Hematology

- Histology
- Biochemistry
- **Pathology**
- Pharmacology
- Physiology
- Microbiology

- Handout
- Slide
- Sheet 2

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  2
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(وَأَنْ لَيْسَ لِلْإِنْسَانِ إِلَّا مَا سَعَى * وَأَنَّ سَعْيَهُ سَوْفَ يُرَى)

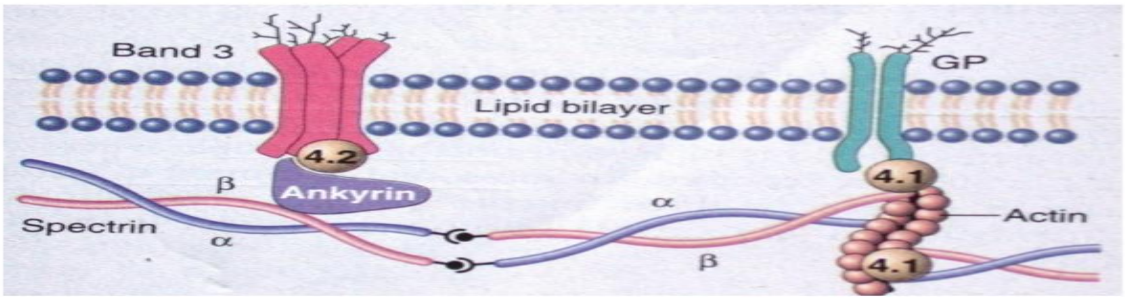
HEMOLYTIC ANEMIAS

* This sheet was written according to section two's record . Four hemolytic diseases will be discussed . Hope you'll find it an easy one ☺

Let's start !

- 1st disease : Hereditary Spherocytosis :

** Etiology : Results from a frameshift mutation in a gene that encodes structural proteins inside RBC membrane . Normal RBC cell membrane has structural proteins that keep the integrity of the cell membrane (the cytoskeleton) . In this disease , there is a mutation in genes encoding one of these proteins **(the vertical ones) .** These proteins are **: spectrin (alpha or beta unit may be affected) , ankyrin , band 4.2 and band 3** . The mutation may affect one or more of these proteins .



** Remember from PBL lecture that symptoms vary in each of the diseases we are concerned with from mild to severe . The same concept applies here as follows :

- Total loss of one protein or more ⟶ Severe symptoms .
- Abnormal protein (protein is still present but it is not at its perfect / complete state) ⟶ Mild symptoms .

** Inheritance : The disease is inherited as AD (autosomal dominant) . There's 50% chance to transmit the disease for each sibling .

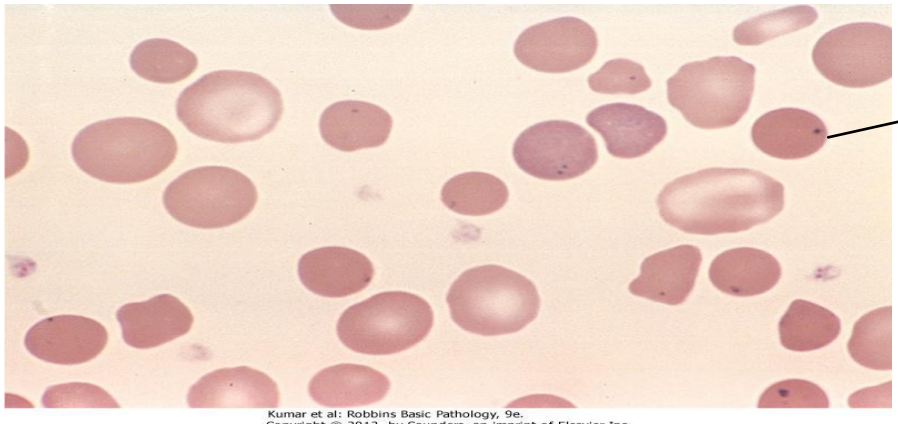
**Prevalence : The disease is common in North Europe (Not common in our area).

** Age – specificity : The disease is not age-specific . It can appear early in life or later on according to the severity .

