Pathology: Hematology

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Leukocytes: nucleated blood cells
- **Granulocytes**: neutrophils, eosinophils, basophils
- **Lymphocytes**: B-cells, T-cells, NK cells

### Neutrophils

**Development**: (morphology mirrors functional develop.)

Myeloblast → promyelocyte → myelocyte → metamyelocyte → band → PMN

1. **Myeloblast**: large, round nucleus; 2+ nucleoli; scant basophilic cytoplasm, no granules
2. **Promyelocytes**: blue/violet (azurophilic) 1° granules, nucleus eccentric, nucleolus
3. **Myelocyte**: clumped nuclear chromatin with no nucleolus, cytoplasm has 2° and 1° granules
4. **Metamyelocyte**: cytoplasm pinker, more 2° granules, nucleus bean-shaped
5. **Band**: nucleus elongated like sausage; width of nucleus smaller then length of indentation
6. **Segmented neutrophil**: fine filament separating 2+ lobes

**Phases**:
- **Bone marrow mitotic phase**: blast → myelocyte (7.5d)
- **Bone marrow post-mitotic phase**: meta → mature gran (16.5d)
- **Blood**: (6hrs) → tissue (1-2d)

**Note**: PMNs spend very little time in circulation
- (only 8% in intravascular space)
- Circulating PMNs equilibrate with marginating PMNs (50/50 of those 8% in circulation)

**Myeloid growth factors**
- **G-CSF** and **GM-CSF** are primary ones (granulocyte ± macrophage colony stimulating factor)
  - Used clinically to increase PMN production (e.g. post chemo)
- **Expand** committed progenitors; **shorten** bone marrow transit time → **INCREASE OUTPUT**
- **Enhance function** of mature PMNs to inflammatory signals

### Neutropenia (ANC < 1800)

- **ANC** (abs. neutrophil ct) < **1800/μL** (Mild 1000-1800, Moderate 500-1000, severe <500)
- ↓PMNs, ↑risk infection
- **DDx**:
  - ↓production (congenital – cyclic, like Kostmann’s Syndrome / acquired – drugs, vitamin B12, leukemia; viral causes)
  - ↑destruction (autoimmune/sepsis)
  - sequestration / splenomegaly
  - pseudoneutropenia (increased marginal pool)

### Neutrophilia (ANC > 8000)

- Associated with **wide variety of physiologic stresses** (inflammation, infection, metabolic disturbances, etc)
- If associated with **infection**: mainly **bacterial**; look for toxic granulation, vacuolization, Dohle bodies
  - **Dohle bodies**: mRNA in cytoplasm; indicative of cellular activation
- Also drugs (corticosteroids, more), hematologic disorders (**myeloproliferative** – see below)
I’ve got too many WBC: Is it CML or Leukemoid reaction (malignant or benign)?

<table>
<thead>
<tr>
<th></th>
<th>LEUKEMOID</th>
<th>CML</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC &gt; 100K</td>
<td>Rare</td>
<td>Often</td>
</tr>
<tr>
<td>BASOPHILIA</td>
<td>Rare</td>
<td>Often</td>
</tr>
<tr>
<td>CHROMOSOMAL CHANGES</td>
<td>None</td>
<td>BCR-ABL</td>
</tr>
<tr>
<td>SPLENOPEGALY / HEPATOMEGLY</td>
<td>Rare</td>
<td>Often</td>
</tr>
<tr>
<td>CLINICAL COURSE</td>
<td>Resolves</td>
<td>Progresses</td>
</tr>
<tr>
<td>WBC (LEUKOCYTE) ALKALINE PHOSPHATASE</td>
<td>High (LAP&gt;100)</td>
<td>LAP&lt;10</td>
</tr>
</tbody>
</table>

**Neutrophil Defects**

**Qualitative PMN Defects**
- Recurrent infections & abscesses; receptors for opsonins / integrins are frequent targets
- Leukocyte adhesion defects (integrins, selectins) – PMN stay in circulation
- Defective motility (inherited/acquired): cytoskeletal defects → ↓ chemotaxis
- Defective cell killing: e.g. **Chronic granulomatous disease**
  - genetic disorders, defects in generating ROS, can’t kill some organisms (no respiratory burst)
  - can ID absence of superoxide production (nitroblue tetrazolium (NBT) dye test)
  - granulomas at site of infection instead (bacteria proliferate inside cell, can’t clear infection)

