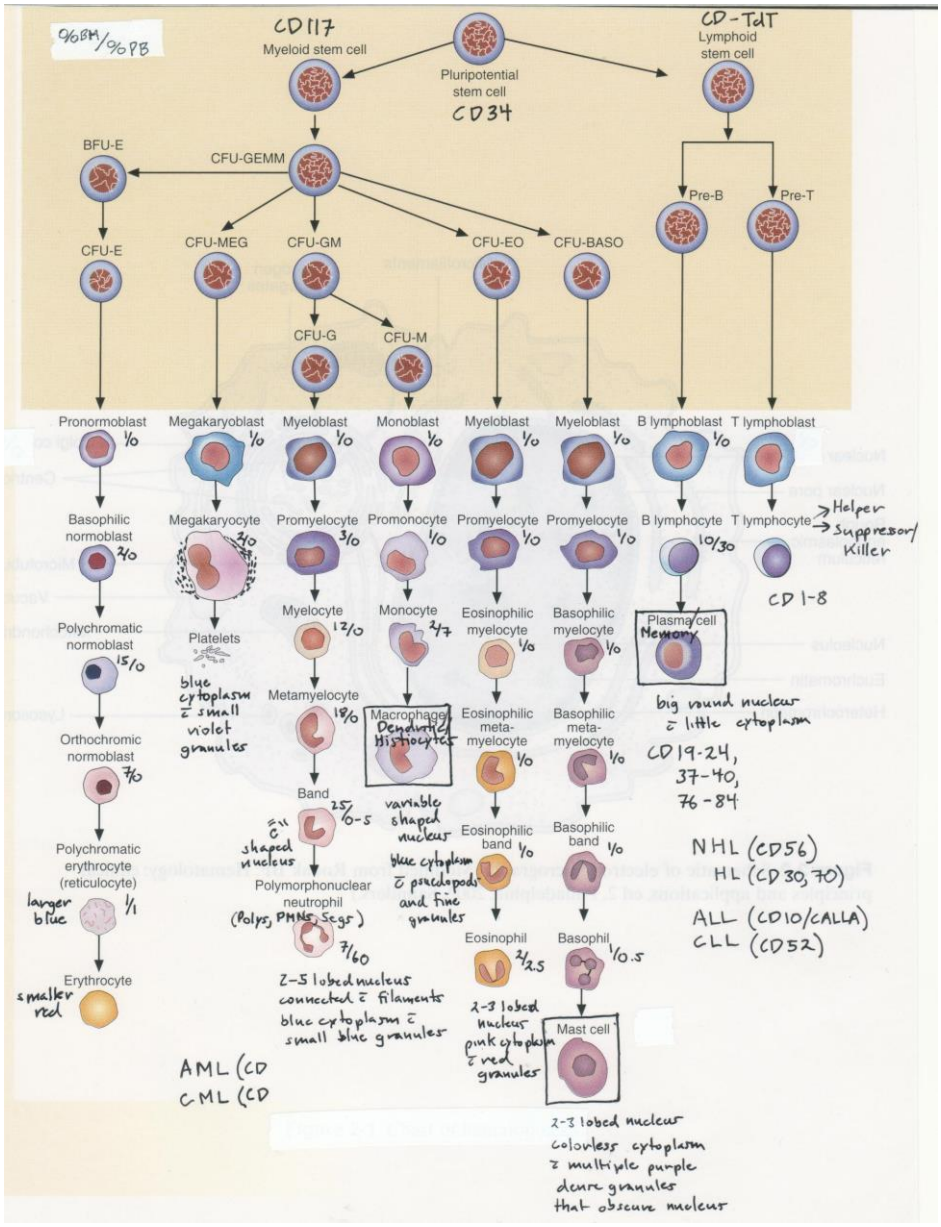
New Topics

- You can do flow cytometery on peripheral blood

General

- Blood
  - 55% Plasma (Serum + Clotting Factors) vs 45% Formed Elements aka Cells (RBC + Buffy Coat (white layer of WBCs + yellow layer of Plts))
  - NB draw whole blood in non-AC tube and spin it creating <u>plasma</u> and formed elements (2) Draw whole blood in AC tube and mix allowing AC to bind clotting factors and then spin it creating <u>serum</u> and formed elements w/ clotting factors
  - NB as cells mature their (1) cytoplasm becomes smaller and less basophilic as it loses RNA and (2) nucleus becomes smaller and more dense
  - NB PBS (Peripheral Blood Smear) start at 10x then oil immersion at 100x, look before feather edge



