

The Laboratory Diagnosis of Platelet Disorders

An Algorithmic Approach

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• **Objective.**—To provide both a detailed description of the laboratory tests available in the diagnosis of platelet disorders and a testing algorithm, based on platelet count, that can be used to direct the evaluation of platelet disorders.

Data Sources.—A literature search was conducted using the National Library of Medicine database.

Study Selection.—The literature on laboratory testing of platelet function was reviewed.

Data Extraction and Data Synthesis.—Based on the literature review, an algorithm for platelet testing was developed.

Conclusions.—A history of mucocutaneous bleeding often indicates abnormal platelet function that can be associated with a normal, increased, or decreased platelet

count. Multiple laboratory procedures can now be used to determine the underlying pathologic condition of platelet dysfunction when other deficiencies or defects of the coagulation cascade or fibrinolysis are ruled out. Simple procedures, such as platelet count, peripheral blood smear, and a platelet function screening test, will often lead the investigator to more specific analyses. Although platelet function testing is often limited to larger medical centers with highly trained technologists, newer technologies are being developed to simplify current procedures and make platelet function testing more accessible. This review provides an algorithm for platelet testing that may be of benefit to pathologists and physicians who deal with hemostatic disorders.

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Platelets are small (2- μ m-diameter), nonnucleated blood cells produced in the bone marrow from megakaryocytes. Platelets are activated rapidly after blood vessel injury or blood exposure to the artificial surfaces of implanted devices, and they are a crucial component of the primary hemostatic response. In their inactivated state, platelets are roughly discoid in shape and contain cytoplasmic organelles, cytoskeletal elements, invaginating open-canalicular membrane systems, and platelet-specific granules, called alpha and dense granules. Platelets have numerous intrinsic glycoproteins embedded in the outer surface of their plasma membrane that are receptors for ligands ranging from fibrinogen, collagen, thrombin, and thrombospondin to von Willebrand factor (VWF) and fibronectin.^{1–3} Platelets promote hemostasis by 4 interconnected mechanisms: (1) *adhering* to sites of vascular injury or artificial surfaces, (2) *releasing* compounds from their granules, (3) *aggregating* together to form a hemostatic platelet plug, and (4) providing a *procoagulant* surface for activated coagulation protein complexes on their phospholipid membranes (Figure 1).

Platelet *adhesion* to the subendothelium is the initial step in platelet activation. The subendothelium is composed of extracellular matrix proteins, such as collagen, fibronectin,

VWF, thrombospondin, and laminin,⁴ many of which are ligands for receptors on the platelet surface. These adhesive proteins are exposed when the endothelial layer is disrupted. Because of the large number of extracellular matrix proteins and a high density of platelet surface receptors, platelet adhesion to areas of vascular injury is extremely rapid. VWF, a large, multimetric protein secreted into the extracellular matrix from endothelial cells, facilitates platelet adhesion by binding to platelet surface glycoprotein Ib/IX/V, especially at high shear rates.^{5–7} Platelets can also adhere to vascular wall-associated fibrin or fibrinogen through interaction with platelet surface glycoprotein IIb/IIIa.^{8,9}

After adhering to the subendothelium, platelets undergo a cytoskeletal activation that leads to a shape change with development of pseudopods. Intracellular signaling processes lead to increased cytoplasmic calcium and then initiate a secretory *release* reaction, whereby products from the alpha granules (platelet factor 4, β -thromboglobulin, thrombospondin, platelet-derived growth factor, fibrinogen, VWF) and dense granules (adenosine diphosphate [ADP], serotonin) are released into the surrounding milieu.¹⁰ The granule membranes contain many integral glycoproteins on their inner leaflet, such as P-selectin (CD62p) in the alpha granule and gp53 (CD63) in the lysosome, which become expressed on the outer platelet membrane after the release reaction.¹¹ The release of ADP from the dense granules, together with calcium mobilization, leads to a conformational change of the fibrinogen receptor, the glycoprotein IIb/IIIa receptor complex (integrin $\alpha_{IIb}\beta_3$).¹² This conformational change of the fibrino-

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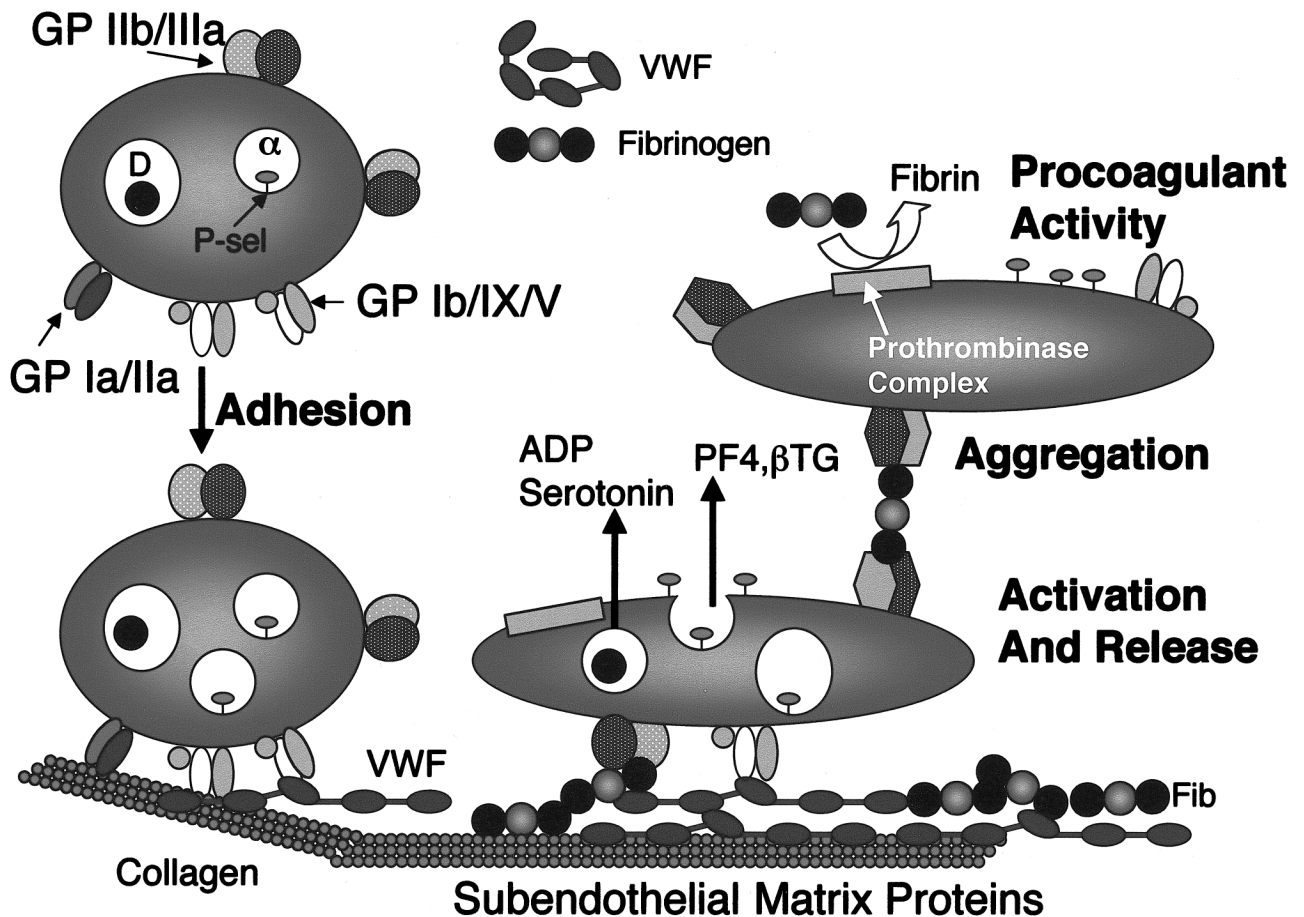


Figure 1. Platelet activation is stimulated by vascular injury through exposure of platelets to extracellular matrix proteins and adsorbed plasma proteins, including von Willebrand factor (VWF) and fibrinogen (Fib). Platelets adhere to VWF through the surface glycoprotein Ib/IX/V and to fibrinogen through the glycoprotein IIb/IIIa receptor. Platelet adhesion stimulates intracellular signaling, leading to degranulation of alpha granules (platelet factor 4 [PF4] and β -thromboglobulin [β TG]), and phospholipid reorganization with formation of coagulation complexes and fibrin formation. Adenosine diphosphate (ADP) release from dense granules and activation of the glycoprotein IIb/IIIa receptor also occur, leading to aggregation of platelets to the adherent layer.

gen receptor initiates the process of *aggregation*, whereby a glycoprotein IIb/IIIa receptor on one platelet is bound in a homotypic fashion to the same receptor on adjacent platelets via a central fibrinogen molecular bridge. Beside ADP, other agonists, such as epinephrine, thrombin, collagen, and platelet-activating factor, can initiate platelet aggregation by interaction with membrane receptors. This platelet release reaction and aggregation lead to the recruitment of many other platelets to the vessel wall with the formation of a hemostatic platelet plug.

