3 Hemostasis

contents

I. INTRODUCTION TO THE VASCULAR SYSTEM

A. Vascular Structure and Function

1. **Endothelium**
   a. Vascular permeability and blood flow rate are controlled by a single layer of endothelial cells that line the vessel wall.
   b. Vascular lining is nonreactive to platelets and plasma proteins until damaged.
   c. Upon injury, increased vascular permeability occurs, allowing leakage of plasma proteins and blood cell migration to site of injury.
   d. Damage causes vasoconstriction to minimize blood loss; allows interaction among vessels, platelets, and plasma proteins.

2. **Subendothelium**
   a. Composed of smooth muscle cells and connective tissue with collagen fibers
   b. Exposure of collagen causes platelet activation; activates the intrinsic pathway of secondary hemostasis.

3. **Vascular endothelium** produces or releases substances important in hemostasis.
   a. **Produces von Willebrand factor (vWF),** necessary for platelet **adhesion** to collagen; carrier protein for coagulation factor VIII:C
   b. **Tissue factor** in vessels is **exposed** during vessel damage and activates the **extrinsic pathway** of secondary hemostasis.
   c. **Tissue plasminogen activator** is released during vessel damage and activates the **fibrinolytic system.**
   d. Produces **prostacyclin,** a platelet aggregation inhibitor and vasodilator
   e. The endothelial surface receptor **thrombomodulin** forms a complex with **thrombin** to inhibit factors V and VIII in secondary hemostasis through the protein C system.

II. DISEASES AND CONDITIONS ASSOCIATED WITH THE VASCULAR SYSTEM

A. Hereditary Vascular Defects

1. **Hemorrhagic telangiectasia:** Thin vessel walls cause mucous membrane bleeding.
2. **Ehlers-Danlos syndrome:** Abnormal collagen production causes hyperelastic skin and joint abnormalities.

B. Acquired Vascular Defects

1. **Vitamin C deficiency:** Vitamin C is needed for proper collagen synthesis and vessel integrity. Deficiency causes **scurvy.**
2. Drug-induced (steroids) or age-induced (senile purpura)
3. Inadequate platelet support because of quantitative or qualitative platelet defects

C. Vascular Defect Bleeding Symptoms

1. Superficial, resulting in easy bruising and petechiae

III. INTRODUCTION TO THROMBOCYTES

A. Thrombocyte Maturation

1. **Megakaryoblast**
   a. Committed **myeloid progenitor cell,** in response to growth factor **thrombopoietin,** gives rise to megakaryocytes.
   b. Earliest thrombocyte stage where the nucleus divides without cytoplasmic division; process known as **endomitosis**
   c. Results in the formation of **giant cells,** with a size range of 20–50 μm
   d. Round nucleus contains 2–6 nucleoli and fine chromatin.
   e. The scant basophilic cytoplasm contains **no granules;** irregularly shaped with **cytoplasmic tags** (blunt extensions of cytoplasm)
2. **Promegakaryocyte**
   a. **Increases** size with a range of 20–80 μm
   b. Indented or lobulated nucleus contains variable number of nucleoli with coarsening chromatin.
   c. Basophilic cytoplasm with **granules** beginning to appear; cytoplasmic tags present
   d. **Demarcating membrane system (DMS)** begins to form.
      1) DMS is an invagination of the plasma membrane that becomes the future site of platelet fragmentation.
3. **Megakaryocyte**
   a. **Increases** in size up to 100 μm; **largest cell in the body**
   b. It contains a **multilobulated nucleus** with very **coarse chromatin** and variable number of **nucleoli.**
   c. Cytoplasm has many **small granules** that stain purple with Wright's stain.
   d. Represents 1% of nucleated bone marrow cells with a reference range of 5–10 megakaryocytes on low (10×) power
   e. Increased number indicates increased demand for platelets; acute bleeding episodes
   f. Approximately 2000–4000 platelets per megakaryocyte are shed into the marrow sinuses and enter circulation as cytoplasmic fragments. The nucleus remains in marrow and is phagocytized by marrow macrophages.
4. **Mature platelets (thrombocytes)**
   a. **2–4 μm** in size, appearing as **pale blue** cells with **azurophilic granules**
   b. Mature **platelets** have **no nucleus.**
   c. **Platelet zones**
      1) **Peripheral zone**
         a) Glycocalyx is the exterior coat and contains glycoprotein receptor sites.

        b) Submembrane area contains the phospholipid membrane (PF3), which serves as a surface for interaction of coagulation factors in **secondary hemostasis.**

    2) **Sol-gel (structural) zone** contains microtubules, cytoskeleton, actin, and myosin.

    3) **Organelle zone** contains the granules, lysosomes, mitochondria, peroxisomes, and glycogen. It controls platelet function in response to coagulation.

        a) **Alpha granules** predominate and contain a number of different proteins, with some of the most prominent being fibrinogen, von Willebrand factor, beta thromboglobulin, platelet-derived growth factor (PDGF), and PF4 (platelet factor 4).

        b) **Dense bodies** (delta granules) contain ADP, ATP, serotonin, and calcium.

        c) **Lysosomes** (third type of granule) contain hydrolase enzymes.

  d. Membrane systems

    1) **Dense tubular system** (DTS): Regulator of intracellular calcium concentration

    2) **Open canalicular system** (OCS): Releases granular contents through channels leading to the surface of the platelet

B. Platelet Characteristics

1. The reference range for healthy individuals is **150–450 $\times$ 10^9/L** or approximately 7–21 per high power field. Two-thirds of available platelets are in **circulation;** one-third is stored in the **spleen.**

2. **Life span** of 8–12 days; shorter in certain disease states

3. With Wright's stain, platelets stain gray-blue with purple granules.

4. Platelets are found in the bone marrow, spleen, and blood vessels; in the blood vessels platelets function in hemostasis.

5. Originate from the same progenitor cell as the erythroid and myeloid series

6. **Giant platelets** indicate premature release from the bone marrow and result from increased demand.

7. **Immature platelets** are found in the peripheral blood in certain diseases (e.g., acute megakaryocytic leukemia, myelodysplastic syndrome).

C. Thrombocyte Function

1. Platelet function is dependent on **platelet secreted proteins, ATP, ADP, calcium, and platelet factors.**

2. **Platelet-secreted proteins**

  a. **Serotonin** stimulates vasoconstriction when vessel injury occurs.

  b. **Thromboxane A$_2$** stimulates platelet aggregation and vasoconstriction.

  c. **Actomyosin** contracts the thrombus at the end of the coagulation process.

3. **Platelet factors**
   a. **PF4:** Neutralizes heparin
   b. **PF3:** Platelet phospholipid needed for proper platelet function and coagulation
      1) Needed in the production of **thromboxane A$_2$**
      2) Provides a surface for fibrin formation, limiting the hemostatic response to the site of injury
4. Proper platelet function involves **adhesion, release of granule contents, aggregation, and clot retraction.**
   a. **Adhesion**
      1) Platelets undergo a shape change and adhere to vascular surfaces.
      2) Response to collagen exposure in subendothelium caused by vascular injury
      3) Dependent on binding of **von Willebrand factor** at the GPIb receptor site
      4) Can be activated by thrombin
   b. The **contents** of the platelet **storage granules** are **released** into the open canalicular system in response to internal, cellular contraction.
   c. **Aggregation**
      1) **Fibrinogen** attaches at the **IIb/IIIa receptor** of adjoining platelets, forming the initial platelet plug.
      2) Platelets release nonmetabolic **ADP** (platelet agonist), **serotonin, and PF4.**
      3) During aggregation, **PF3** is released to provide the phospholipid surface needed for binding of clotting factors in secondary hemostasis.
   d. **Clot retraction**
      1) Follows clot formation
      2) Dependent on **thrombasthenin** and **glycoprotein receptors IIb/IIIa**
      3) Restores normal blood flow to the vessel.

D. **Laboratory Analysis of Platelets**
   1. Quantitative
      a. Platelet numbers: Automated instrumentation, hemacytometer counts, blood smear estimates
   2. Qualitative
      a. **Bleeding time** will detect defects in adhesion, release, and aggregation.
      b. **Platelet aggregation studies** detect platelet function abnormalities. Aggregating agents used include ADP, epinephrine, collagen, thrombin, and ristocetin.
      c. **vWF:Ag (antigenic)** and **vWF:RCo (activity) assays** are used to assess von Willebrand factor.

IV. DISEASES AND CONDITIONS ASSOCIATED WITH THROMBOCYTES

A. Hereditary Adhesion Defects

1. **von Willebrand disease**
   a. Lacks von Willebrand factor, which is needed for platelets to adhere to collagen in damaged vessels and is a carrier protein for coagulation factor VIII:C
   b. Decreased platelet adhesion causes mucous membrane bleeding that is variable in severity.
   c. **Laboratory:** Normal **platelet count,** prolonged **bleeding time,** decreased **aggregation response to ristocetin,** variable **aPTT,** normal **PT,** decreased **vWF:RCo, vWF:Ag, and VIII:C**
   d. **Most common hereditary hemorrhagic disorder;** autosomal-dominant inheritance

2. **Bernard-Soulier syndrome**
   a. Giant platelets (increased MPV) that **lack glycoprotein Ib receptor;** adhesion defect due to faulty binding of the platelet to von Willebrand factor
   b. **Laboratory:** Variable **platelet count, platelet anisocytosis** (increased PDW), prolonged **bleeding time,** decreased **aggregation response to ristocetin,** normal **aPTT and PT,** normal **vWF:RCo, vWF:Ag, and VIII:C**

B. Hereditary Aggregation and Clot Retraction Defect

1. **Glanzmann thrombasthenia**
   a. **Hemorrhagic disorder** seen in populations where consanguinity is prevalent
   b. Lack of **glycoprotein IIb/IIIa,** the fibrinogen binding receptor
   c. Inability of fibrinogen to bind with platelets causes aggregation defect; lack of thrombasthenin/actomyosin causes clot retraction defect.
   d. **Laboratory:** Decreased **aggregation response** with ADP, epinephrine, and collagen, normal response with ristocetin

C. Storage Pool Defects: Deficiency of One or More Types of Storage Granules

1. **Gray-platelet syndrome** is characterized by large platelets, thrombocytopenia, and an absence of **alpha granules.** Patients are prone to lifelong mild bleeding tendencies.
2. **Wiskott-Aldrich syndrome** is characterized by small platelets (low MPV), thrombocytopenia, and a decreased amount of **alpha granules and dense bodies.** Patients are prone to hemorrhage and recurrent infections.
3. **Hermansky-Pudlak syndrome** is characterized by a lack of **dense body granules.** Patients exhibit oculocutaneous albinism and are prone to hemorrhage.

D. Acquired Defects

1. **Drugs**
   a. Aspirin and nonsteroidal anti-inflammatory drugs interfere with the cyclooxygenase enzymes, preventing **thromboxane A$_2$** synthesis and subsequent aggregation.
   b. Clopidogrel bisulfate (Plavix®) and ticlopidine are adenosine diphosphate (ADP) receptor inhibitors. The blockage of this receptor inhibits platelet aggregation.
   c. Eptifibatide and similar antiplatelet medications block IIb/IIIa glycoprotein receptors, preventing aggregation.
2. **Myeloproliferative disorders and uremia** are examples of diseases that can cause platelet dysfunction.

E. Quantitative Platelet Disorders

1. **Primary thrombocytosis**
   a. Uncontrolled, **malignant proliferation** of platelets, **not** in response to thrombopoietin; can be caused by **essential thrombocythemia, polycythemia vera,** and **chronic myelocytic leukemia**
   b. Platelet counts can be **>1000 $\times$ 10^9/L.**
   c. Associated with either hemorrhagic or thrombotic complications
2. **Secondary (reactive) thrombocytosis**
   a. It is characterized by increased platelet production, usually in response to **thrombopoietin.** Platelet count is elevated, but usually **<1000 $\times$ 10^9/L.** Can result from:
      1) Chronic and acute **inflammatory disease** (e.g., tuberculosis, cirrhosis)
      2) **Iron deficiency:** Iron regulates thrombopoiesis by inhibiting thrombopoietin; deficiency causes increased thrombopoietin and stimulates thrombopoiesis.
      3) **Rapid blood regeneration** due to hemolytic anemia and acute blood loss
      4) **Exercise, prematurity, and response to drugs**
      5) **Other conditions:** Cytotoxic drug withdrawal, post-operative state from tissue damage, and splenectomy
3. **Thrombocytopenia**
   a. **Decrease** in the **number of platelets,** which can result from the following:
      1) **Megakaryocyte hypoproliferation:** Caused by chemotherapy, marrow replacement by malignant cells, aplastic anemia, drug and alcohol abuse
      2) **Ineffective thrombopoiesis:** Caused by megaloblastic anemias
      3) **Increased loss/destruction**
         a) **Nonimmune** loss is due to severe hemorrhage, extensive transfusion (dilution loss), and increased consumption seen in the microangiopathic hemolytic anemias (e.g., DIC, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura [ADAMTS 13 deficiency]).

  b) **Immune** loss can be due to neonatal purpura, post-transfusion purpura, immune/idiopathic thrombocytopenic purpura, and heparin-induced thrombocytopenia.

 4) **Splenic sequestration**

  a) **Hypersplenism** may result in up to 90% of platelets being sequestered.

  b) Increased destruction of damaged and normal platelets

  c) **Splenomegaly** occurs in leukemia, lymphoma, Gaucher and other storage diseases, cirrhosis of the liver, and sarcoidosis.