**Morphologic changes:**
- Reduced DNA (but not RNA) synthesis
- MCV > 120 = think megaloblastic anemia
- Cells of any type that have rapid turnover are affected

**Rare examples of changes (USMLE, not for exam)**
- May-Hegglin anomaly: aut-dom, large dohle bodies, giant platelet, thrombocytopenia
- Pelger-Huet anomaly: aut-dom, bi-lobed/dumbbell-shaped nuclei, normal function
- Alder-Reilly anomaly: large azurophilic granules, seen in Huler’s syndrome & other metabolic disorders, metachromatically-staining mucopolysaccharide deposits
- Chediak-Higashi syndrome: Aut-recessive, lysosomal trafficking regulator protein messed up, large cytoplasmic lysosomal inclusions, defects in T/NK cytotoxicity & killing activity of PMNs; occulocutaneous albinism, recurring infections, more

**Eosinophils**
- Regulate hypersensitivity reactions
- Innate defense against helminthes/ticks
- IL-5 is major regulatory growth factor

**Hypereosinophilia:** worms, wheezes, weird diseases
- Can cause significant tissue damage, sequelae

**Basophils**
- Mystery! Granules have histamine, heparin, PAF but no known deficiency disorder
- For both basophils & eosinophils, you can have zero on WBC + diff and it’s fine

**Basophilia:** chronic infection, cancer, iron deficiency, myeloproliferative disorders

**Monocytes**

Roles: Phagocytosis, immunomodulation, antigen presentation

Chronic monocytosis: usually chronic infection or inflammatory disease

<table>
<thead>
<tr>
<th>CLINICAL CONDITION</th>
<th>DIFFERENTIAL COUNT FINDING</th>
<th>ABSOLUTE CELL COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>acute infection</td>
<td>granulocytosis</td>
<td>&gt; 9000/UL</td>
</tr>
<tr>
<td>chronic inflammation</td>
<td>monocytes</td>
<td>&gt; 1000/UL</td>
</tr>
<tr>
<td>parasitic infection</td>
<td>eosinophilia</td>
<td>&gt; 700/UL</td>
</tr>
<tr>
<td>viral infection</td>
<td>lymphocytosis</td>
<td>&gt; 3500/UL</td>
</tr>
<tr>
<td>aplastic anemia</td>
<td>neutropenia</td>
<td>&lt; 1500/UL</td>
</tr>
<tr>
<td>acute leukemia</td>
<td>immature cells or blasts</td>
<td>can see bands or myelocytes, blasts are not a normal finding</td>
</tr>
</tbody>
</table>
Introduction to Hemostasis & Platelet Biology

Hemostasis: physiologic process that leads to stopping of bleeding
- Platelets & plasma clotting proteins

Primary hemostasis: injury to blood vessel → constriction → aggregation of platelets → platelet plug
Secondary hemostasis: inactive coagulation proteins → active (coagulation cascade; thrombin; fibrinogen→fibrin)
- Platelet plug becomes more stable (protein + fibrin backbone)

Platelets: the basics

- Made in bone marrow: multinucleated megakaryocytes stimulated by thrombopoieten (TPO, a growth factor)
  o Platelets are not cells: fragments of megakaryocyte cytoplasm
- 150-300K /μL is normal, circulate for 10 days (120d for RBC, a few hrs for PMNs)
- Spleen stores 1/3 body platelet mass (like a reserve for trauma)

Histology:
- Canalicular system (CS) lets molecules in/out
- Dense granules (DG): ADP, ATP, serotonin
- Alpha granules (AG): fibrinogen, von Willebrand Factor (vWF), immunoglobulin
  o all taken up from plasma; importance not clear
- Lots of mitochondria (M)
- Microtubules (MT): maintain discoid shape
  o Rearrange in activation (discoid → smaller sphere with long pseudopodia → more SA to contact other platelets)

Function

1. Adhesion
   Injury → subendothelium exposed → vWF (glycoprotein) binds to subendothelium
   a. vWF released from Weibel Palade Body (large organelle in endothelial cells, circulates in plasma; binds fast (1-3s postinjury!))
   b. vWF is ligand for platelet glycoprotein Ib-IX (receptor on platelets) → platelets bind vWF at injury site