** Pathogenesis : Loss of structural protein(s) ⟶ abnormal **weak** cell membrane ⟶ while RBCs are moving , any minor physical trauma can cause **cell membrane loss** ⟶ with time , many bubbles are lost from the cell membrane ⟶ normal morphology of RBCs changes , they are no longer

biconcave , they become **spherical** (resembles a ball) i.e. they become **spherocytes** which is **abnormal** and hence the name of the disease . **Histiocytes** (spleen macrophages) identify these spherocytes and take them out , this causes ⟶ **EXTRAvascular hemolysis** .

** One point that you should keep in mind : After an RBC has undergone this abnormal alteration (from normal to spherocyte) , it becomes smaller **(Low MCV).** However , what's inside the cell (Hb content) is not affected **(Normal MCH) .**
Hb stands for hemoglobin , MCV stands for Mean Cell Volume and MCH stands for Mean Cell Hemoglobin . I'll be using these abbreviations till the end of the sheet .



This is a spherocyte . Notice that it's smaller than normal and hyperchromatic . The small blue dot inside is what we call (Howell-Jolly body) .

** Explanation of morphology : In a blood film , a lot of rounded RBCS (spherocytes) are seen . They appear **smaller than normal** (lower-than-normal MCV) and **hyperchromatic** (no central pallor) . Another feature is the appearance of **Howell-Jolly bodies** which are small looped dots that appear after splenectomy . These dots indeed are DNA remnants. Remember that normal RBCs do NOT have DNA , if there's DNA in an RBC , the spleen identifies it (the DNA) and takes it out . Since the spleen is the site of destruction of abnormal RBCs which causes extravascular hemorrhage , the only treatment for this disease is to correct the anemia by **splenectomy (spleen removal)** , that's why Howell-Jolly bodies appear markedly after splenectomy (Spleen is not there , so DNA remnants will not be taken out of RBCs and they'll stay) . **The remnants of DNA of RBCs after spleen has been removed form Howell-Jolly bodies .**

- *In another words ; (for clarification) :*
  Howell-Jolly bodies are nuclear remnants which are present in some normal red cells in the bone marrow but are removed or 'pitted' by the spleen during the first few hours the cells spend in the circulation, so absence of splenic function, either following splenectomy, or occasionally due to splenic atrophy, results in the appearance in the peripheral blood of red cells containing Howell-Jolly bodies.

** MCHC = Ratio of MCH/MCV (Ratio of weight/size)

We said previously that in this disease , MCH is normal as cell content of Hb is not affected , and MCV will be small , this results in **high MCHC value** (ratio) and this is a numeric indicator for hereditary spherocytosis .

** Another indicator for the disease is **high reticulocyte count** since it's extravascular hemolytic anemia ; reticulocytes will increase in an attempt to compensate RBCs loss .

** Spherocytes have abnormal shape and are fragile (weak , break easily) . A test is done to check for this fragility (an important **diagnostic** tool) which is **osmotic fragility test .** In this test , hypotonic solution is added to blood so that water goes inside RBCs . Normal RBCs can tolerate water entry into them to a certain level before they blast (break) . Due to their fragility , spherocytes cannot tolerate water entry , they lyse before normal cells do .

Early lysis of RBCs in osmotic fragility test indicates they're spherocytes . This occurs in hereditary spherocytosis and in another disease that also gives spherocytes (discussed later) .

