HEMATOLOGY
&LYMPH SYSTEM

Pathology sheet

Number	10
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Before we start...

●This sheet was written according to the recording that belongs to section 1.
●This is the last pathology lecture in the hematology and lymph system.
●This sheet discusses three subjects (diseases of blood vessels, platelets disorder, clotting factor deficiency).

When tissue injury occurs, the body response with hemostasis which involves blood vessels, primary hemostasis and secondary hemostasis.
Tissue injury stimulates the contraction of the endothelial cells and the exposure of the subendothelial von willebrand factor (v-WF) and collagen **(Blood Vessels)** resulting in platelet adhesion, activation and aggregation forming primary plug **(Primary Hemostasis)**.
Primary plug is good for a while but not very stable so we need clotting factors of either the extrinsic or intrinsic pathway or both **(Secondary Hemostasis)**.
Ends results of clotting is fibrinogen polymerization forming *Fibrin*.

Remember:
Fibrinogen = clotting factor I
Thrombin = clotting factor II
Fibrin polymers are stabilized by clotting factor XIII

The clot should not extend to the whole blood vessel; it has to be limited by *plasmin* which causes fibrinolysis at the edge of the clot.

When we talked about anemia, we've mentioned some tests (CBC, MCV, Hb, and Hct); the same is applied for bleeding, we have many tests:

1- Platelet count

- Same as RBC count using an automated machine.
- Normally platelet count should be between (150,000- 450,000) _ actually it's different from one lab to another thus there is a reference range with each test.
- Below 150,000 by definition is thrombocytopenia but bleeding doesn't occur in the cases of mild depression in platelet count.
- Bleeding occurs only when platelets drop to 20,000 – 50,000.
- Spontaneous bleeding doesn't occur unless platelets count below 5,000.
- The most dangerous thing in the spontaneous bleeding is intracranial hemorrhage (Here is the risk of death).

2- <u>Platelets function</u>

- Normal platelets count but dysfunctional platelets.
- The most common cause of acquired dysfunction platelets is aspirin (patients who take aspirin have normal Platelet count but they bleed if they undergo surgery due to their dysfunctional platelets.

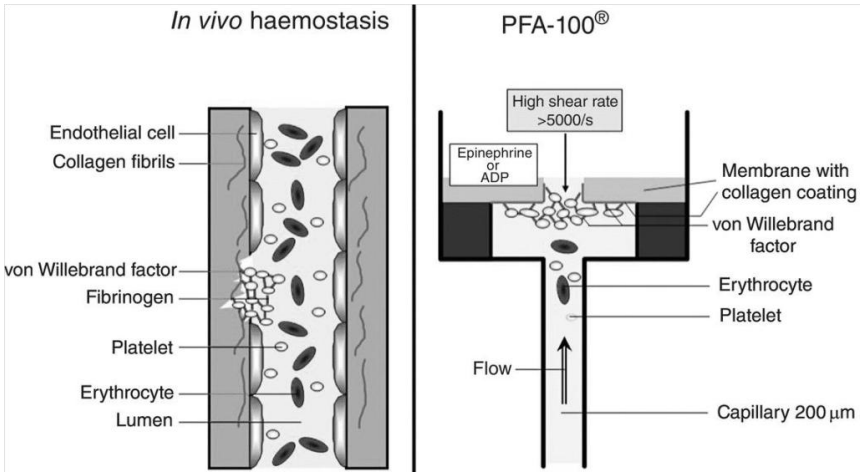
Tests for platelet function:

1- Bleeding time :

- Old, outdated, should not be used, not easy to standardize and doesn't correlate well with the risk of post surgical hemorrhage.
- Simply injure the volar aspect of the patient's forearm then smear it to get how long it takes the blood to clot. However, a patient with normal bleeding time doesn't mean that he is not going to bleed during the surgery so we have other new tests (2&3 mentioned below).

2- Platelets function assay

- Machine similar to the normal blood vessel (tube with small hole containing ADP and epinephrine as activators for platelets), allow the blood to flow through it at a certain speed then get how long does it take to close the hole.
- More accurate and more correlative than bleeding time.



