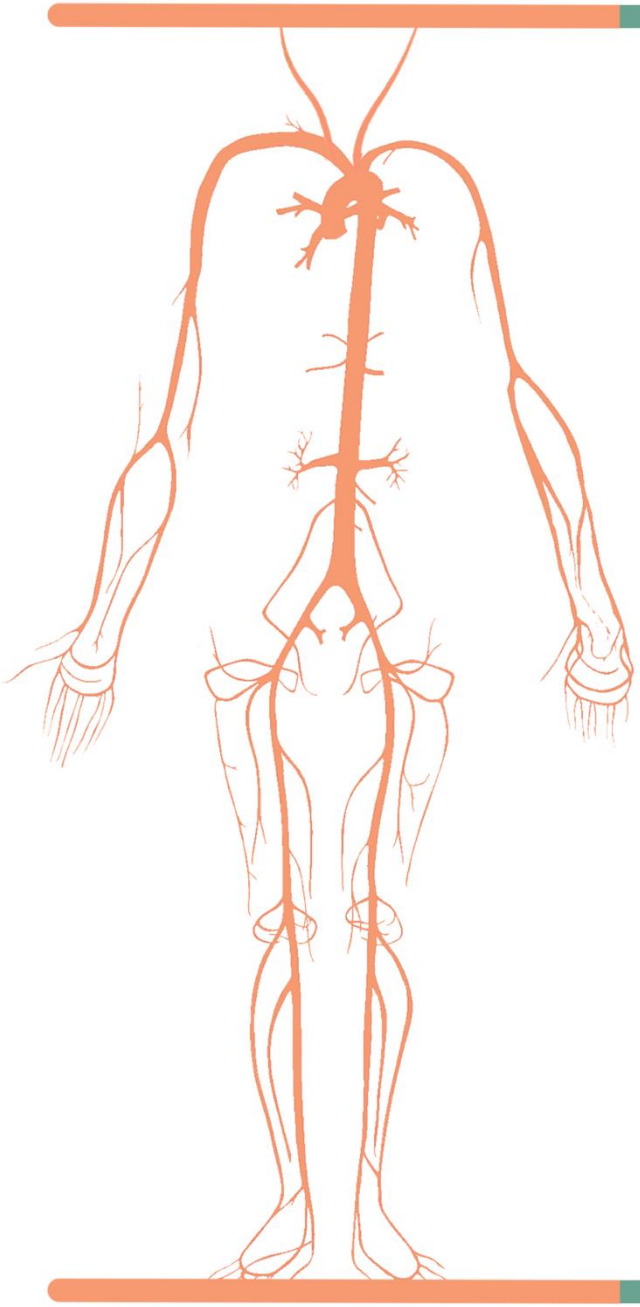
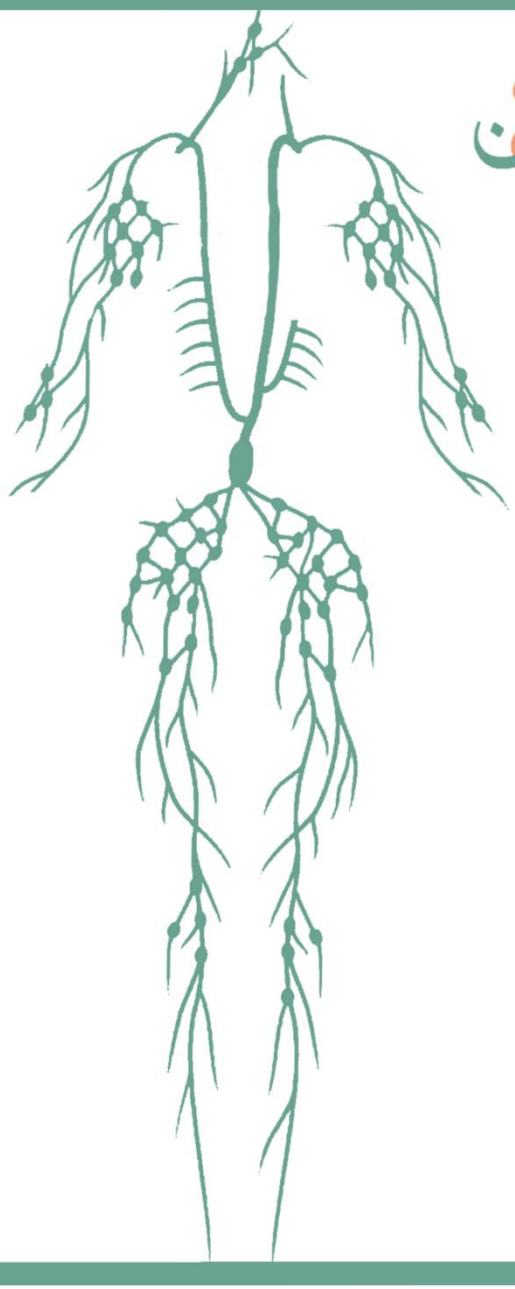
Pathology