2. Platelet Activation
   Initial platelets adhere → release ADP & thromboxane (TxA2) → other platelets activated → more platelets incorporated into developing plug
   a. ADP: adenosine diphosphate, from platelet dense granules, released → binds ADP receptors on other platelets
   b. Thromboxane: made by platelets, causes platelet activation & vasoconstriction.
     i. Two thromboxanes: A2 = active but unstable, 30s half-life → hydrolyzed to inactive B2
     ii. Derived from arachadonic acid; related to prostaglandin (aspirin inhibits TxA2 production)
   c. Platelets change shape too (bigger SA, more clotting proteins deposited, localize to needed areas
d. Clotting cascade being activated by tissue factor (subendothelial Mϕ) at the same time
   i. Thrombin activates platelets (cleaves thrombin receptor on platelets, which undergoes shape change & signals after cleaveage! Crazy!) Also cleaves fibrinogen \( \rightarrow \) fibrin to form mesh for plug

3. Aggregation
Platelets adhering to each other = aggregation. Glycoprotein Iib-IIa (integrin on platelet) undergoes conformational change during activation; binds fibrinogen
   a. Fibrinogen (water soluble) \( \rightarrow \) fibrin (water insoluble polymer, forms supporting structure for blood clot)
   b. Platelet plugs fill in like concrete poured on steel bars

“Bleeding time” is how long it takes for all this to happen (usually 3-7m)

Platelet Dysfunction

Congenital / Inherited disorders: can lead to life-long bleeding.
- Tons of etiologies: absent agonist receptors, adhesion / receptor / aggregation / secretion / signaling defects

Acquired dysfunction: more common than inherited
- Renal failure: toxins build up, platelet function ↓
- Medications: aspirin is most common

Testing for abnormal platelet function
1. Bleeding time: small controlled forearm incision, measure time to clotting
2. Platelet aggregation: add agonist (ADP, collagen, etc.) to stimulate platelets) compare to control, measure degree of aggregation. Different agonists to test different receptions

What keeps platelets from adhering to endothelium/subendothelium normally?

Passive factors: negative charge on endothelial cell surface

Active processes:
- Prostacycline (PGI2): vasodilator, inhibits platelet activation by raising cyclic AMP levels
- ADPase destroys ADP (decreases platelet activation)
- Nitric oxide (endothelial-derived releasing factor, EDRF): inhibits platelet function
- Tissue plasminogen activator: promotes clot lysis
- Thrombomodulin: inhibits thrombin (inactivates clotting cascade; inhibits platelet activation)

Quantitative changes in platelets (low platelets!)

Thrombopoiesis: Thrombopoieten (TPO)
1. Functions:
   a. Megakaryocyte-stimulating factor (along with cytokines, e.g. IL-6)
   b. Also primes platelets to be more sensitive to platelet agonists
   c. Can give recombinant form to increase platelets in thrombocytopenic patients
2. Made in liver
3. Thrombopoietin receptor (on platelets & megakaryocytes): receptor binds & internalizes growth factor
   a. inverse relationship between TPO levels & platelet / megakaryocyte mass (sucking it out of plasma!)
**Causes**: decreased production, increased destruction, sequestration.

1. **Sequestration**: splenic enlargement can sequester large #s platelets, lowering circulating number

2. **Decreased production**
   - Marrow infiltration (tumor, leukemia)
   - Chemotherapy / toxins
   - Hypoplasia (myelodysplasia)
   - Aplasia (aplastic anemia)
   - Thrombopoietin deficiency

3. **Increased destruction**
   - **Immune** (autoimmune / alloimmune disorders, drug-induced, infectious mono)
     - **Autoimmune thrombocytopenia**: #1 immune cause
       - Auto-Ab to platelet glycoprotein receptors (glycoprotein IIb-IIIa)
       - Cause unknown; destruction takes place in spleen/liver after Ab-mediated phagocytosis of RBC
       - Severe thrombocytopenia & bleeding
       - Ab can also bind megakaryocytes / impair platelet production
       - Splenectomy can be tx (stop destruction)
     - **Non-immune**
       - DIC with excessive clotting intravascularly / sepsis
       - Thrombotic thrombocytopenia purpura (TTP) pathologic increase in platelet adhesion

   - Bone marrow: adequate / increased # megakaryocytes = normal production, increased destruction
   - Peripheral destruction is most common mechanism

