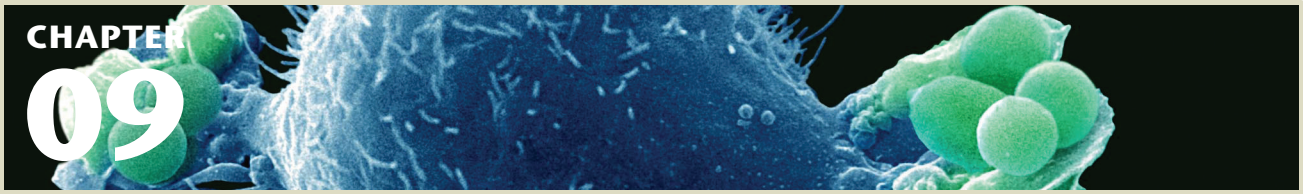

CHAPTER 09

Bleeding disorders

Jorge A. Di Paola and Amy D. Shapiro

Overview of hemostasis

Hemostasis is the process through which bleeding is controlled at a site of damaged or disrupted endothelium and is a dynamic interplay between the subendothelium, endothelium, circulating cells, and plasma proteins. Immediately after vessel injury, plasma and cellular components are recruited and activated to reduce bleeding and initiate tissue repair. The hemostatic process often is divided into three phases: the vascular, platelet, and plasma phases. Although it is helpful to divide coagulation into these phases for purposes of understanding, in vivo, they are intimately linked and occur in a continuum. The *vascular phase* is mediated by the release of locally active vasoactive agents that result in vasoconstriction at the site of injury and reduced blood flow. Vascular injury exposes the underlying subendothelium and procoagulant proteins, including tissue factor and collagen that then come into contact with blood. Platelets bind to von Willebrand factor (vWF) incorporated into the subendothelial matrix through their expression of glycoprotein Iba (GPIba). Platelets bound to vWF form a layer across the exposed subendothelium, a process termed *platelet adhesion*, and subsequently are activated via receptors, such as the collagen receptors glycoprotein (GPVI) and integrin $\alpha_2\beta_1$, resulting in calcium mobilization, activation of the fibrinogen receptor, integrin $\alpha_{IIb}\beta_3$, and subsequent platelet aggregation (Figure 9-1). For a detailed discussion of platelet function, please see Chapter 10.

The *plasma phase* of coagulation is initiated through the exposure of tissue factor (TF) in the subendothelium and on damaged endothelial cells. TF binds to the small amounts of circulating activated factor VII (FVIIa), resulting in formation of the TF:FVIIa complex, also known as the extrinsic tenase complex; this complex binds to and activates factor X (FX) to activated FX (FXa). The TF:FVIIa:FXa complex converts a small amount of prothrombin to thrombin, resulting in an initial thrombin burst sufficient to cleave factor VIII (FVIII) from vWF and to generate an amplification loop through activation of clotting factors, including FVIII, factor IX (FIX), and factor XI (FXI). These reactions include platelet activation, resulting in the expression of surface platelet factor V (FV) and activation of FV to FVa; activated FIX (FIXa) generated through these noted reactions binds to the surface of activated platelets. Activated FVIII complexed with FIXa forms the potent intrinsic tenase complex, resulting in the conversion of large amounts of FX to FXa, which in association with FVa on the activated platelet surface, results in a thrombin burst sufficient to convert fibrinogen to fibrin (Figure 9-2) and to result in subsequent normal clot formation. The formed clot is stabilized by the thrombin-mediated activation of factor XIII (FXIII) and thrombin-activatable fibrinolysis inhibitor (TAFI). Ultimately, the clot

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Off-label drug use: *Dr. Di Paola:* rFVIIa for management of bleeding in hemophilia at doses and regimens that are not approved and for other off-label indications; prothrombin complex concentrates for treatment of warfarin overdose, vitamin K deficiency, and liver disease. *Dr. Shapiro:* rFVIIa for high-dose therapy and rare disorders.

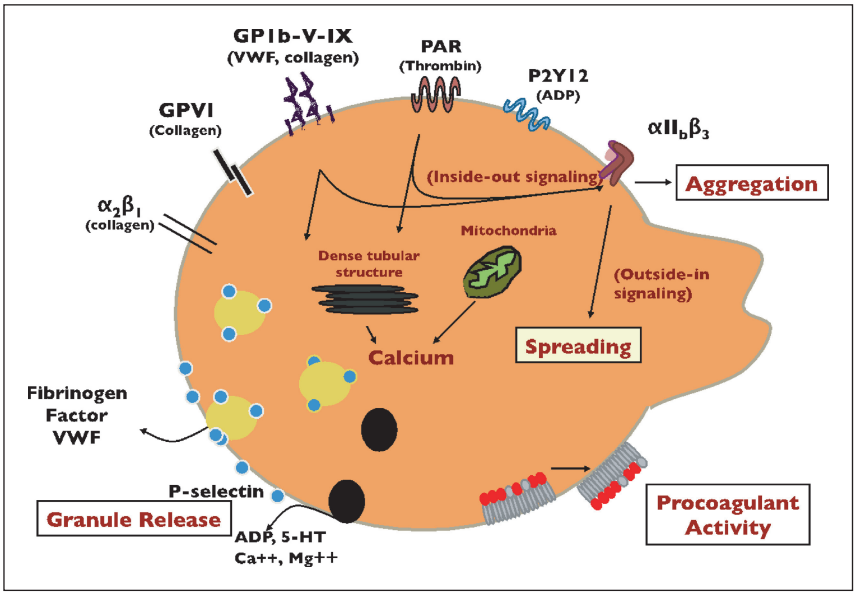

Figure 9-1 Platelet activation. Platelets can undergo activation through stimulation by soluble agonists, such as thrombin, or by contact (adherence) to the subendothelial matrix. This simplified cartoon shows several platelet components, including receptors and granules as well as the pathways of activation and the effect on platelet responses, such as aggregation, spreading, granule release, and procoagulant activity.

undergoes fibrinolysis, resulting in the restoration of normal blood vessel architecture. The fibrinolytic process is initiated by the release of tissue plasminogen activator (tPA) near the site of injury. tPA converts plasminogen to plasmin, which (via interactions with lysine and arginine residues on fibrin) cleaves the fibrin into dissolvable fragments.

Both the hemostatic and fibrinolytic processes are regulated by inhibitors that limit these processes to the site of injury and quench the reactions to prevent systemic activation and pathologic propagation. The hemostatic system has three main inhibitory pathways mediated through antithrombin: the protein C–protein S complex and the tissue factor pathway inhibitor (TFPI). TFPI is a protein produced by endothelial cells that inhibits the TF:FVIIa complex and FXa. Binding to FXa is required for the inhibitory effect on TF:FIIa. Antithrombin released at the margins of endothelial injury binds in a 1:1 complex with thrombin, inactivating thrombin not bound by the developing clot. Excess free thrombin at the clot margins binds to thrombomodulin, a receptor expressed on the surface of intact endothelial cells that when complexed with thrombin activates protein C; activated protein C complexes with protein S and inactivates activated FVa and FVIIIa. This negative feedback results in reduced subsequent thrombin generation and quenching of fibrin generation. The fibrinolytic system also includes inhibitors, principally plasminogen activator inhibitor-1 (PAI-1), which as its name implies, inhibits tPA, and α_2-antiplasmin (α_2AP), which inhibits plasmin.

This chapter is devoted to a discussion of the pathophysiology, clinical presentation, diagnosis, prognosis, and treatment of hemostatic abnormalities, hereafter referred to as bleeding disorders. The first section will review the approach

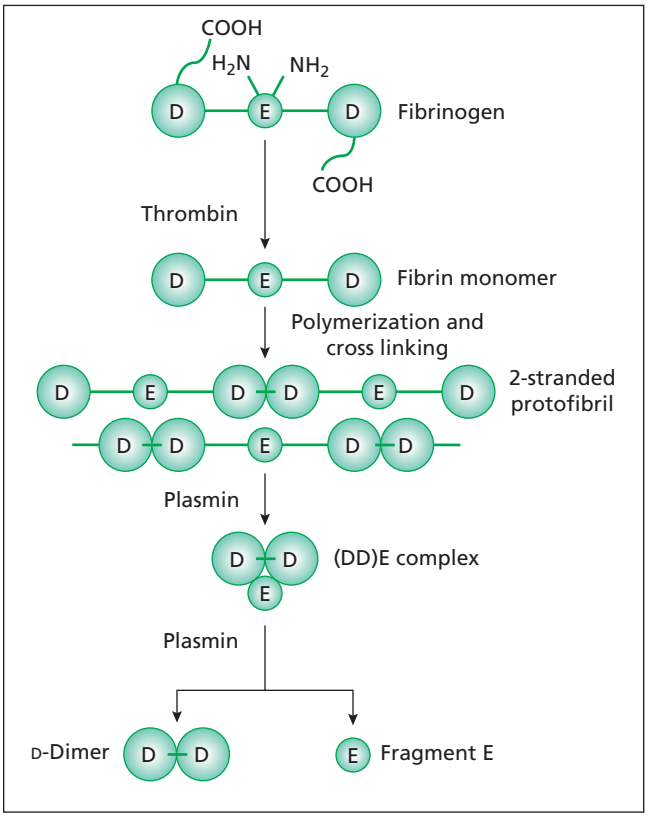

Figure 9-2 Fibrin formation and degradation. Fibrinogen has a trinocular structure with a central E and two D domains. Thrombin cleaves fibrinopeptides A and B from the NH_2 terminal of the A1 and BJ chains, respectively, located in the E domain. The resultant fibrin monomers polymerize nonenzymatically forming protofibrils. Factor XIIIa cross-links the D domains of adjacent fibrin monomers. Plasmin degrades cross-linked fibrin, thereby generating (DD)E complexes composed of an E fragment noncovalently bound to D-dimer. With further plasmin attack, the (DD) E complex is degraded into fragment E and D-dimer.

to a patient with excessive bleeding followed by a discussion of the specific disorders.

Key points

- Hemostasis is a complex and highly regulated process involving the subendothelium, endothelial cells, circulating cells, and plasma proteins that include both positive and negative feedback mechanisms.
- Defects in primary hemostasis (platelets and vWF) typically result in mucocutaneous bleeding symptoms.
- Defects in coagulation factors cause variable symptomatology but may result in deep tissue bleeding, including intramuscular hematomas, hemarthroses, retroperitoneal, and, occasionally, central nervous system bleeding events.

Approach to the patient with excessive bleeding

Excessive bleeding may occur in both male and female patients of all ages and ethnicities. Symptoms can begin as early as the immediate newborn period (uncommonly even in utero) or anytime thereafter. The bleeding symptoms experienced are related in large part to the specific factor and level of deficiency; bleeding can be spontaneous, that is, without an identified trigger, or may occur after a hemostatic challenge, such as delivery, injury, trauma, surgery, or the onset of menstruation. Furthermore, bleeding symptoms may be confined to specific anatomic sites or may occur in multiple sites. Finally, bleeding symptoms may be present in multiple family members or may occur in the absence of a family history. All of this information is important to arrive at a correct diagnosis rapidly and with minimal yet correctly sequenced laboratory testing. Thus, a detailed patient and family history is a vital component of the approach to each patient with a potential bleeding disorder.

Importance of medical history

Obtaining a detailed patient and family history is crucial regardless of prior laboratory testing. The history includes a detailed discussion of specific bleeding and clinical symptoms. Information regarding bleeding symptoms should include location, frequency, and pattern as well as duration both in terms of symptom appearance and time required for cessation. The location may suggest the part of the hemostatic system affected; patients with disorders of primary hemostasis (platelets and vWF) often experience mucocutaneous bleeding, including easy bruising, epistaxis, and menorrhagia in women of childbearing age, whereas patients with disorders of secondary hemostasis (coagulation factor deficiencies) may experience deep-tissue bleeding, including the joints, muscles, and central nervous system. The bleeding pattern and duration of each episode, particularly for mucus membrane bleeding, assist in the determination of the likelihood of the presence of an underlying bleeding disorder. The onset of symptoms can suggest the presence of a congenital versus acquired disorder. Although congenital conditions can present at any age, it is more likely that patients with a long history of symptoms or symptoms that begin in childhood have a congenital condition, whereas patients whose onset occurs at an older age are more likely to have an acquired condition. Congenital clotting factor deficiencies that do not present until later in life do occur and include mild factor deficiencies and coagulation factor deficiencies associated with variable bleeding patterns, most notably FXI deficiency. Additional important information to be collected includes the current use of medications and herbal supplements as these may affect the hemostatic system; the presence or absence of a family history of bleeding; a history of hemostatic challenges, including surgery, dental procedures, and trauma; and a menstrual history in females. The goal at the end of the history is to establish the likelihood of a bleeding disorder, as this will guide the direction of the laboratory investigation. Quantification of clinical bleeding is a challenge, particularly in the outpatient setting. In recent years, several bleeding assessment tools have been developed to more accurately differentiate bleeding phenotypes in healthy individuals and in patients with bleeding disorders. It is likely that in the future these assessment tools will significantly affect the evaluation of patients. These assessment tools, however, require validation in prospective studies. For now, the personal bleeding history is critical to guide laboratory testing; in addition, a positive family history serves as supportive evidence for a hereditary bleeding disorder, but its absence does not rule this out.