Activated platelets also play a vital *procoagulant* role that serves as a link between platelet function and coagulation activation. Platelet membrane phospholipids undergo a rearrangement during activation with a transfer of phosphatidyl serine from the inner table to the outer table of the platelet membrane, providing a binding site for phospholipid-dependent coagulation complexes that activate both factor X and prothrombin.¹³

LABORATORY TESTS USED IN THE EVALUATION OF PLATELET FUNCTION

Clinical History

A careful clinical and family bleeding history should be taken before beginning a laboratory evaluation of platelet function. The history should include an assessment of the

duration, pattern, and severity of bleeding problems, including whether the bleeding is spontaneous or associated with trauma or surgery. A lifelong bleeding diathesis may suggest a congenital platelet dysfunction, but an onset in adulthood does not necessarily exclude a congenital problem. In obtaining a history of bleeding pattern, it is necessary to determine whether a true hemorrhagic disorder exists. In this regard, it is often helpful to assess if the bleeding is out of proportion to the degree of trauma, or whether blood transfusions were required for relatively minor surgical procedures, such as tooth extractions.

Platelet-mediated bleeding disorders usually result in a mucocutaneous bleeding pattern, with ecchymosis, petechiae, purpura, epistaxis, and gingival bleeding commonly observed.⁴ This pattern is in contrast to that observed with coagulation protein disorders, in which deep tissue bleeding and hemarthroses are more common. Von Willebrand disease, an abnormality of VWF, has bleeding symptoms very similar to platelet dysfunction, and evaluation for von Willebrand disease should be included in the initial evaluation of a possible platelet disorder.¹⁴ Bleeding diatheses due to vascular malformations may give a bleeding pattern similar to platelet disorders, but is often more focal than diffuse. Acquired purpuras, such as those seen with disseminated intravascular coagulation, vasculitis, or in-

Table 1. Drugs That Affect Platelet Function*

Nonsteroidal anti-inflammatory drugs (NSAIDs)	Psychotropics and anesthetics
Aspirin	Tricyclic antidepressants (ie, imipramine)
Ibuprofen	Phenothiazines (ie, chlorpromazine)
Mefenamic acid	Local and general anesthesia (ie, halothane)
Indomethacin	
Cox-2 inhibitors	Chemotherapeutic agents
Antimicrobials	Mithramycin
Penicillins	Daunorubicin
Cephalosporin	Carmustine
Nitrofurantoin	
Hydroxychloroquine	Miscellaneous agents
Amphotericin	Dextran
Cardiovascular agents	Radiographic contrast
β-Adrenergic blockers (ie, propranol)	Quinidine
Vasodilators (ie, nitroglycerin)	Ethanol
Diuretics (ie, furosemide)	
Calcium channel blockers	Foods
Anticoagulants	Caffeine
Heparin	Garlic
Coumadin	Cumin
Lepirudin	Turmeric
Argatroban	Antiplatelet drugs
Bivalirudin	Phosphodiesterase inhibitors
Thrombolytic agents	Dipyridamole
Streptokinase	Cilostazole
Urokinase	Adenosine diphosphate receptor antagonists
Tissue plasminogen activator	Ticlopidine
	Clopidogrel
	Glycoprotein IIb/IIIa antagonists
	Abciximab
	Eptifibatide
	Tirofiban

* Adapted from George and Shattil.¹⁶

fections, can usually be distinguished from platelet dysfunction, because platelet disorders usually cause bleeding from mucous membranes ("wet" purpura), whereas vascular purpura is usually confined to the skin ("dry" purpura).¹⁵

Many drugs and foods can affect platelet function (Table 1), so a complete drug history should be obtained.¹⁶ It is important to remember that aspirin, an irreversible inhibitor of platelet function, is an ingredient of many over-the-counter and prescription medications, such as cold or flu remedies. Platelet dysfunction is associated with many systemic disorders, such as renal disease, hepatic failure, connective tissue disorders, myeloproliferative disorders, myelodysplastic disorders, malignancy, and cardiovascular disease. Additionally, some clinical features, such as albinism, deafness, nephritis, and susceptibility to infections, may help in the differential diagnosis of the inherited platelet disorders.¹⁷

Platelet Count and Peripheral Blood Smear

The accepted normal range of the platelet count is generally between 150 to 400 × 10³/μL of blood, although values much lower than this can be quite adequate for hemostasis. Many clinicians will refrain from platelet repletion in a stable patient until counts drop below 10 to 20 × 10³/μL.¹⁸ Initial evaluation of the platelet count must take into consideration any pseudothrombocytopenia. Pseudothrombocytopenia is often due to cold-reacting platelet agglutinins or platelet binding to neutrophils (platelet satellitism). The agglutinins are often seen in patients with high immunoglobulin levels or infections and usually only bind platelets when calcium is chelated, such

as in an EDTA blood collection tube.¹⁹ A pseudothrombocytopenia associated with the glycoprotein IIb/IIIa antagonist drug abciximab has also been reported.²⁰ Pseudothrombocytopenia can be diagnosed by examining a peripheral smear, where large aggregates of platelets are observed, often around the feathered edge. A more accurate platelet count can be established by collecting the blood sample in either citrate or heparin anticoagulants. Giant platelets observed with macrothrombocytopenia syndromes can give false low platelet counts, because the large platelets may be counted as leukocytes by automated cell counters.²¹

The mean platelet volume (MPV) is an indication of platelet size. Normal MPV ranges are approximately 7 to 11 fL. The MPV can be an indication of platelet turnover, because platelets newly released from the bone marrow are larger and tend to decrease in size with age in the circulation.²² In patients with rapid turnover, the platelets will, in general, be larger because of the larger size of newly produced platelets. True congenital macrothrombocytopenias usually have uniformly large platelets; often the platelets are at least twice the normal size and may be as large as erythrocytes. Newer techniques based on messenger RNA detection in platelets (reticulated platelets) may also be helpful to indicate the rate of thrombopoiesis.^{23,24}

Platelet disorders can be associated with varying platelet appearances. In von Willebrand disease, Glanzmann thrombasthenia, and myeloproliferative disorders, the platelets have typical morphologic features, whereas giant platelets are seen in Bernard-Soulier disease and other ma-

crothrombocytopenia syndromes.^{17,25} In patients with Wiskott-Aldrich syndrome, the platelets may be small.²⁶ Platelets in the gray platelet syndrome, an alpha granule deficit, are characteristic for being pale, gray, and hypogranular on a Wright-stained blood smear.²⁷ Some platelet storage pool disorders (SPDs) may have morphologically normal platelet counts by light microscopy, but may have decreased alpha and/or dense granules by electron microscopy.²⁸

Platelet Function Screening Tests or Bleeding Time

In the initial evaluation of platelets, it is desirable to perform a screening test to evaluate platelet function. For nearly a century, the bleeding time was the only platelet function screening test available.²⁹ The bleeding time is a test that is fraught with variability and involves the creation of a standardized cut in the skin and measurement of the time it takes for bleeding to stop. The initial Duke bleeding time used a small incision in the earlobe, and the similar Ratnoff method uses an incision in the ball of the finger.^{29,30} The most commonly used bleeding time is the Ivy bleeding time, where a standardized incision is made on the volar surface of the forearm with a spring-loaded device, using venostatic pressure applied on the upper arm by a sphygmomanometer.³¹ The bleeding time result depends not only on platelet number and function, but also on fibrinogen concentration, adequate vascular function, orientation and size of the incision, site of the incision, skin quality, skin temperature, operator technique, and patient cooperation.

Although procedural variability affects the bleeding times, bleeding time has been included traditionally as a screening test for suspected bleeding disorders.³² The bleeding time has little use as a presurgical screen for hemostatic competence in individuals without a history of bleeding and is not useful in discerning platelet dysfunction in thrombocytopenic patients.³³ Many laboratories have stopped performing the bleeding time test completely because of its variability, poor reproducibility, and lack of correlation with intraoperative bleeding. Newer automated whole blood platelet function screening assays, such as the Platelet Function Analyzer-100 (PFA-100, Dade Behring, Marburg, Germany), are gaining popularity as initial screens for platelet function even though they do not measure the vascular component of the bleeding time.³⁴ These are described later in the section entitled "Newer Methods of Platelet Evaluation."

