بسم الله الرحمن الرحيم

Physiology

Final | Lecture 11

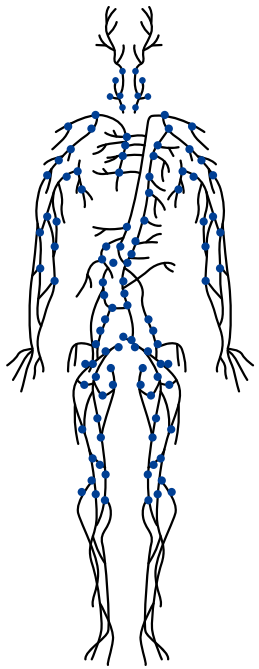
Hemostasis & Blood Coagulation (pt.3) Clinical lab & Tests

﴿ وَقُل رَّبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا ﴾

ربنا آتنا من لدنك رحمة وهيئ لنا من أمرنا رشدًا

Written by: Abdulrahman Khw
Zaid Bassam

Reviewed by:
Mahmoud Aljunaidi



Clinical Perspective Physiology/Hemostasis

Ebaa M Alzayadneh, PhD

Associate Professor of Physiology

Causes of Excessive Bleeding

- Any **clotting factor deficiency** leads to deficient hemostasis and prolonged bleeding.

❖ **Most commonly:**

- Hepatocellular disease.
- Vitamin K deficiency – slide 4.
- Hemophilia – slide 6.
- Low platelet count (thrombocytopenia) – slide 8.

Vitamin K Deficiency

- Vitamin K **is essential to carboxylate glutamic acid** in five important clotting factors; therefore, it serves as an important **cofactor** for the synthesis of:
  - Prothrombin, in the liver, and factors VII, IX, X, and protein C (1972).
- In this process vitamin K is **oxidized and inactivated**.
- Vitamin K epoxide **reductase** complex 1 (**VKOR c1**) **reduces vitamin K and reactivates it.**
- Both **vitamin K production** in the small intestine and the activity of **the enzyme VKORC1** are essential for the normal **synthesis of prothrombin** and other vitamin K–dependent clotting factors.

Vitamin K

- **Produced** in the intestine by **bacteria**.
  - Vitamin K deficiency is **rare in adults** but relatively **common in newborns**, as their intestinal flora is **not yet fully developed.**

❖ Causes of vitamin K deficiency:

- It's **Fat-soluble**: malabsorption of fats can lead to deficiency.
  - Remember fat-soluble vitamins: A, K, D and E.
- **Lack of bile production or delivery** can cause fat malabsorption and vitamin K deficiency
- In patients with **liver or biliary disease** such as viral diseases and hepatitis, vitamin K can be injected 4-8 hours before surgery.

Hemophilia

- One of the most studied **bleeding disorders**, it has two types both leading to excessive bleeding:
  1. **Hemophilia A** – Deficiency of factor **VIII,** previously called **anti-hemophilic** factor.
     - 85% of hemophilia cases
     - **It's more common in males,** 1 / 10,000 males.
  2. **Hemophilia B** – Deficiency of factor **IX**
     - 15% of cases
     - About 1 / 60,000 males.
- Both **impair Intrinsic Pathway activation,** to which factors VIII and IX are needed.
- Both genes are on the **X chromosome.** Because males only get **one copy** of the X chromosome, they are **more susceptible** to developing the disorder.
- Clinically: **excessive bleeding after minor trauma**.

Factor VIII Deficiency

- Factor VIII has a partner - **von Willebrand factor** (vWF).
- Deficiency of factor VIII causes hemophilia A. Which results in excessive bleeding upon injury, treat bleeding with factor **VIII replacement.**
- Deficiency of Von Willebrand factor causes **von Willebrand disease**, which resembles **decreased platelet function**.
- **Treatment** is always with the **deficient factor** or – in emergencies- with **fresh frozen plasma**, which contains both factors, factor VIII and vWF.

The Underlined word is hyperlinked

Thrombocytopenia

- Definition: **Low** numbers of **platelets**.
- Bleeding from **small venules or capillaries,** unlike hemophilia, where bleeding occurs in large vessels.
- Causes Petechiae, thrombocytopenic purpura*.
  - **Petechiae**: Tiny red or purple spots on the skin caused by minor capillary bleeding.
- Often idiopathic, unknown cause.

