Vascular Injury: Overview

- Normal vessel function
  - Hemostasis
- Vessel Injury
  - Hemorrhage
  - Thrombosis

Hemostasis

- The arrest of bleeding
  - Hemo = Blood
  - Stasis = Halt, slow
- A normal physiological response to localized vascular injury

Hemostasis

- The process is a complex, balanced interaction between:
  - Blood vessels
  - Platelets
  - Coagulation factors
  - Fibrinolytic and thrombolytic factors
- The goal is a rapid return to normal blood flow and fluidity following vascular injury

Blood vessels: Normal function

- Normal vessels provide unimpeded movement of blood to areas of need
  - Neuro-chemical regulation of flow
- Endothelium is a critical blood vessel component influencing vascular homeostasis
  - Multifunctional and complex:
    - Forms the vascular lining
    - Secretes mediators

Endothelial functions

- Physical barrier
- Regulation of blood flow and vascular tone
  - Normal endothelium is anti-thrombotic
  - Activated/injured endothelium is pro-thrombotic
- Fluid distribution
- Inflammation
- Healing/repair
- Hemostasis

Endothelial mediators: Vascular tone

- Prostacyclin
  - Enhances vascular relaxation and inhibits platelet adhesion and activation
- Nitric oxide
  - Maintains vascular relaxation and inhibits platelet activation. Participates with Protein C and antithrombin to suppress thrombin production
- Endothelium-derived hyperpolarizing factor
  - Induces vascular relaxation
- Endothelin-1
  - Induces vasoconstriction
Endothelial mediators: Anticoagulant

- **Prostacyclin**
  - Enhances vascular relaxation and inhibits platelet adhesion and activation

- **Nitric oxide**
  - Maintains vascular relaxation and inhibits platelet activation. Participates with Protein C and antithrombin to suppress thrombin production

- **Thrombomodulin**
  - Binds thrombin to initiate protein C activation

- **Protein S**
  - Cofactor in Protein C pathway and independently inhibits activation of Factors VIII and X

Endothelial mediators: Anticoagulant

- **Heparan sulfate proteoglycans**
  - Bind and concentrate antithrombin on endothelial surfaces

- **Tissue Plasminogen activator**
  - Activates fibrinolysis

- **Ectoenzyme adenosine-diphosphatase**
  - Degradation of ADP

- **Annexin V**
  - Competitive binding inhibitor of phospholipid-dependent coagulation factors

- **Tissue Factor Pathway Inhibitor**
  - Inhibits the Tissue Factor/Factor VIIa complex

Endothelium in hemostasis:

- Normal endothelium generally induces vascular relaxation and anti-clotting properties
  - Nitric oxide
  - Prostacyclin
  - Endothelium-derived hyperpolarizing factor

Nitric Oxide

- Produced by endothelium, macrophages and neurons
- These have many similarities to O₂ metabolites
- Properties include:
  - Vascular smooth muscle relaxation and vasodilation
    - Increases cyclic GMP concentrations via guanylate cyclase activation
  - Inhibition of platelet adhesion and aggregation
  - Inhibition of leukocyte adhesion and chemotaxis
  - Antimicrobial killing

Endothelial mediators: Procoagulant

- **Tissue Factor**
  - Released following endothelial injury, possibly by activated endothelium

- **Von Willebrand Factor**
  - Forms bridges for platelet adhesion and aggregation

- **Plasminogen Activator Inhibitor-1**
  - Inhibits fibrinolysis

Endothelial mediators: Repair

- **Platelet-derived growth factor (PDGF)**
  - Mitogenesis of smooth muscle and fibroblasts

- **Fibroblast growth factor (FGF)**
  - Fibroblast proliferation

- **Transforming growth factor – β (TGF-β)**
  - Modulation of vascular repair (cell proliferation inhibition)

- **Epidermal growth factor (EGF)**
- **Endothelial cell growth factor (ECGF)**
Nitric Oxide

- A product of arachidonic acid metabolism produced mainly by endothelium
- Properties include:
  - Vascular smooth muscle relaxation and vasodilation
    - Increases cyclic AMP concentrations via adenylate cyclase activation
  - Inhibition of platelet adhesion and aggregation

Prostacyclin

- A product of endothelium that induces hyperpolarization and relaxation of vascular smooth muscle
- It's effect is most pronounced on small compared to large arteries
- The precise mechanism of action is still unclear
  - It involves activation of K⁺ channels
  - It is different than PGI₂ and NO

Endothelium-Derived Hyperpolarization Factor

- Damaged endothelium has predominately pro-clotting properties
  - Release of Tissue Factor from activated endothelium and subendothelial tissues
  - Exposure of underlying collagen and other subendothelial components provide sites for platelet adhesion

Mediators of Vascular Relaxation
Causes of Endothelial Injury

- Inflammation (Vasculitis)
  - Infectious (Bacteria, viruses)
  - Non-infectious (Immune-mediated)
- Necrosis
  - Toxins
  - Infectious agents (viruses)

Hemostatic Process

- Primary hemostasis
- Secondary hemostasis

Primary Hemostasis

- The primary vascular and platelet response to vascular injury
  - Vascular contraction
  - Endothelial activation
    - Pro- and anti-clotting activity
    - Platelet plug formation
  - Most effective for minor vascular injury

Primary Hemostasis: Vascular changes

- Contraction of muscle layers of the blood vessel cause transient vasoconstriction
  - Neurogenic stimuli
  - Endothelial and platelet products
- Endothelial activation
  - Pro-coagulation to limit bleeding
  - Anti-coagulation to limit clotting

Primary Hemostasis: Platelets

- Platelets are the principle mediators of primary hemostasis
- Platelets bind to damaged endothelium or subendothelium to form a primary hemostatic platelet plug to prevent blood loss
Platelets: Normal function

- Platelets are membrane-bound cytoplasmic fragments derived from megakaryocytes in the bone marrow
  - Thrombopoietin is the main regulator of production
    - Colony stimulating factors, IL-3, IL-6, IL-11 also participate