3- Aggregation studies

Tube containing serum and rich in platelets (by now its color is opaque), we add activators for the platelets – ADP, Epinephrine, Collagen, Ristocetin – normally platelets aggregation and precipitation should happen so the tube turns more transparent which means before platelets aggregation the optical density is high but after their aggregation and precipitation what remains above is only the serum so

optical density decreases. In the cases of platelets dysfunction the optical density will not decrease since no aggregation is taking place.

Eq: someone is taking Plavix (clopidogrel) which acts on ADP, when you perform this assay for him; ADP will not work since its receptors on the platelets are blocked by Plavix so optical density remains high.

Tests for clotting factor pathway:

1- *PT (prothrombin time):* for extrinsic pathway (tissue factor and VII) and common pathway (factor I, II, V, X).

Remember:
Clotting factor II, VII, IX, X are vitamin K dependent so PT is a good measure for vitamin K deficiency.

2- *PTT (partial thrombopalstin time):* for intrinsic pathway (factor XII, XI, IX, VIII) and common pathway (factor I, II, V, X).

3- *D dimer and fibrin split products*
- Sensitive for DIC but not specific.
- End results of clotting is Fibrin polymers and for the clot to be limited where it should be, Plasmin is released causing fibrinolysis at the edge of the clot resulting in something called **Fibrin Degradation Products** (FDP) or **Fibrin Split Products** (FSP) which are sensitive for DIC.

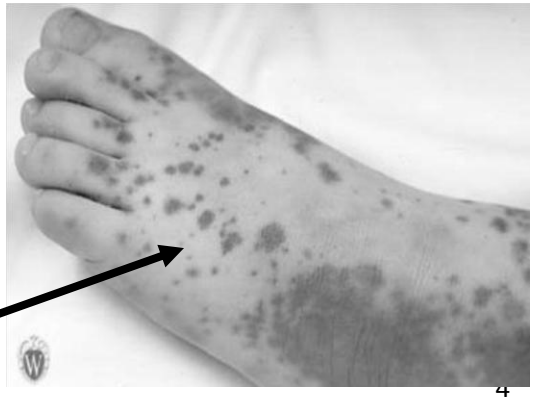
Remember

sensitive means if the test is negative then the disease is NOT there, but if positive, then we cannot tell for sure, on the other hand a specific test means if the test is positive then the disease is there, however if the test is negative then we cannot rule the disease out.

In other words a se**N**sitive test is useful when **N**egative and s**P**ecific test is useful when **P**ositive.

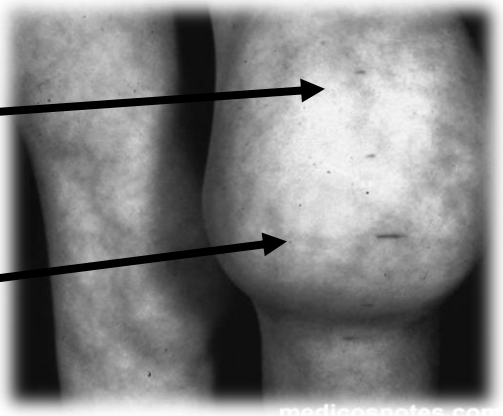
Clinically bleeding present in 2 major ways:

1- Mucocutaneous seen in disorders of the vessels and platelets.
Present as Petechiae and ecchymosis in the skin, mucous membrane, GI.



The pin point hemorrhages called Petechiae.
Large hemorrhages called ecchymosis.

2- Deep bleeding in muscles and joints seen in clotting factor deficiency.



Knee joint bleeding in patients with Hemophilia.
Hemarthrosis (bleeding in a joint).

Disorders related to blood vessels:

1- *Increased vascular fragility* which is caused by:

- •Vitamin C deficiency (scurvy)
- •Amyloidosis

- Amyloid is abnormal protein that will deposit in tissues specifically blood vessel, kidney, brain, as it deposited in blood vessels, they will not be as flexible as they should so changes in blood pressure result in blood vessel damage.
- Localized amyloidosis in the brain is the most common cause of spontaneous intracranial hemorrhage in hypertensive elderly patients.