❖ Severity based on platelets counts:

- **< 50,000** platelets / µL – usually **modest bleeding.**
- **< 10,000** platelets / µL – **life-threatening.**

- Treated with **platelet infusions**, which is effective for 1 - 4 days each time.

* Larger purple patches on the skin due to extensive capillary bleeding under the skin.

Thrombi and Emboli

- An **abnormal/unwanted clot** is a **thrombus**.
- When it dislodges, it **floats** in the bloodstream, therefore it's an **embolus.**

❖ Caused by:

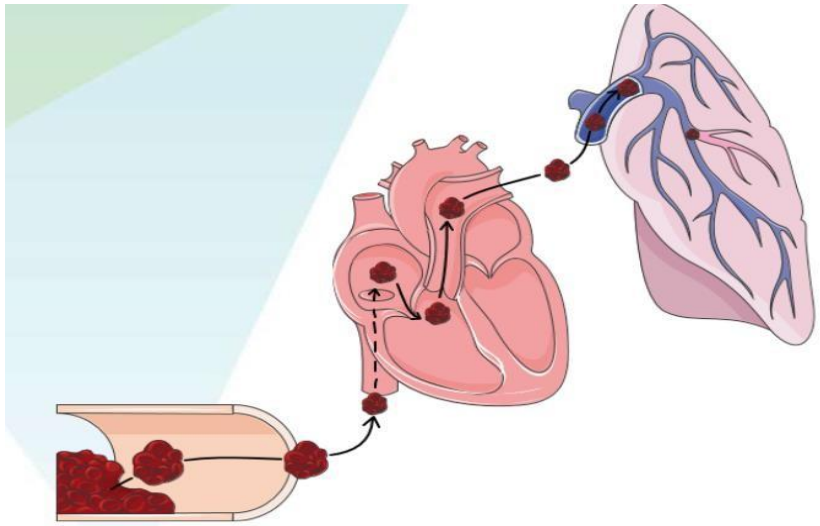
- **Endothelial surface roughening** (e.g. atherosclerosis).
- **Slow blood flow** (e.g. prolonged air travel).

❖ Treatment:

- **tPA**, **t**issue **P**lasminogen **A**ctivator.
  - ➢ A drug that breaks down blood clots quickly by converting plasminogen → plasmin, which dissolves fibrin in the clot.
- **Embolectomy.**

Pulmonary Embolus

- A critical condition that usually occurs from a thrombus in **deep leg veins.**
- **Part of thrombus disengages** (~10% of the time), and travels to the **pulmonary arteries.** Where it can **Occlude pulmonary arteries**.
- If it **blocks the blood supply to a large area of the lung**, it is potentially **fatal.**
- **tPA can be life-saving**, by applying it through **catheters** within one hour to dissolve and remove the clot.



Disseminated Intravascular Coagulation (DIC)

- Another **serious condition** that commonly occurs alongside **septic shock**.
- Occurs in the setting of **massive tissue damage** or **sepsis**.
  - Septic shock triggers wide-spread inflammation and endothelial injury. This can activate the **coagulation cascade**, leading to **DIC.**
- The coagulation cascade can cause a **wide-spread coagulation** in small vessels and the resulting **blockage of blood flow** to tissues may contribute to **circulatory shock.**
- Also, it manifests as **bleeding from multiple sites because of depletion of clotting factors** because of the wide-spread coagulation.

Clinically Useful Anticoagulants

Heparin

- Binds, potentiates **antithrombin III,** an inhibitory protein, and also affects the function of other clotting factors.
- Works **rapidly**, generally **used acutely.**

Coumarins

- **Oral medication.**
- **Inhibit VKOR c1,** the enzyme necessary for vit. K reactivation.
- Deplete active vitamin K → deplete active prothrombin, factors VII, IX, X → slower clot formation and increase bleeding tendency.
- **Slower acting,** takes days to deplete already the present factors; used **chronically**, action stays for 1-3 days after cessation of treatment.
- **Over-anticoagulation** – Treat with FFP and vitamin K supplements.