Platelets: Normal function

- Hemostasis
- Non-hemostatic functions
  - Vascular diameter and permeability
    - Release vasoactive products (serotonin and PAF)
  - Inflammation
    - Produce cytokines (eg: IL-1)
    - Interact with neutrophils
    - Phagocytic and bactericidal
    - Facilitate vascular repair

Platelets: Structure

- Phospholipid membrane containing numerous invaginations (canalicular system)
  - This increases membrane surface area and allows for rapid movement of substances out of the platelet

Platelets: Structure

- Cytoskeleton
- Endoplasmic reticulum
- Primary and secondary granules
- Glycogen and mitochondria
- No nucleus

Platelets: Structure

- Major membrane receptors:
  - GP Ib
    - Binds to immobilized von Willebrand factor at sites of vascular injury
    - Binds thrombin to enhance its response

Platelets: Structure

- Major membrane receptors:
  - GP IIb-IIIa
    - High affinity binding site for fibrinogen
    - Also binds vWF, fibronectin and vitronectin
Platelets: Activation

- Platelet activators include:
  - ADP
  - Thrombin
  - Platelet activating factor
  - Collagen

Platelets in Primary Hemostasis

- Sequential activities in primary hemostasis:
  - Adhesion
  - Aggregation
  - Secretion
  - Contraction

Platelets in Primary Hemostasis: Adhesion

- Platelets adhere to subendothelial substances at sites of vascular injury

Platelets in Primary Hemostasis: Adhesion

- Coating of subendothelial collagen by von Willebrand factor accelerates adhesion by a receptor-mediated process
  - Platelet GPIb binds to vWF on the damaged surface

Von Willebrand Factor

- A large, multimeric plasma glycoprotein
  - Produced mainly by endothelial cells
    - Secreted constitutively or stored and released upon endothelial activation
    - Also produced by megakaryocytes and is present in platelets
  - Secreted vWF complexes with and stabilizes coagulation factor VIII
Platelets in Primary Hemostasis: Aggregation

- Platelets stick to each other to build up an adequate platelet mass for primary hemostasis.

Platelets in Primary Hemostasis: Aggregation

- Conformational change induces expression of GPIIb-IIIa.
  - Fibrinogen forms bridges between platelets.

Platelets in Primary Hemostasis: Aggregation

- Conformational change induces expression of GPIIb-IIIa.
  - vWF can also enhance aggregation through binding to GPIIb-IIIa.

Platelets in Primary Hemostasis: Secretion

- Conformational change induced by adhesion/aggregation results in granule release.
  - Platelet granules contain numerous preformed substances.

Platelet α-granule content

- Factor V
- Fibrinogen
- Thrombospondin
- Fibronectin
- Transforming growth factor-beta (TGF-beta)
- Epidermal growth factor
- Albumin
- B-Thromboglobulin
- Platelet Factor 4
- Platelet-derived growth factor (PDGF)
- Endothelial cell growth factor (ECGF)

Platelet Dense granule content

- Adenosine
- Triphosphate (ATP)
- Guanidine triphosphate (GDP)
- Calcium
- Serotonin
- Adenosine diphosphate (ADP)
- Guanidine diphosphate (GDP)
- Magnesium
- 5’ hydroxytryptamine
Platelets in Primary Hemostasis: Secretion
- Adhesion also stimulates production of platelet membrane-associated substances involved in clotting
  - Thromboxane
  - Platelet Factor 3 (anionic membrane phospholipids including phosphatidylserine)

Arachidonic Acid Metabolites
- Two major pathways are described:
  - Cyclooxygenase
    - Thromboxane synthetase in platelets generates thromboxane
  - Lipooxygenase
    - Thromboxane mediates vasoconstriction and platelet aggregation

Platelet membrane products
- Anionic membrane phospholipids, including phosphatidylserine (PF3) move from the inner to outer membrane
  - These membranes act as cofactors in coagulation

Platelets in Primary Hemostasis: Secretion
- Secretion is a critical event that:
  - Allows primary hemostasis to proceed
  - Provides products that are essential for initiation and progression of secondary hemostasis
    - The platelet surface provides receptors for coagulation factors and cofactors

Platelets in Primary Hemostasis: Contraction
- Contraction minimizes the size of the primary hemostatic plug
  - Platelet actin and myosin and interplatelet fibrinogen bridges are the major mediators

Platelets in Primary Hemostasis: Contraction
- This occurs during the resolution stages of primary hemostasis
  - Lysis of fibrin formed by secondary hemostasis (fibrinolysis) will occur concurrently
  - Contraction and fibrinolysis minimize the size of the platelet/fibrin plug and initiates vascular repair
Secondary Hemostasis: Coagulation

- Coagulation pathways are activated to result in the production of fibrin
  - Fibrin forms a meshwork that is incorporated into the platelet plug to help seal the damaged area (secondary hemostatic plug)
  - Vascular injury and platelet aggregation are the major stimuli of coagulation
  - A more solid clot for more extensive vascular injury compared to the primary clot

Coagulation

- A highly regulated cascade of reactions that form a variety of products involved in hemostasis
- Participants include:
  - Enzymatic coagulation factors
  - Non-enzymatic co-factors

Enzymatic Coagulation Factors

- Proenzymes
  - Plasma proteins that circulate in inactive forms
    - Produced mainly in hepatocytes
  - Upon activation, they gain the suffix “a”
    - Prekallikrein is an exception, the activated form is called kallikrein
    - These include Factors II, VII, IX, X, XI, XII, XIII, and Prekallikrein

Enzymatic Coagulation Factors

- Production of some proenzyme factors is vitamin-K dependent
  - Factors II, VII, IX, X
  - These factors bind Ca\(^{2+}\) to allow critical interactions with phospholipid membranes

Enzymatic Coagulation Factors

- Vitamin K is a cofactor for carboxylation of glutamate
  - This reaction is necessary to allow Ca\(^{2+}\) to bind to the coagulation factor

