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Coagulation and Bleeding Disorders: Review and Update

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Hemostasis is initiated by injury to the vascular wall, leading to the deposition of platelets adhering to components of the subendothelium. Platelet adhesion requires the presence of von Willebrand factor and platelet receptors (IIb/IIIa and Ib/IX). Additional platelets are recruited to the site of injury by release of platelet granular contents, including ADP. The "platelet plug" is stabilized by interaction with fibrinogen. In this review, I consider laboratory tests used to evaluate coagulation, including prothrombin time, activated partial thromboplastin time, thrombin time, and platelet count. I discuss hereditary disorders of platelets and/or coagulation proteins that lead to clinical bleeding as well as acquired disorders, including disseminated intravascular coagulation and acquired circulating anticoagulants.

Primary Hemostasis

Platelets are anuclear cellular fragments derived from bone marrow megakaryocytes. They have a complex internal structure, which reflects their hemostatic functions *(1, 2)*. Two major intracellular granules are present, the α granules and dense bodies (Fig. 1) The α granules contain platelet thrombospondin, fibrinogen, fibronectin, platelet factor 4, von Willebrand factor (VWF),[1] platelet-derived growth factor, β-thromboglobulin, and coagulation factors V and VIII. The dense granules contain ADP, ATP, and serotonin (5-hydroxytryptamine). When platelets are stimulated, both the α granules and dense bodies are released through the open canalicular system.

Platelets and endothelial cells have biochemical pathways involving the metabolism of arachidonic acid (AA; Fig. 2) *(3–5)*. AA is released from membrane phospholipids by phospholipase A_2. Subsequently, cyclooxygenase converts AA to cyclic endoperoxides *(6)*. The endoperoxides are then converted by thromboxane synthetase to thromboxane A_2. Thromboxane A_2 is a potent agonist that induces platelet aggregation *(7)*. Endothelial cells also contain an AA pathway. However, endothelial cells preferentially convert cyclic endoperoxides to prostacyclin *(8)*, which is a potent inhibitor of platelet aggregation.

Primary hemostasis is a process whereby platelets interact with elements of the damaged vessel wall, leading to the initial formation of a "platelet plug". The platelet/injured vessel wall interaction involves a series of events that includes platelet adhesion to components of the subendothelium, activation and shape change, release of platelet granular contents (dense bodies and α granules) with subsequent formation of fibrin-stabilized platelet aggregates, and clot retraction *(8)*. In this process, the activation of platelets with exposure of negatively charged phospholipids (e.g., phosphatidylserine and phosphatidic acid) facilitates the assembly of coagulation factors on the activated platelet membrane, leading to generation of thrombin and subsequent fibrin deposition. The platelet plug and fibrin are analogous to the cork in a bottle of champagne that is stabilized by a wire mesh.

Platelet adhesion is accentuated by increased shear rate. For platelets to adhere to a damaged vascular surface, both fibrinogen and VWF are necessary *(8)*. The platelet glycoprotein (GP) receptor (Ib/IX and V) is the principal receptor for VWF *(9)*. VWF also binds to GP IIb-IIIa, which is expressed when platelets are activated. Both fibrinogen and VWF interact with GP IIb-IIIa. In addition to VWF, other proteins (laminin, thrombospondin, and vitronectin) may be involved in platelet adhesion.

Platelet activation results from exposure of the platelet to damaged endothelium or underlying components of the vessel wall *(8, 9)*. Other biological agonists are in-

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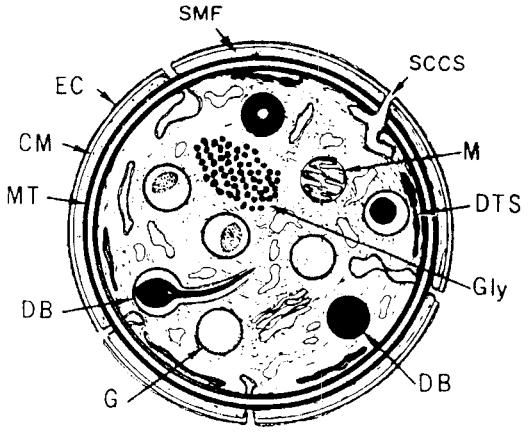
[1] Nonstandard abbreviations: VWF, von Willebrand factor; AA, arachidonic acid; GP, glycoprotein; BT, bleeding time; VWD, von Willebrand disease; DDAVP, desmopressin (1-desamino-8-D-arginine vasopressin); TF, tissue factor; APC, activated protein C; APTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time; LA, lupus anticoagulant; and APA, anti-phospholipid antibody.



SCCS = Open Canalicular System

MT = Microtubules

SMF = Submembrane Filaments

M = Mitochrondria

DB = Dense Bodies

G = Alpha Granules

EC = Exterior Coat; Glycocalyx

CM = Trilaminar Unit Membrane

SMF = Submembrane Area

DTS = Dense Tubular System

Fig. 1. Schematic of electron micrograph of equatorial section of platelet.

