Hemostasis: arrest of bleeding.

4 major players: -Platelets -Blood vessels

-Coagulation factors -Fibrinolytic system (Anticoagulation factors)

Steps of hemostasis:

1- Vasoconstriction → 2 methods → Production of endothelin from endothelial cells

→ Neural reflex → smooth muscle contraction

2- Primary hemostasis: formation of primary platelet plug by adhesion of platelets to collagen and traces of thrombin.

3- Secondary hemostasis: conversion of the primary plug into permanent plug supported by fibrin clot. This is achieved by coagulation factors which will produce fibrin meshwork.

4- Lysis of fibrin: digestion of fibrin clot, stopping the process from spreading to other site and limit it to the site of injury.

→ Platelets

- Adherence to subendothelial collagen by GpIb/IX facilitated by vWF.
- Aggregation with fibrinogen which binds GpIIb/IIIa.
- Normal count is 140,000-400,000/µl.
- Life span: 8-10 days.
- The platelets have a set of canals called "Open Canalicular system", they also have microtubules, glycogen, granules (dense and alpha granules).
- **Alpha granules** contain fibrinogen, PDGF, vWF, PF4, factor V, P-selectin.
- **Dense granules** contain ADP, ATP, Ca^{+2}, Serotonin.
- Membrane lipids on the outer surface are neutral at rest, but upon activation they flip and the negative part switch to the outer surface → coagulation.
- Also when platelets are activated they change their shape from spherical to irregular with spikes, they secrete their granular contents, they produce TX-A2 from arachidonic acid by cyclooxygenase enzyme (TX-A2 promotes platelets aggregation).

→ Coagulation Cascade

- In the lab we have two pathways, intrinsic and extrinsic.

- **Intrinsic pathway**: XII activates XI which activates IX. IXa with the help of VIIIa activates X. Xa will work on Prothrombin to form thrombin, thrombin will activate fibrinogen to become fibrin which will form the clot.

- **Extrinsic pathway**: Tissue factor (thromboplastin) activates factor VII, VIIa with TF activates X and Xa activates prothrombin to thrombin.

- The physiologic cascade: begins with activation of factor VII not XII.
  Once VII is activated with the help of TF, it activates factor IX and X at the same time, and then Xa will activate prothrombin to thrombin (with the help of Va), and thrombin will turn fibrinogen to fibrin.

➔ **Functions of thrombin**:

- Converts fibrinogen into fibrin.
- Activates platelets, endothelial cells, inflammatory cells.
- Activates factors V, VIII, XI, XIII (Factors V + VIII are cofactors, factor XIII is responsible for cross-linking of fibrin).
- Activates protein C (which is an anticoagulant).

➔ Vit.K is important to produce factors **II, VII, IX and X** in the liver, there are some anti-coagulant drugs (Coumadin "Warfarin") that inhibit Vit.K so these factors won't be produced.

➔ **Lab tests**:

- **Prothrombin time (PT)**: measures extrinsic + common pathways
- Normally it's 13-14 sec
- If prolonged → Think about factors VII, X, I , II
- Used for follow up with Warfarin.

- **Partial Thromboplastin Time (PTT)**: measures intrinsic + common pathways.
- Normally it's 25 sec.
- If prolonged → Think about all factors except VII, XIII
- Used for follow up with Heparin.

- **Thrombin Time (TT):** measures common pathway only (measures conversion of fibrinogen into fibrin).

  Ex: If PT is prolonged but TT is normal → rule out fibrinogen (factor I).
  If PT is prolonged and PTT is prolonged → problem in common pathway.
- **Platelet count** (the most common test).

- **Bleeding time:** this is dependent on the platelet count and function only.
- **INR (international normalized ratio)**: similar to PT but given as ratio, normal value is ≤ 1 (if >2 then PT is prolonged). [Note: ISI stands for international standardized index]

$$INR = \left(\frac{PT_{Patient}}{PT_{mean-normal}} \right)^{ISI}$$

➔ **Endothelial cells**: they induce thrombosis at one site and inhibit thrombosis at the other site. In a normal condition they work as anti-coagulant, once there is an injury then the process is reversed and this will induce thrombosis and coagulation at the injury site.

Bleeding Disorders:

1) Vascular disorders → bleeding in skin and mucous membranes.

Acquired vascular defects:

-Senile purpura: atrophy of perivascular connective tissue.

-Scurvy: Vit.C deficiency.

-Steroid purpura. –Hereditary hemorrhagic telangiectasia: Weak blood vessels.

-Ehlers Danlos syndrome: collagen defects.

-Vasculitis: either **hypersensitivity** (Henoch-Schoenlein purpura) or **Septic** (Infections, dengue, measles, bacterial endocarditis, ricketsial infections,…).