Lymphoid Lineage

Lymphocytosis

Primary

- Malignancy
  - **Acute:** ALL
  - **Chronic:** LPD

Secondary/Reactive

- Infection w/ Atypical Lymphs (all viral, some bacteria including TB, Syphilis, Brucellosis, etc)
- Metabolic (Addison's, HyperTH, etc)
- Inflammatory States (UC, RA, etc)
- Other (trauma, seizure, post-splenectomy, etc)

Lymphocytopenia

- Immunodeficiency
- Metabolic (Cushing's, etc)
- Any Severe Debilitating Dz

Myeloid Lineage

Malignancy

- **Acute:** AML
- **Chronic:** MPD

Erythrocytosis

Primary (low epo level)

- Malignancy (PV)

Secondary/Reactive (high epo level)

- Chronic Hypoxia (high altitude, smoking, chronic lung dz, cyanotic heart dz, etc)
- Tumor Epo Production (RCC, adrenal, bronchial, hepatic, uterine, cerebellar, etc)
- Other (adult polycystic kidney disease, post-renal transplant, cirrhosis, hepatitis, androgens, etc)

Relative (nl epo level)

- Dehydration

Anemia

Production Problem (Low Retic)

- **Impaired Hemoglobin Synthesis (Microcytic)**
  - IDA/Hemoglobinopathy/Sideroblastic
  - NB Hemoglobinopathy also hemolyze so there will be some high retic at times
- **Impaired BM Function (Normocytic)**
  - AoA/CD
  - Anemia of Renal Failure
  - Bone Marrow Failure (refer to cytopenias below but a specific one is "Pure Red Cell Aplasia" 2/2 congenital problem, Parvo-B19, paraneoplastic thymoma or CLL, autoimmune dz, drugs)
- **Impaired DNA Synthesis (Macrocytic)**
  - Folate+VitB12 Deficiency
  - Hypometabolic States (HypoTH, Liver Dz, Adrenal Insufficiency, Hypogonadism, GH Deficiency)
  - Drug Induced (alcohol, MTX, hydroxyurea, zidovidine, cytosine, Azathioprine, Imatinib)
  - MDS (consider if all is negative)

Increased Destruction/Loss (High Retic)

- **Destruction (Hemolysis Labs)**
  - Extrinsic/Acquired
  - Intrinsic/Genetic
- **Lost (H&P)**
  - Hypersplenism, GI/Menstrual Bleed, Phlebotomy, Deep Compartment Bleed like retroperitoneum, thigh, etc

Thrombocytosis (refer to Hemostasis Notes)

Thombocytopenia (refer to Hemostasis Notes)

Neutrophilia Granulocytosis

Primary (>50k, low LAP)

- Malignancy: CML

Secondary/Reactive/Leukemoid (<50k, high LAP (leukocyte alkaline phosphatase))

- Infection (bacterial, etc) NB very high leukocytosis can be seen specific infection esp C.diff colitis and E.coli sepsis
- Inflammation (vasculitis, etc)
- Metabolic (Cushing, uremia, DKA, hyperTH, etc)
- Drugs (steroids, epi, Li, etc)
- Malignancy (paraneoplastic, carcinomas, etc)
- Other (tissue damage, physical/emotional stress, smoking, asplenic states, etc)

Neutropenia Agranulocytosis

- Few Infections (overwhelming infection, protozoa like malaria, few bacteria like typhoid, viruses like HBV, CMV/EBV, HIV, etc)
- Non-Infectious Inflammation (SLE, Felty Syndrome, Chronic Autoimmune Neutropenia, etc)
- Drugs (anti-inflammatories, anti-bacterials, AEDs, antithyroids, etc)
- Metabolic (liver dz, etc)