Screening tests

The laboratory evaluation for bleeding includes performance of initial screening tests. Specific factor analyses are performed after mixing studies reveal a correction of prolonged coagulation screening test(s) indicative of a deficiency state or in the face of normal screening tests with a positive history. Screening tests are not sensitive to all abnormalities associated with a bleeding disorder, including vWF, mild FIX, FXIII, PAI-1, and α_2AP deficiencies; therefore, a patient history strongly suggestive of a bleeding disorder may warrant testing for such deficiencies, including rare abnormalities regardless of screening test results. The most common screening tests utilized include the platelet count, prothrombin time (PT), and activated partial thromboplastin time (aPTT). When the PT or aPTT are prolonged, mixing studies are required via a one-to-one mix of patient plasma with known normal standard plasma. Test correction in the mixing study indicates a deficiency state,

whereas lack of correction indicates an inhibitor, either one directed against a specific factor or a global inhibitor as best exemplified by a lupus anticoagulant.

Screening tests also are utilized to identify individuals with a high likelihood of von Willebrand disease (vWD) or platelet disorders. The bleeding time, once widely used, has become obsolete because of the lack of sensitivity and specificity. The PFA–100® (platelet function assay) has been proposed to have a role in screening individuals with suspected platelet dysfunction or vWD. Initial studies demonstrated the efficacy of the PFA-100® in the evaluation of patients with known severe platelet disorders or vWD. The PFA-100® induces high shear stress and simulates primary hemostasis by flowing whole blood through an aperture with a membrane coated with collagen and either adenosine diphosphate (ADP) or epinephrine. Platelets adhere to the collagen-coated surface and aggregate forming a platelet plug that enlarges until it occludes the aperture, causing cessation of blood flow. The time to cessation of flow is recorded as closure time (CT). The sensitivity and specificity of the CT of the PFA-100® were reported as 90% for severe platelet dysfunction or vWD, with vWF plasma levels below 25%. The utility of the PFA-100® as screening tool, however, has been challenged based on the reported low sensitivity (24%-41%) of the device in individuals with mild platelet secretion defect or storage pool disorders.

It is likely that by the time patients are referred to a hematologist that some, if not all, of the previously mentioned tests may have been performed. Screening tests are sensitive to handling, may vary in reliability based on laboratory, and may be influenced by medications. Repeating these laboratory tests often is required; if possible, it is best to discontinue medications known to affect their results. Therefore, although screening tests are used widely to identify hemostatic abnormalities associated with bleeding, they are not perfect. Therefore, the clinical suspicion for a bleeding disorder is critical to determine extent of the laboratory investigation.

Key points

- Patients with bleeding disorders occasionally may present for evaluation before symptom onset, especially in the presence of a known family history or abnormal screening laboratory tests.
- Patients with bleeding disorders can present at any age with bleeding in a variety of sites. The more severe disorders tend to present earlier in life and with bleeding symptoms that often are spontaneous or in such areas as the joints, muscles, or central nervous system.
- The approach to patients with a potential bleeding disorder requires a detailed personal and family history and involves the use of screening laboratory tests, mixing studies when results are abnormal, and subsequent further specific coagulation factor testing.
- Some patients with a history or physical examination indicative of a bleeding disorder may have a normal laboratory evaluation.

Disorders of primary hemostasis

Platelet function disorders

Pathophysiology

Platelets play a key role in primary hemostasis both by constituting the cellular structure for the primary hemostatic plug and by providing a phospholipid surface upon which plasma coagulation proteins bind and form complexes. Low platelets or impaired platelet function may result in bleeding; thrombocytopenic and platelet function defects are reviewed in detail in Chapter 10. Abnormalities in platelet function can occur in any of the multitude of processes required for normal platelet function, including defects in receptor number or function, signaling, and granule content and secretion. An overview of platelet pathophysiology is important to the understanding of described platelet function defects.

A simplified cartoon with the platelet major receptors and activation responses is shown in Figure 9-1. Platelet activation is the result of multiple signaling pathways that culminate into the activation of the fibrinogen receptor integrin $\alpha_{IIb}\beta_3$, an integrin that normally exists in a resting (low-affinity) state but that transforms into an activated (high-affinity) state when stimulated by the appropriate signal transduction cascade. Activated $\alpha_{IIb}\beta_3$, then mediates platelet aggregation and promotes stable thrombus formation. This activation occurs following vascular injury when subendothelial collagen engages $\alpha_2\beta_1$ and GPVI receptors, and turbulent shear stress promotes vWF binding to GPIba-IX-V. A process known as inside-out signaling follows this platelet surface receptor stimulation, leading to activation of $\alpha_{IIb}\beta_3$ and resulting in affinity modulation during thrombus initiation. This conformational change allows engagement of bipolar fibrinogen by multiple $\alpha_{IIb}\beta_3$ integrins, resulting in platelet aggregation. Subsequently, outside-in signaling is initiated when ligand-occupied $\alpha_{IIb}\beta_3$ integrins cluster during aggregation by binding fibrinogen, fibrin, or vWF and trigger signals that stabilize the aggregate, leading to activation responses, including granule release, platelet spreading, and clot retraction. During this multistep process, platelets also become activated through binding of agonists, such as ADP or thrombin, and secrete granular contents that enhance vasoconstriction and further platelet aggregation. Finally, the platelet membrane exposes negatively charged phospholipids, the surface upon which the plasma clotting factors bind and form the fibrin meshwork.

Etiology

Although this section briefly encompasses some of the most well-described defects, a full review of platelet function

defects is included in Chapter 10, and a number of excellent review articles addressing this topic are available.

Defects at any stage of the platelet activation process can result in platelet dysfunction and subsequent bleeding. For example absence or functional defects in GPIba results in Bernard-Soulier syndrome, whereas a gain of function mutation in the same receptor is associated with excess binding of vWF, resulting in platelet-type vWD, a rare bleeding disorder. Defects in the production, storage, and secretion of vasoactive and hemostatic molecules result in excessive bleeding. Such disorders are exemplified by the δ-storage pool defect, which is associated with reduced secretion of ADP, and the gray platelet syndrome, a defect in α-granule formation. A defect in or absence of $\alpha_{IIb}\beta_3$ results in Glanzmann thrombasthenia, a severe platelet function defect. Most platelet function defects are diagnosed via standard assays. Identification of the causative defect or its presence in multiple family members implies a genetic abnormality.

Acquired platelet defects are most commonly the result of medications or herbal supplements, chronic medical conditions such as uremia, or the result of medical interventions such as cardiopulmonary bypass. The list of medications associated with platelet dysfunction is vast. The most commonly used medications that result in platelet dysfunction, many of which are over the counter, include aspirin and other nonsteroidal anti-inflammatory drugs, antihistamines, guaifenesin, certain anticonvulsants (valproic acid in particular), antibiotics, and antidepressants, including most commonly selective serotonin reuptake inhibitors. Commonly used supplements, such as garlic, ginger, omega-3 fatty acids, vitamin E, and gingko biloba, also have been reported to affect platelet function. Thus, when obtaining a medical history, it is imperative to ask not only about prescribed medications but also over-the-counter and herbal supplements. Most of these medications and supplements will not lead to a clinically apparent bleeding disorder, but instead they often exacerbate clinical bleeding associated with a mild disorder or confound results of platelet function tests. Therefore knowledge of all medications and supplements is critical to interpret laboratory tests.

Clinical presentation

Patients with platelet function disorders present with similar symptoms regardless of the specific defect. The severity of symptoms is dictated by the specific condition and clinical situation. Patients with platelet function defects exhibit mucocutaneous bleeding similar to patients with vWD. Severe hemorrhage can occur in patients with profound thrombocytopenia or Glanzmann thrombasthenia. Patients may present to the hematologist as a result of abnormal bleeding, a known family history of bleeding either with or without a personal bleeding history, or an abnormal screening test such as the PFA-100® obtained before a planned procedure.

Diagnosis

The diagnosis of platelet disorders is covered in Chapter 10. Briefly, the platelet count must be determined and the smear reviewed; platelet aggregation assays will be abnormal in the setting of significant thrombocytopenia (ie, $<100 \times 10^9$), and the PFA-100® will be abnormal with significant thrombocytopenia or anemia. Thus, a complete blood count (CBC) should be performed before obtaining platelet-specific studies. The two commonly available tests to screen for platelet function disorders both have limitations. The original screening test was the bleeding time; as previously stated, the bleeding time has fallen out of favor because of its limitations, particularly its inability to predict clinical bleeding.

PFA-100®

The PFA-100® is a widely available laboratory test that may be abnormal in some congenital and acquired platelet function disorders and commonly in types 2 and 3 vWD. The usefulness of the PFA-100® CT in the diagnosis of type 1 vWD is controversial (see discussion in the section on vWD). A significant limitation of the PFA-100® is the fact that the CT is affected by the platelet count and hemoglobin levels. The CT will be abnormal if the platelet count is less than 100,000/μL and the hemoglobin is <10 g/dL. Patients with severe platelet function defects, such as Bernard-Soulier and Glanzmann thrombasthenia, also will have abnormal results. The CT is often abnormal in patients on aspirin, clopidogrel, and ticlopidine. The effects of other medications known to affect platelet function, such as valproic acid, are not clear. The utility of the CT is limited by insufficient sensitivity, such that it rarely obviates the need for further testing, and its inability to distinguish between the two most common bleeding disorders (ie, platelet function defects and vWD). These aspects significantly limit its use as a screening test. The CT may be abnormal in mild disorders, such as common platelet secretion defects; however, its sensitivity for these disorders is insufficient to rule out such defects in the face of a normal result.

Platelet aggregometry

The most specific assay of platelet function is platelet aggregometry. This assay uses platelet-rich plasma (PRP) and evaluates platelet aggregation via light transmission after the addition of a variety of agonists, such as ADP, epinephrine, ristocetin, arachidonic acid, collagen, and thrombin-related activation peptide. Patients with a variety of both

severe and mild platelet function disorders exhibit abnormal platelet aggregation profiles, and furthermore, the spectrum of abnormalities can be diagnostic of specific disorders. For example, if results demonstrate absent aggregation to all agonists except ristocetin, the pattern is diagnostic of Glanzmann thrombasthenia, whereas normal aggregation to all agonists and absent response to ristocetin is consistent with Bernard-Soulier syndrome. In addition, a pattern of aggregation followed by disaggregation with ADP is consistent with secretion defects. Luminometry, commonly used in combination with platelet aggregation, provides a sensitive evaluation of ATP release from dense granules. ATP released by the platelets provides energy for the added light-producing enzyme luciferase, and a light burst is recorded. In patients with a dense granule deficiency or platelet release defect, this burst is impaired. A more detailed discussion of platelet aggregation can be found in reviews of platelet function disorders. Platelet aggregation testing is labor intensive and expensive.

As with the PFA-100® CT, many medications and supplements have been reported to affect platelet aggregation studies; therefore, if possible, the assay should be performed when patients are no longer receiving these medications or supplements for approximately 10 days. This assay can be performed in anemic and even thrombocytopenic patients (if one suspects a platelet function defect in addition to thrombocytopenia) as it is performed on PRP. For thrombocytopenic patients, the amount of blood required may be prohibitive, and consultation with the coagulation laboratory is recommended before ordering the assay in this circumstance. Although most laboratories in the United States use PRP for aggregometry studies, whole blood aggregometry is also available in some centers with reliable reported results.

Flow cytometry

Flow cytometry may be employed to quantify levels of platelet surface receptors and can confirm the diagnosis of Bernard-Soulier and Glanzmann thrombasthenia. In some institutions, these assays are available and have become the method of choice for diagnosis.

Some platelet function defects lead to easily identifiable platelet ultrastructural changes visualized by electron microscopy. In particular, patients with a deficiency or absence of dense bodies (δ-storage pool deficiency) or α-granules (gray platelet syndrome) can be diagnosed by this method. Finally, and because most of the genes responsible for these disorders have been identified, genetic testing is available for selected families and may guide future therapeutic strategies as well as provide information for genetic counseling.

Treatment

Congenital platelet function defects may benefit from medical modalities for hemostatic control, although ultimately, platelet transfusions may be required if medications or local measures are ineffective. In acquired conditions, treatment or reversal of the underlying condition will resolve the platelet dysfunction; however, this is not always possible. In such situations, the approach to management of bleeding is similar to that for congenital disorders.

Several medications enhance hemostasis nonspecifically and are useful in the face of platelet dysfunction. These include desmopressin, antifibrinolytic agents, estrogen, and recombinant FVIIa (rFVIIa). Desmopressin may improve platelet function in many congenital disorders, uremia, and during cardiopulmonary bypass; the specific mechanism of action is not clear. Desmopressin may be administered intravenously, subcutaneously, and, for home management, intranasally. Intranasal use requires the highly concentrated solution (Stimate®; CSL Behring, King of Prussia, PA) as the intranasal formulation commonly used to manage diabetes insipidus or enuresis is ineffective as a hemostatic agent. In some circumstances, it may be useful to perform a desmopressin challenge test before its clinical use. The challenge test entails assessment of platelet function before and approximately 90 minutes after administration; however, it is recognized that a poor correlation between the results of platelet function tests and clinical outcomes exists, and thus, the value of this approach is uncertain. Desmopressin is a safe agent, although its use can lead to vasodilation, resulting in facial flushing with rare reductions in blood pressure sufficient to result in clinical symptoms. Moreover, as an analog of an antidiuretic hormone, desmopressin can result in water retention and hyponatremia. Although this rarely occurs in adults and older children, the risks are increased in young children and in those receiving intravenous fluids. Therefore, an experienced care provider should oversee its use. Repeated use at short intervals should be limited because of the development of tachyphylaxis. Desmopressin should not be used in children under 2 years of age because of the high risk of hyponatremic seizures.