2) Platelets defects:

-**Quantitative problems (Thrombocytopenia)**: when platelets count is below 5,000 fatal spontaneous bleeding may occur in the brain, eyes or lungs. Prophylactic platelet transfusion is advised when platelet count between 10,000-20,000. Classification of thrombocytopenia:

- **Failure of production**: either decreased BM megakaryocytes, or Increased BM megakaryocytes (Ineffective megakaryopoiesis).
- **Increased destruction**: in the spleen, autoimmune destruction (Idiopathic thrombocytopenic purpura, SLE, HIV, CLL), Drug related (Quinidine, Heparin –Heparin induced thrombocytopenia-), post-transfusion (Alloantibodies), Neonatal (Alloantibodies or autoantibodies).
  - Idiopathic thrombocytopenic purpura: Autoantibodies against GpIIb/IIIa or GpIb
    More common in women of child bearing age, reduced lifespan of platelets to hours, decreased platelets count, increased megakaryocytes, petechia, bruises, menorrhagia, normal Hb and WBCs, diagnosed by presence of antiplatelet antibodies and anti-nuclear antibodies, or Coomb's test in Evan's syndrome.
    Treatment: steroids, splenectomy, high dose IV Igs against antiplatelet Abs.
    Acute idiopathic thrombocytopenic purpura in children: preceded by infection or vaccination, 90% spontaneous remission, treated by steroids or IV Igs.

- **Increased consumption (Thrombotic microangiopathies)**: presence of mini thrombi in blood vessels, consuming coagulation factors and platelets causing thrombocytopenia and coagulation deficiency.
  - Thrombotic Thrombocytopenic Purpura (TTP)
    ADAMTS13 deficiency (which is normally needed to cleave HMWK) → accumulation of HMWK → Thrombi → consumption of platelets and coagulation factors.
    Symptoms: fever, CNS and renal microangiopathies, hemolytic anemia.
    Normal PT, PTT. Treatment by plasma exchange.
  - Disseminated Intravascular Coagulation (DIC)
    Causes: sepsis or endothelial damage → release of phospholipids or tissue factor → activation of extrinsic coagulation cascade → consumption of platelets and factors.
- **Hypersplenism (sequestration)** → trapping of all blood cells → Pancytopenia.
- **Dilutional thrombocytopenia**.

- **Qualitative problems**: deficiency of platelet membrane glycoproteins.
  Gp Ia/IIa: adhesion to collagen
  Gp Ic/IIa: laminin receptor
  Gp IIb/IIIa: binding to fibrinogen
  Gp Ib/IX: adhesion to subendothelial tissue via vWF
  - **Bernard Soulier syndrome**: deficiency of Gp Ib/IX → adhesion defect
    AR, characterized by giant platelets.
  - **Glanzman's thrombasthenia**: deficiency of Gp IIb/IIIa → aggregation defect
    AR, failure to aggregate in response to ADP, collagen, Epinephrine, Thrombin.

3) Coagulation disorders:

- Acquired: Vit. K deficiency (drop in factors II, VII, IX, X) or liver disease.

-Congenital: (1) **vWF**: carries and stabilizes factor VIII, bridges collagen to platelets receptor GpIb.

- **Von willebrand disease type 1**: mild deficiency of vWF, most common type, AD, no symptoms due to sufficient amount of factor VIII, all sizes of vWF multimers are present, slightly prolonged PTT, platelets fail to agglutinate by ristocetin.
- **Von willebrand disease type 2**.
- **Von willebrand disease type 3**: absent vWF, marked reduction in factor VIII, AR, hemophilia-like due to factor VIII deficiency.
  In vWD: bleeding in mucous membranes (GI bleeding), easy bruising, menorrhagia, Post-operative bleeding.

(2) **Hemophilias**

- **Hemophilia A**: factor VIII deficiency, X-linked, Females are usually carriers, 1/3 cases have no family history.
  Bleeding in large joints and soft tissue (hemarthrosis and hematoma), bleeding in GIT, UT, brain and nose.
  Prolonged PTT, normal PT and normal TT, Low factor VIII assay.
- **Hemophilia B (Christmas disease**): factor IX deficiency, X-linked, Bleeding sites similar to type A, prolonged PTT, normal PT, normal TT, normal factor VIIII assay.

4) Excessive fibrinolytic system.

Clinical cases

Notes: Normal MCV (80-96), <80 → microcytosis , >96 → macrocytosis
Red cell Distribution Width (RDW): normally it's 12-15%, if it's high → anisocytosis
Low Hb, Low MCV, Normal RDW → Thalassemia trait.
Low Hb, Low MCV, High RDW → Iron deficiency anemia.

(1) **Multiple Myeloma**: **elderly** with low back pain
Acute renal failure → **elevated total plasma protein**
Rouleaux formation → then it's a plasma cell disorder → perform serum protein electrophoresis (SPEP) → M spike (elevated gamma globulins)
If narrow spike → monoclonal
If wide-based spike → polyclonal
Do immunofixation → IgG kappa myeloma.
Diagnostic criteria: **CRAB** → High **C**alcium, **R**enal failure, **A**nemia, **B**one lesions + Plasmacytoma + elevated serum or urinary monoclonal proteins.