**Platelet function** can be impaired too:
- e.g. absence or markedly reduced platelet aggregation in response to epinephrine

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**Thrombocytopenia**

<table>
<thead>
<tr>
<th>Platelet Count</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 100,000</td>
<td>No excessive bleeding (even with major surgery)</td>
</tr>
<tr>
<td>50-100,000</td>
<td>Can bleed longer than normal with severe trauma</td>
</tr>
<tr>
<td>20-50,000</td>
<td>Bleed with minor trauma, petichiae, no spontaneous bleeding</td>
</tr>
<tr>
<td>&lt; 20,000</td>
<td>May have spontaneous bleeding</td>
</tr>
</tbody>
</table>

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**Thrombocytosis**

**Elevated** platelet count

**Etiologies**:
- Bone marrow disorders of essential thrombocytois
- Polycythemia rubra Vera (excess RBC + WBC/platelets produced from bone marrow)
- Chronic myelogenous leukemia

**Thrombocytosis**: can see
- Thrombotic complications (too many platelets)
- Hemorrhagic complications (paradoxical: absorbing out all the vWF, etc.)
Blood Transfusion

Indications for transfusion

- **Restore blood volume** in patient with major blood loss (saline, etc. also used for volume expansion if possible)
- **Restore O₂-carrying capacity** (anemia from bleeding, hemolysis, inadequate RBC) – usually with Hgb < 7 g/dL
- **Replace cellular elements / plasma proteins**

Comes from: **healthy altruistic blood donors** (volunteers)

Whole blood:

- Provides: **volume expansion, red cell mass**, some **coagulation factors** (but low content of platelets, factor VII)

Components are often preferable (spin down & separate parts: see picture)

- **Why?** Avoids circulatory overload, ↓ harmful metabolic materials, concentrate required material, ↓ risk of dz transmission, maximize use of donated blood
- **General parts:**
  - Plasma (albumin, IgGs, Factor VII/IX/other coagulants, etc.)
  - Buffy coat (platelets, leukocytes)
  - RBCs

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood (1 unit)</td>
<td>Blood (450 mL)</td>
</tr>
<tr>
<td></td>
<td>Preservative (63 mL)</td>
</tr>
<tr>
<td>Packed RBC (1 unit)</td>
<td>RBC (200 mL)</td>
</tr>
<tr>
<td>Fresh Frozen Plasma</td>
<td>Plasma (220 mL)</td>
</tr>
<tr>
<td>(1 unit = 10-15mL)</td>
<td>Factor VIII (100U)</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen (250 mg)</td>
</tr>
<tr>
<td></td>
<td>vWF (40-70% of what was in 1 U FFP)</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>4x10¹¹ platelets</td>
</tr>
<tr>
<td>(1 unit = 10-15mL)</td>
<td>(6-8 random donor platelet units)</td>
</tr>
<tr>
<td>Platelets (1 pheresis unit = 200mL)</td>
<td></td>
</tr>
</tbody>
</table>

Preservation

- **RBC** during storage
  - ↓ ATP levels, ↓ 2,3-DPG (corrects quickly in vivo)
  - ↑ metabolites (K accumulation can be a problem in **newborns**)
- 75% @ 24 hrs is acceptable RBC survival (related to ATP levels)
- Storage possible for **5-6 wks** with modern preservatives (older = ~3-4 wks)

**Red Cells**

Different **components** available:

1. **Red blood cells** (reduced volume)
2. **Leukocyte-depleted RBC** (leukocytes removed)
3. **Washed RBC** (plasma removed; e.g. if patient allergic to plasma protein)
4. **Frozen RBC** (increase storage period, almost indefinite, use for **really** rare blood types)
Platelets

- **Pooled concentrates** (from lots of donors, like a blended whiskey):
  give you lower cost and more readily available supply

- **Apheresis** (from individual donors; the “single-malt”):
  gives you lower donor exposure to disease, lower reaction rates but has a more limited donor pool and costs more $$

< 100k platelets = ↑ bleeding time

<10k = start giving prophylactic transfusions (spontaneous bleeding)

<5k = ↑ GI hemorrhage risk

Platelet Alloimmunization

- Pts. with previous transfusions / pregnancies: refractory (don’t respond / response decreases each time)
- HLA Ab destroy incompatible platelets (20-50% pts)
- HLA matching can help, need to prevent refractoriness
  - Treat with HLA matching, platelet crossmatching (same efficacy)
  - Prevent by limiting donor exposure, leukodepleting platelets

Leukodepleted components

Good for:
- ↓ transfusion reactions, ↓ alloimmunization
- ↓ risk viral transmission (viruses that hang out in RBC: CMV, HTLV-1, etc.)
- ↓ immunomodulation? Some evidence that WBC suppress immune system?