SCCS, open canalicular system; *M*, mitochondria; *DTS*, dense tubular system; *Gly*, glycogen. Reprinted with permission from White JG, Gerrard JM. Ultrastructural features of abnormal platelets. A review. Am J Pathol 1976;83:589–632.

volved in platelet activation, including thrombin, epinephrine, ADP, and thromboxane A_2. With activation, platelets transform from a disk to a "spiny sphere" with long pseudopodia. The initial generation of trace amounts of thrombin leads to amplification of the coagulation response. Thrombin activates factor XI in the contact system and coagulation cofactors V and VIII *(10)*. The initial formation of fibrin at the site of vascular injury is unstable. Factor XIII (fibrin-stabilizing factor) is activated by thrombin, causing cross-linking of fibrin strands and stabilization of the fibrin/platelet plug.

Hereditary and Acquired Disorders of Platelet Function

Abnormalities of platelet function are characterized by clinical bleeding of varying severity. In most cases, patients present with mucocutaneous bleeding or excessive hemorrhage following surgery or trauma. A platelet count and careful examination of the peripheral smear is essential in the initial evaluation of patients with mucocutaneous bleeding. When examining the peripheral smear, it is important to evaluate the relative size of platelets. Large platelets may be seen as a result of accelerated marrow production of platelets attributable to a hemorrhagic event or recovery from bone marrow suppression as a result of infections or drugs. Large platelets are also encountered in the setting of patients with accelerated platelet turnover (idiopathic thrombocytopenic purpura) *(11)*.

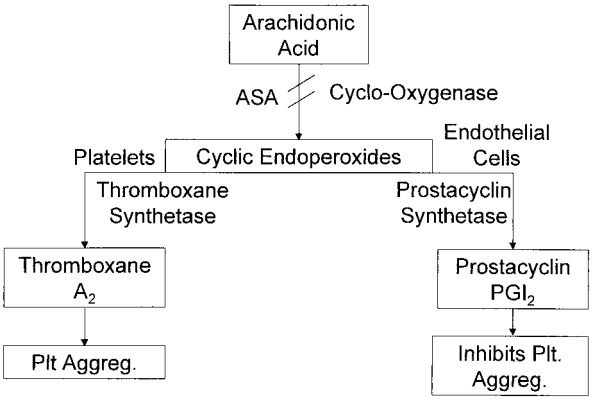


Fig. 2. AA pathway.

Platelets and endothelial cells contain pathways for metabolism of AA. When platelets or endothelial cells are activated, an enzyme, phospholipase A_2, is activated, liberating AA. AA is then converted to thromboxane A_2 by cyclooxygenase and thromboxane synthetase. Thromboxane A_2 is a potent activator of platelets, leading to platelet aggregation (Plt Aggreg). In the endothelial cells, prostacyclin synthetase converts cyclic endoperoxides to prostacyclin (*PGI_2*). Prostacyclin inhibits platelet aggregation. Aspirin (*ASA*) inhibits cyclooxygenase. Modified from Pallister C. Blood, physiology and pathophysiology. Butterworth-Heinemann Ltd., 1994:452.

The bleeding time (BT) test has also been widely utilized as a means of accessing primary hemostatic response (platelet-injured vessel wall interaction) *(12)*. Unfortunately, the BT is relatively insensitive and, in many cases, nonspecific with respect to identifying abnormalities of primary hemostasis *(13)*. The major variables are the inherent differences between individuals performing the BT and the various BT devices. The introduction of BT devices designed to decrease the variability of the depth of the induced wound was a major advance over the traditional Ivy BT test *(12, 13)*. Despite the introduction of the newer devices, there remains substantial variability between individuals performing BTs as well as the possible complication of scar formation at the test site (typically, the anterior-lateral aspect of the arm).

There are several variables in the BT in addition to the technical aspects of performing the test. BTs tend to be longer in females and decrease with aging. One cosmetic complication frequently seen in elderly patients who have experienced extensive sun exposure is the formation of a somewhat symmetrical subepidermal hemorrhage, which is attributable to blood dissecting into the subepidermis as opposed to exiting onto the surface of the skin at the site of the BT incision. The BT is also affected by the hematocrit and platelet mass. Patients with chronic renal disease

and decreased hematocrit often have a prolonged BT *(14, 15)*. Increasing the hematocrit to >30% often will correct a prolonged BT in a patient with chronic renal disease *(16)*. Abnormalities of connective tissue (e.g., Ehlers-Danlos syndrome) may produce abnormal BTs *(17)*.

The BT together with the Rumpel-Leede test were the first attempts to evaluate platelet/vascular response to injury *(18)*. The Rumpel-Leede test involved inflating a blood pressure cuff midway between systolic and diastolic pressure and leaving the cuff on for a period of time, which was variable depending on the patient's tolerance for the procedure. The arm distal to the blood pressure cuff was evaluated for the presence of petechiae.