FFP: fresh frozen plasma

Outside the body

In vitro Anti-coagulation

- **Siliconized containers prevent activation of factor XII and platelets.**
  - They prevent blood from clotting by **inhibiting** any surface interactions that could activate **the intrinsic pathway.** The smooth silicone surface ensures that the blood remains stable and non-reactive during handling or transport.
- **Heparin** – used in blood collection, heart-lung and kidney machines.

- **Calcium chelators** (citrate, EDTA) used in blood collection, blood storage.
  - Oxalate can prevent clotting, but **citrate** is preferred because it's **safer** and **less toxic**.
    - It **binds calcium** to keep the blood from clotting, and later the **liver can remove it, so it doesn't accumulate.**
  - **EDTA** also works by **binding calcium**, but it's **not** used for plasma collection, only in specific blood tubes for tests.

Blood Coagulation Tests

Now rarely used due to poor reliability.

Clotting

Clotting time

- Invert tube every 30 seconds
- Normally **6 – 10 minutes**
- **Not reproducible**, high variability, generally not used

Bleeding

Bleeding Time
(from small cut)

- normally **1 - 6 minutes**
- Largely **reflects platelet function**

Bleeding time

- A **bleeding time** is used to evaluate the second phase of hemostasis, which involves adherence of the platelets to the injured vessel, platelet activation and aggregation (formation of a plug).

✓ The time measures **how long it takes for a platelet plug to form.**

✓ It **increases when** the platelets count is low (**thrombocytopenia**), **platelet function is abnormal or with the use of aspirin.**

- **Aspirin** acts as an anticoagulant because it **blocks the synthesis of prostaglandins and thromboxane A_2**, which are normally **required for platelet activation** and **aggregation**.

- **Disadvantages:** Insensitive, Invasive & operator dependent.
- **Advantages:** A simple good test to evaluate the platelet's function and structural abnormalities.

We will learn this concept in the labs

The Duke Method

1. **Clean** the tip of the finger or the earlobe with alcohol.
2. **Puncture** the skin with a special lancet. The wound should be 3-4 mm deep.
3. **Wipe the blood drop by a filter paper every 30 seconds.**
4. **Repeat** until no more blood is absorbed by the filter paper. Which indicates a platelet plug has formed.

5. **Multiply** the number of blood drops **by 30 seconds.**
   - Or divide the number of spots of blood by 2, and that will give you the bleeding time in minutes.
   - **Normal** value: **less than 5 minutes.**

We will learn this concept in the labs

Clotting Time

- It measures the time required for a blood sample to coagulate in vitro. Clotting time depends on the **availability of coagulation factors.**

- Many techniques are used; the one we use in our lab depends on using **non-heparinized capillary tubes**.

- Clotting time is **prolonged** in conditions like **hemophilia**, **vitamin K deficiency**, **liver diseases**, and **warfarin overdose**.

Common Clotting tests

- Clotting is mainly assessed by:
  - **Prothrombin Time (PT):** Measures the **extrinsic pathway** and common pathway (factors I, II, V, VII, X).
    - *mixing a sample of the patient's blood with a reagent that contains tissue factor and calcium.*
  - **Partial Thromboplastin Time (PTT):** Measures the **intrinsic pathway** and common pathway (factors I, II, V, VIII, IX, X, XI, XII).
    - Calcium and activating substances are added to the plasma to begin the intrinsic pathway of the coagulation cascade. The activating substances such as kaolin, which activates the contact-dependent factor XII (hydrated aluminum silicate), and cephalin, which substitutes for platelet phospholipids.

