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Hematology ~ Platelets

I. Homeostasis refers to the maintenance of the fluid state of the blood. This requires a delicate balance between procoagulation (clot formation), anticoagulation and fibrinolysis (clot dissolution). Dozens of plasma proteins are involved, as well as tissue proteins, blood platelets, and the surfaces of endothelial cells that line vessels.

The Five Phase of Coagulation

Phase I: Vasoconstriction
Phase II: Formation of a Hemostatic Plug
Phase III: Formation of a Blood Clot
Phase IV: Control of Coagulation and Clot Retraction
Phase V: Fibrinolysis

Coagulation disorders can arise from a decrease in number or function of platelets, a deficiency in a coagulation factor, or by enhanced fibrinolytic activity. Defects in any of these components may contribute to hemorrhage or thrombosis (clotting).

Phase I: Vasoconstriction. Damage to vessel walls triggers vasoconstriction and exposes collagen and “tissue factor” to the blood; this triggers a series of events that lead to the formation of a platelet plug at the site of injury.

Phase II: Formation of a Hemostatic Plug. Platelets are circulating, anuclear cells that are fragments of megakaryocytes; they are released from the bone marrow.

Platelets do not possess DNA or protein synthetic capacity. They do contain mitochondria and a variety of granules that contain glycogen, ADP, serotonin (5-HT), and platelet factors that play important roles in vasoconstriction, the formation of a platelet plug, and clotting.

Megakaryocytes typically produce ~ 35,000 platelets/µl blood per day but can produce over 8 times more under stress. Average circulation time is 8 – 12 days with ~ 10% turnover per day.
**Adhesion, Activation and Aggregation.** Circulating platelets bind to the exposed collagen fibers, an interaction that is enhanced by the von Willebrand factor. This interaction stimulates the release of granules containing ADP, serotonin (5-HT), thromboxane A₂ (TxA₂) and “platelet factors”. These compounds mediate further vasoconstriction and the transition to a “sticky” platelet. The released compounds promote further vasoconstriction, platelet activation and platelet aggregation to form a hemostatic plug at the site of injury.
Phase III: Formation of a Blood Clot.

1. Activation. There are 12 plasma proteins, or “factors” involved in coagulation:
   i. The Vitamin K Dependent Factors ~ thrombin* (a.k.a., II), VII*, IX*, X*
   ii. Contact Activation Factors ~ XI and XII, prekallikrein, high-molecular-weight kininogen (HMWK)
   iii. Thrombin Sensitive Factors ~ V*, VIII*, XIII, and fibrin* (a.k.a., I)

Aside: Remember the relationship between kallikrein, bradykinin, angiotensin converting enzyme (ACE) and blood pressure?

**Thromboplastin** is a lab reagent usually derived from placenta that contains phospholipids and tissue factor. This reagent can activate the extrinsic coagulation pathway *in vitro* (PT test)

A derivative, partial thromboplastin, contains the lipids only and is used to activate the intrinsic coagulation pathway *in vitro* (aPTT test).
**Phase IV: Control of Coagulation and Clot Retraction.** The initiation of the coagulation cascade immediately activates proteins involved in anti-coagulation.

**Tissue Factor Pathway Inhibitor**
- TFPI is a reversible inhibitor of thrombin and Xa; The Xa•TFPI complex further inhibits VIIa

**Anti Thrombin (a serpin)**
- AT inactivates several factors, predominantly thrombin and Xa; the inhibitory activity of AT is strongly stimulated by heparin.

**Protein C\* and Protein S\***
- Protein C is activated by the thrombin-thrombomodulin complex; the latter is an integral membrane protein found on the surface of endothelial cells.
- Activated Protein C binds to Protein S and the ProteinC•ProteinS complex inhibits Va and VIIIa

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**Phase V: Fibrinolysis.** After a clot has formed, the underlying problem (mechanical injury, infection, etc.) can be repaired. At some point, the clot must be removed to restore normal blood flow through the vessel; this is the function of the fibrinolytic pathway.

**Plasmin\*** is a protease that degrades fibrin to small, soluble fragments ~ fibrin degradation products (FDP) and ultimately D-dimer\*.
II. Lab Tests

A. Platelet Tests (Phase II)

1. Platelet Count (PLT)*. Normal Range: 150,000 – 400,000 cells/µl

i. **Thrombocytosis** (thrombocytemia) refers to an increase platelet count. It can be triggered by stress or infection and is associated with essential thrombocythemia and chronic myelogenous leukemia.