Platelet aggregation is an important component of laboratory testing in a patient with clinical findings suggestive of a primary hemostatic abnormality *(19, 20)*. The addition of an agonist (e.g., ADP, epinephrine, or collagen) to normal platelet-rich plasma produces an aggregation pattern characterized by a biphasic response when epinephrine is used as the agonist. The primary wave results from the addition of exogenous epinephrine, and the secondary wave reflects the "release reaction" of the dense bodies. With release, granular components are excreted through the open canalicular system. Abnormalities of the release reaction may be seen in patients with storage pool disease (characterized by loss of platelet nucleotides and serotonin from the dense granules; Table 1) *(21)*. Dense body storage pool abnormalities have been described in Hermansky-Pudlak, Chédiak-Higashi, and Wiskott-Aldrich syndromes and thrombocytopenia with absent radii *(22–26)*. Patients with afibrinogenemia or Glanzmann thrombasthenia (abnormalities of the GP IIb-IIIa receptor) lack both primary and secondary responses to various platelet agonists *(27)*. Glanzmann thrombasthenia is an autosomal recessive defect that frequently is encountered in patient populations in which there is a high incidence of consanguinity.

There are numerous reports of patients with selectively impaired aggregation response to various platelet agonists *(28)*. Lack of response to epinephrine has been reported in patients with decreased α_2 adrenergic receptors *(29)*. Isolated collagen receptor defects have been reported (decreased platelet GP Ia) *(30)*. It is important to appreciate the variability one may see in platelet aggregation studies. Often a lack of a secondary response is attributable to drugs (classically aspirin) that inhibit cyclooxygenase. The pharmaceutical industry is intensively developing various inhibitors of ADP receptors (ticlopidine and clopidogrel) and IIb-IIIa receptors (Table 2 and Fig. 3) *(31)*.

Other tests that have been used in evaluating platelet function include the prothrombin consumption test (a test to evaluate the platelet contribution of activated phospholipids), flow cytometry to quantify surface GPs, receptor occupancy, and electron microscopy for evaluating ultrastructural anatomy *(32–35)*.

Several recently developed instruments have been designed to assess the global platelet response. Examples include Xylum®, PFA-100® (Dade-Behring), and test systems marketed by Array and Medtronics *(36–39)*. The Xylum and PFA-100 represent instruments that attempt to simulate the in vivo response of platelets to vascular injury *(40)*. In the case of the PFA-100, two collagen-impregnated membranes, one with ADP and the other with epinephrine, are used to evaluate the platelet re-

Table 1. Hereditary disorders of platelet function.[a]

Abnormalities of adhesion (platelet vessel wall interaction/adhesion)
VWD
Bernard Soulier syndrome (abnormal or absent GP Ib)
Abnormalities of platelet aggregation
Congenital afibrinogenemia
Glanzmann thrombasthenia (abnormal GP IIb/IIIa)
Disorders of platelet release/signal transduction
Storage pool disease
Deficient dense bodies
Hermansky-Pudlak syndrome
Chediak-Higashi syndrome
Wiskott-Aldrich syndrome
Thrombocytopenia with absent radii
Deficient α granules
Gray platelet syndrome
Deficiency of α granules and dense bodies
Signal transduction defects
Abnormal AA pathways
Impaired AA release
Cyclooxygenase deficiency
Thromboxane synthetase deficiency
Abnormalities of platelet membrane response
Scott syndrome

[a] From Rao *(2)*.

Table 2. Antagonists of platelet IIb/IIIa receptors.[a]

Abciximab (c7E3)
Chimeric compound
RGD[b] sequence
Kistrin
Echistatin
Cyclic peptides
Integrelin
Peptidomimetics (i.v. & oral)
Tirofiban (i.v.)
Lamifiban (i.v.)
Eptifibatide (i.v.)
Xemilofiban (oral)
DMP 802 (oral)
SR 121787 (oral)

[a] Several inhibitors of platelet IIb/IIIa receptors have been reported. Included are a number of synthetic small molecular inhibitors, peptidomimetics. They may be given either intravenously (lamifiban, tirofiban, and eptifibatide) or orally (e.g., xemilofiban).

[b] RGD, critical peptide sequence necessary for binding; i.v., intravenous.

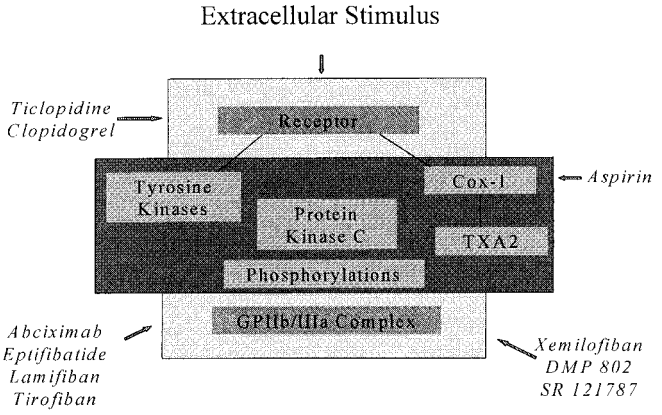


Fig. 3. Targets for platelet inhibitors.