Fresh Frozen Plasma

Frozen within 6 hours of collection

Components: VIII, prothrombin complex (X, IX, VI, V, prothrombin) with concentration the same as in fresh plasma

- Problem: putting lots of volume into the patient

Cryoprecipitate

- Helps fix volume problem of FFP to help with hemostasis (only 10-15 mL per unit; fibrinogen + factor VII + vWF)
- Slowly thaw FFP at 4° C, centrifuge, precipitate resuspended; not used as much anymore

Concentrates

- Mostly recombinant (e.g. coagulation cascade factors); give instead of cryoprecipitate
  - After pooled concentrates ➔ HIV (90-95% hemophiliacs infected in 1980s), recombinant much safer
- Patients (e.g. hemophilia) can give themselves at home instead of having to come in

Irradiated Blood

- Helps eliminate risk of transfusion-associated graft-vs-host dz
  - 98% FATAL!
  - Donor WBC can attack recipient; normally recipient WBC win the fight but not if immunodeficient!
- Give for pts with congenital immunodeficiency, BMT / solid organ transplant, neonates

**COMPONENTS FOR HEMOSTASIS**

- Platelets
- Cryoprecipitate
- Factor VIII concentrates
- Factor IX concentrates
- Fresh frozen plasma

**Indications for platelet transfusion**

- Thrombocytopenia (bleeding or prophylaxis)
  - More useful if ↓ production of platelets
  - Less useful when ↑ destruction (DIC, ITP)
- Dysfunctional platelets (aspirin, etc.)
Immunohematology

ABO Blood Groups

- **Transferases** put sugar chains on RBC antigens
  - **Genotype** (which transferases you have determines what those antigens look like)
- **Bacteria** have similar polysaccharides, so via natural exposure to those, body will develop Abs against all the RBC ones that aren’t “self”
  - **High density** of ABO antigens on RBC
  - **High titer IgMs** → intravascular hemolysis
- **ABO Groups**
  - Group A has anti-B, etc.
  - **O** = universal donor, **AB** = universal recipient
  - Prevalence depends a lot on population tested
- **ABO typing**: mix cells & plasma
  - (one from patient, one of known type)
  - **Agglutinate** if plasma has Ab against RBC Ag

![Sugar Configurations of the H, A, B Antigens](image)

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>O</td>
</tr>
<tr>
<td>A</td>
<td>O+Galactose</td>
</tr>
<tr>
<td>B</td>
<td>O+Galactose</td>
</tr>
<tr>
<td>AB</td>
<td>O+Galactose</td>
</tr>
</tbody>
</table>

**ABO Grouping**

<table>
<thead>
<tr>
<th>Cells Tested with</th>
<th>Serum Tested with</th>
<th>Interpretation</th>
<th>Frequency in U.S. Population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B cells</td>
<td>A cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>AB</td>
<td>45 49</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>A</td>
<td>40 27</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>B</td>
<td>11 20</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>AB</td>
<td>4 4</td>
</tr>
</tbody>
</table>

![Rh Blood Groups](image)

**Rh = “rhesus factor”, types of RBC antigens (Very immunogenic)**
- 15% US pop Rh-; D>c>E

**Problem scenario:**
1. **Father Rh+** and **Mother Rh-**
2. **If fetus is Rh+**, then mother can develop **anti-Rh Ab** (usually during exposure to fetal blood at delivery)
3. **Subsequent pregnancy**: mother’s **IgG** cross placenta; destroy **fetal RBC** → **hydrops fetalis** (fetal loss)

**Solution**: if mom is Rh- and dad is Rh +,
- Give **Rh IG** at 28 wks and **delivery** (coat fetal RBC in maternal circulation & prevent maternal immune reaction)

**Other Blood Groups**
- 300+ described; many can be clinically significant (hemolysis + transfusion reactions); others aren’t
- **Give blood if the patient needs it!**
  (match as best you can, but even if the match isn’t perfect, sometimes a patient needs blood no matter what)
Managing Transfusions: Pretransfusion Testing

