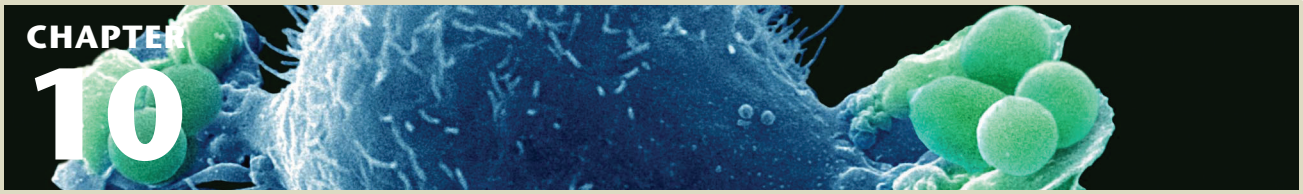

CHAPTER 10

Disorders of platelet number and function

Keith R. McCrae and A. Koneti Rao

Platelet biology: structure and function

Hemostasis encompasses a series of interrelated and simultaneously occurring events involving the blood vessels, platelets, and coagulation system. Defects affecting any of these major participants may lead to a hemostatic defect and a bleeding disorder. This chapter will focus on the disorders related to platelet number and function.

Platelet structure

Blood platelets are anucleate fragments derived from bone marrow megakaryocytes. The platelet diameter ranges from 1.5 to 3.0 μm, roughly one-third to one-fourth that of erythrocytes. Platelet volume is approximately 7 fL. Electron microscopy reveals a fuzzy coat (glycocalix) on the platelet surface composed of membrane GPs, glycolipids, mucopolysaccharides, and adsorbed plasma proteins. The plasma membrane is a bilayer of phospholipids in which cholesterol, glycolipids, and GPs are embedded. The phospholipids are asymmetrically organized in the plasma membrane; the negatively charged phospholipids (such as phosphatidylserine [PS]) are present almost exclusively in the inner leaflet, whereas the others are more evenly distributed. Platelets have an elaborate channel system, the open canalicular system, which is composed of invaginations of the plasma membrane and extends throughout the platelet and opens to the surface. The discoid shape of the resting platelet is maintained by a well-defined cytoskeleton consisting of the spectrin membrane skeleton, the marginal microtubule coil, and the actin cytoskeleton. The microtubule coil, present below the platelet membrane, is made up of α-β-tubulin dimers and plays a role in platelet formation from megakaryocytes, in addition to maintaining the discoid platelet shape. In proximity to the open canalicular system is the dense tubular system, a closed-channel network derived from the smooth endoplasmic reticulum; it is considered the major site of platelet prostaglandin and thromboxane synthesis.

Platelets contain a variety of organelles: mitochondria and glycogen stores, lysosomes, peroxisomes, dense granules, and α-granules. The lysosomes contain acid hydrolases; the dense granules contain calcium (which gives them the high electron density), adenosine triphosphate (ATP), adenosine diphosphate (ADP), magnesium, and serotonin (5-hydroxytryptamine). Serotonin is taken up by platelets from plasma and incorporated into the granules. The α-granules contain a large number of proteins, including β-thromboglobulin (βTG) and platelet factor 4 (PF4), which are considered platelet specific; several coagulation factors (eg, fibrinogen, factor V, factor XIII); von Willebrand factor (vWF); growth factors (eg, platelet-derived growth factor [PDGF], vascular endothelial growth factor [VEGF]); vitronectin; fibronectin; thrombospondin; the factor V binding protein multimerin; P-selectin; albumin; and immunoglobulin G (IgG). Some of these (eg, vWF, PF4, βTG) are synthesized by megakaryocytes, whereas others (eg, albumin, IgG) are incorporated into the granules from plasma.

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Off-label drug use: *Dr. Rao*: Desmopressin for management of patients with inherited platelet function defects and renal failure. Recombinant VIIa for management of patients with inherited platelet function defects. *Dr. McCrae*: Rituximab for ITP and TTP.

Platelet function in hemostasis

Following injury to the blood vessel, platelets adhere to exposed subendothelium by a process (adhesion) that involves, among other events, the interaction of a plasma protein, vWF, and a specific glycoprotein (GP) complex on the platelet surface, GP Ib-IX-V (GPIb-IX) (Figure 10-1). This interaction is particularly important for platelet adhesion under conditions of high shear stress. Adhesion is followed by recruitment of additional platelets that form clumps, a process called aggregation (cohesion). This involves binding of fibrinogen to specific platelet surface receptors, a complex composed of GPIIb-IIIa (integrin αIIbβ3). GPIIb-IIIa is platelet specific and has the ability to bind vWF as well. Although resting platelets do not bind fibrinogen, platelet activation induces a conformational change in the GPIIb-IIIa complex that leads to fibrinogen binding. Activated platelets release the contents of their granules (secretion), including ADP and serotonin from the dense granules, which causes the recruitment of additional platelets. Moreover, platelets play a major role in coagulation mechanisms; several key enzymatic reactions occur on the platelet membrane lipoprotein surface. During platelet activation, the negatively charged phospholipids, especially PS, become exposed on the platelet surface, an essential step for accelerating specific coagulation reactions by promoting the binding of coagulation factors involved in thrombin generation (platelet procoagulant activity).

A number of physiologic agonists interact with specific receptors on the platelet surface to induce responses, including a change in platelet shape from discoid to spherical (shape change), aggregation, secretion, and thromboxane A_2 (TxA_2) production. Other agonists, such as prostacyclin, inhibit these responses. Binding of agonists to platelet receptors initiates the

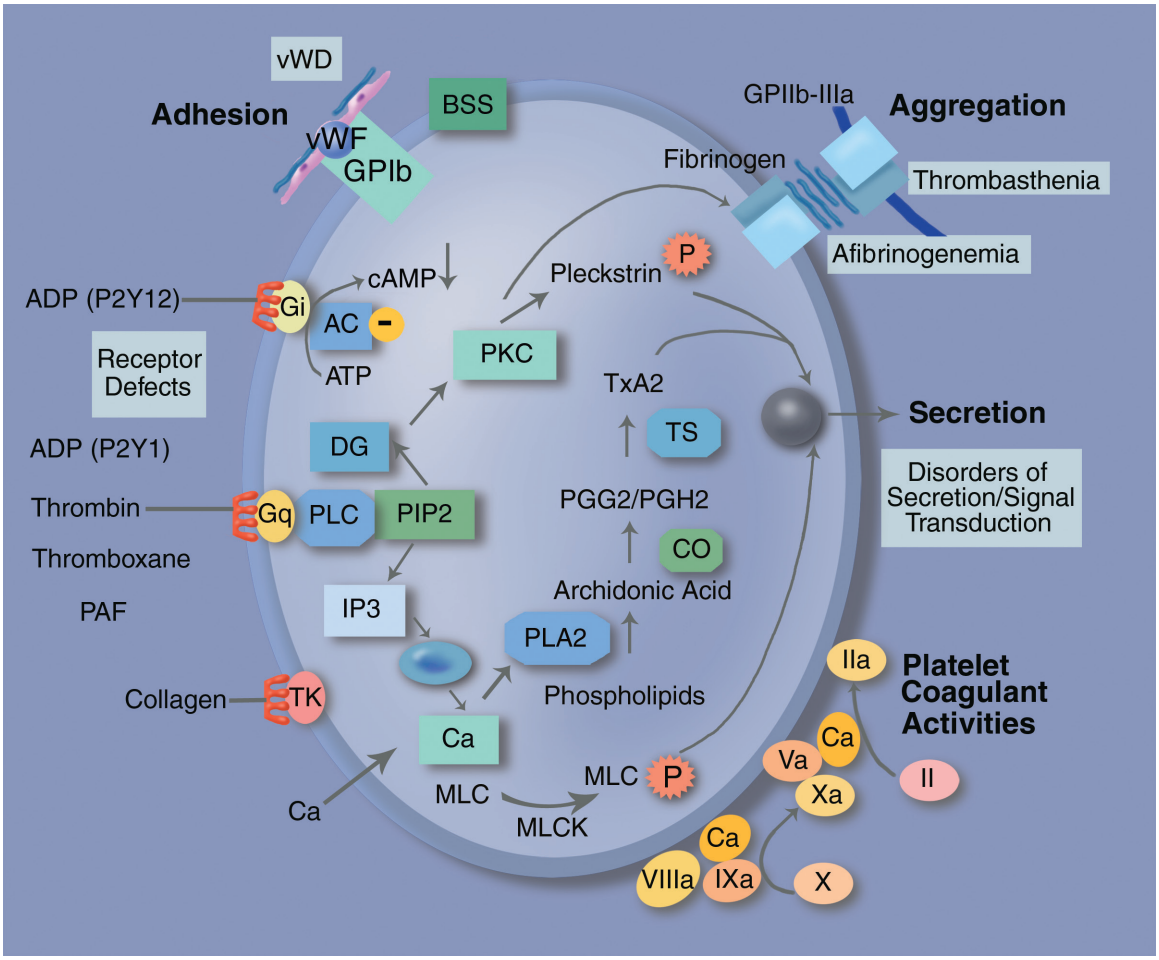

Figure 10-1 Schematic representation of selected platelet responses to activation and inherited disorders of platelet function. The Roman numerals in the circles represent coagulation factors. Modified with permission from Rao AK. Congenital disorders of platelet function: disorders of signal transduction and secretion. *Am J Med Sci.* 1998;316:69-76. AC = adenylyl cyclase; ADP = adenosine diphosphate; BSS = Bernard-Soulier syndrome; CO = cyclooxygenase; DAG = diacylglycerol; G = guanosine triphosphate–binding protein; IP3 = inositol trisphosphate; MLC = myosin light chain; MLCK = myosin light chain kinase; PAF = platelet activating factor; PIP2 = phosphatidylinositol bisphosphate; PKC = protein kinase C; PLC = phospholipase C; PLA2 = phospholipase A2; TK = tyrosine kinase; TS = thromboxane synthase; TxA2 = thromboxane A2; vWF = von Willebrand factor; vWD = von Willebrand disease.

production or release of several intracellular messenger molecules, including products of hydrolysis of phosphoinositide (PI) by phospholipase C (diacylglycerol and inositol 1,4,5-triphosphate [$InsP_3$]), TxA_2, and cyclic nucleotides (cyclic adenosine monophosphate) (Figure 10-1). These induce or modulate the various platelet responses of Ca^{2+} mobilization, protein phosphorylation, aggregation, secretion, and thromboxane production. The interaction between the platelet surface receptors and the key intracellular enzymes (eg, phospholipases A_2 and C, adenylyl cyclase) is mediated by a group of proteins that binds and are modulated by guanosine triphosphate (G proteins). As in most secretory cells, platelet activation results in an increase in cytoplasmic ionized calcium concentration; $InsP_3$ functions as a messenger to mobilize Ca^{2+} from intracellular stores. Diacylglycerol activates protein kinase C (PKC), and this results in the phosphorylation of several proteins. PKC-activation is considered to play a major role in platelet secretion and in the activation of GPIIb-IIIa. Numerous other mechanisms, such as activation of tyrosine kinases and phosphatases, also are triggered by platelet activation. Either inherited or acquired defects in these platelet mechanisms may lead to impairment of the platelet role in hemostasis.

Regulation of platelet number

Overview

The platelet count is regulated by the relative rates of platelet production and clearance. Kinetic studies have demonstrated that the average platelet life span is 7-10 days. Platelets that are lost through senescence, activation, or other processes are replaced by new platelets derived from bone marrow megakaryocytes. Platelet production from megakaryocytes, in turn, is driven by the hormone thrombopoietin (TPO) and its cellular receptor, c-Mpl.

Thrombopoietin and the thrombopoietin receptor c-Mpl

A healthy adult produces 1-3 $\times$ 10^{11} platelets per day, although production can increase tenfold during times of high demand. The number of circulating platelets is regulated by TPO, which binds to megakaryocytes and hematopoietic stem cells via c-Mpl. c-Mpl is a member of the class I hematopoietic growth factor receptor superfamily and activates several signaling pathways in megakaryocytes, resulting in megakaryocyte proliferation and differentiation, ultimately resulting in platelet production. c-Mpl also is expressed on platelets, which bind and clear TPO from the circulation. TPO is secreted constitutively from the liver, and although its production from liver and bone marrow may increase slightly during thrombocytopenic states, its overall production is relatively constant. As a consequence, the level of free TPO is regulated primarily by the number of circulating platelets, the platelet life span, and the megakaryocyte mass. In conditions such as aplastic anemia, which is characterized by a low platelet count and decreased bone marrow megakaryocyte mass, free TPO levels are high. In disorders such as immune thrombocytopenia, however, although the platelet count is low, platelet life span is diminished and the megakaryocyte mass may be expanded. This results in enhanced TPO clearance and plasma TPO levels that usually fall within the normal range despite thrombocytopenia. The role of TPO as the principal physiologic regulator of platelet production has been confirmed in studies of TPO and c-Mpl deficient mice, which have 5%-15% of the normal levels of circulating platelets, megakaryocytes, and megakaryocyte progenitor cells. TPO alone, however, does not fully support megakaryocyte polyploidization in vitro, suggesting that additional factors, such as stem cell factor, interleukin 3 (IL-3), interleukin 6 (IL-6), and interleukin 11 (IL-11), are required for optimal megakaryocyte development.

Normal platelet production

Megakaryocyte proliferation and differentiation involves endomitosis and polyploidization, a process in which the nucleus divides but the cell does not. In the process of maturation, megakaryocytes form secretory granules and a demarcation membrane system that permeates the cytoplasmic space. This extensive membrane system eventually projects multiple filamentous pseudopodial structures called proplatelets. This process utilizes the entire repertoire of cytoplasmic granules, macromolecules, and membranes. Ultimately, fragmentation of the pseudopodial projections leads to the release of new platelets. Each megakaryocyte produces 1,000-3,000 platelets before the remaining nuclear material is phagocytosed by resident macrophages. Released platelets circulate for 7-10 days before undergoing senescence and clearance by phagocytic cells in the reticuloendothelial system.