HematoLymphatic





Title: Sheet 5 – Bleeding Disorders

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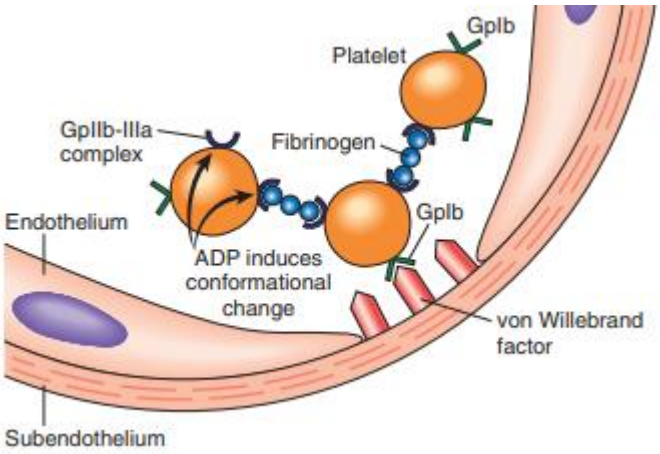
Quick Review

- Intact, normal endothelial cells help to maintain blood flow in **blood vessels** by inhibiting the activation of platelets and coagulation factors.
- Endothelial cells stimulated by injury or inflammatory cytokines (e.g., TNF or IL-1) upregulate expression of **clotting** factors (e.g., tissue factor) that promote clotting, and downregulate expression of anticoagulant factors.
- After vascular injury, platelets undergo a series of events:
- **1.Platelet Adhesion:** When the **endothelium** is injured, this brings **platelets** into contact with the subendothelial ECM, which includes among its constituents **von Willebrand factor (vWF)**, a large multimeric protein that is synthesized by Endothelial Cells. vWF is held fast to the ECM through interactions with collagen. It binds tightly to platelets through **Gp1b**, a glycoprotein found on the surface of platelets. These interactions allow vWF to act as a sort of molecular glue that binds platelets tightly to denuded vessel walls.



- **2.Platelet Activation**: Leads to irreversible shape change (by ADP release) and secretion.
- **3.Platelet Aggregation**: It is promoted by bridging interactions between fibrinogen and **GpIIb/IIIa receptors** on adjacent platelets.

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- In normal physiology, in order to stop bleeding, there should be an integration of the function of 4 main factors: **clotting factors**, **platelets**, **blood vessels** and **endothelium**.
- Pathologic bleeding that occurs spontaneously or after trauma (prolonged bleeding) is caused by defect in either of these factors.

1.BLOOD VESSEL-RELATED BLEEDING

Blood vessels can be associated with bleeding in several diseases (that do not only affect the blood vessels):

-Connective tissue diseases: A group of diseases that can be inherited, sometimes acquired, or associated with autoimmune diseases (like rheumatologic diseases).

-Chronic steroid intake: Weakens the blood vessels and increases the chance of their rupture.

-Systemic amyloidosis: The amyloid protein can infiltrate through any organ and cause physical damage, including in the blood vessels.

-Vasculitic infections: Some bacterial and fungal infections, like spirochetes and some fungi, can infect the blood vessels, causing vasculitis and sometimes aneurysm which results in rupture and bleeding.

An **aneurysm** refers to a weakening of an artery wall that creates a bulge, or distention, of the artery.

-Vitamin C deficiency (scurvy): It was common in the past, but now it's almost an outdated disease. Vitamin C is important for the structure of the collagen of blood vessels, thus when it's deficient, it will weaken the bloods vessel and this results in bleeding.

* Patients with these diseases will manifest bleeding in the **superficial** part of the body (skin) and in the superficial mucous membranes (such as the oral cavity). In the skin, we call it either **petechia** if the bleeding is in a small area or **ecchymoses** when it is a large bruise.

