in at least seven chromosome 17 genes individually can give rise to Hermansky-Pudlak syndrome. Although bleeding associated with most dense granule deficiencies is mild, Hermansky-Pudlak syndrome is an exception. In one series, hemorrhage accounted for 16% of deaths.³²

Chédiak-Higashi syndrome is an autosomal recessive disorder characterized by partial oculocutaneous albinism, pyogenic bacterial infections, dense granule deficiency with mild hemorrhage, and giant lysosomal granules in neutrophils and lymphocytes. A series of chromosome 13 mutations yield a truncated protein that encourages fusion of cytoplasmic granules. The disorder progresses to an accelerated phase in 85% of Chédiak-Higashi syndrome patients marked by lymphocytic proliferation in the liver, spleen, and marrow and macrophage accumulation in tissues. Pancytopenia worsens, resulting in thrombocytopenia-related hemorrhage and ever-increasing susceptibility to infection leading to early death.³¹

Wiskott-Aldrich syndrome is an X-linked recessive disease characterized by severe eczema, with immune deficiency causing recurrent infections and thrombocytopenia. Bleeding episodes are moderate to life-threatening. A combination of ineffective thrombocytopoiesis and increased platelet sequestration and destruction accounts for the thrombocytopenia.³³⁻³⁴

In Wiskott-Aldrich platelets, the number of both α - and dense granules is decreased, and the platelets are small, a feature of diagnostic importance. As in all storage pool deficiency, the platelets show a decreased secretion response to thrombin ADP, collagen, and epinephrine, though aggregation is demonstrable, if suppressed. Bone marrow transplantation has been attempted with some success.

INHERITED THROMBOXANE PATHWAY DISORDERS: ASPIRIN-LIKE DEFECTS

Platelet secretion (release) requires the activation of several biochemical pathways, including one that leads to thromboxane A_2 (TXA₂) formation.³⁵ This is called alternatively the arachidonic acid, prostaglandin, cyclooxygenase, or eicosanoid synthesis pathway. Phospholipase catalyzes the release of arachidonic acid from membrane phospholipids. Arachidonic acid is converted to intermediate prostaglandins by cyclooxygenase and to TXA₂ by thromboxane synthase. TXA₂ bind activation receptors TP α and TP β to mobilize ionic calcium from internal stores into the cytoplasm, resulting in aggregation and secretion. TXA₂ is required for the in vitro secretion and aggregation response to epinephrine, ADP, and low concentrations of collagen.

Several acquired or congenital platelet secretion disorders are traced to modifications of arachidonic acid pathway enzymes. For instance, inhibition of cyclooxygenase occurs on ingestion of drugs such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), reducing TXA₂ production.

Hereditary abnormalities of eicosanoid synthesis enzymes are termed *aspirin-like defects* because the clinical and laboratory manifestations resemble those that follow aspirin ingestion. Platelet aggregation and secretion responses to arachidonic acid, one $\mu\text{g/mL}$ and five $\mu\text{g/mL}$ collagen, and ADP are reduced, whereas the response to thrombin is normal. In contrast to dense granule deficiency, granular contents and numbers are normal. Deficiencies of the enzymes cyclooxygenase and thromboxane synthase are well-documented, and dysfunction or deficiency of thromboxane receptors is known.³⁵

ADDITIONAL INHERITED DISORDERS OF PLATELET MEMBRANE RECEPTORS

The $\alpha_2\beta_1$ (GP Ia/IIa) integrin is a collagen receptor. A deficiency has been reported in a patient who lacked an aggregation response to collagen, whose platelets did not adhere to collagen, and who had a lifelong mild bleeding disorder.^{36,37} A deficiency in a second collagen receptor, GP VI, also has been reported in patients with mild bleeding. The platelets of these patients failed to aggregate in response to collagen. A family with gray platelet syndrome (α -granule deficiency) and defective collagen adhesion has been described. Affected members of the family have a severe deficiency of GP VI.

Platelets have at least three ADP receptors. P2X₁ is linked to an ion channel that facilitates calcium ion influx, P2Y₁ may mediate calcium mobilization and shape change and P2Y₁₂

is thought to be responsible for platelet aggregation.³⁸ Some patients have decreased platelet aggregation in response to ADP but normal platelet shape change and calcium mobilization. These patients have an inherited deficiency of the P2Y₁₂ receptor.^{39,40} Bleeding problems are usually mild, but the only treatment for severe bleeding is platelet transfusion.