Key points

- The primary mediator of platelet production is TPO, produced primarily by the liver.
- TPO production is largely constitutive; thus TPO levels are regulated by the platelet and megakaryocyte mass through binding of TPO to its receptor, c-Mpl.
- TPO levels are normal in immune thrombocytopenia (ITP) because of enhanced clearance of TPO bound to platelets but are elevated in bone marrow failure syndromes.
- The normal platelet life span is 7–10 days.

Immune causes of thrombocytopenia

Clinical case

A 68-year-old man is referred for evaluation of increased bruising, primarily on his forearms, for the last 3 months. He restores old cars for a hobby and believes that trauma associated with this work may have caused his bruises, although he cannot recall specific instances during which he injured himself. He denies epistaxis, melena, or other evidence of systemic bleeding. He is in otherwise good health other than mild hypertension treated with an angiotensin converting enzyme inhibitor; he does not take other prescription medications but takes fish oil and vitamin C supplements. On physical examination, he looks well but several 2.0 cm bruises are noted on the distal upper extremities and back of the hands. Complete blood count reveals a hemoglobin of 12.8 gm/dL, white blood cell (WBC) count of 6.9×10^9/L, and platelet count of 22×10^9/L.

Immune thrombocytopenia

ITP is an autoimmune disorder characterized by thrombocytopenia and a variable risk of bleeding. An international working group recently proposed standard terminology and definitions for ITP. The term *immune* is now used instead of idiopathic and the term *purpura* has been abandoned, because bleeding symptoms, including purpura, are not necessarily present. Thus, the working group recommended the term *immune thrombocytopenia*, although the abbreviation ITP is preserved. In this classification scheme, *primary* is used to denote ITP with no precipitating cause, while *secondary ITP* refers to all other forms of immune-mediated thrombocytopenia (Table 10-1). These recommendations are consistent with the guidelines developed by the American Society of Hematology working group.

ITP is a common cause of thrombocytopenia in adults and children. Estimates of prevalence vary widely, ranging between 3 and 20 per 100,000 persons, with an estimated incidence of 2-10 cases per 100,000 patient-years. In childhood, the highest incidence is in children <5 years old, with a gradual decrease toward adolescence. Most studies find the incidence to be equal in girls and boys, although some reports suggest a higher incidence in boys <5 years. In adults, the incidence and prevalence of ITP is greatest in the elderly, with a female preponderance in the middle-adult years and a slight male preponderance in patients >70 years. In most children, ITP is self-limited and often is detected after an antecedent viral or infectious illness, whereas approximately 90% of cases of ITP in adults become persistent or chronic and cannot be linked to an obvious precipitating event. Although patients with more severe thrombocytopenia may present with mucocutaneous bleeding, those diagnosed with thrombocytopenia on a routine blood count are often asymptomatic. There is no gold-standard laboratory test for ITP, and thus, the diagnosis is made by excluding nonimmune causes of thrombocytopenia and investigating potential secondary causes.

Secondary ITP occurs in the setting of drugs, such as quinine or sulfa-containing drugs (see section on drug-induced thrombocytopenia), lymphoproliferative disorders, systemic lupus erythematosus or other autoimmune disorders, antiphospholipid antibody syndrome, and infections with hepatitis C, HIV, and *Helicobacter pylori*. Nonimmune causes of thrombocytopenia, including hypersplenism, hereditary thrombocytopenias, and type 2B von Willebrand disease (vWD), also should be included in the differential diagnosis of ITP (Table 10-2). Occasional patients with myelodysplastic syndromes may present with isolated thrombocytopenia.

Table 10-1 International Working Group proposed definitions of disease.

Primary ITP	Isolated thrombocytopenia Platelets $<100 \times 10^9$/L No other apparent causes of thrombocytopenia No secondary cause of ITP present
Secondary ITP	All other forms of immune-mediated thrombocytopenia except primary ITP Designate with presumed cause, in parentheses, following secondary ITP (eg, secondary ITP; lupus-associated)
Phases of the disease	Newly diagnosed: within 3 months of diagnosis Persistent: between 3 and 12 months of diagnosis Chronic: lasting >12 months Severe: presence of bleeding at presentation sufficient to mandate treatment, or occurrence of new bleeding symptoms requiring additional intervention

Adapted from Rodegheiro F et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386-2393.
ITP = immune thrombocytopenia.

Clinical features of ITP

Clinical features of primary and secondary ITP are generally similar, although in secondary ITP clinical manifestations related to the underlying disorder may be prominent. International guidelines recommend that a platelet count below 100×10^9/L is required for the diagnosis of ITP,

Table 10-2 Differential diagnosis of immune thrombocytopenia.

Previously diagnosed or high risk of conditions that may be associated with autoimmune thrombocytopenia (eg, HIV, hepatitis C virus, or other infection; other autoimmune or immunodeficiency disorders; malignancy; recent vaccination)
Liver disease, including cirrhosis from any cause
Drugs (prescription or nonprescription), alcohol abuse, consumption of quinine (tonic water), environmental toxins
Bone marrow disorders, including myelodysplastic syndromes, leukemias, other malignancies, fibrosis, aplastic anemia, and megaloblastic anemia
Recent transfusions (posttransfusion purpura) and recent immunization
Inherited thrombocytopenia: thrombocytopenia-absent radii syndrome, radioulnar synostosis, congenital amegakaryocytic thrombocytopenia, Wiskott-Aldrich syndrome, *MYH9*-related disease, Type IIb von Willebrand disease, Bernard-Soulier syndrome

Adapted from Rodegheiro F et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386-2393.

because mild thrombocytopenia may occur normally in non-Caucasians, and rarely results in the development of more severe thrombocytopenia or other autoimmune disease. The most common symptom of ITP is mucocutaneous bleeding, which may manifest as petechiae, purpura, ecchymosis, epistaxis, menorrhagia, oral mucosal, or gastrointestinal bleeding. The most feared complication is intracranial hemorrhage, which occurs only rarely. Bleeding because of thrombocytopenia is uncommon at platelet counts $>30 \times 10^9$/L. There is significant variability in bleeding among patients with similar platelet counts, however, and some individuals with counts $<10 \times 10^9$/L bleed infrequently. The risk of fatal bleeding is greatest in elderly patients with persistent and severe thrombocytopenia (platelets $<20 \times 10^9$/L).

Physical examination should focus on typical bleeding sites. Dependent areas and skin underneath tight clothing should be examined for petechiae and purpura, and oral mucous membranes should be examined for hemorrhagic bullae, which may be associated with an increased risk of severe bleeding at other sites. In a patient with ITP, the remainder of the general physical examination is normal. The presence of lymphadenopathy or splenomegaly should prompt investigations for underlying infection or lymphoproliferative disease. Skeletal, renal, or neurologic abnormalities suggest a familial cause of thrombocytopenia.

Recent studies suggest that fatigue is a common symptom in patients with ITP, occurring in >20% of children and up to 40% of adults. Fatigue correlates with a platelet count $<100 \times 10^9$/L and treatment with steroids, but not with duration of ITP, age, or gender. Fatigue usually resolves with successful treatment of ITP. Finally, epidemiologic studies suggest that ITP is associated with a relative risk of thrombosis of approximately 1.5-2.0. The mechanism of thrombosis in these individuals is not well established, although the risk of thrombosis does not correlate directly with the platelet count.

Pathophysiology of ITP

Primary ITP is a syndrome that results from several different pathophysiologic mechanisms. Classic experiments performed in the 1950s and 1960s demonstrated a critical role for antiplatelet antibodies in mediating the enhanced clearance of platelets in patients with ITP. These antibodies recognize platelet glycoproteins, most commonly GPIIb-IIIa and GPIb-IX. These antibodies may recognize the same targets on megakaryocytes, leading to impairment of megakaryocyte proliferation and differentiation, and proplatelet production. In most patients, both enhanced platelet destruction and impaired platelet production contribute to the development of thrombocytopenia. As noted, plasma levels of TPO generally are not elevated in patients with ITP.

Dysregulated T-cells in patients with ITP may enable the development of platelet autoantibodies, have a direct cytotoxic effect on platelets, and impair platelet production by megakaryocytes. Recent interest has focused on decreased levels of regulatory T-cells (T_{reg}) in patients with ITP; successful ITP treatment has been associated with restoration of T_{reg} levels.

The pathogenesis of *secondary ITP* may share similar mechanisms as primary ITP. For example, the thrombocytopenia that occurs in patients with antiphospholipid antibodies may reflect the concurrent presence of antibodies against platelet GPs. Unique pathogenic mechanisms, however, have been identified in some types of secondary ITP. For example, antigen mimicry, in which antibodies directed to a foreign (viral) protein cross-react with specific epitopes on platelet GPIIb-IIIa has been observed in hepatitis C–associated ITP. A similar pathophysiology may underlie the pathogenesis of ITP in patients with *H. pylori* infection and HIV.

Diagnosis of ITP

The diagnosis of ITP rests on a consistent clinical history, physical examination, and the exclusion of other causes. Leukocyte counts and hemoglobin are normal unless significant

thrombocytopenic bleeding has resulted in anemia. Examination of the peripheral blood film should be performed to exclude pseudothrombocytopenia (ethylenediaminetetraacetic acid–dependent platelet agglutinating antibodies), microangiopathic hemolytic anemia, or abnormalities suggestive of other disorders. The mean platelet volume (MPV) may be increased in patients with ITP. Some 15%-25% of ITP patients have detectable antinuclear or antiphospholipid antibodies; these generally have no prognostic importance, although one report suggested an increased incidence of thrombosis in ITP patients with antiphospholipid antibodies.

Bone marrow examination is not required routinely, but it should be performed to exclude other causes of thrombocytopenia when atypical features such as unexplained anemia, lymphadenopathy, or splenomegaly are present. Because approximately 80% of patients with ITP respond to initial therapy with corticosteroids, intravenous immunoglobulin (IVIg) or Rh-immune globulin (anti-D), failure to respond to these agents should prompt consideration of bone marrow examination. Bone marrow examination also may be warranted in elderly patients in whom myelodysplasia is suspected and should be considered in patients scheduled to undergo splenectomy. Megakaryocyte number is typically normal or increased in the marrow of patients with ITP.

With increased appreciation that secondary causes of ITP may be more common than previously believed, additional laboratory studies, such as screening for hepatitis C and HIV, and evaluation for combined variable immunodeficiency should be considered. Table 10-3 contains a list of suggested screening studies proposed by the ITP International Working Group.

Management of primary ITP in children

Because spontaneous recovery is expected in most children with primary ITP, families of children generally need counseling and supportive care rather than specific drug therapy. Severe hemorrhage occurs in ~1 in 200 children with newly diagnosed ITP, and intracerebral hemorrhage occurs in <1 in 500, usually in the first month after diagnosis. For those in whom treatment is considered necessary, a short course of corticosteroids, IVIg, or anti-D (in Rh-positive individuals) generally results in rapid recovery of the platelet count. Adverse effects of therapy in children include behavioral changes from corticosteroids, headache from IVIg, and hemolysis from anti-D, which rarely may be severe. Patients (adults and children) with a positive Coombs test should not receive anti-D because of an increased risk of severe hemolysis.

Recovery of the platelet count ultimately occurs in 80% of children even without therapy, usually within 6 months but occasionally over a year or more. The remaining 20% have persistent thrombocytopenia, yet even in this group, major bleeding is uncommon. Splenectomy generally is reserved for severe persistent thrombocytopenia and bleeding and results in complete remission in ~75% of children. The risk for overwhelming sepsis after splenectomy is greater in young children, and therefore, splenectomy generally is deferred until at least 5 years of age. Vaccination against *Streptococcus pneumoniae*, *Neisseria meningitides*, and *Haemophilus influenzae* type b should be given before splenectomy in children and adults, and penicillin prophylaxis is recommended until adulthood. Rituximab is another effective therapy in children, with a long-term remission rate of 22% in retrospective analyses. Thrombopoietic agents are

Table 10-3 International Working Group recommendations for the diagnosis of ITP in adults.

Basic evaluation	Test of potential utility	Tests of uncertain benefit
Patient and family history	Glycoprotein-specific antibodies	TPO levels
Physical examination	Antiphospholipid antibodies	Reticulated platelets
CBC and reticulocyte count	Antithyroid antibodies and thyroid function	Platelet-associated IgG
Peripheral blood film	Pregnancy test in women of childbearing potential	Platelet survival study
Bone marrow exam (in selected patients)	PCR for parvovirus and CMV	Bleeding time
Blood group (Rh)		Complement levels
Direct antiglobulin test		
H. pylori, HIV, HCV (suggested by majority regardless of geographic region)		
Quantitative Immunoglobulins (consider in children with ITP, recommend in children with persistent or chronic ITP)		

Adapted from Provan D et al. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood*. 2010;115(2):168-186.

CBC = complete blood count; CMV = cytomegalovirus; HCV = hepatitis C virus; IgG = immunoglobulin G; ITP = immune thrombocytopenia; PCR = polymerase chain reaction; TPO = thrombopoietin.

also effective in refractory childhood ITP, although the safety of long-term treatment with these agents in the pediatric population has not been established.

Management of primary ITP in adults

In contrast to children, ITP in adults evolves into a chronic disease in approximately 90% of patients. Given this realization, the goal of ITP management in adults is to maintain a safe platelet count while minimizing the toxicity of therapy. Therapy should not be dictated by the platelet count alone, but it also should consider other factors that modulate the risk of bleeding. There are no controlled studies demonstrating the superiority of any specific sequential treatment algorithm and significant variability exists among the treatment approaches advocated by different hematologists.