 5) **Hereditary conditions:** May-Hegglin anomaly, Bernard-Soulier and Wiskott-Aldrich syndromes

 6) **Falsely low platelet counts**

  a) **Platelet satellitosis:** Platelets can adhere to neutrophils when exposed to EDTA. Redraw in sodium citrate to correct; multiply obtained platelet count by 1.1 to correct for dilution factor in sodium citrate tube.

  b) **EDTA-dependent platelet agglutinins:** Platelets can adhere to each other when exposed to EDTA. Correction of the problem is the same as for platelet satellitosis.

F. Vessel and Platelet Defect Bleeding Symptoms

1. Superficial, resulting in easy bruising, petechiae, ecchymoses, purpura, epistaxis, mucous membrane, or gingival bleeding

V. INTRODUCTION TO HEMOSTASIS

A. Primary Hemostasis

1. **Vascular system and platelets** are involved; primary hemostasis starts when platelets come in contact with exposed collagen, microfilaments, and the basement membrane of endothelial tissue.

2. **Small blood vessels constrict,** allowing platelets to adhere to exposed tissue, which causes **ADP/ATP** release (promotes platelet aggregation, acts as an energy source) and synthesis of **thromboxane A_2** from arachidonic acid (promotes activation, release, and aggregation).

3. **Platelets** begin to **aggregrate,** which causes the release of additional ADP, ATP, and **serotonin** (substance that promotes vasoconstriction).

4. **Platelet receptor sites** are **exposed,** which allows binding of coagulation proteins from **secondary hemostasis** (e.g., fibrinogen binds at the glycoprotein IIb/IIIa receptor).

B. Secondary Hemostasis (see Figure 3–1■)

1. The goal is **generation** of sufficient **thrombin** to **convert fibrinogen to fibrin clot.** Secondary hemostasis involves **activation** of **intrinsic, extrinsic,** and **common coagulation pathway factors.**
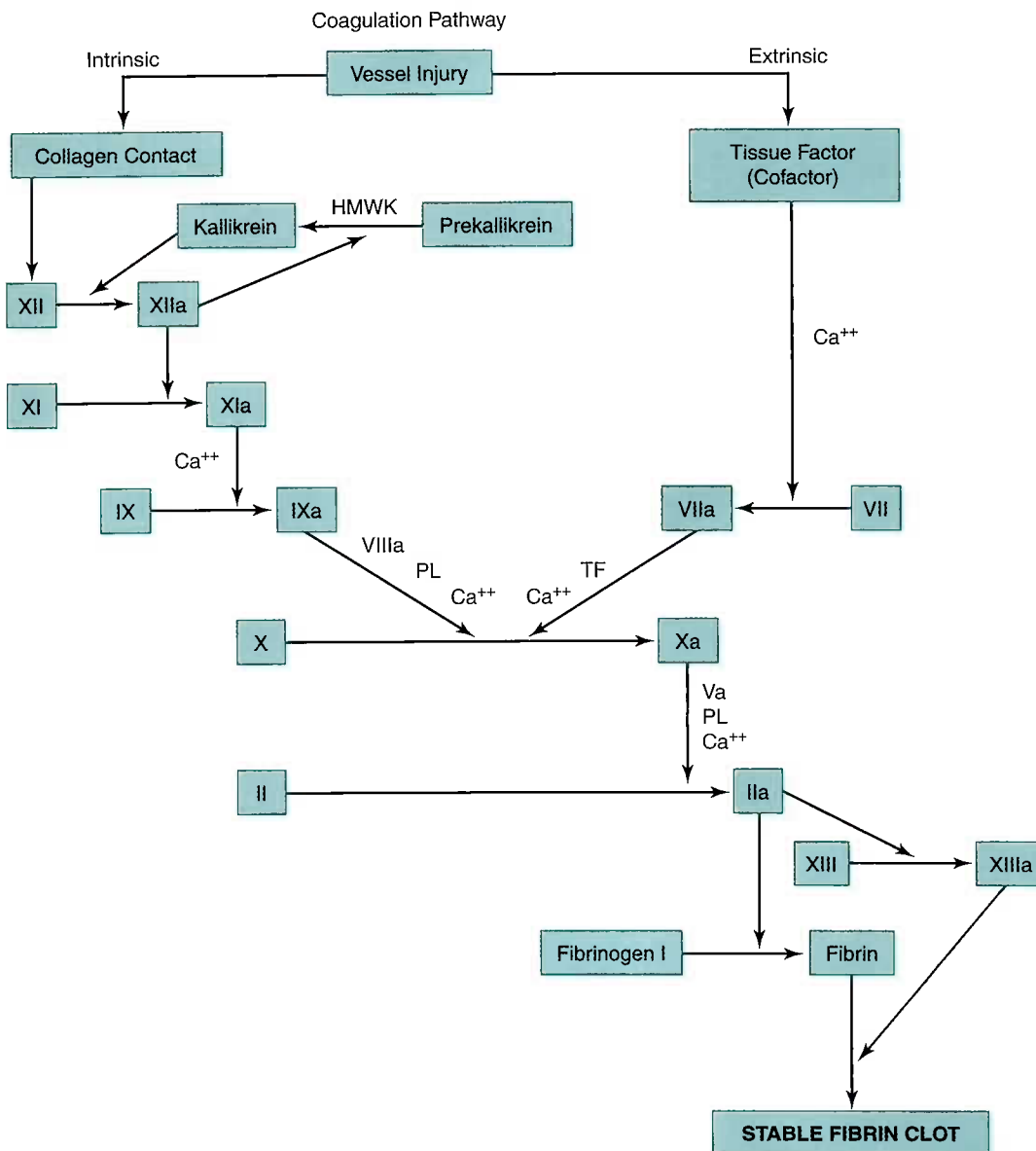
FIGURE 3-1 ■ Coagulation pathway.

2. **Fibrin clot** includes the **platelet plug** formed in **primary hemostasis** and **fibrin** formed in **secondary hemostasis.**
3. **Intrinsic pathway** is activated when coagulation proteins are exposed to subendothelial collagen. The intrinsic pathway includes factors **XII** (Hageman), **XI** (plasma thromboplastin antecedent), **prekallikrein** (Fletcher), **HMWK** (Fitzgerald), **IX** (plasma thromboplastin component/Christmas factor), and **VIII** (antihemophiliac).

4. **Extrinsic pathway (dominant *in vivo* pathway)** starts with the **release of tissue factor** from injured blood vessel endothelial cells and subendothelium. Tissue factor is found in most tissues, organs, and large blood vessels. **Factor VII** (stable factor) is in this pathway.
5. **Common pathway** begins with **factor X activation** by either the extrinsic (main *in vivo*) or intrinsic pathway. It includes factors **X** (Stuart-Prower), **V** (proaccelerin/labile factor), **II** (prothrombin), and **I** (fibrinogen).
6. Alternative pathways link the extrinsic, intrinsic, and common pathways.
7. Additional synonyms include **tissue factor** (III), **calcium** (IV), **fibrin stabilizing factor** (XIII), and **ristocetin cofactor** (von Willebrand factor).

C. Coagulation Factors (Coagulation Proteins)

1. **Coagulation factors** are also known as **enzyme precursors** or **zymogens.** They are found in the plasma, along with nonenzymatic cofactors and calcium.
2. **Zymogens** are substrates having **no biologic activity** until converted by enzymes to **active forms** called **serine proteases.**
   a. The **zymogens** include **II, VII, IX, X, XI, XII,** and **prekallikrein.**
   b. The **serine proteases** are **IIa, VIIa, IXa, Xa, XIa, XIIa,** and **kallikrein.**
3. **Cofactors** assist in the **activation of zymogens** and include **V, VIII, tissue factor,** and **high molecular weight kininogen (HMWK).**
4. In its active form, **factor XIII** is a **transglutaminase.**
5. **Fibrinogen** is the only substrate in the cascade that does **not** become **an activated enzyme.**

D. The Coagulation Groups

1. **Contact group**
   a. Includes **prekallikrein, HMWK,** and **factors XI** and **XII**
   b. Produced in the **liver**
   c. Requires contact with a foreign surface for activation (e.g., collagen *in vivo,* kaolin *in vitro*)
   d. Functions of the contact group:
      1) **XII** and **prekallikrein** reciprocally **activate each other; HMWK** is a **cofactor** for this process.
      2) All play a **role in intrinsic coagulation activation.**
      3) **XIIa, kallikrein,** and **HMWK** play a role in the inflammatory response, intrinsic fibrinolytic activation, kinin formation, and activation of the complement system.
2. **Prothrombin group**
   a. Includes factors **II, VII, IX,** and **X**
   b. Produced in the **liver**
   c. **Vitamin K** is required for synthesis of functional factors, with **calcium binding sites** necessary for binding to phospholipid (PF3) surfaces.

    d. Causes for synthesis of **nonfunctional factors:**
- 1) **Vitamin K deficiency** or **antibiotics** that kill the intestinal bacterial flora responsible for vitamin K synthesis
- 2) **Oral anticoagulants** (warfarin) that interfere with the metabolism of vitamin K (vitamin K antagonists)

3. **Fibrinogen group**
   - a. Includes factors **I, V, VIII,** and **XIII**
   - b. Produced in the **liver**
   - c. Consumed in the clotting process
   - d. **Thrombin feedback** on fibrinogen group factors depends on its concentration.
     - 1) **Low thrombin levels activate** factors **V, VIII** (positive feedback on the cascade), and **XIII** and induce platelet aggregation.
     - 2) When **thrombin levels** are **high,** thrombin **binds to thrombomodulin** on the endothelial cell surface and **activates** the **protein C pathway.**
     - 3) **Activated protein C** and its cofactor, **protein S,** inhibit factors V and VIII (negative feedback on the cascade).
   - e. Factors I, V, and VIII serve as substrates for the fibrinolytic enzyme plasmin.
   - f. Factors I and V are found in platelets.
   - g. Conversion of **fibrinogen to fibrin** is a three-step process.
     - 1) **Fibrinogen alpha and beta fibrinopeptides** are cleaved by thrombin, forming soluble fibrin monomers.
     - 2) **Fibrin monomers** spontaneously polymerize, forming **soluble fibrin polymers.** This is the endpoint for clot-based tests.
     - 3) **Clot stabilization** occurs, requiring thrombin activation of **XIII** and **calcium.**
   - h. **VIII/vWF complex**
     - 1) Factor **VIII** is synthesized in the **liver** and is composed of two fractions.
       - a) **VIII:C** (antihemophilic factor) is the coagulation portion that acts as a cofactor in the intrinsic coagulation pathway.
       - b) **VIII:Ag** is the antigenic property of factor VIII.
       - c) Both **VIII:C** and **VIII:Ag** are deficient in hemophilia A.
     - 2) **von Willebrand factor** (vWF) is synthesized by **endothelial cells** and **megakaryocytes** and is composed of two fractions.
       - a) **vWF:RCo** (ristocetin cofactor) is needed for **platelet adhesion** to collagen *in vivo*; it is needed for a normal response to ristocetin on aggregation studies *in vitro.*
       - b) **vWF:Ag** is the **antigenic property** of vWF.
       - c) Both **vWF:RCo** and **vWF:Ag** are **deficient** in **von Willebrand disease.**
     - 3) **vWF subunits** polymerize to form **multimers** of varying sizes that complex with and act as the **carrier protein for factor VIII:C.**

E. **Complement System and Coagulation System Interaction**

1. The **complement system** is activated during coagulation and fibrinolysis.
2. Contains more than 30 circulating blood proteins, primarily to mediate inflammatory response and immune and allergic reactions.
3. Complement functions in **lysing antibody-coated cells.**
4. **Plasmin** (in association with antibody-antigen complexes) **activates C1** and causes **cleavage of C3** to C3a and C3b. **C3a** increases vascular permeability, and **C3b** causes immune adherence of erythrocytes to neutrophils, which enhances phagocytosis.
5. Complement activation is regulated by **C1 inactivator,** which also inhibits several coagulation factors.

F. **Kinin System and Coagulation System Interaction**

1. The **kinin system** contains four plasma proteins: factors **XII** and **XI, prekallikrein** (Fletcher factor), and **HMWK** (Fitzgerald factor).
2. Generates **bradykinin,** an active peptide, and **kallikrein,** a proteolytic enzyme
3. Involved in chemotaxis and pain sensation
4. **Function:** Mediate inflammatory responses, promote vasodilatation, and activator of intrinsic coagulation and complement pathways

VI. THE FIBRINOLYTIC SYSTEM

A. **Fibrinolytic System:** Keeps blood vessels clear and is important in **clot dissolution.** During this process, **plasminogen** is activated to **plasmin.**

B. **Plasminogen**

1. Glycoprotein produced in the **liver**
2. **Zymogen** (inert) found in the plasma
3. **Converted to plasmin** by plasminogen activators:
   a. **Intrinsic activators** are **XIIa, kallikrein,** and **HMWK.**
   b. **Extrinsic activators** are **tissue-type plasminogen activator** (t-PA) and **urokinase-type plasminogen activator** (u-PA).
   c. **Exogenous activators** (therapeutic agents) include **t-PA, streptokinase,** and **urokinase.** They are administered to **lyse** existing clots.

C. **Plasmin**

1. **Not normally found in circulation;** the precursor plasminogen is found in circulation
2. **Degrades** fibrin clots (fibrinolysis), fibrinogen (fibrinogenolysis), factors V and VIII
3. **Activates** the complement system

VII. REGULATORY PROTEINS OF COAGULATION AND FIBRINOLYSIS

A. Antithrombin (AT)
1. Produced in the **liver**
2. Principal **inhibitor of coagulation**
3. **Inhibits** the **serine proteases**
4. Therapeutic **heparin enhances** the action of antithrombin.