Antifibrinolytic agents (aminocaproic acid [EACA] and tranexamic acid [TXA]) are commonly used adjunctive hemostatic therapies. These agents, which are lysine analogues, inhibit plasmin-mediated thrombolysis and exert their effect through clot stabilization and prevention of early dissolution. Thus, these agents may be effective in prevention of rebleeding, a common problem in individuals with bleeding disorders especially in areas with increased fibrinolysis, such as the gastrointestinal tract. These agents may be administered intravenously, orally, or topically in amenable circumstances. These agents are used either therapeutically

for bleeding or prophylactically as part of perioperative management. Treatment of mucosal bleeding commonly includes the use of antifibrinolytic agents in conjunction with desmopressin; this combination is also effective in bleeding from other sites, for example, in the management of menorrhagia. Antifibrinolytic agents have been used widely for many years, have a documented safety profile, and are well tolerated in most patients. Commonly reported side effects include headache and abdominal discomfort; however, these symptoms do not preclude its continued use if ameliorated with other agents, such as acetaminophen. Antifibrinolytic agents should be used with caution in patients with a history of thrombosis or atherosclerosis and are contraindicated when hematuria is present as obstructive uropathy secondary to ureteral clots may develop.

Estrogens have documented effectiveness in the management of excessive menstrual bleeding. The mechanism of action is not well elucidated, although their use is associated with an increase in procoagulants, including vWF and FVIII, and a decrease in naturally occurring coagulation inhibitors, particularly protein S. Conjugated estrogens also are used for the management of severe menorrhagia with both the previously mentioned hemostatic effects and with the additional local effect of reduced uterine blood flow. Estrogen in combination with progestins, as in oral contraceptive agents, is useful for home management of menorrhagia in patients with bleeding disorders, including platelet function disorders and vWD. The positive effects of these agents are likely similar to conjugated estrogens in conjunction with progestin-induced stabilization of the endometrial lining. The risks associated with estrogens include thrombosis; thus, these agents should be avoided in patients with a history of thrombosis or who are deemed at high risk for thrombosis.

Although rFVIIa has been shown anecdotally to be effective for the management of severe bleeding in patients with platelet function defects, its value in this setting is not clearly defined. This agent is licensed in the European Union for the management of bleeding in patients with Glanzmann thrombasthenia refractory to platelet transfusions. rFVIIa is costly and may be associated with adverse events, including thrombosis; therefore, its use should be supported by evidence of its efficacy and judicious utilization. Although off-label, the use of rFVIIa in patients with severe bleeding in whom standard therapeutic measures have failed is a reasonable guideline adopted by many institutions. For severe bleeding unresponsive to the previously mentioned measures, especially in patients with Bernard-Soulier and Glanzmann thrombasthenia, platelet transfusion should be administered to provide normally functioning platelets. The general risks associated with platelet transfusion common to all patients include the risk of transfusion reactions and potential transmission of infectious agents (see Chapter 11 for details on risk of platelet transfusions). A more important specific risk associated with Bernard-Soulier and Glanzmann thrombasthenia is alloimmunization because of the formation of antibodies against the absent receptor. Once antibodies develop, future platelet transfusions are likely to be ineffective and may be associated with unusual reactions. Thus, judicious use of platelet transfusions is imperative in these patients.

The benefits of local measures in the management of bleeding episodes for which these approaches are applicable should be emphasized. Application of direct pressure is an effective measure for epistaxis, oral bleeding, and cutaneous bleeding. For accessible bleeding sites, including the nose, mouth, and skin, the use of topical adjunctive agents are also effective and safer than systemic therapy.

Prognosis and outcomes

The majority of commonly encountered platelet function disorders are associated with mild intermittent bleeding episodes that do not significantly interfere with daily life. Disorders like Glanzmann thrombasthenia, however, can be associated with significant bleeding that profoundly affects quality of life. In some patients, bleeding is so severe that bone marrow transplantation has been undertaken to correct the defect by replacing the population of megakaryocytes. This extreme approach is reserved only for the most severe patients in whom an unaffected human leukocyte antigen (HLA)–compatible sibling is available. Patients with platelet function disorders should receive thorough education regarding their condition and its management so that bleeding episodes are recognized early, managed at home, or prevented through appropriate measures or interventions. Important, patients should be advised to report their condition to physicians before undergoing invasive procedures so that appropriate prophylactic measures are used to prevent bleeding; in addition, all new medications should be checked for their ability to interfere with platelet function.

Gaps in knowledge

The complexity of establishing a correct diagnosis cannot be underestimated as the first and most important step in the appropriate management of patients with platelet function disorders. Although current laboratory assays are helpful, patients may be left without a more specific diagnosis other than the broad category of a platelet function defect. The complexity of platelet structure and function makes identification at a molecular or cellular level impractical or impossible in many patients outside of specialized research centers. Therefore, an important area for future research is the development of widely available laboratory assays with

increased sensitivity and specificity that are able to unravel platelet function defects into better defined categories. Some promising approaches, such as the use of platelet proteomics and platelet adhesion assays under flow conditions, are being developed and improved. Although these assays presently are used only in a research setting, it is feasible that further work will allow development of clinically useful versions. In addition, the ongoing development of global hemostatic assays may allow for identification of a patient's defect despite their previous evaluations being poorly defined or unrevealing. At present, a number of assays are under evaluation; it is hoped that in the relatively near future, these may become a part of the armamentarium available in the coagulation laboratory.

Key points

- Platelet function disorders can be congenital or acquired and typically present with mucocutaneous bleeding symptoms.
- Screening test for platelet disorders have limited value. The gold standard laboratory evaluation for platelet function disorder involves platelet aggregation studies.
- Glanzmann thrombasthenia is the most severe platelet function defect and has the potential to result in significant bleeding requiring blood transfusion. Platelet transfusions in this disorder are reserved for life-threatening bleeding because of the risk of developing alloantibodies that render further transfusions ineffective.
- Secretion defects are among the most common platelet function defects and typically cause mild to moderate mucocutaneous bleeding symptoms that are managed with desmopressin, antifibrinolytic agents, and hormonal therapy for menorrhagia.

von Willebrand disease

Pathophysiology

vWD is the most common bleeding disorder with a reported prevalence of symptomatic disease that ranges from 1/100 to 1/10,000. The transmission of vWD is autosomal dominant for most types but also can be rarely inherited in a recessive manner.

vWD is caused by the quantitative or qualitative deficiency of vWF, which is a large, multimeric glycoprotein produced in megakaryocytes and endothelial cells. Therefore, two pools of vWF are available for normal hemostasis. Plasma vWF, mostly released from stored vWF in Weibel-Palade bodies in endothelial cells, and platelet vWF that is stored in α-granules and released upon platelet activation. The main roles of vWF in hemostasis are to promote platelet adhesion to the exposed subendothelium and to serve as a chaperone for factor VIII (FVIII) in plasma, protecting it from proteolytic degradation. vWF undergoes dimerization in the endoplasmic reticulum (ER), glycosylation in the ER and Golgi complex, and multimerization in the Golgi complex and is packed into storage granules after cleavage of the vWF propeptide (vWFpp). The vWFpp is released in equimolar concentrations to the mature vWF molecule. The vWFpp therefore is useful to measure the rate of clearance of mature vWF. When multimers are secreted into the blood, the largest (also called ultralarge) vWF multimers are cleaved by the metalloprotease adisintegrin and metalloprotease with thrombospondin (ADAMTS13). Recent data suggest that vWF clearance is led in part by macrophages in the liver and spleen.

Classification of vWD

vWD often is categorized into quantitative or qualitative vWF defects. Although vWD type 1 and type 3 represent partial and absolute quantitative defects respectively, vWD type 2 is characterized by a qualitative vWF defect. Following is a brief description of the different subtypes and the molecular mechanisms that define them. Figures 9-3 and 9-4 illustrate these mechanisms and how they lead to the current classification. Table 9-1 describes the subtypes in more detail.

vWD type 1

vWD type 1 is defined by partial quantitative deficiency of vWF and bleeding symptoms. A family history of the disease or others with clinical symptoms is usually present, yet their absence does not preclude the diagnosis. Those patients with vWF levels <20 IU/dL usually have identifiable mutations in the vWF gene (*vWF*) and commonly are associated with significant bleeding symptoms. Approximately 75% of cases of vWD type 1 result from mutations that exert a dominant negative effect by impairing the intracellular transport of vWF subunits and causing subsequent decrease in vWF secretion. A second recently identified mechanism is the rapid clearance of vWF from the circulation because of specific mutations in the vWF gene. Therefore, impaired secretion and increased clearance are likely the two most common molecular mechanisms that lead to vWD type 1. The variant of vWD type 1 that is due to increased clearance is called type 1C. Because vWF is synthesized on a 1:1 ratio with vWFpp, an alteration of the ratio in favor of the propeptide suggests increased vWF clearance. This, plus the presence of unusually large multimers is indicative of vWD type 1C. Patients with type 1C vWD have a robust initial response to desmopressin, but they exhibit an abrupt vWF level decrease within 2-4 hours.

A consistent diagnostic criterion is difficult to achieve as not all individuals that inherit a mutation in vWF show signs

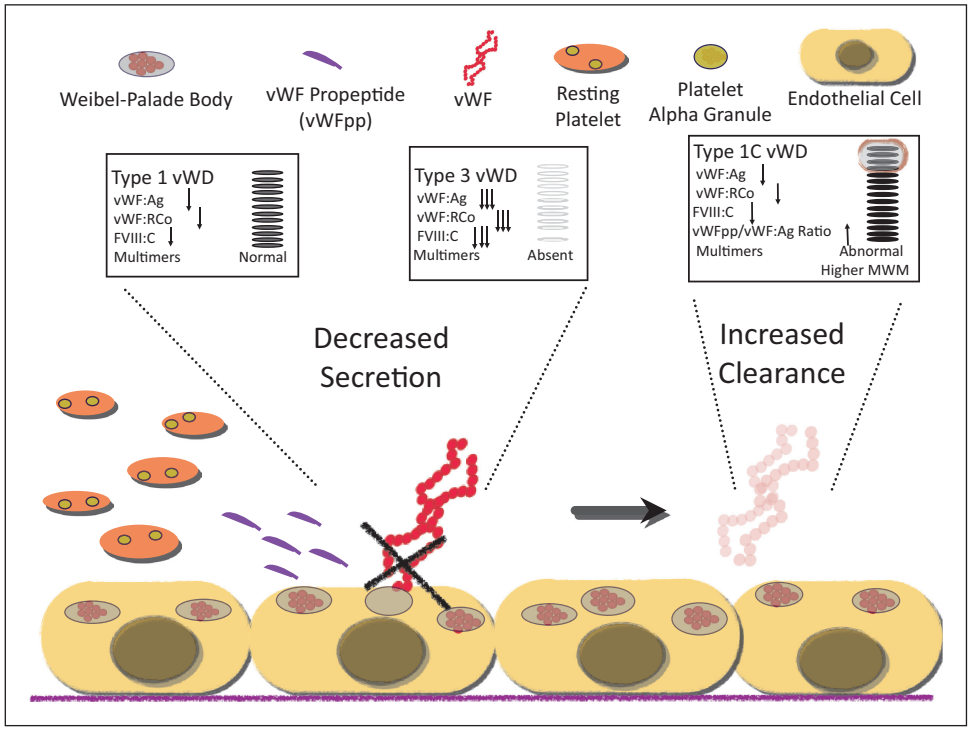

Figure 9-3 Mechanisms of disease for vWD types 1 and 3. Note that in boxes are shown the most common laboratory findings for these types. From Hematology 2012, the ASH Education Program.

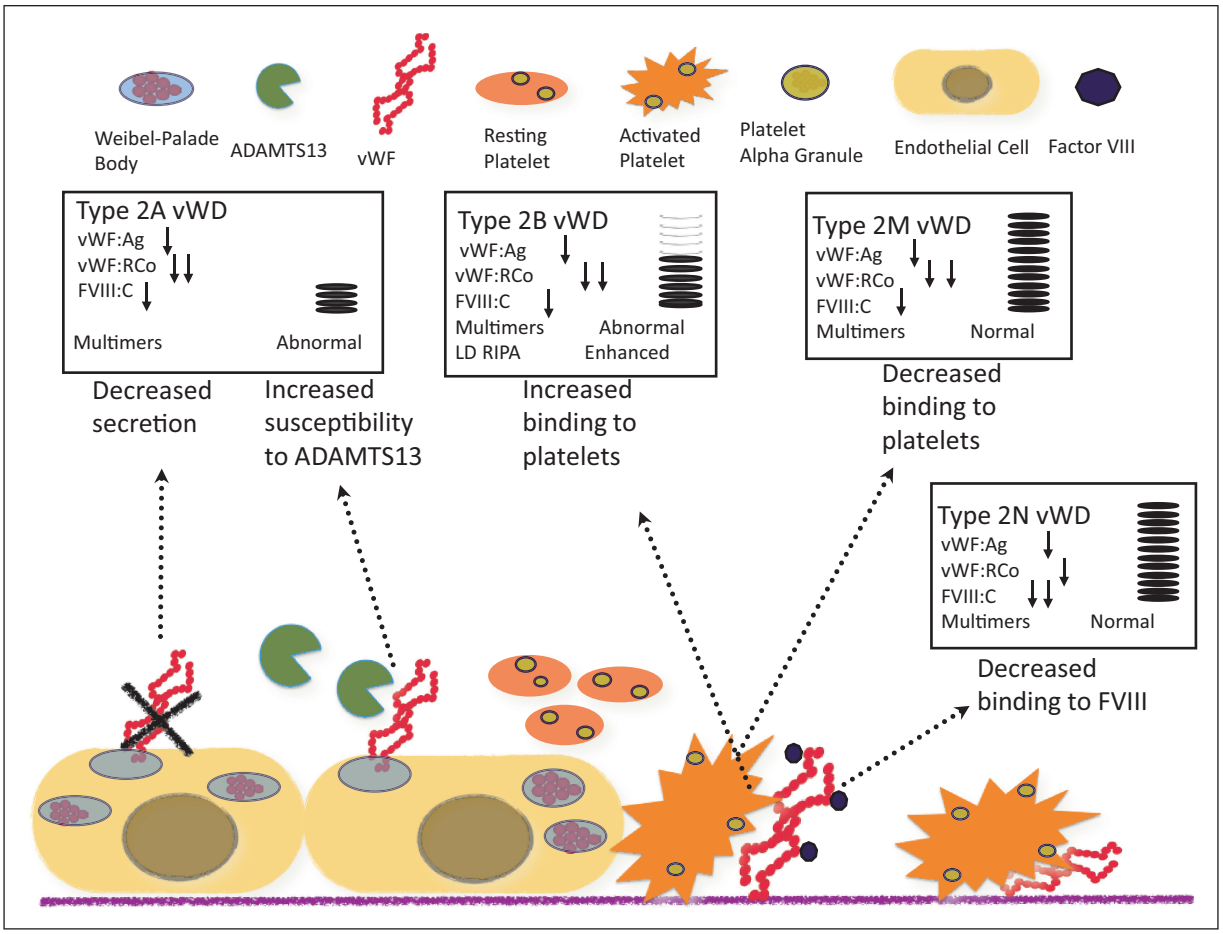

Figure 9-4 Mechanisms of disease for vWD types 2. Note that in boxes are shown the most common laboratory findings for the different subtypes. From Hematology 2012, the ASH Education Program.