- •Chronic steroid use

- Patients with Cushing syndrome/disease develop thin skin thus bleed easily.

- •Vasculitis

Lab Findings: **Normal platelet count, function, Normal PT and PTT.**

2- *Endothelial Damage* which is caused by:

- DIC (the most common complication of many diseases such as obstetric birth, sepsis, massive tissue trauma among many others – even what kills in Snake bite is DIC.
- Overwhelming damage to the endothelial cells converting them to prothrombotic surfaces.
  - •Consumption of platelets and coagulation factors (consumptive coagulopathy).

Disseminated Intravascular Coagulopathy (DIC):

DIC occurs as a complication of a wide variety of disorders; it is caused by the systemic activation of coagulation and results in the formation of thrombi throughout the microcirculation, consumption of platelets and coagulation factors and severe bleeding.

Causes of DIC:

1-Widespread endothelial damage.
2- Release of tissue factor or thromboplastic factor.

Diseases associated with DIC:

1- *Obstetric complication, placental damage*
Sometimes after delivery, tissue of placenta remains in the uterus or in the cases of fetal demise in which the baby dies within the uterus and remains for long period of time, here the necrotic tissue releases prothrombotic factors into the circulation.

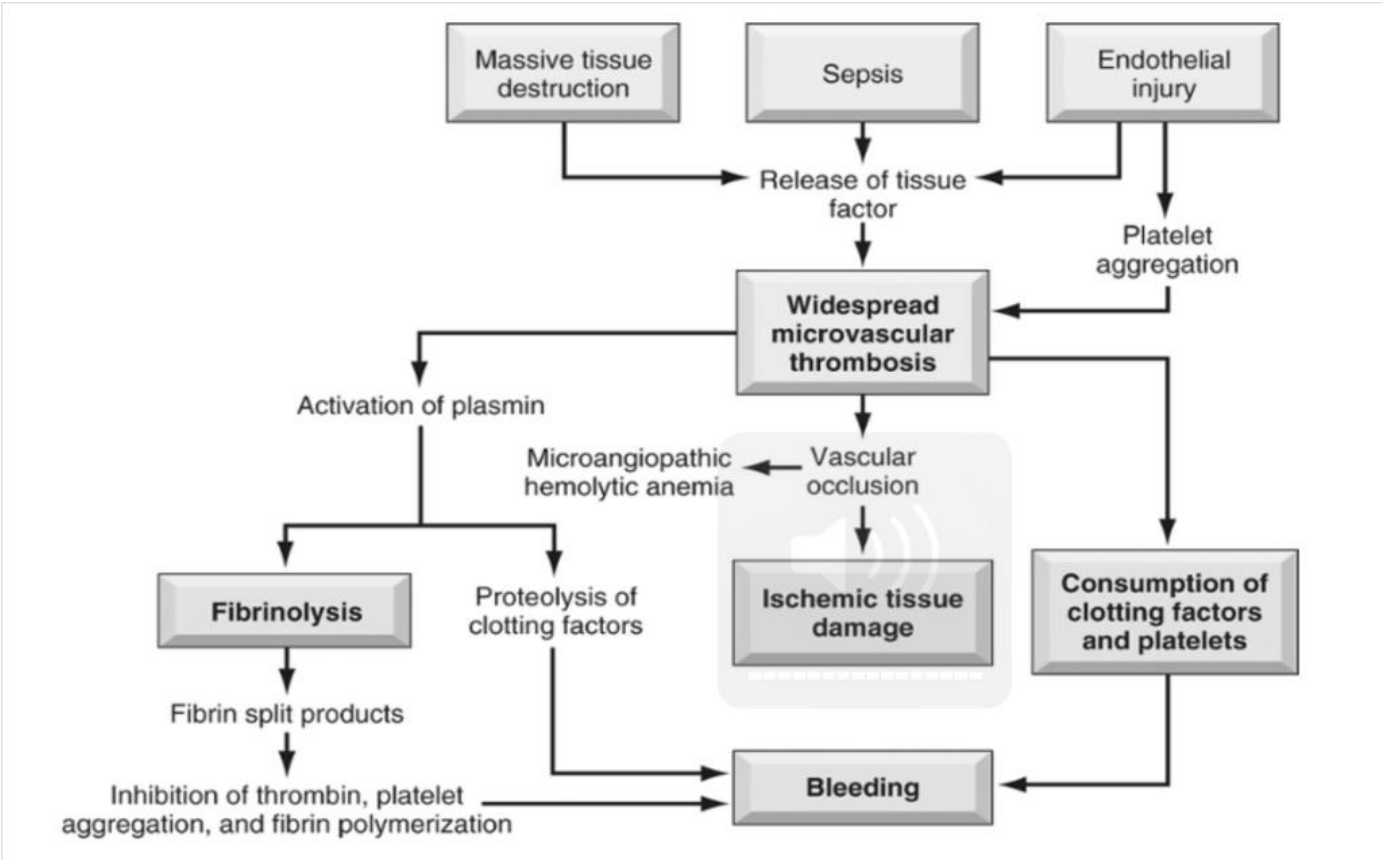
2- *Cancer*
APL (acute promyeloplastic leukemia) and adenocarcinomas specially pancreatic adenocarcinomas causing DIC by:
- Releasing proteolytic enzymes
- Releasing tissue factor