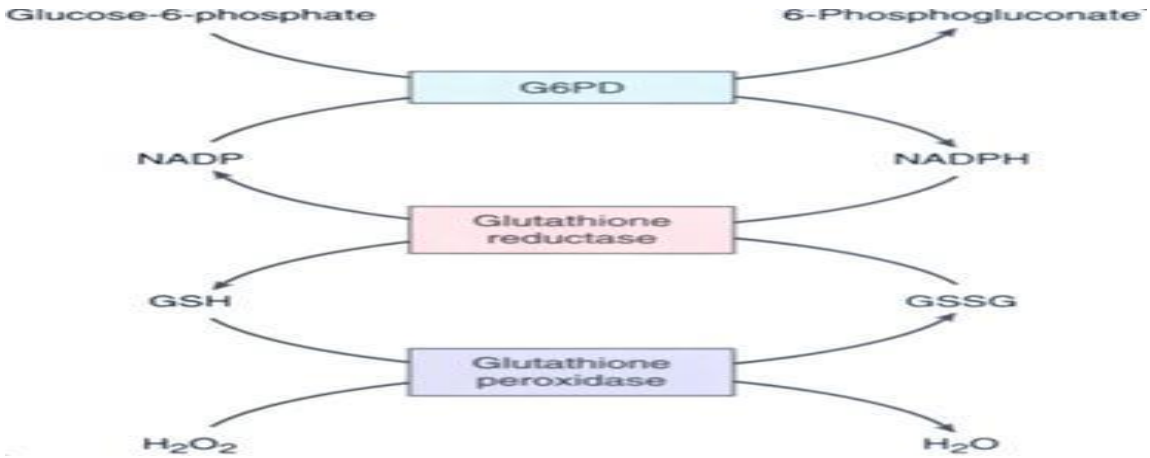
** **Family history** is important . Because this disease is AD (50% transmission chance to a sibling) , you'll mostly find another person in the patient's family having the disease .

** Clinical coarse (signs and symptoms) is **very variable** according to the severity of the mutation . Signs and symptoms are those of extravascular hemolytic anemia including anemia , jaundice and splenomegaly . The disease can appear early in life (neonatal onset) and sometimes it appears late (adult onset) .

** Treatment : **Splenectomy** (Abnormally shaped RBCs will go and there'll be no anemia) .

- **2nd disease : Glucose-6-Phosphate Dehydrogenase Deficiency :**

** A metabolic disease (deficiency in an enzyme in RBCs which is G6PD).



From the chart above , notice the two functions of G6PD in RBCs :

First : It's involved in **glycolysis** . Actually , G6PD is the main enzyme in glucose metabolism in RBCs . It converts glucose-6-phosphate to 6-phosphogluconate . Remember from biochemistry that though oxygen is there , glycolysis in RBCs is only anaerobic due to the absence of mitochondria . *This function of G6PD is not related to anemia pathogenesis .*

Second : *This is what we are concerned with when talking about anemias .* **conversion of NADP to NADPH .** NADPH is involved in the production of glutathione (GSH) which is important for neutralizing free radicals . Free radicals are toxic molecules that are generated inside the cells so that they must be neutralized by other molecules , otherwise they cause injury to cell structure . The function of glutathione here is to neutralize H2O2 (Hydrogen Peroxide) which is harmful to cells . Following the sequence of reactions above , you can conclude **that G6PD deficiency results in generating large amounts of the toxic compound hydrogen peroxide .** Hydrogen peroxide causes injury to RBCs .

** Etiology : This disease is **inherited .** Mode of inheritance is **X-linked recessive** (one mutated copy is enough to cause the disease) , that's why most patients are males as they have only one X chromosome (higher chance for disease symptoms to appear , no carrier state) . Females have 2 X chromosomes , if one is mutated and the other is normal , the normal one will be sufficient to achieve the function in producing enough amounts of G6PD . The female in this case is carrier but asymptomatic .

Sometimes , normally **, random X inactivation** in females occurs . In this case, one X chromosome will be inactivated in some cells (these cells are normal as we explained) , while the other X chromosome will be also inactivated in other cells (both chromosomes are inactivated , these cells are abnormal expressing the disease) . Since not all RBCs are affected but only some of them , symptoms will appear but mildly .

So this disease is very common in males . Females can be affected but with less severity and milder symptoms .

A student asked if another enzyme deficiency (other than G6PD) in the sequence of reactions we mentioned can cause the same result . The doctor said yes but it is very rare, usually it is G6PD that becomes deficient .