Eosinophilia

Primary

- Malignancy: MPD

- Hyper-Eosinophilic Syndrome (HES) very rare, >6mo of eosinophilia, very similar to chronic eosinophilic leukemia, can affect any organ esp heart (CHF 2/2 endocardial fibrosis, thromboembolism), skin (Eosinophilic fasciitis/cellulitis/folliculitis), CNS (esp psych, mononeuritis multiplex), PDGFRA and FIP1L1 gene mutations resulting fused tyrosine kinase that responds to TKI, Dx: eosinophilia >1500eos/mcl x>6mo + DOE + organ damage, 25% 3yr mortality

Secondary/Reactive

- Infections (Parasite)
- Atopic Dz (Asthma, Eczema, AR, Drug Rxn)
- Pulm (Simple/Acute/Chronic Eosinophilic Pneumonia, ABPA)
- Other (Drug Reaction, Eosinophilic GI Dz, HIV, CVD, Vasculitis esp Churg-Strauss, Addison's, Cholesterol Emboli Syndrome, etc)

Basophilia

Primary

- Malignancy: MPD

Secondary/Reactive

- Chronic Hypersensitivity States
- Other (Mast Cell Dz, etc)

Monocytosis

Primary

- Malignancy : MPD

Secondary/Reactive

- Certain Infection (TB, SBE, Hepatitis, Syphilis, Listeria, Brucella, Fungal, etc)
- Granulomatous Dz (Sarcoid, IBD, etc)
- Collagen Vascular Dz

Pan/Bi/Uni Lineage Cytopenia (NB don't forget dilutional)

- **Hypocellular BM:** aplastic anemia, hypoplastic MDS, PNH, severe megaloblastic anemia
- **Eu/Hypercellular BM:** eu/hyperplastic MDS, aleukemic leukemia, mets to BM
- **Myelophthisic BM aka BM replacement:** myelofibrosis, granulomatous dz, storage dz

Aplastic Anemia

- Mech: hematopoietic stem cell failure (even though called "anemia" it is actually a pancytopenia)
- Epidemiology: bimodal peak in adolescence/elderly
- Tx: 80% mortality at 2yrs therefore you must treat if young w/ HSCT (80% complete response) or if there is no donor and pt is old then immunosuppression (65% complete response) w/ ATG (anti-thymocyte globulin), ALG (anti-lymphocyte globulin), cyclosporine, steroids, etc
- Complication: acute leukemia
- Etiology
    - Idiopathic (70%) 2/2 intrinsic defect w/ stem cells
        - Truly Idiopathic
        - Genetic
            - **Immunodeficiency Syndromes (refer)**
            - **hTERT Telomerase Mutation**
            - **Fanconi's Anemia:** AR disorder presenting usually at ~8yo in which chromosomes become fragile thus affecting every cell in body but in particular blood cells = abnormal skin pigmentation, stunted growth, PCT dysfxn, skeletal deformities (absent thumbs, absent first metacarpal, absent radius) pancytopenia, cancer (AML, skin, GI, GU)
            - **Diamond-Blackfan Syndrome:** same as Fanconi's except that it presents during first few months of life and only has pancytopenia no physical abnormalities
            - **Kostmann Syndrome**
            - **Cyclic Neutropenia**
    - Stem Cell Destruction (30%) 2/2 immune mediated destruction of stem cells
        - Drugs: XRT, immunosuppressants, chemo, idiosyncratic drug reactions (NSAIDs, AEDs, antithyroid, acetazolamide, abs including beta-lactams/sulfa/bactrim/chloramphenicol, etc)
        - Infection: virus (Parvovirus B19, HHV-6, HIV, HCV/HBV, EBV/CMV), bacteria (TB), sepsis
        - Immune: SLE, GVHD, Thymoma
        - Toxins: alcohol, chemicals (benzenes, insecticides)
        - Nutritional: folate, vitB12

Plt (life span = 10d)

WBC (life span = 17d)