2. PLATELETS-RELATED BLEEDING

-Bleeding secondary to platelet disorders can result from numerous problems:

- Thrombocytopenia: Decrease in the number of platelets. The most common disease is called **IMMUNE THROMBOCYTOPENIC PURPURA (ITP)** (will be discussed later in this lecture). AIDs patients have **damaged Megakaryocytes** in the bone marrow and this also results in thrombocytopenia.

precursor of platelets

- Thrombocytosis (dysfunctioning platelets): There is an increase in platelet number but they can't function properly. This is common in myeloproliferative neoplasms (we are going to talk about it in the neoplastic part of the WBCs disorders) So, the platelets don't function properly although they're large in number and patients have a chance of bleeding.

How do we test the platelets according to their function?

-Bleeding time test: Done

by making a small cut in the superficial part of the body like the ear and we wait until the bleed stops, (it's obsolete and rarely used these days).

Instead, we use these two tests:

-Platelets aggregation test

-Von Willibrand factor (vWF) tests

Both tests are done at the same time in order to know whether the abnormality is that (1) the platelets can't function well OR (2) vWF is missing.

Firstly, in the platelet aggregation test, we add an antibiotic called ristocetin, which causes artificial platelet aggregation. If there is no aggregation, it is either the platelets are dysfunctional OR the vWF factor is missing.

So, in order to find out where is the abnormality, we do the vWF test. If the vWF factor is present, then the problem is that the patient has dysfunctional platelets.

REMEMBER! The vWF factor is needed for platelet adhesion to the site of injury.

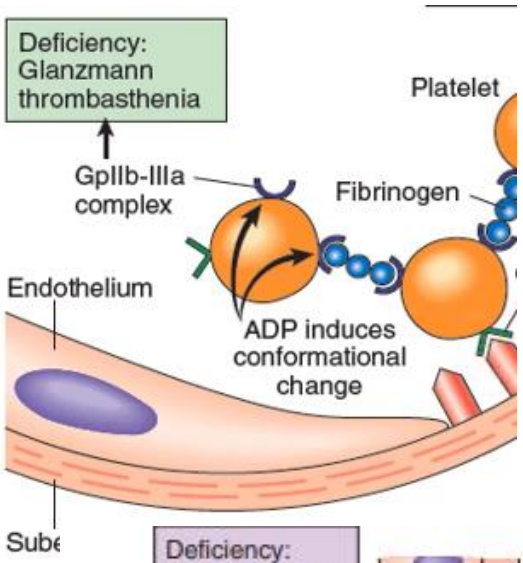
-GLANZMANN THROMBASTHENIA

-Rare, autosomal recessive disease.

-Acquired (autoimmune disease)

-Deficiency/blockage of platelets glycoprotein IIb-IIIa receptors (CD41/CD61 complex).

- Fibrinogen cannot bind platelets and this will result in prolonged hemorrhage.

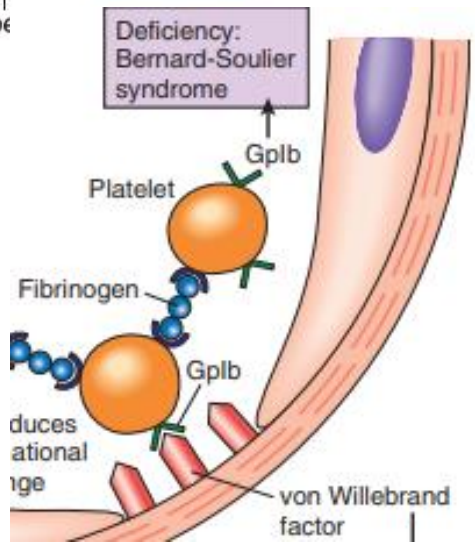


-BERNARD SOULIER SYNDROM

-Very rare, autosomal recessive disease.

-Deficiency is platelets membrane glycoprotein Ib (CD42b) receptor, which binds to vWF.

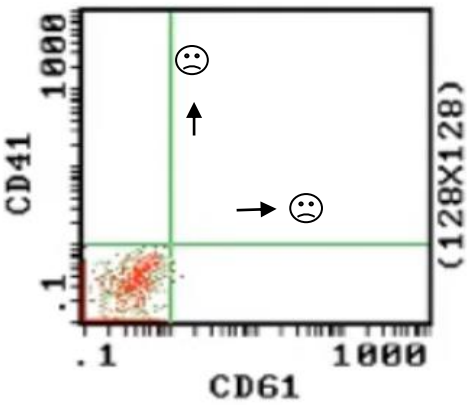
-Platelets are large, can show thrombocytopenia.

