

Hematology Pathophysiology Notes

Hematopoiesis

- occurs in bone exclusively
- all cellular elements are derived from the PPSC – it retains the ability to both replicate itself and to differentiate
- Types of differentiation determined by the influence of various cytokines
 - o GM-CSF, TPO, EPO, IL-3, IL-2 etc.
- The Erythroid progenitor line
 - o PPSC → Pronormoblast (this step takes 5-7 days)
 - o Pronormoblast → Basophilic normoblast (start to see condensing of nucleus, crumping of chromatin) → Polychromatophilic Normoblast (decreasing RNA in the cytoplasm shows less, lighter blue) → Orthochromatophilic Normoblast → Reticulocyte → Mature RBC
 - These steps, from Pronormoblast → Reticulocyte takes about 5-7 days
 - o Total 10-14 days for production
 - o Reticulocyte counts give you an idea of how hard the Bone marrow is working to pump out the RBCs
 - Stains more blue than RBC and still may have small amounts of RNA present in them
 - Undergoes removal of RNA on passing through the spleen on 1st day of life
 - can be detected using supravital stain
- RBC properties
 - o Anucleated, highly flexible, biconcave disks, 80-100 fl in volume
 - o Flexibility is essential for passage through the capillaries
 - o Carries O₂ to and CO₂ away from cells
 - o ~ the size of the nucleus of a lymphocyte
 - o half-life of about 120 days
- evaluation of RBCs
 - o Total #, MCV, Shape (aniso, poikilocytosis, spherocytes) and color
 - o Normal Hct
 - Female → 35 - 47 %
 - Male → 40-52% (higher because androgens stimulate more than estrogens)
 - o Normal Hb
 - Female → 12-16 gm/dl
 - Male → 13.5-17.5 gm/dl
 - o Reticulocyte Count → 0.2 – 2.0%
 - o MCV can help categorize anemia for a differential diagnosis
 - o Reticulocyte count →
 - Retic % x RBC count, normal is 100,000
 - More accurate than regular percentage in assessing the cause of anemia
 - Why? Because if you had something like ACD, then you can have normal % of reticulocytes, but if you calculated the total number, then you'd see that it is depressed
- Considering different types of Anemia and their etiologies
 - o Anemia due to RBC destruction
 - Extravascular markers
 - Heme is metabolized to bilirubin in M-phages and globin is metabolized intracellularly → Unconjugated bilirubin is excreted into plasma, bound to albumin, goes to the liver → Bilirubin is conjugated in the liver and excreted into bile and then into the upper GI tract → Conjugated Bilirubin then passes to the lower GI tract and is metabolized into urobilinogen which is excreted in stool and urine.

- Intravascular Markers
 - ↓ in plasma Haptoglobin – free Hb in the circulation leads to binding of Hb to haptoglobin (a protein made in the liver for the purpose of scavenging free Hb from circulation), resulting in low plasma haptoglobin
 - Hb is filtered by the kidney and reabsorbed by the tubules, leading to hemosiderinuria
 - Capacity of the tubules to reabsorb protein is exceeded leading to hemoglobinuria
 - You usually need a few days for the urine hemosiderin to increase after an acute hemolytic event because the tubule cells have a half life of a few days so they do not slough off until later, unless you have chronic hemolysis
- Test results from Hemolytic anemia
 - ↑ Reticulocyte count, unconjugated Bilirubin, LDH (since RBCs do not have mitochondria, this is their main energy source)
 - ↓ haptoglobin
 - hemoglobinuria and hemosiderinuria
- Stimulator of Erythropoiesis – EPO (cytokine produced in kidney – peritubular interstitial cells of the outer cortex – in response to low O₂ delivery)
 - Necessary for differentiation and proliferation of the myeloid cell line
 - Accelerates maturation into reticulocytes, and stimulates the proliferation at the level of BFU-E and CFU-E
 - Absence results in apoptosis of erythroid committed cells
 - Renal failure can lead to anemia due to the lack of EPO
 - MOA – binds specifically to its receptor on progenitors
 - The receptor is a transmembrane protein of the cytokine receptor superfamily (JAK-STAT pathway)
 - Binding leads to dimerization of the receptor and this activates the tyrosine kinase activity
 - EPO levels correlate to Hb levels

Iron Deficiency Anemias (for more detail look at transcript)

- characterized by microcytic, hypochromatic cells (however, note that microcytic cells are only present in ACD about 30% of the time, normally, they are normocytic)
- Types
 - Iron deficiency anemia
 - ↓ serum iron, ↑ transferrin, ↓ ferritin
 - Anemia of Chronic Disease (this is a mild anemia)
 - ↓ serum iron, ↓ transferrin, ↑ ferritin
 - Iron deficiency anemia and ACD concurrently
 - Ferritin will be somewhere in the normal to low range as will transferrin levels

Megaloblastic Anemias

- characterized by macrocytic, ovalocytes
- Caused by impaired DNA synthesis (delayed mitosis) while RNA is not impaired → resulting in a nuclear-cytoplasmic asynchrony that affects all rapidly proliferating cell lines, including cells of the bone marrow, GI, and GYN
- Blood smears can show hypersegmented neutrophils (98%), pancytopenia (if the anemia is severe enough), reticulocytopenia, elevated LDH (90%)
- normal or elevated serum iron
- serum B12 or Folate are low