Bone Marrow Examination

Examination of the bone marrow can be performed to evaluate both thrombocytopenia or thrombocytosis, but it has little role in the evaluation of platelet dysfunction with a normal platelet count. The bone marrow examination may be helpful to ascertain whether thrombocytosis is due to reactive or myeloproliferative disorders. Thrombocytosis can often accompany iron deficiency; evaluation of serum iron studies together with the erythrocyte indices on the complete blood cell count can usually diagnose the condition and obviate the need for a bone marrow evaluation. In a thrombocytopenic patient, when no other reason for low platelet counts can be determined, the bone marrow examination is useful for determining the presence or absence of megakaryocytes; absence indicates dysfunctional marrow, whereas increased numbers suggest peripheral destruction with attempted bone marrow com-

ensation. Bone marrow examination can also detect myelophthistic disorders, such as acute leukemia, lymphoma, or metastatic malignancy, which could explain a patient's thrombocytopenia.

Platelet Aggregation

Platelet aggregation studies measure the ability of agonists to cause in vitro platelet activation and platelet-platelet binding. Platelet aggregation studies can be performed in whole blood by an impedance technique or in platelet-rich plasma by a turbidimetric technique.^{35,36} Platelet aggregation techniques using a microtiter plate or flow cytometer have also been described, but are not widely performed.^{37,38} Whole blood platelet aggregation can be combined with studies of dense granule adenosine triphosphate release with a lumiaggregometer.³⁹ Turbidimetric platelet aggregation studies require platelet-rich plasma prepared from a whole blood specimen. Many factors can affect the platelet aggregation results, such as the platelet count, processing temperature, stirring rate, and processing time (testing should be completed within 4 hours of phlebotomy).⁴⁰ In addition, clinicians who order the tests should advise patients to discontinue using, if possible, any medication that may interfere with the results of the test (Table 1).

In the turbidimetric platelet aggregation assay, platelet aggregation is measured spectrophotometrically by the increase in light transmission after addition of an aggregation agonist.³⁵ The agonists typically used in the assay include ADP, collagen, arachidonic acid, and epinephrine. Optimal platelet aggregation shows a biphasic pattern for the agonists ADP and epinephrine; the initial increase in aggregation is due to primary aggregation in response to activation of the glycoprotein IIb/IIIa platelet membrane receptor, whereas the second wave of aggregation is the result of platelet degranulation with recruitment of additional platelet aggregates. Other agonists, such as arachidonic acid, thrombin receptor agonists, and collagen, usually show only a single wave of aggregation.

Another important reagent used in the evaluation of platelet function by aggregation is the antibiotic ristocetin, which facilitates the binding of VWF to the glycoprotein Ib/IX/V complex.⁴¹ Ristocetin-induced platelet aggregation evaluates aggregation after the addition of various concentrations of ristocetin. This dose response allows testing for both increased and decreased sensitivity to ristocetin. For a normal result, the patient requires the presence of both functional VWF and normal glycoprotein Ib/IX/V, so ristocetin-induced platelet aggregation is an assay that can detect both von Willebrand disease and some platelet dysfunctions, such as Bernard-Soulier syndrome.

Coagulation Testing and von Willebrand Assays

Platelet dysfunction does not directly affect the coagulation proteins; however, the laboratory evaluation of platelet dysfunction should also include some basic coagulation assays, such as the prothrombin time (PT) and activated partial thromboplastin time (APTT), to exclude a coagulopathy as the reason for bleeding.

Von Willebrand disease is not strictly a platelet dysfunction, but it is often considered in the differential diagnosis of bleeding disorders with long bleeding times or abnormal platelet function screening test results.¹⁴ Patients with von Willebrand disease will usually have a family history of a bleeding diathesis and present with muco-

cutaneous bleeding. The underlying pathologic features of von Willebrand disease are due to decreased levels or defective function of VWF. Von Willebrand disease is protean in manifestation, but the various types have been grouped into 3 general categories (types 1, 2, and 3). Patients with von Willebrand disease may have decreased VWF antigen, decreased ristocetin cofactor activity, decreased ristocetin aggregation, decreased factor VIII levels, normal or increased APTT, and increased bleeding times with normal platelet counts.¹⁴

Newer Methods of Platelet Evaluation

New assay systems to assess platelet function have recently become clinically available.⁴² These include the PFA-100, the Ultegra (Accumetrics, San Diego, Calif), and the Plateletworks (Helena, Beaumont, Tex). Most of these devices are small, stand-alone devices that can be used at the patient's bedside or in laboratories that otherwise could not perform platelet function studies.

The PFA-100 is a device that measures platelet-related primary hemostasis in a citrated whole blood specimen.³⁴ It uses 2 disposable cartridges that contain a membrane with a central aperture (147 μm) coated with aggregation agonists (collagen and epinephrine and collagen and ADP), through which platelets are passed at high shear rates (5000–6000 s^{-1}). The instrument measures the "closure time" required for platelets to adhere to the membrane, aggregate, and occlude the aperture. The collagen-epinephrine cartridge is the primary screening cartridge; it detects platelet dysfunction induced by intrinsic platelet defects, von Willebrand disease, or platelet-inhibiting agents. The collagen-ADP cartridge usually produces abnormal results with platelet disorders and von Willebrand disease, but produces a normal closure time with aspirin-like drugs because of the high ADP concentration. Von Willebrand disease, intrinsic platelet dysfunction, and nonaspirin drugs may produce an abnormal closure time with both cartridges.³⁴ Samples collected for analysis on the PFA-100 are stable for up to 5 hours. The PFA-100 results can be affected by low platelet counts and low hematocrits, but are not affected by heparin.⁴³

The Ultegra, a rapid platelet function assay, is an automated turbidimetric whole blood assay designed to assess platelet aggregation based on the ability of activated platelets to bind fibrinogen.⁴⁴ Fibrinogen-coated polystyrene microparticles agglutinate in whole blood in proportion to the number of available platelet glycoprotein IIb/IIIa receptors.⁴⁴ This test uses a whole blood specimen and can be used at the patient's bedside without highly trained laboratory personnel. Because of the use of agonist-activated platelets and fibrinogen-coated microparticles, the Ultegra is designed to measure specifically the effect of glycoprotein IIb/IIIa antagonist drugs, such as abciximab, tirofiban, or eptifibatide, and may be of use to monitor the effect of these potent antiplatelet drugs in the cardiac catheterization laboratory or intensive care unit.^{42,45,46} It is not sensitive to drugs such as aspirin or the thienopyridines (clopidogrel and ticlopidine), and it is not designed to detect platelet functional disorders or von Willebrand disease.

Recently, a rapid platelet aggregometer has become available (Plateletworks), which is designed to determine the percentage of platelet aggregation in fresh whole blood samples taken during interventional cardiac procedures. It measures the change in the platelet count due to

the aggregation of functional platelets in the blood sample. This is the first bedside test to simultaneously measure both platelet count and platelet aggregation.

An experimental device, a dynamic clot retractometer (Hemodyne), measures platelet force development by exerting a tensile force on a platelet clot.⁴⁷ The device is based on the principle of clot retraction and measures both the tensile properties of the platelet cytoskeleton and the integrity of the glycoprotein IIb/IIa receptor—fibrinogen linkage. Decreased platelet force development has been detected with this device in uremia and with glycoprotein IIb/IIIa antagonists,⁴⁸ and increased force development has been detected in patients with coronary artery disease.

Flow cytometry has been used to study platelet structure and function, but this technique is used only in specialized centers. Flow cytometric analysis is based on the detection of cell surface proteins with fluorescently labeled antibodies. It has been used in the detection of platelet activation by using antibodies to proteins newly expressed on the platelet surface during activation, such as P-selectin or thrombospondin, or by detecting new epitopes on glycoprotein IIb/IIIa induced by binding fibrinogen (ligand-induced binding sites).⁴⁹ Flow cytometric evaluation of P-selectin (CD62P) expression has been used to distinguish heparin-induced thrombocytopenia (HIT) from HIT with thrombosis, which may allow for early intervention for prevention of thrombotic complications.⁵⁰ Flow cytometry, using fluorescently labeled abciximab, can be used to determine the number of inhibited glycoprotein IIb/IIIa receptors after the infusion of glycoprotein IIb/IIIa inhibitors.⁵¹ It can also measure platelet activation, which may correlate with thrombotic risk in certain clinical situations.⁵² Platelet flow cytometry can be used to diagnose deficiencies of platelet surface glycoproteins. It has been used to detect the absence of glycoprotein IIb/IIIa receptors in patients with Glanzmann thrombasthenia and has been used to study deficiencies of glycoprotein Ia, Ib, IIb, IV, and IX.⁵³ Flow cytometric methods have also been used to measure dense granules (mepacrine uptake or release), aggregation, microparticle formation, and platelet procoagulant activity.⁵⁴