The activation of platelets occurs when platelets adhere to subendothelial components of the vessel wall. After adhesion, release of platelet granular contents leads to platelet aggregation. Various drugs have been used to inhibit platelet activation. Ticlopidine and clopidogrel inhibit the ADP-induced activation pathway. Aspirin irreversibly blocks cyclooxygenase enzyme (*Cox-1*). This prevents the generation thromboxane A_2 (*TXA2*), which is a potent activator of platelets. Various inhibitors of GP IIb/IIIa complex prevent platelet aggregation. Among the inhibitory drugs that have been developed recently are Abciximab (c7E3 Fab fragments), Eptifibatide (a cyclic peptide), and two peptidomimetics (lamifiban and tirofiban). Oral inhibitors include xemilofiban, DMP 802, and SR 121787. These three drugs are oral inhibitors of platelet function. Reprinted with permission from Nurden AJ, Poujol C, Darrieu-Jacs C, Nurden P. Platelet glycoprotein IIb/IIIa inhibitors. Basic and clinical aspects. Arterioscler Thromb Vasc Biol 1999;19:2835–40.

sponse *(40)*. The patient's citrated blood sample is aspirated under high shear rates (5000–6000 dyn/cm^2) through a 150-μm diameter aperture in the center of a collagen-impregnated membrane. The endpoint is obtained when the flow of blood ceases. This test system is extremely sensitive to the presence of aspirin (epinephrine abnormal/ADP normal). The PFA-100 may be used to monitor antiplatelet drug therapy. Other new instruments under development offer promise in the monitoring of patients who are increasingly exposed to a greater variety of platelet antagonists *(41)*. The PFA-100 has been used to screen patients for von Willebrand disease (VWD) and has been very effective in identifying these patients *(42)*.

Therapeutic Options in Management of Platelet Disorders

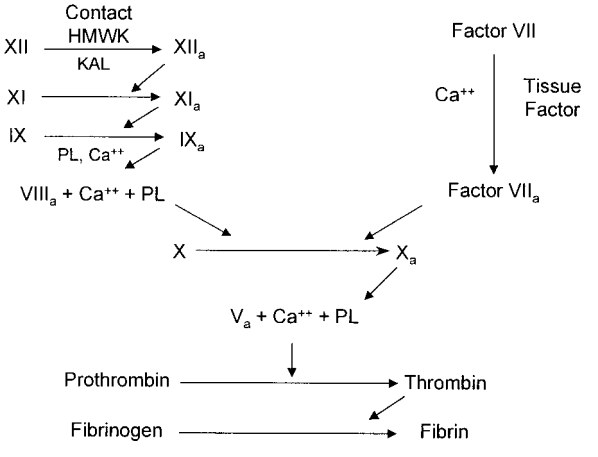
Increasingly, desmopressin (DDAVP) is being used to manage patients with abnormalities of primary hemostasis, e.g., VWD, patients exposed to aspirin, and cirrhotic patients with bleeding complications *(43)*. DDAVP triggers the release of VWF from Weibel-Palade bodies of vascular endothelium. DDAVP has also been used in the management of patients with mild to moderate hemophilia A (deficiencies of factor VIII) *(44)*.

Recombinant human erythropoietin has been used to manage bleeding in uremic patients *(45)*. In cases of severe thrombocytopenia or iatrogenic inhibition of platelet function, the use of platelet concentrates is indicated.

In renal failure patients with hemorrhagic complications, correction of the hematocrit to >30% often will alleviate bleeding problems *(45, 46)*. The red cell mass is instrumental in "marginating" platelets to the endothelial-blood interface. The proximity of platelets to endothelium facilitates the primary hemostatic response after vascular injury.

Coagulation Factors: Formation of Fibrin Clot

Fibrin is critical in stabilizing the initial platelet plug. The formation of fibrin involves several enzymatic steps culminating in the generation of thrombin, which converts fibrinogen to fibrin. Fig. 4 represents a simplified coagulation cascade incorporating the intrinsic and extrinsic pathways *(47)*. The coagulation proteins may be classified into three groups: fibrinogen family, vitamin K-dependent proteins, and the contact family (Table 3). The fibrinogen family of proteins includes fibrinogen, factor V, factor VIII, and factor XIII. This family of proteins has relatively large molecular weights, and its members are substrates for thrombin. The formation of a stable fibrin clot is dependent on the ability of thrombin to convert fibrinogen to fibrin and simultaneously activate factor XIII to $XIII_a$, which stabilizes the fibrin clot. The initiation of coagulation begins with exposure of tissue factor (TF) to the circulating blood *(48)*. TF binds factor VII, producing a TF-VII_a complex. The TF-VII_a complex triggers the final common pathway by converting factor X to factor X_a in the presence of factor VIII. In addition, TF-VII_a activates factor IX to IX_a. This dual activation mediated by TF-VII_a explains the initiation of coagulation following tissue damage. Once trace amounts of thrombin are generated, there is marked amplification through thrombin feedback to activate factors V, VIII, and XI.



PL = Phospholipid

Fig. 4. Simplified coagulation cascade.

HMWK, high molecular weight kininogen; *Kal*, kallikrein; *PL*, phospholipid; *Ca*$^{++}$, ionic calcium; subscript a denotes the activated form of the coagulation factors (e.g., factor XII_a, factor VII_a). The initiation of coagulation involves two separate pathways: intrinsic and extrinsic. Physiologically, the extrinsic pathway, which is initiated by tissue damage and exposure of tissue factor, is the most important pathway to initiate the hemostatic response. Tissue factor forms a complex with factor VII_a, leading to activation of the final common pathway at factor X. The extrinsic pathway is evaluated in vitro by PT. The intrinsic pathway involves exposure of factor XII to activated cellular surfaces or subendothelium. Activation of factor XII (Hageman factor) initiates the intrinsic pathway.