1. **Verify patient identity**: the wrong sticker is the biggest cause of reactions! (no way for blood bank to test)
   a. Need **Full Name, History #, Phlebotomist ID** all perfectly correct or request will be **REJECTED**

2. **Blood compatibility testing**
   a. **Type & Screen**: ABO + Rh type of patient (“type”); “screen” for unexpected Ab in patient’s serum (indirect Coomb’s)
   b. **Type & Cross**: ABO + Rh and **crossmatch** (“cross”) patient serum with donor RBC (indirect Coomb’s)

### Adverse Effects of Transfusion

#### Acute Hemolytic Transfusion Reaction
- Pt has Ab (especially IgM) against donor RBC →
- immediate reaction (hemolysis)
- **IgM** → fix C’
  o Usually naturally occurring (e.g. ABO), T-cell-indep
  o Intravascular hemolysis
    - Hb in urine (hemoglobinuria) & plasma
- **IgG**-coated RBC → phagocytosis via RES
  o Usually alloantibodies via preg or transfusion (e.g. Rh system)
  o Extravascular hemolysis
    - anemia w/o plasma/urine color change

**Signs & Sx:**
- fever, chills/rigors, anxiety (sense of impending doom), nausea/vomiting
- dyspnea, flushing, hypotension, pallor
- hemoglobinuria/hemoglobinemia, bleeding, DIC, jaundice
- Renal failure (40-50%) and Death (10%) can result

**Investigation**: If you **suspect** hemolytic transf. reaction:
- **STOP TRANSFUSION & RE-CHECK**
- Re-check: Obtain blood samples, check for clerical error, re-do direct coomb’s, check plasma

**Treatment**: if you **know** there’s one going on
- **STOP TRANSFUSION** but leave IV in (need access)
- Start dieresis, start fluids, control BP, watch renal function, follow coagulation status
- **Avoid antigen-positive blood** for future transfusions

#### Delayed Hemolytic Transfusion Reaction
- Alloantibody-mediated, extravascular hemolysis
  - Amnestic response (>72h after transfusion or >10d if primary alloantibody response)
- ↓Hct, icterus, fever
- + direct antiglobin test (alloantibodies in pt serum); + antibody screen

### Adverse Effects of Transfusion

<table>
<thead>
<tr>
<th>HEMOLYTIC</th>
<th>INFECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Intravascular</td>
<td>• Hepatitis</td>
</tr>
<tr>
<td>• Extravascular</td>
<td>• CMV</td>
</tr>
<tr>
<td>NONHEMOLYTIC</td>
<td>• Malaria</td>
</tr>
<tr>
<td>• Fever</td>
<td>• AIDS</td>
</tr>
<tr>
<td>• Allergic</td>
<td>• West Nile Virus</td>
</tr>
<tr>
<td>GRAFT-VS-HOST DISEASE</td>
<td>• Bacteria</td>
</tr>
<tr>
<td>TRALI</td>
<td>• CHAGAS</td>
</tr>
<tr>
<td>(Transfusion Related Acute Lung Injury)</td>
<td>• CJD variant</td>
</tr>
</tbody>
</table>

### Indirect Coomb’s (antiglobulin) Test:
1. mix screening RBC & pt’s serum
2. add anti-human-IgG Abs
3. If patient has anti-RBC Abs, the anti-human-IgGs will link them and the RBC will fall out of solution (used for type & screen / type & cross)

### Direct Coomb’s (antiglobulin) test:
1. use pt’s RBC & serum
2. add anti-human-IgG Abs
3. If pt has autoantibodies, they’ll fall out of solution (used for autoimmune hemolytic anemia, for instance)

### Severity depends on:
- **degree** of incompatibility
- **rate** of transfusion
- **amount** of transfusion
**Febrile, Non-Hemolytic Reactions**

**Signs / Sx:** Temperature elevation of > 1° C

**Etiology:** WBC are the problem
- WBC Ab in pt. serum vs donor WBC / platelets
- Cytokines generated by stored WBC

**Prevent** with leukocyte-depleted or washed blood

**Ddx vs:** hemolytic / septic reactions
(could grow some bacteria in bag if skin plug gets in)

**Allergic Reactions**

**Most common** type of reaction by far

**Pathogenesis:** Histamine release *(immune-mediated)*

**Management:** Antihistamines, steroids/ephinephrine if severe

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**Infectious Complications / Transfusion Transmitted Diseases (TTD)**