** Prevalence : This disease is common in our region (The Middle East) and also in Africa .

** **Normal G6PD is called G6PD –B .** Mutations result in different enzymatic abnormalities in quantity and/or quality of the enzyme ; the enzyme could be totally absent , it could be present but with abnormal function , etc., that's why G6PD variant is designated by a specific letter in each case for differentiation as follows :

G6PD-A : A stands for Africa where this type of deficiency is common . Here the enzyme is deficient ; **quantity is low** (less than normal but not zero) with different structure but it's still functioning , so disease symptoms appear every now and then **(not that severe) .**

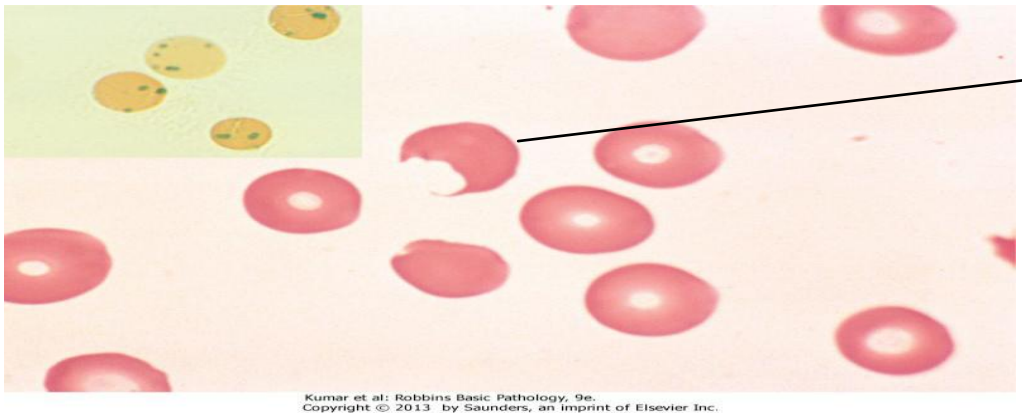
G6PD-M : M stands for Mediterranean Region where this type of deficiency is common . The enzyme is present in **normal quantity** but it's not functioning **(poor quality)** so that symptoms here are **more severe , blood transfusion is needed .**

** Pathogenesis : Though G6PD is present in all mammalian cells , the disease will mostly affect RBCs markedly with high potency/severity , why is that ? This is because RBCs are **non-nucleated** . Other mammalian cells are able to synthesize G6PD once it becomes deficient since they have nuclei (DNA) that's able to code for the deficient protein/enzyme and synthesize it again . RBCs lack nuclei so they lack this ability . They have to adapt to live with the limited amount of enzymes they're released with . Remember that RBCs have long life-span (120 days) compared to life-span of other cell types , so they are more prone to lysis and damage . When RBCs are released from bone marrow where they're synthesized , they have limited amount of G6PD to live with . With time , RBCs lose this amount gradually , so logic wise **; RBCs that lyse due to G6PD deficiency are the older (aged) ones .** Newly formed RBCs still have enough amount of G6PD , they're less prone to lysis due to G6PD deficiency . This is important to understand some features of the disease .

** Degree of deficiency : Normally , RBCs have extra reserve due to their long life-span , that's why G6PD deficiency lytic symptoms appear when G6PD is below 20% of normal amount **(20% is the cutoff point for lytic symptoms to appear) .** From this we conclude that **symptoms vary** (mild-moderate-severe) according to the degree of deficiency . For example , a person with 50% degree of deficiency is probably a carrier of the mutation , though he's asymptomatic .

** What exactly occurs ? Hydrogen peroxide is a free radical , it has extra electrons (high energy) that's why it moves fast . While moving , if it strikes any cellular structure , it damages it . Hydrogen peroxide increases due to many causes including increase in oxygen for any reason (as H_2O_2 is a metabolite of O_2) . It also increases due to G6PD deficiency as mentioned .

Increase of H2O2 due to any cause alters the normal configuration of globin chains in Hb , these chains become **more condensed** and the cell in that region will be harder than normal . These condensations are visualized by a special stain **(crystal violet , supravital stain)** as bodies known as (Heinz Bodies) **so Heinz Bodies are altered condensed globin chains secondary to G6PD deficiency .** When RBCs containing Heinz bodies reach the spleen , histiocytes are able to sense the bodies as they're hard . They take them out **(pinch/bite them) only** (not the whole RBC is taken by histiocytes) . RBCs look as if they were bitten in a blood film (**diagnostic tool** when G6PD deficiency is suspected).



** Crisis : As mentioned previously , hemolysis occurs in old-aged RBCs (not in new ones) . G6PD deficiency patients live normally as long as metabolism is normal when there's acceptable amount of G6PD . However , sometimes crisis occurs , why ?