3- *bacterial sepsis*
Endotoxins damage the endothelial cells activating the monocytes that release TNF, tissue factor, IL1. End results of monocytes activation is both increase tissue factor and decrease thrombomodulin (antithrombotic mechanism to limit the clot).

4- Deposition of antigen-antibody complex such as in SLE.
5- Extremes of temperature (either heat or cold).
6- Major trauma such as severe head trauma, burns, severe limb injury.

Mechanism of DIC:

Whatever is the cause (sepsis, endothelial injury, massive tissue trauma), the end result is massive release of tissue factor which cause widespread thrombosis which means also activation of plasmin that causes thrombolysis which releases FSP (sensitive for DIC as mentioned previously), FSP release worsens the situation by inhibition of fibrin polymerization so continued bleeding, also FSP causes proteolysis of factor V and VIII thus bleeding that is unable to counteract by clotting, in addition to the consumption of clotting factor. All of this will result in the formation of micro thrombi and microangiopathic hemolytic anemia (Remember we see schistocytes under the microscope) and can trigger ischemia and ischemic changes during the surgery.



Consequences of DIC:

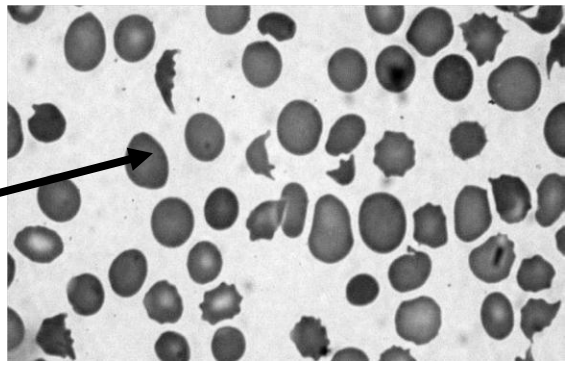
1- *Widespread micro thrombi and fibrin deposition*
   – Ischemic infarcts
   – Microangiopathic hemolytic anemia

2- Bleeding tendency due to
   – Consumption of platelets and clotting factors
   – Widespread activation of plasmin which cleaves factors V and VIII further decreasing their concentration in the blood

3- – Release of FDP which inhibit platelets and fibrinogen polymerization

Morphology of DIC:

- Intravascular hemolysis with schistocytes
- Micro thrombin in the small vessels and capillaries



Clinical manifestations of DIC:

- Variable
- Could be acute (as in postpartum DIC) or chronic (as in cancer)
- Can be minimal or severe

(Shock, acute renal failure, dyspnea, cyanosis, convulsions, coma and death).