- **Neutrophils**
    - Left Shift
    - Nucleus (nl 3.2 lobes)
        - **Hypersegmentation >5** (VitB12/Folate Deficiency, Chronic Infections)

        - **Hyposegmentation <3** usually two looking like "eye-glasses" (Pelger-Huet Syndrome (AD), MDS = pseudo Pelger Huet anomaly, MPD)
    - Intracytoplasmic Changes
        - Dirty Cytoplasm w/ **Toxic Granules** (multiple small dark purple granules looking like dirty cytoplasm), **Toxic Vacuoles**, **Dohle Bodies** (single very faint blue bodies bigger than granules): sepsis
            - NB other granules are seen in Chediak-Hegashi Syndrome, May-Hegglin Syndrome, etc
        - **Degranulation** (clear cytoplasm): infection, MDS
        - **Auer Rods** (few fine red rods): AML
        - **Smudge Cells** (disintegrating nucleus and ruptured cells): CLL
- **Lymphocytes**
    - **Atypical/Reactive** (vacuolated cytoplasm, irregulary shaped large nucleus, azurophilic granules)

RBC (life span = 120d)

- General
    - NB no one looks at Hgb g/dL (?), RBC #/mcL (number or RBCs), MCH pg (Mean Corpuscular Hemoglobin), Chromasia aka Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dL)
    - Hemoglobin = Heme (Protophorphyrin Ring + Iron) + Globin
- **Hct** (percentage of total blood volume occupied by RBCs, blood is spun down separating into packed RBCs and buffy-coat/plasma, this ratio is the Hct)
- **Red cell Distribution Width (RDW)** (variation in RBC width calculated by SDx100 / MCV = 13%)
- **Reticulocyte Index (RI)** ([RC x (Pt's Hct / Nl Hct)] / Maturation Factor = 2%) where Maturation Factor = 1 if Hct 45%, 1.5 if 35%, 2 if 25%, etc
- **Size aka Mean Corpuscular Volume (MCV)** (average volume of an RBC calculated by Hct/RBC x 10 = fL)
    - **Micro vs Normo** (70fL = lymphocyte nucleus) **vs Macro**
    - **Anisocytosis** (variation in size, any severe anemia)
- **Shape**
    - **Poikilocytosis** (variation in shape, any severe anemia)
    - **Spherocytes**: hereditary spherocytosis, hemolysis, transfused cells, severe burns
    - **Ovalocytes/Elliptocytes** (egg/cigar shaped): hereditary ovalocytosis/eliptocytosis, thalassemia, IDA, megaloblastic anemia, myelopthysis
    - **Stomatocytes** (instead of circular central pallor there is a slit): hereditary stomatocytosis, hemolysis, alcoholism, liver dz, artifact
    - **Drepanocyte aka Sickle Cell**
    - **Codocyte aka Target Cell:** any hemoglobinopathy esp thalassemia, IDA, splenectomy, liver dz
    - **Schistocytes aka Helmet Cells** (irregularly contracted cells): any hemolytic process
    - **Echinocytes aka Burr/Crenated Cells** (regularly spaced spinous processes): uremia, hemolysis, infants, gastric cancer, pyruvate kinase deficiency, artifact
    - **Acanthocytes aka Spur Cells** (IRregularly spaced spinous processes): liver dz, abetalipoproteinemia, splenectomy, malabsorption, hypothyroidism, vitE deficiency
    - **Dacryocytes aka Tear Drop Cells:** any BM infiltrative disorder (you will also see immature RBC/RBC called "leukoerythroblastic" change)
    - **Bite Cells** (removed Heinz Bodies) G6PD
    - **Rouleaux** (poker chip stacking in same field as solitary RBCs): any hyperproteinemia state vs **Autoagglutination** (clumped together): Ab/Ag reaction
- **Intra-RBC Abnormalities**
    - **Nucleated RBC**
    - **Pathogens** (Malaria, Babesios, etc)
    - **Howell-Jolly Bodies** (single cytoplasmic blue body representing nucleic acid): hyposplenism, folate/vitB12 def, hemolytic anemias
    - **Heinz Inclusion Bodies** (single membrane bound large blue body representing denatured Hgb): enzymopathies
    - **Pappenheimer Bodies aka Siderotic Granules** (cluster of small blue granules bodies representing iron): iron overload states
    - **Cabot Rings** (single large purple ring/figure eight representing remnants of mitotic spindle): MDS, folate/VitB12 def
    - **Basophilic Stippling** (multiple small round light blue granules representing ribosome aggregates that have not been lost representing premature RBCs): sideroblastic anemia, thalassemia
    - **Polychromasia** (diffuse blue color representing diffuse RNA that has not been lost representing premature RBCs aka reticulocytes)
    - **Hemoglobin C Crystals** (very large hexagonal crystals)

