Hemostasis I and II

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Lecture Outline

I. Introduction
II. Normal Hemostasis
III. Components of the Hemostatic System
    A. Role of Endothelium
    B. Platelets
    C. Coagulation System
    D. Fibrinolytic System
IV. Thrombosis
If you compare the two legs, the right leg shows a difference in color, swelling and vascular deficit (gangrene). This is typical of DVT.

Pulmonary Embolism
Note the clots

Traveling Embolus

Thrombus
Embolus
Introduction
The purpose of blood is to carry oxygen and nutrients to various parts of the body.

1. How does blood remain fluid?
2. How does blood clot when a blood vessel is injured to prevent escape of the blood from the vessel?

The answers lie in a complex process which we will study in the next two lectures.

Normal Hemostasis

- Normal hemostasis is the complex process by which ruptured vessels undergo changes which prevent blood loss.
- The major event is the formation of a hemostatic plug that fills the leakage site in the injured vessel.

Hemostasis

- is dependent upon three major entities:
  1. Blood vessel wall: endothelium and sub endothelial substances
  2. Platelets
  3. Coagulation and fibrinolytic systems

* Diseases and pathologic conditions such as cancer, sepsis and congenital coagulation defects may lead to bleeding disorders.
REGULATION OF HEMOSTASIS

- Under normal conditions blood remains fluid and the cellular components of blood, (erythrocytes, leukocytes and platelets) are not activated or physiologically altered.
- Similarly the endothelial cells also remain inert.
- Only in pathologic conditions is the functional state of these components altered.
- Endothelial damage, activation of platelets and release of tissue factor from cells results in thrombogenesis.
- Once a thrombus is formed it can obstruct blood flow and produce an inflammatory response.

Summary of Hemostatic Responses

1. Vasoconstriction
   - occurs immediately and briefly through reflex neurogenic mechanisms.
   - may mediate humoral factors released from endothelium such as endothelin.
   - serves to reduce blood loss.
A. Endothelin Release- Endothelin is a potent vasoconstricting agent released from the endothelial cells in distress.
B. Reflex Vasoconstriction- Release of various mediators.
C. Extracellular Matrix - Contractile fibers

Summary of Hemostatic Responses (continued)

2. Primary hemostasis
   • Platelet adherence
     • The injury damages endothelial cells and exposes subendothelial collagen. Platelets quickly adhere to the collagen and become activated.
   • Activation
     • The platelets change in shape and release chemicals such as adenosine diphosphate, thromboxane A2 and serotonin which recruit additional platelets to the site of injury and promote aggregates to form, resulting in a hemostatic plug.

A. Platelets adhere to the damaged vessels (GP Ib binding to vWF)
B. Platelets undergo shape change, from discoid formation (extending pseudopods)
C. Light granules (alpha) release PF4, PDGF and other proteins. Dense granules (beta) release ADP, Ca2+, histamine, serotonin and epinephrine
D. Recruitment. Activated platelets recruit other platelets
E. Hemostatic plug formation. Several platelets aggregate and form a plug
Summary of Hemostatic Responses (continued)

3. Secondary hemostasis:
   • Simultaneously, tissue factor is released at the site of injury from the endothelial cells which combine with platelet factors to initiate the plasma coagulation cascade ultimately forming thrombin.
   and
   • The coagulation proteins form complexes on the platelet surface utilizing the phospholipids of the platelet membrane.

A. Tissue Factor: Procoagulant released from various cells. Promotes coagulation.
B. Phospholipid complex expression: Surface phospholipids are expressed. Promotes the coagulation process.
C. Thrombin generation: By the activation of coagulation cascade, thrombin is generated.
D. Fibrin polymerization: The formed fibrin is polymerized by Factor XIIIa.

Summary of Hemostatic Responses (continued)


The consolidated platelet-fibrin clot (thrombin) forms a permanent plug which seals the hole in the vessel wall. Erythrocytes and leukocytes become part of the thrombus.
Once the clot is formed, it is subjected to endogenous lysis by fibrinolytic enzymes. Clot size can also be increased due to cellular recruitment. The composition of the clot depends on the vascular sites and the patients own pathophysiologic state. The stationary clot (Thrombus) can also break apart and travel to another location in the vasculature (Embolus).

Components of the Hemostatic System

Role of Endothelium

1. Endothelial cells modulate elements of the hemostasis-coagulation sequence. There are two possible pathways depending on the circumstances.
   A. Antithrombotic effect (normal state).
   B. Prothrombotic effect (response to injured endothelium).