Lab Findings in DIC:

–Low platelets due to increased consumption
–High PT and PTT due to decrease clotting factors
–Elevated FDP

- Prognosis depends on the severity of presentation and the underlying condition
- Treatment with heparin or replacement of coagulation factors (directed at the underlying condition.

Thrombocytopenia

- Low platelet count
  –Less than 15000

- Bleeding occurs when platelet count between 20000-50000
  –20000-50000 posttraumatic bleeding
  –Less than 5000 spontaneous bleeding

- Mucocutaneous bleeding
  – If thrombocytopenia is severe enough, the risk of Brain hemorrhage is high and it's a major factor for mortality

⟹Thrombocytopenia due to either decreased production or decreased survival, also certain medications are an important risk factor.

Types of Thrombocytopenia:

1- Immune Thrombocyopenic Purpura (ITP)

- Same as immune hemolytic anemia; antibodies usually IgG attacking antigens on platelets coating them then they are destroyed by the spleen as it can detect anything coated.
- Antigen is usually attacked are IIb/IIIa (for fibrin) and Ib/IX.

- Spleen causes damage to those antibody coated platelets.
  - Benefits of splenectomy (respond nicely)
- **Treatment:** Steroids, immunosuppressive therapy or splenectomy.

We have 2 clinical syndromes caused by ITP:

1- Acute: children after a viral infection, self-limiting (we can give the patient platelets if severe).

2- Chronic: affects women 20-40 years of age, has nothing to do with viral infection.

NOTE: *Patient with petechial hemorrhages and normal RBC count, normal Hb, severely depressed platelets; simply by CBC, you can confirm that it's likely not leukemia since it usually causes pancytopenia.*

Clinical presentation of ITP:

Mucocutaneous bleeding, Brain hemorrhage is rare

Lab Findings

– Low platelets
– Normal PT
– Normal PTT
– normal platelets function
–Bone marrow shows megakaryocytic hyperplasia since the bone marrow is trying to compensate.

2- *Heparin induced thrombocytopenia (HIT)*

- It is the only thrombocytopenic disease that doesn't cause bleeding but thrombosis.
- Occurs in 3-5% of patients using unfractionated heparin (Heparin can be unfractionated or LMWH and its given in the hospital as IV to the patients with venous thrombosis not arterial (stroke)
- Develops after 1-2 weeks of therapy
- Results in thrombosis associated with low platelets
- IgG antibodies against platelet factor 4 (PF4) that presents on the platelets resulting in their activation thus thrombosis and platelets consumption
- Cessation of heparin breaks the cycle
- Use of low molecular weight heparin has less risk of developing HIT

So the problem and the risk of HIT is thrombosis in a patients already seek treatment for thrombosis.

3- *Thrombotic Thrombocytopenic Purpura (TTP)* has 5 clinical findings:
–Fever
–Thrombocytopenia
–Microangiopathic hemolytic anemia
–Neurological manifestations
–Renal failure

4- *Hemolytic Uremic Syndrome (HUS)* has 5 clinical findings:
–Fever
–Thrombocytopenia
– Renal failure
– NO Neurological manifestations
– Frequently in children secondary to E. coli infection

NOTE: *Difference between TTP and HUS is the neurological symptoms (in the case of TTP) and E- coli infection in children (in the case of HUS) BUT both has widespread micro thrombi.*

NOTE: *TTP and HUS are different from DIC as they show NO significant consumption of clotting factor and Normal PT and PTT. (TTP & HUS are thrombocytopenic disorders with NORMAL PT &PTT).*

Coagulation disorders

- Hereditary or acquired (acquired is more common than hereditary).

- *The most common causes of acquired clotting factor deficiency:*

1- Vitamin K deficiency in newborns (should be given Vitamin K injection after birth to prevent bleeding) and patients with fat malabsorption such as cystic fibrosis.
- Vitamin K deficiency affects clotting factors II, VII, IX, and X.

2- Liver disease since all clotting factor are synthesized by the liver except vWF which is synthesized by the endothelial cells.

3- DIC due to its massive clotting factor consumption.