- Marrow can be noted for classic megaloblastic changes – megaloblasts in the bone marrow lead to macrocytes in the peripheral blood
 - Autohemolysis in the bone marrow (due to ineffective erythropoiesis) will cause an ↑ bilirubin and lactate dehydrogenase (LDH)
 - Also see WBC changes → giant metamyelocytes in bone marrow, hypersegmented neutrophils (more than 5 lobes) in the peripheral blood
 - Platelets are NOT increased in size
- Results in a megaloblastic anemia and a neurologic deficit
- Types
 - Vitamin B12 deficiency →
 - Absorption → dietary B12 binds to R proteins in the saliva and stomach → IF is secreted by the gastric parietal cells → in the duodenum, the increased pH and pancreatic enzymes, the B12 is broken away from the R proteins and then the free B12 binds to IF → in the ileum, the B12-IF complex is absorbed by the ileal mucosal epithelial cells → B12 is transported in the blood bound to transcobalamin II (these levels can be looked at in assessing the type of megaloblastic anemia)
 - Function of B12 in the body are
 - Folate metabolism – required for de-methylation of methyl THF
 - Methylation of myelin
 - Conversion of methylmalonyl CoA to succinyl CoA
 - Conversion of homocysteine to methionine
 - Due to dietary deficiency – this is rare since Vit B12 is stored in the liver and so takes years to be deficient in it
 - Due to ↓ absorption resulting from:
 - ↓ IF associated with gastrectomy (pernicious anemia)
 - Pancreatic insufficiency (since pancreatic proteases normally breakdown B12-R complexes in the duodenum so that IF in the more basic environment can bind to the Vit. B12)
 - Intestinal malabsorption due to parasites (fish tapeworm *Diphyllobothrium latum*), bacteria (blind-loop syndrome – bacteria eat up the Vit B12 before it has a chance to be taken up by the ileum), Crohn’s disease of the ileum
 - Signs and Symptoms
 - Weakness due to the anemia (megaloblastic anemia)
 - Sore tongue, atrophic glossitis due to generalized epithelial atrophy
 - Subacute combined degeneration of the spinal cord – demyelination of the posterior and lateral portion of the spinal cord
 - Loss of position sense (touch and proprioception)
 - Lateral portion degeneration effects dorsal spinocerebellar tracts (arms and legs dystaxia) and corticospinal tracts (spastic paralysis)
 - Lab results
 - Low serum B12
 - ↑ serum homocysteine (risk factor for atherosclerosis)
 - ↑ methylmalonic acid in urine (since it can not be broken down to succinylCoA)
 - no Vit B12 to be a cofactor for the enzymatic actions
 - Schilling test – radioactive oral Vit. B is given with a concurrent IM injection of non-radioactive B12 at the same time and then measure urine for radioactive B12 – this test is done in order to determine the cause of the anemia
 - Pernicious anemia – abnormality corrected with IF administration
 - Bacterial overgrowth – not corrected for even with IF, but is corrected if given antibiotic therapy

- Ileal dysfunction – low radioactive B12 in urine even with IF, without IF and after antibiotic therapy
- Tx
 - First draw blood before anything
 - Then begin therapy with B12 and folate
 - Avoid transfusions unless the patient has some heart disease or unless hemodynamic compromise is present
 - Post IM injection of Vit. B12, reticulocytes will increase in number in about 5 days
- Folate deficiency
 - Folic acid is important for thymine and purine biosynthesis
 - Critical for DNA synthesis
 - Critical for one carbon transfer metabolic reactions
 - The primary transport for of folic acid in the blood is N5 methyl THF
 - Can result from:
 - ↓ intake, dietary deficiency in this case only takes months to develop - Seen more in chronic alcoholics and the elderly
 - ↓ absorption (folate is absorbed in the duodenum) by the deconjugation of poly-Glu)
 - conditions where the need for folate ↑ such as
 - pregnancy (can be a cause of neural tube defects, will cause increase in α -fetoprotein can result in myeloceles, spina bifida, etc)
 - conditions where there is ↓ use of folate
 - folate antagonists are used in chemotherapy (methotrexate)
 - Signs and Symptoms
 - Megaloblastic anemia
 - No neurologic symptoms
 - Lab results
 - ↓ serum folate levels
 - ↑ serum homocysteine
 - Tx – start with Folate and B12 until you know which one it is, otherwise, if you have B12 deficiency and are just given folate, the neurological deficit can progress further

Hemolytic Anemias

- general characteristics
 - absent haptoglobin, hemoglobinuria, hemoglobinemia
 - normocytic, normochromic peripheral blood
 - shortened RBC survival, reticulocytosis due to increased destruction
 - ↑ LDH and ↑ indirect bilirubin
- Intracorpuscular Hemolysis (defects in the RBC itself)
 - causes:
 - Membrane abnormalities – Hereditary Spherocytosis, Hereditary Stomatocytosis (Membrane permeability defects), Paroxysmal Nocturnal Hemoglobinuria (increased sensitivity to complement)
 - Metabolic abnormalities – G6P deficiency
 - Hemoglobinopathy
 - Signs
 - Release of Hb into the blood causes Hemoglobinemia and hemoglobinuria
 - ↑ bilirubin from the RBC causes jaundice and an increased risk of pigment gall stones

- Hb may be oxidized to methemoglobin which causes methemoglobinuria and methemoglobinemia
 - Markedly decreased Hb binding proteins in the blood such as Haptoglobin and hemopexin
 - No splenomegaly
- Extracorporeal Hemolysis
 - Splenomegaly can result if the extravascular hemolysis occurs in the spleen
 - Hepatomegaly results if the extravascular hemolysis occurs in the liver
 - ↑ bilirubin and ↓ haptoglobin occurs (just not as much as IV hemolysis)
 - there is no hemolobinemia, hemoglobinuria and methemoglobin formation
 - causes
 - Non-immune – Mechanical, infectious, chemical, thermal, osmotic
 - Immune – auto-antibody mediated
- Specific Types
 - Hereditary Spherocytosis – defective or absent spectrin molecule leading to loss of the RBC surface membrane leading to spherocytosis.
 - Autosomal dominant disorder
 - ↓ deformability and flexibility of the cell resulting in increase osmotic fragility (at a given difference of osmolarity, its is more prone to hemolysis than a normal RBC) and clearance by spleen Macrophages which can result in symptoms of extracorporeal hemolysis
 - filtered and hemolysis in the spleen can occur leading to splenomegaly, increased bilirubin and increased risk for jaundice and pigment gallstones
 - Lab results
 - Increased osmotic fragility, normal MCH with an increased MCHC
 - Tx - Splenectomy
 - Paroxysmal nocturnal hemoglobinuria
 - Caused by decreased GPI proteins especially DAF
 - DAF usually binds to GPIs on the RBC surface to breakdown complement components from lysing the cell (specifically C3 convertase). DAF deficiency result sin increased complement activity
 - Clonal cell disorder (so effects all cell lines), with ongoing intravascular and extravascular hemolysis, classically at night (this may be due to the fact that people become slightly acidotic during sleep and complement components are more active in the ↓ in pH (so exercise can also cause this to occur)
 - Symptoms – breathelessness at night
 - Lab diagnosis – acid hemolysis (Ham Test), sucrose hemolysis, CD 59 negative (their RBCs are without CD 59 – a product of the PIG-A gene)
 - Peripheral blood can be noted for pancytopenia, anemia, leucopenia, thrombocytopenia
 - The complications are an increased risk for aplastic anemia, leukemia, and venous thrombosis
 - G6PD deficiency
 - G6PD deficiency results in decreased levels of the antioxidant glutathione (GSH), and thus RBCs become sensitive to oxidant stresses leading to hemolysis (times of oxidative stress are infection, medication, fava beans?)
 - G6PD is the rate-limiting enzyme in the hexose-monophosphate shunt
 - G6PD also produces NADPH which keeps glutathione reduced and then glutathione can protect by breaking down hydrogen peroxide