Table 3. Families of coagulation proteins.

Fibrinogen	Vitamin K-dependent	Contact
Fibrinogen	Factor II	Factor XII
Factor V	Factor VII	HMWK[a]
Factor VIII	Factor IX	Prekallikrein[b]
Factor XIII	Factor X	Factor XI

[a] HMWK, high-molecular weight kininogen, also known as Fitzgerald factor.
[b] Also known as Fletcher factor.

The enzymatic reactions involved in the generation of thrombin occur on the surface of damaged cells (e.g., endothelial cells, monocytes, platelets, and tumor cells) *(49)*. With activation of platelets, phosphatidylserine is mobilized from the inner leaflet of the platelet membrane and exposed on the external surface *(50)*. Table 4 summarizes the key procoagulant complexes necessary for normal coagulation. A cofactor for the activation of factor IX by factor XI_a has not been identified. The TF-VII_a complex is capable of activating both factors IX and X.

There are physiologic inhibitors in plasma that serve to localize procoagulant activity to the site of injury and maintain hemostatic balance (Table 5). Antithrombin (known as antithrombin III in old terminology) is the principle inhibitor of thrombin, factor X_a, and the other serine proteases (Table 6) *(51)*. Antithrombin serves as a "pseudosubstrate" for thrombin and "traps" thrombin in an antithrombin-thrombin complex, which is cleared from the circulation *(52)*. In the presence of heparin, there is marked acceleration of the antithrombin-thrombin interaction, leading to anticoagulation of the patient. Heparin cofactor II is also an inhibitor of thrombin. Heparin cofactor II inhibition is accentuated by dermatan sulfate *(53, 54)*. Other members of the serpin (serine protease inhibitors) family inhibit thrombin but to a substantially lesser degree than antithrombin.

Thrombin also binds to an endothelial receptor, thrombomodulin. As the name implies, thrombomodulin binds thrombin with resulting loss of thrombin's procoagulant activities (ability to convert fibrinogen to fibrin, activation of platelets, and factors V, VIII, XI, and XIII). As a result of binding to thrombomodulin, the thrombin-thrombomodulin complex activates protein C to activated protein C (APC) *(55)*. The protein C/protein S pathway is one of the most important regulatory systems. Once protein C is converted to APC, it becomes an inhibitor of coagulation *(56)*. This inhibition is mediated by the inactivation of activated factors VIII and V (Fig. 5). Protein S, a vitamin K-dependent protein, is an important cofactor for this reaction. The formation of anticoagulant complexes is analogous to procoagulant complexes described above (Table 7).

Table 4. Procoagulant complexes.[a]

	IXase	Int[b] Xase	Ext Xase	IIase
Enzyme	XI_a	IX_a	VII_a	X_a
Cofactor	?[c]	VIII	TF	V
Surface	Plts/Endoth	Plts/Endoth	Plts/Endoth	Plts/Endoth
Substrate	IX	X	X	II

[a] For the coagulation process to occur, it is necessary to assemble procoagulant complexes on the surface of activated cells (e.g., platelets, endothelial cells, monocytes) or the subendothelial matrix of the vessel wall. In the intrinsic pathway, the contact factors [factor XII, Fitzgerald factor (high-molecular weight kininogen), prekallikrein (Fletcher factor), and factor XI] interact to initiate the contact phase of coagulation. With the generation of factor XI_a, factor IX is activated. The extrinsic pathway of coagulation involves tissue damage with exposure of tissue factor, which binds factor VII_a. In vivo, the most important pathway to initiate coagulation is the extrinsic pathway. The complex of tissue factor-factor VII_a activates factor X and also factor IX. Complexes involved in the procoagulant process include an enzyme, cofactor, and appropriate cellular surface as well as a substrate protein.
[b] Int, intrinsic; Ext, extrinsic; Plts, platelets; Endoth, endothelial cells.
[c] Not identified.

Table 5. Hemostatic balance.[a]

Procoagulant	Anticoagulant
Enzymes (IIa, Xa, and others)	AT[b]
Cofactors (V, VIII)	PC, PS
PL surfaces	β_2 GPI
Extrinsic system (TF)	TFPI

[a] To confine the procoagulant response to the localized site of injury, there are several "regulatory pathways", which serve to limit activation of coagulation to the site of injury. Physiologic anticoagulants include antithrombin (formerly known as antithrombin III), which inhibits the serine protease enzymes of coagulation (e.g., thrombin, factor X_a, and factor IX_a). The kinetics of antithrombin inhibition of serine proteases are enhanced in the presence of unfractionated heparin. The cofactors of coagulation include factor V_a and factor $VIII_a$. The protein C system is designed to down-regulate these two critical cofactors. The down-regulation of activated cellular surfaces remains a matter of conjecture. There have been suggestions that β_2-glycoprotein I may be a physiologic inhibitor by virtue of its ability to bind to activated phospholipid cellular surfaces. The regulation of the extrinsic pathway (TF mediated) is modulated by the TF inhibitor. Patients with hereditary disorders of the anticoagulant pathways (antithrombin, protein C/protein S system) have a predisposition to thrombosis. Deficiencies of these proteins are inherited in an autosomal fashion.
[b] AT, antithrombin; PC, protein C; PS, protein S; PL, phospholipid; β_2 GPI, anti-β_2-glycoprotein I; TFPI, tissue factor inhibitor.