Non-enzymatic Coagulation Factors

- Cofactors
  - Non-enzymatic participants that are necessary for enzymatic coagulation reactions
  - Cofactors include Factors I, III, V, VIII, High Molecular Weight Kininogen, Ca\(^{2+}\), and phospholipids
  - Ionized free calcium is required
Hemostasis: Coagulation

- Coagulation has been described by several different models:
  - Classical Coagulation pathways
    - Extrinsic pathway
    - Intrinsic pathway
    - Common pathway
  - Integrated model of coagulation

Hemostasis: Extrinsic Pathway

- This pathway is activated by the release of Tissue Factor (TF, Factor III) by damaged endothelial surfaces
  - Also referred to as the Tissue Factor Pathway
- Factor III reacts with Factor VII to cause the activation of Factor X

Extrinsic Pathway participants

- Factor III (Tissue Factor: TF)
  - Cofactor released at sites of vascular injury
    - Released by activated monocytes/macrophages, vascular smooth muscle cells, other extravascular cells and probably activated endothelium
    - A transmembrane cellular receptor
  - Factor VII (Proconvertin)
    - Proenzyme; forms a complex with TF
  - Calcium
    - Cofactor for VIIa

Extrinsic Pathway

- A complex of III, VIIa and Ca\(^{2+}\) is the critical component
  - This activates Factor X of the common pathway
  - It also activates Factor IX of the intrinsic pathway
- Generation of thrombin (via Xa) is a major outcome

Classical coagulation: Extrinsic

Hemostasis: Intrinsic Pathway

- Activation of Factor XII (Hageman Factor) is the key step in the process
  - Factor XIIa initiates the cascade leading to activation of Factor X
  - Factor XIIa also initiates some important non-coagulant pathways
Intrinsic Pathway Participants

- Factor XII (Hageman Factor)
  - Proenzyme, initiates the pathway
- Prekallikrien (Fletcher Factor)
  - Proenzyme, forms complex with XII and HMWK
- High molecular weight kininogen (Fitzgerald factor)
  - Cofactor, circulates in a complex with Factor XI and Prekallikrien

Intrinsic Pathway

- Contact factors (factor XII, HMWK and PK) initiate the pathway by binding negatively charged surfaces, like collagen
  - HMWK circulates in association with PK and Factor XI
  - Binding of HMWK brings reactants into close association on the activating surface
  - XIIa facilitates binding of HMWK to the activating surface

Intrinsic Pathway

- Factor XIa initiates the formation of the Xase complex* by activating Factor IX
  - *A complex of Factors IXa and VIIIa, Ca^{2+} and a phospholipid surface
  - This complex converts Factor X to Factor Xa

Intrinsic Pathway

- Factor XIIa initiates the formation of the Xase complex* by activating Factor IX
  - *A complex of Factors IXa and VIIIa, Ca^{2+} and a phospholipid surface
  - This complex converts Factor X to Factor Xa

Intrinsic Pathway

- Intrinsic coagulation is probably initiated secondary to extrinsic and common pathway activation
  - It propagates and amplifies thrombin formation initiated by the extrinsic pathway
- Intrinsic coagulation likely plays a secondary role in vivo
  - Individuals with deficiencies of Factor XII, HMWK, and PK don't have bleeding tendencies

Intrinsic Pathway Participants

- Factor XI (Plasma thromboplastin antecedent)
  - Proenzyme, activated by XIIa
- Factor IX (Christmas Factor)
  - Proenzyme, activated by XIa
- Factor VIII (Antihemophilic Factor)
  - Cofactor for IXa; circulates non-covalently bound to vWF; it accelerates attachment and localization of coagulation factors
  - Calcium

Other Hageman Factor Pathways

- In addition to initiating intrinsic coagulation activated Factor XII, also:
  - Activates prekallikrein
    - Kallikrein cleaves kininogen into bradykinin
      - Functions of bradykinin include:
        » Increased vascular permeability
        » Vasodilation
        » Extravascular smooth muscle contraction
        » Pain
  - Activates plasminogen
    - Plasmin is a major fibrinolytic agent
    - Plasmin activates complement (plasmin cleaves C3 and C5)
Classical coagulation: Intrinsic

Hemostasis: Common Pathway

- This pathway is initiated by activated Factor X
  - Factor Xa can be generated by both intrinsic and extrinsic pathways
- A major step in the pathway is the conversion of prothrombin (Factor II) into thrombin (Factor IIa)

Common Pathway Participants

- Factor X (Stuart-Prower Factor)
  - Proenzyme, activated by both extrinsic and intrinsic pathways
- Factor V (Proaccelerin)
- Factor II (Prothrombin)
  - Proenzyme, activated by Factor Xa
  - Multifunctional enzyme

Common Pathway Participants

- Platelet Factor 3 (Phosphatidylserine)
  - Surface for localizing coagulation reactions (Factors Xa and IIa)
- Factor I (Fibrinogen)
  - Protein, converted to fibrin by Factor IIa; a positive acute phase protein
- Factor XIII (Fibrin-stabilizing factor)
  - Proenzyme, stimulates cross-linking of fibrin

Hemostasis: Common Pathway

- Prothrombin (Factor II) is converted to thrombin by the prothrombinase complex
  - This complex consists of Factors Xa and Va, and calcium on a phospholipid surface

Common Pathway: Thrombin

- Thrombin activities include:
  - Cleavage of fibrinogen to fibrin
  - Activates Factor XIII
  - Activates cofactors V and VIII
    - Va amplifies the common pathway
    - Enhances XI activation
    - Activates protein C when bound to thrombomodulin
Common Pathway: Thrombin

- Thrombin activities include:
  - Activates thrombin activatable fibrinolysis inhibitor through interaction with thrombomodulin
  - Platelet activation
  - Effects are concentration-dependent
    - High thrombin concentrations destroy rather than activate Factors V and VIII
  - Inhibition of fibrinolysis

Secondary hemostasis

- Fibrin formed by coagulation combines with platelets to form a firm, secondary hemostatic plug