B. Proteins C and S
1. **Vitamin K–dependent** regulatory proteins
2. Activated when thrombin binds to thrombomodulin on the endothelial cell surface
3. Inhibit factors V and VIII to provide **negative feedback** on the cascade

C. Tissue Factor Pathway Inhibitor: Inhibits factor VIIa–tissue factor complex

D. α_2-Macroglobulin: Inhibits thrombin, Xa, kallikrein, and plasmin

E. α_1-Antitrypsin: Inhibits XIa and inactivates plasmin

F. C1 Inhibitor: Inhibits C1 from the complement cascade, and XIIa, XIa, kallikrein, and plasmin

G. α_2-Antiplasmin: Principal inhibitor of fibrinolysis; neutralizes plasmin

H. PAI-1 (plasminogen activator inhibitor-1)
1. Important **inhibitor** of fibrinolysis
2. Prevents activation of plasminogen by t-PA; released from endothelial cells upon damage

VIII. THROMBOTIC DISORDERS

A. Primary Thrombotic Disorders
1. **Deficiency in regulatory proteins**
   a. **Antithrombin (AT) deficiency**
      1) **Genetic deficiency** occurs about 1:2000 in the general population; associated with **deep vein thrombosis** and **pulmonary embolism**
      2) Serine proteases not inhibited; negative feedback to cascade impaired
      3) **Laboratory:** Antithrombin activity assay (antigenic testing less common)
   b. **Protein C or Protein S deficiencies**
      1) Vitamin K–dependent **regulatory proteins** that **inactivate** factors V and VIII

        2) Can **cause** superficial and deep vein **thrombosis** and/or pulmonary **embolism**

        3) **Laboratory:** Immunologic and functional testing to diagnose

  2. **Decreased activation of the fibrinolytic system**

    a. **XII, prekallikrein,** and **HMWK** are contact factors in secondary hemostasis, but their most important role is the **intrinsic activation** of the **fibrinolytic system. Deficiencies** are associated with thrombosis, **not** hemorrhage.

    b. All have an autosomal recessive inheritance pattern.

    c. **Factor XII (Hageman factor) deficiency** causes a **prolonged aPTT;** factor XII assay confirms.

    d. **Prekallikrein (Fletcher factor) deficiency** causes a **prolonged aPTT** that shortens in patient plasma incubated with **kaolin.**

    e. **HMWK (Fitzgerald factor) deficiency** causes a **slightly prolonged aPTT.**

    f. **Plasminogen deficiency** is characterized by thrombosis due to an inability to generate plasmin.

  3. **Genetic mutations**

    a. **Factor V Leiden** (Activated Protein C Resistance—APCR)

      1) **Most common hereditary cause of thrombosis;** caused by an amino acid substitution

      2) **Protein C is incapable of inactivating factor V Leiden,** causing thrombin generation and subsequent fibrin clot formation.

      3) **Laboratory:** PCR-based molecular assay to single-point mutation in the gene for factor V

    b. **Prothrombin gene mutation 20210**

      1) **Second most common hereditary cause of thrombosis;** caused by an amino acid substitution

      2) May have slightly elevated prothrombin level

      3) **Laboratory:** PCR-based molecular assay

    c. **Dysfibrinogenemia**

      1) Autosomal-dominant trait; abnormal structure of fibrinogen; caused by gene mutations

      2) Associated with either bleeding or thrombosis; dependent on the specific gene mutation

B. Secondary Thrombotic Disorders

  1. **Lupus anticoagulant** and **anticardiolipin antibodies:** The body develops autoantibodies against platelet phospholipids; etiology is unknown.

  2. **Post-operative status:** Thrombotic event starts after tissue factor release during surgery, activating the extrinsic coagulation (dominant *in vivo*) pathway.

  3. **Malignancy:** Risk of malignancy increases because of the release of thromboplastic substances by neoplastic cells.

4. **Pregnancy**
   a. The placenta is rich in tissue factor, which may enhance thrombosis during pregnancy, especially high-risk patients having caesarian section delivery.
   b. Factor V and VIII levels increase, contributing to clot formation.
5. **Estrogen/oral contraceptives:** Increase risk of venous thrombosis and renal artery thrombosis
6. **Morbid obesity:** Results in decreased AT levels and increased PAI-1, causing thrombosis
7. **Hyperhomocysteinemia:** This disorder is linked to atherosclerosis, resulting in arterial and venous thromboembolism. Mechanisms are not fully understood but may be associated with a reduction in the localized activation of the protein C pathway.

IX. HEMORRHAGIC DISORDERS

A. **Inherited Disorders:** Generally affect only **one** hemostatic component (e.g., factor VIII)

B. **Acquired Disorders:** Involve **multiple** hemostatic components or pathways (e.g., warfarin therapy, liver disease)

C. **Hemorrhagic Symptoms:** Associated with defects in secondary hemostasis; include bleeding into deep tissues, joints, abdominal and other body cavities

D. **Inherited Intrinsic Pathway Hemorrhagic Disorders**
   1. **von Willebrand disease**
      a. Autosomal-dominant trait
      b. **Most common hereditary bleeding disorder;** abnormalities in **both** primary and secondary hemostasis
      c. Caused by a defect in von Willebrand factor that is needed for **platelet adhesion** to collagen in primary hemostasis. vWF is also the **carrier protein for factor VIII:C** in secondary hemostasis.
      d. **Clinical:** Mild to moderate bleeding dependent of vWF and VIII:C levels; menorrhagia common symptom in women
      e. **Laboratory:** Decreased **vWF:RCo, vWF:Ag,** and **VIII:C;** abnormal **platelet aggregation with ristocetin,** variable **aPTT** (often prolonged because of decreased VIII:C), and prolonged **bleeding time**
      f. **Treatment:** Factor **VIII** concentrates; **DDAVP** (deamino-D-arginine-vasopressin) used to raise plasma levels of vWF and VIII:C
   2. **Factor VIII:C (hemophilia A, classic hemophilia) deficiency**
      a. Sex-linked disorder transmitted on the **X chromosome** by carrier women to their sons

b. Accounts for 80% of the hemophilias; **second most common hereditary bleeding disorder**

c. Many new cases of hemophilia A result from spontaneous mutations.

d. **Clinical:** Bleeding symptoms are proportional to the degree of the factor deficiency. Spontaneous bleeding occurs often and is especially bad in joint regions (hemarthrosis).

e. **Laboratory:** Prolonged **aPTT** only, factor VIII:C assay to confirm

f. **Treatment: Cryoprecipitate** and factor **VIII** concentrates are used; in mild cases, DDAVP can be used to stimulate the release of VIII:C and vWF from stored reserves.

g. About 15–20% of patients will develop a factor VIII inhibitor; it is associated with a bleeding tendency and worse prognosis.

3. **Factor IX (hemophilia B, Christmas disease) deficiency**

a. Sex-linked recessive trait

b. Accounts for 20% of the hemophilias; **third most common hereditary bleeding disorder**

c. **Clinical:** Bleeding symptoms are similar to those seen in hemophilia A.

d. **Laboratory:** Prolonged **aPTT** only; factor IX assay to confirm

e. **Treatment: Fresh frozen plasma** (FFP) or factor **IX** concentrates

f. Between 1 and 3% of patients will develop a factor IX inhibitor; it is associated with a bleeding tendency and worse prognosis.

4. **Factor XI (hemophilia C) deficiency**

a. Mainly seen in the Ashkenazi Jewish population

b. Characterized by clinical bleeding that is asymptomatic until surgery or trauma

c. **Laboratory:** Prolonged **aPTT** only; factor XI assay to confirm

5. Deficiencies of factors XII, prekallikrein, and HMWK in the intrinsic pathway have already been discussed with the thrombotic disorders.

E. **Inherited Extrinsic and Common Pathway Hemorrhagic Disorders**

1. **Factor VII (stable factor) deficiency**

a. Autosomal-recessive trait

b. **Clinical:** Soft tissue bleeding

c. **Laboratory:** Prolonged **PT** only

2. **Factor X (Stuart-Prower) deficiency**

a. Autosomal-recessive trait

b. **Clinical:** Soft tissue bleeding and chronic bruising

c. **Laboratory:** Prolonged **PT** and **aPTT**

3. **Factor V (Owren disease, labile factor) deficiency**

a. Autosomal-recessive trait

b. **Clinical:** Mild to moderate bleeding symptoms

c. **Laboratory:** Prolonged **PT** and **aPTT**

4. **Factor II (prothrombin) deficiency**
   a. Autosomal-recessive trait
   b. **Clinical:** Mild bleeding symptoms
   c. **Laboratory:** Prolonged **PT** and **aPTT**
5. **Factor I (fibrinogen) deficiency**
   a. Autosomal-recessive trait; results from the following inherited disorders:
      1) **Afibrinogenemia:** Inherited **lack** of fibrinogen; severe bleeding symptoms
      2) **Hypofibrinogenemia:** Inherited **deficiency** of fibrinogen; bleeding symptoms correlate with fibrinogen concentration
   b. **Clinical:** Spontaneous bleeding of mucosa, intestines, and intracranial sites
   c. **Laboratory:** Prolonged **bleeding time** (fibrinogen bridges do not form; platelet aggregation defect), decreased **fibrinogen** concentration, and prolonged **PT, aPTT,** and **thrombin time**
6. **Factor XIII (fibrin-stabilizing factor) deficiency**
   a. Autosomal-recessive trait
   b. **Clinical:** Spontaneous bleeding, delayed wound healing, and unusual scar formation; increased incidence of spontaneous abortion
   c. **Laboratory: 5.0 M urea test** abnormal, **PT** and **aPTT** normal, enzymatic and immunologic studies can be done

F. **Acquired Disorders of Coagulation and Fibrinolysis**
   1. **Hepatic disease**
      a. The **liver** is the major site of **hemostatic protein synthesis.**
      b. Hepatic disease can result in **decreased synthesis** of coagulation or regulatory proteins; it also causes **impaired clearance** of activated hemostatic components.
      c. **Laboratory:** Prolonged **PT, aPTT, bleeding time,** and possibly decreased **platelet counts** because of hypersplenism, alcohol toxicity, and disseminated intravascular coagulation (DIC)
   2. **Vitamin K deficiency**
      a. **Vitamin K** is needed for **liver synthesis** of functional factors **II, VII, IX, and X.**
      b. Vitamin K is produced by normal intestinal flora.
      c. **Deficiencies** in vitamin K can result from oral antibiotics, vitamin K antagonists (warfarin), or decreased absorption resulting from obstructive jaundice.
      d. Breast-fed babies are more prone to vitamin K deficiency because breast milk is sterile, which allows no bacterial intestinal colonization to occur.
      e. **Laboratory:** Prolonged **PT** (VII, X, II) and prolonged **aPTT** (IX, X, II)
   3. **Disseminated intravascular coagulation with secondary fibrinolysis**
      a. Predisposing condition triggers **systemic clotting;** leads to systemic **fibrinolysis** and **bleeding**

      b. Triggering events include gram-negative septicemia, acute promyelocytic leukemia (FAB M3), obstetrical complications, massive tissue damage.

      c. Fibrinogen group factors (I, V, VIII, XIII) and platelets are consumed in clotting.

      d. **Laboratory**

        1) **PT, aPTT,** and **thrombin time** are prolonged.

        2) **Platelet count, antithrombin,** and **fibrinogen** concentrations are decreased.

        3) **Fibrin** and **fibrinogen degradation products** are present (abnormal **D-dimer** and **FDP** tests).

        4) **Schistocytes** form when RBCs are fragmented by intravascular clots.

      e. **Clinical:** A systemic thrombotic event causes multiple organ failure; systemic lysis ultimately leads to severe hemorrhage.

      f. **Treatment:** Treat the underlying condition with **FFP, platelet transfusions, antithrombin concentrates,** and **heparin** to stop systemic clotting.

   4. **Primary fibrinogenolysis**

      a. Plasminogen is inappropriately activated to plasmin in the **absence** of clot formation. **Plasmin circulates** free in plasma and destroys factors I, V, and VIII.

      b. Caused by certain malignancies (e.g., prostate cancer) or massive tissue damage that causes release of plasminogen activators

      c. **Laboratory**

        1) **PT, aPTT,** and **thrombin time** are prolonged, and **fibrinogen** concentration is low (plasmin degrades fibrinogen, V, and VIII).

        2) **Platelet** count, **RBC morphology,** and **antithrombin** concentration are normal because there is no clot formation.

        3) **Fibrinogen degradation products** are present (abnormal FDP test), but **fibrin degradation products** are **absent** (normal D-dimer because there is **no clot formation**).

      d. **Clinical: Hemorrhagic** symptoms occur that may resemble DIC.

      e. **Treatment:** Epsilon aminocaproic acid (**EACA**) is used to turn off inappropriate systemic lysis.

   5. Inhibitors to factors VIII and IX in the intrinsic pathway have already been discussed with factor VIII and IX deficiencies. These inhibitors are associated with bleeding.

X. SAMPLE COLLECTION, HANDLING, AND PROCESSING FOR COAGULATION TESTING

   A. Nontraumatic Venipuncture: It is *essential* that **trauma be avoided** because it may introduce tissue thromboplastin that would activate coagulation.

B. Order of Draw: It is *important* that proper order of draw be followed. Collect tube for coagulation testing **before** any tubes containing heparin, EDTA, sodium fluoride, or clot-promoting additives.