Another use of flow cytometry is the detection of platelet autoantibodies in patients with idiopathic thrombocytopenic purpura and drug-induced thrombocytopenias, which is sensitive but not specific.⁵⁵ This test can be made more specific for drug-induced antibodies by incubating the platelets in the presence of the drugs in question or by using activation-dependent tests, such as ⁵¹Cr release⁵⁶ or ¹⁴C serotonin release.⁵⁷ Antigen capture assays, such as monoclonal antibody immobilization of platelet antigens (MAIPA), have improved specificity further by being able to detect antibody binding to specific platelet surface glycoproteins.⁵⁸

Platelets with increased RNA content (reticulated platelets) can be measured by flow cytometry using the dye thiazole orange, which binds to RNA and DNA.^{23,59} This technique is gaining acceptance as a diagnostic tool to evaluate whether thrombocytopenia is due to increased platelet destruction or decreased platelet production, since platelets newly released from bone marrow have increased RNA content. This assay has recently been automated on the Cell Dyn instruments (Abbott, Abbott Park, Ill). It is anticipated that implementation of reticulated

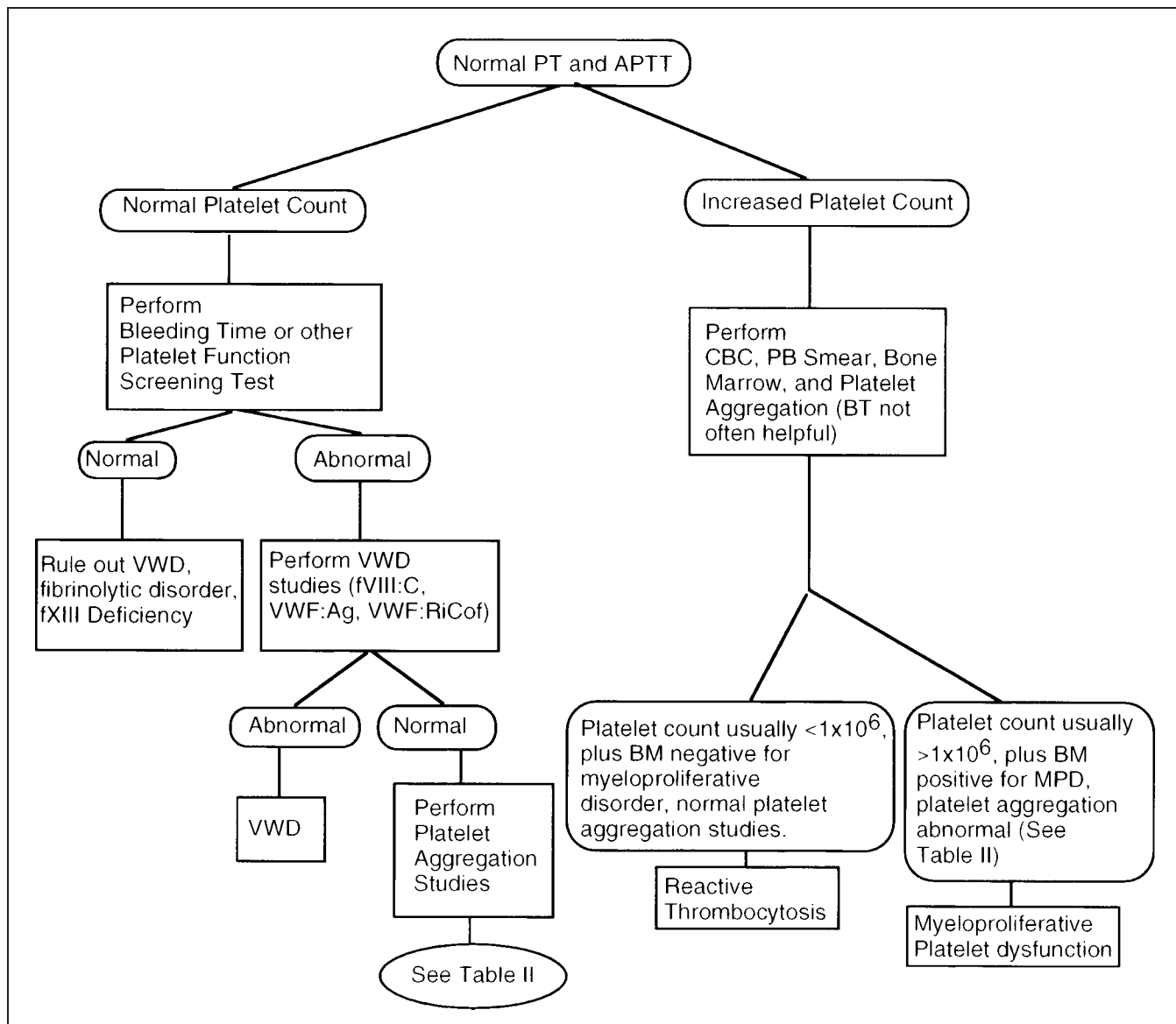


Figure 2. Algorithm for evaluation of a platelet dysfunction that leads to a bleeding disorder with a normal or elevated platelet count. In a patient with a normal platelet count and a normal prothrombin time (PT) and activated partial thromboplastin time (APTT), the algorithm indicates to start with a platelet function screening test. If abnormal, von Willebrand disease (VWD) should be excluded (using factor VIII:C, von Willebrand factor antigen [VWF:Ag], and ristocetin cofactor [VWF:RiCof] assays), followed by platelet aggregation studies. If the platelet count is increased, a reactive thrombocytosis should be distinguished from a myeloproliferative disorder (MPD), based on complete blood cell count (CBC), peripheral blood (PB) smear, bone marrow (BM) evaluation, and platelet aggregation.

platelet counts may help to avoid bone marrow examination in some individuals with thrombocytopenia.

Electron microscopy may be used for the ultrastructural evaluation of platelets, particularly in patients with suspected SPDs, showing a decrease or absence of the organelles (cytoplasmic dense granules) that store adenine nucleotides, serotonin, and calcium.²⁸ Giant platelet disorders also have characteristic electron microscopic findings.^{28,60} Other specialized methods of platelet evaluation, such as crossed immunoelectrophoresis, will be discussed in the following section with the individual disorders for which they are useful.

DIAGNOSTIC CATEGORIES OF PLATELET-DERIVED BLEEDING DIATHESIS

Platelet disorders may be divided into 3 major categories: platelet dysfunction associated with *normal*, *decreased*,

or *increased* platelet counts, as shown in the algorithms in Figures 2, 3, and 5. In all of the disorders discussed herein, the results of the coagulation screening tests PT and APTT should be considered normal.

Platelet Dysfunction With Normal Platelet Count

Platelet dysfunction with a normal platelet count usually indicates a qualitative platelet disorder. In following the algorithm in Figure 2, these disorders would be evaluated in a patient with a normal PT, APTT, and platelet count. The results of a platelet function screening test would be abnormal and test results for von Willebrand disease would be normal. Platelet aggregation studies would then be used to distinguish the following disorders, followed by more specific tests, if required. Most drug-induced platelet dysfunction will also demonstrate platelet