Hemostasis: Integrated Model

- There are many points of interaction between each of these classical pathways
- A web-like integrated model may more appropriately demonstrate the integration and amplification that occur in vivo

Common Pathway

- Fibrin monomers cleaved by thrombin from fibrinogen self-polymerize
  - Factor XIII helps to cross-link and stabilize the fibrin polymers

Classical coagulation: Common
Hemostasis: Integrated Model

• Key points of integration:
  – TF-VIIa activates X (common) and IX (intrinsic)
  – Thrombin-initiated activation of Factors V, VIII and XI amplifies intrinsic and common pathways
  – Activation of extrinsic Factor VII by Factors XIIa and IXa and kallikrein

Hemostasis: Fibrinolysis

• Dissolution of clots is important to maintain flow and fluidity of blood through the damaged area
  – It is activated simultaneously with coagulation
• Plasmin is the major mediator of fibrinolysis

Fibrinolysis Participants

• Tissue plasminogen activator (T-PA)
  – Produced by activated endothelium
  – Binds to plasminogen-fibrin complex to activate plasminogen
    • Most active when attached to fibrin clot
• Urokinase
  – Present in plasma
  – Activates plasminogen in the fluid phase
• Fibrin Degradation Products; FDPs
  – Biologically active fibrin fragments generated during fibrinolysis

Fibrinolysis Participants

• Factor XIIa/HMWK/Kallikrein
  – Activated contact group coagulation factors cleave plasminogen to plasmin
• Plasmin
  – Derived from plasminogen
  – Plasmin degrades fibrin, fibrinogen, Factors Va and VIIIa, vWF, HMWK and other pro-thrombotic factors (eg- Factor XIII)
  – Plasmin also activates Factor XII and the complement system

Hemostasis: Fibrinolysis

• Fibrinolysis must be well balanced and timed:
Hemostasis: Fibrinolysis

- If excessive or too rapid, the clot may degrade before vascular repair occurs
- If minimal or too slow, clot persistence may lead to permanent vessel alteration and reduced blood flow

Fibrinolysis: FDPs

- Fibrin degradation products produce an anti-thrombotic, pro-hemorrhagic state
  - Major fragments include X, D, Y, and E
  - They impair platelet function
  - They compete with fibrinogen for binding sites on thrombin and platelets
  - They interfere with fibrin polymerization
  - They increase during coagulation, DIC, inflammation, hemorrhage, or decreased clearance due to liver or kidney disease

Hemostasis: Regulation

- Hemostasis is a fine balance between pro- and anti-clotting mechanisms
- A major function of regulatory pathways is to confine hemostasis to only those locations it is needed

Hemostasis: Regulation

- Factors that regulate hemostasis include:
  - Depletion of activated coagulation factors
  - Clearance of activated coagulation factors
    - Most activated factors are complexed by inhibitors and then cleared from the circulation by hepatocytes or the mononuclear/phagocyte system
  - Inactivation of activated coagulation factors or products

Hemostasis: Regulation

- Coagulation inactivators/inhibitors include:
  - Antithrombin (Antithrombin III)
  - Protein S: Protein C: Thrombomodulin
  - Tissue Factor Pathway Inhibitor
- Fibrinolytic inactivators/inhibitors include:
  - Plasminogen activator inhibitor -1
  - Antiplasmins
  - C1 inhibitor

Antithrombin

- Antithrombin is the major circulating anticoagulant
  - It is a serine protease with a wide range of activity
  - It accounts for about 80% of the thrombin-inactivating activity of plasma
**Antithrombin**

- Antithrombin degrades all activated coagulation factors except for Factor VIIa.
  - Its most important function is to degrade Factors IIa, IXa and Xa.
  - It also degrades plasmin and kallikrein to inhibit fibrinolysis, kinin formation and complement activation.

**Antithrombin**

- Antithrombin is most effective in the presence of heparin or heparan sulfate.
  - Heparin/Heparan sulfate causes a conformation change in AT to increase its affinity for thrombin.
  - They act as catalysts which increase the rate of inactivation by 2,000 – 10,000-fold.
  - AT binds heparan sulfate on the surface of normal endothelium and platelets.
  - AT/heparin do not inhibit coagulation factors bound to platelets or fibrin.
    - This allows localized coagulation to continue.

**Inactivators: Protein C**

- Protein C is a Vitamin K-dependent anticoagulant and pro-fibrinolytic agent produced mainly by hepatocytes.
  - Protein C is activated by thrombin, especially when thrombin is bound to thrombomodulin.

**Inactivators: Protein C**

- Thrombomodulin is an endothelial receptor for thrombin.
  - When thrombin is bound to thrombomodulin, it enhances activation of protein C nearly 20,000-fold.
  - Binding of thrombin to thrombomodulin results in loss of the procoagulant properties of thrombin.
  - Thrombin bound to thrombomodulin can’t cleave fibrinogen and is internalized into endothelial cells.

**Protein C**

- When activated Protein C complexes with Protein S on phospholipid surfaces it inactivates Factors Va and VIIa.
Inactivators: Protein S

- Protein S is another Vitamin-K-dependent glycoprotein that is a cofactor with Protein C and can independently inhibit Factors VIIIa, Xa and Va
  - Protein C/S also inhibits plasminogen activator inhibitor
  - This promotes plasmin activation and fibrinolysis

Inactivators: Tissue Factor Pathway Inhibitor

- TFPI circulates in plasma and platelets
  - Produced by endothelium, smooth muscle monocytes/macrophages and hepatocytes
- Complexes with TF-VIIa-Xa on the endothelial surface to inhibit subsequent Factor X activation

Coagulation inactivators

Fibrinolytic Inactivators

- Plasminogen activator inhibitor - 1
  - Inhibits Tissue plasminogen activator and urokinase to prevent conversion of plasminogen to plasmin