- *The most common Hereditary clotting factor deficiency:*

1- Von-Willebrand disease
2- Hemophilia A
3- Hemophilia B

NOTE: *As mentioned previously platelets and vessel bleeding is mucocutaneous and clotting factor deficiency causes deep bleeding EXCEPT von willebrand disease. WHY!! (Mentioned below)*

- *Von Willebrand Factor has 2 functions:*
  1- It adheres to the platelets in the cases of injury (vWF is subendothelial when the injury occurs it will be exposed to the platelets thus bind to them by Ib platelets antigen.
  2- It protects factor VIII (found in blood) from being degraded.

So its deficiency cause dysfunction in platelet adhesion and factor VIII deficiency BUT platelets dysfunction is more important and that's why vWF deficiency causes mucocutaneous bleeding rather than deep bleeding.

1- Von-Willebrand disease
- Autosomal dominant
- Mucocutaneous bleeding
- The most common inherited bleeding disorder
- 1% of the US population (3 million or more are affected)
- Frequently asymptomatic and under recognized
- symptoms can be severe or minimal
- specific study to diagnose (platelets aggregation study in which ristocetin – similar to vWF – is added to the platelets resulting in no response).

Lab Findings:
- Normal platelet count
- Abnormal platelet aggregation studies
- Elevated PTT due to factor VIII deficiency (since vWF protects factor VIII)
- Normal PT

2- Hemophilia A
- Deficiency in factor VIII
- More common than Hemophilia B
- X-linked disorder affects males
- Deep muscle and joint hemorrhage
- Deficiency can be either quantitative (low level of factor VIII) or qualitative (normal

level with abnormal function).
- Most cases are associated with low factor VIII level
–10% are associated with normal level but reduced activity

Lab Findings:
–Normal platelet count and function
–Normal PT
–Elevated PTT that corrects with mixing studies

3- Hemophilia B
- Also called Christmas disease
- Factor IX deficiency
- Deep muscle and joint bleeding (hemarthrosis)
- X-linked disorder
- Affects males
- It is less common than hemophilia A

Lab Findings:
- same as hemophilia A

•It is important to distinguish between Hemophilia A and B (different treatment) by doing specific assays (antigen assay) for factors VIII, and IX.

- Treatment for both Hemophilia A and B is achieved by the replacement for the deficient factor.

Examples for the Exam Question mentioned throughout the lecture:

- 10 years old child develop petechiae over his body, his lab results showed normal platelet count, function, PT and PTT, which of the following is the most likely diagnosis?

1- DIC 2- Vitamin K Deficiency 3- Thrombocytopenia 4- Vasculitis

Key point to answer *is to know that all his bleeding tests are normal so the problem is structural in blood vessels.*

- 30 years old patients came to the hospital complaining from fatigue, weakness, shortness of breath, CBC revealed pancytopenia, bone marrow examination showed

50% blast cells, genetic tests showed t(15;17). After 3 days of treatment in the hospital, patient suffered from severe shock, coma, acute renal failure. Histological examination showed several microthrombi. Which one of the following is expected to be found in his bleeding lab studies?

Whatever the choices were, the key point to answer *is to know that the patient suffers from acute promyelocytic leukemia (t(15;17)) , acute leukemia (50% blasts) , DIC (shock, coma, acute renal failure and microthrombi) so you have to know about DIC.*

- Female patient suffered from Deep Vein Thrombosis, ***uFH*** has been prescribed for her, after 10 days she starts complaining from sever leg pain, ultrasonography showed thrombosis, severe infarction. What is the diagnosis? HIT

- 5 years old Child has overwhelming sepsis by E- coli, Lab results showed decrease platelets count but normal PT and PTT. What is the diagnosis?

  1- DIC 2- ITP 3- HUS

Key point *is to remember that DIC has abnormal PT and PTT while HUS has normal PT and PTT*.

- 15 year old patient develop petechiae and ecchymosis in the skin and mucous membrane, Lab results showed normal platelet count, normal PT, elevated PTT. What is the diagnosis?

Key point to answer *is to know that the only mucocutaneous bleeding with normal platelets count and elevated PTT is vWF deficiency.*