Asymptomatic patients with mild or moderate thrombocytopenia and no bleeding require no specific treatment. Platelet counts $<30 \times 10^9$/L may be associated with an increased bleeding risk, and although there is significant variability in bleeding among individual patients, this platelet count threshold has been suggested as a cutoff for considering treatment of ITP. Although several first-line therapies are available, prednisone (1 mg/kg daily) remains the initial treatment of choice because of its efficacy and low cost. Approximately 75% of patients initially respond to corticosteroids, although tapering usually precipitates relapse, and ultimately only 10%-15% of patients are able to maintain a safe platelet count after steroid discontinuation. High-dose dexamethasone (40 mg daily for 4 days, repeated in biweekly or monthly cycles) provides an alternative for the initial treatment of patients with ITP, with some studies suggesting that high-dose corticosteroids used early in the treatment course induce more durable remissions. A still more aggressive approach that employed dexamethasone and rituximab in the initial treatment of ITP demonstrated a significantly higher sustained response rate at 6 months after treatment initiation in patients that received this combination versus those receiving dexamethasone alone (63% vs. 36%, $n = 52$, p <0.004, 95% confidence interval [CI] 0.079-0.455), although these differences appear to be lost on longer term follow-up. Up to 5%-10% of patients with ITP may achieve a durable remission, usually within the first year after presentation; however, it is uncertain whether these are spontaneous or related to treatment. This observation has led to a recommendation by the International Working Group that splenectomy be deferred until at least 1 year after presentation, if possible.

For patients who do not achieve a durable response after initial treatment with corticosteroids, intermittent IVIg or anti-D may be effective. Both of these agents are associated with response rates similar to those of corticosteroids; however, the duration of response generally is only 2-4 weeks and thus frequent, intermittent dosing is required if these agents are used as chronic therapy. One uncontrolled study of 28 Rh-positive, nonsplenectomized adults reported that repeated dosing of anti-D for platelet counts $<30 \times 10^9$/L was an effective maintenance therapy and that 43% of patients treated in this manner ultimately entered a durable remission. Nevertheless, both IVIg and anti-D generally are considered to be bridging agents used to maintain platelet counts in a safe range until more definitive therapy can be initiated.

There are several additional options for therapy after steroid failure, specifically rituximab, thrombopoietic agents, or splenectomy. Splenectomy has been a popular therapy for decades, although the availability of alternative treatments, concerns about long-term adverse events following splenectomy, and the realization that some patients with newly diagnosed ITP ultimately may improve over time has led to decreased utilization (20%-25% of patients) in contemporary cohorts compared with older series (50%-60% of patients). Although both the ITP International Working Group and the revised American Society of Hematology (ASH) guidelines consider splenectomy an acceptable second-line therapy for ITP, the former group weights splenectomy equally to more than 10 other options, whereas the ASH guidelines *recommend* splenectomy (grade 1B evidence) for patients who fail corticosteroids while *suggesting* rituximab or thrombopoietic agents (grade 2C evidence). Splenectomy leads to a high rate of durable remissions. In a systematic review, 1,731 (66%) of 2,623 adults with ITP achieved a complete response following splenectomy with a median follow-up of 28 months (range 1 to 153 months), and ~65% of patients remained in complete remission 10 years after splenectomy. Splenectomy does not jeopardize subsequent responses to other ITP therapies (other than anti-D) and may reduce long-term costs of ITP management. Disadvantages of splenectomy include a lack of validated predictors of response, surgical risk with a 30-day mortality and complication rate of 0.2% and 9.6% for laparoscopic splenectomy and 1.0% and 12.9% for open splenectomy, an increased risk of postsplenectomy infection, and a potentially increased risk of vascular thrombosis compared with the general population (although whether this is increased compared with nonsplenectomized age-matched ITP controls is unknown). The incidence of infection may be reduced by presplenectomy vaccination; repeat immunization or monitoring of antibody titers every 5 years may further reduce infection rates. Aggressive treatment of fever in splenectomized ITP patients is indicated.

Rituximab, an anti-CD20 monoclonal antibody that rapidly depletes $CD20^+$ B lymphocytes, provides another treatment option that many hematologists consider before splenectomy in patients who fail initial corticosteroid

therapy. In a systematic review of 313 ITP patients, half of whom were not splenectomized, 62.5% achieved a platelet count response (platelet increment of 50×10^9/L), with a median time to response of 5.5 weeks (range, 2 to 18 weeks) and a median duration of response of 10.5 months (range, 3 to 20 months). In a single-arm study of 60 nonsplenectomized ITP patients, 40% achieved a platelet count $\geq 50 \times 10^9$/L with at least a doubling from baseline at 1 year, and in 33.3%, this response was sustained for 2 years. A recent pilot randomized, placebo-controlled trial that assessed a composite endpoint of any platelet count $<50 \times 10^9$/L, significant bleeding, or rescue treatment once standard treatment was stopped failed to demonstrate a treatment advantage for rituximab within 6 months of therapy initiation. Complete responses and overall platelet responses, however, were observed in 46.2% and 73.1% of placebo treated patients, respectively. An appealing aspect of rituximab therapy is the induction of long-term responses in a subset of patients; in one recent series, 21% of adults treated with rituximab achieved treatment-free responses of at least 5 years. Adverse effects of rituximab include infusion reactions (eg, hypotension, chills, rash), serum sickness, and cardiac arrhythmias. Reactivation of latent JC virus causing progressive multifocal leukoencephalopathy has been reported, but it appears to be extremely uncommon. Reactivation of hepatitis B after rituximab has been described, and active hepatitis B infection is a contraindication for treatment.

The TPO receptor agonists romiplostim and eltrombopag are approved in the many countries for patients with ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy. These agents bind and activate the TPO receptor, c-Mpl, leading to increased platelet production; however, they have no structural similarity to endogenous TPO and do not stimulate cross-reactive TPO antibodies. The response rates to these agents range from 59% to 88%, and loss of response while on continued therapy is uncommon. These agents are effective before and after splenectomy and usually allow decreases in dosage or discontinuation of concomitant ITP therapy. The short-term safety and tolerability of these agents was demonstrated in clinical trials and confirmed through Food and Drug Administration–mandated postmarketing surveillance, although safety data beyond 5 years is only beginning to emerge. A disadvantage of these agents includes the potential need for long-term therapy, although anecdotal reports describe patients in whom these drugs have been discontinued with maintenance of hemostatic platelet counts. Increased bone marrow reticulin develops in approximately 5% of patients treated with TPO receptor agonists, but there is no evidence for development of progressive or irreversible bone marrow fibrosis. Eltrombopag carries a warning because of the potential for hepatotoxicity. Occasional patients treated with either agent may develop more severe thrombocytopenia following discontinuation than existed before treatment.

Additional treatment options for refractory ITP include azathioprine, danazol, dapsone, and other immunosuppressant medications; however, evidence from randomized controlled trials of these agents is limited. Thrombocytopenia in patients with secondary ITP often responds to treatment of the underlying disease, for example, eradication of hepatitis C with antiviral therapy or treatment of HIV with highly active antiretroviral therapy (HAART). Treatment of *H. pylori* infection has led to resolution of ITP in >50% of cases in certain regions, particularly Japan, although generally it has not been effective in North America. This may reflect differences in endemic *H. pylori* strains in different geographic regions.

Emergency treatment of ITP

Patients with new-onset, severe thrombocytopenia ($<20 \times 10^9$/L) and bleeding should be hospitalized. Examination of the peripheral blood smear to exclude thrombotic microangiopathy and a careful medication history to exclude drug-induced thrombocytopenia should be undertaken. Once a presumptive diagnosis of ITP has been reached, management of bleeding may require platelet transfusions in combination with high doses of parenteral corticosteroids (methylprednisolone 1 g intravenously daily for 2-3 days) or IVIg (1 g/kg for 1-2 days). Increases in the platelet count may become apparent within 3-5 days, although complete responses may require 1-2 weeks. Bleeding manifestations sometimes may improve before notable improvements in the platelet count. Emergency splenectomy may be required for patients with refractory thrombocytopenia and persistent bleeding.

Key points

- ITP may exist as a primary disorder or secondary to a number of other illnesses.
- The diagnosis of primary ITP is made by excluding other causes of thrombocytopenia.
- ITP in children is usually self-limited; conversely, ITP in adults develops into a chronic disease in ~90% of patients.
- The pathogenesis of ITP involves accelerated platelet destruction and decreased platelet production.
- Corticosteroids are first-line therapy for ITP, although rarely induce a durable remission.
- There is no universally accepted scheme for treatment of corticosteroid-resistant ITP, although rituximab, splenectomy, thrombopoietic agents, and other drugs are all effective

Drug-induced immune thrombocytopenia

More than 200 drugs have been implicated in drug-induced immune thrombocytopenia (DITP), including quinine and quinidine (present in tonic water, bitter melon, and certain medications), nonsteroidal anti-inflammatory agents, trimethoprim-sulfamethoxazole, vancomycin, rifampin, anticonvulsants, sedatives, and acetaminophen as well as the platelet GPIIb-IIIa inhibitors tirofiban, eptifibatide, and abciximab. A case-control study of drug use among patients with acute reversible thrombocytopenia compared with nonthrombocytopenic controls showed that trimethoprim-sulfamethoxazole was most frequently implicated. A systematic review of individual patient data found that the most commonly reported drugs with a definite or probable causal relation to thrombocytopenia were quinidine, quinine, rifampin, and trimethoprim-sulfamethoxazole. George et al. developed an online database of implicated drugs (Platelets on the Web; available at http://www.ouhsc.edu/platelets). Heparin-induced thrombocytopenia (HIT) is discussed separately because of its unique clinical manifestations and pathophysiology.

Mechanisms of DITP

DITP develops approximately 7 days after drug exposure, although when induced by the GPIIb-IIIa antagonists, eptifibatide, tirofiban, and abciximab may present within hours and even on the first exposure to the drug. Several mechanisms, specific for individual drugs, underlie the development of DITP. Quinine-induced thrombocytopenia was described >140 years ago and serves as a prototype. In this disorder, the binding of naturally occurring antibodies to platelet GPs is greatly enhanced in the presence of sensitizing drug. This may result from binding the drug to specific GPs, such as GPIIb-IIIa or GPIb-IX, and perhaps to the antibody itself. Affinity maturation of B-cells producing such antibodies may result in the generation of antibodies that can destroy platelets in the presence of drug. Another mechanism of DITP involves the induction of autoantibodies by drugs such as gold, procainamide, sulfonamides, and interferon-α or -β, leading to development of a syndrome that resembles ITP. An often-overlooked cause of DITP is that which follows vaccinations, including diphtheria-pertussis-tetanus (DPT), and measles-mumps-rubella (MMR), which reflects the development of true autoantibodies similar to those described in ITP.

Tirofiban and eptifibatide ("fibans") are small molecule mimetics of the RGD region of fibrinogen that inhibit fibrinogen binding to activated GPIIb-IIIa and block platelet aggregation. Thrombocytopenia may occur because of preexisting antibodies that recognize conformation-dependent neoepitopes (mimetic induced binding sites [MIBS]) induced in GPIIb-IIIa following mimetic binding. Abciximab, a chimeric (mouse–human) Fab fragment to GPIIb-IIIa, causes acute profound thrombocytopenia in 0.5%-1.0% of patients on their first exposure because of preexisting antibodies that recognize the murine portion of abciximab. Thrombocytopenia caused by GPIIb-IIIa antagonists may be severe, with platelet counts $<10 \times 10^9$/L. Patients may require platelet transfusions to treat hemorrhagic complications, which are exacerbated by the concomitant use of heparin, aspirin, and other antiplatelet agents.

Diagnosis of DITP

Clinical criteria for levels of evidence for DITP have been proposed that may be used to judge the likelihood of drug being implicated in DITP. These include the temporal association between drug exposure and thrombocytopenia, the exclusion of other causes of thrombocytopenia, and recurrence upon drug rechallenge. In practice, however, patients are often on many drugs and have concurrent illnesses, such as infections, that may make the diagnosis of DITP difficult. The detection of an antibody that binds tightly to normal platelets in the presence of the drug establishes the diagnosis in many cases; however, such testing is available only in specialized laboratories and results frequently are not available in time to aid with acute treatment decisions. Moreover, drug-dependent platelet antibodies may be missed when the antibodies recognize a metabolite of the drug instead of the drug itself, as with naproxen and acetaminophen.

Treatment for DITP involves discontinuation of the drug and administration of platelet transfusions for severe bleeding. Resolution of thrombocytopenia may require 4-8 days, although bleeding symptoms usually improve more rapidly. Corticosteroids or IVIg have been beneficial in anecdotal cases, although their efficacy has not been assessed in controlled studies.

Key points

- DITP is caused by many drugs.
- Quinidine, quinine, and trimethoprim-sulfamethaxazole are commonly implicated.
- Thrombocytopenia caused by tirofiban, eptifibatide, and abciximab may occur soon after exposure in patients not previously exposed to these drugs.
- DITP can be confirmed in some cases by the demonstration of a drug (or drug metabolite)-dependent, platelet-reactive antibody in vitro.

Heparin-induced thrombocytopenia

HIT is an idiosyncratic drug reaction caused by antibodies against multimolecular complexes of PF4 and heparin.