- Oxidative stress leads to Heinz body formation which can be seen with methylene blue and crystal violet stains → ultimately, these cells get eaten up by the splenic macrophages (extravascular hemolysis) which can form bite cells
- The actual deficiency is not due to the absence of the enzyme, but rather a defective protein folding, resulting in the protein having a decrease half-life, so towards the later stages of an RBCs life (more than 20 days), the functional levels of the enzyme start to decline
- The enzyme regenerates NADPH which allows glutathione to get regenerated so it can have antioxidant effects
- Shows an X-linked inheritance commonly found in African Americans and Mediterraneans
 - Mediterranean version is associated with fava bean ingestion and shows a more severe hemolysis because all the RBCs have decreased G6PD activity due to decreased synthesis and stability
 - African American version is associated with intermittent hemolysis since old the older RBCs have decreased levels of G6PD and usually occurs in response to oxidative states such as infections
- Microangiopathic anemia
 - Caused by vascular abnormalities (such as AV fistula, Cavernous hemangioma), renal lesions, vasculitis
- Immune hemolytic anemia
 - General principles
 - All require antigen-Ab interactions, and the types of reactions depend on the class of Ab, amount of antigen on the cell surface, complement availability, functional status of the reticuloendothelial system
 - Can manifest as intravascular or extravascular hemolysis
 - Ab combines with RBC to activate complement (results in intravascular hemolysis) or opsonize the RBC for cell mediated endocytosis (Macrophages recognize the Fc portion of the Ig and/or C3b and then this results in extravascular hemolysis)
 - Coombs test
 - Direct Test – looks for Ig and/or complement on the surface of the RBC
 - Coombs reagent has anti-human Ig and anti-human complement
 - Mix it with patient, and if those immune components are on the cell surface, then, you will get agglutination due to the Coombs reagent
 - Indirect Test – looks for Anti-RBC Ab in the patients serum, using a panel of RBCs with known surface Ags
 - Combine patient's serum with cells from a panel of RBCs with known antigens
 - Add combs reagent to the mixture
 - If anti-RBC antigens are in serum, agglutination occurs
 - Subtypes
 - Drug related hemolysis, alloimmune hemolysis (transfusion reaction, disease of the newborn), Autoimmune hemolysis (warm and autoimmune hemolysis)
 - Drug-related –
 - Immune complex mechanism (quinidine, quinine, Isoniazid)
 - Drug and Ab bind in the plasma
 - Immune complexes activate complement in the plasma, or sit on the RBC, and that is recognized by Macrophages and results in

- destruction of the immune complex, and the RBC lyses for being an innocent bystander
 - Haptenic Immune mechanism – penicillins, cephalosporins
 - Drug binds to and reacts with red cell surface proteins, antibodies recognize the altered protein/drug as foreign and initiate immune processes
 - True Auto-immune Mechanism – Methyldopa, L-dopa, Procainamide, Ibuprofen
 - Not known etiology, but the drug causes antibodies to for against antigens found normally on the RBC surface
- Allo-Immune hemolysis
 - Hemolytic transfusion reaction – caused by recognition of foreign antigens on transfused blood cells - Several subtypes
 - Immediate intravascular hemolysis (occurs in minutes) in reaction to pre-formed anti-bodies – this is life-threatening
 - Can results in symptoms such as back pain, fever, HTN and DIC
 - Slow extravascular hemolysis (occurs over days) in reaction to repeat exposure to a foreign antigen to which there was previous exposure – usually with only mild symptoms
 - Delayed sensitization (occurs over weeks) as a result of 1st exposure to an antigen; this is usually asymptomatic
 - Need to pre-test ABO and Rh type for both the donor and recipient
 - Antibody screen of donor and recipient including indirect Coombs
 - Hemolytic disease of the newborn
 - Occurs due to incompatibility between the mother negative for an antigen and fetus/father positive for that antigen. Rh and ABO incompatibilities are most common causes
 - Usually occurs in the 2nd or later pregnancy
 - Requires maternal IgG antibodies vs. RBC antigens in fetus
 - Note that IgM doesn't cross the placenta
 - Can cause severe anemia in the fetus with erythroblastosis and heart failure
 - Hyperbilirubinemia can lead to severe brain damage (kernicterus) if not promptly treated
 - Can be prevented if caused by an Rh incompatibility by administration of anti-Rh D to Rh negative mothers after each pregnancy
- Autoimmune hemolysis
 - Occurs due to formation of Abs that attack self RBCs
 - Auto Abs allow for complement fixation on the RBC
 - Associated with lymphoproliferative disease or collagen vascular disease
 - Types
 - Warm type – usually due to IgG Abs, with complement fixation to level of C3, if at all. Ig binding occurs at all temperatures, Fc receptors and C3b receptors on Macrophages cause extravascular hemolysis. 70% of cases are associated with other illnesses, and it is responsive to steroids and splenectomy
 - Cold Type – usually due to IgM mediated with Abs binding at 30 degrees or lower. Complement is responsible for this with the formation of the MAC leading to RBC lysis intravascularly. 90% is associated with other

illnesses and it is poorly responsive to steroids or splenectomy but is responsive to plasmapheresis