C. Use Plastic- or Silicone-Coated Glass Tubes: Plain glass tubes will **activate** the **intrinsic pathway,** including the activation of the **contact factors** prekallikrein, XI, and XII.

D. Ratio of Blood to Anticoagulant: The ratio in blood collection tubes is **critical,** and it must be maintained at a **9:1** ratio of **blood** to 3.2% sodium citrate **anticoagulant** or excess citrate will bind calcium chloride in the reagents for PT and aPTT, causing falsely long coagulation times.

E. Specimen Processing: Specimens must be processed as soon as possible following blood collection. Recommendations include processing within **4 hours** for **aPTT** and **24 hours** for **PT.** Centrifuge to obtain platelet-poor plasma, and remove plasma from cells; can freeze plasma at –20°C.

F. Temperature: Testing must be performed at **37°C.** Enzyme reactions work best at 37°C. Labile factors V and VIII will break down at temperatures above 37°C. Factors VII and XI will be activated at cold temperatures.

XI. EVALUATION TESTS FOR SECONDARY HEMOSTASIS

A. Activated Partial Thromboplastin Time (aPTT)
   1. Screening test for factors **XII, XI, prekallikrein, HMWK, IX, VIII, X, V, II, and I (intrinsic/common pathways)**
   2. Monitors unfractionated **heparin therapy**
   3. Two reagents needed:
      a. **Platelet phospholipid substitute** with an **activator** (kaolin, celite, silica, or ellagic acid)
      b. **Calcium choride**
   4. **Principle:** Add phospholipid/activator reagent to citrated platelet-poor plasma and incubate to allow for contact factor activation. Add calcium chloride; measure the time required for clot formation.
   5. Run normal and abnormal controls (essential for quality control).
   6. **Reference range:** 23.0–35.0 sec; established by each institution
   7. **Prolonged aPTT** can indicate:
      a. **Factor deficiencies** in the **intrinsic/common pathways;** factor activity less than 25–30% will prolong
      b. **Acquired** circulating inhibitor: Heparin, lupus inhibitor, or antibody to a specific factor

8. **Sources of error**
   a. Improper sample collection, preparation, and inherent patient problems
      1) **Falsely long aPTT:** Blood collection tube not full, large clot in tube, heparin contamination from line draw, hematocrit >55.0%, and lipemia/icterus only if optical method used
      2) **Falsely short aPTT:** Hemolysis, small clot in tube, and plasma containing platelets (not platelet poor)
   b. Incorrect **reagent** preparation: Incorrect dilution, water impurities, or improper storage
   c. **Instrumentation:** Problems with temperature, light source, bubbles in sample

B. Prothrombin Time (PT)

1. Screening test for factors **VII, X, V, II, and I (extrinsic/common pathways)**
2. **Monitors** anticoagulation **therapy** by vitamin K antagonists **(warfarin/coumarin)**
3. **Reagents: Thromboplastin** source (tissue factor/TF) with **calcium chloride**
4. **Principle:** Add thromboplastin reagent containing calcium chloride to citrated platelet-poor plasma; measure the time required for clot formation.
5. Run normal and abnormal controls (essential for quality control).
6. **Reference range:** 10.0–14.0 sec; established by each institution
7. **INR:** International normalized ratio
   a. Means of **standardizing PT reporting** worldwide; not dependent on thromboplastin reagent or instrument used
   b. **INR** values are used to **monitor warfarin/coumarin therapy.** There is no reference range. The therapeutic range is dependent on the condition being treated, but it is generally considered to be between **2.0 and 3.0.**
   c. Formula

$$INR = \left[\frac{\text{Patient PT (in seconds)}}{\text{Control PT (in seconds)}} \right]^{ISI}$$

   d. **ISI** is the **international sensitivity index** for the thromboplastin reagent; this number is provided by the manufacturer and is lot number and instrument specific.
   e. The most sensitive thromboplastin reagents have an ISI value of 1.00, based on World Health Organization (WHO) standards.
8. **Prolonged PT** can indicate **factor deficiencies** in the **extrinsic/common pathways;** factor activity less than 25–30% or warfarin therapy will prolong the PT.

9. **Sources of error**
   a. Improper sample collection, improper preparation, and inherent patient problems
      1) **Falsely long PT:** Same as for aPTT
      2) **Falsely short PT:** Small clot in tube
   b. **Reagent** preparation and **instrumentation** problems are the same as for aPTT.

C. Other Laboratory Tests

1. **Mixing study** is performed when the PT or aPTT is prolonged to **differentiate a factor deficiency from a circulating inhibitor.** Patient plasma is mixed with normal pooled plasma and test(s) is(are) repeated.
   a. **Shortening of the time** into the reference range (correction) indicates a **factor deficiency** (hereditary, or acquired causes such as warfarin therapy or liver disease).
   b. **Partial or no correction** indicates a **circulating inhibitor** (heparin, lupus inhibitor, VIII inhibitor, IX inhibitor).
2. **Fibrinogen level** is a quantitative test for fibrinogen. **Thrombin reagent** is added to **diluted** citrated patient plasma. Thrombin clotting time obtained is read using a standard curve and is inversely proportional to fibrinogen concentration.
3. **Thrombin time** is a qualitative/quantitative **test for fibrinogen. Thrombin reagent** is added to **undiluted** patient plasma and result is reported in seconds. Presence of **heparin, degradation products,** or **low fibrinogen** level will **prolong** the result.
4. **Factor assays** are used to **confirm** a suspected **factor deficiency,** as suggested by a mixing study that shows correction. Test measures the ability of patient plasma to correct the PT or aPTT result obtained with plasma known to be factor deficient (compared to known standards). The **factor activity percent** is reported.
5. **5.0 M urea clot solubility test:** The unstable clot that forms in factor XIII deficiency dissolves in 5.0 M urea; a factor XIIIa-stabilized clot remains intact in 5.0 M urea for at least 24 hours.
6. **Dilute Russell viper venom test** is a sensitive test that uses snake venom as the reagent to activate factor X in the cascade. If the **lupus inhibitor** is present, the venom is neutralized, and the test is prolonged.
7. **Activated clotting time (ACT)**
   a. Whole blood is placed in a glass tube containing activator. Determine time it takes the clot to form; blood is kept at 37°C during testing.
   b. Point-of-care test performed at a clinic, cardiac catheterization laboratory, or surgical suite. Most often used to **monitor high-dose heparin therapy** during coronary artery bypass surgery.

XII. EVALUATION TESTS FOR THE FIBRINOLYTIC SYSTEM

A. Fibrin Degradation Products (FDPs): Latex particles are coated with antibody against **fibrinogen** and are mixed with patient **serum.** Macroscopic agglutination indicates degradation products. This is a **nonspecific test** that will be **abnormal** when either **fibrin degradation products or fibrinogen degradation products are present** (DIC and primary fibrinogenolysis).

B. D-Dimer Assay: Latex particles are coated with antibody against **D-dimer.** Highly **specific** measurement for **fibrin degradation products;** does **not** detect fibrinogen degradation products. Abnormal result indicates a clot has formed, been stabilized by factor XIIIa, and is being lysed by plasmin (abnormal in **DIC,** but normal in primary fibrinogenolysis).

XIII. ANTICOAGULANT THERAPIES

A. Unfractionated Heparin Therapy

1. Treatment of choice to **prevent extension of existing clots** due to acute thrombotic events (e.g., venous and arterial thrombosis, pulmonary embolism, thrombophlebitis, acute myocardial infarction)
2. Therapy involves a bolus of **heparin,** followed by continuous infusion.
3. **Antithrombin** must be present with levels of 40–60% of normal for heparin to work.
4. The **antithrombin/heparin** complex **inhibits serine proteases,** including XIIa, XIa, IXa, Xa, IIa, and kallikrein. **Inhibition is immediate.**
5. It **inhibits** the conversion of fibrinogen to fibrin, platelet aggregation, and activation of factor XIII.
6. Heparin activity can be immediately **reversed by** administration of **protamine sulfate.**
7. Monitor with **aPTT;** therapeutic range is approximately 1.5–2 times patient's baseline aPTT value prior to treatment. Dosage is adjusted accordingly.
8. Daily **platelet counts** should be performed on heparinized patients to **monitor for heparin-induced thrombocytopenia** (HIT). If detected, heparin therapy is immediately halted and different anticoagulant therapies are considered.

B. Warfarin (Coumadin®/Coumarin) Therapy

1. This oral anticoagulant is prescribed on an outpatient basis to prevent extension of existing clots and recurrence of thrombotic events, and prophylactically it is often prescribed postsurgery to prevent thrombosis.
2. **Vitamin K antagonist**
3. Warfarin inhibits liver synthesis of functional prothrombin group factors II, VII, IX, and X. Factor VII is affected first (short half-life) and to the greatest extent.

4. Overlap with heparin therapy is common, because full anticoagulant action of warfarin is not achieved for 4–5 days. Warfarin is often used for **up to 6 months** or longer.

5. **Monitor** with **PT** and **INR;** INR therapeutic range is 2.0–3.0 for most conditions. If INR is higher with serious bleeding, **vitamin K** can be administered to **reverse affects.**

C. Other Medications Used in Hemostasis

1. **Low-molecular-weight heparin** (e.g., enoxaparin sodium), subcutaneous injection, requires antithrombin to work
   a. Fixed dose response reduces the need for laboratory monitoring.
   b. Lower risk of heparin-induced thrombocytopenia (HIT)
   c. It is mainly an anti-Xa inhibitor; anti-IIa response is reduced.
   d. If monitoring is needed, perform anti-Xa assay.

2. **Direct thrombin inhibitor** (e.g., argatroban, lepirudin, bivalirudin) inactivates thrombin only; does not require presence of antithrombin to work
   a. Used in place of unfractionated or low-molecular-weight heparin when HIT suspected
   b. These medications will prolong the PT, aPTT, and thrombin time.

3. **Fibrinolytic therapy:** Tissue plasminogen activator, streptokinase or urokinase, can be used to **lyse existing clots** and reestablish vascular perfusion.
   a. These medications convert plasminogen to plasmin.
   b. Plasmin destroys the fibrin clot, factors I, V, and VIII.
   c. Affected tests include PT, aPTT, thrombin time, fibrinogen, FDP, and D-dimer (also bleeding time because of low fibrinogen).

4. **Antiplatelet medications** (e.g., aspirin, Plavix®, ticlopidine, and nonsteroidal anti-inflammatory drugs/NSAIDS) may be used in conjunction with other anticoagulant therapies to prevent recurrence of thrombotic events.

review questions

Principles of Coagulation

1. The hemorrhagic problems associated with scurvy are due to a deficiency of _____, which is a cofactor required for collagen synthesis.
   A. Vitamin C
   B. Prothrombin
   C. Vitamin K
   D. Protein C

2. The number of platelets an average megakaryocyte generates is approximately
   A. 25–50
   B. 50–200
   C. 200–500
   D. 2000–4000

3. Which of the following is *not* a cause of thrombocytopenia?
   A. Splenomegaly
   B. Chemotherapy
   C. Increased thrombopoietin
   D. Aplastic anemia

4. Platelets interacting with and binding to other platelets is referred to as
   A. Adhesion
   B. Aggregation
   C. Release
   D. Retraction

5. In platelet aggregation studies, certain aggregating agents induce a biphasic aggregation curve. This second phase of aggregation is directly related to
   A. Formation of fibrin
   B. Changes in platelet shape
   C. Release of endogenous ADP
   D. Release of platelet factor 3

6. A platelet aggregation agent that characteristically yields a biphasic curve when used in optimal concentration is
   A. Arachidonic acid
   B. Collagen
   C. Epinephrine
   D. Ristocetin

7. The platelet aggregation pattern drawn below is characteristic of the aggregating agent
   A. ADP
   B. Collagen
   C. Ristocetin
   D. Thrombin

Addition of aggregating agent
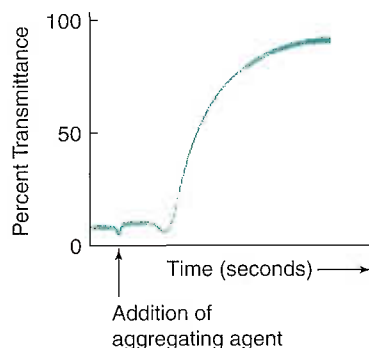
8. The operating principle of a platelet aggregometer is best described as
   A. Aggregation on a foreign surface: Platelet aggregation is directly proportional to the difference in platelet counts performed before and after platelet-rich plasma is passed through a column of glass beads.
   B. Change in optical density: As platelets aggregate, the optical density of the platelet-rich plasma decreases.
   C. Electrical impedance: Platelet aggregates are counted as they pass through an aperture, temporarily interrupting the flow of current between two electrodes.
   D. Pulse editing: Editing electronically generated pulses can differentiate the number of free platelets versus platelet aggregates.