Laboratory Evaluation of Coagulation Pathways

The laboratory tests for evaluation of platelet function have been discussed above. Evaluation of the coagulation pathways relies on four relative simple tests: the activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), and fibrinogen assays *(57–61)*.

Table 6. Serine proteases inhibited by antithrombin.[a]

Coagulation	Fibrinolysis	Kinin system
Factor XIIa	Plasmin	Kallikrein
Factor XIa		
Factor IXa		
Factor VIIa		
Factor Xa		
Thrombin		

[a] Antithrombin inhibits the activated enzymes involved in coagulation, fibrinolysis, and kinin system. In the presence of heparin, the interaction between antithrombin and the various enzymes is accelerated.

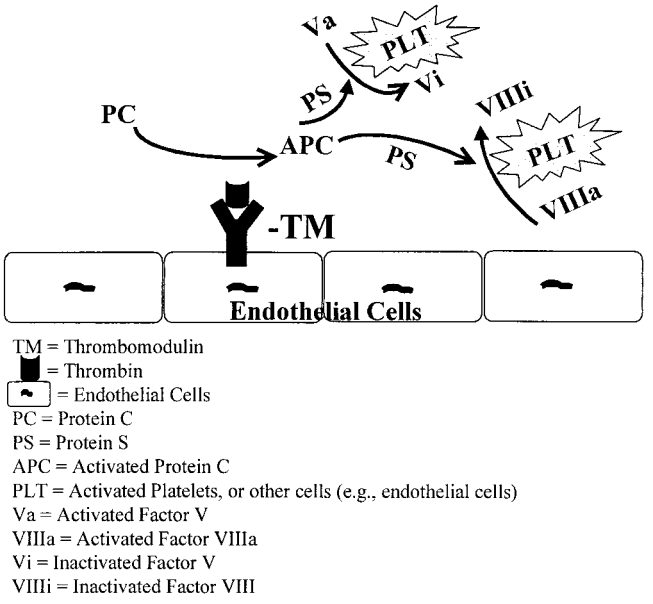


Fig. 5. Down-regulation of activated factors V and VIII by protein C/protein S system.

Protein C (*PC*) and protein S (*PS*) are vitamin K-dependent proteins that participate in the down-regulation of hemostasis. When thrombin is generated in vivo, it converts fibrinogen to fibrin. In addition, thrombin may also bind to an endothelial receptor, thrombomodulin (*TM*). As the name implies, once thrombin binds to thrombomodulin, it loses it procoagulant activity and actively participates in the protein C/protein S anticoagulant pathway. Thrombin-thrombomodulin converts protein C to APC. APC in the presence of protein S (a cofactor) will inactivate the activated forms of factors V and VIII, thus inhibiting the generation of thrombin. Patients who have hereditary deficiencies of protein C and protein S are predisposed to venous thromboembolic events.

In addition, the availability of a test to monitor D-dimer is of considerable value. D-Dimer is increased in fibrinolysis; in addition, it is used as a negative predictor to rule out deep vein thrombosis *(62)*. Laboratories must carefully select the correct test (sensitivity) and apply this test in an appropriate clinical situation.

The APTT is a test that evaluates the intrinsic pathway of coagulation. The APTT reagents comprise an activator (e.g., ellagic acid, celite, or kaolin) and phospholipids. The phospholipids may be either synthetic or derived from animal tissue (e.g., rabbit brain). With the exception of factors VII and XIII, the APTT evaluates all of the coagulation factors. The most common cause of a prolonged APTT is incorrect collection of the blood sample. Most frequently, this is attributable to obtaining blood through an indwelling line that has been flushed with heparin. In addition, a traumatic venipuncture may produce an abnormal APTT. A polycythemic blood sample may yield an abnormal APTT because of the excess amount of citrate available to chelate calcium in a polycythemic blood sample. Other causes of a prolonged APTT include factor deficiencies [VWD, hemophilia A (factor VIII deficiency), and hemophilia B (factor IX deficiency)] and the presence of circulating anticoagulants (also known as inhibitors). The most common circulating anticoagulant is the lupus anticoagulant (LA) *(63, 64)*. Antibodies to factor VIII are also encountered in both hemophilia A patients and adults who occasionally have an acquired autoantibody against factor VIII *(65, 66)*. Hereditary and acquired factor deficiencies often produce an abnormal APTT. Most reagent manufacturers provide reagents that will yield an abnormal APTT when the concentration of factor VIII is <30–35% (0.30–0.35 kilounits/L). However, there is substantial variability between reagents. There may also be lot-to-lot variability of APTT reagents from the same manufacturer. Therefore, it is imperative when any aspect of the "system" (e.g., reagent-instrument combination or collection tubes) is changed to recalculate the reference interval and the relative sensitivity of the system to factor deficiencies and heparin.