Other plasma cell disorders:

MGUS → SMM → Multiple Myeloma (MM)

-MGUS (Monoclonal Gammopathy of Unknown Significance): elevated serum proteins only [M proteins, <3g/dL] (no CRAB, no increase in plasma cells, no evidence of another B-cell proliferative disorder).
-SMM (Smoldering Multiple Myeloma): slightly higher M protein levels than MGUS [≥3g/dL and/or 10-60% Plasmacytoma], no symptoms of MM [No end organ damage].

(2) **Vit. B12 deficiency anemia**
High MCV + hypersigmented neutrophils + neurological symptoms.
Two common causes in Jordan:

Causes of Macrocytosis:

-Alcoholism -Liver disease -Hypothyroidism

-Megaloblastic anemia (B12 or folate def.)

-Myelodysplastic syndrome

-Reticulocytosis -Antimetabolites

-Drugs: Hydroxyurea, Zidovudine, Methotrexate, Imatanib, …

- Metformin: reduces intestinal absorption of Vit.B12 and lowers serum Vit.B12 concentration, rarely causes megaloblastic anemia.
-Proton pump inhibitors [Omeprazole]: impairs release of cobolamin (Vit.B12) from food.

(3) **Iron deficiency anemia** → most common among pregnant females.
Low MCV, Anisocytosis (high RDW), increased central pallor, low serum iron, high total iron binding capacity.
If elderly → perform endoscopy to make sure there's no internal bleeding from gastric or colonic cancers.
Treatment by oral iron supplement or IV iron infusion.

(4) **Thrombotic Thrombocytopenic Purpura (TTP)**
Low platelets, fever +confusion, renal impairment, schistocytes, low ADAMTS13 (less than 10%).
When we see schistocytes → 4 possible diagnosis → TTP or DIC or heart valve hemolysis or hemolytic uremic syndrome. To confirm TTP diagnosis we do the ADAMTS13 test.
Treatment: Plasma exchange or Fresh-Frozen Plasma.
-Drug-induced TTP: caused by 6 drugs that should be memorized! [**Quinine, Mitomycin C, Gemcitabine, Cyclosporine, Tacrolimus, Sirolimus**]

(5) **CLL (Chronic Lymphocytic Leukemia)**
Elevated WBCs, lymphadenopathy, **Smudge cells**, above the age of 50, to confirm diagnosis perform flow cytometry.
Flow cytometry → CD5+ only → Mantle cell lymphoma
→ CD5+ and CD23+ → CLL
If CD103+ then it's hairy cell leukemia.

Remember that:

(8,14) →Burkitt's lymphoma→C-MYC to heavy chain Ig gene.

(11,14) → Mantle cell lymphoma→cyclin D overexpression, older male.

(14,18)→ Follicular cell lymphoma →bcl2 overexpression, indolent and difficult to cure.

(6) **Factor XII deficiency**
Normal PT, very high PTT, **no history of bleeding**!

(7) **Acute Myelogenous Leukemia (AML)**
Leukocytosis, Thrombocytopenia, Anemia, **Auer Rods** in myeloblasts.

(8) **Chronic Myelocytic Leukemia (CML)**
Anemia, Leukocytosis, Thrombocytosis, Splenomegaly
Blood film shows **all stages of WBC maturation**.
Translocation (9,22) → Philadelphia chromosome
Treatment by Imatanib and Desatinib.

(9) **Myelofibrosis**

Anemia**, Massive splenomegaly**, Nucleated RBCs, **BM fibrosis**.

[Other possibilities for nucleated RBCs: sickle cell anemia, thalassemia, myelofibrosis, metastatic cancers, sepsis, severe trauma].

(10) **Autoimmune hemolytic anemia**

Anemia, **high reticulocyte count** (this indicates hemolysis), **spherocytosis** (when we see spherocytes there are two possibilities: Autoimmune hemolytic anemia or hereditary spherocytosis).

(11) **Non-Hodgkin lymphoma**

Ant. Mediastinal mass, BM failure (anemia, zero reticulocytes, low hematocrit).

(12) **Hodgkin lymphoma**

B-symptoms (Fever, Malaise, Night sweats, Weight loss), Lymphadenopathy, **Reed-Sternburg cells.**

(13) **Giant platelet disorder (Bernard Soulier syndrome**)

Normal WBCs count, normal RBCs count, thrombocytopenia, normal PT, normal PTT, prolonged bleeding time (19 min), Giant platelets.

(14) **Deep Vein Thrombosis** → best treatment option → Warfarin.

(15) **Tumor Lysis syndrome**

High K^{+}, High phosphates, very high LDH, high uric acid, Low Ca^{+2}

Avoided by IV hydration + Allopurinol + Rasburicase.