Table 9-1 Classification and diagnosis of von Willebrand disease.

Condition	Description	vWF:RCo (IU/dL)	vWF:Ag (IU/dL)	FVIII	vWF:RCO/ vWF:Ag
Type 1	Partial quantitative vWF deficiency (75% of symptomatic vWD patients)	<30*	<30*	↓ or Normal	<0.5-0.7
Type 2A	Decreased vWF-dependent platelet adhesion with selective deficiency of high–molecular weight multimers	<30*	<30-200* †	↓ or Normal	<0.5-0.7
Type 2B	Increased affinity for platelet GPIb	<30*	<30-200* †	↓ or Normal	Usually <0.5-0.7
Type 2M	vWF-dependent platelet adhesion without selective deficiency of high–molecular weight ↓ multimers	<30*	<30-200* †	or Normal ↓	<0.5-0.7
Type 2N	Markedly decreased binding affinity for FVIII	30-200	30-200	↓↓	<0.5-0.7
Type 3	Virtually complete deficiency of vWF (severe, rare)	<3	<3	↓↓↓ (<10 IU/dL)	Not applicable
Low vWF		30-50	30-50	Normal	<0.5-0.7
Normal		50-200	50-200	Normal	<0.5-0.7

↓ refers to a decrease in the test result compared to the laboratory reference range.

* <30 IU/dL is designated as the level for a definitive diagnosis of vWD; some patients with type 1 or type 2 vWD have levels of vWF:RCo or vWF:Ag of 30-50 IU/dL.

† The vWF:Ag in the majority of individuals with type 2A, 2B, or 2M vWD is <50 IU/dL.

** This does not preclude the diagnosis of vWD in patients with vWF:RCo of 30-50 IU/dL if there is supporting clinical or family evidence for vWD, nor does this preclude the use of agents to increase vWF levels in those who have vWF:RCo of 30-50 IU/dL and who may be at risk for bleeding.

of clinical disease (phenomenon known as low penetrance) and not all individuals that inherit the same mutation show the same clinical signs (known as variable expressivity). More than 50% of individuals with vWF levels in the mildly decreased range (30-50 IU/dL) are asymptomatic or have minimal bleeding symptoms. Therefore, the presence of plasma vWF levels between 30 and 50 IU/dL does not automatically define vWD type 1. Individuals with blood group O have 25%-30% lower vWF levels as compared with those who have blood group A; therefore, 14% of blood group O individuals in the United States are expected to have vWF levels equal to or lower than 50 IU/dL. On the basis of the fact that mild bleeding commonly is reported in the healthy population, it is possible that many individuals diagnosed with mild vWD type 1 may not have genetically inherited vWD but rather an association of mild decreased vWF levels (within the established range for blood group O) and mild bleeding symptoms. Regardless of causality, most individuals in this category likely benefit from similar therapeutic measures used for patients with vWD type 1.

vWD type 2

vWD type 2 is subclassified into type 2A (loss of intermediate– and high molecular– weight multimers because of decreased secretion or increased susceptibility to ADAMTS 13), type 2B (gain-of-function mutation resulting in spontaneous vWF-platelet binding under physiologic shear conditions, resulting in clearance of the highest–molecular weight multimers and mild thrombocytopenia), type 2M (loss of function mutations that decrease the interaction of vWF with its platelet receptor and decreased ristocetin cofactor activity), and type 2N (mutations in vWF causing reduced binding to FVIII allowing for increased clearance).

vWD type 3

vWD type 3 is inherited in an autosomal recessive mode and is characterized by complete lack of vWF protein with undetectable vWF antigen assay (vWF:Ag) and ristocetin cofactor assay (vWF:RCo) levels, and resultant very low FVIII:C levels (<5%), representing the steady state of factor VIII in the absence of its vWF chaperone. Multimers are absent and the bleeding pattern is usually severe.

The clinical presentation of vWD includes mucocutaneous bleeding—specifically easy and excessive bruising and bleeding from mucosal surfaces, including the nose, mouth, and gastrointestinal and genitourinary tracts. The extent, location, and nature of bruising are important clinical points. Multiple bruises of various ages in a variety of locations are suggestive of a disorder of primary hemostasis. Epistaxis or oral-pharyngeal bleeding sufficient to result in anemia suggests the presence of a hemostatic disorder.

Menorrhagia, particularly at onset of menarche, also is suggestive of a mucocutaneous bleeding disorder. Excessive bleeding following procedures involving the mucus membranes may unmask a previously unknown bleeding disorder. The most common of these events include childbirth, oral surgery, including dental work, tonsillectomy or adenoidectomy, and sinus surgery. Some patients present to the hematologist as a result of a documented family history of bleeding without an individual specific bleeding event. Less commonly, patients may present because of abnormal screening tests ordered before a planned procedure. Clinical manifestations may range from mild to severe. Type 3 vWD may be associated with similar bleeding events observed in severe hemophilia, likely because of the extremely low FVIII levels. Severe menorrhagia resulting in early hysterectomy has been observed in females with a variety of subtypes including types 1, 2, and 3. Because bleeding manifestations of vWD include commonly observed symptoms in the normal population, such as bruising, epistaxis, and menorrhagia, clinical suspicion is important for timely and accurate diagnosis.

Diagnosis of vWD

Screening tests

Screening tests have several limitations in predicting the diagnosis of a bleeding disorder. These limitations are especially relevant to vWD. For example, the aPTT, which has been proposed as a screening test for vWD, is often normal in most cases of types 1 and 2 vWD. The aPTT yields abnormal results only if the FVIII is reduced sufficiently to be detected by the assay. Therefore, the aPTT is only noticeably abnormal in patients with types 2N and 3 vWD, and sometimes in type 2B. The PFA–100® CT also has been proposed as a screening test for individuals with suspected vWD; as previously discussed, this test is sensitive only to very low vWF levels and therefore patients with vWD often are missed. In summary, screening laboratory methods have limited value when a diagnosis of vWD is suspected. Therefore, in clinical practice, in the face of a significant history of mucocutaneous bleeding, specific laboratory assays for vWD are required.

Diagnostic tests

Diagnostic assays for vWD include quantitative measurement of vWF (vWF:Ag), the platelet-binding function (vWF:RCo, in which the agglutination of fixed platelets in response to patient plasma is measured in the presence of ristocetin), and the FVIII activity and binding to vWF (FVIII activity and FVIII-binding assay). Also the vWF multimers distribution is used to differentiate subtypes. A potential role for assays that measure the binding of vWF to collagen has been described. On the basis of these results, further testing, described in further detail later, may be pursued. Limitations exist with several of these assays. Both vWF and FVIII are acute-phase reactants and may increase two to five times above baseline because of a variety of conditions or circumstances, including but not limited to elevated estrogen levels, as with oral contraceptive agents or due to pregnancy. These increased levels elevate low baseline levels to within the normal range, obscuring diagnosis. Therefore, normal levels do not completely rule out vWD, especially in the face of a suspicious clinical history, and must be interpreted with caution. Performance of these assays requires an experienced coagulation laboratory. It is common for patients who have undergone serial testing to have moderate variations in levels over time. Because of the difficulty in ruling out this disorder with one normal evaluation, it is not uncommon for patients to undergo repeated testing. When local laboratory results are inconsistent, a useful strategy is to perform testing in a reference hemostasis laboratory. Finally, many preanalytic variables must be considered to accurately interpret laboratory testing. For example, refrigeration of whole blood samples before separation can result in reduced plasma vWF levels; in addition, platelet contamination of the separated plasma may result in protease-induced vWF alterations, causing decreased activity.

vWF:RCo is widely used and is accepted as the gold standard for vWF activity. The assay can be difficult to interpret as it exhibits a high coefficient of variability when the vWF:RCo is lower than 15 IU/d, and it may affect differentiation between type 1 and type 2 vWD. Latex immunoassay and ristocetin enzyme-linked immunoadsorbent assay (ELISA) currently are under evaluation for their clinical utility as alternatives for the traditional vWF:RCo assay. A recent report suggests that the vWF:RCo assay can be abnormal in a subset of otherwise-healthy African Americans due to the presence of a common single nucleotide polymorphism (SNP) in exon 28 of vWF. This SNP appears to affect ristocetin binding without conferring an evident hemorrhagic risk and potentially may lead to a false diagnosis of vWD type 2. Newly developed functional assays utilizing the platelet ligand for vWF, GPIb, may allow for assessment of vWF activity without the need for ristocetin, thus improving discrimination of type 2 variants without the noted pitfalls. Low-dose ristocetin-induced platelet aggregation (LD-RIPA) is used to identify abnormally increased binding of vWF to platelets. vWF multimers usually are run on an agarose gel to evaluate the full range of molecular weight multimers present within the mature vWF molecule. Multimeric analysis is required to differentiate between various subtypes of vWD type 2, and their absence easily identifies vWD type 3 (Figure 9-5). The FVIII activity level and FVIII-binding assay

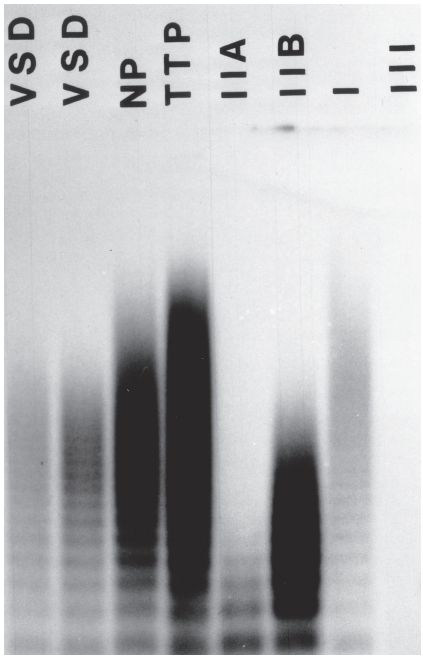

Figure 9-5 Representation of a vWF multimer analysis. The third column from the left represents normal plasma as indicated by the NP at the top of the column. In type 2A vWD, there is a loss of high- and intermediate-weight multimers as indicated by the loss of the bands in the gel under the heading. In type 2B vWD, there is a loss of HMWM. In type 1, all the multimers are present but in reduced amounts as can be seen by the presence of all the bands but with more faint staining than seen in normal plasma. In type 3 disease, there is a complete absence of multimers, and no staining of bands is visible. The labeled columns VSD and TTP stand for ventricular-septal defect, a condition that results in AWS with the loss of multimers of all sizes, and thrombotic thrombocytopenic purpura in which ultralarge multimers can be observed.

provide a more accurate diagnosis of vWD type 2N. Finally the collagen-binding assay measures binding of large vWF multimers to collagen and represents an additional method to assess vWF functional activity. The collagen-binding assay does not require the use of ristocetin, but studies have reported that the type of collagen employed influences the results.