1) If we have **high amount of oxygen** due to any cause (this definitely leads to production of high amount of hydrogen peroxide that causes RBC lysis) . Metabolism of some drugs leads to production of free radicals including hydrogen peroxide , these drugs include : Sulfonamides (antibiotics) , antimalarial drugs , vitamin K and large aspirin doses . Deficiency in this case is reversible ; after stopping the drug , patient will get back to normal .
2) **Infections** (especially bacterial ones) . When there's a bacterial infection, neutrophils destroy the bacteria by a special enzyme found in their lysosomes which is myeloperoxidase (lysosomes in this case are high in number in order to fight the bacteria efficiently) . The problem is that myeloperoxidase produces oxygen so there'll be increased O2 and thus increased free radicals production . This eventually leads to cell lysis . Deficiency here is also reversible and things get back to normal when infection goes away .
3) Diet also plays a role ; **fava beans** produce increased amount of oxygen . يدعى المرض في هذه الحالة (التفول) .

Under the effect of these causes , symptoms **will not appear instantaneously** after exposure to the initiating factor . **It takes 1-2 days** till metabolism occurs and crisis symptoms appear . For example , if a patient eats fava beans , crisis won't appear in an hour , that's why when **taking history** of a patient , you should ask about the few previous days and not only about the few previous hours . Symptoms usually depend on the causal factor .

*** A student asked if a person with GDPD deficiency due to drug intake can eat fava beans and the doctor said yes .*

** Severity and symptoms : Usually it's **severe** as it's **INTRAvascular hemolysis** ; Heinz bodies can break cell membrane and cause cell lysis . Symptoms are those of intravascular hemolysis including hypoxia all over the body and met-hemoglobinemai which lead to bone pain , red urine , etc.

** Diagnosis : In addition to morphologic changes , enzyme assay is done to check for its quality and quantity . NADPH is measured , if G6PD is deficient or abnormal , NADPH won't be produced . **When do we test the enzyme ?**

It is wrong to do the test immediately after the crisis onset because after hemolysis bone marrow produces large amount of new functional RBCs with good amount of G6PD so deficiency won't be detected . What we should do is to wait awhile (one month perhaps) till the patient becomes stable i.e. till defective cells become old-aged with decreased amount of G6PD so that deficiency can be detected .

- 3rd disease : Paroxysmal Nocturnal Hematuria (PNH) :

** Though the name of the disease is horrible :p , it is not very severe . Let us analyze the name :

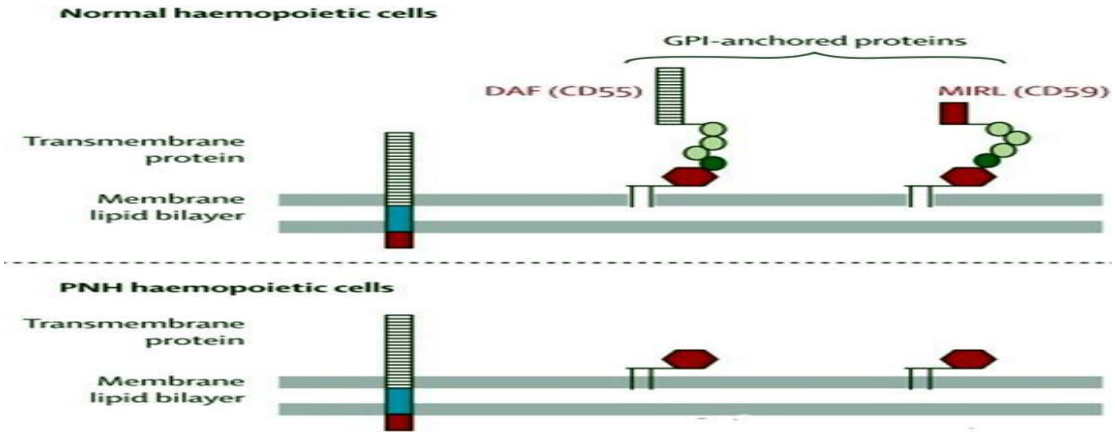
Paroxysmal ⟶ sudden onset .

Nocturnal ⟶ at night .

Hematuria ⟶ blood in urine .