Fibrinolytic Inactivators

- Antiplasmins
  - α₂-antiplasmin is the first to bind and neutralize plasmin
    - There is rapid inhibition of circulating plasmin so fibrinolysis remains localized
  - α₂-macroglobulin binds excess plasmin after α₂-antiplasmin becomes saturated
  - α₁-antitrypsin binds plasmin after α₂-macroglobulin becomes saturated
Antiplasmins

- Antiplasmins also have non-fibrinolytic activity
  - $\alpha-2$-macroglobulin can bind to certain activated factors (e.g., thrombin), and entraps but does not inactivate them
  - $\alpha-1$ antitrypsin is a potent inhibitor of Factor Xla
  - $\alpha-1$ antitrypsin and $\alpha-2$-macroglobulin are the major plasma inhibitors of activated Protein C

C1 Inhibitor

- C1 inhibitor modulates the complement, coagulation, kinin, and fibrinolytic pathways
  - C1 inhibitor inhibits:
    - Activation of C1
    - Cleavage of C2 and C4
    - Coagulation factors Xla, and Xla
    - Activation of plasminogen
    - Activation of kallikrein

Hemostasis: Regulation

- The endpoint of hemostasis is when the damaged vessel is repaired and the platelet/fibrin clot contracts and is lysed

Hemostasis and other host responses

- Prothrombotic environments are pro-inflammatory
  - IL-1 and TNF activate endothelium
    - Activated endothelium produces TF and expresses leukocyte adhesion molecules
  - Intrinsic pathway contact factors generate fibrin, activate fibrinolysis, produce vasoactive kinins, and activate complement

Hemostasis and other host responses

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Abnormal Hemostasis

• Hemostasis is a complex balance that once disturbed, can lead to various problems

Abnormal Hemostasis

• Inadequate hemostasis can lead to hemorrhage
• Excessive or inappropriate hemostasis can lead to thrombosis

Abnormal Hemostasis: Hemorrhage

• Hemorrhage is the loss of blood from the vessel into extravascular sites
  – By rhexis: Active blood loss due to tears or rents in the blood vessel
  – By diapedesis: Passive blood loss through endothelial gaps

Hemorrhage: Causes

• Vascular injury
• Platelet disorders
• Coagulation disorders

Hemorrhage: Vascular Injury

• Any disruption of the endothelium or blood vessel wall will result in hemorrhage
• Hemostatic processes are activated to attempt to control the hemorrhage

Causes of Vascular Injury

• Trauma
• Inflammation (Vasculitis)
  – Infectious (Bacteria, viruses)
  – Non-infectious (Immune-mediated)
• Secondary invasion
  – Inflammatory or neoplastic
• Necrosis
  – Toxins
  – Infectious agents (viruses)
• Endothelial degeneration
  – Endotoxin
Hemorrhage: Platelet disorders

- Abnormal platelet function (Thrombocytopenia)
- Platelet deficiency (Thrombocytopenia)
  - Decreased production
    - Bone marrow injury or suppression
  - Excessive utilization
    - Widespread injury or Disseminated Intravascular Coagulation (DIC)
  - Premature destruction
    - Damage due to viruses or other infectious agents
    - Immune-mediated

Abnormal Platelet function

- Most platelet function defects are associated with an inability to adhere or aggregate at a site of injury
- Other functional defects can affect granule content or the degranulation process

Causes of Thrombocytopenia

- Inherited problems of adhesion and/or Aggregation include:
  - GpIb deficiency
    - Bernard-Soulier syndrome of humans
  - Defective GpIIb and GpIIia
    - Glanzmann’s thrombasthenia of humans
    - This has been rarely reported in Otterhounds and Great Pyrenees dogs and horses
      - The Ca+2 binding domain of GpIIb is defective

Von Willebrand Disease

- Most common inherited bleeding disorder of dogs
  - Corgi, Doberman Pinscher, German Shepherd Dog, German Shorthaired and Wirehaired Pointers, Golden Retriever, Shetland Sheepdog, and Standard Poodle are most commonly affected

Causes of Thrombocytopenia

- Inherited problems of adhesion and/or Aggregation include:
  - von Willebrand Factor deficiency

Von Willebrand Disease

- Abnormal primary hemostasis due to a functional deficiency of vWF
  - Platelets do not efficiently bind to damaged endothelium
- Signs include mucosal hemorrhage, bruising and prolonged bleeding
Von Willebrand Disease

- There are three major forms of vWD
  - Type 1
    - Most common form
    - All multimeric forms of vWF are present, but in decreased concentrations
    - Affects multiple dog breeds
    - Variable bleeding

- Type 2
  - Rare
  - Decrease in large multimeric forms of vWF
  - German shorthair and wirehair pointers
  - Bleeding is severe

- Type 3
  - All multimeric forms are absent
  - Bleeding is severe
  - Chesapeake Bay Retrievers, Scottish Terriers, Shetland Sheepdogs

Causes of Thrombocytopathy

- Inherited problems of granules include:
  - Platelet degranulation defects
    - Abnormal synthesis or release of granule products has been reported in Simmental cattle, dogs (Spitz, Basset hound, American foxhounds), cats and fawn-hooded rats
    - Some of these are due to Ca\(^2\) diacylglycerol guanine nucleotide exchange factor I mutations
  - Chediak-Higashi syndrome
    - Defective platelet storage of ADP
    - Occurs in Aleutian mink, cattle, Persian cats, killer whales

- Some other inherited problems of platelet function include:
  - Cyclic hematopoiesis
    - Grey Collie
  - Platelet procoagulant defect
    - German Shepherd
  - Dense-granule storage pool disease
    - American Cocker Spaniel

- Acquired platelet function problems include:
  - Drugs
    - Anti-inflammatories, anesthetics and antibiotics
  - Uremia associated with renal failure
  - Increased FDPs
  - Hepatic disease
  - Immune-mediated thrombocytopenia
  - Megakaryocytic neoplasia
  - Infection
    - BVDV and FeLV
Acquired Platelet Dysfunction