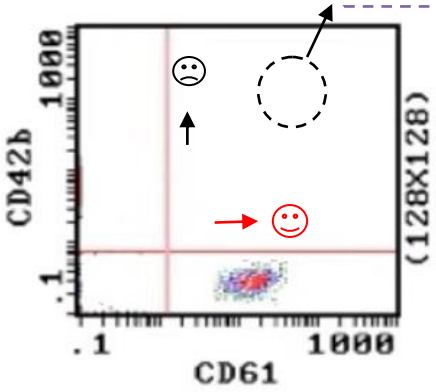


IF it was located here, then the patient is healthy.

-***How to diagnose Glanzmann and Bernard Soulier diseases***? By flow cytometry

*Remember: this test examines the antigen on the surface of cells in fluid. *





Left patient: These are the platelets as you can see, it doesn't move to any of the markers CD61 and CD41 (they didn't go to either direction) which means he/she is deficient of both of them and has Glanzmann Thrombasthenia.

Right patient: In this patient we examine CD42b and CD61. Ss you can see the platelets move to the CD61 (normal) while CD42b is deficient so this patient has Bernard Soulier syndrome.

-IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

- Purpura means bleeding of the skin.

*The underlined points here are the most important ones as they are the only points mentioned by the doctor.

-Patients have isolated thrombocytopenia (and sometimes anemia of blood loss).

-Most bleeding occurs in the skin, mucosal surfaces (petechiae and ecchymoses), and in the GIT, urinary tract and CNS.

-Acute ITP: Affects children, commonly follows viral infection, and is self-limited.

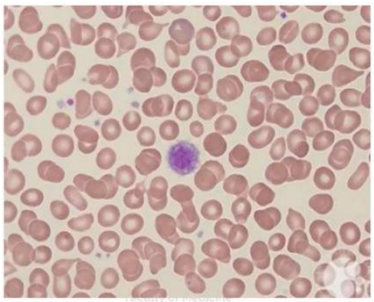
-Chronic ITP: Affects middle age adults (F>M).

-IgG auto-antibodies are made against platelets membrane glycoprotein IIb/IIIa thus platelets will aggregate (chronic ITP).

-Coated platelets are engulfed by macrophages in spleen.

-Splenomegaly is not always present but patients benefit from splenectomy.

**Peripheral blood shows large platelets, the bone marrow shows increased number of megakaryocytes to compensate thrombocytopenia, and the spleen shows large aggregates of B-lymphocytes and plasma cells which are responsible for secreting this autoantibody.



▪ ITP: thrombocytopenia, mean platelets volume (MPV): high

-HEPARIN-INDUCED THROMBOCYTOPENIA

-Acquired, appears in 5% of patients who receive unfractionated heparin.

Heparin is commonly used in medical practice as an anticoagulant to stop coagulation. It has 2 forms: (1) Unfractionated/Large molecular weight form (2) Fractionated, an improved form that only consists of the active site of the heparin. Also called low molecular weight heparin.

-This syndrome most commonly affects those who take unfractionated heparin, and rarely can develop in fractionated (low molecular weight) heparin.

- For unknown reasons, heparin induces synthesis of IgG antibodies that target a protein in the cell membrane of platelets called **platelet factor-4.** These immunoglobulins will bind to these antigens on the surface of the platelets, causing platelet aggregation inside the body and formation of thrombus.

-Here the antibody will cross react the platelets making a spontaneous thrombus, so the patient has paradoxical thrombocytopenia with thrombosis. (remember we mentioned this in paroxysmal nocturnal hemoglobinuria. The difference here is platelets are lysed and torn and their content will promote the formation of the thrombus).

10:20

-THROMBOTIC MICROANGIOPATHIES

Microangiopathies: Microvascular diseases, i.e. disease of small blood vessels

-Includes thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). They have different pathogenesis but end up with similar findings.

-Do not mix between ITP and TTP since in TTP we have thrombosis all over the body. Platelets are consumed as thrombi are made and this results in thrombocytopenia.