So the disease appears suddenly , it is characterized by hemolysis that occurs at night .

This disease is **acquired (not inherited) . Remember from the previous lecture that causes of hemolytic anemia are either extrinsic (such as malarial infection) or intrinsic in which the defect is in the RBC itself . **All intrinsic causes are hereditary except in paroxysmal nocturnal hematuria ,** which is acquired intrinsic hemolytic disease .



** What occurs in the disease ? A **mutation in the gene** phosphatidylinositol glycan group A **(PIG-A)** occurs . This gene encodes cell membrane protein which is glycosylphosphatidylinositol **(GPI)** . GPI (red in the figure above) is a structural protein that's found normally in cell membrane of all hematopoietic cells and not only RBCs (it's found in WBCs , RBCs and platelets) . It is an anchoring protein i.e. its function is to carry another proteins . The 2 proteins we are concerned with in this disease are **CD55 and CD 59** (numbers are important) . They're anchored by GPI . Another names for these proteins (not very important) :
DAF (Decay-accelerating factor) for CD55 and MIRL (membrane inhibitor of reactive lysis) for CD59.
These 2 proteins function normally to antagonize (neutralize) complement system . So what's the complement system ? The complement system refers to proteins (not cellular elements) that move inside blood plasma . These proteins are involved in defense against infections . Normally , if there is no infection , the complement system is inert (not active, not functioning) . If an infection arises , complement system proteins are activated, they adhere to each other on the cell surface of micro-organisms and start to make pores in their cell membrane (like stabbing wounds) until the fluid inside the cell goes out which leads to micro-organism's lysis and death .

How do WBCs , RBCs and platelets protect themselves from complement system ? by CD55 and CD59 .

In PNH , GPI is deficient ⟶ CD55 and CD59 are no longer present on cell surface ⟶ protection against complement system is lost .

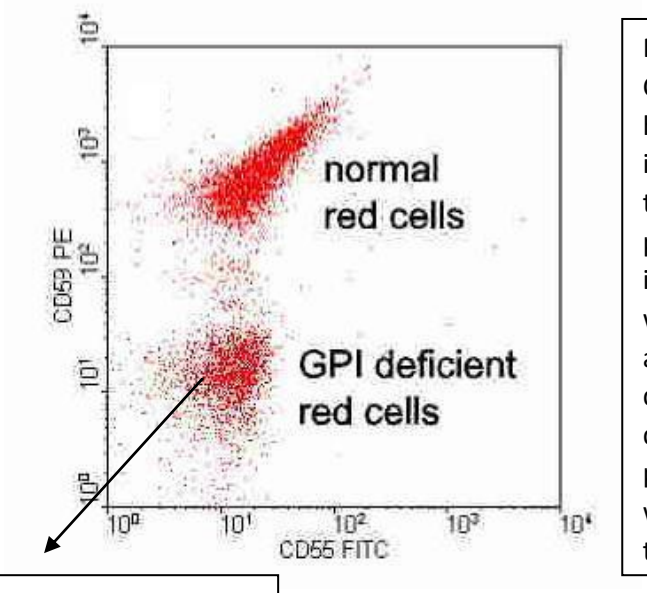
** This mutation occurs in stem cells (in early cells , not in mature cells) in the bone marrow . Since it is acquired , not all stem cells are mutated (some

cells acquire the mutation , others don't) so the mutation may be present but the disease isn't active (normal person , asymptomatic) . The disease becomes active (symptoms appear) if the mutated stem cell becomes predominant (when other stem cells die for some reason) . In this case , larger number of hematopoietic cells carrying the mutation will be produced , CD55 and CD59 will be absent and symptoms appear .

** How do CD55 and CD59 inactivate the complement system ? **By inhibiting C3-convertase** that's necessary for activation of the complement system .

Deficiency in CD55 and CD59 ⟶ activation of C3-convertase ⟶ activation of complement system ⟶ tri-lineage cell lysis (3 cell lines are affected ; RBCs , WBCs and platelets resulting in anemia , neutropenia and thrombocytopenia respectively) .

** Diagnosis : A special test is done . It's called **flow cytometry test** . In this test , viable (alive) blood cells are taken , then antibodies against CD55 and CD59 are added to them , shadows of antibody-protein reactions can be seen .