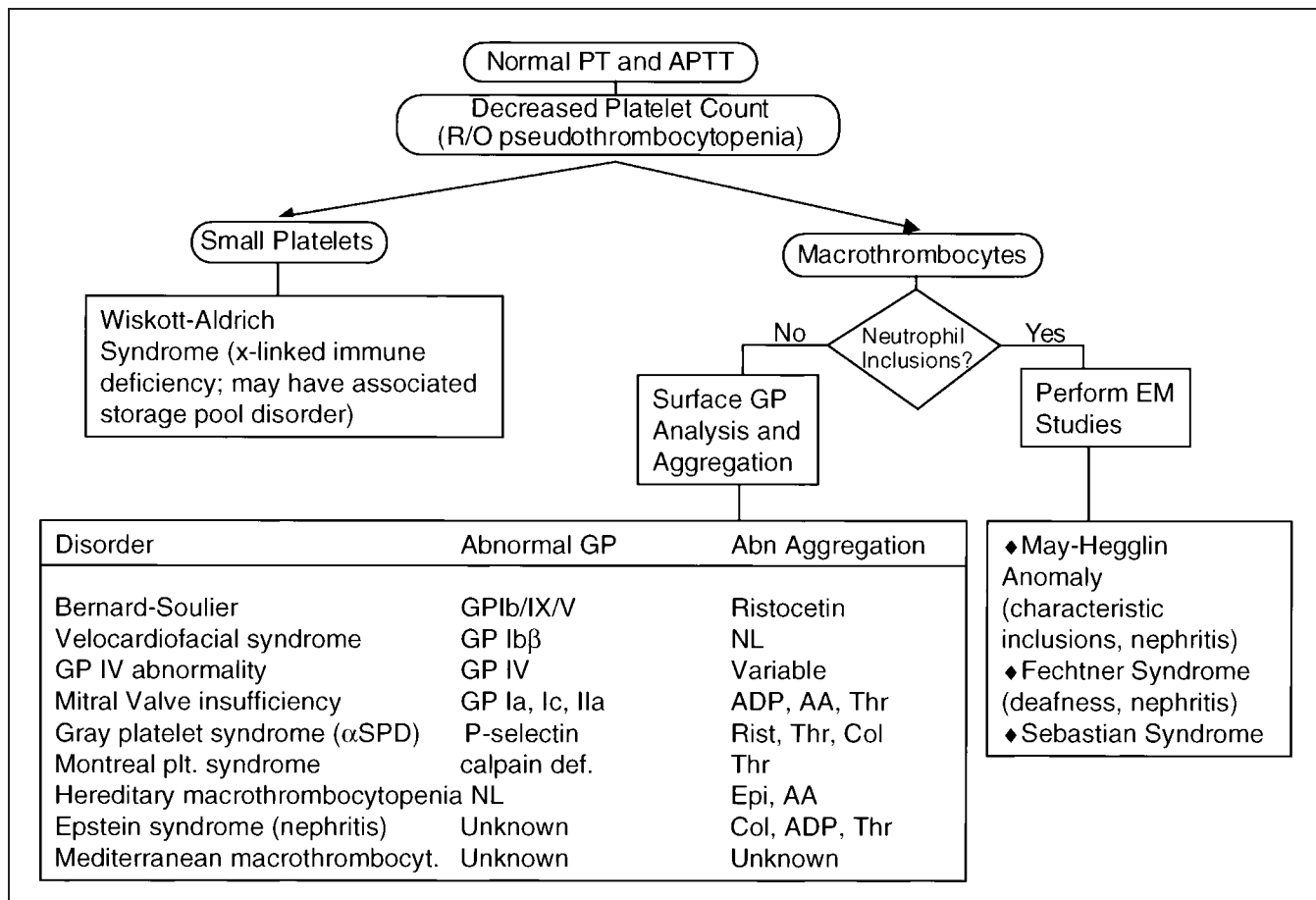


Figure 3. Algorithm for evaluation of a platelet dysfunction that leads to a bleeding disorder in a patient with a decreased platelet count, but either small or large platelets. Small platelets and immune deficiency may suggest Wiskott-Aldrich syndrome. Large platelets would suggest a macrothrombocytopenia syndrome. Neutrophilic inclusions should prompt electron microscopic (EM) studies, which can be used to differentiate May-Hegglin anomaly, Fechtner syndrome, and Sebastian syndrome. Lack of neutrophilic inclusions would prompt surface platelet glycoprotein (GP) analysis and platelet aggregation studies, which will distinguish the disorders in the table at the bottom left of the figure. Abn indicates abnormal; NL, normal; ADP, adenosine diphosphate; AA, arachidonic acid; Thr, thrombin; Rist, ristocetin; Col, collagen; Epi, epinephrine; and def, deficiency.

dysfunction with a normal platelet count, so it is extremely important to take a careful drug history.¹⁶ A list of drugs that cause platelet dysfunction can be found in Table 1. Platelet aggregation abnormalities typically found with drugs such as aspirin, glycoprotein IIb/IIIa antagonists, or the thienopyridines can be found in Table 2.

Glanzmann thrombasthenia is a congenital deficiency or dysfunction of glycoprotein IIb/IIIa, the receptor for fibrinogen responsible for mediating platelet aggregation.^{61,62} It is an autosomal recessive disorder that manifests in lifelong mucocutaneous bleeding. Glanzmann thrombasthenia can be classified according to the amount of glycoprotein IIb/IIIa: type I, 0% to 5% of normal; type II, 6% to 20% of normal; and variant disease, 50% to 100% of normal with abnormal fibrinogen binding.⁶³ Mutations of both glycoprotein IIb and glycoprotein IIIa have been implicated.⁶³ In patients with Glanzmann thrombasthenia, the bleeding time or platelet function screening test results will be abnormal. No aggregation response will be seen on addition of ADP, collagen, epinephrine, and arachidonic acid–aggregating agents, whereas the ristocetin-induced aggregation is normal.⁴⁰ This finding is virtually diagnostic of Glanzmann thrombasthenia, but the disorder can be confirmed by platelet flow cytometry or crossed immu-

noelectrophoresis of platelet membrane proteins (Table 2).⁶⁴ Afibrinogenemia, a rare deficiency of fibrinogen, can present with similar initial platelet aggregation results, but the aggregation defect in afibrinogenemia is restored with addition of fibrinogen to the specimen. Additional laboratory studies in patients with Glanzmann thrombasthenia will show decreased platelet-associated fibrinogen, defective fibrinogen binding to platelets, and decreased clot retraction.⁶⁵

Bernard-Soulier disease is a congenital deficiency of the platelet glycoprotein Ib α /Ib β /IX/V receptor, the surface receptor for VWF-mediated platelet aggregation.⁶⁶ The disorder is inherited as an incompletely recessive autosomal trait with severe bleeding. Many patients with Bernard-Soulier disease have moderately severe thrombocytopenia with large platelets, and this disorder is included with the macrothrombocytopenia syndromes discussed herein. Most of the Bernard-Soulier genetic defects are due to mutations of the *GPIb α* gene, but may also be due to defects of the *GPIb β* or *GPIX* genes.^{67,68} Glycoprotein Ib is expressed on the demarcation membrane system in the megakaryocytes that is responsible for platelet fragmentation, so it is postulated that glycoprotein Ib plays a role during megakaryopoiesis and maintenance of platelet

Table 2. Aggregation Characteristics*

Disorder	ADP		AA	EPI	Collagen	Ristocetin	Other Studies
	Primary	Secondary					
VWD	N	N	N	N	N	↓, N or ↑	Factor VIII:C, VWF antigen, VWF ristocetin cofactor, VWF multimers
Glanzmann thrombasthenia	↓ or Abs	↓ or Abs	↓ or Abs	↓ or Abs	↓ or Abs	N	Deficiency of GP IIb and/or GP IIIa by flow cytometry
Bernard-soulier syndrome	N	N	N	N	N	↓ or Abs	Macrothrombocytopenia Deficiency of GP Ib/IX/V (one or more) by flow cytometry
Dense granule platelet storage pool disorder (δ-SPD)	N	↓	N	N or ↓	N or ↓	N	Decreased ATP release by lumiaggregometry ↓ Dense granule mepacrine uptake and release by flow cytometry Decreased dense granules by TEM Abnormally high ATP/ADP ratio Acquired SPD "exhausted platelets" (CPB, DIC, TTP, HUS, MPD)
α-SPD	Var	Var	N	N	Var ↓	N	Albinism in Hermansky-Pudlak and Chediak-Higashi Infections, small platelets seen with Wiskott-Aldrich Pale platelets on smear, ↓ alpha granules by TEM, ↓P-selectin
Signal transduction disorders	N	↓	Var ↓	Var ↓	Var ↓	N	Decreased granule release with normal number of granules Receptor defects may show ↓ aggregation to EPI, collagen only ↓G-protein activation, phospholipase C activation, calcium mobilization, pleckstrin, or tyrosine phosphorylation
Aspirin-like drug or defects of thromboxane synthesis	N	↓↓	↓ or Abs	↓	↓ or Abs	N	Normal aggregation with prostaglandin G ₂ seen with aspirin or cyclooxygenase deficiency Decreased or absent prostaglandin G ₂ aggregation with thromboxane synthetase deficiency
Myeloproliferative disorder	N	N	N	↓ or Abs delayed lag	N	N	Other abnormalities: α or δ-SPD, cyclooxygenase abnormality, other surface GP derangements, ↓ or ↑ aggregation to ADP, collagen, spontaneous aggregation
Scott syndrome	N	N	N	N	N	N	Defective platelet procoagulant activity (ie, PF3) Defective microparticle formation
Uremia	N	↓	↓	N/↓	N/↓	N	Abnormal creatinine, BUN Decreased PF3
Thienopyridines (ticlopidine and clopidogrel)	↓	Abs	N	N	N	N	History of clopidogrel or ticlopidine therapy
GP IIb/IIIa antagonists	↓ or Abs	↓ or Abs	↓ or Abs	↓ or Abs	↓ or Abs	N	History of treatment; abciximab, tirofiban, or eptifibatide Increased receptor occupancy by flow cytometry

* ADP indicates adenosine diphosphate; AA, arachidonic acid; EPI, epinephrine; VWD, von Willebrand disease; Abs, absent; GP, glycoprotein; ATP, adenosine triphosphate; TEM, transmission electron microscopy; CPB, cardiopulmonary bypass; DIC, disseminated intravascular coagulation; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; MPD, myeloproliferative disease; Var, variable response; PF3, platelet factor 3; and BUN, blood urea nitrogen. Up and down arrows indicate increased and decreased, respectively.

size.¹⁷ Normal platelet aggregation is noted with exposure to ADP, collagen, epinephrine, and arachidonic acid, but aggregation is absent with the addition of ristocetin.⁶⁵ Adhesion of platelets to subendothelium or immobilized VWF is markedly reduced at all shear rates in patients with Bernard-Soulier syndrome; this finding may have direct clinical consequences.⁶⁹ The glycoprotein abnormality can be confirmed with flow cytometry or crossed immunoelectrophoresis.⁷⁰ Additional laboratory studies show normal VWF antigen and ristocetin cofactor activity to distinguish Bernard-Soulier syndrome from von Willebrand disease.