Prothrombin Time

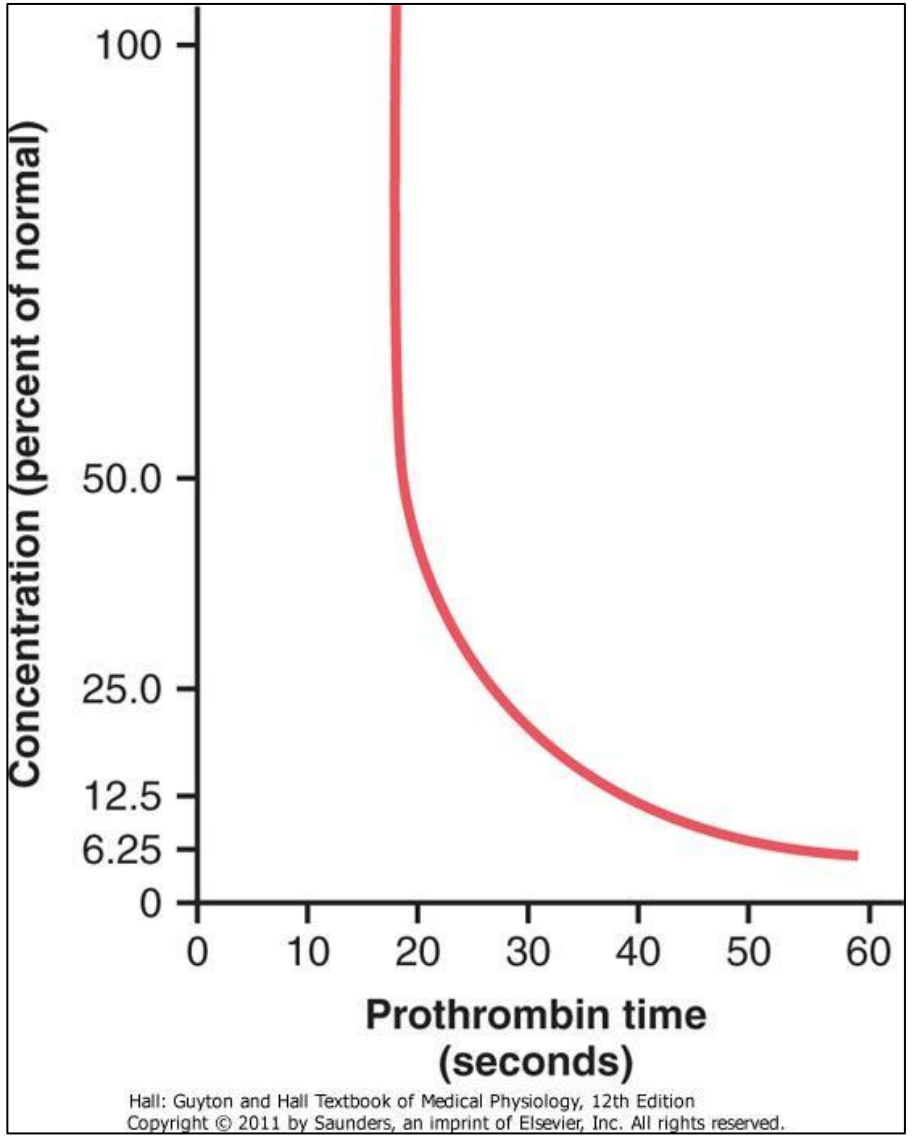
- **Add excess calcium and tissue factor** to oxylated blood, measure time to clot.
  - Blood is usually treated with citrate to prevent clotting. Calcium is added to activate clotting factors, along with tissue factor to trigger the extrinsic pathway.
- **Assesses Extrinsic and Common Pathways.**
- Usually about **12 seconds**
  - If the clotting time is prolonged, it may indicate a problem with prothrombin activity or the effect of anticoagulant drugs.
- **Tissue factor** batches have to be **standardized** activity expressed as **"International Sensitivity Index (ISI)"**
  - Tissue factor (TF) is added to specifically **activate the extrinsic coagulation pathway**.
  - Because TF activity can vary between reagent batches, results are standardized using the International Sensitivity Index (ISI).
- The prothrombin time (PT) **measures the time it takes to form thrombin**, which then converts fibrinogen into fibrin, resulting in a stable clot.

Prothrombin Concentration and Function

- Prothrombin time (PT) can be used to **estimate the percentage of prothrombin** present in plasma.
  - This is because the **PT value correlates with the concentration of prothrombin.**

- By measuring PT in seconds, we can assess the concentration of prothrombin in the blood, with higher PT values indicating a lower concentration of prothrombin and vice versa.

- This method allows detection of **prothrombin deficiency** or monitoring patients undergoing **anticoagulant therapy**, which can alter prothrombin levels.