Hemoglobinopathies and Thalessemias

- HbA ($\alpha_2\beta_2$)
- HbA2 ($\alpha_2\delta_2$)
- HbF ($\alpha_2\gamma_2$)
- Hb Barts (γ_4)
- Hb H (β_4)
- hemoglobinopathy is the qualitative change in the Hb resulting from a mutation in nucleotide sequence of globin-chains
 - Hb S – (sickle cell anemia with a β -globin chain which has a valine (neutral) substituted for glutamate (-) at the 6th position of the chain)
 - With low O₂ tension, the RBC sickles, but with repeated sickling, Ca enters the RBC and H₂O and K⁺ leave the RBC resulting in irreversible sickling which will result in chronic extravascular hemolysis – this will result in erythroid hyperplasia in the bone marrow, jaundice and \uparrow bilirubin levels
 - Hypoxia, dehydration (Increasing HbS concentrations inside the RBC will induce sickling, while thalessemia will make it better), acidosis (\downarrow pH decreases O₂ affinity and will induce sickling) and infection triggers sickling
 - The chronic hemolysis will result in reticulocytosis and hyperbilirubinemia
 - Capillaries blocked by sickled cells will cause a vaso-occlusive crisis (painful crisis), hand-foot syndrome (swelling in children), Autosplenectomy due to chronic infarcts and subsequent ischemic necrosis and fibrosis of the spleen (this will result in increased susceptibility to infections by encapsulated organisms and Howell Jolly bodies in peripheral blood smear)
 - Note that the spleen and bone marrow are particularly prone to getting sickling events due to the sluggish nature of the blood flow through these areas
 - Note also that the anemia and vascular stasis leads to fatty changes in the heart, liver, and renal tubules \rightarrow erythropoiesis is increased in the bone marrow, and the expansion in the bone marrow will lead to the appositional bone growth and the crewcut appearance of the skull on X ray
 - Other complications – avascular necrosis of the femoral head, increased incidence of salmonella oteoyelitis (getting leg pain), leg ulcers, risk for aplastic crisis (especially with parvovirus B19 infection of erythroblasts – this can result in sudden cessation in erythropoiesis resulting in worsening anemia and \downarrow reticulocytes), acute chest syndrome (necrotic bone marrow makes fat emboli to the lung – which eventually exacerbates sickling) and priapism (due to blood vessel occlusion), CNS stroke,
 - Heterozygous (AS) trait \rightarrow patients are normal until they reach high altitudes
 - About 8% of African Americans are heterozygotes for Hb S
 - These patients have about 40% HbS and since most (60%) is Hb A which does not interact well with HbS, sickling does not occur too often
 - Hb C is another β globin mutant which interacts well with HbS, and so this can be problematic because it will induce sickling
 - Hb F does not interact well with Hb S and so perinatal infants will be asymptomatic until about 6 months
 - Patients with sickle trait have fewer symptoms than those with sickle disease and they also have resistance to P. Falciparum (malaria)
 - Homozygotes (SS) patients have full blown sickle cell anemia
 - Tx – hydroxyurea in order to induce more Hb F which will increase O₂ in the blood and will also reduce inflammation which reduces RBC stasis and sickling prone situations

- Thalessemia is the quantitative change in the Hb resulting from a decreased or absent globin chain, can be α or β chain
- Thalessemia provides a protective advantage to carriers such as protection against malaria
 - α -thalessemia has decreased α globin chains with excess β -chains
 - genetically, there are a total of 4 α globin chain genes – these α chains are expressed pre-natally and post-natally so disease can present during both of these times
 - α thalessemia is due to gene deletions
 - clinically, there are a number of different possible disease states
 - normal people have 4 α genes and 100% α chains
 - silent carriers have 1 deletion → total of 3 α genes, which produces 75% of normal α chains
 - these individuals are completely normal and asymptomatic with normal lab values as well
 - α -Thalessemia minor patients have 2 α genes either in a cis (on same gene missing – more common in Asians) or trans (on different loci missing – more common in African Americans)
 - since blacks have the trans form, their off spring can never get H disease or hydrops while Asians can have offspring with hydrops fetalis
 - Hb H disease patients have 3 deletions (if there is a newborn with sever anemia, run gel electrophoresis and look for Hb H)
 - These patient have 1 α gene which results in 25% of the α chains being produced
 - Results in increased Hb H which has 4 β subunits, and forms Heinz bodies which can be seen with crystal blue stain
 - Hydrops fetalis – these patients have 4 deletions and the condition is lethal in utero, they have 0 α genes and thus, 0% α chains
 - This condition results in increased γ 4 (Bart's Hemoglobin)
 - Hb H and Hb Bart are better than the free α globin chains seen in β thalessemia, however, they have an increased affinity for O₂ which results in a poorer O₂ delivery
 - β -thalessemia has decreased β globin chains with excess α -chains – too much α chains result in extra-vascular hemolysis
 - eventually results in a iron overload
 - is due to a point mutation forms either some β -chains (β^+) or none (β^0) – usually a result of a point mutation to form a stop codon, or a point mutation which results in an abnormal mRNA processing at the splice junctions
 - genetically there are a total of 2 β -globin chain genes which are expressed only post-natally and therefore no pre-natal disease occurs
 - β -thalessemia minor is an asymptomatic condition with increased HbA₂ (8%) and increased Hb F (5%)
 - the HbA₂ level here is diagnostic
 - β -thalessemia intermedia has a severe anemia, but no transfusions are needed
 - β -thalessemia (Cooley's anemia)
 - patients are initially normal at birth because HbF levels are high enough to compensate, but at about 6 months, when Hb F levels start to fall, patients start to show symptoms
 - the decreased RBC lifespan results in severe hemolytic anemia – intermedullary destruction (within the bone marrow) result in ineffective erythropoiesis, and the hemolysis increases bilirubin levels and increases the likelihood of jaundice and gall stones

- these patients will require life long transfusions which can result in secondary hemochromatosis so Tx will rely on chelator (desferrioxamine) use concurrently, and agents such as hydroxyurea to increase HbF
- CHF is the most common cause of death
- Erythroid hyperplasia in the bone marrow will result in crew-cut skull X-rays and increase sizes of the maxilla which result in a “chipmunk face”
- Peripheral blood smear will show a microcytic/hypochromic (due to ↓ β globin) anemia, numerous target cells (which are due to increased RBC membrane) and increase reticulocytes
- Hb electrophoresis will show ↑ Hb F (90%), ↑ Hb A2 and ↓ Hb A