The PT is the most commonly performed test of hemostasis. The PT evaluates the extrinsic pathway of coagulation (factors VII, X, V, II, and fibrinogen). The PT is used to monitor patients on oral anticoagulant therapy. With the recent introduction of sensitive PT reagents, the use of the international normalized ratio has become the standard reporting format for PT results *(67)*. Patients receiving oral anticoagulant therapy in most cases have a targeted therapeutic range of an international normalized ratio of 2.0–3.0 *(68)*. There are exceptions, including mechanical valves, patients who re-thrombose when in the therapeutic range of 2.0–3.0, and patients with antiphospholipid antibody syndrome. The PT may be prolonged in patients with disseminated intravascular coagulation, liver disease, or vitamin K deficiency.

The TT is a simple test. Thrombin (bovine or human) is added to citrated plasma. There are two variations of the TT: one uses calcium and the other does not. In individuals with fibrinogens <1000 mg/L, the TT will be prolonged. Other causes of a prolonged TT include the presence of heparin in a blood sample, dysfibrinogenemias, antibodies to thrombin, and gammopathies (e.g., multiple myeloma and Waldenström macroglobulinemia).

A functional assay for fibrinogen is part of the initial analysis of patients with bleeding disorders. Often, the TT is not prolonged in patients with hypofibrinogenemia until it is <1000 mg/L. A discrepancy between the functional assay and antigenic assay is encountered in patients with dysfibrinogenemia *(69)*.

Table 7. Anticoagulant complexes.[a]

	PC[b] activation	APC activity
Enzyme	Thrombin	APC
Cofactor	TM	PS
Surface	Endothelium	Plts/Endothelium
Substrate	Protein C	V_a, $VIII_a$

[a] The protein C/protein S system is involved in down-regulating the two key cofactors involved in the activation of factor X (factor $VIII_a$) and in the activation of prothrombin (factor V_a). Patients with acquired or hereditary deficiencies of protein C and protein S are at increased risk of thromboembolic events.

[b] PC, protein C; TM, thrombomodulin; PS, protein S; Plts, platelets; V_a, activated factor V; $VIII_a$, activated factor VIII.

Hereditary Disorders of Coagulation Proteins

VWD is the most common inherited disorder of hemostasis *(70)*. The incidence of VWD in the population is ~1%. It is found in all ethnic groups, and in many cases, patients remain undiagnosed. VWD is an autosomal dominant disorder affecting both males and females *(71, 72)*. Before puberty, easy bruising and epistaxis are the most frequently encountered clinical presentations. At the time of puberty, the frequency of epistaxis tends to decrease. In affected females, the chief complaint becomes one of menorrhagia *(73)*. It is estimated that ~10% of hysterectomies performed in the United States are the result of underlying occult VWD *(71)*. With appropriate diagnosis and patient management, many unnecessary surgeries could be eliminated.

The diagnosis of VWD requires a careful patient/family history. Many patients with VWD are first diagnosed following an accident/trauma or surgery. Prolonged bleeding following surgery is often encountered in VWD patients *(71, 72)*. However, the laboratory diagnosis may be very difficult because of the "fluctuation" of VWF in the patient's plasma. VWF responds to stress similar to other acute phase proteins, e.g., fibrinogen, fibronectin, and vitronectin *(72)*. Therefore, it is not appropriate to test the patient for VWD in the setting of acute bleeding or stress.

VWF is synthesized in endothelial cells and megakaryocytes. In the endothelial cells, it is stored in the Weibel-Palade bodies with a range of molecular masses from 0.5 to >20 million Da *(72)*. VWF is also found in the α granules of platelets. VWF will bind to collagen, particularly in situations of high shear stress. In addition, as discussed above, VWF will bind platelet receptors GP IIb/IIIa and GP Ib/IX/V. Many variants of VWD have been described. These include both qualitative and quantitative abnormalities as well as combinations of both defects. Table 8 summarizes the current classification of VWD *(72)*.

Laboratory testing includes a BT or other means of analyzing platelet function. Recent reports utilizing the PFA-100 suggest that this system or similar new platelet analyzers are preferable to the classical BT *(42)*. Not infrequently, one may encounter variability of the APTT in patients with VWD.

Tests to classify VWD include quantification of VWF. Initially, this was determined by Laurell rocket immunoelectrophoresis. More recently, ELISA assays as well as flow cytometry have been used with a greater degree of sensitivity. The ristocetin cofactor is a test to assess VWF activity. Ristocetin-induced platelet agglutination is the most widely used procedure *(74, 75)*. However, there are recent reports on the use of antibodies to the collagen binding site as a means of testing for VWF function. This assay system uses an ELISA format *(76)*. A factor VIII:C (coagulant activity) assay is also a part of the evaluation for VWD. Multimeric analysis of VWF by agarose gel electrophoresis is very helpful in identifying variants of

Table 8. Classification of VWD.[a]