Laboratory test results are compatible with vWD type 1 if the levels of both vWF:RCo and vWF:Ag are greater than 2 standard deviations below the population mean and the plasma vWF multimer distribution is normal. Additionally the vWF:RCo/vWF:Ag ratio approximates 1. In patients with vWD type 1C, the vWF:Ag and vWF:RCo are low and the multimer assay is characterized by the presence of abnormally large high–molecular weight forms. As this subtype is characterized by rapid vWF clearance, a vWFpp level allows for discrimination of vWD type 1C through the vWFpp/vWF:Ag ratio.

vWD type 2 is a qualitative defect caused by mutations in vWF that result in abnormal interactions with several of its ligands. The diagnosis of type 2A is made in the presence of a low vWF:Ag and a disproportionately low vWF:RCo with pronounced loss of high–molecular weight multimers (HMWM). The vWF:RCo/vWF:Ag ratio approximates 0.5. Type 2M is caused by mutations in the platelet glycoprotein 1bα (GPIb) binding site, with resultant decreased binding of vWF to GPIb, and subsequent impairment of platelet-dependent function. The multimer structure and distribution in vWF is normal. Type 2B results from gain-of-function mutations in the binding site for GPIb, leading to the formation of rapidly cleared platelet–vWF complexes. LD-RIPA is employed to confirm this subtype. A level of ristocetin insufficient to promote platelet binding with normal vWF causes enhanced platelet agglutination in these gain-of-function mutations. This phenomenon is also seen in patients with platelet-type vWD (also known as pseudo-vWD), a rare disorder caused by mutations in platelet GPIb. It is important to differentiate these two entities as treatment approaches are significantly different. vWD type 2B is treated with vWF concentrates as the molecular defect is in vWF, whereas pseudo-vWD is treated with platelet transfusions as it is caused by mutations in platelet GPIb. If pseudo-vWD is suspected, the patient's platelets are tested with a normal exogenous vWF substrate for evaluation in a ristocetin-induced platelet agglutination-based mixing study. Enhanced binding confirms the diagnosis. Finally, type 2N is characterized by mutations in the FVIII-binding site of vWF disturbing the normal interaction of these two proteins. Patients with vWD type 2N may exhibit normal or decreased vWF:Ag and vWF:RCo with disproportionately decreased FVIII:C. Patients with this diagnosis may be misclassified as mild factor deficiency. Specific FVIII-binding assays are used to confirm the diagnosis of type 2N. Symptomatic patients are either homozygous or compound heterozygous for mutations in the vWF gene. Patients with a prior diagnosis of mild FVIII deficiency who do not respond well to FVIII infusions or belong to families for whom the inheritance appears to be autosomal dominant should be evaluated for vWD type 2N.

vWD type 3 is characterized by undetectable vWF:Ag and vWF:RCo levels, FVIII:C levels commonly <5%, and lack of multimers. A description of the laboratory pattern for each subtype is shown in Table 9-1

Genetic testing

Sequencing of vWF gene is challenging due to its large-size, highly polymorphic structure, and presence of a homologous partial pseudogene in chromosome 22. Therefore, gene sequencing for diagnosis currently is reserved for specific cases in which these test results will likely contribute signifi-

cantly to diagnosis and management. Genetic testing may be justified in vWD type 3 as large deletions may predispose to the development of inhibitory antibodies and anaphylactic reactions. Also, gene sequencing could be useful in cases in which treatment options vary based on diagnosis, such as in vWD type 2N.

Acquired von Willebrand syndrome

Acquired von Willebrand syndrome (AWS) is a rare disorder in which vWF is synthesized normally but cleared from the circulation more rapidly. Several conditions have been associated with AWS. Three mechanisms are associated with the observed increased clearance: (i) autoantibodies against vWF and immune complex formation (eg, hypothyroidism due to Hashimoto's thyroiditis), (ii) vWF binding to cancer cells (eg, Wilms tumor, lympohproliferative disorders), and (iii) increased proteolytic activity of HMWM under pathological high–shear stress conditions (eg, congenital heart disease, aortic stenosis, angiodysplasia). The laboratory diagnosis and management of AWS does not differ significantly from the congenital forms. Treatment of the underlying disorder leading to AWS often resolves the defect.

Treatment

The principles of management of vWD are to increase or replace vWF to achieve hemostasis. This is accomplished with either medications that cause the release of endogenous stores of vWF into the circulation (desmopressin) or the use of vWF-containing concentrates derived from human plasma. Mild to moderate bleeding associated with type 1 vWD often is managed with desmopressin, most commonly with the intranasal preparation, and antifibrinolytic agents as required. Desmopressin's mechanism of action is based on the secretion of stored vWF from Weibel-Palade bodies in endothelial cells into the plasma. A desmopressin challenge test, as described in the platelet section, should be performed to document a hemostatic response; in vWD, the vWF:Ag, vWF:RCo, and FVIII levels are performed before and 60-90 minutes after the dose, depending on the route of administration. Repeat laboratory evaluation at 4 hours post dose may be appropriate when an altered half-life of the native protein is suspected, as observed in type 1C. Approximately 90% of patients with type 1 vWD respond with hemostatic levels; however, the response varies and should be measured to determine its adequacy for specific hemostatic challenges. Repeated administration of desmopressin in proximity may lead to tachyphylaxis, with decreased response levels with repeated use likely resulting from depletion of the storage pool. Thus, use of desmopressin no more than once daily and no more than on 2-3 consecutive days serves as an acceptable clinical guideline for home use. There are some reports of the benefits of desmopressin in type 2 vWD; in general, it is less effective in these subtypes and has been reported to precipitate thrombosis or result in significant thrombocytopenia as a result of in vivo platelet aggregation in type 2B or platelet-type vWD. For these reasons, patients with type 2 vWD most commonly are treated with exogenous normal vWF replacement via a concentrate. Desmopressin is ineffective in type 3 vWD, and treatment is dependent on the use of replacement therapy via concentrate.

Several products available in the United States contain intact vWF, including Humate-P (CSL Behring, King of Prussia, PA), Alphanate (Grifols Biologicals, Los Angeles, CA), Koate DVI (Talecris, Research Triangle Park, NC), and Wilate (Octapharma, Lachen, Switzerland), with other similar products available in other countries. These plasma-derived concentrates contain vWF and FVIII in varying ratios and with variable amounts of multimer size or distribution. Humate-P, Alphanate, and Wilate are approved by the U.S. Food and Drug Administration (FDA) for the treatment of vWD. Although these products are manufactured via processes that include viral attenuation and inactivation steps, a theoretic risk of transmission of infectious agents exists. As with all human plasma products, a potential for allergic reactions also exists; however, these are infrequently reported with these products. Administration of the first dose in a hospital or clinic setting may be considered.

Antifibrinolytic agents are useful adjunctive therapies and are used in a similar fashion as described for platelet defects. Conjugated estrogens and oral contraceptive agents are effective therapies for the management of menorrhagia. Topical measures also are useful in some situations. The benefits and risks of these agents are identical to those described in the "Treatment" section of Platelet function disorders. Case reports exist in the literature regarding the use of rFVIIa in vWD; these are limited to patients with type 3 disease with inhibitors to vWF.

Gaps in knowledge

The most challenging aspect in the management of vWD is the establishment of an accurate diagnosis, particularly in type 1 disease. This can be especially difficult because vWF levels may appear to be normal because of the associated clinical circumstances, despite a clinical history suggestive of this disorder. Recently published data used a Bayesian analysis of laboratory data and personal and family history to predict the probability of diagnosis of vWD. Future research aimed at the development of laboratory assays with improved performance characteristics to decrease variability and diagnostic dilemmas is needed. A wide variation in bleeding symptoms exists among patients within the same disease subtype, likely because of genetic modifiers of the bleeding

phenotype. Overall, currently available therapies are effective; however, it is not completely clear under what circumstances specific therapies are best applied to achieve an optimal outcome. There are few prospective comparative therapy studies to guide physicians in determining the risks and benefits of available therapies; recently published treatment guidelines published by the National Heart, Lung, and Blood Institute are based on the best available evidence and expert opinion.

Key points

- vWD is the most common inherited bleeding disorder in the general population.
- vWD is divided into several subtypes. Type 1 is the most common encompassing two-thirds of cases.
- Laboratory diagnosis of vWD may be difficult, especially in type 1.
- vWD treatment is based on the subtype; the most common agents used for treatment include desmopressin, antifibrinolytics, hormonal therapy for menorrhagia, and vWF concentrates for severe bleeding or in types 2 and 3.

Disorders of secondary hemostasis

Hemophilia A and B (FVIII and FIX deficiency)

Pathophysiology

The previous review of the physiology of hemostasis reveals the critical roles played by FVIII and FIX in thrombin generation and ultimately normal fibrin clot formation. Absence or decreased amounts of either FVIII or FIX results in reduced thrombin generation on the surface of activated platelets at injured sites. Inadequate thrombin generation lead to a clot with poor structural integrity visualized by electron microscopy; formation of large, coarse fibrin strands as opposed to normal thinner strands that form a tight network are observed. In addition, reduced thrombin generation results in decreased generation of activated FXIII required for cross-linking of fibrin monomers and decreased TAFI generation, both of which result in a clot less resistant to normal lysis. Therefore, deficiencies of FVIII or FIX result in poorly formed clots that are more susceptible to normal fibrinolysis, clinically observed as the bleeding manifestations in hemophilia.

Etiology

Congenital deficiencies of FVIII and FIX occur as a result of genetic mutations in F8 and F9, respectively, both located on the long arm of the X chromosome. These deficiencies commonly are observed in males due to their hemizygous state. Heterozygous females may have factor levels observed in the mild hemophilia range as a result of nonrandom X chromosome inactivation. These women may be more appropriately classified as having mild hemophilia and treated accordingly for bleeding episodes. Rarely, females may have levels in the severe or moderate deficient range because of skewed lyonization or the presence of other genetic abnormalities, such as Turner syndrome or X-autosomal translocations. A wide range of mutations result in hemophilia, and the mutation type (deletion, inversion, missense, or nonsense) and specific area of the protein affected determine the severity of disease. In approximately 25% of cases, no family history is identified. In such cases, either the affected individual has a de novo mutation arising in either the patient's or—in the case of the intron 22 inversion (the most common mutation causing hemophilia A)—the maternal grandfather's germ cells during meiosis likely due to single unpaired X chromosome. F8 intron 22 inversions account for ~45% of severe hemophilia A cases.

Rarely, hemophilia can be acquired as a result of the development of autoantibodies most commonly directed against FVIII. This condition, also known as acquired hemophilia, has been associated with a variety of conditions, including pregnancy, malignancies, and advanced age. In ~50% of cases, no known associated disorder can be identified. These autoantibodies inhibit the functional activity of endogenous FVIII, resulting in a bleeding diathesis. Although some bleeding symptoms are similar to congenital hemophilia, the incidence of hemarthroses in acquired hemophilia is small, whereas soft tissue, abdominal, and retroperitoneal hemorrhage are more frequent.

Clinical presentation

The clinical presentation of congenital hemophilia is highly variable and is correlated with the level of deficiency. In infants born to known female carriers, the diagnosis most often can be established at birth by assaying FVIII or FIX from umbilical cord blood. Prenatal testing is available if the genetic defect has been identified within the family; this testing may not be required when the knowledge gained would not alter the course of pregnancy or the planned mode of delivery. The presentation of symptoms leading to diagnosis in patients either without a family history or not tested at birth is quite variable and dependent on the severity of disease.

Severe hemophilia, defined as a factor activity level <1%, may present in the newborn period with intra- or extracranial bleeding; prolonged bleeding from venipuncture or heel stick or after circumcision; or with excessive bruising. Infants with severe hemophilia who do not develop symptoms in the newborn period often present during the first year of life

with abnormal bruising, muscle hematoma especially with immunization, or bleeding in the joint or muscle due to activity or intercurrent injury. Although the precise prevalence of intracranial hemorrhage is not known, it likely approximates 1%-3%. Moderate hemophilia (factor activity levels between 1% and 5%) has a variable age of presentation; diagnosis may be established due to a known family history, in the newborn period due to bleeding, or later in life, even as an adult, with a bleeding event associated with intercurrent injury or a procedure. Bleeding symptoms include deep tissue, muscle, or joint bleeding; mucocutaneous bleeding is a common presentation due to increased fibrinolysis in the oropharynx and the inability to form a stable clot. Mild hemophilia (factor activity levels between 5% and 40%) may be diagnosed at ages similar to moderate hemophilia. For patients without a documented family history, the age of presentation is highly variable; excessive bleeding always is associated with injury or surgery. Patients with mild hemophilia typically present later in childhood or during the teenage or adult years.

Joint disease, or hemophilic arthropathy, remains a major morbidity. Although preventive therapy is effective (see Treatment section for details), patients occasionally may present with recurrent hemarthroses, ultimately leading to joint disease. It is not uncommon for patients who have not received optimal treatment, such as those who emigrated from developing nations, to present with hemophilic arthropathy.

Acquired hemophilia may present with the dramatic onset of either mucocutaneous or internal bleeding. Hemarthroses are uncommon. Life-threatening bleeding with associated significant morbidity and mortality are observed.

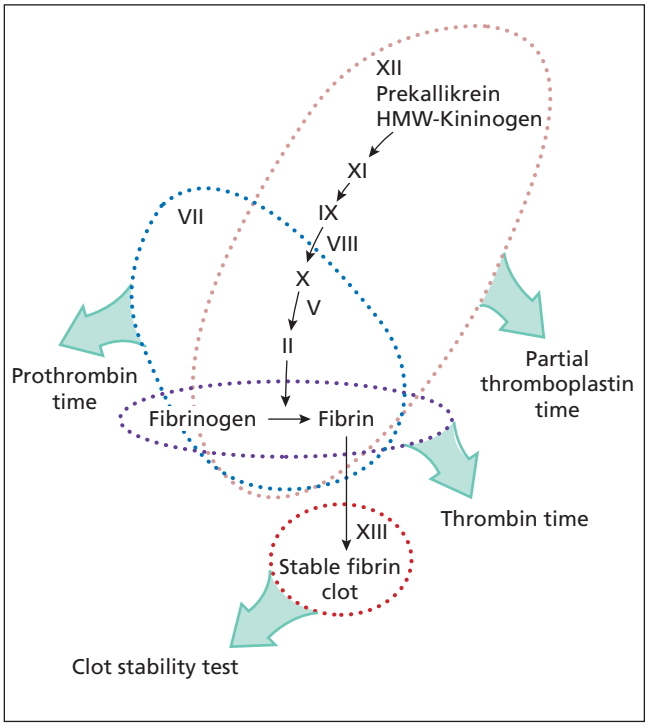

Figure 9-6 Plasma coagulation reactions in in vitro laboratory assays Factor XII, prekallikrein, and high–molecular weight kininogen are required for a normal partial thromboplastin time but not for normal in vivo hemostasis. Also, plasma factor XI may not always be required for normal in vivo hemostasis. Platelets and tissue factor are required for normal in vivo hemostasis but are supplied by exogenous reagents in the laboratory assays. This diagram outlines the coagulation factors required for each of four basic tests.