Platelets and Coagulation

- When thinking of bleeding disorders, think of etiology as being a problem with platelets as opposed to a problem with the coagulation cascade, and then ask yourself if it is a problem with production or with increased destruction
- What happens in a bleeding situation?
 - Vasoconstriction mediated by Endothelin
 - Thrombogenesis
 - Changes in blood flow and turbulence favors clot formation
 - TF released from injured cells activated Factor VII (the extrinsic pathway)
 - Subendothelial collagen exposure activates Factor XII (the intrinsic pathway)
 - Release of vWF binds to the exposed collagen and facilitates platelet adhesion
 - Damaged endothelium has decreased production of anti-thrombogenic substances such as prostacyclin, NO₂, tPA and thrombomodulin
 - Note that platelets can stop the bleed, but fibrin from the coagulation cascade is needed to form the plug
 - Hemostasis is divided into 3 phases
 - Primary – platelet plug formation
 - Secondary – activation of clotting and desposition/stabilization of fibrin
 - Tertiary – dissolution of fibrin clot (dependent on plasminogen activation)
- Platelets
 - Derived from megakaryocytes in the bone marrow – anucleate cellular fragments, with multiple granules and multiple organelles
 - Synthesis is controlled by IL-6, IL-3, IL-11 and thrombopoietin (this one is critical for final release and maturation of the platelet from the bone marrow)
 - Circulates as inactive, non-binding concave disks but undergoes major shape change upon binding into spherical stellate form, also developing receptors for clotting factors and each other and subendothelium and releasing their granules
 - They form a non-covalent but nonetheless tight bond
 - Steps in platelets stopping bleeding
 - Step I – adhesion (first vWF binds exposed collagen, and then platelets adhere to the WF via their Gp Ib)
 - Step II – shape change and degranulation
 - The α granules contain fibrinogen, fibronectin, Factor V, vWF, PDGF, Platelet Factor 4 and the δ granules contain ADP (a potent platelet aggregator), Ca, Histamine and serotonin and Epinephrine
 - TXA₂ release aids in platelet aggregation
 - Membrane expression of a phospholipids complex which functions as a platform for the coagulation cascade
 - Step III – aggregation

- Additional platelets are recruited from the bloodstream, ADP and TXA2 cause aggregation
 - Fibrinogen cross links platelets by binding Gp IIb-IIIa
- Laboratory tests for platelets and their function
 - Platelet count (normally between 150-400)
 - Bleeding time (cut the arm and check out how long it takes to stop – normally 2-7 minutes – greater than 7 minutes indicated a platelet count < 100)
 - Platelet aggregation studies which sort out abnormal platelet function
- Generally speaking, platelet disorders present with superficial bleeding symptoms such as petechiae, nose bleeds (mucous membranes), ecchymoses, hematomas
- Platelet function defects present with an increased bleeding time
 - Platelet function defects – common symptoms are spontaneous bleeding from mucosal membranes, prolonged bleeding from wounds, menorrhagia in young females
 - Adhesion defects
 - Bernard Soulier Disease – very rare → abnormal GpIb-IX complex (vWF receptor) on platelets so does not bind vWF
 - The vWF receptor is the only adhesion mediator in high shear stress
 - Von Willebrand disease (pretty common) results from reduced or dysfunctional vWF (prevalence 1-2%) which is normally produced by endothelial cells and megakaryocytes
 - Autosomal dominant inheritance with variable penetrance, generally a mild bleeding disorder with variable test results
 - Factor VIII is stabilized by vWF, and without it, the half life of Factor VIII is minutes, but binding to vWF makes it 12 hrs
 - Often see lab results with normal platelet count, prolonged bleeding time, normal PT but with prolonged aPTT, abnormal platelet response to ristocetin (which causes thrombocytopenia)
 - Tx is with Desmopressin (an ADH analog which releases vWF from Weibel Palade bodies)
 - Platelet function defects – release defects resulting in a mild bleeding disorder and a definite decrease in platelet aggregation
 - Δ granule storage pool disease – failure to form dense granules so no ADP, Ca, Serotonin is release upon activation so you also fail in recruiting platelets for aggregation
 - Gray-platelet syndrome – failure to package α granules so you don't release fibrinogen and other platelet aggregatory factors
 - Aggregation defects – congenital
 - Glanzmann's thrombasthenia – autosomal recessive – lack of fibrinogen receptor Gp IIb/IIIa – platelets can not aggregate and bleeding can be severe
 - Acquired defects – Drug Induced
 - Alcohol (a lot is needed, about 1 liter everyday for 2 months), Prostaglandin Synthetase Inhibitors (i.e. NSAIDS, Dipyridamole), ADP receptor inhibitors (clopidogrel, ticlopidine), β-lactam antibiotics, Heparin
 - Tx for platelet function defects
 - Platelet transfusion, desmopressin (which causes release of vWF from endothelial cells), cryoprecipitate (same as desmopressin), dialysis

- Thrombocytopenia –
 - decreased production
 - decreased number of megakaryocytes – good response to platelet transfusion
 - neoplastic causes such as aplastic anemia, leukemia, metastatic carcinoma, drugs, radiotherapy, or primary bone marrow disorders such as megaloblastic anemias, myelodysplastic or myeloproliferative disorders
 - increased destruction
 - shortened platelet lifespan, increase megakaryocytes, macroplatelets and bad response to platelet transfusion
 - causes can be immune – ITP, lymphoma, drugs, or ↑ consumption, or septicemia (since infection causes endothelial cells to expose subendothelial collagen)
 - ITP (immune thrombocytopenic purpura)
 - Antiplatelet antibodies made in the spleen against platelet antigens such as Gp IIb/IIIa and Gp Ib/IX
 - Platelets are destroyed upon reaching the spleen in circulation because of the Fc receptors on M-phages that bind to IgG coated platelets
 - Acute form – occurs in children after viral infection and is self-limited
 - Chronic form usually seen in women of childbearing years, may be the first manifestation of SLE, symptoms include petechiae, ecchymoses, menorrhagia and nosebleeds
 - Lab results show a decreased platelet count and prolonged bleeding time
 - Normal PT and aPTT time
 - Peripheral blood smear shows thrombocytopenia and enlarged immature platelets
 - Bone marrow shows increased numbers of megakaryocytes with immature forms
 - Tx – corticosteroids (to ↓ Ab production), Ig therapy which flood Fc receptors on splenic M-phages or splenectomy (removing the site of platelet destruction and Ab production)
 - HIV associated thrombocytopenia
 - Early on – immune mediated, marrow is within normal limits and Tx is with anti-retroviral therapy
 - Later on – virus infiltrates marrow, get pancytopenia, results in association with infection or neoplasm, and is poorly responsive to all treatments