Binding of HIT antibodies to Fc receptors on monocytes and platelets causes cellular activation; HIT antibodies also activate endothelial cells by binding endothelial cell-associated PF4. The net result is elevated levels of circulating microparticles and an intensely prothrombotic state. HIT occurs most commonly in patients receiving unfractionated heparin (UFH), with a reported incidence of 0.2%-5.0%; the risk of HIT associated with low–molecular weight heparin (LMWH) is five- to tenfold lower. Thrombosis develops in 40%-50% of patients with HIT despite the occurrence of thrombocytopenia; bleeding is rare. Although the diagnosis of HIT in the acute setting is clinical, confirmation depends on correlative laboratory testing. Transient thrombocytopenia following the administration of heparin (previously called type I HIT, or nonimmune HIT) is an innocuous syndrome that is uncommonly diagnosed and caused by direct platelet agglutination by heparin.

Clinical features

HIT is uncommon in patients <40 years of age and is more common in females (odds ratio 2.37). The incidence of HIT is approximately threefold greater in surgical than medical patients. Of the surgical patients, those undergoing orthopedic surgery have the highest incidence of HIT (5%); cardiac surgery patients have a lower incidence of HIT (2%-3%) despite a higher seroconversion rate in the heparin-PF4 enzyme-linked immunoadsorbent assay (ELISA). Clinical features consistent with HIT include a platelet count decrease of 50% or more that begins 5-10 days after starting heparin (or sooner in patients with recent heparin exposure), the presence of thrombosis, and the exclusion of other causes. Absolute thrombocytopenia (platelet count $<150 \times 10^9$/L) is not required for a diagnosis of HIT; rather a decrease in the platelet count from baseline is required. Uncommonly, HIT may develop 2-3 weeks after prior heparin exposure (delayed onset HIT). Several clinical scoring systems have been developed to assist with determining the pretest probability of HIT. The most commonly used is the 4T system (thrombocytopenia, timing, thrombosis, and other; see Table 10-4). This system has been shown to have a high negative predictive value (ie, a low score is useful in ruling out HIT), but its effectiveness is limited by modest interobserver agreement and a relatively low positive predictive value. Recent studies have demonstrated that this system also is of limited utility in intensive care patients, a setting in which HIT is uncommon. Another system, the HIT expert probability score also been has developed, although the clinical experience with this system is not extensive. The impact of either scoring system on patient outcomes has not been determined.

Thrombosis is present in ~50% of newly diagnosed cases of HIT, and it develops in ~40% of patients with asymptomatic thrombocytopenia resulting from HIT within the first 10 days following heparin discontinuation. Venous thrombosis occurs twice as frequently as arterial thrombosis, although limb artery thrombosis, myocardial infarction, and microvascular thrombosis have been described. Phlegmasia due to occlusion of the lower-extremity venous system resulting in arterial insufficiency may be difficult to discern from arterial thrombosis. Adrenal infarction, skin necrosis at the heparin injection site, and anaphylactoid reactions after an intravenous heparin bolus also may occur as a result of PF4/heparin antibodies. Thrombosis in unusual sites, such as cerebral sinuses, vascular grafts, and fistulas, and visceral vessels also may develop. HIT-associated thrombosis occurs with increased frequency at sites of vessel injury, thus vascular

Table 10-4 4Ts scoring system for HIT.

4Ts	2 points	1 point	0 point
Thrombocytopenia	Platelet count decrease of >50% and platelet nadir $\geq 20 \times 10^9$/L	Platelet count decrease of 30%-50% or platelet nadir of $10\text{-}19 \times 10^9$/L	Platelet count fall of <30% or platelet nadir $<10 \times 10^9$/L
Timing of platelet count fall	Clear onset of thrombocytopenia 5-10 days after heparin administration; or platelet decrease within 1 day, with prior heparin exposure within 30 days	Consistent with day 5-10 decrease but not clear (eg, missing platelet counts) or onset after day 10; or decrease within 1 day, with prior heparin exposure 30-100 days ago	Platelet count decrease <4 days without recent exposure
Thrombosis or other sequelae	New thrombosis (confirmed); skin necrosis (lesions at heparin injection site); acute systemic reaction after intravenous unfractionated heparin bolus	Progressive or recurrent thrombosis; nonnecrotizing skin lesions; suspected thrombosis (not proven)	None
Other causes for thrombocytopenia	None apparent	Possible	Definite

Adapted from Lo G et al., Evaluation of pretest clinical score (4T's) for the diagnosis of heparin-induced thrombocytopenia in two clinical settings. *J Thromb Haemost.* 2006;4:759-765.

interventional procedures and placement of intravascular devices such as vena caval filters should be avoided.

HIT testing

Two types of tests are available for detection of HIT antibodies: quantitative PF4/heparin immunoassays (PF4/heparin ELISA) and functional assays demonstrating the ability of HIT antibodies to activate platelets, such as the serotonin release assay (SRA), generally considered the gold standard for diagnosis, or heparin-induced platelet activation (HIPA).

The sensitivity of the PF4/heparin ELISA approaches 100%, and thus a negative test is useful in excluding HIT. Difficulties concerning its use include long turnaround time in institutions in which it is not performed daily, and its low specificity and positive predictive value, particularly in the postcardiac surgery setting; the latter reflects a significant incidence of false-positive results. Specificity may be increased by considering the level of positivity. High ELISA reactivity correlates closely with the presence of platelet-activating HIT IgG in some studies, whereas positive platelet activation studies were uncommon in patients with weakly positive ELISA values (0.4-0.9). The use of an ELISA that detects only anti-PF4/heparin IgG as opposed to the polyspecific ELISA that detects IgG, IgA, and IgM antibodies also may increase specificity, as may the addition of a confirmatory step performed in the presence of high heparin concentrations.

Functional assays have improved specificity compared with the ELISA. These assays are technically difficult, however, requiring washed donor platelets, and for the SRA, radioisotope. Because of these considerations, the performance of functional assays is limited primarily to specialized reference labs, and their results generally are not available at the time the diagnosis of HIT must be considered.

Treatment of HIT

Although previously underdiagnosed, increased appreciation of HIT and the frequent use of highly sensitive tests has led to overdiagnosis in the current era, with the attendant costs and increased bleeding risks associated with inappropriate anticoagulation therapy. Current guidelines of the American College of Chest Physicians suggest that routine monitoring of the platelet count in patients on heparin therapy should be performed every 2-3 days for patients with a risk of HIT of >1% and that routing monitoring is unnecessary for those in whom the risk of HIT is <1% (Table 10-5).

The cornerstone of HIT therapy is immediate discontinuation of heparin when the disease is suspected, usually before laboratory diagnosis. All individuals with suspected HIT should receive ultrasound evaluation of the extremities.

Table 10-5 Incidence of HIT according to patient population and type of heparin exposure.

Patient population (minimum 4 days' exposure)	Incidence of HIT (%)
Postoperative patients	
Heparin, prophylactic dose	1-5
Heparin, therapeutic dose	1-5
Heparin, flushes	0.1-1.0
LMWH, prophylactic or therapeutic dose	0.1-1.0
Cardiac surgery patients	1-3
Medical	
Patients with cancer	1.0
Heparin, prophylactic or therapeutic dose	0.1-1.0
LMWH, prophylactic or therapeutic dose	0.6
Intensive care patients	0.4
Heparin, flushes	<0.1
Obstetric patients	<0.1

Adapted from Linkins LA et al. Treatment and prevention of heparin-induced thrombocytopenia. *Chest.* 2012;141(2)(suppl):e495S-e530S.
HIT = heparin-induced thrombocytopenia; LMWH = low–molecular weight heparin.

Anticoagulation using a nonheparin anticoagulant should be initiated even in patients with no thrombosis because of the continued high risk of thrombosis after heparin discontinuation. Alternative anticoagulation should be continued until the platelet count has normalized; some advocate for a longer duration of anticoagulation (eg, 30 days), although no controlled data demonstrating the benefit of this approach are available. Patients with HIT and no thrombosis should receive at least 1 month of full-dose anticoagulation, and those with thrombosis should receive at least 3 months. LMWH should not be used because of cross-reactivity with most heparin-dependent antibodies. Initiation of warfarin without coverage by an alternative anticoagulant may lead to hypercoagulability because of the inhibition of protein C γ-carboxylation, and patients who develop HIT while on warfarin or who have been started on warfarin alone should be treated with vitamin K in addition to a nonheparin anticoagulant.

Currently available nonheparin anticoagulants available in the United States include argatroban and bivalirudin, both of which are direct thrombin inhibitors. Argatroban is hepatically cleared and approved for treatment of HIT with or without thrombosis, as well as percutaneous coronary interventions in patients with HIT or at risk for HIT. The use of argatroban in HIT is associated with a hazard ratio of 0.3 for the development of new thrombosis. Argatroban is monitored using the activated partial thromboplastin time (aPTT), but in conjunction with warfarin, it may have significant effects on the PT. Thus, transitioning patients from argatroban to warfarin should be performed by following the guidelines suggested by

the manufacturer. Bivalirudin is approved for percutaneous coronary interventions in patients with HIT or a history of HIT and has the advantage of a short half-life of only 25 minutes. Other anticoagulants such as Danaparoid and Lepirudin are no longer available in the United States. A number of reports have described the use of the synthetic pentasaccharide fondaparinux in patients with HIT, although this agent has not been studied in a controlled manner.

Key points

- HIT occurs in 0.2%-5% of adults exposed to UFH, approximately 40% of whom develop thrombosis.
- HIT antibodies are directed against a large, multimolecular complex of PF4/heparin.
- ELISA tests for HIT antibodies are highly sensitive, but they have low specificity and thus are frequently positive when confirmatory functional assays, including the ^{14}C-serotonin release assay, are not.
- When HIT is suspected, heparin must be discontinued and a nonheparin anticoagulant initiated.
- Anticoagulation with a nonheparin anticoagulant generally is continued for 30 days in patients with HIT without thrombosis and for at least 3 months in those who develop thrombosis.
- Delayed-onset HIT may develop several weeks after heparin exposure.

Other causes of thrombocytopenia

Thrombotic microangiopathies

Clinical case

A 17-year-old female is referred for evaluation of renal insufficiency and anemia. She and her siblings were placed in foster care while they were very young, and she has no information on the health of her parents or older relatives. Her renal function was first noted to be abnormal 1 year ago and over the last 2 months she has developed profound fatigue. Her 22-year-old sister is married and in good health. Her 15-year-old brother also has been noted to have mildly abnormal renal function, as well as significant anemia. On examination she appears fatigued and pale. There is no organomegaly. The complete blood count reveals hemoglobin of 8.5 gm/dL, WBC of 9.1×10^9/L, and a platelet count of 77×10^9/L. The lactic dehydrogenase (LDH) is elevated at 632 IU/L. Peripheral blood film reveals 1-2 schistocytes per high-power field. Subsequent evaluation included sequencing of complement regulatory genes and revealed a mutation in factor H.

Clinical features

The thrombotic microangiopathies discussed in this chapter include thrombotic thrombocytopenic purpura (TTP) and the typical and atypical hemolytic uremic syndrome (HUS and aHUS, respectively). Each of these disorders is characterized by microangiopathic hemolytic anemia (MAHA) and thrombocytopenia, with a variable component of neurologic or renal dysfunction and fever. This pentad of symptoms was once common at the time of presentation, but increased awareness of these disorders has led to earlier diagnosis. Currently, the presence of MAHA and thrombocytopenia without another apparent cause is sufficient for the diagnosis of thrombotic microangiopathy (TMA).

TTP occurs in both a rare inherited form due to mutations in the vWF-cleaving protease, ADAMTS13 (a disintegrin **a**nd **m**etalloprotease with **t**hrombospondin-1-like repeats), as well as a more common acquired form in which ADAMTS13 deficiency is caused by autoantibodies. Patients with TTP generally present acutely or subacutely with fatigue and malaise, with variable neurologic symptoms that may range from mild personality changes to obtundation. aHUS may present in a similar manner, but it also may demonstrate a more chronic presentation with progressive renal insufficiency, low-grade MAHA, and thrombocytopenia. Typical HUS follows infection with enteropathogenic *E. coli*, may occur in epidemics, and often is preceded by bloody diarrhea and abdominal pain (the frequent presence of diarrhea has led to the designation of this disorder as D^+, as opposed to aHUS, referred to as D^-). Not all patients with typical HUS have diarrhea, however, whereas up to 30% of aHUS patients may provide such a history; thus, the presence or absence of diarrhea does not always distinguish these disorders. Renal insufficiency is usually the most prominent component of typical HUS.

Distinguishing between these TMAs may be difficult because of extensive overlap in symptoms. Although some features, such as neurologic manifestations may be more frequent in TTP, renal failure is more common in HUS and aHUS; however, these characteristics alone do not allow discrimination. Because of these overlapping symptoms, it has been difficult to develop unambiguous classification schemes for these disorders. Recent scientific advances have led to new information concerning the pathogenesis of these diseases. For example, although most cases of TTP are associated with deficiency of ADAMTS13, activation of the alternative pathway of complement resulting from mutations in complement regulatory proteins underlie approximately 70% of cases of atypical aHUS. These differences have allowed for the development of pathogenesis-based classification schemes for TMAs; an example of one scheme developed by the British Committee for Standards in Haematology and the British Transplantation Society is depicted in Table 10-6.

Pathogenesis

TMAs cause microvascular thrombi in critical organs, leading to ischemia and organ damage. These thrombi induce

Table 10-6 Classification scheme for thrombotic microangiopathies.

Disorders in which etiology is established
ADAMTS13 abnormalities
ADAMTS13 deficiency secondary to mutations
Antibodies against ADAMTS13
Disorders of complement regulation
Genetic disorders of complement regulation
Acquired disorders of complement regulation (eg, factor H antibody)
Infection induced
Shiga and verotoxin (Shiga-like toxin) producing bacteria
Streptococcus pneumoniae
Defective cobalamin metabolism
Quinine induced
Disorders in which etiology is not well understood
HIV
Malignancy
Drugs
Pregnancy
Systemic lupus erythematosus and antiphospholipid antibody syndrome

Adapted from Taylor CM et al. Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom. *Br J Haematol.* 2009;148:37-47.
ADAMTS13 = adisintegrin and metalloprotease with thrombospondin.