Abnormalities of platelet secretion can be due to either deficiency of platelet granules or defects in the signal transduction events that regulate secretion or aggregation.⁷¹ Platelet SPDs can be congenital or acquired and are the result of either a deficiency of granules (alpha and/or dense granules) or defective granule release on platelet activation.⁷² Dense granule SPDs (δ -SPDs) can be seen as a singular clinical entity or as part of other hereditary disorders, such as Chediak-Higashi, Hermansky-Pudlak syndrome, thrombocytopenia-absent radius syndrome, or Wiskott-Aldrich syndrome.⁷²⁻⁷⁴ Often δ -SPD shows decreased aggregation response to ADP, epinephrine, and collagen with normal aggregation to arachidonic acid and ristocetin. Decreased adenosine triphosphate release by lumiaggregometry and decreased mepacrine uptake or release by flow cytometry are observed. Ultrastructural abnormalities in these disorders usually show decreased dense granules. In addition, α -SPD (Gray platelet syndrome) has decreased alpha granules and is usually considered a macrothrombocytopenia.⁷⁵ A rare α/δ -SPD has been described that has features of both disorders.⁷² Acquired platelet storage pool disorders can be seen with underlying myeloproliferative disorders in which the platelet degranulation is defective as a result of the disease. Circulating "exhausted" platelets simulating SPDs can be observed in clinical scenarios where there is ongoing in vivo platelet activation, such as cardiopulmonary bypass, disseminated intravascular coagulation, and thrombotic thrombocytopenic purpura or hemolytic uremic syndrome.

In addition to the SPDs, platelet release defects can be seen with defects of platelet signal transduction. In general, these disorders are poorly defined, but may constitute a significant percentage of patients with abnormal secondary wave of aggregation and decreased granule release, in whom alpha and dense granules are not deficient.⁷¹ Defects of the platelet receptors for thromboxane A₂, collagen, ADP, and epinephrine are included in this category.⁷⁶⁻⁷⁸ Defects of the collagen and epinephrine receptors can be distinguished because usually they demonstrate a selective defect in aggregation to a single agonist.^{76,77} These disorders can be confirmed by flow cytometry, where a deficiency of a surface glycoprotein is identified. Defects of the signaling pathways, including G protein activation, phospholipase C activation, calcium mobilization, pleckstrin phosphorylation, and tyrosine phosphorylation, have also been described.⁷¹ In general, these patients show decreased primary aggregation and decreased granule release without granule deficiency. Identification of the exact defect requires detailed biochemical studies, which are not available in most laboratories. Defects of thromboxane A₂ synthesis have been described, including defective liberation of arachidonic acid

from the platelet membrane, cyclooxygenase enzyme deficiency, or thromboxane synthase deficiency.⁷⁹ These individuals will display an aspirin-like defect in aggregation despite having never used aspirin therapy.

Platelets play an important procoagulant role with assembly of coagulation complexes on activated platelet membranes that are rich in phosphatidyl serine. A rare congenital platelet functional disorder is Scott syndrome, due to defective "flip" of phosphatidyl serine to the outer table of the platelet membrane.⁸⁰ These patients will have normal platelet aggregation studies, but have abnormal platelet procoagulant activity (platelet factor 3) and micro-particle formation.

Other significant disorders of platelet function with platelet counts in the normal range are usually acquired with the presence of another disease or drug therapy. These are, by far, more common than the disorders described herein. Platelet dysfunction is often observed with chronic renal failure or liver disease, in patients experiencing a variety of myeloproliferative and lymphoproliferative disorders (ie, polycythemia vera, myelofibrosis, paroxysmal nocturnal hemoglobinuria, acute myelogenous leukemia, and hairy cell leukemia). Platelet dysfunction also may be associated with a variety of clinical scenarios, such as previous cardiopulmonary bypass, implantation of prosthetic materials such as vascular grafts and prosthetic heart valves, and ventricular assistance devices.^{81,82} Platelet dysfunction in these disorders is usually difficult to characterize because nonspecific defects of platelet aggregation are usually observed.

Platelet Disorders With Thrombocytosis

Patients with elevated platelet counts may have clinical bleeding, but may also be asymptomatic or have thrombosis. In these patients, laboratory evaluation should be primarily aimed at elucidating the cause of the thrombocytosis and should include a complete blood cell count, peripheral blood smear, bone marrow evaluation, cytogenetic study, and platelet aggregation study. In general, platelet function screening tests have little usefulness in evaluating these disorders and do not necessarily correlate with further platelet function tests. In patients with thrombocytosis, the differential diagnosis is primarily between a reactive thrombocytosis and a myeloproliferative process (essential thrombocytosis, chronic myelogenous leukemia, polycythemia vera, and myelofibrosis). The algorithmic approach to the diagnosis of thrombocytosis is shown in Figure 2. In general, patients with a myeloproliferative disorder often have platelet counts greater than $1 \times 10^6/\mu\text{L}$, and patients with reactive thrombocytoses have counts less than this, but there is a great deal of overlap. For myeloproliferative disorders, characteristic features of a specific disease can be discerned by examination of the peripheral blood smear, bone marrow, and cytogenetic studies.

Platelet aggregation studies alone can suggest an underlying myeloproliferative disorder, particularly when epinephrine-induced aggregation alone is reduced or absent.^{83,84} The decreased epinephrine-induced aggregation is thought to be due to down-regulation of α_2 -adrenergic receptors.⁸³ This pattern of platelet aggregation is also observed in patients with a congenital defect of the α_2 -adrenergic receptors,⁷⁶ but these patients usually have a normal platelet count. Other patterns of platelet dysfunction with myeloproliferative disorders include decreased

platelet aggregation to ADP or collagen, dense-granule storage pool pattern, abnormal platelet morphologic structure, abnormalities of the arachidonic acid pathway, and decreased receptors for prostaglandin D₂.^{83,85} Additionally, some patients may show increased aggregation with various agonists or may have spontaneous aggregation without added agonists.⁸⁶ In the clinical evaluation of patients with myeloproliferative disorders, both bleeding and thrombosis can be observed in these patients, and the results of the platelet functional tests will not necessarily distinguish whether the patient is at risk for bleeding or thrombosis.⁸⁷

In contrast to patients with myeloproliferative disorders, patients with reactive thrombocytosis usually have normal platelet function. A reactive, or secondary, thrombocytosis can be associated with many clinical entities, such as iron deficiency, inflammatory and infectious disorders after splenectomy in malignancies such as carcinomas or lymphomas, myelodysplastic disorders, smoking, or exercise. It can also be observed as a rebound thrombocytosis following splenectomy, treatment for idiopathic thrombocytopenic purpura, pernicious anemia, or cessation of myelosuppressive drugs.

Platelet Disorders With Thrombocytopenia

Disorders in which the platelet count is *decreased* can be divided, for evaluation purposes, by the size of the platelets. Thrombocytopenias can be congenital or acquired, but they have been grouped by platelet size in this discussion. See Figure 3 for an algorithmic approach to small and large platelets and Figure 5 for an approach to the diagnosis of normal-sized platelets.

Thrombocytopenia with small platelets can be seen in patients with Wiskott-Aldrich syndrome. This is an X-linked recessive disorder characterized by recurrent infections, eczema, and thrombocytopenia. These individuals will have absent immunologic responses to polysaccharide antigens and progressive decline in T-lymphocyte function. The MPV is often low (approximately half the normal size) and lymphocytes are deficient in CD43 (sialophorin).⁸⁸ Platelet dysfunction is severe; the platelets are unable to aggregate and a storage pool-like pattern is often seen.⁸⁹ Patients with thrombocytopenia due to marrow aplasia may also have small platelets, but the MPV is usually low to normal not decreased.

The rare macrothrombocytopenia disorders are all congenital in nature and most are inherited in an autosomal dominant fashion. They are usually due to congenital defects in platelet production by megakaryocyte or demarcation membrane systems, although the structural or genetic abnormalities are known in only a few disorders (Figure 3).¹⁷ Some patients with acquired platelet destruction and turnover, such as idiopathic thrombocytopenic purpura, may have high MPVs due to the rapid release of new platelets, but, in general, the macrothrombocytopenia syndrome platelets are much larger and more uniform in size.