Subtype	Frequency	Genetic transmission	Clinical findings	FVIIIC[b]	VWF:Ag	VWF:Act	Multimers	DDAVP response
Type 1	70% of VWD	Autosomal dominant	Mild to moderate bleeding	Decreased	Decreased	Decreased	Normal distribution	+++[c]
Type 2A	10–15% of VWD	Autosomal dominant	Mild to moderate bleeding	Decreased	Decreased	Decreased	High and intermediate multimers absent	±
Type 2B	<5% of VWD	Autosomal dominant	Mild to moderate bleeding	Variable, decreased	Variable, decreased	Enhanced, RIPA	Absent large multimers	May be contraindicated
Type 2M	Rare	Autosomal dominant, missense mutation	Variable bleeding	Variable, decreased	Variable, decreased	Variable, decreased	Large and intermediate multimers present	–
Type 2N	Uncommon	Autosomal dominant, missense mutation	Variable bleeding	↓↓[d] VIIIC	Variable	Variable	Normal	–
Type 3	Rare	Gene deletion, missense, nonsense mutation, autosomal recessive	Severe bleeding	Markedly decreased ↓↓↓	Markedly decreased ↓↓↓	Markedly decreased ↓↓↓	Absent	No response

[a] Modified from Nichols and Ginsburg *(72)* and Triplett *(75)*.
[b] FVIIIC, factor VIII activity; VWF:Ag, von Willebrand factor antigen; VWF:Act, von Willebrand factor activity; RIPA, ristocetin-induced platelet aggregation.
[c] +++, excellent response to DDAVP; ±, unpredictable response to DDAVP; –, no response to DDAVP.
[d] ↓↓, moderate decrease of factor VIII activity; ↓↓↓, marked decrease of factor VIII activity.

VWD *(74)*. In many cases, this assay is not readily available. There are several reference centers nationwide that have substantial expertise in multimeric analysis of VWF.

Management of clinical bleeding in patients with VWD in many cases is relatively simple *(70, 72)*. DDAVP is used to manage epistaxis and provide prophylaxis for minor surgery. Blood product replacement therapy in the past relied primarily on cryoprecipitate. However, because of the risk of infection (e.g., hepatitis and HIV), the recommended replacement therapy of choice is Humate-P® or other factor VIII concentrates with significant amounts of VWF *(74)*. There is a VWF concentrate available in France. Other therapeutic modalities include ε-aminocaproic acid (Amicar®) and tranexamic acid in the management of mucous membrane bleeding. Estrogens are also helpful in the management of VWD-related menorrhagia.

Acquired VWD may be seen in a variety of settings, including immunologic disorders, hypothyroidism, cardiac defects, and uremia *(77)*.

hemophilia (factor viii, ix, xi deficiencies)

Hemophilia A is the oldest recognized hereditary bleeding disorder *(78)*. It is sex-linked in transmission. The gene for hemophilia A is located on the long arm of the X chromosome. The gene spans 186 kb of DNA, and many mutations have been described. The inversion mutation accounts for 25% of mutations in hemophilia A patients. Fifty percent of patients with severe hemophilia A (<1% activity) carry the inversion mutation *(79)*. There are several different variants of this mutation: type I distal (a3), type II proximal (a2), and type III. Hemophilia A is classified based on the amount of factor VIII activity. Patients with severe hemophilia A (<1% factor VIII activity) have joint bleeding with resulting hemarthroses as well as deep intramuscular bleeding. One of the major complications seen in the recent past was transmission of HIV in replacement blood products (factor VIII concentrates and cryoprecipitate). As a result, in the early 1980s, a large portion of the hemophilic population developed HIV positivity and AIDS. The recent introduction of recombinant factor VIII replacement therapy has immensely improved the management of hemophilia patients *(80)*. One complication of replacement therapy, however, continues to present a challenge: the development of factor VIII inhibitors in a large percentage of severe hemophilia A patients. In these patients, replacement therapy or management of an acute bleed presents a challenge. Porcine factor VIII and activated factor VII are new products for this type of patient, and prothrombin complex concentrates (Autoplex® and Feiba) are used *(79)*.

Hereditary factor IX deficiency (hemophilia B) and hereditary factor XI deficiency (hemophilia C) are relatively common hereditary hemostatic disorders. Factor IX deficiency is very heterogeneous. Factor XI deficiency is primarily encountered in the Jewish population.

Circulating Anticoagulants (Inhibitors)

The most common acquired inhibitor of coagulation is the LA *(81)*. LA is a member of the anti-phospholipid antibody (APA) family. When evaluating patients for potential APAs, it is necessary to do both coagulation testing to identify LA as well as ELISA assays to identify "anti-cardiolipin antibodies" and antibodies to β_2-glycoprotein I *(82)*. APAs may be seen in many patient populations, e.g., after infection and in patients with autoimmune disease *(63)*. Most APAs seen in the setting of infections have no clinical complications. However, a large percentage of APA patients with underlying autoimmune disease present with thrombotic complications involving both the arterial and venous circulation, as well as recurrent fetal loss/spontaneous abortion in women. APA syndrome is diagnosed based on the presence of clinical complications (e.g., thrombosis or recurrent spontaneous abortion) and positive laboratory testing for LA and/or anti-cardiolipin antibodies.

The laboratory diagnosis of LA requires a well-coordinated work-up using three screening procedures as recommended by the Scientific Subcommittee of the International Society of Thrombosis and Hemeostasis *(83)*. The three most commonly used tests are a LA-sensitive APTT reagent, Staclot LA®, and a dilute Russell viper venom time. More recently, the dilute PT has been used.

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