Blood Products/Procedures

- **Packed Red Blood Cells (PRBC)**
    - **1U = 3% Hct**
    - transfuse 2 Units PRBCs if $\leq$23 if no other problems or $\leq$25 if CAD (the prior practice of Hct<30 is based on a study in 1942)
    - darbopoetin (Aranesp) 2.25mcg/kg SC Qwk (comes as 25, 40, 60, 100, 150, 200, 300, 500 mcg) otherwise if not chemo related then just 0.45mcg/kg SC Qwk (NB epoietin (Epogen/ProCrit) shorter t1/2 and is not available at BUMC), there was

a big meta analysis in 2007 showing that RBC stimulating agents stimulate cancer progression and increase r/o CAD/CVA/DVTE, so use when Hgb<12g/dL and not anemia of cancer)
    - **T&S&C** (takes 15min, "type" aka determine the recipient's major RBC antigens, "screen" aka check the recipient's serum for antibodies to major RBC antigens, "cross" aka test recipient's serum for antibodies to minor antigens on the donor's RBCs and is performed right before blood is given)
    - **Alloimunity** (formation of antibodies from sensitization like transfusions, pregnancy, etc (antigen negative pt (A, B, O, Rh-) to foreign antigens (B, A, AB, Rh+)))
    - **leuko-poor/reduced aka LP/R** (eliminates WBCs that may cause a fever transfusion reaction and thus is used in pts who have a h/o this transfusion reaction)
    - **washed** (eliminates plasma proteins that may cause an allergic-to-anaphylactic transfusion reaction and thus is used in pts who have a h/o this transfusion reaction)
    - **irradiated** (eliminates immunologically active T-lymphocytes and thus is used in pts who are immunocompromised following a transplant to prevent GVHD)
    - **CMV negative** (eliminates CMV virus and thus is used in pts who are immunocompromised following a transplant)
    - all PRBCs contain citrate which binds Ca acting as an AC, phosphate which retards the breakdown of 2,3-DPG, and dextrose which serves as an energy source
    - PRBC is 500mL of whole blood that is centrifuged and made void of 250mL of plasma and platelets thus 1U PRBC = 250mL
    - 250mg of iron per unit (Sx can develop after 20U of PRBC!!!)
    - you can warm blood products to increase infusion rate or b/c of hypothermia
- **Platelets**
    - 1U = 50mL (should raise by 10k)
    - if <10 or <50 and actively bleeding / pre-op
    - no need for lasix as plts are not volume expanders
    - if plts become refractory (check plt level 1hr after transfusion not next morning) to transfusions then check HLA Typing and in meantime transfuse LR/P+IR+SD (Single Donor) not RD (Random Donor or Pooled) Plts and when available transfuse the HLA matched, check PRA to determine need for HLA matching
    - if you are suspecting dysfunctional platelets give ddAVP at 0.3mcg/kg IV q12-24hrs x2
    - contraindicated in HUS/TTP, HELLP, HIT
- **CSF**
    - NO WBC transfusion but note that PRBCs have WBCs nevertheless must use CSF
    - filgastrim (Neupogen) SC Qd x7d or pegfilgastrim (Neulasta) SC Qd x1d after successful induction
- **Fresh Frozen Plasma (FFP)**
    - contains all factors
    - 10-20mL/kg which should increase [clotting factor] by >20% (just give 3units, where 1unit = 250mL)
    - Uses: deficiency of multiple factors as in coumadin reversal, microvascular bleeding as in DIC, liver failure with elevated coags and concomitant bleeding, specific factor defect when no other specific factor exists, etc
    - Called fresh frozen b/c "frozen" "w/in 6hrs" of phlebotomy
    - **Plasmapharesis:** removing plasma vs **Plasma Exchange:** plasmapharesis then replacement w/ FFP
- **Cryoprecipitate (Cryo)**
    - contains only vWF, factor 8, factor 13, factor 1 (fibrinogen)
    - 1unit/10kg which should increase fibrinogen by 5-10mg/dL (just give a "10pack" as Cryo comes only in 10packs where a 10pack = 10units and 1unit = 20mL)
    - Uses: quantitative (<100mg/dL) fibrinogen deficiency (e.g. DIC), qualitative fibrinogen problem (e.g. acquired dysfibrinogenemia associated with liver disease), hemophilia A (factor 8 deficiency), Factor 13 deficiency, von Willebrand disease, bleeding due to uremia and HD not available
    - has been supplanted by the use of specific factor products that are safer and more efficacious
    - called "cryo-precipitate" b/c when FFP is thawed vWF, Factor 8, Factor 13, and fibrinogen are still solid aka "cryo" and thus separated aka "precipated"
- **IVIG**
    - polyvalent IgG from thousands of donors which saturate Fc receptors on macrophages thus decreasing macrophages from binding auto-antibodies on normal cell
    - 2g/kg IV divided over 5d per pharm protocol
- **Transfusion Related Problems**
    - Infectious Risks per unit
        - **HTLV (1:3million)**
        - **HIV (1:1.8million) first started testing in 1989**
        - **HepC (1:1.6million) first started testing in 1990**
        - **HepB (1:200k) first started testing in 1960**
        - **CMV (common)**
        - **Bacteria: Skin Flora, *Yersinia*, *E.Coli, Psuedomonas* (rare) occurs b/c of bacteremia or skin plugs are drawn into collection bag during venipuncture) NB more common in plts b/c kept at room temp**
        - **Other: Syphilis, Trypanozomi, Babesia, Borrelia, West Nile, Prion (3 cases of CJD)**
    - Non Infectious Risks per unit
        - **Acute Hemolysis (1:50k)**
            - Mech: preformed Abs against major Ags (A, B, Rh)