Several macrothrombocytopenia disorders are characterized by the presence of neutrophilic inclusions. May-Hegglin anomaly is the most common macrothrombocytopenia and is an autosomal dominant disorder characterized by Dohle body inclusions in neutrophils with a mild bleeding disorder.^{17,90} The Dohle bodies are blue, spindle-shaped inclusions in the periphery of the neutrophil cytoplasm (Figure 4). The thrombocytopenia is usu-

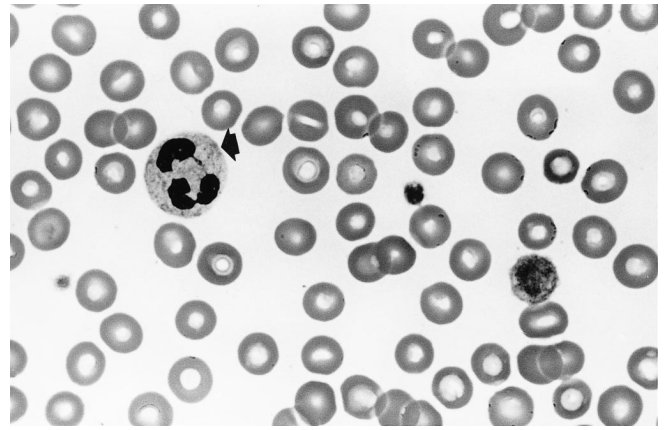


Figure 4. Photomicrograph of a peripheral blood smear from a patient with May-Hegglin anomaly. The platelets are uniformly increased in size and some are as large as erythrocytes. The neutrophil has basophilic cytoplasmic inclusions (arrow) (Wright stain, original magnification $\times 100$).

ally moderate, with platelet counts of 60 to 100 $\times 10^3/\mu\text{L}$, and the mean MPV is approximately 12.5 fL, but is often much larger. Laboratory studies will usually show normal platelet aggregation and a normal bleeding time,⁹¹ attesting to the increased functionality of the larger platelets. Electron microscopic analysis of platelets in the May-Hegglin anomaly will often show a disorganization of the microtubules.^{17,91} Electron microscopic analysis of the neutrophilic inclusions shows them to lack a limiting membrane, be free of specific granules, and contain parallel bundles of ribosomes, microfilaments, and segments of endoplasmic reticulum.¹⁷ Platelet surface glycoproteins are usually normal.⁹²

The 2 other macrothrombocytopenia disorders with neutrophilic inclusion are Fechtner syndrome and Sebastian syndrome. Fechtner syndrome can be distinguished by hereditary nephritis, deafness, cataracts (Alport syndrome), and macrothrombocytopenia with a mild-to-moderate bleeding disorder.⁶⁰ The MPV may be as large as 20 fL, and the peripheral smear shows uniformly giant platelets with pale blue, irregularly shaped inclusions in the neutrophil cytoplasm.¹⁷ Platelet aggregation studies and platelet surface glycoprotein studies are normal. Sebastian syndrome⁹³ has no clinical associations like Fechtner syndrome.

There are several rare macrothrombocytopenia syndromes without neutrophil inclusions, which are generally characterized by either surface glycoprotein abnormalities or platelet functional defects. Bernard-Soulier syndrome lacks glycoprotein Ib/IX/V on platelet surfaces, as has been discussed herein. Patients who are *heterozygous* for the disease will show only giant platelets on the blood smear without hypoplatelet function, thrombocytopenia, or bleeding. These heterozygous patients may have associated velopharyngeal insufficiency, conotruncal heart disease, and learning disabilities together with an abnormality of glycoprotein Ib β , and are classified as having the velocardiofacial syndrome.⁹⁴ The Gray platelet syndrome is an autosomal dominant α -SPD characterized by mild bleeding symptoms, reticulin fibrosis of the bone marrow, variable thrombocytopenia, and large (mean, 13 fL), gray-appearing platelets on the peripheral blood smear due to decreased alpha granules.⁷⁵ Pale platelets can also be seen

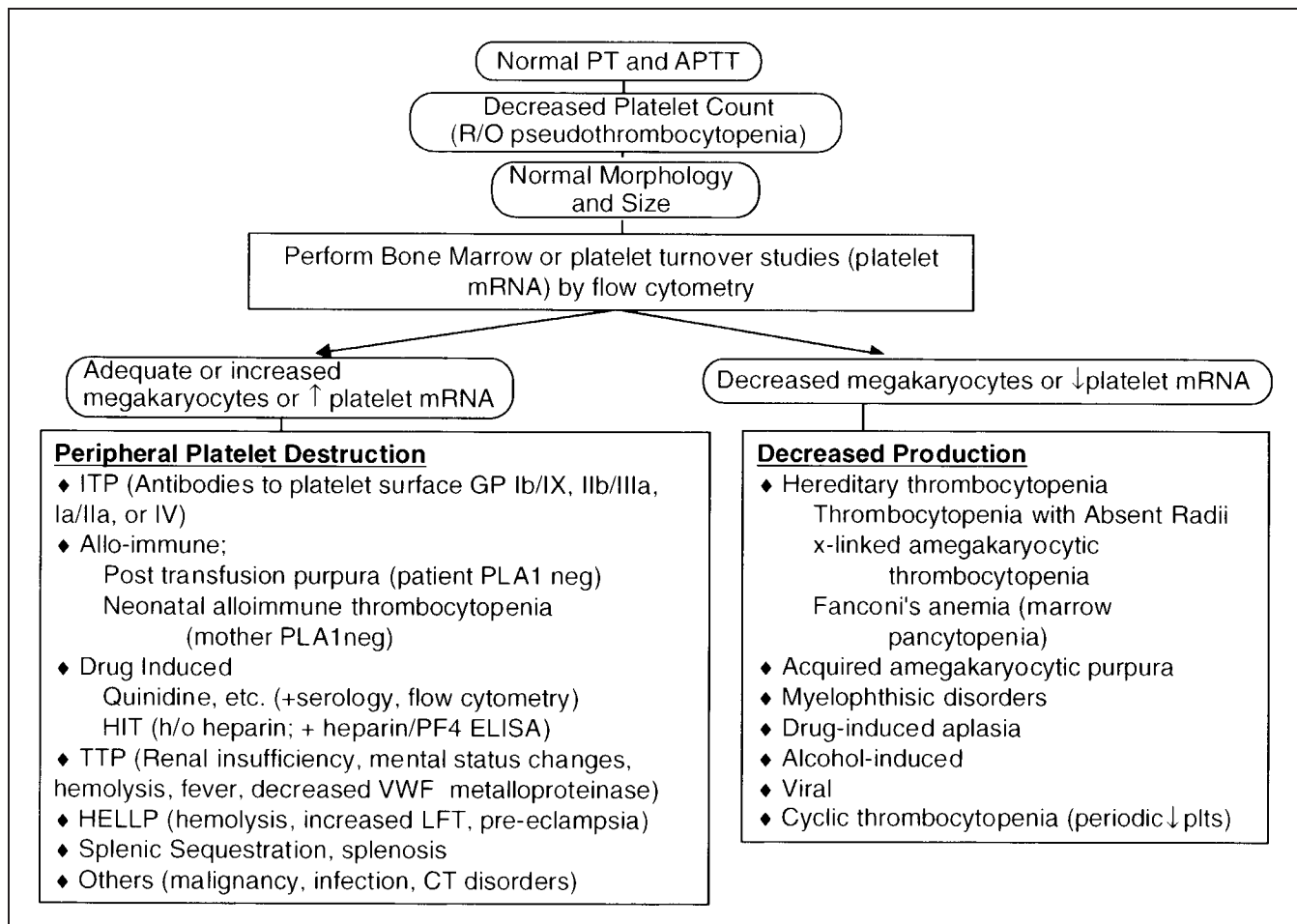


Figure 5. Algorithm for evaluation of a platelet dysfunction that leads to a bleeding disorder in a patient with a decreased platelet count and platelets of normal size and morphologic structure. In patients meeting this criteria, the differential diagnosis is between peripheral platelet destruction and decreased production. A bone marrow evaluation is useful in distinguishing the diagnostic possibilities. Platelet turnover studies, such as evaluation of platelet messenger RNA by flow cytometry, may be useful before a bone marrow examination to suggest peripheral platelet destruction. PT indicates prothrombin time; APTT, activated partial thromboplastin time; R/O, rule out; ITP, idiopathic thrombocytopenic purpura; GP, glycoprotein; HIT, heparin-induced thrombocytopenia; PF4, platelet factor 4; ELISA, enzyme-linked immunosorbent assay; TTP, thrombotic thrombocytopenic purpura; HELLP, hemolysis elevated liver enzymes and low platelets; LFT, liver function tests; and CT, connective tissue.

with ongoing platelet activation and circulating "exhausted" platelets, but in these patients there will be a mixture of normal and pale platelets. It is unclear whether the pathophysiologic origin of the Gray platelet syndrome is due to premature release of alpha granules from the cell or abnormal signal transduction or calcium flux.¹⁷ Platelet aggregation study results may be abnormal for thrombin and collagen, and flow cytometry studies have shown increased surface P-selectin, but decreased alpha granule P-selectin.⁷⁵ Other rare macrothrombocytopenias are listed in Figure 3. Those that have known surface glycoprotein abnormalities include the glycoprotein IV abnormality and mitral valve insufficiency with abnormalities of glycoproteins Ia, Ic, and IIa.¹⁷

Bone marrow examination may be helpful in differentiating the underlying causes in thrombocytopenic platelet disorders with normal platelet morphologic structure and size. This group of disorders includes both congenital and acquired thrombocytopenias that are usually due to either decreased platelet production or increased platelet destruction (Figure 5). The number of megakaryocytes on the bone marrow can help to distinguish between these

origins, but analysis of platelet turnover by messenger RNA analysis may also be helpful.