• Antiplatelet drugs
  – Aspirin
    • Irreversible inhibition of the cyclooxygenase pathway of arachidonic acid metabolism in platelets
    • Thromboxane A2 synthesis is inhibited
  – Thienopyridine derivatives (clopidogrel)
    • Inhibits dense granule degranulation by blocking ADP interactions with platelets

Acquired Thrombocytopathy

• Increased FDPs
  – FDPs compete with fibrinogen for platelet binding sites and inhibit platelet aggregation
  – FDPs bind to platelet GP IIb-IIIa sites, preventing fibrinogen binding and cross-linking between adjacent platelets
• Uremia associated with renal failure
  – Urea and other nitrogenous compounds (eg: guanidinosuccinic acid and phenols) contribute to decreased platelet aggregation
  – FDPs can be elevated with uremia

Decreased platelet numbers

• Adequate numbers of platelets are necessary for successful response to vascular injury
  – For mild, localized injury, an animal may be slightly thrombocytopenic and still respond adequately
  – For severe, widespread injury, even an animal that originally had adequate platelet numbers may rapidly deplete the number necessary for a successful response

Causes of Thrombocytopenia

• Decreased production
  – Myelophthisis
    • Bone marrow neoplasia, myelofibrosis
  – Chemicals
    • Estrogen, bracken fern, trichothece mycotoxins
  – Drugs
    • Chloramphenicol, sulfonamides, phenylbutazone
  – Radiation and chemotherapy

Causes of thrombocytopenia

• Increased destruction
  – Immune mediated
    • Primary
    • Secondary
      – Infectious agent or chemical (drug)-induced (various antibiotics and anti-inflammatories)
  – Infection
    • BVD virus, canine distemper virus, canine parvovirus, Ehrlichia sp., FIV, FeLV, EIA, Anaplasma sp., Histoplasma capsulatum, Babesia sp.
    • Thrombocytopenia is often associated with endotoxemia
Causes of thrombocytopenia

• Increased consumption
  – Endothelial activation
    • Vasculitis: Infectious agents (RMSF, canine herpes, canine adenovirus, Dirofilaria)
    • Endocarditis
  – Localized intravascular coagulation
    • Vascular neoplasia, hemorrhage, thrombosis
  – Disseminated Intravascular Coagulation
    • Endotoxemia, shock

Hemorrhage: Coagulation Disorders

• Inherited deficiency of coagulation factors
• Acquired coagulation defects
  – Decreased production due to liver disease
  – Vitamin K antagonism or deficiency
    • Rodenticides, sweet clover poisoning, biliary or bowel disease
  – Increased use (consumption; DIC)
  – Inhibition of coagulation factors
    • Heparin, FDPs, antiphospholipid antibody, antibody to coagulation factors

Causes of Coagulation Disorders

• Inherited Coagulation Factor Deficiencies in animals include:
  – Extrinsic pathway
    • Factor VII
  – Intrinsic pathway
    • Prekallikrein and Factors XII, XI, IX, VIII
  – Common pathway
    • Factors X, II, I

Inherited Coagulation Factor Deficiencies: Extrinsic

• Factor VII
  – Dogs, mainly in beagle colonies
    • Also reported in Alaskan malamute, boxer, bulldog, miniature schnauzer
  – Mild, increased bruising

Inherited Coagulation Factor Deficiencies: Intrinsic

• Factor XII
  – Reported in cats and dogs (also marine mammals, birds, reptiles and fish)
    • A periodic random finding in cats
      – Asymptomatic
  – Prekallikrein
    – Miniature and Belgian horses and dogs
    – Mild mucosal bleeding

• Factor XI
  – Cattle (Holstein and Japanese black) and dogs (English springer Spaniels, Kerry blue terriers, Great Pyrenees)
    • Most common inherited coagulopathy of cattle
  – Spontaneous bleeding is rare, most cases are mild but severe hemorrhage can follow surgery or trauma
    • Bleeding is delayed ½ to 4 days after trauma
  – Factor XI has a dual role: sustaining thrombin generation and downregulating fibrinolysis
Inherited Coagulation Factor Deficiencies: Intrinsic

• Hemophilia A
  – Factor VIII deficiency, x-linked recessive
  – Most common inherited coagulopathy in animals
    • Reported in dogs, cats, horses, cattle
    • Best documented in dogs (eg: German shepherd)

Inherited Coagulation Factor Deficiencies: Intrinsic

• Hemophilia A
  – Factor VIII circulates in close association with vWF
    • Factor VIII activity may be slightly decreased in some forms of vWD

Inherited Coagulation Factor Deficiencies: Intrinsic

• Hemophilia A
  – There is considerable variability in the degree of loss of Factor VIII activity
    • Mild (>5% activity): no spontaneous bleeding and usually maintain normal hemostasis
    • Moderate (2-5% activity): Can have serious hemorrhage after trauma, achieving hemostasis is prolonged
    • Severe (<2% activity): Spontaneous bleeding may occur

Inherited Coagulation Factor Deficiencies: Intrinsic

• Hemophilia A
  – Severity of bleeding depends on the degree of deficiency and exposure to trauma and site of hemorrhage
    • Outcome is location dependent (ie subdural vs. subcutaneous hematoma)

Inherited Coagulation Factor Deficiencies: Intrinsic

• Hemophilia B (Christmas disease)
  – Factor IX deficiency, x-linked recessive
  – Reported in dogs and cats
  – Similar signs to Hemophilia A
  – In most cases, Factor IX activity is very low
    • It lacks the variability in activity associated with Factor VIII in hemophilia A

Inherited Coagulation Factor Deficiencies: Intrinsic

• Hemophilia A and B
  – In these conditions the female is an unaffected carrier
  – The male may or may not be affected
  – When a female carrier is mated to an unaffected male, 50% will get a defective X chromosome, so 50% of female offspring will be carriers and 50% of male offspring will be affected
**Inherited Coagulation Factor Deficiencies: Common**

- **Factor X**
  - Rare
  - American cocker spaniels, Jack Russell terriers
  - Asymptomatic (30-70% activity) to mildly severe mucosal bleeding (5-30% activity)