9. Of the following therapeutic agents, those considered to be antiplatelet medications are
   A. Aspirin and Plavix®
   B. Coumadin® and heparin
   C. Heparin and protamine sulfate
   D. Tissue plasminogen activator and streptokinase

10. A potent inhibitor of platelet aggregation released by endothelial cells is
    A. Epinephrine
    B. Prostacyclin
    C. Ristocetin
    D. Thromboxane A_2

11. The reference value for mean platelet volume (MPV) is approximately
    A. 2–4 fL
    B. 5–7 fL
    C. 8–10 fL
    D. 11–14 fL

12. The platelet parameter PDW refers to the
    A. Average platelet volume
    B. Cell weight versus density
    C. Capacity to adhere to foreign surfaces
    D. Variation in platelet cell size

13. A normal histogram showing platelet size distribution is best described as
    A. Bimodal, nonskewed peaks
    B. Left-skewed single peak
    C. Right-skewed single peak
    D. Single peak, Gaussian distribution

14. Which of the following is *not* a normal maturation stage for platelets?
    A. Megakaryoblast
    B. Promegakaryocyte
    C. Micromegakaryocyte
    D. Megakaryocyte

15. The recommended type of microscopy for the performance of manual platelet counts is
    A. Electron
    B. Dark field
    C. Light
    D. Phase contrast

16. Twenty microliters of blood are diluted in 1.98 mL of diluent. This dilution is plated on both sides of a Neubauer-ruled counting chamber. A total of 356 cells is seen when both large center squares are counted. The platelet count *expressed in SI units* is
    A. $178 \times 10^9/L$
    B. $178 \times 10^3/\mu L$
    C. $356 \times 10^9/L$
    D. $712 \times 10^9/L$

17. The size threshold range used by electrical impedance methods to count particles as platelets is
    A. 0–10 fL
    B. 2–20 fL
    C. 15–40 fL
    D. 35–90 fL

18. In storage pool disease, platelets are primarily deficient in
    A. ADP
    B. Platelet factor 3
    C. Thrombasthenin
    D. Thromboxane A_2

19. The anticoagulant required for routine coagulation testing is
    A. Sodium heparin
    B. Sodium citrate
    C. Acid citrate dextrose
    D. Sodium fluoride

20. Which of the following is *not* synthesized in the liver?
    A. Factor VIII
    B. Plasminogen
    C. Protein C
    D. von Willebrand factor

21. When thrombin binds to thrombomodulin on the endothelial cell surface, thrombin can
    A. Activate the protein C pathway
    B. Activate factor V and factor VIII
    C. Convert fibrinogen to fibrin
    D. Stimulate platelet aggregation

22. The coagulation factors having a sex-linked recessive inheritance pattern are
    A. Factor V and factor VIII
    B. Factor VIII and factor IX
    C. Factor IX and factor X
    D. von Willebrand factor and factor VIII

23. Prekallikrein deficiency is associated with
    A. Prolonged aPTT that does not correct with a mixing study
    B. Autosomal dominant inheritance
    C. Increased risk of thrombosis
    D. Delayed bleeding at the incision site following surgery

24. Which of the following will *not* cause the thrombin time to be prolonged?
    A. Fibrin degradation products
    B. Heparin
    C. Factor I deficiency
    D. Factor II deficiency

25. The expected screening test results for a patient with a fibrin stabilizing factor deficiency are
    A. Prolonged prothrombin time
    B. Prolonged activated partial thromboplastin time
    C. Prolonged prothrombin time and activated partial thromboplastin time
    D. Normal prothrombin time and activated partial thromboplastin time

26. A patient on therapeutic warfarin will most likely have a(n)
    A. Normal PT/INR, increased aPTT, prolonged bleeding time, low platelet count
    B. Increased PT/INR, increased aPTT, normal bleeding time, normal platelet count
    C. Normal PT/INR, normal aPTT, normal bleeding time, normal platelet count
    D. Increased PT/INR, normal aPTT, prolonged bleeding time, low platelet count

27. Which of the following complexes is *not* needed for blood coagulation to occur?
    A. VIIa, tissue factor, Ca^{2+}
    B. IXa, VIII, Ca^{2+}, PF3
    C. Xa, V, Ca^{2+}, PF3
    D. XIIa, kallikrein, HMWK

28. von Willebrand factor is a
    A. Phospholipid required for multiple reactions in the coagulation sequence
    B. Plasma protein that binds platelets to exposed subendothelial collagen
    C. Plasma protein with procoagulant activity in the intrinsic coagulation system
    D. Platelet membrane glycoprotein that attaches the platelet to the injured vessel wall

29. Fibrin strands are cross-linked and the fibrin clot is stabilized by the activity of
    A. α_2-Antiplasmin
    B. Factor XIIIa
    C. Plasmin
    D. Thrombin

30. Which of the following does *not* contribute to the activation of the fibrinolytic system?
    A. XIIa
    B. XIa
    C. Kallikrein
    D. Tissue plasminogen activator

31. Which of the following enzymatically degrades the stabilized fibrin clot?
    A. Plasminogen
    B. Plasmin
    C. Prothrombin
    D. Thrombin

32. The activity of the lupus anticoagulant and anticardiolipin antibodies appears to be directed against
    A. Factor V
    B. Factor VIII
    C. Factor IX
    D. Phospholipid

33. Heparin inhibits clotting by
    A. Chelating calcium ions
    B. Preventing activation of prothrombin
    C. Causing liver synthesis of nonfunctional factors
    D. Enhancing the action of antithrombin

34. The main regulatory protein of secondary hemostasis is
    A. Antithrombin
    B. Protein C
    C. α_2-Antiplasmin
    D. Tissue plasminogen activator

35. Why is the activated partial thromboplastin time (aPTT) *not* the procedure of choice for detecting a platelet factor 3 (PF3) deficiency?
    A. Platelet-rich plasma is used for this test.
    B. The reagent contains a phospholipid substitute for PF3.
    C. PF3 is unstable in the reagent used for this test.
    D. PF3 does not function in the system being tested.

36. Measurement of the time required for fibrin formation when thrombin is added to plasma evaluates the
    A. Fibrinogen concentration
    B. Prothrombin concentration
    C. Extrinsic clotting system
    D. Intrinsic clotting system

37. A fibrinogen assay is performed on the fibrometer using the standard 1:10 dilution with Owren's buffer. The seconds obtained do not read on the standard curve. An alternate 1:20 dilution is performed and is 400 mg/dL when read off the curve. The concentration of fibrinogen to be reported in mg/dL is
    A. 160 mg/dL
    B. 200 mg/dL
    C. 400 mg/dL
    D. 800 mg/dL

38. Which of the following is *not* true of the international normalized ratio (INR)?
    A. INR is dependent on reagents and instrumentation used.
    B. INR is calculated using the PT ratio taken to the power of the ISI value.
    C. The World Health Organization recommends reporting the INR on patients on stable oral anticoagulant therapy.
    D. A therapeutic INR for a patient on Coumadin® is between 2.0 and 3.0, but may be higher depending on the cause of the patient's underlying disease state.

39. A prolonged aPTT result is obtained on a patient diagnosed with acute disseminated intravascular coagulation (DIC). The patient has not yet been treated for this disorder. The most likely cause of the prolonged aPTT is
    A. In addition to DIC, the patient is deficient in a factor required for the extrinsic pathway.
    B. DIC is characterized by synthesis of less stable coagulation factors, which deteriorate rapidly in the circulation.
    C. Systemic activation of the coagulation system depletes some factors more rapidly than the liver can synthesize them.
    D. The patient has been misdiagnosed; a prolonged aPTT indicates that the problem is deficient, not excessive, coagulation.

40. Which of the following test results is *not* characteristic of DIC?
    A. Decreased fibrinogen concentration
    B. Positive test for degradation products
    C. Decreased platelet count
    D. Increased antithrombin

41. The principle of _____ methods depends on cleavage of synthetic substrates by an active serine protease.
    A. Chromogenic
    B. Photo-optical
    C. Mechanical
    D. Immunodiffusion

42. Epsilon aminocaproic acid is the treatment of choice for
    A. von Willebrand disease
    B. Hemophilia A
    C. DIC with secondary fibrinolysis
    D. Primary fibrinogenolysis

43. A clot retraction defect is most likely due to
    A. Lack of platelet receptor glycoprotein Ib
    B. Lack of platelet receptor glycoprotein IIb/IIIa
    C. Insufficient ADP in dense bodies
    D. Absence of von Willebrand factor

44. Thrombocytosis is a characteristic of
    A. Disseminated intravascular coagulation
    B. Splenomegaly
    C. Polycythemia vera
    D. Idiopathic thrombocytopenic purpura

45. In which of the following functions are the products released by vascular endothelial cells *not* involved?
    A. Inhibition of platelet aggregation
    B. Activation of the fibrinolytic system
    C. Conversion of thrombin from a procoagulant to an anticoagulant
    D. Cross-linkage of fibrin monomers

46. If a physician suspects a qualitative platelet defect, the most useful test to order is the
    A. Platelet count
    B. Prothrombin time
    C. 5.0 M urea solubility test
    D. Bleeding time

47. The coagulation factors referred to as "vitamin K-dependent" are
    A. I, V, VIII, XIII
    B. II, V, IX, XII
    C. II, VII, IX, X
    D. XI, XII, Fletcher, Fitzgerald

48. A patient on warfarin therapy will be deficient in a functional amount of
    A. Fibrinogen and prothrombin
    B. Stable and labile factors
    C. Protein C and protein S
    D. Fletcher and Fitzgerald factors

49. A 25-year-old male presents to his physician complaining of leg pain. The physician diagnoses a deep vein thrombosis and wants to determine the cause of the thrombotic episode. Which of the following conditions would *not* be associated with such a thrombotic episode?
    A. Factor V Leiden and Prothrombin 20210 mutations
    B. Hypofibrinogenemia and hyperhomo-cysteinemia
    C. Lupus anticoagulant and anticardiolipin antibodies
    D. Antithrombin and protein C deficiencies

50. An 85-year-old male with slurred speech and paralysis on the right side of the body is seen in the emergency department. A stat D-dimer is ordered and is very high. The physician suspects a thromboembolic event based on the D-dimer, and needs to institute clot-dissolving therapy immediately. The most likely diagnosis and appropriate therapy for the patient is
    A. Myocardial infarction; treat with aspirin
    B. Pulmonary embolism; treat with warfarin
    C. Deep vein thrombosis; treat with heparin
    D. Stroke; treat with tissue plasminogen activator

51. Reversal of a heparin overdose can be achieved by administration of
    A. Vitamin K
    B. Protamine sulfate
    C. Antithrombin
    D. Warfarin

52. Which of the following best describes protein C?
    A. Vitamin K-dependent inhibitor to clotting
    B. Activator of factors V and VIII:C
    C. Inhibitor of fibrinolysis
    D. Synthesized by endothelial cells

53. The prothrombin time will detect deficiencies in the _____ pathway(s) when calcium and a tissue factor source such as rabbit brain are added to plasma.
    A. Extrinsic
    B. Extrinsic and common
    C. Intrinsic
    D. Intrinsic and common

54. A 65-year-old patient in the emergency department has a normal D-dimer and an elevated FDP result. These results are consistent with the presence of degradation products of
    A. Non-cross-linked fibrin
    B. Cross-linked fibrin
    C. Fibrinogen
    D. Plasmin

Specimen Acceptability

55. A specimen is received for a prothrombin time and activated partial thromboplastin time. The 5 mL tube has 2.5 mL of blood in it. Expected test results are
    A. PT and aPTT both falsely short
    B. PT and aPTT both falsely long
    C. PT and aPTT both unaffected
    D. PT unaffected, aPTT falsely short

56. A microtainer EDTA sample obtained during a fingerstick puncture is run on an automated cell counter, yielding a platelet count of 178×10^9/L. In the erythrocyte monolayer of the stained peripheral blood smear, an average of 9 platelets per field is seen under 1000× magnification. Based on these data, you should
    A. Report the results because the platelet count and platelet estimate correlate.
    B. Recollect a specimen for a repeat platelet count because the platelet count and estimate do not correlate.
    C. Examine the periphery of the blood smear for clumping because the platelet count and estimate do not correlate.
    D. Rerun the platelet count on the available specimen to confirm the results.

57. Blood for an aPTT was collected from a 5-year-old boy. During the venipuncture, he had to be restrained by several people and still managed to be a moving target. The result of the child's aPTT was 18.0 sec (reference range 22.0–38.0 sec). The aPTT controls were in range. Which of the following interpretations would apply to the aPTT result?
    A. aPTT is abnormal because of a hereditary factor deficiency.
    B. aPTT is invalid because of contamination with tissue factor.
    C. Tube is probably not full, resulting in a falsely short time.
    D. Result is within reference range for a patient of this age.

58. Laboratory tests requested on a patient scheduled for early morning surgery include a CBC with platelet count. An automated platelet count performed on the specimen is 57×10^9/L. In the monolayer area of the peripheral blood smear there are approximately 12 platelets per oil immersion field, many of which are encircling neutrophils. Controls are in range. Based on this information, the best course of action is
    A. Report all the results because the instrument is functioning properly.
    B. Alert the physician immediately so cancellation of surgery can be considered.
    C. Thoroughly mix specimen and repeat platelet count; if results remain the same, report all results and indicate that platelet count has been confirmed by repeat testing.
    D. Have the specimen redrawn using 3.2% sodium citrate as the anticoagulant.

59. Phlebotomist Forgetful Frank collected a tube of blood for an aPTT on John Smith at 10:00 A.M. The blood was collected in a sodium citrate tube. At 4:30 P.M., Frank was getting ready to leave for the day when he discovered Mr. Smith's blood specimen on his blood collection tray. So before leaving, Frank delivered the tube of blood to the laboratory for testing. Which of the following best describes the expected results?
    A. Sodium citrate is a preservative as well as an anticoagulant, so the aPTT result should be accurate.
    B. An aPTT collected in sodium citrate will give falsely long results because some factors are unstable in this anticoagulant.
    C. A falsely long aPTT is expected because some factors deteriorate rapidly at room temperature.
    D. Exposure of the plasma to erythrocytes for several hours has probably activated the factors, so the aPTT will be falsely short.