Antithrombotic Effect

a. Antiplatelet effect:
   • Intact endothelium prevents platelets and coagulation proteins from coming into contact with subendothelial collagen.
   • Normal endothelial cells secrete prostacyclin and nitric oxide that prevent platelet aggregation.
Antithrombotic Effect

b. Anticoagulant effect:

• The endothelial cell membrane contains receptors which play an indirect role in anticoagulation.
• Heparin-like molecules, combine with a naturally occurring anticoagulant protein, antithrombin
• Thrombomodulin, combines with thrombin creating a complex that activates protein C.
• The endothelium also secretes protein S which is a cofactor for protein C activation

c. Fibrinolytic effect:

• Endothelial cells also secrete plasminogen activators (t-PA) which promote fibrinolysis.
• Plasminogen is converted to plasmin and dissolves the clot.

Activated protein C mediates proteolytic degradation of Factor Va and VIIIa.
3. Prothrombotic effect:
   • Normal endothelial cells inhibit platelet adherence and prevent blood clotting.
   • Injury causes a loss of these anticoagulant mechanisms.

Prothrombotic Effect

a. Endothelial cells:
   • secrete von Willebrand factor, a protein, which forms a molecular bridge between platelets and sub endothelial collagen.
   • Platelet adhesion to endothelial cells occurs.
Prothrombotic Effect

b. Simultaneously, endothelial cells:
   • Synthesize and secrete tissue factor, which activates the extrinsic sequence of the coagulation cascade.
   • Cytokines released by injured endothelial cells can stimulate cells to synthesize more tissue factor.

Prothrombotic Effect
c. Tissue factor:
   • promotes the generation of thrombin and formation of a clot.
   • Once the clot is formed it traps other cells such as erythrocytes and leukocytes.

Platelets

• Definition: Platelets are discoid, anuclear cells which play a major role in hemostasis.
Platelets

Structure of platelets:
• The plasma membrane contains many **glycoprotein receptors**
• which play a role in the attachment of platelets to subendothelial proteins (via von Willebrand factor)
• inter-adherence between platelets (via fibrinogen) and secretion of substances from intra-cytoplasmic platelet granules.

b. Platelet cytoplasm contains **two types of granules** which contain substances which play a role in hemostasis.

Platelets

(1). Light granules (alpha):
- Contain fibrinogen, fibronectin, coagulation factors V and VIII, platelet factor 4 (heparin-binding chemokine) and growth factors, PDGF (platelet derived growth factor) and TGFβ (transforming growth factor beta).

(2). Dark granules (beta):
- Contain ADP, ATP, ionized calcium, histamine, serotonin and epinephrine.

Platelets

Platelet receptors

i. Glycoprotein IIb/IIIa
ii. Glycoprotein Ib
iii. Thrombin
iv. Serotonin
v. ADP
Function of Platelets

- With vessel injury, circulating platelets are exposed to subendothelial proteins (e.g. collagen, proteoglycans, fibronectin) which causes platelets to undergo three reactions.

Adhesion

- Platelets attach to the subendothelial collagen through a molecular bridge.
- The platelets plasma membrane, glycoprotein receptor (GP Ib) attaches to the von Willebrand factor which in turn attaches to collagen.
- Adhesion is a critical reaction because it prevents the blood flow from dislodging the adherent platelets and unplugging the defect in the vessel wall.
Activation and Secretion

- Activation of platelets is initiated by molecules binding with platelet membrane GPIIb/IIIa receptors.

- Upon activation platelets release other granular content including such substances as coagulation factors, ADP, calcium and thromboxane A2.

- The phospholipid complex is activated when negatively charged phospholipids become exposed on the platelet surface.

- This complex serves as a site on which coagulation factors combine with ionized calcium to activate the intrinsic pathway.
Aggregation

- The release of ADP and thromboxane A2 from activated platelet granules initiates a reaction which serves to recruit, activate and aggregate platelets.
- Serotonin and thromboxane A2 released by platelet granules, vasoconstrict the vessel, decreasing the size of injury, reducing blood flow and the likelihood of the plug detaching from the vessel wall.

Aggregation (cont).

- Simultaneously, thrombin is formed by the activation of the intrinsic pathway (ADP, calcium, coagulation factors, phospholipid complex). Thrombin, ADP and thromboxane A2 accelerate platelet aggregation.
- Thrombin also converts fibrinogen to fibrin. Fibrin surrounds and structurally holds platelets in a secondary (irreversible) hemostatic plug.

Coagulation System

- The blood coagulation system is comprised of a network of proenzymes, which are activated to their functional form.
- Once thrombin is formed, it is capable of converting a soluble, namely fibrinogen into fibrin.
The fibrin clot is stabilized by a transamidase enzyme (XIIIa) and TAFIa.
Coagulation Factors

- The coagulation system is composed of a complex network of proenzymes (zymogens), cofactors, activators and inhibitors.

- Initiation of either the extrinsic or intrinsic pathways results in the formation of thrombin which transforms fibrinogen to fibrin.

- Factor X plays a central role in the generation of thrombin by the intrinsic and extrinsic pathways.