Diagnosis

The laboratory diagnosis of hemophilia begins with screening coagulation studies, including the PT and aPTT; the aPTT is almost always abnormal. It is important to be cognizant of circumstances in which the aPTT may be normal, especially in mild deficiencies (Figure 9-6). After identification of a prolonged aPTT, a mixing study with normal plasma is performed. Correction of the prolongation points to a factor deficiency, and therefore, specific factor analyses are performed, including FVIII and FIX. The type and level of severity of hemophilia are thereby established. As previously discussed, appropriate specimen procurement and handling are critical to obtain accurate results. In newborns where cord blood is tested due to a known family history, levels may be altered based on sample procurement, level of deficiency, and neonatal variations as seen with decreases in vitamin K–dependent clotting factors. Therefore, repeat testing may be required based on cord blood results and their concordance with expected results and clinical symptoms. In addition, assaying factor activity levels at the lowest range of the curve is technically difficult, and sample analysis through a reference laboratory may aid differentiation of the severe from moderate forms. Finally, because FVIII is an acute-phase reactant, obtaining a true baseline level may be difficult in patients with moderate and mild deficiencies based on their clinical circumstances. In addition, mild FIX and FVIII deficiency may be associated with a normal aPTT in some laboratories or circumstances; therefore, if a clinical suspicion for hemophilia exists, FIX and FVIII activity levels should be obtained in addition to the normal screening test.

Treatment

The mainstay of hemophilia treatment is replacement of the deficient coagulation factor. There are a number of commercially available factor concentrates to treat both FVIII and FIX deficiency (Tables 9-2 and 9-3). The choice of the specific product used includes consideration, among other things, of availability, cost, and method of manufacture. Both recombinant and plasma-derived products are

Table 9-2 Factor VIII concentrates currently available in the United States.

Brand name	Generation	Pd versus R	Presence of human proteins	Stability at RT
Monoclate	NA	Pd	Albumin	No
Hemophil M	NA	Pd	Albumin	No
Recombinate	1st	R	Albumin	No
Kogenate FS	2nd	R	Albumin in processing, not final product	Yes: 3 months
Helixate FS	2nd	R	Albumin in processing, not final product	Yes: 3 months
Advate	3rd	R	None	Yes: 6 months
Xyntha (B-domain deleted)	3rd	R	None	Yes: 6 months

Other FVIII concentrates approved for use in FVIII deficiency; these may also contain vWF and are not in general use. NA = not applicable; RT = room temperature; PD = plasma derived; R = recombinant.

available, and decisions of product used should be made in consultation with the patient and family. Typically, 1 IU/kg of FVIII will increase the FVIII level by 2%; doses can be repeated as needed approximately every 8-12 hours. With FIX, dosing depends on the product used—plasma-derived FIX (pdFIX) or recombinant FIX (rFIX). With pdFIX, 1 IU/kg increases the FIX level by 1%, whereas with rFIX, the level increases by 0.6%-0.8%, with children exhibiting a lower recovery compared with adults. FIX doses can be repeated every 12-24 hours as needed.

Treatment approaches are divided into two main categories: prophylaxis and on demand. Prophylaxis is the regular infusion of factor replacement to prevent or suppress bleeding events. Primary prophylaxis is the initiation of replacement therapy before or shortly after the first hemarthrosis and has been proven to be the most effective approach to prevent the development of joint disease. Therefore, primary prophylaxis should be considered the optimal therapy for severe hemophilia; however, when it should be instituted and when or if it should be stopped remain controversial. In Sweden, where prophylaxis was pioneered, therapy is initiated before the first joint bleed commonly between 9 and 12 months of age. In the United States, a common approach is to wait until 1-2 hemarthroses have occurred because some patients even with severe hemophilia may not experience a hemarthrosis until several years of age, thereby limiting invasive therapy until required. Prophylaxis is time and resource intensive and requires adequate venous access often necessitating a central venous catheter; therefore, there may be a benefit to institute therapy after hemarthrosis has occurred to demonstrate its necessity. The negative effect of this approach is that even one significant hemarthrosis may result in joint damage; in addition, this approach allows subclinical bleeding, a potential although as yet not well-defined contributor to joint disease. Once primary prophylaxis has been instituted, it should be continued throughout childhood. The topic of continued prophylaxis into adulthood is an ongoing area of research.

Secondary prophylaxis is the regular infusion of replacement therapy as described earlier but after the onset of significant hemarthroses or joint disease to interrupt a bleeding pattern or prevent further joint damage through suppression of bleeding episodes. Joints with repeated bleeding develop acute or chronic synovitis, followed by articular damage; the process of repeated bleeding in a joint is termed target joint. The bleeding pattern in target joints has been documented to be amenable to secondary prophylaxis. Prophylaxis may be administered for specific patients in circumstances that require adequate hemostatic coverage, such as before sports. In other situations, limited prophylactic therapy is reasonable and is reviewed in cited references.

Although primary prophylaxis is used most frequently in patients with severe disease, some individuals with moderate deficient hemophilia require this therapy because of their bleeding pattern. Secondary prophylaxis and limited prophylaxis are used in all severities of hemophilia based on circumstances that warrant adequate hemostatic coverage. Issues related to prophylaxis include adherence, cost, and the need for adequate venous access; prophylaxis and the associated issues have been reviewed. Several prophylactic and general treatment-dosing approaches exist and are detailed in Table 9-4. Although prophylaxis is effective in the prevention of the majority of spontaneous bleeding events, patients who experience breakthrough bleeding episodes require immediate treatment according to the recommendations in Table 9-4.

Table 9-3 FIX concentrates currently available in the United States.

Brand	Pd versus R	Presence of human proteins	Stability at RT
Mononine	Pd	FIX and others	No
Alphanine	Pd	FIX and others	No
Benefix	R	w	Yes (for 6 months)

Pd = plasma derived; R = recombinant.

Table 9-4 Typical dosing for FVIII and FIX deficiency in different clinical circumstances.*

Factor	Joint/muscle	Life or limb threatening	Preoperative	Prophylaxis
FVIII	25 IU/kg Repeat as needed	50 IU/kg Multiple doses required	50 IU/kg	25-40 IU/kg three times weekly or every other day
pdFIX	50 IU/kg Repeat as needed	100 IU/kg Multiple doses required	100 IU/kg	50 IU/kg twice weekly
rFIX	60-70 IU/kg Repeat as needed	120-140 IU/kg Multiple doses required	120-140 IU/kg	60-70 IU/kg twice weekly

*Represent general dose guidelines, practice varies. The volume of distribution of infused rFIX is ~1.2 in adults, and ~1.4 in children. Therapy duration for intracranial hemorrhage varies but is minimally ~2 weeks; in children intracranial hemorrhage should prompt consideration for ongoing prophylactic therapy. Prophylaxis regimens vary; listed doses typically utilized in Swedish regimens.

Episodic treatment for bleeding episodes is referred to as on-demand therapy (ie, the use of factor replacement therapy after bleeding occurs). This treatment approach does not require regular infusions with their associated issues and is less expensive in the short run, but is ineffective in the prevention of joint disease. This mode of therapy now is used primarily for patients with moderate and mild deficient hemophilia due to the infrequency of bleeding events and the associated low risk of joint disease. On-demand therapy may be used by adults with severe disease who experience fatigue with the requirements of prophylaxis or who feel it is not required. The typical initial dosing for bleeding episodes can be found in Table 9-4. Infusion therapy for hemophilia, regardless of the regimen used, is best delivered in the home setting to allow for prophylaxis or prompt therapy. Family members and patients are trained to administer the factor concentrate at home without the need for a medical facility.

Adjunctive therapy for hemophilia is similar to that discussed for platelet defects and vWD. Patients with mild FVIII deficiency may be treated with desmopressin after a challenge dose demonstrates a hemostatic response; the response level dictates the type of bleeding events that may be treated with this agent. Antifibrinolytic agents are efficacious for mucosal bleeding and commonly are used in conjunction with factor concentrate or desmopressin. For women with hemophilia with menorrhagia, hormonal suppressive therapy can be used as well as antifibrinolytic therapy.

Complications of treatment: inhibitors

A significant complication of hemophilia after exposure to replacement therapy is the development of neutralizing antibodies termed *inhibitors*. Inhibitors render standard treatment with replacement therapy ineffective and result in hemorrhagic episodes that are prolonged and more difficult to control, with associated increased risk of morbidity and mortality. The incidence of inhibitors is between 20% and 35% in severe, previously untreated, FVIII-deficient patients and <5% in severe FIX-deficient patients. The present inhibitor prevalence is approximately 10% in FVIII deficiency and 3%-5% in FIX deficiency. Risk factors for inhibitor development include both patient- and environmental-related issues. Among the patient-specific risk factors, the most important is hemophilia severity, with patients with severe disease at highest risk. The specific genetic mutation, ethnicity, and family history of inhibitors also have been shown to affect the expression of this complication. Mutations resulting in major disruptions of the gene, such as large deletions, are associated with increased risk. In addition, patients of African or Hispanic ethnicity have a significantly higher rate of inhibitor development. Environmentally related risk factors have been purported to include the source of the factor product used (plasma derived vs. recombinant); these data remain controversial. A recent systematic review suggested that the rate of inhibitor formation in severe FVIII deficiency is twofold higher in patients who received recombinant FVIII versus those who received plasma-derived FVIII. A prospective study is under way to confirm or refute this finding.

Inhibitors are divided into two categories: low titer (also known as low-responding inhibitors) and high titer (high-responding inhibitors). A low-responding inhibitor is characterized as one with a titer, measured in the Bethesda assay of <5 Bethesda units (BU) despite repeated exposure or stimulation, whereas high-responding inhibitors are those that achieve a titer >5 BU at any time regardless of present titer. Patients with high-responding inhibitors may exhibit a decrease in or an undetectable titer with complete withdrawal of the specific clotting factor. Despite this, with subsequent exposure to the deficient factor, these patients mount a memory response and will demonstrate an increase in inhibitor titer in 7-10 days after exposure. Stimulation and increase of inhibitor titer is termed anamnesis. Therefore, it is clear that high-responding inhibitor patients who achieve an undetectable inhibitor titer have not had the inhibitor response ablated and should not be challenged again unless experiencing life- or limb-threatening bleeding episodes.

Patients with low-responding inhibitors commonly are managed with higher doses of standard replacement therapy

calculated to overcome the inhibitor titer and achieve a hemostatic level. A minority of patients have low-titer inhibitors that resolve without intervention (often within a few weeks) and are termed transient inhibitors; therefore, ongoing measurement of titers is important to document persistence and for dose calculation. Patients with high-responding inhibitors are not able to achieve a hemostatic level with standard replacement therapy and thus are treated with alternative hemostatic products termed bypassing agents.

The three important strategies for the management of patients with high-responding inhibitors include: (i) management of bleeding episodes, (ii) prevention of bleeding, and (iii) eradication of the inhibitor. Inhibitor eradication, also called immune tolerance induction (ITI), requires regular administration of the deficient factor to reset/tolerize the immune system. An international prospective ITI study in good-risk patients recently was completed and published (Hay et al., 2012). This study compared daily high-dose FVIII (200 IU/kg/d) to lower dose FVIII (50 IU/kg/d) three times weekly. The study was stopped before reaching the planned endpoint because of an increased rate of bleeding observed in patients on the low-dose arm. Typical ITI regimens may include either of these infusion schedules or a regimen of 100 IU/kg given once daily. Retrospective registries have identified several factors affecting ITI success, including the peak inhibitor titer, titer at start of therapy (<10 BU associated with improved outcome), age at initiation, and time from inhibitor development to ITI start. It is best to initiate ITI when the titer is <10 BU, although this must be balanced against the risk of delaying tolerance. Inhibitor development in FIX deficiency is far less common and has associated unusual complications. Patients with FIX deficiency may develop anaphylactoid reactions to infused FIX concentrate before or at the time of inhibitor emergence. For such patients, ITI may not be possible or, if undertaken, requires desensitization to FIX. FIX-deficient patients with inhibitors undergoing ITI are at risk for developing nephrotic syndrome. ITI-associated nephrosis is more likely to occur in patients with a history of an anaphylactoid reaction. The etiology of nephrosis in these patients is unclear, although it is thought to be related to immune complex formation. The overall success rate of ITI in FIX deficiency is 35%, far lower than the 75% achieved in FVIII deficiency. Thus, although fewer FIX inhibitor patients exist, they represent a significant treatment challenge for practitioners.

The management of bleeding episodes in inhibitor patients is challenging, with the majority of hemophilia-related morbidity in the United States occurring in patients with high-responding inhibitors. Bypassing agents are used to treat bleeding episodes in patients with high-responding inhibitors. Two bypassing agents are available for the management of bleeding in inhibitor patients, activated prothrombin complex concentrate (APCC; FEIBA, Baxter, Westlake Village, CA) and rFVIIa (NovoSeven, Novo Nordisk, Bagsvaerd, Denmark). APCC is a plasma-derived concentrate consisting of the vitamin K–dependent clotting factors both in nonactivated and activated forms. The mechanism of action of APCC largely is ascribed to the presence and action of FXa and prothrombin, although FIXa and FVIIa also are contained; small quantities of nonactivated FVIII may be present. rFVIIa contains FVIIa as its sole agent and is genetically engineered. The mechanism of action of rFVIIa is through thrombin generation on the surface of activated platelets through tissue factor–dependent and –independent mechanisms. Both APCC and rFVIIa have been demonstrated to be safe and effective, with variable response rates ranging from 70% to 90%. Two prospective studies compared these products and revealed essentially similar response rates. Both products have considerable data supporting their safety (>30 years for APCC and >10 years for rFVIIa) with few reported thrombotic events in hemophilic inhibitor patients. In addition, APCC as a plasma-derived product has an excellent safety record without documented viral transmission.