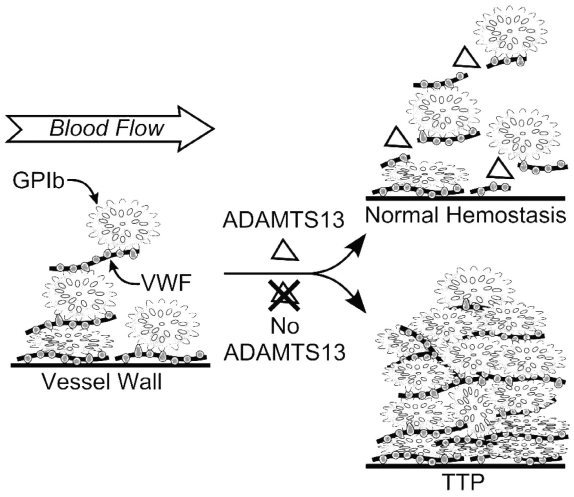

Figure 10-2 Pathogenesis of idiopathic TTP caused by ADAMTS13 deficiency. Multimeric vWF adheres to endothelial cells or to connective tissue exposed in the vessel wall. Platelets adhere to vWF through platelet membrane GPIb-IX. In flowing blood, vWF in the platelet-rich thrombus is stretched and cleaved by ADAMTS13, limiting thrombus growth. If ADAMTS13 is absent, vWF-dependent platelet accumulation continues, eventually causing microvascular thrombosis and TTP. Reproduced from Sadler JE. von Willebrand factor, ADAMTS-13 and thrombotic thrombocytopenic purpura. *Blood.* 2008;112 (1):11-18. ADAMTS13 = a disintegrin and metalloprotease with thrombospondin; GP = glycoprotein; TTP = thrombotic thrombocytopenic purpura; vWF = von Willebrand factor.

shearing of red blood cells, leading to the characteristic schistocytic anemia, which also may be caused by oxidative stress. Endothelial cell activation or damage also promote TMA, leading to the elaboration of unusually large vWF multimers that enhance platelet agglutination and microvascular occlusion.

Most cases of TTP result from an inherited or acquired deficiency of ADAMTS13, leading to elevated levels of unusually large vWF multimers that induce platelet aggregation in the microvasculature. ADAMTS13 regulates vWF activity by cleaving high–molecular weight multimers; failure to do so may result in the microvascular thrombosis and ischemia characteristic of TTP (Figure 10-2). The observations that some patients develop apparent TTP despite normal levels of circulating ADAMTS13, whereas other patients with congenital ADAMTS13 deficiency may not develop TTP until adulthood, suggests that factors other than ADAMTS13 deficiency, such as endothelial damage or activation, also are needed to trigger TTP. Other TTP-like syndromes can be caused by drugs, including quinine, ticlopidine, clopidogrel, cyclosporine, tacrolimus, mitomycin C, and gemcitabine, and also may occur in the setting of bone marrow transplantation, systemic lupus erythematosus, disseminated malignancy and HIV infection. The pathogenesis of these syndromes is diverse, whereas some are associated with antibodies to ADAMTS13, others are not, and may result from direct endothelial cell toxicity.

HUS results from infection by enteropathogenic *E. coli*, most commonly serotype O157:H7. The capacity of these organisms to cause HUS reflects their production of two 70 kD bacterial exotoxins named verotoxins. Verotoxin-1 is homologous to a Shigella toxin and therefore generally is referred to as Shiga-like toxin 1 (SLT-1 or Stx1). Most strains of pathogenic *E. coli* produce a second toxin, Stx2, which is associated with a higher risk of developing HUS. The intact, 70 kD Stx holotoxin consists of a 32 kD A subunit and five 7.7 kD B receptor-binding subunits that bind globosyltriaosylceramide (Gb3; CD77) receptors expressed on capillary endothelium. Following binding to Gb3, the toxin is internalized. The A subunit is proteolyzed to a 27 kD A1 subunit that binds the 60s ribosomal subunit, inhibiting protein synthesis and inducing endothelial cell apoptosis. Recent studies have demonstrated that signal transduction initiated through cross-linked Stx B subunit/Gb3 complexes induce the release of vWF from endothelial cells. Finally, Stx acts in concert with lipopolysaccharide to trigger a procoagulant state that involves platelet activation, tissue factor induction, and the release of unusually large vWF multimers.

The pathogenesis of aHUS reflects increased activation of the alternative complement pathway (AP) because of

mutations resulting in loss or functional impairment of complement regulatory proteins, or less frequently, activating mutations in complement proteins themselves. aHUS is transmitted in an autosomal manner, accounting for the familial inheritance, although penetrance is only 50%. Under normal conditions, the AP is constitutively activated because of ongoing C3 hydrolysis (Figure 10-3), and thus tight regulation of the AP by complement inhibitory proteins is required to prevent complement-mediated injury. AP activation leads to the generation of the C5b-C9 lytic complex on cell surfaces, and in the case of aHUS, endothelial cell damage is the primary consequence, resulting in characteristic microvascular thrombotic lesions. Complement activation is regulated primarily by the plasma protein, factor H, and the membrane-associated membrane cofactor protein (MCP; CD46), each of which binds membrane-bound C3b and promotes its inactivation by factor I. Several mutations in complement regulatory proteins underlie the development of aHUS. Most common are mutations in factor H, which impair the interactions of factor H with membrane-bound C3b, and account for 30% of cases; an additional 5%-10% of cases of aHUS result from acquired antibodies to factor H. Mutations in MCP, usually impairing membrane expression, are observed in 15% of patients with aHUS. Factor I mutations occur in 12% of aHUS patients. Activating mutations in factor B or C3 occur in 5%-10% of patients with aHUS. Mutations in thrombomodulin, another complement regulatory protein, have been described.

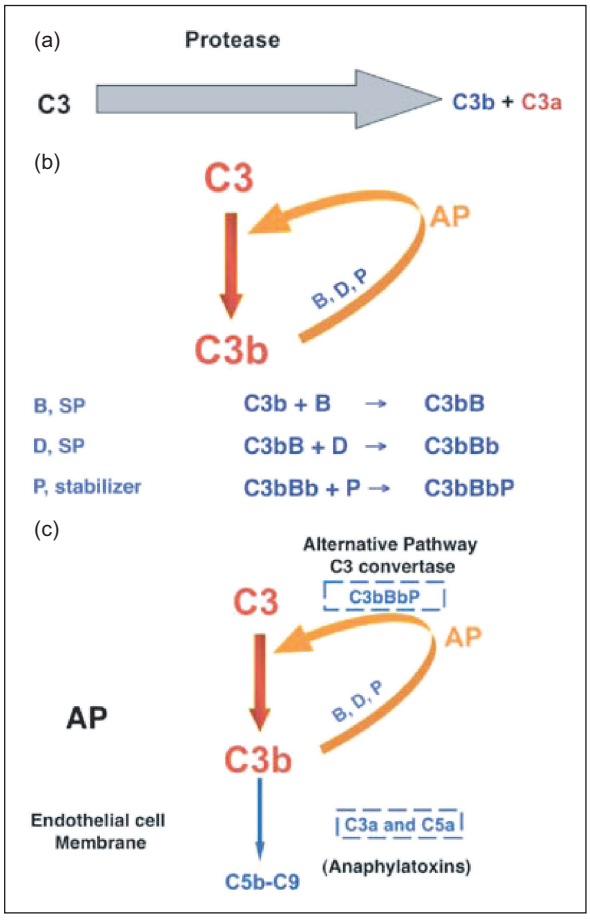

Figure 10-3 The alternative pathway of complement activation. (a) The AP of the complement system originally consisted of a serine protease that cleaved C3 to the opsonin C3b and the proinflammatory anaphylatoxin C3a. (b) An amplification loop was next evolved to more efficiently deposit C3b on a target and liberate C3a into the surrounding milieu. B indicates factor B, D indicates factor D, a serine protease; P, properdin, a stabilizer of the enzyme. (c) Development of a C5 convertase. The same enzyme that cleaves C3 (AP C3 convertase) can cleave C5 to C5a and C5b with the addition of a second C3b to the enzyme complex (AP C5 convertase). Reproduced from Liszewski MK, Atkinson JP. Too much of a good thing at the site of tissue injury: the instructive example of the complement system predisposing to thrombotic microangiopathy. *Hematology: Am Soc Hematol Educ Program.* 2011;2011:9-14. AP = alternative complement pathway; GP =glycoprotein.

Diagnosis

The diagnosis of TMA requires clinical awareness and prompt recognition of symptoms. TTP is more common in females, with a peak incidence in the fourth decade; other risk factors include obesity and African ancestry. The diagnosis of TTP can be assumed in patients with MAHA and thrombocytopenia without another apparent etiology, such as malignant hypertension, vasculitis, scleroderma renal crisis, tumor emboli, or disseminated intravascular coagulation. Fever and neurologic symptoms are less common, although evidence of renal involvement even in the absence of renal insufficiency sometimes can be obtained through examination of the urinary sediment. Schistocytes are invariably present and are accompanied by elevation of the LDH, which may be striking; levels of unconjugated bilirubin also may be increased. Nucleated red blood cells frequently are present. The PT, PTT, and fibrinogen levels are normal, and the D-dimer is normal or only mildly increased. The direct antiglobulin test is negative. Consideration of secondary causes of TTP should include a detailed drug history, HIV testing, and a focused search for autoimmune disease and malignancy. TTP often presents during pregnancy, particularly in the second and third trimesters. ADAMTS13 assays may be useful in confirming the diagnosis of TTP when severe deficiency (<5%) is present in the appropriate clinical setting, and provide prognostic information, with lower levels of ADAMTS13 and higher levels of anti-ADAMTS13 antibodies associated with higher relapse rates. Many patients with TMA and detectable or even normal ADAMTS13 levels, however, also respond to plasma exchange, and thus this therapy should not be withheld from such individuals. Moreover, recovery of ADAMTS13 levels during initial

plasma exchange may lag behind clinical responses and are not useful in determining the duration of plasma therapy.

Patients with aHUS may present acutely, mimicking TTP, or in some cases more insidiously with renal insufficiency as the primary symptom. Thrombocytopenia may be less severe in aHUS than TTP. A family history of similar disease may be apparent, although the low penetrance of complement inhibitor mutations may make such a history difficult to dissect. Exacerbations of disease may follow upper-respiratory infections and may be accompanied by fatigue and malaise. aHUS commonly presents in association with pregnancy, most commonly at 3-4 weeks postpartum. Complement levels in patients with aHUS may be decreased, but normal levels do not exclude aHUS. Sequencing of complement inhibitor proteins, factors B or C3, are useful in confirming a clinical impression of aHUS, but this sequencing is performed only in specialized laboratories and therefore not useful in the acute setting.

Typical HUS is the most common cause of acute renal failure in children and is most common in the pediatric population. This disorder, however, increasingly has been recognized in adults. The disease begins with abdominal pain and watery diarrhea 2-12 days after toxin exposure. This presentation may be difficult to differentiate from inflammatory bowel disease, appendicitis, ischemic colitis, or intussusception. Bloody diarrhea generally ensues on the second day, though up to one-third of patients do not report blood in the stool. Fever is typically absent or mild. The definitive diagnosis is made by culture of *E. coli* O157:H7 on sorbitol-MacConkey agar. The presence of Shigatoxin or its structural genes may be detected by enzyme immunoassay or PCR of the stool. Serologic studies demonstrating an increase in convalescent antibody titer to Shigatoxin or *E. coli* lipopolysaccharide may be useful in confirming the diagnosis.

Management

Plasma exchange is the standard of care for treatment of TMAs, particularly TTP. Untreated, TTP is associated with a mortality of approximately 85%, although 90% of patients with TTP treated with plasma exchange survive. The superiority of plasma exchange over infusion was demonstrated in a randomized Canadian trial of 103 adults with TTP, although patients randomized to the plasma exchange arm received more plasma. The exchange of 1 to 1.5 plasma volumes is standard initial treatment; however, larger volume exchanges may have additional benefit in patients with an inadequate response. Plasma exchange is continued daily until the platelet count reaches normal levels ($>150 \times 10^9$/L), LDH normalizes, and symptoms have resolved. Neurologic symptoms improve most rapidly. No evidence suggests a benefit of either abrupt discontinuation or tapering of plasma exchange. Antiplatelet agents have not been shown to be beneficial and may increase bleeding, although some guidelines advocate their use in patients in whom the platelet count increases rapidly during plasma exchange. Corticosteroids are used initially in most patients with TTP because of the presence of ADAMTS13 antibodies, although a significant benefit has not been demonstrated consistently in randomized studies. In recent years, the potential utility of rituximab in TTP has been revealed. In a single-arm study, the use of rituximab in patients who did not respond rapidly to plasma exchange (with plasma exchange continued), led to more rapid resolution of TTP and a lower incidence of relapse compared with historical controls. Other studies have demonstrated the apparent efficacy of rituximab in relapsed TTP and the disappearance of ADAMTS13 antibodies following treatment. Other adjunctive therapies for refractory TTP include immunosuppressive agents, such as cyclosporine and vincristine, as well as splenectomy, which may decrease relapse rates. Platelet transfusion has been associated with a rapid decline in clinical status in occasional patients, although a retrospective analysis could not identify a clear association of platelet transfusion with poor outcomes.