Classification of Blood Coagulation Factors

A. The fibrinogen group
   Factors I, V, VIII and XIII

B. The prothrombin group
   Factors II, VII, IX and X
   All of these proteins contain γ-carboxy glutamic acid which is needed for the binding of calcium.

C. The Contact group
   Factors XI, XII, Fletcher factor (Prekallikrein), Fitzgerald factor (HMW Kininogen).

D. Other Factors
   Protein C, Protein S, Fibronectin
Inhibitors of the Coagulation System

These inhibitors are plasma proteins which are capable of inhibiting the formed serine protease enzymes involved in the regulation of the clotting process.

a. Antithrombin III (AT)
b. Heparin cofactor II (HC II)
c. Tissue factor pathway inhibitor (TFPI)
d. Inhibitors to clotting factors
e. Lupus anticoagulant and antiphospholipid antibodies
f. Antibodies to coagulation factors (rare).

COMPARISON OF THE STRUCTURES OF TISSUE FACTOR WITH TISSUE FACTOR PATHWAY INHIBITOR

Inhibitors of the Coagulation System (cont)

• Antithrombin is a plasma inhibitor which also mediates the anticoagulant actions of heparin.

• Heparin cofactor II is a weak inhibitor of thrombin.

• Tissue factor pathway inhibitor is a potent inhibitor of tissue factor.
The Fibrinolytic System

- The fibrinolytic system is a network of enzymes that are responsible for the dissolution of a formed clot.
- This system is comprised of proenzymes which when activated are converted to their enzymatic forms.
- When activated these enzymes can also facilitate the digestion of fibrinogen.

The Fibrinolytic System

<table>
<thead>
<tr>
<th>Fibrin (clot)</th>
<th>Plasmin</th>
<th>Fibrin split products</th>
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<tbody>
<tr>
<td></td>
<td>Plasmin</td>
<td>(Fibrinolysin)</td>
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Fibrinolytic System

- Plasminogen (profibrinolysis) is converted by into plasmin.
- Several physiologic and pharmacological plasminogen activators can convert plasminogen into plasmin.
- Several natural and acquired inhibitors can oppose plasminogen activation.
- The important inhibitors of the fibrinolytic system are given below
  - Plasminogen activator inhibitor (PAI)
  - $\alpha_2$-antiplasmin
  - $\alpha_2$-macroglobulin
  - Thrombin activatable fibrinolytic inhibitor (TAFI)
Abnormal Hemostasis

- Abnormal hemostasis, thrombosis, is the process by which blood forms a clot within intact blood vessels (vessels which have not ruptured).
- Abnormal hemostasis is a pathologic process that represents the activation of the clotting system when there are no ruptured vessels.

Bleeding occurs when a blood vessel ruptures.

- Hemostasis prevents excessive loss of blood from a ruptured blood vessel.
- Abnormal hemostasis can also result in the loss of blood into surrounding soft tissues, into a body cavity or from the body.
Thrombosis

- Thrombosis can be defined as a pathologic transition of the state of blood from fluidity to non-fluidity.
- The stationary clot (thrombus) may progress and eventually break into smaller pieces which when released into blood circulation are called emboli (embolus).
- Many factors are responsible for the process of thrombogenesis.

Thrombosis

- The thrombogenic mechanisms differ in the arterial and venous systems.
- Such conditions as blood flow, endothelial cell composition, size of the blood vessel and the degree of oxygenation influences this process.

1. Arterial Thrombosis
2. Venous Thrombosis and Pulmonary Embolism
3. Microvascular Thrombosis
Pathogenesis of Thrombosis (Virchow’s Triad)

- The following three major factors contribute to thrombosis.
  1. Injury to endothelium resulting in the release of tissue factor
  2. Alterations in blood composition
  3. Stasis

Hypercoagulable State

- Imbalance of the blood coagulation mechanisms leading to thrombotic transitions.

- Age, smoking, oral contraception and diet also play important roles in contributing to a hypercoagulable state. Thrombotic stroke, myocardial infarction and peripheral arterial thrombosis result from this syndrome.
Molecular Thrombophilia

- Factor V Leiden (APC resistance)
- Prothrombin 20210
- Methylene tetrahydrofolate reductase (MTHFR mutations), hyperhomocysteinemia
- Abnormal Antithrombin (mutant forms)

Acquired Thrombophilias

- Sepsis
- Cancer
- Trauma
- Pregnancy
- Hyperlipidemia
- Drugs

Reference: Basic Pathology (Kumar) 9th Edition, Chapter 3, PP. 79-90
Which of the following coagulation factors is activated by contact with surfaces and is capable of triggering the intrinsic pathway of coagulation?

A. Factor IX
B. Factor VIII
C. Tissue factor
D. Factor XIII
E. Factor XII

Which of the following factors complexes with tissue factor to trigger extrinsic pathway of coagulation?

A. Factor II
B. Factor V
C. Factor VII
D. Factor X
E. Factor XII