- Coagulation

- Coagulation factors – zymogens, some form complexes requiring Ca, Phospholipid surface or cofactors – majority are produced by the liver
 - Intrinsic pathway is activated by contact factors such as contact with subendothelial collagen, HMW kininogen or kallikrein
 - Extrinsic pathway is activated by the release of tissue factor (from injured cells)
 - Thrombin is a serine protease which cleaves fibrinogen to fibrin, activates V to Va and VIII to VIIIa, activates platelets by cleaving the thrombin receptor, activates XIII to XIIIa which aids in cross linking fibrin and in the presence of thrombomodulin → activates Protein C to APC
 - Fibrin has highly hydrophilic tails which allow it to travel in blood, however, thrombin cleaves off the hydrophilic tails so that the fibrin becomes extremely hydrophobic and then, factor XIIIa functions in X-linking the fibrin molecules
 - Vit. K Dependent Carboxylase – converts first 7-12 glutamic acids to γ -carboxyglutamic acid and confers calcium binding and lipid binding capability on these proteins, and

without Vit. K, you would produce factors II, VII, IX, X and Protein C and S, but they would just be inactive in coagulation

- Lab tests for coagulation
 - PT – prothrombin time
 - Tests the extrinsic and common coagulation pathways – factors VII, X, V, prothrombin and fibrinogen (really measures the formation of the fibrin monomer)
 - aPTT – partial prothrombin time
 - tests the intrinsic and common coagulation pathways – factors XII, XI, IX, VIII, X, V, prothrombin, fibrinogen
 - TT – Thrombin time assesses adequate fibrinogen levels
 - FDP – Fibrin degradation products – tests fibrinolytic system (increased in DIC)
- Clotting assays is used to distinguish deficient plasma for specific factors
 - Mix the patient's plasma with factor deficient plasma, 50:50 – those which are normal will have clotting times WNL, those missing factors will have prolonged clotting times
 - If more than one clotting assay is prolonged – it implies an inhibitor to clotting protein
- vW disease and hemophilia are congenital clotting disorders
 - Hemophilia – incidence is .02% - not the most common, but most serious disorder
 - Type A lacks factor VIII, type B lacks IX
 - Type A is treated with replacing Factor VIII
 - When treating type B, you need to double the doses because factor IX distributed into total body water also
 - Sex-linked recessive disease, types A and B are clinically indistinguishable except by factor analysis and is genetically lethal without replacement therapy
 - The incidence rate does not change although many who have it do not survive to reproduce → this means that new, current mutations can lead to this
 - Clinical severity correlated with factor level → Mild hemophilia is with a >5% factor level (bleeding only occurs with significant trauma or surgery, Moderate is 1-5% factor level (bleeding with mild trauma, hemarthroses with trauma or spontaneously – occasionally), Severe is < 1% factor level – often see spontaneous mucosal and spontaneous bleeding
 - Tx rules – treat first, ask later, if there is bleeding into closed spaces let it be, avoid emergent procedures if possible
 - Hemophilia A vs. vWF deficiency
 - Factor VIII functions for fibrin clot formation
 - vWF functions for platelet adhesion and stabilization of factor VIII
 - in vWF deficiency, you will have low vWF and factor VIII → bleeding time and aPTT are prolonged
 - in hemophilia, you have normal vWF, but low factor VIII → bleeding time is normal but aPTT is prolonged
- Acquired Bleeding Disorders
 - Vit. K deficiency – usually in hospitalized patients because ↓ dietary consumption and concurrent ↓ in normal gut flora is needed for this to happen
 - PT goes up 1st, secondary to factor VII's short half life
 - Tx is to replace Vit. K and a response should be noted in 24-48 hours
 - Liver disease – one of the major causes of bleeding disorders
 - ↓ synthesis of Vit. K dependent proteins
 - ↓ clearance of activated clotting factors so you can see ↑ clotting in some cases
 - ↑ fibrinolysis secondary to ↓ anti-plasmin
 - dysfibrinogenemia secondary to synthesis of abnormal fibrinogen

- ↑ fibrin split products due to impaired clearance which can result in inhibition of the clotting process
- ↑ PT, aPTT, TT
- ↓ platelets (hypersplenism)
- Tx – replacement therapy but is reserved for a bleeding procedure since the half life of the factors is very short, normally a very high volume and frequency is needed in order to use it otherwise
- Coumadin therapy
- Heparin therapy
- DIC

Coagulation regulation and DIC

- Coagulation inhibitors
 - TFPI – tissue factor pathway inhibitor, LACI – lipoprotein associated coagulation inhibitor, EPI – extrinsic pathway inhibitor → all work by complexing with factors VIIa/TF/Xa and inactivating Xa which doesn't allow prothrombin to be cut into thrombin
 - ATIII/Heparin Cofactor II/Heparin → binds and inactivates enzymes (so these factors decrease in number XIa, IXa, Xa and Thrombin)
 - ATIII – inactivates XIa, IXa, Xa and Thrombin
 - Heparin enhances the activity of ATIII, dose is determined by monitoring the aPTT
 - Complications with heparin are bleeding, mild platelet dysfunction, thrombocytopenia (in 1-5% of patients), osteoporosis is common with long-term use, and heparin is a heterogenous mixture so dosing can be problematic
 - LMWH however is less heterogeneous than heparin, you have less platelet dysfunction because it is smaller than heparin, it has a longer half life and also results in less thrombocytopenia
 - Protein C/Protein S/Thrombomodulin → cleaves and inactivates cofactors (Va and VIIIa)
 - Thrombomodulin is on the surface of endothelial cells and functions to change the affinity that thrombin has for Va and fibrinogen
 - Plasminogen (functions in tertiary hemostasis to cleave fibrin)
- Anti-coagulation deficiency diseases
 - Heterozygous Protein Deficiency
 - Increased venous thrombosis
 - Occasional increased arterial thrombosis
 - Warfarin induced skin necrosis (protein C deficiency)
 - Homozygous Protein Deficiency
 - Neonatal purpura fulminans
 - Fibrinogenolysis
 - Chronic DIC
- Activated protein C resistance
 - Normally, APC cleaves and inactivates Va and also VIIIa
 - Failure for activated protein C to prolong aPTT (98% of the time due to a mutation of Arg 506 to glutamine which is the cleavage site where APC cleaves Va)
 - Extremely common (5-20% of the white population with this mutation)
 - 4X increased risk in heterozygotes for thromboemboli and even more so in homozygotes
 - can exist in combination with other disorders and when they do exist, their effects have a synergistic effect
- acquired hypercoagulable states
 - C4b Binding protein – acute phase reactant synthesized under inflammatory states
 - Increases in inflammatory diseases