- **Factor II (prothrombin) deficiency**
  - Rare
  - Reported in boxers, otterhounds and English cocker spaniels
  - Hypoprothrombinemia
  - Bleeding can range from epistaxis and umbilical bleeding in neonates to mild mucosal bleeding in young adults

- **Factor I (fibrinogen) deficiency**
  - Uncommon
  - Saanen goats, Bernese mountain dogs, Lhasa apso, Vizsla and Collie
  - May occur as afibrinogenemia, hypofibrinogenemia or dysfibrinogenemia (abnormal fibrinogen)
  - Hemorrhage can range from severe (afibrinogenemia in goats) to mild

- **Combined vitamin-K-dependent factors**
  - Factors II, VII, IX and X are deficient
  - Defective vitamin K-dependent carboxylase decreases affinity for vitamin K
  - Rare: Devon rex cats
  - Bleeding ranges from severe and fatal in kittens to asymptomatic

**Inherited Coagulation Factor Deficiencies: Combined**

- Combined vitamin-K-dependent factors
  - Factors II, VII, IX and X are deficient
  - Defective vitamin K-dependent carboxylase decreases affinity for vitamin K
  - Rare: Devon rex cats
  - Bleeding ranges from severe and fatal in kittens to asymptomatic

**Inherited Coagulation Factor Deficiencies**

- Many deficiencies except for Hemophilia A and B are autosomal recessive (or incomplete penetrance)
- The degree of severity varies based on the inheritance pattern
  - Homozygous animals typically have 5-10% enzyme activity
  - Heterozygous animals typically have 40-60% enzyme activity

**Acquired Coagulation Disorders**

- Decreased Production
  - Extensive liver disease
  - Vitamin K deficiency
- Increased utilization
  - Widespread endothelial injury
  - Severe trauma or burns
  - Disseminated Intravascular Coagulation
- Inhibition of coagulation factors
  - Heparin, FDPs, antiphospholipid antibody, antibody to coagulation factors
Acquired Coagulation Disorders

- Liver disease
  - Decreased production of both pro- and anticoagulant factors
  - Bleeding is uncommon unless liver disease is severe or associated with DIC

- Vitamin K antagonists inhibit conversion of oxidized vitamin K to reduced (active) form
  - Reduced vitamin K is required to interact with Vitamin K-dependent carboxylase
- Antagonists include:
  - Moldy sweet clover
    - bis-hydroxycoumarin
  - Rodenticides
    - Coumarins (eg: warfarin) or indanediones (eg: bromadiolone)
    - Sulfafquinoxaline and other drugs

- Warfarin inhibits recycling of Vitamin K at two thiol-dependent steps

- Vitamin K deficiency can be induced by:
  - Anorexia
  - Enteric antimicrobials
  - Decreased fat digestion or absorption
    - Malabsorption
    - Cholestasis
    - Pancreatic insufficiency

- Increased utilization
  - Widespread endothelial injury
  - Severe trauma or burns
  - Disseminated Intravascular Coagulation

- Inhibition of coagulation factors
  - Heparin, FDPs, antibody to coagulation factors
Hemorrhage: Morphology

• Red irregular foci in tissues characterized by extravascular erythrocytes
• Classifications of hemorrhage
  – Petechia
  – Ecchymosis
  – Suffusive

Petechial Hemorrhage

McGavin and Zachary: Mosby

Hemorrhage Morphology

• Petechia
  – Pinpoint (1-2 mm) hemorrhage usually associated with mild injury and diapedesis
• Ecchymosis
  – Medium (2-3 cm) hemorrhage associated with more severe vascular injury
• Suffusive
  – Large localized hemorrhage (eg: paintbrush hemorrhage)

Ecchymotic Hemorrhage

Noahs Archive

Suffusive Hemorrhage

McGavin and Zachary: Mosby

Hemorrhage: Morphology

• Hemorrhage into body cavities
  – Hemopericardium
  – Hemothorax
  – Hemoperitoneum

McGavin and Zachary: Mosby
Hemorrhage: Morphology

• Hemorrhage into tissue or interstitium
  – Hematoma
  • An extravascular coagulum of blood

Hemorrhage Pathogenesis

• Petechia and ecchymoses
  – Platelet problems (e.g., thrombocytopenia), or mild vascular injury
• Suffusive
  – Coagulation factor problems or more severe vascular injury
• Hematoma and bleeding into body cavities
  – Coagulation factor problems or severe vascular injury

Hemorrhage: Significance

• Hemorrhage can be insignificant to life-threatening
• Factors influencing clinical outcome:
  – Location
    • Vital vs. non-vital tissues or organs
  – Volume
    • Loss of large blood volumes can lead to shock
  – Rate of loss
    • Slow rates of loss can have some compensation

Abnormal hemostasis: Thrombosis

• Thrombosis is the formation of a solid mass of blood components within a blood vessel or the heart
• Thrombosis is a reflection of excessive or inappropriate hemostasis

Thrombosis: Causes

• A shift in the normal hemostatic balance towards thrombosis
  – Endothelial activation/injury
  – Platelet activation
  – Coagulation pathways activated
  – Stasis
  – Decreased fibrinolysis
  – Abnormal anti-coagulant proteins

Thrombosis: Causes

• “Virchows Triad”
  – Alterations in blood vessels
  – Alterations in blood flow
  – Alterations in blood coagulability

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### Thrombosis: Alterations in blood vessels

- **Endothelial injury**
  - Trauma
  - Chemical injury
  - Drugs
  - Inflammation
  - Immune reactions
  - Toxins
- **Normal endothelium is anti-thrombotic**
  - Platelets do not adhere and coagulation is not activated

- **Endothelial injury can be caused by:**
  - Viruses (eg: Canine adenovirus-1)
  - Bacteria (eg: Salmonella)
  - Fungi (eg: Aspergillus)
  - Nematode parasites (eg: Dirofilaria)
  - Immune-mediated vasculitis
  - Endotoxin
  - Vitamin E/Selenium deficiency
  - DIC