60. An aPTT and PT are requested on a patient scheduled for emergency surgery. On an optical density clot detection system, normal and abnormal controls for both tests are within range, but the patient's results exceed the upper limit of linearity. The patient's aPTT and PT have been performed in duplicate, but there still is sufficient plasma, which is grossly lipemic, to repeat the tests. What is the best course of action to follow?
    A. Report the results immediately by phone, emphasizing that the tests were run in duplicate and the controls are within range.
    B. Request a new specimen and repeat the aPTT and PT using freshly diluted controls.
    C. Repeat the aPTT and PT on an instrument that detects clot formation electromechanically.
    D. Inform the physician that accurate results are impossible.

61. A sodium citrate tube is received in the laboratory for PT and aPTT testing. Results are as follows:

Prothrombin time	>100.0 sec (control 12.0 sec)
aPTT	>200.0 sec (control 32.0 sec)

On examination, a large clot is discovered. The abnormal test results are due to deficiencies of factors
    A. I, V, VIII, IX
    B. I, II, V, VIII, XIII
    C. II, VII, IX, X
    D. VIII, IX, XI, XII

Case Studies

62. A 30-year-old female is admitted to the hospital with neurological symptoms. The following results are obtained:

Hemoglobin	60 g/L
Hematocrit	0.19 L/L
Platelet count	25×10^9/L
RBC morphology	Many schistocytes
ADAMTS-13	Markedly decreased

The most likely diagnosis for the patient is
A. Thrombotic thrombocytopenic purpura
B. Idiopathic thrombocytopenic purpura
C. Hemolytic uremic syndrome
D. von Willebrand disease

63. A 4-year-old child is seen in the emergency department with petechiae and a platelet count of 15×10^9/L. She has no previous history of bleeding problems. Three weeks earlier she had chicken pox. The physician advises the parents to keep the child off the playground to avoid injury, and says the child will recover within 2–4 weeks with no further treatment. What condition does this child most likely have?
A. Essential thrombocythemia
B. Idiopathic thrombocytopenic purpura
C. Thrombotic thrombocytopenic purpura
D. Glanzmann thrombasthenia

64. Laboratory results on a 16-year-old female with frequent nosebleeds and severe menorrhagia are as follows:

Platelet count	250×10^9/L
Bleeding time	>15 min (reference range ≤8.0 min)
Prothrombin time	13.0 sec (control 12.0 sec)
aPTT	75.0 sec (control 32.0 sec)
Platelet aggregation	Normal response to ADP, collagen, epinephrine; no response with ristocetin

These results are consistent with
A. Christmas disease
B. Hemophilia A
C. Glanzmann thrombasthenia
D. von Willebrand disease

65. Laboratory results on a 6-year-old female with petechiae and severe epistaxis are as follows:

Platelet count	145×10^9/L
Bleeding time	>15 min (reference range ≤8.0 min)
MPV	16.0 fL (reference range 8.0–10.0 fL)
Platelet aggregation	Normal response to ADP, collagen, epinephrine; no response with ristocetin
Prothrombin time	11.5 sec (control 12.0 sec)
aPTT	33.0 sec (control 32.0 sec)

These results are consistent with
A. Bernard-Soulier syndrome
B. von Willebrand disease
C. Glanzmann thrombasthenia
D. Ehlers-Danlos syndrome

66. A clot retraction defect is suspected in a newborn male experiencing severe bleeding following circumcision. The following results are obtained:

Platelet count	320×10^9/L
Bleeding time	>15 min (reference range ≤ 8.0 min)
Platelet aggregation	Normal response to ristocetin; weak response to ADP, collagen, epinephrine
Prothrombin time	12.0 sec (control 12.0 sec)
aPTT	31.0 sec (control 32.0 sec)

These results are characteristic of
A. von Willebrand disease
B. Glanzmann thrombasthenia
C. Storage pool disease
D. Christmas disease

67. Results on a 35-year-old male presenting with sudden severe hemorrhagic problems are as follows:

Platelet count	225×10^9/L
Bleeding time	6.5 min (reference range ≤ 8.0 min)
Prothrombin time	12.8 sec (control 12.0 sec)
aPTT	85.0 sec (control 32.0 sec)
aPTT 1:1 mixing study	65.0 sec

These clinical manifestations and laboratory results are consistent with
A. Lupus anticoagulant
B. von Willebrand disease
C. Hemophilia A
D. Factor VIII inhibitor

68. An 80-year-old man suffered a heart attack 1 month ago, and after the hospital stay was discharged with instructions to follow an outpatient treatment plan. He arrives at the cardiology clinic today for lab work to monitor the treatment plan. The following results are obtained:

PT	52.0 sec (control 12.0 sec)
INR	5.5 (therapeutic range 2.0–3.0)
aPTT	50.0 sec (control 32.0 sec)

This patient is most likely on a
A. Nontherapeutic dose of unfractionated heparin
B. Nontherapeutic dose of coumarin
C. Nontherapeutic dose of both unfractionated heparin and coumarin
D. Fibrinolytic agent such as tissue plasminogen activator

69. The following results are obtained on a 60-year-old male patient:

WBC	24.7×10^9/L
RBC	6.67×10^{12}/L
Hgb	200 g/L
Hct	0.61 L/L
Plt	79×10^9/L
PT	19.3 sec (control 12.0 sec)
aPTT	81.2 sec (control 32.0 sec)

The WBC, RBC, Hgb, Hct, and Plt were performed on blood collected in an evacuated tube containing EDTA. The PT and aPTT were performed on blood collected in an evacuated tube containing 3.2% sodium citrate. The standard collection procedure was followed, and all tests were performed within the appropriate time limits. Based on this information, the statement that best explains the prolonged coagulation test results is

A. Coagulation reactions require platelet factor 3; availability of this component is insufficient when the platelet count is below 100×10^9/L.

B. The ratio of anticoagulant to blood is critical; the volume of anticoagulant must be decreased when the Hct is greater than 55%.

C. The PT and aPTT evaluate the extrinsic and intrinsic pathways, respectively; prolongation of both tests indicates a deficiency of a factor common to both systems.

D. Coagulation reactions are inhibited by a product released by leukocytes; this inhibitory activity becomes significant when the leukocyte count is greater than 20.0×10^9/L.

70. The following results are obtained on a 3-year-old boy with sudden severe hemorrhagic problems:

Bleeding time	5.0 min (reference range ≤ 8.0 min)
Prothrombin time	13.0 sec (control 12.0 sec)
aPTT	95.0 sec (control 32.0 sec)
aPTT 1:1 mixing study	35.0 sec
Platelet aggregation	Normal with ristocetin, ADP, collagen, and epinephrine

These clinical manifestations and laboratory results are consistent with

A. Aspirin therapy

B. von Willebrand disease

C. Hemophilia A

D. Heparin therapy

71. Screening tests for a 46-year-old male patient admitted for minor surgery follow:

Platelet count	325×10^9/L
Bleeding time	4.5 min (reference range ≤ 8.0 min)
Prothrombin time	13.0 sec (control 12.0 sec)
aPTT	95.0 sec (control 32.0 sec)
aPTT 1:1 mixing study	32.0 sec

The patient has no clinical manifestations of a bleeding problem and has no personal or family history of bleeding problems, even following dental extraction. Several family members have been treated for deep vein thrombosis. Based on these laboratory results and the clinical history, the most likely cause of the prolonged aPTT is

A. Heparin present in the sample
B. Factor VIII deficiency
C. Factor XII deficiency
D. Factor XIII deficiency

72. A 25-year-old obstetrical patient at 35 weeks gestation is admitted through the emergency room. She has bleeding in the genitourinary tract, and there are visible petechiae and ecchymoses. The following laboratory results are obtained:

Platelet count	Markedly decreased
Prothrombin time	Prolonged
aPTT	Prolonged
Fibrinogen	Decreased
Thrombin time	Prolonged
D-dimer	Positive
FDP	Positive
Antithrombin	Decreased
RBC morphology	Schistocytes present

These laboratory results are consistent with

A. Primary fibrinogenolysis
B. DIC with secondary fibrinolysis
C. Factor II deficiency
D. Heparin therapy

73. A 57-year-old man with prostate cancer is admitted to the intensive care unit with severe bleeding problems. The following laboratory results are obtained:

Platelet count	Normal
Prothrombin time	Prolonged
aPTT	Prolonged
Fibrinogen	Decreased
Thrombin time	Prolonged
D-dimer	Negative
FDP	Positive
Antithrombin	Normal
RBC morphology	Schistocytes absent

These laboratory results are consistent with
A. Primary fibrinogenolysis
B. DIC with secondary fibrinolysis
C. Factor II deficiency
D. Coumadin® therapy

74. A patient in the hospital for an acute myocardial infarction is placed on standard unfractionated heparin therapy and aspirin. Laboratory results are performed before instituting therapy and then daily as shown:

	Before Therapy	Day 2	Day 3
Prothrombin Time	Normal	Normal	Normal
aPTT	Normal	Prolonged	Prolonged
Platelet Count	325×10^9/L	160×10^9/L	42×10^9/L

The most likely complication by Day 3 is
A. Disseminated intravascular coagulation
B. Primary fibrinogenolysis
C. Aspirin-induced thrombocytopenia
D. Heparin-induced thrombocytopenia

75. A 24-year-old female with painful swelling in her left leg is seen by her physician, who orders laboratory testing for PT and aPTT. The PT is normal. The aPTT is prolonged, but shortens with a 10-minute incubation of patient plasma with partial thromboplastin reagent that uses kaolin as the activator. A 1:1 aPTT mixing study corrects to normal. The most likely diagnosis is
A. Factor II deficiency
B. Factor VIII inhibitor
C. Factor XIII deficiency
D. Prekallikrein deficiency

Principles of Coagulation

1.

A. Vascular integrity is influenced by vitamin C intake. In a deficiency or absence of vitamin C, collagen production is insufficient or abnormal. Vitamin C deficiency is associated with capillary fragility and the primary hemostasis bleeding symptoms of petechiae and mucosal bleeding.

2.

D. Each megakaryocyte produces approximately 2000–4000 platelets. A single megakaryocyte can generate this large number of cells because platelets are nonnucleated fragments of their cytoplasm. The number of platelets generated by a megakaryocyte depends on its cell size, which is directly related to the number of endomitotic divisions before cytoplasmic fragmentation.

3.

C. Thrombopoietin is the major humoral factor involved in platelet production. Increased thrombopoietin results in thrombocytosis; decreased thrombopoietin results in thrombocytopenia. Two-thirds of platelets, once released from the bone marrow, are in circulation. The other one-third of platelets is sequestered in the spleen.

Splenomegaly is a cause of thrombocytopenia due to increased sequestration. Chemotherapeutic agents destroy both normal and malignant cells, causing thrombocytopenia, anemia, and leukopenia. Aplastic anemia occurs when the bone marrow fails to produce any of the three cell lines.

4.

B. "Adhesion" refers to platelets interacting with something other than platelets. *In vivo* platelets adhere to collagen that is exposed when vessel damage occurs. "Aggregation" refers to attachment of platelets to other platelets. Release is the process by which platelet granule contents are secreted. Retraction describes one of the final steps in coagulation in which the fibrin-platelet plug contracts, restoring normal blood flow to the vessel.

5.

C. In platelet aggregation studies, the addition of the aggregating agent may induce an initial aggregation phase followed by a secondary wave. The initial phase is due to the interaction of the aggregating agent with the platelet. The second phase is due to release of nonmetabolic ADP from platelet granules, which promotes the additional wave of aggregation.

6.

C. Epinephrine is the only aggregating agent listed that typically gives a biphasic pattern. ADP and thrombin also give biphasic patterns when used in optimal concentrations. Arachidonic acid causes a rapid monophasic platelet aggregation. Collagen and ristocetin also induce monophasic aggregatory responses.

7.

B. Collagen is the only aggregating agent that includes a single wave response preceded by a lag phase. During the lag phase collagen stimulates platelets to release their granule contents. Endogenous ADP released from the platelets then initiates irreversible platelet aggregation.

8.

B. When an aggregating agent is added to an optically dense suspension of platelet-rich plasma (PRP), the platelets normally stick to each other, forming platelet aggregates. As additional platelets aggregate, the cell suspension becomes clearer and has a few large clumps of cells. At maximum aggregation the specimen is relatively clear, allowing light transmission that is only partially obstructed by a few large platelet aggregates.

9.

A. Aspirin inhibits the enzyme cyclooxygenase in the prostaglandin pathway, preventing platelet aggregation. Plavix® (clopidogrel bisulfate) blocks the IIb/IIIa fibrinogen-binding platelet receptor, preventing platelet aggregation. Coumadin® and heparin inhibit clotting factors in secondary hemostasis. Protamine sulfate can be used to neutralize heparin. Tissue plasminogen activator and streptokinase are fibrinolytic system activators.

10.

B. Prostacyclin, also referred to as PGI_2, is the most potent inhibitor of platelet aggregation known. Injured endothelial cells release prostacyclin. Epinephrine and ristocetin are potent stimulators of platelet aggregation. Thromboxane A_2, generated by platelets via the prostaglandin pathway, also stimulates platelets to aggregate.

11.

C. The average volume of normal platelets is approximately 8–10 fL. This platelet parameter is equivalent to the erythrocyte parameter MCV (mean corpuscular volume). MPV is increased in the hereditary Bernard-Soulier syndrome and May-Hegglin anomaly; also in acquired disorders with increased need for platelet release from the bone marrow.