Seen in PNH patient .

Notice the figure , the vertical line represents CD59 antibody intensity whereas the horizontal line represents CD55 antibody intensity . Normal cells have high amounts of these two proteins i.e. positive antibody-protein reactions will be noticed with high intensity values (around 1000 for CD59 that's why the shadow is directed upwards and around 100 for CD55 that's why the shadow is directed to the right) . In contrast , GPI deficient cells have low amount of both proteins (intensity is around 10 only) that's why the shadow is directed downwards and to the left) .

** Clinical features vary from patient to another . Though the name of the disease indicates sudden onset (i.e. acute pattern) , clinically , **only ¼ cases (25%) have acute symptoms** ⟶ patient wakes up at night suffering from **severe pain** , blood appears in urine .

The predominant pattern of the disease (75% of cases) is **chronic intravascular hemolysis** . In chronic cases , symptoms are **milder** but persistent . As mentioned before , patients will have anemia , neutropenia and thrombocytopenia . Neutropenia makes the patient more prone to infections (reduction in immunity occurs) . **Thrombocytopenia is important** in this

disease. **Although platelets are low , patients usually have thrombosis !** This is a characteristic of PNH .

How come ? Usually , thrombosis occurs in accompany with thrombocytosis which is increased platelets count . In this case , thrombosis occurs although the number of platelets is low because when platelets lyse , their contents are released . These contents include proteins that promote thrombus formation such as **Thromboxane A2** .

** As you know , one of cancer cells features is their tendency to acquire many mutations . **In 10% of PNH cases** , stem cells acquire more mutations and progress into **neoplasm** (MDS , AML / will be discussed later) .

** Note about the complement system **: Complement system is activated efficiently when there's acidosis** . In infections , acidosis is increased that's why complement system is highly activated .

** A student asked about a case in which a person who has acquired the disease works at night and sleeps during the day . Symptoms in this case will appear during sleeping time (i.e. during the day) . Time is not the issue , the idea is that during sleeping , respiration is decreased resulting in high amounts of CO_2 in the body as a medium , this leads to increased acidosis and thus increased activity of the complement system and accordingly symptoms appear. The disease is named nocturnal since most people sleep at night :p

** Another student asked about patient's age . Since the disease is acquired , it usually appears later in life and not during childhood .

- 4th disease : Thalassemia :

فقر دم البحر الأبيض المتوسط – Mediterranean anemia

** It's not a single disease . It's a big **spectrum**/group of many disease . They're very common and important . Thalassemia is **inherited** .

** Etiology : The disease is caused by a **mutation** that's transmitted (inherited) in **an autosomal recessive** mode .
Adult Hemoglobin (Hb A) consists of 2 alpha globin chains and 2 beta globin chains . Thalassemia means deficiency in Hb A .The problem is that **red bone marrow can't synthesize HB A in normal amount .** The problem can be either in :
Alpha chains ⟶ alpha thalassemia **(deletion mutation)** or in
Beta chains ⟶ beta thalassemia (**point mutation** , one nucleotide is changed) .

** Prevalence : Endemic areas are the Mediterranean Region , Middle East , Tropical Africa , India and South-East Asia . So it's very common globally .

** 2 genes encode beta globin chain while 4 genes encode alpha globin chain (this is the total number of genes from the 2 alleles) . Depending on this , **alpha thalassemia is milder** because more genes encode the chain , if one is lost , the three residual genes can still encode the globin chain . Because beta chain has only two encoding genes , beta thalassemia will be more symptomatic .

** Clinical classification : The spectrum of thalassemia diseases vary from asymptomatic-mild-moderate-severe-very severe .

** Molecular classification of beta thalassemia :

1) Beta thalassemia **minor** : **loss of one gene** , the other gene functions normally , patient is **asymptomatic carrier** of the disease .
2) Beta thalassemia **major** : **total loss of the 2 genes** , patients will have anemia very early in life , blood transfusion is needed since infancy . The disease here is **very severe** .
3) Beta thalassemia **intermedia** : In the middle between the 2 previous cases. Sometimes , one gene is lost , in other cases , the 2 genes are affected but the mutation is not that severe . The quantity of the beta chain produced is somehow good/enough so symptoms appear but mildly , no regular need of blood transfusion .