Clinical consequences are primarily thrombosis (cerebrovascular accidents (CVA), myocardial infarction (MI), deep venous thrombosis (DVT), pulmonary embolism (PE), etc.), microcirculatory disturbances (headache (HA), parathesia), and ironically hemorrhage.

ii. **Thrombocytopenia** refers to a decrease in platelet count below 100,000/µl. It can be associated with aplastic anemia, leukemia, thrombocytopenic purpura, and splenomegaly. Also with radiation therapy and many drugs, notably heparin.

<table>
<thead>
<tr>
<th>Heparin-induced Thrombocytopenia (HIT) and Thrombosis. This results from the development of IgG Abs against the heparin•platelet factor 4 complex (a hapten)</th>
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<tbody>
<tr>
<td>This results in platelet activation and the formation of platelet microparticles, which initiate the formation of blood clots (thrombosis). In addition, the platelets are removed from circulation by the spleen, which depletes circulating platelets (thrombocytopenia).</td>
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"HIT" typically occurs ~ five days after the initiation of therapy. The IgG Abs circulate for about 3 months and patients given heparin within this time period may experience symptoms within hours.

Idiosyncratic drug-induced thrombocytopenia has been observed with sulfonamides, quinine, and chloramphenicol.

Important cut-offs:  

- > 500,000/µl -> risk of thrombosis  
- < 50,000/µl -> risk of echymosis and petechiae  
- < 20,000/µl -> risk of CNS hemorrhage

Platelet transfusion is indicated with PLT < 20,000/µl
2. Mean Platelet Volume (MPV). Normal Range: 7 – 10 fL.

Though available as part of the CBC w/ Dif, it is not routinely utilized. MPV is useful for differentiating hypoproducive vs. hyperdestructive thrombocytopenia; the platelet size is larger during increased platelet synthesis.

3. Platelet Function Tests

i. Bleeding Time (BT). Normal Range: < 10 minutes

This test assesses platelet and capillary function. A “standardized” skin cut is made and the time required to stop bleeding monitored.

Patients with an increased bleeding time and normal platelet count are qualitatively diagnosed as having abnormal platelet function.

ASA may increase BT as much as minutes.

ii. Platelet Aggregation. A partially purified platelet preparation is warmed and aggregation initiated with collagen, ADP, arachidonic acid, or other agents. Aggregation is measured by light scattering.

B. Coagulation Tests (Phase III)

1. Prothrombin time (PT, or protime). Normal Range: 10 – 13 seconds (variable)

PT measures the time required to generate fibrin after the addition of Ca\(^{2+}\) and thromboplastin (phospholipids and tissue factor; see above) to the patient’s serum.

This test assesses deficiencies in the extrinsic and common coagulation pathways (activity of clotting factors VII, X, V, prothrombin (II) and fibrinogen (I)).

PT measurements are variable due to variability in the test reagents (obtained from placenta) and are now normalized as the International Normalized Ratio*:

\[
\text{INR} = \left( \frac{\text{Patient PT}}{\text{Mean Lab PT}} \right)^{\text{ISI}}
\]

where ISI is the International Sensitivity Index, which indicates how a particular batch of tissue factor compares to an international reference standard.
The ISI is usually between 1.0 and 2.0 and is used to determine the clotting tendency of blood, liver damage, and vitamin K status. It is also routinely used to monitor warfarin therapy.

Normal INR ~ 1
Target ~ 2 - 3 for warfarin anticoagulant therapy

2. Activated Partial Thromboplastin Time* (aPTT). \textit{Normal Range} (varies)

aPTT also measures the time required to generate fibrin, but after addition of Ca$^{2+}$, \textit{partial} thromboplastin (phospholipids \textit{without} tissue factor) and an “activator” (silica, diatomaceous earth) to the patients plasma (see above).

This test assesses deficiencies in the \textit{intrinsic} and common coagulation pathways (activity of factors XII, HMWK and prekallikrien, XI, IX, VIII, X, V, prothrombin (II), and fibrinogen (I)).

aPTT is often used to monitor anticoagulation therapy with heparin.

\begin{itemize}
  \item Normal Value ~ 24-36 seconds
  \item Target is 1.5 – 2 times the control value.
\end{itemize}

(note that PT can also increase with high doses of heparin)

The use of \textit{low molecular weight heparins} (e.g., enoxaparin*) precludes routine aPTT monitoring in most cases; exceptions include patients with renal insufficiency and who are severely obese.