12.

D. PDW is an abbreviation for platelet distribution width. This parameter measures the uniformity of platelet size. It is the platelet equivalent of the red cell parameter RDW. It represents the coefficient of variation of the platelet population.

13.

C. A histogram showing platelet size distribution is made by plotting platelet size (x axis) versus number (y axis). The resulting curve is usually a single, right-skewed peak. This reflects a larger number of platelets in the lower size range with a "tail" of larger cells to the right of the majority.

14.

C. Micromegakaryocytes, also known as dwarf megakaryocytes, are thought to be megakaryocytes that have lost their ability to undergo endomitosis. They can be seen in the peripheral blood of patients with myelodysplastic syndromes or myeloproliferative disorders. They may resemble lymphocytes, but cytoplasmic blebs can help to identify them as micromegakaryocytes.

15.

D. Phase microscopy is currently recommended for manual platelet counts. This allows satisfactory discrimination between platelets and debris, a major problem in manual counts. Light microscopy may also be used; however, differentiating between platelets and debris is more difficult than with phase microscopy.

16.

A. Twenty microliters of blood (0.02 mL) added to 1.98 mL of diluting fluid gives a dilution of 1:100; the dilution factor is 100. The standard platelet counting area is the center mm^2 on both sides of the chamber. The standard formula for hemacytometer counts expressed in mm^3 is

$$\frac{\text{Total number cells counted} \times \text{Dilution factor}}{\text{Total area counted} \times \text{Depth}}$$

The correct equation for this problem is

$$\frac{356 \times 100}{2 \text{ mm}^2 \times 0.10 \text{ mm}} = 178,000$$
$$= 178 \times 10^3/\text{mm}^3$$

When expressed in SI units, the platelet count is $178 \times 10^9/\text{L}$. Answer B is expressed in conventional units, not SI units.

17.

B. In the electrical impedance method for counting platelets, particles between 2 and 20 fL will be classified as platelets by the analyzer's computer. The normal average platelet volume is 10 fL. One dilution is used for counting and sizing of platelets and red blood cells. In the electrical impedance method, size thresholds differentiate the two.

18.

A. Platelets in storage pool disease are deficient in dense granules. The platelets in this disorder lack nonmetabolic ADP found in dense granules and normally released when the platelets are stimulated. This accounts for a poor response to aggregating agents.

19.

B. The Clinical and Laboratory Standards Institute (CLSI) recommends 3.2% (0.109 M) sodium citrate for coagulation testing. Sodium heparin is used for many chemistry tests, but will cause times that exceed linearity if used for coagulation tests. Acid citrate dextrose (ACD) is used for HLA phenotyping, DNA analysis, and paternity tests. Sodium fluoride is used for glucose testing.

20.

D. The liver produces most of the clotting factors as well as inhibitors to clotting. A patient with liver disease has impaired synthesis of these clotting factors and inhibitors. One of the few hemostatic proteins not produced by the liver is von Willebrand factor, which is produced by endothelial cells and megakaryocytes.

21.

A. Thrombomodulin, an endothelial cell receptor, has the ability to change the specificity of thrombin from a procoagulant to an anticoagulant. Once bound to thrombomodulin, thrombin has anticoagulant properties because of its activation of protein C. Protein C, along with its cofactor protein S, then exerts negative feedback on the clotting system by inactivating factor V and factor VIII.

22.

B. Factor VIII and factor IX are the sex-linked recessive hemostatic defects. von Willebrand factor deficiency and dysfibrinogenemia are inherited as autosomal dominant disorders. Factor V, factor X, and most of the other inherited hemostatic disorders have an autosomal recessive inheritance pattern.

23.

C. Prekallikrein (Fletcher factor) deficiency is one of the many autosomal recessive disorders. The aPTT will be prolonged and will correct with a mixing study because it is a factor deficiency. Because prekallikrein is an activator of the fibrinolytic system, prekallikrein-deficient patients cannot lyse clots efficiently and are prone to thrombosis. Fibrinolytic and anticoagulant therapies are indicated in patients who develop thrombosis. Delayed post-operative bleeding at the incision site is characteristic of a factor XIII deficiency.

24.

D. The thrombin time is a test that measures fibrinogen. Thrombin reagent is added to undiluted patient plasma, and the time it takes for fibrinogen conversion to fibrin is measured. Anything that interferes with the ability of thrombin to convert fibrinogen to fibrin will prolong the test. Heparin and the degradation products X and E inhibit thrombin. Factor II cannot be measured in the thrombin time because the reagent used is its active form, thrombin (II_a).

25.

D. Thrombin converts fibrinogen to the fibrin monomer. Fibrin monomers spontaneously polymerize to form the fibrin polymer. This is the endpoint of clot-based PT and aPTT tests. This fibrin polymer is unstable. Once activated, factor XIII, also known as fibrin stabilizing factor, produces strong, covalent bonds to create a stable fibrin polymer. This occurs after the endpoint of the PT and aPTT has been reached. A factor XIII deficiency is suspected when delayed post-operative bleeding occurs at the incision site, and the deficiency can be confirmed with the 5 M urea clot solubility test.

26.

B. Warfarin is a vitamin K antagonist and affects liver synthesis of the prothrombin group factors II, VII, IX, and X. The factors are produced but are nonfunctional. Warfarin therapy is monitored with the prothrombin time (PT), which will detect nonfunctional II, VII, and X. The aPTT, though used to monitor heparin therapy, can detect nonfunctional II, IX, and X caused by warfarin therapy. Warfarin does not affect platelet function or quantity. Aspirin and other antiplatelet medications such as clopidogrel bisulfate (Plavix®) will affect platelet function and prolong the bleeding time.

27.

D. The intrinsic, extrinsic, and common pathways each have a complex that must form for blood coagulation to occur. The intrinsic complex of IXa, VIII, and Ca^{2+} forms on the platelet surface (PF3) and activates factor X. The extrinsic complex of VIIa, tissue factor, and Ca^{2+} activates factor X as well as factor IX and is the dominant pathway *in vivo*. Factor IX activation by the extrinsic complex provides a link between the intrinsic and extrinsic systems and minimizes the importance of the contact factors *in vivo*. The prothrombin-converting complex of Xa, V, Ca^{2+}, and PF3 is responsible for converting prothrombin to thrombin.

28.

B. von Willebrand factor (vWF) is a portion of the plasma protein known as the factor VIII/von Willebrand factor complex. Its function is to bind to platelet membrane glycoprotein Ib and form a bridge between the platelet and exposed subendothelial collagen. vWF is a carrier protein for factor VIII:C, but vWF does not have coagulant activity in secondary hemostasis as factor VIII:C does.

29.

B. Activated factor XIII is a transglutaminase that cross-links fibrin monomers between glutamine and lysine residues. Fibrin monomers that are not cross-linked lack the stability to maintain the hemostatic plug, as evidenced by the bleeding problems experienced by individuals deficient in factor XIII. Thrombin contributes to the formation of the fibrin clot, which is degraded by plasmin. Once the fibrin clot has been lysed and plasmin is free in circulation, α_2-antiplasmin quickly neutralizes plasmin.

30.

B. Once activated, three of the four contact factors activate both the intrinsic clotting system and provide intrinsic activation of the fibrinolytic system. The only contact factor that does not activate the fibrinolytic system is factor XI. Extrinsic activation of the fibrinolytic system is achieved by the release of tissue plasminogen activator by damaged endothelial cells.

31.

B. Plasmin, the active form of plasminogen, is the enzyme responsible for degrading fibrin into several different fragments. The D-dimer test is abnormal when there is excessive fibrinolytic activity. Prothrombin is the inactive precursor of thrombin that cleaves fibrinogen to form fibrin, which is stabilized by the activity of factor XIII.

32.

D. The lupus anticoagulant was first discovered in patients with systemic lupus erythematosus. It is actually seen in more patients without SLE, but the original name remains. Lupus anticoagulant and anticardiolipin antibodies belong to the antiphospholipid antibody family. Their activity appears to be directed against the phospholipid portion of the prothrombinase complex (Xa-V-phospholipid-calcium). The antibodies are usually IgG, but can also be IgM. They are found in autoimmune disorders, neoplasms, and some infections. They can also be medication related and can be found in apparently normal individuals. Their presence is suspected when the patient is experiencing thrombosis and the aPTT is prolonged with no correction of the mixing study.

33.

D. Heparin forms a complex with antithrombin to inhibit coagulation. The heparin-antithrombin complex rapidly inhibits thrombin and other serine proteases. Coagulation may be inhibited by coumarin and related vitamin K antagonists that cause liver synthesis of nonfunctional prothrombin group factors II, VII, IX, and X. Several anticoagulants, such as sodium citrate and EDTA, prevent fibrin formation by chelating calcium ions, which serve as cofactors in several reactions in the coagulation cascade.

34.

A. Antithrombin is the most important naturally occurring inhibitor to clotting and accounts for 80% of negative feedback in the coagulation cascade by inhibiting serine proteases. Protein C and its cofactor, protein S, inhibit cofactors V and VIII. α_2-Antiplasmin is responsible for neutralizing plasmin once the clot has been lysed. Tissue plasminogen activator activates the fibrinolytic system in response to clot formation.

35.

B. The reagent used in the aPTT procedure is a phospholipid extract that substitutes for platelet factor 3 in coagulation reactions. Platelet-poor plasma is used, so thrombocytopenia does not affect the aPTT. Platelet factor 3 functions in the intrinsic and prothrombin-converting complexes that must form for blood coagulation to occur.

36.

A. When thrombin is added to patient plasma, fibrinogen is converted to fibrin. No factors above fibrinogen in the cascade are measured, including prothrombin. Both the thrombin time and fibrinogen test use thrombin reagent; both tests measure only one factor, fibrinogen.

37.

D. A 1:20 dilution is used when the time obtained on a patient sample is less than the shortest time used in preparation of the standard curve. A 1:20 dilution is diluted by a factor of 2 when compared to the usual 1:10 dilution. The value read off the curve must be multiplied by 2 to take into account the alternate dilution used.

$$400 \times 2 = 800 \text{ mg/dL}$$

38.

A. The PT seconds are dependent on the reagent or instrument used. Because of this, the World Health Organization recommends using the INR to monitor patients on stabilized Coumadin® therapy because it is *independent* of the reagent or instrument used. The INR is a means of standardizing the reporting of prothrombin times (PTs) worldwide. The INR is calculated by many instruments and laboratory information systems but can be calculated manually as follows:

$$INR = \left[\frac{\text{Patient PT (in seconds)}}{\text{Control PT (in seconds)}} \right]^{ISI}$$

where
    INR = the International Normalized Ratio
    ISI = the International Sensitivity Index of the thromboplastin source. This value is determined by the manufacturer for each lot number of thromboplastin reagent. The closer to 1.00 the ISI, the more sensitive the thromboplastin reagent is in detecting factor deficiencies.
    Patient PT = the prothrombin time in seconds for the patient.

Control PT is the geometric mean of the reference interval.

39.

C. As coagulation occurs *in vivo,* some factors are consumed just as they are when blood is allowed to clot in a test tube *in vitro.* The factors consumed during coagulation are I, II, V, VIII, and XIII. Results of laboratory procedures relying on one or more of these factors will be affected. All these factors except factor XIII contribute to the reactions evaluated in the aPTT procedure.

40.

D. The PT and aPTT are prolonged in DIC because of consumption of factors I, II, V, and VIII. Platelets are trapped in forming clots and are removed from circulation. The fibrinolytic system is activated by systemic intravascular coagulation; fibrin and fibrinogen degradation products are elevated. FDP and D-dimer tests will both be positive. The regulatory proteins antithrombin, protein C, and protein S are depleted trying to turn off systemic clotting.

41.

A. The proteolytic activity of antithrombin after activation to a serine protease can be assayed via methods that employ synthetic substrates. The cleavage of the synthetic substrate by an active serine protease will yield a chromogenic compound. Chromogenic methods can also be used to assay plasminogen, protein C, and heparin.

42.

D. Epsilon aminocaproic acid (EACA) is a specific inhibitor of plasmin and is used to turn off inappropriate lysing that occurs in primary fibrinogenolysis. Fibrinolysis seen in DIC is an appropriate body response to systemic clotting. If EACA is administered to a patient in DIC, clots that form will not be lysed, and this could be quickly fatal to the patient.

43.

B. Glanzmann thrombasthenia is a disorder characterized by absent or defective GP IIb/IIIa platelet receptors for fibrinogen binding and subsequent platelet aggregation. Clot retraction in these patients is abnormal due to the lack of the contractile protein actomyosin/thrombasthenin. Neither insufficient ADP in dense bodies, absence of von Willebrand factor, nor absence of the platelet receptor glycoprotein Ib affects clot retraction. Lack of glycoprotein Ib, the von Willebrand factor receptor site, causes the platelet adhesion defect seen in Bernard-Soulier disease.

44.

C. Polycythemia vera, a hemopoietic stem cell disorder characterized by excessive production of erythrocytic, granulocytic, and megakaryocytic cells in the bone marrow, is usually accompanied by thrombocytosis. ITP, DIC, and splenomegaly are all characterized by thrombocytopenia. In ITP, platelet destruction is mediated by immune mechanisms. Platelets are consumed in DIC and sequestered in an individual with an enlarged spleen.

45.