        - S/S: F, chills, N, hypoTN, back/flank pain, SOB, chest pain, AKI, hemoglobinuria, jaundice that occurs <24hrs after transfusion usually within a few minutes
        - Tx: stop transfusion and send bags and pt's blood to lab for testing (T&C, Coombs's Test, CBC, BMP, DIC Panel, Total Bilirubin), call blood bank, transfuse compatible blood products, fluids/pressors, furosemide to protect kidneys, bicarbonate to alkalinize urine to prevent hemoglobin crystallization, closely monitor renal function, steroids, epinephrine (1:1,000) 0.5-1.0mL IM x1, diphenhydramine
    - **Transfusion Related Acute Lung Injury (TRALI) (1:5000)**
        - Mech: donor Abs bind recipient WBCs in pulmonary vasculature causing pulmonary sequestration and subsequent release of mediators causing pulmonary damage
        - S/S: just like ARDS (resolves in 1wk)
        - Tx: just like ARDS
    - **Delayed Hemolysis (1:1000)**
        - Mech: Abs against minor antigens (Kidd, Duffy, Kell, MNS)
        - S/S: less severe form of acute hemolysis that occurs 7-10d after transfusion
        - Tx: similar to acute hemolysis
    - **Allergic Reaction (1:150) to Anaphylaxis (1:35k)**
        - Mech: reaction to plasma proteins in transfusion
        - S/S: itching, hives, flushing, bronshospasm, laryngeal edema, hypoTN (usually NO fever) more immediate than delayed hemolysis
        - Tx: diphenhydramine 50mg PO x1 to epi and steroids and use washed PRBC in future
    - **Fever (1:100)**
        - Mech: Abs against WBCs Ag from prior transfusions and esp pregnancies
        - S/S: F, chills, dyspnea, anxiety (Sx hours after transfusion)
        - Tx: acetaminophen 650mg PO x1 and use LP/R PRBC in future
    - Other Risk: **fluid overload** (give lasix), **iron overload** (esp if pt has received >20units), **hypothermia** (b/c blood is cold), **hypocalcemia** (b/c of citrate in blood), **hyperkalemia/academia** (b/c of lysis during infusion), **transfusion associated immunomodulation** (it has been shown that getting transfusions increases incidence of nosocomial infections b/c PRBC is immunosuppressive if pts need chronic transfusions as in Thallassemia)