** Molecular classification of alpha thalassemia :

1) If **one gene is lost** , the person will be totally normal because the other three genes are able to produce enough amount of alpha globin chain . The person here is a **silent carrier (asymptomatic)** . RBCs are slightly smaller than normal but their function is good so there will be no anemia. A problem appears if two silent carriers get married . It's expected in this case that more severe forms of thalassemia appear in the siblings. For this reason , a **pre-marital test** for thalassemia is done .In this test , MCV is checked , If it is slightly decreased then this person is probably a silent thalassemia carrier .
2) If **two genes are lost** , alpha thalassemia **minor** results . There'll be very mild anemia that doesn't affect life quality .
3) If **three genes are deleted** , there'll be only one functional gene that produces very few amount of alpha globin chains that bind beta chains later to form very few amount of Hb A . In this case , a specific disease **(Hemoglobin H disease)** appears . Because alpha globin chains are extremely low , there'll be excess beta and gamma chains that are still working and appear early in life . 4 beta chains combine to form a

tetramer , similarly, 4 gamma chains combine to form a tetramer . **These tetra-gamma molecules are referred to as Hb Barts .** Hb Barts can be measured to know if the disease is there (**diagnostic** feature for the disease). Symptoms are intermediate , the patient will have chronic persistent symptomatic anemia but there's no need for blood transfusion .

4) If the **4 genes are deleted** , there'll be no alpha globin and thus no Hb at all . This is alpha thalassemia **major** . Patients in this case **die in utero (born dead) .**

Remember : Hb A ⟶ **2 alpha** + 2 beta
Hb A2 ⟶ **2 alpha** + 2 delta
Hb fetal ⟶ **2 alpha** + 2 gamma
(All Hb types require alpha chain)

So it's clear that if there's no alpha globin , none of hemoglobin types can be produced .

** Symptoms of thalassemia in symptomatic cases appear after the age of **six months** because fetal Hb is active in the first 6 months of life . It starts to decrease after this age and when this occurs , symptoms start to appear .

** Pathogenesis (will be repeated and cont'd next lecture) : Very important , a lot of mechanisms and symptoms are involved , you should know how it occurs stepwise .

Due to decreased production of Hb A , RBCs don't have enough amount of Hb that's why they appear smaller in size and paler **(hypochromic microcytic anemia)** . This anemia causes **hypoxia** that's not corrected **(life-long , persistent) .**

In **Beta thalassemia** , the mutation results in decreased copies of beta globin and relatively increased copies of alpha globin with each DNA replication . Decreased beta globin leads to **decreased Hb A** and eventually to hypoxia . **Excess alpha globin** also causes problems because excess alpha chains bind delta chains which results in **increased amount of Hb A2** . They also bind to gamma chains which results in **increased fetal Hb .**

Excess alpha chains that are altered by combination with other chains cause diseases . They are abnormal , they combine and alter the normal structure of RBCs , this eventually leads to **RBCs hemolysis** and this is the mechanism of hemolysis in thalassemia . So thalassemia is anemia secondary to decreased amount of Hb in RBCs but there's another factor that promotes anemia which is the significant hemolysis . **Again , hemolysis in thalassemia is due to excess globin chains that accumulate in RBCs .** These RBCs are identified in the spleen and lysed there which is known as extravascular hemolysis . In addition , intravascular hemolysis might occur if the amount of excess globin

chains in RBCs is very high . Also , they might die early in bone marrow before going to the circulation **. So thalassemia patient has combined intra- and extra- vascular hemolysis .**

Chronic hypoxia in thalassemia major and intermedia stimulates erythropoietin . There's **persistent increase in erythropoietin** in these patients ⟶ persistent bone marrow activation (bone marrow is always active) ⟶ a lot of **normoblasts** which are nucleated RBC precursors are produced . These normoblasts are not functioning , the process is called **ineffective erythropoiesis.**

AND THE DOCTOR STOPPED HERE !

Feel free to give me your feedback if you find any mistake or if you have any comment ☺

Don't forget me from your Duaa .

GOOD LUCK !