The most important consideration when choosing a product in an inhibitor patient is its ability to achieve rapid bleed control and thereby limit morbidity and mortality. Thus product choice is individualized. Because APCC is an FIX-based product, its use in FIX inhibitor patients with infusion-associated reactions is contraindicated. Another consideration is that rFVIIa does not stimulate either the FVIII or FIX inhibitor titer and may be preferred if trying to allow the inhibitor to reach a low level before ITI initiation. APCC may contain small quantities of FVIII and result in continued stimulation of the inhibitor titer in FVIII-deficient patients. Management of acute bleeding is critical; therefore, inhibitor stimulation is not an absolute contraindication to APCC use during this time if any bleeding episode is unresponsive to rFVIIa. Dosing regimens for both products have been established (Table 9-5). Occasionally, patients present with bleeding events refractory to both agents. In such cases, the use of combination APCC and rFVIIa has been reported using an alternative sequential regimen. Although the approach has been demonstrated to be effective and safe in a small number of young children, the reports remain anecdotal.

Historically, the prevention of bleeding in inhibitor patients was confined to prevention of bleeding during invasive procedures. Because of obvious concerns for hemostatic control during surgery and postoperatively with bypassing agents and concern for thrombotic events with repeated use in a high-risk setting, inhibitor patients were not offered elective surgery until fairly recently. Few studies demonstrating successful hemostatic strategies for inhibitor patients in

Table 9-5 Typical dosing for currently available bypassing agents.

Agent	Joint/muscle	Life or limb threatening	Preoperative	Prophylactic
APCC*	50-75 U/kg Repeat every 8-12 hours as needed	75-100 U/kg Repeat every 12 hours	50-75 U/kg	75 U/kg three times weekly
rFVIIa Standard dose†	90-120 mcg/kg Repeat every 2-3 hours as needed	90-120 mcg/kg Repeat every 2-3 hours	90-120 mcg/kg	90 mcg/kg/day
rFVIIa High dose	270 mcg/kg Data not available on follow-up doses required	270 mcg/kg Data not available on follow-up doses required	No data	270 mcg/kg/day

*Doses >200 U/kg/day contraindicated per prescribing information. APCC is licensed for the treatment of bleeding, not for surgery or prophylaxis.

†The licensed dose of rFVIIa in the United States is 90-120 mcg/kg for treatment and prevention of bleeding during surgery; not approved for prophylaxis.

the surgical setting have been performed. Over the past decade, several prospective studies have demonstrated the successful use of rFVIIa for both minor and major surgery (see Table 9-5 for dosing recommendations). This has led to an increased availability of required surgical procedures in inhibitor patients, most notably orthopedic procedures for amelioration of hemophilic arthropathy. APCCs have been used in the surgical setting, but the body of reports supporting their use, dosing, and safety is smaller compared with rFVIIa.

Recently, prophylaxis with bypassing agents to prevent bleeding episodes in inhibitor patients has gained attention as a potentially feasible approach. Several case reports of rFVIIa used prophylactically led to the performance of a prospective study that demonstrated an approximately 50% reduction in bleeding episodes during prophylaxis in patients with a high frequency of bleeding. A number of case series have demonstrated the use of APCC for prophylaxis with mixed results; a prospective study is under way. Currently, several new agents are in development with potential improved hemostatic properties and longer half-lives that may improve the overall treatment of inhibitor patients and make prophylaxis more effective and feasible in the future.

The management of bleeding episodes in acquired hemophilia is similar in many respects to that of congenital hemophilia with inhibitors, and the principles outlined earlier largely apply. An exception of note is that patients with acquired hemophilia often are elderly and at increased risk for thrombosis; thus, bypassing agents, although often required for control of bleeding, may have an associated higher rate of thrombotic complications. Inhibitor eradication in acquired hemophilia is different than in congenital hemophilia complicated by inhibitors. Because acquired hemophilia is due to the development of autoantibodies that result from loss of self-tolerance, they tend to respond to immunosuppressive medications effective in autoimmune disorders in general. Although these patients are too few to allow for well-designed prospective studies, a number of reports have demonstrated the effectiveness of glucocorticoids, cyclophosphamide, and more recently rituximab, with order of use as listed respectively. Although ITI has been reported in acquired hemophilia, it is more cumbersome than immunosuppression alone and usually is not required.

New therapies

Recently, new approaches to prolong the half-life of exogenously administered coagulation concentrates have been reported. Different strategies have been employed, including approaches to alter clearance, such as sialic acid residues addition or hydrophilic polymer conjugation, including coagulation factors encapsulated in polyethylene glycol (PEGylated) liposomes. Additionally, fusion proteins rendered resistant to normal clearance pathways are being explored. The use of products that increase the half-life of exogenous clotting factors has the potential to decrease dosing frequency in prophylactic regimens, positively improving compliance and quality of life, and also may improve on-demand therapy options through a decreased requirement for repeated dosing. These products are being explored actively in clinical trials. Finally, a major breakthrough in the field of hemophilia was recently reported. Nathwani et al. (2011) reported in the *New England Journal of Medicine* the result of a trial in which six patients with hemophilia B were treated with one infusion of an adenovirus-associated virus (AAV) vector expressing FIX. All patients showed expression of coagulation FIX for >6 months and their exogenous factor infusions were reduced significantly.

Prognosis and outcomes

Currently, patients with severe hemophilia without inhibitors treated on a prophylactic regimen have an excellent

prognosis and lead near-normal lives commonly without the development of hemophilic arthropathy. The Swedish cohort followed for nearly 40 years substantiates these outcomes. For patients with inhibitors, the outcome is more variable and the risk of morbidity is significant. When ITI is successful, the outcome can be converted to that of a noninhibitor patient, yet the morbidity experienced depends on the amount of joint disease and other bleeding events that occurred before ITI success. It is likely that many of these patients will have experienced hemarthroses, muscle, or even intracranial hemorrhage and that some of these bleeding events will be associated with permanent sequelae. For inhibitor patients in whom ITI was not successful or not performed, significant musculoskeletal morbidity is common, resulting in permanent disability and poor quality of life. With improved hemostatic coverage available for surgical interventions, even hemophilic patients with inhibitors now may undergo procedures to reduce pain and increase functionality. Combined with the increased use of prophylaxis, it is possible now to develop treatment strategies to ameliorate the consequences of recurrent bleeding and allow patients to lead more productive lives.

Gaps in knowledge

The greatest challenge with the potential for significant reward lies with gene therapy, a potentially curative approach. Development of improved therapeutic approaches for inhibitor patients who still experience increased morbidity and mortality compared with noninhibitor patients are required. One approach deserving of future work is the prevention of inhibitor formation. An improved understanding of the immunologic pathways involved in inhibitor formation and development of tolerance would open avenues to prevent inhibitor development or increase the rate of tolerance achieved. It is conceivable that an approach could be developed to program the immune system to induce tolerance before or in association with exposure to exogenous normal factor concentrate. Future research efforts could lead to the development of replacement products that are less or perhaps not immunogenic. In inhibitor patients, methods to perform ITI in FIX deficiency lag behind those for FVIII deficiency. For patients with anaphylactoid reactions, options for desensitization and subsequent ITI are limited, with an overall poor outcome, although rare success has been reported. The FIX-deficient inhibitor population with anaphylactoid reactions represents a small vulnerable population with only one therapeutic agent presently available for the management of bleeding episodes; new approaches and treatments clearly are required.

Key points

- Hemophilia is an X-linked disorder resulting from deficiencies of FVIII or FIX and is categorized as mild, moderate, and severe depending on the factor level.
- Patients with severe hemophilia are at risk for the development of joint disease termed hemophilic arthropathy that can be prevented by regular factor infusions begun at an early age (prophylaxis).
- Factor replacement therapy is available to treat bleeding episodes and is highly effective.
- Patients with hemophilia, most notably those with severe disease, may develop neutralizing antibodies directed against the deficient or replaced factor-termed inhibitors; inhibitors are divided into high- and low-responding types, and the presence of an inhibitor may render standard substitutive therapy ineffective.
- Inhibitors can be eradicated through treatment regimens termed ITI.
- Patients with high-responding inhibitors are treated with bypassing agents to manage their bleeding episodes; overall, bypassing products are not as effective as standard factor replacement in noninhibitor patients, and as such, inhibitor patients have an increased risk of hemorrhage-associated morbidity and mortality.

Rare factor deficiencies

Pathophysiology

Deficiencies of other coagulation factors that play a role in thrombin generation, cross-linking, and stabilization of the fibrin clot or down-regulation of fibrinolysis may lead to a bleeding diathesis. Deficiencies of fibrinogen, factor II (FII), FV, FVII, FX, and FXIII result in bleeding disorders in cases in which the severity of the bleeding most often is related to the factor levels, with the exception of FXI deficiency, in which case even patients with severe deficiencies may exhibit a variable bleeding tendency. Although FVIII and FIX deficiency are defined as rare disorders affecting <200,000 Americans, deficiencies of these other coagulation factors are far less common. Therefore, the clinical presentation related to any specific level and the range of symptoms experienced are less well described than in hemophilia A and B. For detailed discussion of these disorders, see the special issue of the journal *Hemophilia* (volume 14, issue 6, November 2008).

Etiology

As with hemophilia, rare factor deficiencies can result from a genetic defect or can be due to an acquired condition. The genes for these coagulation factors are located on somatic chromosomes. Affected individuals may be homozygous or compound heterozygotes. Because the number of genetic

mutations causing most of these rare disorders may be large, the ability to predict a level or phenotypic presentation is difficult.

Acquired factor deficiencies may be associated with a wide range of conditions, including commonly encountered liver dysfunction and uncommon circumstances, such as acquired FV deficiency due to exposure to bovine thrombin. Acquired disorders may result in multiple-factor deficiencies, as seen in liver dysfunction and vitamin K deficiency, or in single-factor deficiencies, such as in amyloid-associated FX deficiency.

Each acquired clotting factor deficiency may result from a wide range of disorders, and it is beyond the scope of this chapter to review all conditions that may result in any specific coagulation disorder. The more frequently encountered disorders and associated coagulation deficiencies will be highlighted. Hypofibrinogenemia can result from liver disease, use of chemotherapeutic agents such as L-asparaginase, and the Kasabach-Merritt syndrome (hemangioma with consumptive coagulopathy). Other consumptive processes such as disseminated intravascular coagulation lead to multiple coagulation factor deficiencies. FII, FVII, FIX, and FX are vitamin K dependent and are synthesized in the liver and thus become deficient in liver failure, with vitamin K deficiency, and with the use of vitamin K antagonists. A deficiency of FII due to specific factor antibody has been observed as part of the antiphospholipid syndrome. FX deficiency may occur with amyloidosis because of adsorption of the clotting factor onto the abnormal accumulated amyloid. A deficiency of FV may occur due to cross-reacting antibody development after exposure to topical thrombin or after the use of antimicrobials, such as cephalosporins. Acquired specific coagulation factor autoantibodies have been reported for other coagulation factors outside of FVIII, but these are exceedingly rare.

Two genetic multiple-factor deficiencies occur, including combined FV and FVIII and combined vitamin K–dependent coagulation factor deficiency. Combined FV and FVIII deficiency results from mutations in two genes *LMAN1* and *MCFD2* that encode for a protein complex that functions as a cargo receptor transporting FV and FVIII from the ER to the Golgi. The combined vitamin K coagulation factor deficiency is due to a number of mutations in genes that encode for enzymes involved in the vitamin K pathway. Both conditions are rare and have been reported in consanguineous families or individuals from closed small genetic groups. These combined coagulation factor deficiency states commonly are associated with moderate to severe deficiencies and variable bleeding symptoms.

Clinical presentation

The clinical presentation of the congenital rare factor deficiencies is variable and depends on the specific clotting factor and level of deficiency. These deficiency states may be discovered as a result of a known family history, although this is less common in autosomal recessive disorders unless a sibling has been identified. More commonly, affected individuals present with excessive bleeding, ranging from mild mucocutaneous bleeding to catastrophic intracranial hemorrhage. Unique features for each factor deficiency can be found in Table 9-6. Age at presentation is variable and most often is related to the affected coagulation factor and level of deficiency, with severe disorders presenting in childhood, and mild disorders presenting upon hemostatic challenges, such as surgeries. Patients with severe FXIII deficiency may present in the newborn period with significant umbilical stump or intracranial hemorrhage, whereas patients with severe FXI deficiency may present as adults either due to an abnormal aPTT obtained before a planned procedure or due to bleeding associated with trauma or surgery.

Acquired rare factor deficiencies present in the context of selected disorders, although these may not always be apparent during the initial presentation of the bleeding disorder. For example, patients with liver disease–associated coagulopathy often have signs and symptoms of liver dysfunction, including jaundice, ascites, and caput medusa, among others. Vitamin K deficiency may be seen in newborns who did not receive vitamin K at birth or those with malabsorption-related conditions. The resultant bleeding symptoms are similar to those seen in congenital factor deficiencies, although hemorrhagic disease of the newborn is associated with a high rate of intracranial hemorrhage.

Association of factor levels with disease severity

A recent communication of the Scientific Subcommittee of Rare Bleeding Disorders of the International Society of Thrombosis and Hemostasis underscores the importance of the association between coagulation factor levels and clinical bleeding in selected rare bleeding disorders. A thorough review of the literature and an extensive report from the known registries showed a clear correlation of undetectable levels of fibrinogen, FII, FV, and FXIII and severe bleeding. It also showed that levels <10% are associated with severe bleeding in FVII and FX deficiency. As previously reported, there was no correlation between FXI levels and spontaneous bleeding, but patients with levels < 20% appear to be at higher risk for postoperative bleeding.