D. Endothelial cells release prostacyclin, which is a potent inhibitor of platelet aggregation. They also release tissue plasminogen activators, which initiate the fibrinolytic system. When thrombin binds with thrombomodulin on the endothelial cell surface, the specificity of thrombin changes. Rather than converting fibrinogen to fibrin, thrombin complexed with thrombomodulin activates protein C. Protein C along with protein S inhibits clotting by "turning off" factors V and VIII. Factor XIII is responsible for cross-linking fibrin monomers but it is not a component of endothelial cells.

46.

D. The bleeding time test is a primary hemostasis screening test for platelet and vascular function. The platelet count is a quantitative test that cannot be used to determine platelet function. The prothrombin time is a secondary hemostasis screening test and does not evaluate platelet function because platelet-poor plasma is used for testing. The 5 M urea solubility test will detect a factor XIII deficiency.

47.

C. The prothrombin group factors II, VII, IX, and X are called vitamin K–dependent factors. Vitamin K is needed by the liver to synthesize functional circulating forms of these factors. In the absence of vitamin K, the liver synthesizes the prothrombin group factors, but they are nonfunctional because they lack the carboxyl (COOH) groups needed for binding to Ca^{2+} on phospholipid membranes. The oral anticoagulant warfarin is a vitamin K antagonist and causes liver synthesis of these nonfunctional factors.

48.

C. Vitamin K is required for liver synthesis of regulatory proteins C and S and functional clotting factors II, VII, IX, and X. A deficiency of vitamin K decreases the concentrations of these proteins and subsequently affects test results that measure one or more of them. Fibrinogen, Labile, Fletcher, and Fitzgerald factors do not require vitamin K for their synthesis.

49.

B. All of the conditions listed are associated with thrombosis except hypofibrinogenemia. Although hereditary dysfibrinogenemia frequently causes thrombosis, hypofibrinogenemia causes bleeding tendencies. The most common hereditary thrombotic disorder, factor V Leiden, is caused by synthesis of an abnormal factor V molecule that is resistant to the inhibitory affects of protein C. The Prothrombin 20210 mutation, in which an abnormal factor II molecule is synthesized, is the second most common hereditary thrombotic disorder.

50.

D. A high D-dimer level indicates the presence of a thrombus (deep vein thrombosis, pulmonary embolism) but is not useful in determining the location. If a thrombus breaks away and travels to the brain, a stroke occurs, causing the symptoms described in this patient. Tissue plasminogen activator will activate the fibrinolytic system to lyse the clot. It should be administered within hours of onset of symptoms to prevent irreversible brain damage. Because of the small window of treatment time, a D-dimer performed on a possible stroke patient should be done STAT. Aspirin, warfarin, and heparin can be administered to prevent the formation of new clots but will not lyse existing clots.

51.

B. A heparin overdose can result in hemorrhage. If bleeding becomes life threatening, protamine sulfate can be given. Heparin will dissociate from antithrombin if protamine sulfate is administered, because heparin has a higher affinity for protamine sulfate. Vitamin K can be administered in the management of bleeding for patients who overdose with warfarin, which is a synonym for Coumadin®.

52.

A. Protein C, a glycoprotein produced in the liver, is a potent inhibitor of coagulation. The activation of protein C, by the thrombin/thrombomodulin complex, will cause the inactivation of factors V and VIII:C. Protein C and its cofactor, protein S, are vitamin K–dependent proteins.

53.

B. The prothrombin time test measures the coagulant activity of the extrinsic and common pathway factors of I, II, V, VII, and X. The reagent used for the prothrombin time test contains calcium and tissue thromboplastin. Thromboplastin is an extract of tissue such as brain or

placenta. The activated partial thromboplastin time test measures all coagulation factors present in the intrinsic and common pathways except factor XIII. Calcium, a phospholipid source, and an activating agent, such as kaolin, silica, or celite, are present in the reagents used for the activated partial thromboplastin time.

54.

C. The D-dimer is a specific marker of fibrinolysis. A normal D-dimer test can be used to rule out the formation of a clot. The D-dimer test will be elevated when a clot has formed, factor XIII has cross-linked fibrin, and the fibrinolytic system is lysing the clot. The FDP detection test will be abnormal whether fibrin degradation products or fibrinogen degradation products are present. Because the D-dimer is normal in this patient, fibrin degradation products have not formed, but fibrinogen degradation products are present resulting in the elevated FDP result.

Specimen Acceptability

55.

B. A 9:1 ratio of blood to anticoagulant is needed for sodium citrate to bind all available calcium in the blood sample and prevent coagulation. When the 9:1 ratio is not maintained due to the tube not being full, excess sodium citrate present will bind reagent calcium in the test system. This will cause falsely prolonged PT and aPTT results.

56.

A. The results should be reported. A platelet estimate is obtained by multiplying the average number of platelets per oil immersion field (in an erythrocyte monolayer) by 20,000. This number is based on a normal erythrocyte count, which must be considered when comparing the platelet count and estimate. The estimate in this example is 180×10^9/L. This agrees with the platelet count.

57.

B. A clean venipuncture is required for coagulation testing. The description of the traumatic venipuncture indicates that the result might be invalid because of exposure to tissue thromboplastin/tissue factor, resulting in a falsely short test result. A tube that is not full would cause a falsely long time. A factor deficiency causes long clotting times. This 5-year-old would have the same reference range as adults.

58.

D. Platelets encircling neutrophils is a phenomenon referred to as platelet satellitosis. This "pseudothrombocytopenia" occurs when the blood of some individuals is anticoagulated with EDTA. Recollecting the specimen using sodium citrate often corrects this problem. If sodium citrate is used, the platelet count obtained must be multiplied by 1.1 for reporting purposes. Multiplying by 1.1 adds back the 10% loss of platelets seen when sodium citrate, with a 9:1 ratio of blood to anticoagulant, is used.

59.

C. Factors V and VIII are labile and deteriorate rapidly at room temperature. Blood for aPTT testing should be tested within 4 hours of draw. Sodium citrate is the appropriate anticoagulant for coagulation procedures.

60.

C. The lipemic plasma may interfere with the detection of fibrin clot formation by instruments measuring a change in optical density. The change in optical density may be insufficient for detection. The most accurate results in this situation can be obtained by performing the procedure on an electromechanical fibrin clot detection instrument, such as a fibrometer. Requesting a new specimen will be of no use because the redraw will most likely also be lipemic.

61.

B. A synonym for the fibrinogen group of factors is the consumed factors, because they are totally used up in clot formation. In the conversion of fibrinogen to fibrin, all of prothrombin is converted to thrombin. Thus, factors I, V, VIII, XIII, and II are all missing in serum.

Case Studies

62.

A. With the severe anemia and many schistocytes, a microangiopathic hemolytic anemia should be considered (TTP, HUS). HUS is seen in children after a gastrointestinal infection, frequently caused by *E. coli 0157:H7,* and results in renal damage. TTP is seen in young adults and is more common in women than men. TTP causes neurological damage. Patients with TTP have unusually large multimers of von Willebrand factor because they have a deficiency of a metalloprotease, ADAMTS-13, responsible for breaking down the multimers. These large multimers of vWF bind strongly to platelets, causing platelet aggregation and thrombotic complications in multiple organs.

63.

B. Acute idiopathic thrombocytopenic purpura is mainly seen in young children. A viral infection often precedes the onset of symptoms by several weeks. In 90% of patients with acute ITP, there is an increase in IgG immunoglobulin attached to the surface of the platelets. Spontaneous remission occurs in most patients within 2–6 weeks of the onset of the illness. A chronic form of ITP, believed to be a different disease, is seen in adults.

64.

D. The patient's platelet count is within the reference range, but the bleeding time is prolonged. This indicates a platelet function problem. The coagulation tests indicate a problem in the intrinsic clotting system (factors XII, XI, IX, VIII, Fitzgerald, and Fletcher). The one disorder in which both platelet function and the coagulant property of factor VIII:C are affected is von Willebrand disease. A synonym for von Willebrand factor is the ristocetin cofactor. In its absence, platelets will not aggregate with ristocetin. The platelet aggregation pattern confirms this diagnosis.

65.

A. Bernard-Soulier syndrome is a platelet adhesion defect that can be mistaken for von Willebrand disease. Platelets in this syndrome lack the glycoprotein Ib receptor, which is necessary for von Willebrand factor to attach to the platelet. Both disorders give identical platelet aggregation patterns. Bernard-Soulier syndrome is noted for giant platelets (note the increased MPV) and varying degrees of thrombocytopenia. Because von Willebrand factor is present in Bernard-Soulier syndrome, the aPTT is normal.

66.

B. Because the platelet count is within the reference range, the prolonged bleeding time is due to a qualitative platelet disorder. Poor clot retraction is characteristic of Glanzmann thrombasthenia. Clot retraction is normal in storage pool disease and von Willebrand disease. Christmas disease is caused by factor IX deficiency and does not affect platelet function. Glanzmann thrombasthenia can be further differentiated from von Willebrand disease by the platelet aggregation study results. The PT and aPTT results rule out a secondary hemostasis defect.

67.

D. Failure of normal plasma to correct the aPTT indicates the presence of a circulating inhibitor. A factor VIII inhibitor is associated with hemorrhagic problems and would result in noncorrection of the mixing study. Hemophilia A and von Willebrand disease are both caused by factor deficiencies that would result in correction when a 1:1 mixing study is performed. The lupus anticoagulant is a circulating inhibitor that prolongs the aPTT with little or no correction of the mixing study, but it is associated with thrombosis, not severe hemorrhagic problems.

68.

B. Coumarin interferes with the function of vitamin K in the synthesis of prothrombin group factors II, VII, IX, and X. Tests that measure one or more of these factors will be prolonged. These factors are synthesized but are nonfunctional. Of the factors affected by coumarin, IX, X, and II are measured in the aPTT. VII, X, and II are measured in the PT. The patient is on a nontherapeutic dose of coumarin, and the INR demonstrates this. Unfractionated heparin (administered intravenously) and fibrinolytic activators are not used on an outpatient basis.

69.

B. The required blood-to-anticoagulant ratio for coagulation testing is 9:1. If a volume of blood contains an elevated number of RBCs, generally considered to be a hematocrit greater than 55%, this ratio will be affected. Excess sodium citrate in the patient plasma, which acts as an anticoagulant by binding calcium ions, will bind the reagent calcium added back to the test plasma during the procedure. Falsely prolonged results are obtained. The specimen needs to be redrawn using less sodium citrate.

70.

C. Hemophilia A is inherited as a sex-linked recessive disorder of factor VIII:C. Mothers are carriers who pass the disease on to male off-spring. This disorder is strictly a secondary hemostasis defect, so tests for primary hemostasis such as the bleeding time and platelet aggregation studies are normal. Factor deficiencies correct when a 1:1 mixing study is performed; presence of heparin in the sample would result in little or no correction of the mixing study. Aspirin affects platelets in primary hemostasis.

71.

C. An abnormal aPTT that corrects with a 1:1 mixing study, and a normal PT indicate a deficiency in the intrinsic coagulation pathway. Because factor XII is an activator of the fibrinolytic system, a deficiency can result in thrombosis. Presence of heparin in the sample would cause noncorrection of the mixing study. Factor VIII deficiency causes bleeding into muscles and joints, not thrombosis. Factor XIII can be eliminated because it is not measured in the aPTT or PT, and a deficiency would cause a bleeding disorder.

72.

B. DIC is a consumption coagulopathy in which the fibrinogen group of factors I, V, VIII, XIII, as well as II in the prothrombin group, is consumed in systemic clotting faster than the liver can synthesize them. When the clotting system is activated, the fibrinolytic system is simultaneously activated. Degradation products form causing the FDP and D-dimer tests for degradation products to be positive. The thrombin time measures fibrinogen and is prolonged due to low fibrinogen and the presence of degradation products. Antithrombin is quickly depleted in an attempt to stop systemic clotting. Platelets are consumed in clotting, and schistocytes form as red blood cells fragment as they encounter fibrin strands in circulation.

73.

A. Primary fibrinogenolysis is an unusual disorder in which the fibrinolytic system is activated in the absence of clot formation. Plasmin degrades factors V, VIII, and fibrinogen. The D-dimer test is positive if fibrin degradation products are present; they are absent in this disorder. The FDP test is positive in the presence of either fibrin *or* fibrinogen degradation products. Tests that are abnormal in DIC due to the systemic clotting are normal in primary fibrinogenolysis.

74.

D. Up to 5% of patients receiving unfractionated heparin therapy for more than 5 days develop an IgG antibody that can cause platelet activation, leading to thrombosis in the microvasculature. If this occurs, the platelet count drops quickly. Patients receiving heparin therapy should be monitored with daily platelet counts. Direct thrombin inhibitors such as lepirudin, bivalirudin, and argatroban can be used in place of heparin. Enoxaparin sodium, a low-molecular-weight heparin, is contraindicated as a treatment for heparin-induced thrombocytopenia. Aspirin therapy causes a qualitative, not quantitative, platelet defect.

75.

D. The patient's symptoms suggest a possible deep vein thrombosis. An increased incubation time of patient plasma with aPTT reagent using kaolin is considered to be a screening test for prekallikrein deficiency. The additional incubation time allows for increased activation of the contact factors, thus shortening the aPTT. Because prekallikrein is an activator of fibrinolysis, a deficiency causes an increased thrombotic risk in the patient. A factor II deficiency would not give a normal PT result. A factor XIII deficiency can only be detected by performing a 5.0 M urea solubility test. A factor VIII inhibitor would cause noncorrection of the mixing study. All conditions listed except prekallikrein deficiency would cause bleeding symptoms.

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