Bone Marrow Bx

- Call BM Tech at x3326 10min in advance before 4pm
- just done gloves no need for gown
- Get BM Kit, extra sterile lidocaine bottles, gloves
- Order: Routine (Bx and Aspirate) + Flow & Cytometry (if a new dx to checks for CD#s) + Molecular Pathology (PCR of oncogenic genes) + Cytogenetics (if a new dx to check for chromosomal abnl but some also check after Tx b/c cytogenetics can change) + Culture/PCR
- Get Small Syringe, put small needle on it (if you need to do flow cyto then get another syringe and fill it up w/ 1cc of heparin from a bottle given to you by the tech, flush it up and down to coat inside of syringe dont shoot all of it out)
- Find flat spot on posterior iliac crest, inject a wheal of lidocaine, then hit periosteum and inject TONS of lidocaine in a clock wise fashion
- Make a nick in skin with a scalpel
- Take small blue "jabber" remove bottom blue piece but keep stylet and stick into bone (you'll hear a crunch), remove top, remove stylus, attach dry syringe and remove <1cc for tech to check for spicules, once comfirmed attach regular syringe and remove 1cc for routine aspirate and if doing flow cyto / cytogenetics attach heparin coated syringe and draw up 3/3cc and if you want culture tech will ge you another dry syringe from which you will draw up 2cc
- Take big blue "jabber" and stick into BM (you'll hear a crunch), then remove stylus and go down another inch, once at that depth then twist clockwise 10x, counter 10x, wiggle up, wiggle down, wiggle side-to-side, then slowly take it out, use metal wire to poke out BM Bx onto tech's slide thru other end

Starting Coumadin

- Day 1
    - A protocol exists at BUMC
    - Bridge w/ heparin (gtt protocol) / lovenox (1mg/kg SC BID) and only when aPTT is therapeutic then you start Coumadin: Why? t1/2 of Protein C/S (6hrs) is shorter than Factor 2,7,9,10 (66hrs) therefore pt is hypercoagulable before pt is anticoagulated
    - Start Coumadin 5mg PO at 1800 and check INR Qday until therapeutic x2d and if pt requires Coumadin then give what is recommended for each day and then after a few days when therapeutic take total amount of coumadin given and divide by number of days to give per day dose
    - d/c heparin gtt only when coumadin given for >5d and until INR therapeutic >2d
    - pt can be sent home even if subtherapeutic by giving home sc lovenox after education and follow up at INR clinic (arrange w/ care coordinator) in few days
    - follow INR weekly initially and based on how labile the pt's INR is you can range from Qwk to Qmo
- Day 2
    - <1.5 = 6mg
    - 1.5-1.9 = 2.5mg
    - 2.0-2.5 = 1mg
    - >2.5 = No Dose

- Day 3
  - <1.5 = 5-10mg
  - 1.5-1.9 = 2.5-5mg
  - 2.0-2.5 = 0-2.5mg
  - 2.6-3.0 = 0-2mg
  - >3.0 = No Dose
- Day 4
  - <1.5 = 10mg
  - 1.5-1.9 = 5-7.5mg
  - 2.0-3.0 = 0-5mg
  - >3.0 = No Dose
- Day 5
  - <1.5 = 10mg
  - 1.5-1.9 = 7.5-10mg
  - 2.0-3.0 = 0-5mg
  - >3.0 = No Dose
- Day 6
  - <1.5 = 7.5-12.5mg
  - 1.5-1.9 = 5-10mg
  - 2.0-3.0 = 0-5mg
  - >3.0 = No Dose