The finding of adequate or increased megakaryocytes on the bone marrow or increased reticulated platelets suggests peripheral platelet destruction. Platelet functional tests are usually not helpful in differentiating between the entities in this class of disorders, since most functional studies will give abnormal results simply because of the low platelet number. The overall MPV is usually normal with destructive thrombocytopenia, but there is usually a range of platelet size and many large platelets are seen, indicating the rapid platelet turnover. These disorders are invariably acquired and an underlying abnormality should be sought. In general, the clinical scenario is the most helpful in classifying these disorders.

Idiopathic thrombocytopenic purpura is known to be due to platelet sensitization, with autoantibodies leading to platelet destruction in the reticuloendothelial system; peripheral smears may show variable macrothrombocytopenia and autoantibodies to specific surface glycoproteins can be detected by flow cytometry or immunoassay,⁹⁵ although diagnosis is largely from clinical findings. An-

other immune thrombocytopenia is posttransfusion purpura, where immune destruction of both transfused and recipient platelets is seen, usually beginning 5 to 12 days after transfusion. Patients often have abnormal platelet antigens and are frequently PL^{A1} negative (PL^{A2} homozygous or HPA-1b).⁹⁶ Anti-PL^{A1} antibodies can be detected both in plasma and attached to the platelet surface. Neonatal alloimmune thrombocytopenia occurs when the mother lacks a platelet antigen present on the neonate's platelets. Maternal antibodies cross the placenta and cause severe thrombocytopenia shortly after birth. Several platelet antigen systems may be responsible for this scenario, but often the mother is PL^{A1} negative (PL^{A2} homozygote).⁹⁷ Interestingly, this same PL^{A2} genetic polymorphism may impart a risk for coronary artery disease.⁹⁸

The thrombocytopenia of thrombotic thrombocytopenic purpura is thought to be secondary to deficiency of a VWF-cleaving metalloproteinase in many patients, leading to diffuse thrombus formation in small vessels and a decline of circulating platelets.⁹⁹ These patients will show characteristic clinical symptoms with renal failure, mental status changes, fever, and hemolysis with prominent schistocytes on the peripheral blood smear, but normal screening coagulation test results.¹⁰⁰ Thrombotic thrombocytopenic purpura has also been associated with Shiga toxin-producing strains of *Escherichia coli* and with drugs such as cyclosporine, quinine, ticlopidine, and clopidogrel.¹⁰⁰⁻¹⁰³ An assay for the VWF-cleaving metalloproteinase is now available, but because it is based on the VWF multimer assay, is not readily available in most laboratories.⁹⁹

Drug-induced thrombocytopenias due to immunologic platelet destruction can be seen with many drugs, but the most common offenders are quinidine, quinine, heparin, sulfonamide drugs, and gold salts.¹⁰⁴ This syndrome has also been described with the glycoprotein IIb/IIIa antagonist abciximab, but because this drug is based on a monoclonal antibody to the fibrinogen receptor (Fab' fragment), the thrombocytopenia may not be alloimmune in origin.¹⁰⁵ Drug-induced thrombocytopenias can be diagnosed by detecting the presence of platelet-associated antibody by flow cytometry, although this is a nonspecific finding that can also be seen with infections and autoimmune disorders. The drug dependence of the antibody binding can be demonstrated by incubating platelets with patient plasma in the presence of the drug.⁵⁵

Heparin-induced thrombocytopenia is a distinctive drug-induced thrombocytopenia associated with heparin therapy, where antibodies are formed to heparin-platelet factor 4 complexes, leading to platelet aggregation, platelet microparticle formation, endothelial injury, and paradoxical thrombosis.¹⁰⁶ The thrombotic complications can be either venous or arterial and may cause death, limb loss, and pulmonary thrombosis. Bleeding secondary to thrombocytopenia is rare in HIT. The thrombocytopenia is often delayed 5 to 12 days after starting heparin therapy and usually resolves after heparin therapy is stopped. Specific laboratory tests, such as heparin-induced platelet aggregation, serotonin release, and heparin-platelet factor 4 enzyme-linked immunosorbent assay, are available to positively diagnose this disorder.^{106,107}

Thrombocytopenias due to decreased platelet production include both rare congenital and more common acquired causes (Figure 3). Congenital thrombocytopenias due to decreased megakaryocytes with normal platelet size include thrombocytopenia-absent radius syndrome,

X-linked megakaryocytic thrombocytopenia, and Fanconi anemia. This last disorder is distinguished by erythroid hypoplasia and DNA instability. Acquired megakaryocytic hypoplasia is seen with myelophthistic disorders, viral illnesses, and drug-induced hypoplasia.¹⁰⁴

ACTIVATED PLATELET SYNDROMES

Some patients with myeloproliferative disorders will show evidence of activated platelets or hyperplatelet function. Although most patients with essential thrombocythemia and polycythemia vera will display hypoaggregation, some will actually show an increased response to various agonists, *excluding* epinephrine.⁸⁵ Some of these patients will also exhibit spontaneous platelet aggregation *in vitro*. *In vitro* hypoplatelet or hyperplatelet function in patients with myeloproliferative disorders does not necessarily correlate with clinical symptoms of bleeding or thrombosis.

The sticky platelet syndrome is a poorly defined disorder associated with arterial and venous thromboembolic events characterized by hyperaggregability of platelets on exposure to ADP and epinephrine. The molecular mechanism of this disorder is not known, but it has been suggested that emotional stress may be a precipitating factor for systemic thrombosis.¹⁰⁸

Platelet activation is known to occur in patients with concomitant cardiovascular disease,¹⁰⁹ hypertension,¹¹⁰ and diabetes,¹¹¹ most likely due to platelet activation secondary to vascular injury. Additionally, abnormal glycation is thought to contribute to abnormalities of platelet function in diabetic patients.¹¹² Older patients with hypertension are found to have a higher prevalence of thromboembolic tendencies associated with platelet hyperactivity.¹¹³ Genetic polymorphisms of the glycoprotein IIIa platelet membrane receptor gene (the PL^{A2} genotype) may predispose patients with this allele to a higher risk of acute coronary and cerebrovascular events, although the mechanism for the thrombotic tendency is uncertain.⁹⁸ Additional genetic polymorphisms of other platelet surface proteins are being investigated as risk factors for cardiovascular disease.

Various techniques for the diagnosis of *in vivo* platelet activation or increased platelet function are available. Spontaneous *in vitro* platelet aggregation or the finding of increased aggregation response to low concentrations of platelet agonists suggests increased platelet function. The detection of circulating platelet aggregates may be made either by flow cytometry or by collecting a whole blood sample into a fixative.¹¹⁴ Platelet flow cytometry can also be used to detect circulating activated platelets due to neoexpression of new surface markers (ie, P-selectin or CD63) or by detecting proteins bound to platelet surface glycoproteins (ie, fibrinogen, thrombospondin).^{49,52} Some caution must be exercised in the diagnosis of increased platelet activation, because persistent *in vivo* activation can lead to degranulation of platelets with the detection of hypoaggregation in the laboratory.

CONCLUSION

Multiple causes exist for platelet-derived bleeding diatheses. The laboratory evaluation of these disorders can range from simple to complex as outlined herein, but should initially include a thorough evaluation of the patient's medical history, concentrating on personal and familial bleeding disorders and all current medications. With this as a starting point, the clinician and/or pathol-

ogist may find algorithms such as those presented herein helpful to guide investigation toward elucidating the underlying origin for platelet-derived bleeding.

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