Fondaparinux* is a new penta-saccharide that binds to anti-thrombin and inhibits Factor Xa, but not thrombin. It is used to treat DVTs and pulmonary embolisms.

The aPTT can also be used, along with the PT, to test for factor deficiencies:

\begin{itemize}
  \item $\uparrow$ aPTT with normal PT: factors VIII, IX, XI, XII, PK, HMWK
  \item $\uparrow$ aPTT and $\uparrow$ PT: fibrinogen (I), prothrombin (II), V, X
\end{itemize}

An elevated aPTT is also associated with liver dysfunction, vitamin K deficiency, and warfarin therapy; in each case PT is affected earlier and to a greater extent.
3. Activated Clotting Time (ACT). Normal Range: 80 – 130 seconds (variable)

Clotting of whole blood is initiated with an “activator” (celite, glass beads). Probes the intrinsic clotting pathway. Used to monitor heparin therapy during invasive procedures that require moderate to high-dose therapy (i.e., cardiopulmonary bypass surgery).

4. Thrombin Time (TT). Normal Range: 14 – 16 seconds (variable)

This test measures the time it takes for a clot to form in plasma containing anticoagulant, after an excess of thrombin has been added. It is the most sensitive test for fibrinogen deficiency and is also used to monitor fibrinolytic therapy.

5. Fibrinogen (FIBCL). Normal Range: 200 – 400 mg/dL

This assay measures the concentration of fibrinogen in the sample. A high concentration of thrombin is added to a diluted sample of the patient’s plasma and the time required to clot determined; the more fibrinogen in the sample, the shorter the time to clot. This is compared to a reference standard to determine the amount of fibrinogen in the sample.

C. Clot Degradation Tests (Phase V) are use to probe for deficiencies in fibrinolysis.

Plasmin degrades fibrin to fibrin degradation products (FDP) and finally to the D-dimer, which can be detected in the blood.

1. Fibrin Degradation Products (FDP). Normal Range: 2 – 7 µg/mL (varies).

   Note: FDP can increase as a result of fibrinolysis or by degradation of fibrinogen and fibrin in the blood. These products are quantified by immuno assay.

2. D-Dimer*. Normal Range < 200 ng/mL (varies).

D-dimers are peptides that result from fibrin clot degradation. Increases above normal indicate coagulopathy due to DVTs, pulmonary embolism, obstetrical complications (pre-eclampsia), or organ transplant rejection.

D-dimer is quantified by ELISA assay.
III. Effects of Drugs on Coagulation

A. Desired Therapeutic Effects.

1. Heparin* binds to anti-thrombin and the complex strongly inhibits thrombin (II) and Xa (see above). This leads to an increase in aPTT and to a lesser extent PT. The target value is 1.5 – 2.5 times the control value.

2. Warfarin* and Vitamin K*

Factors II (thrombin), VII, IX and X contain multiple glutamate (Glu) residues that have been covalently modified to $\gamma$-carboxyglutamate (Gla) residues (post-translational modification).

These dicarboxylate side chains tightly bind Ca$^{2+}$, which alters their conformation to one that efficiently binds to phospholipids at the platelet surface, thus anchoring the proteins to the damaged site.

Vitamin K is an essential co-factor for $\gamma$-glutamyl carboxylase:

Warfarin inhibits vitamin K reductase. This drug is used clinically as an anticoagulant. Warfarin is also used as a rat poison!

The INR is most often used to monitor therapy. The target for most conditions is an INR ~ 1.5 – 2.5 times the control value.

Vitamin K can be used to counteract the effects of warfarin (it obviates the requirement for KO-reductase). Administration causes a decrease in INR.
3. Aspirin*, clopidogrel* and dipyramidol decrease platelet activity.

Aspirin covalently inactivates COX.

Inactivation of platelet COX1 abrogates TxA2 synthesis and inhibits platelet aggregation.

Inhibition of endothelial COX1 and COX2 shuts down prostacyclin (PGI₂) synthesis, which has the opposite effect.

Platelet COX1 is inactivated at lower ASA concentrations and cannot synthesize more enzyme; in contrast, endothelial COX1/COX2 can be regenerated.

This is the rationale for low-does aspirin therapy post MI and to prevent strokes.