Plasma exchange historically has been the treatment of choice for aHUS as well as TTP, and it remains so in patients with TMA in whom a clear diagnosis of TTP or aHUS cannot be established. Response rates to plasma exchange in patients with aHUS are not as robust as in TTP. Eculizumab, an antibody against complement C5, has shown efficacy in patients with aHUS, leading to its approval for aHUS treatment in 2011. Thus, in established aHUS, eculizumab may be the treatment of choice, though its role in plasma exchange refractory TMA and related syndromes has not been established.

Treatment of *E. coli*–associated typical HUS is generally supportive; the use of antibiotics may lead to increased toxin release and should be avoided. Some patients may require dialysis during the acute phase of their illness. A benefit for plasma exchange in typical HUS has not been demonstrated. The use of Eculizumab in this disorder is under investigation.

Key points

- TTP, atypical HUS (aHUS), and typical (Shiga-like toxin; Stx) HUS share many common features and may be difficult to distinguish from one another.
- The pathogenesis of TTP involves deficiency of ADAMTS13, usually because of acquired autoantibodies that neutralize ADAMTS13 activity. This leads to accumulation of ultralarge vWF multimers that induce platelet agglutination in the microcirculation.
- The pathogenesis of most cases of aHUS involves excessive activation of the AP, leading to complement-mediated damage to vascular cells.

Key points (continued)

- The pathogenesis of typical HUS reflects the toxic effects of Shigatoxin on vascular endothelium and other cell types.
- The treatment of choice for TTP is plasma exchange.
- Plasma exchange is effective in some cases of aHUS, although therapies aimed at inhibition of activation of the AP (ie, Eculizumab) appear more effective.
- Plasma exchange is not effective in typical HUS, which is usually self-limited.
- Pregnancy is associated with an increased frequency of TTP (in the second and third trimesters) and aHUS (postpartum).

Splenic sequestration

Splenic enlargement, usually from advanced liver disease or cirrhosis, results in sequestration of platelets in the splenic vascular network, leading to mild to moderate thrombocytopenia. Typical platelet counts in patients with splenic sequestration are 60-100 $\times$ 10^9/L. Other mechanisms associated with liver disease that may induce thrombocytopenia include viral hepatitis–induced secondary ITP, suppression of platelet production by megakaryocytes resulting from direct viral infection, and decreased production of TPO by the cirrhotic liver. Therapy of chronic hepatitis with interferon-α also may induce thrombocytopenia.

Familial thrombocytopenia

Familial thrombocytopenic syndromes are uncommon, and patients often are misdiagnosed as having ITP. Recognition of these disorders is important to avoid unnecessary and potentially harmful treatments. The diagnosis should be considered in any patient with a family history of thrombocytopenia, or in patients with "ITP" who do not respond to standard therapy. The presence of anatomic defects, including absent radii (thrombocytopenia- absent radii [TAR] syndrome) or right-heart defects (DiGeorge syndrome), and laboratory features, including large platelets and neutrophil inclusions on the blood film (as seen in the *MYH9*-related disorders), support the diagnosis of familial thrombocytopenia.

Autosomal dominant *MYH9*-related macrothrombocytopenic disorders are caused by mutations in the *MYH9* gene, which codes for nonmuscle myosin IIA. These include May-Hegglin, Fetchner, Sebastian, and Epstein syndromes. Associated features include large platelets, Döhle bodies in neutrophils (Figure 10-4), renal failure, hearing loss, and cataracts. Bernard-Soulier syndrome (BSS) is an autosomal recessive familial thrombocytopenic disorder characterized by the absence of the platelet GPIb-IX complex that is associated with large platelets, lack of platelet aggregation by high-dose ristocetin, and bleeding. Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by severe immunodeficiency, small platelets, and eczema. Congenital amegakaryocytic thrombocytopenia (CAMT) is a recessive disorder characterized by severe thrombocytopenia and absence of megakaryocytes in the bone marrow that results from mutations in the Mpl receptor, and may lead to trilineage failure. Inherited thrombocytopenias also occur in association with mutations in specific transcription factors that regulate megakaryocyte and platelet production, including GATA1 (sex-linked inheritance) and RUNX1 (autosomal dominant). Patients with the Paris-Trousseau/Jacobsen syndrome, an autosomal dominant macrothrombocytopenia, have psychomotor retardation and facial and cardiac abnormalities; this syndrome arises because of the deletion of a portion of chromosome 11, 11q23-24, that encompasses the gene encoding the transcription factor friend leukemia integration 1 (FLI-1).

Establishing the diagnosis of familial thrombocytopenia may be difficult. Historically, demonstration of decreased expression of platelet GPIb-IX using flow cytometry has been used to diagnose BSS. Clustering of myosin in granulocytes using an immunofluorescent antibody against nonmuscle myosin heavy chain–type IIa may aid in screening for *MYH9*-related disorders. Improvements in sequencing technologies have allowed for the expansion of genetic analyses for BSS, *MHY9*-related thrombocytopenia, CAMT, GATA1-related thrombocytopenia, TAR syndrome, and WAS-associated thrombocytopenia, and several laboratories in the United States and Europe now provide these services (see http://www.genetests.org).

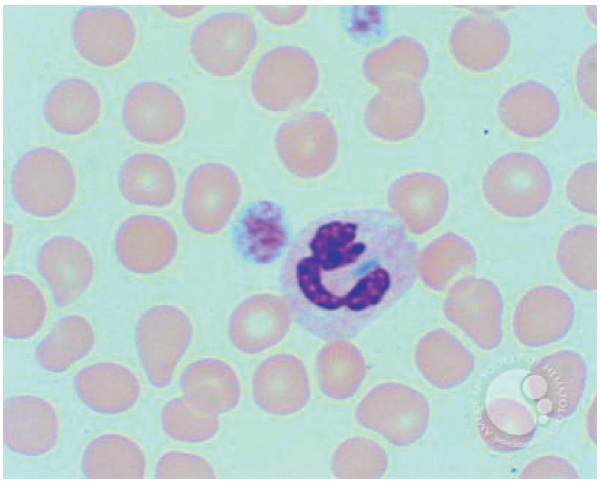

Figure 10-4 May-Hegglin anomaly, an *MYH9*-associated disorder. This peripheral blood film demonstrates a giant platelet, the size of which is similar to neighboring red blood cells. Immediately adjacent is a neutrophil containing a blue Döhle body between the nuclear lobes. Döhle bodies also are seen in infection, where they are located more commonly in the cell periphery, as opposed to the one seen in this figure. From ASH Image Bank #00003385 (submitted by Julia Braza).

Key points

- Splenic sequestration is a common cause of thrombocytopenia in patients with liver disease.
- Failure to respond to standard ITP therapy (corticosteroids, IVIg) should prompt consideration of an inherited thrombocytopenia.
- Genetic diagnosis of inherited thrombocytopenia should be obtained when possible.

Infection-associated thrombocytopenia

Mild and transient thrombocytopenia occurs with many systemic infections. Thrombocytopenia may be caused by a combination of mechanisms, including decreased platelet production, increased destruction, and increased splenic sequestration. In viral infections, infection of megakaryocytes may lead to suppression of platelet production; in rickettsial infections, platelets may be consumed in vasculitic lesions; in bacteremia, platelet consumption may result from DIC or enhanced clearance of immune complex–coated platelets. Thrombocytopenia commonly is associated with HIV and hepatitis C virus infection, both causes of secondary ITP. In HIV, thrombocytopenia usually responds to treatment with highly active antiretroviral therapy.

A rare and unusual manifestation of infection-related thrombocytopenia is the hemophagocytic syndrome, also known as hemophagocytic lymphohistiocytosis (HLH). This disorder may be inherited, occur in conjunction with rheumatologic disease, or occur in response to infection, with Epstein-Barr virus (EBV) being the most common cause. HLH is more common in children and is characterized by persistent activation of macrophages and cytotoxic T-cells, leading to damage of multiple organ systems. Thrombocytopenia occurs in most patients, but usually in the context of bicytopenias and pancytopenia. Diagnosis rests on meeting specific clinical criteria, as well as markedly elevated levels of ferritin and the circulating α-chain of the interleukin-2 (IL-2) receptor. Demonstration of hemophagocytosis on tissue or bone marrow biopsies is useful but not required. Therapy is directed toward eradication of EBV-infected cells, generally using multiagent approaches.

Thrombocytopenia in the critically ill

This topic is covered in greater detail in Chapter 1. Approximately 40% of patients in medical or surgical intensive care units (ICUs) develop a platelet count <150,000/μL; 20%-25% develop a platelet count <100,000/μL, and 12%-15% develop severe thrombocytopenia, with a platelet count <50,000/μL. The development of thrombocytopenia in patients in the ICU is a strong independent predictor for ICU mortality. The spectrum of disorders that cause thrombocytopenia in this setting is extensive and includes drugs, infection, and immune disorders, including ITP, DIC, and TMAs, among others. No high-quality evidence is available to guide the management of such patients, but acutely ill individuals with thrombocytopenia often require platelet transfusion. The optimal threshold for platelet transfusion in this population is uncertain, but platelets generally are given when the platelet count decreases to ~20 × 10^9/L. Bleeding or a planned invasive procedure should prompt consideration of platelet transfusion at a higher platelet count.

Key points

- Infection is a common cause of thrombocytopenia, particularly in ICU patients, that can be induced by a variety of organisms.
- The diagnosis of HLH is based on clinical criteria, as well as elevated levels of ferritin and the circulating IL-2 receptor α-chain

Disorders of platelet function

Disorders of platelet function are characterized by highly variable mucocutaneous bleeding manifestations and excessive hemorrhage following surgical procedures or trauma. Spontaneous hemarthrosis and deep hematomas are unusual in patients with platelet defects. In general, most patients have mild to moderate bleeding manifestations. A majority of patients, but not all, have a prolonged bleeding time. Platelet aggregation and secretion studies provide evidence for the defect but generally are not predictive of the severity of clinical manifestations. Defects in platelet function may be inherited or acquired, with the latter being far more commonly encountered. The platelet dysfunction in these patients arises by diverse mechanisms.

Inherited disorders of platelet function

Clinical case

A 9-year-old girl is referred by her pediatrician for evaluation of long-standing easy bruising and recurrent epistaxis. She has not had any surgery. The physical examination reveals scattered bruises on the lower extremities. The platelet count is 190,000/μL, and the hemoglobin is 11 g/dL. The bleeding time is prolonged at 14 minutes (normal range, 3-7 minutes), and plasma levels of factor VIII, vWF antigen, and ristocetin cofactor are within normal range. Previous blood work had demonstrated normal platelet counts. The hematologist recommends platelet aggregation studies. These studies reveal abnormal platelet aggregation responses upon activation—a primary wave but no secondary wave in response to ADP and epinephrine and decreased aggregation with collagen. The response to ristocetin is normal. The hematologist discusses the diagnosis and management with the parents.

Table 10-7 provides a classification of inherited disorders associated with impaired platelet function, based on the platelet function or responses that are abnormal (Figure 10-1). Of note, not all of these disorders are due to a defect in the platelets per se. Some, such as vWD and afibrinogenemia, result from deficiencies of plasma proteins essential for platelet aggregation or adhesion. Some of these disorders are distinctly rare, but they shed enormous light on platelet physiology. Moreover, in many patients with inherited abnormalities in platelet aggregation responses, the underlying molecular mechanisms remain unknown. In patients with defects in platelet–vessel wall interactions (adhesion disorders), adhesion of platelets to subendothelium is abnormal. The two disorders in this group are vWD, resulting from a deficiency or abnormality in plasma vWF, and BSS, in which platelets are deficient in GPIb (and GPV and GPIX); in both disorders, platelet–vWF interaction is compromised. Binding of fibrinogen to the GPIIb-IIIa complex is a prerequisite for platelet aggregation. Disorders characterized by abnormal platelet–platelet interactions (aggregation disorders) arise because of a severe deficiency of plasma fibrinogen (congenital afibrinogenemia) or because of a quantitative or qualitative abnormality of the platelet membrane GPIIb-IIIa complex, which binds fibrinogen (Glanzmann thrombasthenia). Patients with defects in platelet secretion and signal transduction are a heterogeneous group lumped together for convenience of classification rather than based on an understanding of the specific underlying abnormality. The major common characteristics in these patients, as currently perceived, are abnormal aggregation responses and an inability to release intracellular granule (dense) contents upon activation of platelet-rich plasma with agonists such as ADP, epinephrine, and collagen. In aggregation studies, the second wave of aggregation is blunted or absent.

Table 10-7 Inherited disorders of platelet function.

1. Defects in platelet-vessel wall interaction (disorders of adhesion)
   a. von Willebrand disease (deficiency or defect in plasma vWF)
   b. Bernard-Soulier syndrome (deficiency or defect in GPIb)
2. Defects in platelet-platelet interaction (disorders of aggregation)
   a. Congenital afibrinogenemia (deficiency of plasma fibrinogen)
   b. Glanzmann thrombasthenia (deficiency or defect in GPIIb-IIIa)
3. Disorders of platelet secretion and abnormalities of granules
   a. Storage pool deficiency (δαδ)
   b. Quebec platelet disorder
4. Disorders of platelet secretion and signal transduction
   a. Defects in platelet-agonist interaction (receptor defects) (ADP, Thromboxane A2, Collagen, Epinephrine)
   b. Defects in G-proteins (Gαq, Gαs, Gαi Abnormalities)
   c. Defects in phosphatidylinositol metabolism and protein phosphorylation
      • Phospholipase C-β2 Deficiency
      • PKC-θ deficiency
   d. Abnormalities in arachidonic acid pathways and thromboxane A_2 synthesis
      • Phospholipase A2 deficiency
      • Cyclooxygenase deficiency
      • Thromboxane synthase deficiency
5. Disorders of platelet coagulant-protein interaction (Scott syndrome)
6. Defects related to cytoskeletal/structural proteins
   a. Wiskott-Aldrich syndrome
   b. β1-Tubulin deficiency
7. Abnormalities of transcription factors leading to functional defects
   a. RUNX1 (familial platelet dysfunction with predisposition to acute myelogenous leukemia)
   b. GATA1
   c. FLI-1 (Dimorphic dysmorphic platelets with giant α-granules and thrombocytopenia; Paris-Trousseau/Jacobsen syndrome)

Modified with permission from Rao AK. Congenital disorders of platelet function: disorders of signal transduction and secretion. *Am J Med Sci.* 1998;316:69-77.