Hall: Guyton and Hall Textbook of Medical Physiology, 12th Edition
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International Normalized Ratio (INR)

$$INR = \left(\frac{PT_{\text{test}}}{PT_{\text{normal}}}\right)^{ISI}$$

- Normal INR: 0.9–1.3
- Therapeutic range: 2.0 - 3.0

- The calculation standardizes PT results between different labs and reagent sensitivities.

❖ **Interpretation:**

- INR below normal (<0.9) → tendency to clotting.
- INR above normal (>1.3) → tendency to bleeding.

❖ **Clinical importance:**

- INR is critical for:
  1. Monitoring patients with bleeding disorders.
  2. Adjusting anticoagulant therapy before surgery or during treatment.

Tests of Other Clotting Factors

- Mix the patient's plasma with a large excess of all needed components except the factor being tested.

- Compare time to coagulation with that for pooled plasma of healthy volunteers.

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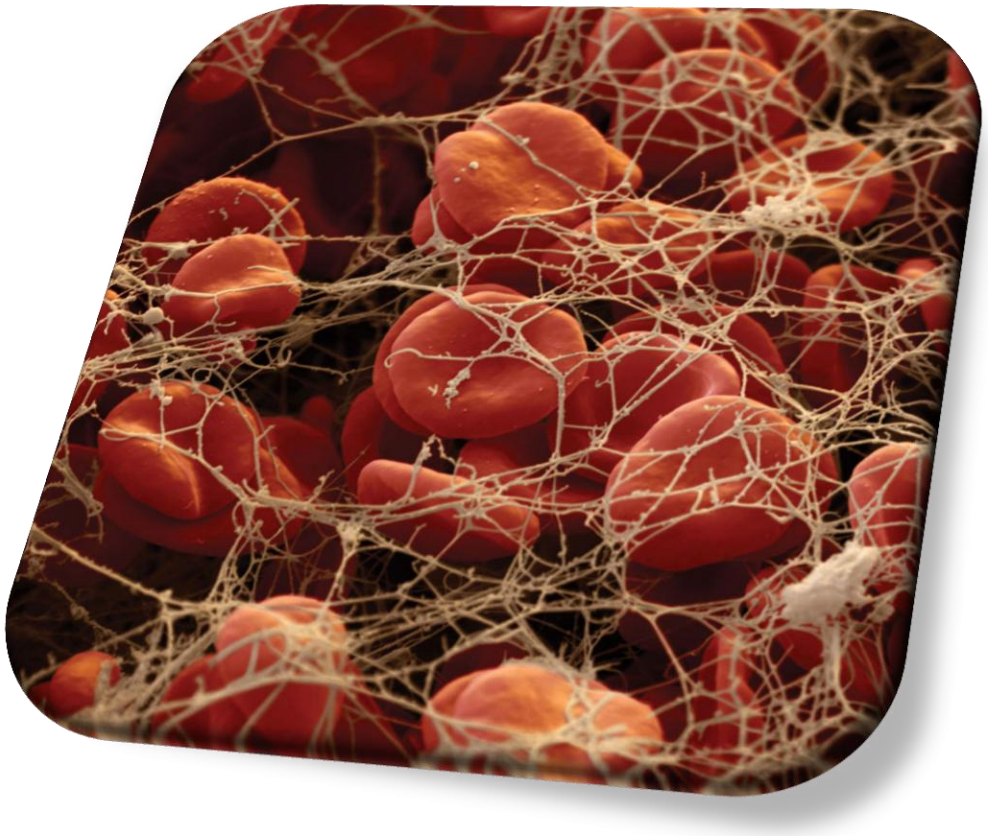
Summary: Bleeding and Clotting tests

Test Name	Assesses	Physiological Basis	Normal Range
Bleeding Time	Primary hemostasis	Platelet adhesion and aggregation	Less than 5 minutes (Duke method)
Prothrombin Time (PT)	Extrinsic & common pathways	Tissue factor activation of clotting cascade	~11–13.5 seconds
Partial Thromboplastin Time (PTT)	Intrinsic & common pathways	Contact activation of clotting cascade	~25–35 seconds

Physiology Quiz 11



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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
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