Diagnosis

Once suspected, the diagnosis of a rare factor deficiency depends on the previously discussed principles of clinical history, physical examination, and an ordered systematic approach to laboratory evaluation. The majority of these deficiencies, when present at a severe or moderate level, result in

Table 9-6 Bleeding sites and symptoms and factor replacement choices for rare factor deficiencies.

Factor deficiency (level associated with major bleeding)*	Bleeding sites	Other symptoms	Factor replacement	Acquired deficiencies
Fibrinogen (<0.1 g/L-1)	No typical sites	Splenic rupture Miscarriage Thrombosis	Fibrinogen concentrate: RiaStap Cryoprecipitate	Liver disease Asparaginase therapy DIC
Factor II (<10%)	No typical sites	None	PCC	Vitamin K deficiency Liver disease Vitamin K antagonists Antiphospholipid syndrome
Factor V (<1%)	No typical sites	None	FFP Platelet transfusion	Topical bovine thrombin exposure, antibiotics
Factor VII (<10%)	Intracranial	Thrombosis	rFVIIa	Vitamin K deficiency Liver disease Vitamin K antagonists
Factor X (<10%)	Intracranial	None	PCC	Vitamin K deficiency Liver disease Vitamin K antagonists Amyloidosis
Factor XI (no clear association between levels bleeding)	Surgery or injury related	None	FFP FXI concentrates available in some countries	Autoantibodies (rare)
Factor XIII (undetectable)	Intracranial Umbilical stump	Poor wound healing Miscarriage	pdFXIII concentrate: Corifact Cryoprecipitate	Cardiopulmonary bypass Inflammatory bowel disease

RiaStap licensed for congenital afibrinogenemia. Recombinant factor VIIa is licensed for the treatment of congenital FVII deficiency. Corifact licensed for congenital FXIII deficiency. Prothrombin complex concentrates (PCC) not licensed for the treatment of rare factor deficiencies and contain variable amounts of factors II, VII, and X with dosing based on FIX units.

* Official Communication of the Scientific Subcommittee on Rare Bleeding Disorders of the International Society of Thrombosis and Haemostasis (ISTH).

DIC = disseminated intravascular coagulation; FFP = fresh frozen plasma; PCC = prothrombin complex concentrate.

prolongation of the PT or aPTT. Important exceptions include deficiencies of FXIII, PAI-1, or α_2AP, in which case the PT and aPTT are normal. The section on fibrinolysis addresses PAI-1 and α_2AP deficiency. On the basis of the results of these screening tests and subsequent mixing studies suggesting a factor deficiency, specific factor assays are performed and may result in diagnosis. If the screening tests are not prolonged, but the clinical history is suggestive of a bleeding disorder, then specific factor assays should be performed for both deficiencies that are known to be associated with normal screening tests and for others that, when present at a mild level, may not prolong these tests. FXIII deficiency is diagnosed via a qualitative assay (clot solubility assay) or via a quantitative assay. The clot solubility assay is abnormal when the FXIII level is <5% and therefore is not consistently sensitive to mild deficiencies; at this time, the clinical phenotype of mild FXIII deficiency is not well described. If suspicion exists that the deficiency is due to an autoantibody, mixing studies will reveal the presence of a time- or temperature-dependent inhibitor.

Treatment

For patients with congenital factor deficiencies, the mainstay of therapy is replacement of the deficient coagulation factor either after bleeding occurs or as prophylactic therapy, as described for severe hemophilia. Table 9-6 lists presently available therapies for factor replacement in the United States. For the majority of patients with rare disorders, standard therapy consists of treatment when bleeding occurs or before procedures or interventions. There are important exceptions to this approach; because severe deficiencies of FX and FXIII frequently result in catastrophic intracranial hemorrhage, these patients receive lifelong prophylaxis. For FX deficiency, this is accomplished through twice-weekly infusions of a prothrombin complex concentrate, whereas for FXIII, this is accomplished via monthly infusions of a plasma-derived FXIII concentrate currently licensed and available in the United States (a recombinant FXIII is in a phase III clinical trial). Severe FVII deficiency may be

associated with intracranial hemorrhage; the clinical phenotype of severe FVII deficiency is more variable than either FXIII or FX deficiencies, therefore, prophylactic treatment should be considered based on patient and family history. Recombinant activated FVIIa is licensed in the United States for the treatment of FVII deficiency.

The approach to management of acquired rare factor deficiencies includes both treatment of bleeding and treatment of the associated condition, if present. Treatment may be as relatively simple as administration of vitamin K in vitamin K deficiency, or it can be complicated as in some cases of liver failure. For patients in whom an associated condition is not identifiable or when present, its treatment is not feasible, the goal of therapy is aimed at intervention for bleeding episodes through either nonspecific therapies, such as fresh frozen plasma, or the use of specific factor concentrates as listed in Table 9-6. An individual approach for each patient's situation and diagnosis is required. Adjuvant therapies, including antifibrinolytic and topical agents, may be used depending on the clinical circumstance. Desmopressin does not have documented efficacy in these rare deficiencies.

Prognosis and outcomes

Congenital rare factor deficiencies are highly heterogeneous conditions both within and between each disorder. Furthermore, acquired conditions that result in rare factor deficiencies are quite varied: An acquired inhibitor may require specific intervention aimed at ablation or may spontaneously remit, as seen with FV antibodies associated with thrombin use; other associated conditions, such as liver failure, may have significant morbidity or mortality. Therefore, prognosis and outcome are related to the specific deficiency, its cause, the availability of an adequate replacement product, and the clinical circumstances. In general, mild to moderate congenital rare factor deficiencies often do not result in major sequelae, and the associated bleeding may be manageable. In those with a severe congenital deficiency, particularly if associated with serious bleeding complications, prophylactic therapy may be an effective approach, if a replacement product is available. These patients may then experience improved outcomes if permanent sequelae resulting from bleeding have not yet occurred. For patients with acquired rare factor deficiencies, outcomes may range from excellent to poor. Those who recover from an underlying condition that caused the coagulopathy may have an excellent outcome if a catastrophic bleed has not occurred. For those whose underlying condition is not treatable, prognosis may be poor and often related to consequences of the underlying disorder, although bleeding may contribute to outcome.

Gaps in knowledge

Large, well-designed prospective studies of congenital rare factor deficiencies are not possible due to the low disease prevalence. Much of current knowledge of these conditions is derived from registry data and small interventional studies. There is a need for both epidemiologic and therapeutic studies in these disorders. Development of international databases is required to establish the natural history and treatment outcomes of these disorders.

A major limitation in some of these conditions is the lack of availability of a specific replacement concentrate for treatment. Presently in the United States, three licensed products for rare disorders are available, specifically for afibrinogenemia, FVII, and FXIII deficiency. A specific concentrate for FXI deficiency is available in the European Union. In the United States, off-label use of products continues, including use of prothrombin complex concentrates for deficiencies of FX and FII. In FV and FXI deficiency, fresh frozen plasma remains the mainstay of therapy; in addition, platelet transfusions are sometimes used in FV deficiency as platelets also contain FV. Even when a concentrate is available, its use in these rare disorders often is guided by personal experience or anecdotal reports. For example, determination of appropriate patients for whom prophylaxis is indicated and the appropriate dosing regimen is largely poorly defined. Also, the peri- and postoperative care of patients with rare disorders is not founded on evidence-based data. There is a clear need for consistent data collection and studies on the clinical management of rare factor deficiencies.

Key points

- Rare factor deficiencies occur as a result of genetic mutations and acquired disorders.
- Treatment of an associated underlying disorder may lead to the resolution of the acquired deficiency.
- Rare factor deficiencies result in highly variable bleeding symptoms, ranging from injury or interventional bleeding (FXI) to severe spontaneous intracranial bleeding (FX and FXIII).
- Few specific factor replacement concentrates are available for patients with rare factor deficiencies.

Disorders of fibrinolysis

Pathophysiology

The fibrinolytic system provides orderly clot remodeling and dissolution. Imbalances in fibrinolysis may lead to excessive fibrinolytic activity through a variety of mechanisms, including increased tPA activity or inadequate

inhibition with PAI-1 or α_2AP deficiencies, and may result in excessive bleeding.

Etiology

Hyperfibrinolysis may result from congenital deficiencies of PAI-1 or α_2AP. PAI-1 deficiency is extraordinarily rare, and in only a few cases has the genetic alteration causing the disorder been identified. Defects in α_2AP also have been described. Both conditions are inherited as autosomal recessive traits. Additionally, hyperfibrinolysis may occur due to a variety of acquired conditions, including liver disease and disseminated intravascular coagulation (DIC); after surgery, particularly cardiac surgery; and some prostatic diseases and cases of acute promyelocytic leukemia. Although these conditions also contribute to bleeding for other reasons (factor deficiencies due to liver disease, consumption of clotting factors in DIC, and platelet dysfunction in cardiac surgery), the possibility of a contributing hyperfibrinolytic state should be considered, as specific therapies are available.

Clinical presentation

The clinical presentation of hyperfibrinolysis is highly variable. Hyperfibrinolytic bleeding may occur in isolation or as a result of a congenital deficiency; most commonly, it occurs as a part of a complex coagulopathy in an acquired disorder. Congenital deficiencies of the fibrinolytic pathway may present with delayed bleeding after injury or intervention and may include mucus membrane, cutaneous, or deep tissue bleeding; however, intracranial hemorrhage has been reported in PAI-1 and α_2AP deficiency. Acquired hyperfibrinolysis presents with bleeding at a variety of sites, and in patients with recent surgery, delayed postoperative hemorrhage often occurs at the surgical site.

Diagnosis

Laboratory investigation of the fibrinolytic system is difficult. The euglobulin clot lysis time (ELT) currently is not available in all laboratories, and interpretation of results is not always straightforward. The ELT assesses the capacity of plasma to lyse a clot formed in patient plasma. Under assay conditions, a clot is expected to dissolve within a set period of time, commonly approximately 2-6 hours, and a shortened ELT suggests hyperfibrinolysis. Several new global hemostatic assays are under evaluation for their ability to more accurately detect hyperfibrinolysis. A currently available global assay is the thromboelastogram and most commonly is used in surgical settings; thromboelastography is a method to assess global hemostasis and can detect hyperfibrinolysis in cases in which the use of antifibrinolytic agents may be helpful to control excessive bleeding.

It is possible to measure a few individual components of the fibrinolytic system, including α_2AP and plasminogen. Although it is possible to measure antigenic levels of PAI-1, the activity assay is problematic as the normal range includes levels of zero, thereby making detection of a dysproteinemic deficiency state impossible. Elevated PAI-1 levels have been associated with atherosclerosis and are not associated with bleeding. PAI-1 levels also exhibit diurnal variation, and any one level may not represent either the highest or lowest physiologic level. A deficiency of α_2AP is measurable; however, the correlation of level of deficiency and risk for bleeding is poorly established. It also is possible to measure the fibrinolytic proteins tPA and plasminogen, with a hyperfibrinolytic state expected to result in increased tPA and decreased plasminogen. Again, the correlation between specific levels and the degree of hyperfibrinolysis has not been established.

Therefore, laboratory diagnosis of the fibrinolytic system presently is not optimal, requiring the clinician to rely on clinical suspicion, including the presence of delayed bleeding, the clinical context, and, at times, response to therapeutic interventions.

Treatment

The treatment of hyperfibrinolytic bleeding is fairly straightforward except when it occurs as a complex coagulopathy when treatment requires careful consideration of thrombotic risk. The control of fibrinolytic bleeding is based on the use of antifibrinolytic agents; although several agents are available, two are most widely used: EACA and TXA. The mechanism of action of both agents involves competition with negatively charged lysine-rich residues in the kringle domain of plasminogen, which render it resistant to activation by tissue or urine plasminogen activators. Thus, these agents are effective in tissues rich in tPA or urine plasminogen activator. Both are available for intravenous and oral administration. Adverse effects and precautions were described previously. When using antifibrinolytic therapy, it is important not to discontinue therapy prematurely because of the risk of delayed bleeding. It is recommended to continue therapy up until the hyperfibrinolysis is felt to have resolved, or possibly on an ongoing basis if a congenital defect is confirmed and ongoing therapy is warranted.

Prognosis and outcomes

Most commonly encountered causes of hyperfibrinolysis are acquired; with trigger resolution, the patient's hemostatic system should return to normal, and provided that catastrophic bleeding has not occurred, patients should recover without sequelae. For rare patients with a confirmed congenital disorder, management with antifibrinolytic agents, even as prophylaxis, can minimize or reduce bleeding symptoms.

Gaps in knowledge

The major gap in knowledge in these conditions is the ability to establish an accurate diagnosis because treatment is less difficult than diagnosis. The fibrinolytic pathway remains the most problematic both in terms of diagnosis of a deficiency state and clearly attributable clinical manifestations. Improved and specific laboratory methods are required. A reliable, easily performed, reproducible screening assay would represent an important first step in the diagnosis of these disorders, followed by development of specific factor assays for all components of the fibrinolytic system. Levels of deficiency correlated with clinical bleeding could then be established. An improved understanding of the genetics of congenital fibrinolytic deficiencies and the associated spectrum of clinical manifestations would assist clinicians in the diagnosis of these rare disorders.

Key points

- Fibrinolytic disorders are the least well-defined hemorrhagic diatheses.
- Hyperfibrinolytic disorders are acquired most often, although rare congenital defects have been documented.
- Laboratory diagnosis of fibrinolytic disorders is difficult and inconsistently precise.
- Treatment of hyperfibrinolytic bleeding is based on the use of antifibrinolytic agents, including EACA and TXA.

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