-TTP: Has **typical pentad** (5) of symptoms:

1. Fever
2. Microangiopathic hemolytic anemia
3. Thrombocytopenia
4. Neurologic deficits
5. Renal failure.

You will find in future that not all of these symptoms are full-blown and very prominent. Most patients will have one of them absent or present in a minor condition

-HUS: Similar symptoms, **dominance of renal failure**, no neurologic symptoms, and common in children. *while **TTP** can affect any age.

-In both diseases, the small circulation in the body is filled with platelet-rich microthrombi (these are small and will obstruct the small circulation). The patient will have thrombocytopenia , clotting factors are intact and inactivated (PT and PTT are **normal**).

(Will be talked about later) Partial thromboplastin time (**PTT**) and the prothrombin time (**PT**) are tests that measure the speed of clotting. Since the clotting factors are inactivated, then the results would be **normal**.

PATHOGENESIS

-TTP: ADAMTS13 is a plasma protein and the function of this protein is to convert the precursor of vWF, which is a large multimer that has a short half-life, into vWF (important in platelet plug formation). In TTP, this plasma protein is deficient so the vWF will stay in its precursor form and this precursor is capable of binding to many platelets, causing aggregation and spontaneous thrombus.

-HUS: Infection by a strain of E.Coli called enterohemorrhagic E.Coli (found in contaminated food) in the gut produces shiga-toxin that reaches kidneys (mostly concentrated here) and causes endothelial damage and thrombosis.

-Blood film for both reveals:

- Schistocytes: Direct/physical damage to RBCs when they try to pass through small thrombi. Appear in a large amount in the peripheral blood.
- Thrombocytopenia: Because platelets were consumed in the formation of thrombi.

3.COAGULATION DISORDERS

-Diseases related to clotting factors.

-Inherited, or **more commonly** acquired.

-Vitamin K deficiency→ Acquired, can be dietary but is **more likely** related to drugs. The most important drug is **warfarin** which blocks the synthesis of clotting factors that need Vitamin K. This deficiency decreases the synthesis of factors:

II (prothrombin),VII,IX,X .....easier way to memorize it as 1972.

1	I
2	II
3	III
4	IV
5	V
6	VI
7	VII
8	VIII
9	IX
10	X

-Liver disease: Since the liver is responsible for producing these factors.

-Disseminated Intravascular Coagulation (DIC): This disease consumes all the clotting factors.*will be explained at the end of the lecture.

-Warfarin (as explained above)

-Auto antibodies: Bind to one or multiple factors. The patient doesn't have a true deficiency of clotting factors. Instead, there is an issue with their function.

How do we test the clotting factors?

-Prothrombin time (PT): Assesses extrinsic (factors V, VII) and common pathways (factors X, prothrombin or fibrinogen).

1	I
2	II
3	III
4	IV
5	V
6	VI
7	VII
8	VIII
9	IX
10	X

-Partial thromboplastin time (PTT): Assesses intrinsic (factors XII,XI,IX,VIII,V) and common pathways.

-For example, if the patient has a prolonged PT, then this patient might have a deficiency in one of these factors --> factors V, VII

-In addition to deficiency, an autoantibody (inhibitor) can interfere with the function of clotting factors. So, in this case we do another test called mixing study.

-Mixing study: Adding a normal serum to the patient's serum then repeating PT and PTT tests. If the issue is corrected/compensated (because the needed clotting factors were added), then the patient has true deficiency. If not corrected, then the patient has an inhibitor autoantibody that blocked the new clotting factors from the normal serum and the tests remain abnormal.

19:30

DISEASES OF THE CLOTTING FACTORS

1.**VON WILLIBRAND DISEASE (vWD) (most common one)**

-Autosomal dominant disorder, has many different subtypes.

-Most common inherited bleeding disorder (1% of population)

-Spontaneous bleeding from superficial parts and mucous membranes (since vWF is important for the function of platelets), wounds and menorrhagia (excessive menstrual period).

-vWF is widespread and vast. It is synthesized in endothelium (in Weibel-palade bodies). It is present beneath endothelium as an anchoring protein and inside platelets.

- It also circulates the plasma and carries factor VIII. (important)

-After endothelial damage, subendothelial vWF binds platelets through glycoprotein Ib (CD42b), forming a platelet plug.

-Ristocetin agglutination test in vitro: it activates vWF to bind to glycoprotein Ib causing platelets clumps. (It's the same test as the platelet aggregation test discussed previously).