FLI-1 = friend leukemia integration 1; GATA1 = sex-linked inheritance; GPIb = glycoprotein Ib; PKC = protein kinase C; RUNX1 = autosomal dominant; vWF = von Willebrand factor.

The patient described in the clinical case at the beginning of this section falls in this heterogeneous large group of "platelet secretion defects"; the platelet dysfunction may arise from a variety of mechanisms. A small proportion of these patients have a deficiency of dense granule stores (storage pool deficiency). In other patients, the impaired secretion results from aberrations in the signal transduction events or in pathways leading to thromboxane synthesis that govern end-responses, such as secretion and aggregation. The findings on the aggregation studies are nonspecific, and it is difficult to establish a specific abnormality from the tracings. Another group consists of patients who have an abnormality in interactions of platelets with proteins of the coagulation system; the best described is the Scott syndrome, which is characterized by impaired transmembrane migration of procoagulant–PS during platelet activation. Defects related to platelet cytoskeletal or structural proteins also may be associated with platelet dysfunction. Recent studies document impaired platelet function associated with mutations in transcription factors (eg, RUNX1, GATA1, FLI-1) that regulate the expression of important platelet proteins. In addition to these groups, some patients have abnormal platelet function associated with systemic disorders, such as Down syndrome and the May-Hegglin anomaly, in which the specific aberrant platelet mechanisms are unclear. The prevalence and relative frequencies of the various platelet abnormalities remain unknown.

Disorders of platelet adhesion

Bernard-Soulier syndrome

BSS, a rare autosomal recessive platelet function disorder, results from an abnormality in the platelet GPIb-IX complex,

which mediates the binding of vWF to platelets and thus plays a major role in platelet adhesion to the subendothelium, especially at the higher shear rates. GPIb exists in platelets as a complex consisting of GPIb, GPIX, and GPV. There are approximately 25,000 copies of GPIb-IX on platelets, and these are reduced or abnormal in the BSS. Although GPV also is decreased in BSS platelets, it is not required for platelet surface GPIb-IX expression. The bleeding time is markedly prolonged, the platelet counts are moderately decreased, and on the peripheral smear, the platelets are markedly increased in size. In platelet aggregation studies, the responses to the commonly used agonists ADP, epinephrine, thrombin, and collagen are normal. Characteristically, the aggregation in platelet-rich plasma in response to ristocetin is decreased or absent, a feature shared with patients with vWD. This is because ristocetin-induced platelet clumping is mediated by binding of vWF to GPIb complex. Unlike in vWD, however, plasma vWF and factor VIII are normal in BSS, and the addition of exogenous vWF (present in plasma cryoprecipitate fractions) does not restore ristocetin-induced agglutination of platelets, because of the GPIb deficiency. Dense granule secretion on activation with thrombin may be decreased in these patients.

The blood film from a patient with BSS may resemble that from some patients with ITP in that the platelets tend to be larger than normal, and there is mild to moderate thrombocytopenia. The diagnosis of BSS is established by demonstrating decreased platelet surface GPIb, which can be performed using flow cytometry.

von Willebrand disease

See the section titled "von Willebrand disease" in Chapter 2.

Disorders of platelet aggregation

Glanzmann thrombasthenia

Glanzmann thrombasthenia is a rare autosomal recessive disorder characterized by markedly impaired platelet aggregation, a prolonged bleeding time, and relatively more severe mucocutaneous bleeding manifestations than most platelet function disorders. It has been reported in clusters in populations in which consanguinity is common. Normal resting platelets possess approximately 50,000-80,000 GPIIb-IIIa complexes on the surface. The primary abnormality in Glanzmann thrombasthenia is a quantitative or qualitative defect in the GPIIb-IIIa complex, a heterodimer consisting of GPIIb and GPIIIa whose synthesis is governed by distinct genes located on chromosome 17. Thus, thrombasthenia may arise due to a mutation in either gene, with decreased platelet expression of the complex. Because of this, fibrinogen binding to platelets on activation and aggregation are impaired. Clot retraction, a function of the interaction of GPIIb-IIIa with the platelet cytoskeleton, also is impaired.

Binding of fibrinogen to the GPIIb-IIIa complex on platelet activation is required for aggregation in response to all physiologic agonists. Thus, the diagnostic hallmark of thrombasthenia is the absence or marked decrease of platelet aggregation in response virtually to all platelet agonists (except ristocetin), with the absence of both the primary and the secondary wave of aggregation; the shape change response is preserved. Platelet-dense granule secretion may be decreased with weak agonists (eg, ADP) but is normal on activation with thrombin. Heterozygotes have approximately half the number of platelet GPIIb-IIIa complexes, but platelet aggregation responses are normal. Although congenital afibrinogenemia also is characterized by a similar absence of platelet aggregation, in this disorder, the prothrombin time, aPTT, and thrombin time are markedly prolonged, whereas they are normal in thrombasthenia. The diagnosis of thrombasthenia can be established by demonstrating decreased platelet expression of the GPIIb-GPIIIa complex using flow cytometry.

Disorders of platelet secretion and signal transduction

As a unifying theme, patients lumped in this remarkably heterogeneous group generally are characterized by impaired dense granule secretion and the absence of the second wave of aggregation upon stimulation of platelet-rich plasma with ADP or epinephrine; responses to collagen, thromboxane analog (U46619), and arachidonic acid also may be impaired. Conceptually, platelet function is abnormal in these patients either when the granule contents are diminished (storage pool deficiency [SPD]) or when the mechanisms mediating or potentiating aggregation and secretion are impaired (Table 10-7).

Deficiency of granule stores

SPD refers to patients with deficiencies in platelet content of dense granules (δ-SPD), α granules (α-SPD), or both types of granules (αδ-SPD).

Patients with δ-SPD have a mild to moderate bleeding diathesis associated with a prolonged bleeding time. In the platelet studies, the second wave of aggregation in response to ADP and epinephrine is absent or blunted, and the collagen response is impaired markedly. Normal platelets possess 3-8 dense granules (each 200-300 nm in diameter). Under the electron microscope, dense granules are decreased in SPD platelets. By direct biochemical measurements, the total platelet and granule ATP and ADP contents are decreased

along with other dense granule constituents, calcium, pyrophosphate, and serotonin.

δ-SPD has been reported in association with other inherited disorders, such as Hermansky-Pudlak syndrome (HPS) (oculocutaneous albinism and increased reticuloendothelial ceroid), Chediak-Higashi syndrome, WAS, TAR syndrome, and Griscelli syndrome. The simultaneous occurrence of δ-SPD and defects in skin pigment granules, as in the HPS, point to the interrelatedness of the two kinds of granules (platelet and melanosome) with respect to genetic control.

In a large group of HPS patients in northwest Puerto Rico, HPS occurs in 1 of every 1,800 individuals. There are at least seven known HPS-causing genes, with most patients having HPS-1 and being from Puerto Rico. These HPS subtypes are autosomal recessive, and the heterozygotes have no clinical findings. In addition to the albinism, most patients have congenital nystagmus and decreased visual acuities. Two additional manifestations in HPS patients are granulomatous colitis and pulmonary fibrosis.

Chediak-Higashi syndrome is a rare autosomal recessive disorder characterized by SPD, oculocutaneous albinism, immune deficiency, cytotoxic T, and natural killer (NK) cell dysfunction, neurologic symptoms, and the presence of giant cytoplasmic inclusions in different cells. It arises from mutations in the lysosomal trafficking regulator (*LYST*) gene on chromosome 1.

Patients with gray platelet syndrome have an isolated deficiency of platelet α-granule contents. The name refers to the initial observation that the platelets have a gray appearance with paucity of granules on the peripheral blood smears. These patients have a bleeding diathesis, mild thrombocytopenia, and a prolonged bleeding time. The inheritance pattern has been variable; autosomal recessive, autosomal dominant, and sex-linked patterns have been noted. Under the electron microscope, platelets and megakaryocytes reveal absent or markedly decreased α-granules. The platelets are severely and selectively deficient in α-granule proteins, including PF4, βTG, vWF, thrombospondin, fibronectin, factor V, and PDGF. In some patients, plasma PF4 and βTG are raised, suggesting that the defect is not in their synthesis by megakaryocytes but rather in their packaging into granules. Platelet aggregation responses have been variable. Responses to ADP and epinephrine have been normal in most patients; in some patients, aggregation responses to thrombin, collagen, and ADP have been impaired.

The Quebec platelet disorder, another disorder affecting the platelet granules, is an autosomal-dominant disorder associated with delayed bleeding and abnormal proteolysis of α-granule proteins (including fibrinogen, factor V, vWF, thrombospondin, multimerin, and P-selectin) resulting from increased amounts of platelet urokinase-type plasminogen activator. These patients are characterized by normal to reduced platelet counts, proteolytic degradation of α-granule proteins, and defective aggregation selectively with epinephrine.

Defects in platelet signal transduction and platelet activation

Signal transduction mechanisms encompass processes that are initiated by the interaction of agonists with specific platelet receptors and include responses such as G-protein activation and activation of phospholipase C and phospholipase A_2 (Figure 10-1). If the key components in signal transduction are the surface receptors, the G proteins, and the effector enzymes, evidence now exists for specific platelet abnormalities at each of these levels.

Patients with receptor defects have impaired responses because the platelet surface receptors for a specific agonist are decreased. Such defects have been documented for receptors for ADP, TxA_2, collagen, and epinephrine. Patients with the ADP receptor abnormalities have had a defect in the P2Y12 ADP receptor, which is coupled to inhibition of adenylyl cyclase and is the receptor targeted by thienopyridines (clopidogrel). Because ADP and TxA_2 play a synergistic role in platelet responses to several agonists, these patients with specific receptor defects manifest abnormal aggregation responses to multiple agonists. Patients described with abnormal platelet responses to collagen have had deficiencies in membrane GPs, GPIa, or GPVI.

G proteins are a link between surface receptors and intracellular effector enzymes, and defects in G protein activation can impair signal transduction. Patients with deficiencies at the level of Gαq, Gαi1, and Gαs have been described.

Patients have been described with impaired signal transduction resulting from defects in phospholipase C activation, calcium mobilization, and pleckstrin phosphorylation. Specific deficiencies at the level of phospholipase C-β2 and PKC-θ have been documented.

A major platelet response to activation is liberation of arachidonic acid from phospholipids and its subsequent oxygenation to TxA_2, which plays a synergistic role in the response to several agonists. Patients have been described with impaired thromboxane synthesis because of congenital deficiencies of phospholipase A_2, cyclooxygenase, and thromboxane synthase.

Disorders of platelet procoagulant activities

Platelets play a major role in blood coagulation by providing the surface on which several specific key enzymatic reactions occur. In resting platelets, there is an asymmetry in the distribution of some of the phospholipids such that PS and phosphatidylethanolamine are located predominantly on the

inner leaflet, whereas phosphatidylcholine has the opposite distribution. Platelet activation results in a redistribution with expression of PS on the outer surface, mediated by phospholipid scramblase. The exposure of PS on the outer surface is an important event in the expression of platelet procoagulant activities. A few patients have been described in whom the platelet contribution to blood coagulation is impaired, and this is referred to as Scott syndrome. In these patients, who have a bleeding disorder, the bleeding time and platelet aggregation responses have been normal.

Other abnormalities

Platelet function abnormalities have been described in association with other entities, such as in WAS, an X-linked inherited disorder affecting T-lymphocytes and platelets characterized by thrombocytopenia, immunodeficiency, and eczema. The bleeding manifestations are variable. Several platelet abnormalities, including dense granule deficiency and deficiencies of platelet GPIb, GPIIb-IIIa, and GPIa, have been reported in WAS. WAS arises from mutations in the gene coding for the WAS protein, which constitutes a link between the cytoskeleton and signaling pathways. Platelet dysfunction also occurs with mutations in tubulin-1, a cytoskeletal protein. More recently, abnormal platelet function has been documented in patients with mutations in transcription factors RUNX1 (also called core-binding factor A_2), GATA1, and FLI-1. Patients with RUNX1 mutations have familial thrombocytopenia, platelet dysfunction, and predisposition to acute leukemia.

Therapy of inherited platelet function defects

Because of the wide disparity in bleeding manifestations, management needs to be individualized. Platelet transfusions are indicated in the management of significant bleeding and in preparation for surgical procedures. Platelet transfusions are effective in controlling the bleeding manifestations but come with potential risks associated with blood products, including alloimmunization in patients lacking platelet GPs. For example, patients with Glanzmann thrombasthenia and BSS may develop antibodies against GPIIb-IIIa and GPIb, respectively, which compromise the efficacy of subsequent platelet transfusions. An alternative to platelet transfusions is intravenous administration of desmopressin (DDAVP), which shortens the bleeding time in some patients with platelet function defects, depending on the platelet abnormality. Most patients with thrombasthenia do not show a shortening of the bleeding time following DDAVP infusion, whereas responses in patients with signaling or secretory defects have been variable, with a shortening of the bleeding time in some patients. More recently, recombinant factor VIIa has been used in the management of bleeding events in patients with Glanzmann thrombasthenia and some other inherited defects. DDAVP and recombinant factor VIIa are not currently approved by the FDA for management of patients with inherited platelet defects; however, factor VIIa is approved in Europe to control bleeding in patients with thrombasthenia.