- Binds to protein S resulting in a decrease in effective Protein S (since bound Protein S is inactive as a cofactor)
- Inflammation → increases IL-1 and TNF- α
 - Both of these cytokines downregulate thrombomodulin by increasing its internalization and decreasing its translation
 - Thrombin as a result only exerts its pro-coagulant functions b/c of the lack of thrombomodulin (so thrombin does not activate protein C at all)
- Heparin or anti-coagulation therapy
 - Lepirudin/Argatroban
 - Direct thrombin inhibitors which inhibit thrombin bound to fibrin
 - Inhibits thrombin activation of platelets so may cause more bleeding than heparin
 - Usually used in place for heparin if there is severe thrombocytopenia
 - Coumadin
 - Inhibits Vit. K dependent Carboxylase activity, preventing the reduction of Vit. K, but does not affect proteins already synthesized
 - Initially may cause a hypercoagulable state because protein C will drop first because of its shorter half life, so you need to give heparin concomitantly for the first 1-2 days
 - Dose is monitored with PT time
 - Multiple drug interactions, toxicity is reversed with high dose Vit. K
 - Thrombolytic therapy
 - Streptokinase – purified from streptococcus and functions in binding to plasminogen and then starts to convert another plasminogen molecule into plasmin
 - Urokinase – purified from urine initially and functions in activating plasminogen directly
 - t-PA – made by endothelial cells with an increased affinity for fibrin bound plasminogen with relative fibrin specificity and activates plasminogen directly – results in lysis of clots
 - thrombolytic therapy has a high incidence of intracerebral hemorrhage
 - results in lowered plasma fibrinogen
 - used in MI to lyse coronary thrombi
 - t-PA has a high incidence of re-occlusion disease
- DIC
 - Always occurs secondary to another disorder
 - Causes
 - Obstetric complications (placental tissue factor activates clotting)
 - Sepsis (LPS), Adenocarcinomas (mucin activates clotting)
 - AML-M3 (cytoplasmic granules in neoplastic promyelocytes activate clotting)
 - Results in widespread microthrombi and the consumption of platelets and clotting factors causes hemorrhage
 - Lab results show
 - ↓ platelets, prolonged PT and aPTT, ↓ fibrinogen, ↑ fibrin split products – fragment D especially since this occurs in patients only with active thrombosis occurring and fragment X can be seen in anyone with fibrinolysis (so the D-dimers is the most conclusive) – this one confirms DIC
 - Tx is to fix the underlying disorder, then give fresh frozen plasma and platelets
 - In acute DIC – bleeding is a concern – a decrease in both coagulants and anti-coagulants
 - chronic DIC – thrombosis is a concern

- defibrination
 - can occur due to release of tissue procoagulants (tumors, fetal, placental, prostatic, pancreatic, Shock), damage to the vascular tree (as in sepsis, aortic aneurysm, hemanigoma, tumor emboli) and ↓ clearance seen with liver disease
- Liver disease
 - result sin factor deficiencies secondary to decreased synthesis
 - abnormal fibrinogen results in excess siaclic acid lysing fibrin, prolonged TT which means that there are abnormally low levels of fibrinogen
 - increase fibrinolysis due to ↓ PAIs and anti-plasmin, and an increase t-PA
 - use vWF to distinguish liver disease from DIC, in liver disease vWF is normal while in DIC, vWF is ↓

White Blood Cell Pathology

- Leukemias in general have evidence of DNA damage seen from gross chromosomal abnormalities
 - o Many result from translocations (APL – 15;17, CML – 9;22, Burkitt's – 8;14) but other causes can be rearrangements, mutations and deletions
 - o These genetic aberrations can be a result of DAN mutagens such as radiation and carcinogens, hereditary disorders or viral infections
 - o Multiple mutation events are thought to play a role in etiology
 - Oncogenes can develop enhanced activity, tumor suppressor genes can get a decrease in activity due to the genetic aberrations
 - o In APL (comprises 7% of ANLL), malignant clones show early differentiation and cells contain Auer rods, DIC is common and the 15;17 translocation is often present, therapy shows sensitivity to arsenical trioxide and retinoic acid (induces remission in APL, cells turn into PMN)
 - The retinoic acid receptor- α gene is on chromosome 17q, (normally a transcription enhancer upon retinoic acid binding, for cell differentiation) and this translocation with the pml gene on 15 causes failure for promyelocytes to differentiate and blocks apoptosis
- Precursor B and T cell Neoplasms
 - o Acute Lymphoblastic Leukemia (ALL) – arises in early progenitor B or T cell (4:1 ratio)
 - Lymphoblasts are positive for terminal deoxytransferase (TdT – determined by using a nuclear stain), PAS and acid phosphatase
 - Immunologic classification for ALL
 - B cell lineage – based on the presence or absence of cytoplasmic or cell surface markers
 - o Surface Ig present \rightarrow mature B cell ALL
 - o Cytoplasmic μ present \rightarrow pre B cell ALL
 - This is the most common type of ALL seen primarily in children
 - Symptoms are due to marrow involvement and pancytopenia (bleeding, infections, weakness)
 - T cell lineage – associated with a mediastinal mass in adult males (Thymic enlargement)
 - Tx – combination therapy, chemo is continued beyond remission, bone marrow transplantation, supportive therapy (RBC, platelet transfusions and antibiotics)
 - o Lymphoblastic lymphoma
 - The majority of cases are T cells and are aggressive and rapidly progressive
 - Clinically see males with a big mediastinal mass
 - The leukemic phase of lymphoblastic lymphoma is similar to T-ALL
- Peripheral B cell neoplasms
 - o Chronic Lymphocytic Leukemia (CLL) and Small Lymphocytic Lymphoma (SLL)
 - CLL is very similar to SLL
 - Patients presenting with blood findings \rightarrow CLL
 - Patients presenting with lymph node finding \rightarrow SLL
 - Lymph node involvement is also common in CLL (50%)
 - SLL is a proliferation of small B lymphocytes, which have B cell markers and one T cell marker (CD5), like B-CLL
 - Classification of CLL
 - B-CLL (98% of cases) \rightarrow has B cell markers like CD 19 and 20, one T cell marker (CD5) is also present \rightarrow cells are CD23+ and CD10 negative
 - T-CLL (1-2% of cases) \rightarrow has T cell markers
 - CLL is a progressive accumulation of neoplastic, immunologically incompetent, clonal lymphocytes