#### Thrombosis: Alterations in blood flow

- **Decreased blood flow or stasis**
  - Blood viscosity increases
  - Endothelium/blood component interactions increase
  - Decreased clearance of activated factors
  - Decreased local tissue oxygenation

- **Turbulent blood flow**
  - Enhances endothelial/blood component interactions

- **Altered blood flow can be caused by:**
  - Gastrointestinal displacement (eg: gastric or intestinal dilation and volvulus)
  - Cardiac disease (eg: Cardiomyopathy)
  - Aneurysm (eg: Strongylus vulgaris)
  - Hypovolemia (eg: shock, diarrhea)

#### Thrombosis: Alterations in blood coagulability

- **Hypercoagulability reflects an increase or decrease in concentrations of activated hemostatic proteins**
  - Coagulation factors or coagulation inhibitors
  - This most commonly occurs due to increased activation or decreased degradation of pro-coagulant factors
- **Enhanced platelet activity can also contribute**
**Thrombosis: Alterations in blood coagulability**

- Hypercoagulability can be caused by:
  - Antithrombin deficiency
  - Hepatic disease
  - Pregnancy
  - Nephrotic syndrome/uremia
  - Anti-phospholipid antibodies
  - Endocrine disease
    - Diabetes mellitus, hyperadrenocorticism, hypothyroidism
  - Neoplasia

**Antithrombin Deficiency**

- Causes of deficiency include decreased production due to liver disease, increased loss due to renal disease or enteropathy (protein-loosing nephropathy or enteropathy)
- Results in a pro-thrombotic state
  - Decreased inactivation of activated factors

**Arterial Thrombi: Morphology**

- These are generally pale and firm
- They consist of alternating layers of fibrin and platelets
  - Red blood cells are washed away due to rapid blood flow
- They often have a head (attached to the endothelium) and a tail that grows downstream

**Thrombosis: Categories**

- **Arterial Thrombi**
  - These form in arteries in association with rapidly flowing blood
- **Venous Thrombi**
  - These form in veins in association with slow moving blood
- **Cardiac Thrombi**
  - These form in the heart chambers or on the heart valves
Venous Thrombi: Morphology

- These are dark red and gelatinous
- They consist of fibrin and platelets intermixed with erythrocytes
- Often occlusive and grow upstream from the point of origin
- They look similar to a postmortem clot

Cardiac Thrombi: Morphology

- Mural cardiac thrombi form in the heart chambers
  - These often mold to the outline of the chamber
- Valvular cardiac thrombi form on the heart valves
  - These are pale and irregular, and are often associated with infection of the valve

Thrombus: Outcomes

- Lysis
- Propogation and Obstruction
- Embolism
- Organization

Thrombus: Lysis

- The thrombus is removed by the dissolution of the fibrin matrix (fibrinolysis) and the platelet plug (thrombolysis)
  - Plasmin is a major participant in the process
- This is most common and efficient with new or small thrombi
- Large more mature thrombi are not easily lysed
**Thrombus: Propagation and Obstruction**
- The thrombus grows until it obstructs the vessel lumen
- This is most common with venous thrombi
  - Rapid blood flow past arterial thrombi makes total obstruction more difficult, particularly in larger vessels
- Dependent tissue is often deprived of oxygen

**Thrombus: Embolism**
- Embolism occurs when a thrombus or portion of a thrombus breaks loose into the circulation and lodges in another blood vessel
- This can occur with arterial, venous and cardiac thrombi
- The embolus can damage and occlude the vessel that it lodges within

**Thrombus: Organization**
- This is the process of resolution and healing for large thrombi that can not be lysed
- Organization reduces the size of the thrombus and converts it to a fibrous scar

**Thrombus: Organization**
- Process:
  - Endothelium grows over the surface of the thrombus
  - Capillaries grow into the thrombus at it's point of attachment
  - Macrophages and fibroblasts enter the site to remove debris and produce collagen
  - New blood vessels can grow into and through the organizing mass (recanalization)
Thrombus: Organization

Thrombus: Significance

• The most significant result of thrombosis is ischemia and infarction
  – Thrombi are the most common cause of infarction
• Clinical significance of thrombi depend on their size, location and type
  – Large thrombi tend to be occlusive and more serious
  – Thrombi in tissues with poor collateral circulation are more serious
  – Venous thrombi tend to be occlusive

Significance: Infarction

Pulmonary infarction
Renal infarction

Hemostatic dyshomeostasis:
Disseminated Intravascular Coagulation

• DIC is a profound disruption of hemostasis
• The major stimulus is widespread (systemic) vascular injury
  – This can occur as a primary event
    • Infectious agents or toxins that causes widespread vascular injury
  – It commonly occurs as a terminal event in shock

Disseminated Intravascular Coagulation

• Causes of DIC include:
  – Necrosis
  – Heat stroke
  – Neoplasia
  – Endotoxemia/septicemia
  – Pancreatitis
  – Hepatic disease
  – Venoms
  – Trauma
  – Burns
Disseminated Intravascular Coagulation

- The fundamental change is accelerated or unbalanced coagulation
  - There are elevated levels of both procoagulant and fibrinolytic substances
- Thrombin plays a central role in DIC
  - It activates platelets and coagulation factors
  - It activates fibrinolysis

- The widespread nature of the response results in rapid consumption of hemostatic proteins
  - Consumption coagulopathy
- Widespread, uncontrolled hemorrhage occurs in later stages
  - There are inadequate platelet or coagulation factors available to seal up the injured areas
  - There are increased levels of FDPs and other degradation products

DIC: Significance

- DIC is a life-threatening and rapidly progressing event
  - It is one of the most dramatic examples of dyshomeostasis in animals

DIC: Morphology

- There are subclinical to severe hemorrhages
  - Often large, widespread hemorrhages
- Shock
- Organ failure
  - Due to thromboembolism or hemorrhage