Finally, Scott syndrome is a rare autosomal recessive disorder in which platelets secrete and aggregate normally, but do not “flip” phosphatidylserine and phosphatidylethanolamine from the inner to the outer leaflet of the plasma membrane.⁴¹⁻⁴⁵ This phospholipid transfer normally occurs upon activation and is essential for the binding of vitamin K-dependent clotting factors. In the membrane of resting platelets, phosphatidylserine and phosphatidylethanolamine are restricted to the inner leaflet of the plasma membrane, and phosphatidylcholine is expressed on the outer leaflet. When platelets are activated, the asymmetry is lost, and phosphatidylserine and phosphatidylethanolamine “flip” to the outer leaflet to facilitate the assembly of clotting factor complexes. The phospholipid “flip” is mediated by a calcium-dependent enzyme, *scramblase*. In Scott syndrome, platelet plug formation occurs normally, but clotting factor complexes do not assemble on the activated platelet surface, leading to anatomic bleeding.

DRUGS THAT INHIBIT PLATELET FUNCTION

Aspirin: inhibition of the eicosanoid synthesis pathway
Aspirin and other NSAIDs inhibit the platelet eicosanoid synthesis pathway. A single 325 mg dose of aspirin irreversibly acetylates 90% of platelet cyclooxygenase. Platelets lack a nucleus and cannot synthesize new cyclooxygenase, so the inhibitory effect is permanent for the circulatory life span of the platelet, seven to ten days. Several test systems are available to determine the optimal dose of aspirin as some patients have, or develop, aspirin resistance. These may also be used to match aspirin dosage to the patient.⁴⁶⁻⁴⁸ Two studies have shown that resistant cardiac patients have a higher rate of a second adverse cardiac event. The reference test is platelet aggregometry. Aspirin suppresses the aggregation response to arachidonic acid and one $\mu\text{g/mL}$ collagen, but the response to five $\mu\text{g/mL}$ collagen is normal. The collagen response may be employed to distinguish aspirin from aspirin-like disorder.

People with a hemostatic defect, such as storage pool deficiency, secretion disorder, receptor disorder, thrombocytopenia, or von Willebrand disease, may experience increased bleeding on aspirin ingestion. These are advised to avoid the use of aspirin, NSAIDs, and other antiplatelet therapy.

Thienopyridines: ADP receptor inhibitors

The thienopyridine derivative clopidogrel and its predecessor ticlopidine are antiplatelet agents used to treat arterial occlusive disease for prevention of secondary myocardial infarction, to decrease the risk of thrombotic stroke in patients with cerebrovascular disease. Clopidogrel is used with aspirin or in patients who are intolerant of aspirin. In contrast to aspirin, the effect of thienopyridines reaches steady state after three to five days, although it can be reached sooner with a loading dose. Thienopyridines interfere with ADP binding to platelet membrane receptor P2Y₁₂.⁴⁹⁻⁵⁰ ADP-induced in vitro platelet secretion and aggregation are inhibited, and responses to collagen and other agonists are reduced. Ticlopidine may rarely produce long-lasting neutropenia, aplastic anemia, thrombocytopenia, gastrointestinal distress, and diarrhea. The incidence and severity of these side effects for clopidogrel are lower so it has become the predominant antiplatelet agent. Clopidogrel and aspirin are used in combination to exploit their synergy as they inhibit platelet function by parallel mechanisms. Like aspirin, the effect of clopidogrel is not readily reversible; platelet function returns to 50% of normal at three days and complete recovery of function occurs at seven days.

Intravenous α_{IIb}/β_3 (GP IIb/IIIa) inhibitors

A third group of antiplatelet agents target the α_{IIb}/β_3 receptor, interfering with its ability to bind fibrinogen and inhibit platelet aggregation.⁵¹ Platelet lumiaggregometry results mimic mild Glanzmann thrombasthenia with poor aggregation response and moderate secretion when challenged with arachidonic acid, collagen, ADP, and epinephrine. The first such agent approved for clinical use in the United States is the Fab fragment of the mouse/human chimeric monoclonal antibody 7E3 (c7E3 Fab; abciximab; RheoPro®). The second agent in this group, eptifibatid or anegrelid, targets an α_{IIb}/β_3 recognition site for fibrinogen's Arg-Gly-Asp (RGD) binding sequence. The agent binds the recognition site and prevents fibrinogen binding. Platelet aggregometry may be applied to detect resistance to clopidogrel and inhibitors to α_{IIb}/β_3 .