-In vWD, the vWF is deficient, and this causes a compound defect, resulting in (1) non-functional platelets so the patient will have **superficial** bleeding and (2) in severe cases, **deficiency of factor VIII** will occur (remember! this factor is carried by vWF).

-Symptoms are **mainly** related **to platelets defect** (superficial bleeds), EXCEPT in the homozygous state which is the severe form and factor VIII will be deficient.

-The severe form resembles **hemophilia A**-clotting factor deficiency, bleed tends to occur in the body cavity not the skin- and the **PTT is prolonged** due to absent factor VIII.

-We have many types of vWD:

- **Type I vWD**: **Most common**, decreased levels of serum vWF so platelets are non-functioning.
- **Type IIA**: Absent high-molecular weight multimers of vWF (the precursor of vWF).
- **Type IIB**: The high molecular weight multimers are present but they have a very short half-life and are hyper-functioning and consume platelets more often. In this subtype, the multimers bind to many platelets causing **mild chronic thrombocytopenia**.

- TTP and Type IIB have similar pathogenesis but in TTP it's widespread and more severe while in Type IIB, it consumes some platelets, causing mild thrombocytopenia without thrombi formation.

2.HEMOPHILIA A

-Second most common inherited bleeding tendency.

-X-linked inheritance*mainly disease of males but can affect females. Also known as classic hemophilia.

-Reduced factor VIII.

-Can affect females (random inactivation of X).

-30% of cases appear as new mutation (no family history) while 70% of cases are due to family history (such as in maternal uncles).

-Normally we have excess amount of factor VIII, and symptoms only appear when there is marked deficiency (around 20% of the normal amount). So, in mild deficiencies excessive bleeding appears mainly after trauma (especially after surgery). Surgery can occur in males early in life in 'circumcision' so if he has hemophilia, he'll develop excessive bleeding after that surgery.

-**Severe** life-threatening bleeding occurs if level drops to **<1%** of normal levels. These patients may die secondary to severe hemorrhage.

-10% of the patients have normal level but non-functioning factor.

-Characteristic of hemophilia A is deficiency of clotting factors not platelets. The bleeding tends to occur **in deep tissues** like soft tissues (ex: muscles) especially with mechanical stress (joints, body cavities). The growing child will develop deformity in joints due to repetitive bleeding.

-Skin petechiae is **absent** (Characteristic of hemophilia A).

-Prolonged PTT, corrected by mixing study. It's an inherited deficiency and not related to an inhibitor antibody.

-Another test is available called specific assay test, which is done by testing the factor VIII itself whether it's available or functioning.

3.HEMOPHILIA B

-AKA Christmas disease.

- Much less common than hemophilia A.

-Deficiency in factor IX (in intrinsic pathway)

-X-linked.

-Clinically similar to hemophilia A:

- Mild → Excessive bleeding after trauma
- Severe → Life-threatening bleeding

-Prolonged PTT, corrected by mixing study.

-Factor assay test is available for factor IX in order to differentiate it from hemophilia A.

4.ENDOTHELIAL-RELATED BLEEDING

-Widespread endothelial damage causes release of **tissue factor**, a prothrombic agent. As a result, microthrombi will be produced that consumes the clotting factors, not only platelets. So, even with **thrombosis** everywhere, patients will complain paradoxically from bleeding (as the clotting factors were consumed in the formation of thrombi), causing disseminated intravascular coagulation (DIC).

-Consumption of clotting factors is the difference between DIC vs. TTP and HUS.

-Rapid consumption of clotting factors (prolonged PT, PTT) and platelets. Consumption exceeds the capability of the liver to synthesize new ones.

-Patients then develop life-threatening bleeding.

-Peripheral blood shows schistocytes, anemia and thrombocytopenia. Same as TTP **BUT** PT and PTT are prolonged in DIC while they are normal in TTP.

Causes of DIC:

- ENDOTHELIAL DAMAGE: By releasing **tissue factor** like in septicemia and viremia. (COVID-19 can cause DIC in patients)
- Snake venom
- Complicated labor (In some situations, the prothrombic tissue factor is not secreted by the endothelium itself, but can come from other sources like the placenta)
- Advanced cancer: Most common one is a type of acute leukemia called acute pro-myelocytic leukemia. These malignant blasts secrete tissue factor in large amounts causing DIC. Another one is epithelial tumors that contain mucin that circulates in blood and activates tissue factor→ DIC
- Severe trauma like surgeries
- Severe inflammation (acute pancreatitis).