- Histologically the affected lymph nodes reveals only a diffuse pattern (not nodular), but proliferation centers are present
- Cytological abnormalities – no specific mutation, but trisomy 21 is common and some involve translocations of the long arm of 14 (14q) which is the site of heavy chain gene, and usually, involvement of this area is prognostic of progressive disease
- Bone Marrow blood findings – numerous normal appearing neoplastic lymphocytes
- Peripheral blood findings - ↑ # of normal appearing lymphocytes, numerous smudge cells (parachute cells) which result from the fact that neoplastic lymphocytes are fragile
 - If B cell monoclonality, then you will only see a κ or a λ chain but not both due to light chain restriction
- Clinical characteristics
 - Mean age at time of diagnosis is 60 – a highly variable presentation , often first presentation is with recurrent infections (an indication of their hypo-Ig condition)
 - Lymphadenopathy (60%), splenomegaly (50%), Hepatomegaly (<40%)
 - Malignant cells are non-functional → patients develop hypogammaglobulinemia → increased risk of infections, some patients develop autoimmune antibodies (10%) and most develop a decrease in one or more Ig types
 - Minor impairments in cell mediated immunity will lead to reoccurrence of infections such as shingles, herpetic lesions, etc.
 - CLL is associated with warm autoimmune hemolytic anemia (AIHA) in 10% of cases and will cause spherocytes to be observed in peripheral blood (a major complication)
 - CLL rarely transforms into a worse disease such as prolymphocytic leukemia or large cell lymphoma (Richter Syndrome)
 - Staging system is indicative of prognosis and depends on the presence of lymphocytosis in addition to lymphadenopathy, splenomegaly, anemia or thrombocytopenia
- Tx – no evidence that therapy prolongs survival, but if you are asymptomatic, you watch and wait, if you are symptomatic, you get radiation for local complications, chemotherapy, monoclonal antibodies or stem cell transplantation
- Multiple Myeloma
 - A clonal malignancy of Plasma Cells
 - increasing incidence with peak age at 70 with Blacks : whites 2:1 (unknown etiology)
 - diagnostic features of multiple myeloma
 - plasmacytosis in marrow – >10% plasma cells (usually much higher) often present in sheets (keep in mind that inflammation, cirrhosis and AIDS can be other sources of plasmacytosis)
 - monoclonal protein in serum or urine
 - lytic disease of the bone
 - blood smear can show multinucleated plasma cells with dysplastic cytoplasm
 - Diagnosis is made from blood test and protein electrophoresis
 - 75-80 % have serum monoclonal Ig, 10-20% make light chains only and rapid renal excretion allows for urine electrophoresis
 - note that CLL, lymphoma, benign monoclonal gammopathy can also have monoclonal Igs
 - Benign Monoclonal Gammopathy is more common than multiple myeloma, but there is no concomitant bone or kidney disease and no anemia, most patients remain stable and about 10% develop multiple myeloma – usually though, monoclonal Ig is an isolated finding

- Patients who present early end up with an incidental diagnosis and are asymptomatic while patients who present later present with lower back pain, recurrent infections and systemic symptoms related to their anemia, renal failure and hypercalcemia
 - Bone disease in Myeloma
 - Results from unbalanced osteoclast activity usually visible on radiograph and ↑ bone breakdown results in hypercalcemia and hypercalciuria
 - Ascites can result from amyloid damage of the kidney
 - Hyperviscosity Syndrome is due to aggregated paraprotein and results in circulatory insufficiency, and abnormal hemostasis
 - Patients can have dyspnea with congestion evident on CXR, encephalopathy and visual disturbances (see fundoscopic abnormalities indicative of hyperviscosity – dilatation and segmentation of the retinal vein) and bleeding
 - Amyloidosis in Myeloma – due to light chain deposition in tissues with a greater incidence of lambda amyloid and frequently manifests in skin, tongue, GI, soft tissue, peripheral nerves, heart, kidneys
 - No effective Tx out there
 - Immunological features of Myeloma
 - Monoclonal Ig and light chains, ↓ levels of normal Ig's (hypogammaglobulinemia), cellular immune responses however are preserved and bacterial infections are common – early (Strep Pneumoniae) and Later (S. Aureus, Gram negative rods)
 - Tx – viphosphonates (pamidronate, zoledronate), Radiotherapy, corticosteroids and conventional chemotherapy, thalidomide (anti-angiogenic), Bortezomib (proteasome inhibitor) and stem cell transplantation
- Lymphoid Neoplasms
- Acute leukemia
 - Peripheral blood shows increase #s of immature blastic cells as well as bone marrow (diagnostic criteria is >30% blastic cells in bone marrow)
 - Acute symptoms are secondary to marrow failure which can result in ↓ RBCs, ↓ WBCs and ↓ platelets

Myeloid neoplasms

- Acute Myelogenous Leukemia
 - Myeloblasts are characterized by intracytoplasmic rods (staining red) called Auer rods
 - Auer rods are abnormal lysosomes (primary granules) that are pathognomonic of myeloblasts and not found in ALL
 - Also stain positive with myeloperoxidase (MPO) or Sudan Black B stain
 - Usually found in promyelocytic leukemia
 - The tissue form of AML is called granulocytic sarcoma (chloroma)
- Chronic Myelogenous leukemia
 - Clonal proliferatin of pluripotent stem cells
 - Unique characteristic is the chromosomal translocation
 - Philadelphia chromosome – t(9;22)
 - Chromosome 9 has *abl* (an oncogene) while 22 has *bcr* (breakpoint cluster region) resulting in formation of a new protein P210 that has tyrosine kinase activity
 - Insidious onset and massive splenomegaly
 - Microscopically – hypercellular bone marrow with all cell lines increased in number
 - Peripherally

- Leukocytosis - ↑ # of neutrophils, band and metamyelocytes, ↑ eosinophils and basophils
 - ↓ leukocyte alkaline phosphatase activity
 - Tx – control with hydroxyurea, bone marrow transplant
 - Prognosis
 - Slow progression (50% develop accelerated phase < 5 years)
 - Blast crisis – non-responsive to chemotherapy
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