DISORDERS THAT AFFECT PLATELET FUNCTION

Myeloproliferative disorders

Chronic myeloproliferative disorders (MPDs) include polycythemia vera, chronic myelocytic leukemia, essential thrombocythemia, and myelofibrosis with myeloid metaplasia. Hemorrhagic complications occur in about one third of MPDs, thrombosis occurs in another third, and although uncommon, some patients develop both. Hemorrhage and thrombosis are least common in chronic myelocytic leuke-

mia. Bleeding is common in myelofibrosis with myeloid metaplasia and thrombosis in essential thrombocythemia and polycythemia vera. A platelet origin for these disorders is supported by the observation that bleeding is usually mucocutaneous, and thrombosis may be arterial or venous. In essential thrombocythemia and polycythemia, thrombosis may occur in unusual sites such as in the mesenteric, hepatic, and portal circulations. Patients with essential thrombocythemia may develop digital artery thrombosis and ischemia of the fingers and toes, occlusions of the microvasculature of the heart, and cerebrovascular occlusions that result in neurologic symptoms.⁵⁴

A variety of platelet function defects have been described in MPDs. Platelets may have abnormal shapes, decreased procoagulant activity, and a decreased number of α - and dense granules. In essential thrombocythemia, platelet survival may be shortened. The risk of thrombosis or hemorrhage correlates poorly with the elevation of the platelet count but may correlate with lumiaggregometry results.

The most common lumiaggregometry abnormalities are decreased aggregation and secretion in response to epinephrine, ADP, and collagen.⁵⁵ Possible causes include loss of platelet membrane α -adrenergic (epinephrine) receptors, impaired release of arachidonic acid from membrane phospholipids, impaired oxidation of arachidonic acid by the eicosanoid synthesis pathway, a decrease in the contents of α - and dense granules, and loss of a variety of membrane receptors for adhesion and activation. There is little correlation between a given MPD and the platelet dysfunction observed with the exception that most patients with essential thrombocythemia lack an in vitro platelet aggregation response to epinephrine. This observation may be helpful in the differential diagnosis.

Multiple myeloma and Waldenström macroglobulinemia

Platelet dysfunction is observed in approximately one third of patients with IgA myeloma or Waldenström macroglobulinemia and in a much smaller percentage of patients with IgG multiple myeloma. Dysfunction results from coating of the platelet membranes by paraprotein. Almost all patients with malignant paraprotein disorders have clinically significant bleeding, and thrombocytopenia with poorly functional platelets is the most likely cause.

Cardiopulmonary bypass surgery

Cardiopulmonary bypass induces thrombocytopenia and a severe platelet function defect that may cause post-surgical

bleeding. Platelets activate and fragment after adherence and aggregation to fibrinogen coating the bypass circuit material. Exposure to hypothermia, bypass pump-priming solutions, blood conservation devices, and the blood-air interface in bubble oxygenators causes degranulation and formation of platelet microparticles. After an uncomplicated bypass, normal platelet function returns in about one hour, although the platelet count does not return to normal for several days. Thrombocytopenia is caused by hemodilution, sequestration of damaged platelets by the reticuloendothelial system, and consumption associated with normal hemostatic processes after surgery.⁵⁶⁻⁵⁹

Liver disease

Mild to moderate thrombocytopenia is seen in approximately one third of patients with chronic liver disease in association with hypersplenism or as a result of alcohol toxicity.⁵⁷ There is reduced platelet aggregation to ADP, epinephrine, and thrombin, perhaps owing to acquired storage pool deficiency.

In chronic alcoholic cirrhosis, the thrombocytopenia and platelet functional deficiencies may result from the toxic effects of alcohol on bone marrow megakaryocytes. Severe end-stage bleeding reflects markedly decreased coagulation factor production, excessive fibrinolysis, dysfibrinogenemia, thrombocytopenia, and occasionally DIC. Upper gastrointestinal bleeding is a relatively common feature of alcoholic cirrhosis, and recombinant factor VIIa has been shown to be effective for some patients.⁶⁰

Renal disease

Platelet dysfunction commonly accompanies uremia, though bleeding is uncommon. Plasma guanidinosuccinic acid (GSA) becomes elevated as a result of urea cycle inhibition. GSA is a nitric oxide (NO) donor, thus NO levels rise. NO diffuses into platelets, activates guanylate cyclase and inhibits platelet activation.⁶¹ GSA is dialyzable: peritoneal dialysis or hemodialysis usually corrects the abnormal platelet function.

Platelet aggregation abnormalities are non-uniform, though there is a consistently deficient ADP-induced secretion response. Anemia is an accompanying cause of bleeding, and uremic patients are typically treated with erythropoietin to normalize the hematocrit.

Bleeding is seen more often in patients who are concurrently taking drugs that interfere with platelet function or who are receiving heparin during hemodialysis. Platelet concentrates are used in an attempt to halt severe hemorrhagic episodes, but usually do not correct the bleeding.

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