Non Steroidal Anti-Inflammatory Drugs (NSAIDs) reversibly inhibit COX*.

There are presently three cyclooxygenase isoenzymes known, most relevant are COX-1 and COX-2; COX-1 is a constitutive enzyme found in most mammalian cells, including platelets. In contrast, COX-2 is an inducible enzyme normally present in low concentrations.

Activated macrophages and other cells strongly express COX2 at sites of inflammation; selective COX-2 inhibitors target the enzyme primarily responsible for inflammation and should have less systemic side effects (e.g., GI bleeding).

B. Effects of Cyp450 Inducers. Many drugs can induce P450s that metabolize warfarin (and other drugs) to inactive products. This decreases the steady state levels of warfarin and a decrease in therapeutic effect, observed as a decrease in PT and INR.

Many anticonvulsant drugs, especially carbamazepine and phenytoin*, are cyp450 inducers*.

A major problem is the addition or discontinuance of inducer drugs while patients are on warfarin, especially if the patient does so without informing the pharmacist. This could result in a major change warfarin levels.
C. **Drugs That Inhibit Anticoagulation**

1. *Drugs that decrease vitamin K levels.* These include laxatives, which interfere with vitamin K absorption and long-term antibiotic therapy, which can alter gut flora and decrease vitamin K synthesis by the bacteria.

   In both cases, a decrease in vitamin K results in an increase in INR and a risk of bleeding.

2. *Drugs that inhibit the metabolism of warfarin.* These include azole antifungal agents, macrolide antibiotics, gemfibrozile, some herbal medications and *grapefruit juice*.
   They act as competitive inhibitors of the P450 enzymes.

3. *Drugs that increase the bioavailability of warfarin.* This results from displacement of warfarin from protein binding sites. These are typically acidic drugs taken in relatively high doses (*penicillins*, fibrates).
**Hematology, Platelets Study Guide**

**Terms You Need to Know**

D-Dimer  
Hemorrhage  
Thrombosis

You should be prepared to describe the relationship between a bone megakaryocyte and a circulation blood platelet.

You should be prepared to describe the events leading to the formation of a platelet plug. What initiates the process and how, in a general sense, do the platelets respond? Unless otherwise indicated, you do not need to memorize specifically what molecules are released during platelet activation but you should know the general outcome.

You should be prepared to discuss the essential features of the clotting cascade. What are the clotting factors and how are they activated?

Be prepared to describe the essential features of the intrinsic (contact) and extrinsic (tissue factor) clotting cascade. What specifically about a damaged vessel initiates each of these processes?

Be prepared to describe the formation of a “hard” fibrin clot *starting from fibrinogen*. What enzymes (there are two) are required to make a hard clot and how are they activated?

Be prepared to describe the proteins involved in controlling the coagulation cascade (there are three inhibitory pathways). How are they activated? What is heparin and what is its role in the process? What clotting factors are inhibited?

Be prepared to describe fibrinolysis. What enzyme is directly involved? How is it activated (there are two proteins)? What are fibrin degradation products? How can you differentiate degradation of a hard fibrin clot vs. a soft fibrin clot and/or circulating fibrinogen?

Be prepared to describe the use of PT and aPTT to probe the extrinsic vs. intrinsic clotting pathways and the TT test for serum fibrin. How are the tests performed? What is the difference between thromboplastin and partial thromboplastin; of what significance is this with respect to the PT and aPTT tests? Which test is most appropriate to monitor warfarin vs. heparin therapy?

Be prepared to describe the problem with the PT test and the relevance of International Normalized Ratio. You do not need to know how to calculate INR, but should understand and be prepared to discuss why it is used.
Be prepared to describe the mechanism of action of heparin. What is the difference between unfractionated heparin and the low molecular weight heparins? How does fondaparinux fit into the picture?

Be prepared to discuss the mechanism of heparin-induced thrombocytopenia and oddly, thrombosis.

Be prepared to discuss the relationship between vitamin K and warfarin and blood coagulation. What enzyme is involved and why is it associated with coagulation?

Be prepared to discuss the role of low dose aspirin in the setting of MI and stroke. What is the mechanism, specifically with respect to platelets vs. endothelial cells.

Be prepared to clearly describe how drugs can inhibit or stimulate the activity of P450 enzymes can increase or decrease the metabolize warfarin (or any drug for that matter). What would their effect be on INR, aPTT and TT tests?