Key points

- Patients with inherited platelet defects typically have mucocutaneous bleeding manifestations; spontaneous hemarthrosis is rare.
- Patients with the BSS have thrombocytopenia, large platelet size, and a defect in platelet GPIb-V-IX complex, leading to impaired binding of vWF and adhesion.
- Patients with Glanzmann thrombasthenia have absent or decreased platelet GPIIb-IIIa, leading to impaired binding of fibrinogen and absent aggregation to all of the usual agonist except ristocetin.
- Patients with δ-storage pool deficiency have decreased dense granule contents; some patients may have associated albinism, nystagmus, and neurologic manifestations.
- Patients with the gray platelet syndrome have decreased α-granule contents.
- In a substantial number of patients with abnormal aggregation responses, the underlying mechanisms are unknown. Some of the patients have defects in platelet activation and signaling mechanisms.

Acquired disorders of platelet function

Alterations in platelet function occur in many acquired disorders of diverse etiologies (Table 10-8). The specific biochemical and pathophysiologic aberrations leading to platelet dysfunction are poorly understood in most of them. In some, such as the myeloproliferative neoplasms (MPN), there is production of intrinsically abnormal platelets by the bone marrow. In others, the dysfunction results from an interaction of platelets with exogenous factors, such as pharmacologic agents, artificial surfaces (cardiopulmonary bypass), compounds that accumulate in plasma due to impaired renal function, and antibodies. In these disorders of platelet dysfunction, the bleeding is usually mucocutaneous with a wide and unpredictable spectrum of severity. The usual laboratory tests that suggest a platelet dysfunction include a prolonged bleeding time and abnormal results in studies of platelet aggregation or the platelet function analyzer (PFA)-100. The bleeding time and the PFA-100 are not reliable discriminators, because these tests may be variably abnormal or normal, even in individuals with impaired platelet aggregation responses. In patients with acquired platelet dysfunction, the correlation between the

Table 10-8 Disorders in which acquired defects in platelet function are recognized.

Uremia
Myeloproliferative disorders
Acute leukemias and myelodysplastic syndromes
Dysproteinemias
Cardiopulmonary bypass
Acquired storage pool deficiency
Acquired von Willebrand disease
Antiplatelet antibodies
Drugs and other agents

abnormalities in platelet aggregation studies and clinical bleeding remains weak.

Myeloproliferative neoplasms

Bleeding tendency, thromboembolic complications, and qualitative platelet defects are all recognized in MPNs, which include essential thrombocythemia, polycythemia vera, chronic idiopathic myelofibrosis, and chronic myelogenous leukemia. The platelet abnormalities result from their development from an abnormal clone of stem cells, but some of the alterations may be secondary to enhanced platelet activation in vivo. The clinical impact of the in vitro qualitative platelet defects, which occur even in asymptomatic patients, often is unclear.

Numerous studies have examined platelet function and morphology in patients with MPN. Under the electron microscope, the platelet findings include reduction in dense and α-granules, alterations in the open canalicular and dense-tubular systems, and a reduction of mitochondria. The bleeding time is prolonged in a minority (17%) of MPN patients and does not correlate with an increased risk of bleeding. Platelet aggregation responses are highly variable in MPN patients and often vary in the same patient over time. Decreased platelet responses are more common, although some patients demonstrate enhanced responses to agonists. In one analysis, responses to ADP, collagen, and epinephrine were decreased in 39%, 37%, and 57% of patients, respectively. The impairment in aggregation in response to epinephrine is more commonly encountered than with other agonists; however, a diminished response to epinephrine is not pathognomonic of an MPN. Platelet abnormalities described in MPN include decreased platelet α_2-adrenergic receptors, TxA_2 production, and dense granule secretion and abnormalities in platelet surface expression of GPIIb-IIIa complexes, GPIb, and GPIa-IIa.

Platelets from patients with polycythemia vera and idiopathic myelofibrosis, but not essential thrombocythemia or chronic myelogenous leukemia, have been shown to have reduced expression of the TPO receptor (Mpl) and reduced TPO-induced tyrosine phosphorylation of proteins. MPN patients have been reported to have defects in platelet-signaling mechanisms.

An acquired decrease in plasma vWF has been documented in MPN patients with elevated platelet counts and may contribute to the hemostatic defect. Plasma vWF, particularly the large vWF multimers, is decreased, is inversely related to the platelet counts, and has improved following cytoreduction. These changes in plasma vWF occur in patients with reactive thrombocytosis as well.

Acute leukemias and myelodysplastic syndromes

The major cause of bleeding in these conditions is thrombocytopenia. In patients with normal or elevated platelet counts, however, bleeding complications may be associated with platelet dysfunction and altered platelet and megakaryocyte morphology. Acquired platelet defects associated with clinical bleeding are more common in acute myelogenous leukemia but also have been reported in acute lymphoblastic and myelomonoblastic leukemias, hairy cell leukemia, and myelodysplastic syndromes.

Dysproteinemias

Excessive clinical bleeding may occur in patients with dysproteinemias, and this appears to be related to multiple mechanisms, including platelet dysfunction, specific coagulation factor abnormalities, hyperviscosity, and alterations in blood vessels because of amyloid deposition. Qualitative platelet defects occur in some of these patients and have been attributed to coating of platelets by the paraprotein.

Uremia

Patients with uremia are at an increased risk for bleeding complications. The pathogenesis of the hemostatic defect in uremia remains unclear, but major factors include platelet dysfunction and impaired platelet–vessel wall interaction, comorbid conditions, and the concomitant use of medications that affect hemostasis. The bleeding time may be prolonged; anemia also contributes to the prolongation, which may shorten following red blood cell transfusion or treatment with erythropoietin.

Multiple platelet function abnormalities are recognized in uremia, including impaired adhesion, aggregation, and secretion. These hemostatic defects may be linked to the accumulation of dialyzable and nondialyzable molecules in the plasma. One such compound, guanidinosuccinic acid, accumulates in plasma, inhibits platelets in vitro, and stimulates generation of nitric oxide, which inhibits platelet responses by increasing levels of cellular cyclic guanosine monophosphate.

Aggressive dialysis ameliorates uremic bleeding diathesis in many patients. Hemodialysis and peritoneal dialysis are equally effective. Platelet transfusions are indicated in the management of acute major bleeds. Other treatments including DDAVP, cryoprecipitate, and conjugated estrogens also have been shown to be beneficial. Elevation of the hematocrit with packed red blood cells or recombinant erythropoietin may shorten bleeding times, improve platelet adhesion, and correct mild bleeding in uremic patients. The beneficial effect of red blood cells has been attributed to rheologic factors whereby the red blood cells exert an outward radial pressure promoting platelet–vessel interactions. Other factors predisposing to bleeding in patients with renal failure include concomitant administration of antiplatelet agents or anticoagulant medications.

Acquired SPD

Several patients have been reported in whom the dense granule SPD appears to be acquired. This defect probably reflects the release of dense granule contents because of in vivo platelet activation or production of abnormal platelets. Acquired SPD has been observed in patients with antiplatelet antibodies, systemic lupus erythematosus, chronic ITP, DIC, HUS, renal transplantation rejection, multiple congenital cavernous hemangioma, MPN, acute and chronic leukemias, and severe valvular disease, and in patients undergoing cardiopulmonary bypass.

Acquired von Willebrand disease

Acquired vWD (AvWD) is an often unrecognized bleeding disorder. Most patients are older (median age 62 years) without previous manifestations or a family history of a bleeding diathesis. The major associated disorders in these patients include lymphoproliferative disorder or plasma cell proliferative disorder, cardiac disease, MPN, and autoimmune disorders. Patients with MPN and reactive thrombocytosis demonstrate an impressive correlation between the plasma vWF abnormalities and elevated platelet counts. AvWD has been documented in patients with severe aortic stenosis and congenital valvular heart disease and in those with left-ventricular assist devices (LVAD), due to shear-stress induced loss of the high–molecular weight multimers of vWF from plasma. Laboratory findings for AvWD have included various combinations of a prolonged bleeding time, decreased plasma levels of vWF and factor VIII, and, most important, a selective reduction in the large vWF multimers in many of these disorders. The goals of treatment in AvWD are first to raise plasma vWF levels (DDAVP or vWF-containing factor VIII concentrates) to treat or prevent bleeding, and second to address the underlying associated conditions.

Antiplatelet antibodies and platelet function

Binding of antibodies to platelets may produce several effects, including accelerated destruction, platelet activation, cell lysis, aggregation, secretion of granule contents, and outward exposure of phosphatidylserine. Patients with ITP have decreased platelet survival and some may have impaired platelet function and prolonged bleeding times even at adequate counts. In ITP patients, the antibodies are directed against specific platelet surface membrane GPs that play a major role in normal platelet function, including GPIb, GPIIb-IIIa, GPIa-IIa, and GPVI, and glycosphingolipids. Some of these antibodies may affect platelet function.

Drugs that inhibit platelet function

Many drugs affect platelet function. For several, the effects on platelets have been studied in vitro, and the relevance of such findings to the drug levels achieved in clinical practice is not well established. Even among those drugs shown to alter platelet responses ex vivo, the impact on hemostasis often remains unclear. Moreover, the impact of concomitant administration of multiple drugs, each with a mild effect on platelet function, is unknown, although this may be clinically relevant. Because of their widespread use, aspirin and nonsteroidal anti-inflammatory agents are an important cause of platelet inhibition in clinical practice. Aspirin ingestion results in the inhibition of platelet aggregation and secretion upon stimulation with ADP, epinephrine, and low concentrations of collagen. Aspirin irreversibly acetylates and inactivates the platelet cyclooxygenase (COX-1), leading to the inhibition of synthesis of endoperoxides (prostaglandin G_2 and H_2) and TxA_2. Typically, it is recommended to wait 5-7 days after cessation of aspirin ingestion to perform studies intended to assess platelet function and elective invasive procedures to ensure that the antiplatelet effect is gone. Several other nonsteroidal anti-inflammatory drugs also impair platelet function by inhibiting the cyclooxygenase enzyme and may prolong the bleeding time. Compared with aspirin, the inhibition of cyclooxygenase by these agents generally is short-lived and reversible (1-2 days). Cyclooxygenase-2 inhibitors do not inhibit platelet aggregation responses.

Ticlopidine, clopidogrel, and prasugrel are orally administered thienopyridine derivatives that inhibit platelet function by inhibiting the binding of ADP to the platelet P2Y12 receptor. These drugs prolong the bleeding time and inhibit platelet aggregation responses to several agonists, including ADP, collagen, epinephrine, and thrombin, to various extents depending on agonist concentrations. GPIIb-IIIa receptor antagonists are compounds that inhibit platelet fibrinogen binding and platelet aggregation. These include a monoclonal antibody against the GPIIb-IIIa receptor (abciximab, ReoPro), a synthetic peptide

containing the RGD sequence (eptifibatide, Integrilin), and a peptidomimetic (tirofiban, Aggrastat). They are potent inhibitors of aggregation in response to all of the usual used agonists (except ristocetin) and prolong the bleeding time. DITP (secondary to drug-dependent antibodies) occurs in 0.2%-1.0% of patients on first exposure to GPIIb-III antagonists.

A host of other medications and agents, including oncologic drugs (eg, mithramycin) and food substances, inhibit platelet responses, but the clinical significance for many is unclear. β-Lactam antibiotics, including penicillins and cephalosporins, inhibit platelet aggregation responses and may contribute to a bleeding diathesis at high doses. These include carbenicillin, penicillin G, ticarcillin, ampicillin, nafcillin, azlocillin, cloxacillin, mezlocillin, oxacillin, piperacillin, and apalcillin. The platelet inhibition appears to be dose dependent, taking approximately 2-3 days to manifest and 3-10 days to abate after drug discontinuation. Cephalosporins also may impair platelet function. Moxalactam has been reported to induce platelet dysfunction associated with prolonged bleeding times and clinical hemorrhage. Other third-generation cephalosporins appear to show little effect on normal platelet function. The clinical significance of the effect of antibiotics on platelet function remains unclear. The general context in which the bleeding events are encountered in patients on antibiotics prevents identification of the precise role played by the antimicrobials because of the presence of concomitant factors (eg, thrombocytopenia, DIC, infection, vitamin K deficiency). Discontinuation of a specifically indicated antibiotic usually is not an option or necessary.

Evidence is growing that selective serotonin reuptake inhibitors (SSRIs) inhibit platelet function, and this has clinical relevance. Serotonin in plasma is taken up by platelets, incorporated into dense granules, and secreted on platelet activation. The SSRIs inhibit the uptake of serotonin and platelet aggregation and secretion responses to activation. In epidemiologic studies, patients on SSRIs have had increased gastrointestinal bleeding and increased bleeding with surgery. Last, given the increasing use of herbal medicines and food supplements, their role and interaction with pharmaceutical drugs need to be considered in the evaluation of patients with unexplained bleeding.

Key points

- Alterations in platelet function are described in many disorders of diverse etiologies; the clinical significance in terms of relationship to bleeding manifestations remains unclear in many.
- A careful drug history should be taken in any patient suspected to have platelet dysfunction.
- Aspirin, nonsteroidal anti-inflammatory agents, and other medications are a major cause of acquired platelet dysfunction.
- Patients with MPN may have altered platelet function that contributes to the bleeding manifestations.

Key points (continued)

- High platelet counts, as observed in MPN patients, may be associated with a loss of high–molecular weight multimers of vWF in plasma.
- Patients with renal failure may have impaired platelet function related to accumulation of substances in plasma that inhibit platelet function. Vigorous dialysis is a major part of management of the platelet dysfunction in these patients.

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