**HIV/AIDS:** 2% AIDS transfusion related prior to 1985 (hemophiliac epidemic)
- screening today: high risk donor deferral, HIV Ab / Ag testing, nucleic acid testing
- Current risk: < 1: 2 million units
  - Residual risk because there’s a small window post-infection where virus is indetectable

**Hepatitis Viruses:** big problem before HCV discovered in 1989 (NANB hepatitis)
- Current risk: 1:2 million units for HCV (same as HIV), about 1:200k for HBV
- HAV: rarely causes TTD
- HBV: TTD usually from Asx carriers; reduced by screening / donor testing
- HCV: nucleic acid testing + Ab screening today, causes cirrhosis & HCC

**CMV:** big problem in immunocompromised patients
- Carried in donor WBC; ubiquitous (75% urban adults)
- problem if donor positive & recipient negative / immunocompromised
- Prevent with donor screening & leukodepletion

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**Transfusions: General Management**

**How safe are blood transfusions?** About 1 death/yr at Hopkins (pretty safe but not without risk)
- **MINIMIZE** homologous transfusion and explore **ALTERNATIVES**

**Perioperative RBC Transfusion:** 7g/dL is more appropriate (10g/dL not justified);
- Moderate perioperative anemia doesn’t affect morbidity / wound healing

**Autologous transfusion:** pre-deposit & use own blood
- More usage post AIDS, esp. elective surgery
- Lots of wastage (don’t crossover into general supply b/c motivation is different, might have high-risk behaviors)

**Intraoperative hemodilution:**
1. remove whole blood during anesthesia, replace with crystalloid / colloid (dilute) →
2. store in OR → washed cells returned after surgery
3. (blood lost in surgery is dilute, so less net RBC loss)

**Intraoperative autologous transfusion** (in general)
- Used for: CV, orthopedic, neurosurgery *(not GI or cancer when contamination would be a problem)*
- Only washed cells returned: might have to use component support too

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**Managing risks with a volunteer blood supply:**
- Minimize exposure
- Use **autologous transfusion** (pre-deposit)
- Use **hemodilution** or intraoperative autologous transfusion
- **Directed** donors (when appropriate)
  - NOT a good alternative in general
  - Family pressure → bad donations
  - Volunteer are safer
- **EPO** & other hematopoietic growth factors
Pharmacologic therapies

- ↓ risk TTD
- **Stimulate production / release of RBC / proteins** (DDAVP for vWF, EPO for RBC); **minimize surg. bleeding**, etc.

Red cell substitutes

- No really good ones available now – maybe 10 years? (expensive, not licensed)
- Would be good for Jehovah’s witnesses, etc.
- Would need to: have normal *in vivo* survival, replace all cellular functions, not have antigenicity / dz transmission / toxicity, be easily prepared & have a long shelf life

### TRANSFUSION CONCLUSIONS

- Avoid transfusions unless specific indications are met
- Use transfusion alternatives if medically and economically prudent
- Use homologous blood components, with recognition that safety has significantly improved
Multiple Myeloma & Plasma Cell Dyscrasias

Plasma cell dyscrasias (= immunosecretory disorders = gammopathies, dyscrasia means "bad temperament")
- Group of diseases characterized by uncontrolled proliferation of plasma cells, synthesizing a homogenous (monoclonal) immunoglobulin (rarely immunoglobulins)

Ig review:
- Ig = Ab, produced by PCs (terminally differentiated B-lymphocytes), each B-cell makes one antigenic Ig (BCR on surface & Ab in circulation), Ig made of heavy + light chains
- 5 classes of Ig by heavy chain (IgG, IgA, IgM, IgD, IgE in order of adult serum levels)
  - 4 subclasses for IgG, 2 subclasses for IgA
  - IgG has longest half-life and is only one that can do placental transfer; IgM is pentamer (biggest)
- 5 isotypes of heavy chains (γ, α, μ, δ, ε); 2 isotypes of light chain (κ, λ)
  - PCs make more light than heavy chains, so free light chains (κ, λ) can be measured in serum/urine

Monoclonal gammopathies:
- MGUS = monoclonal gammopathy of unknown significance; accounts for >50% monoclonal gammopathies
- Multiple Myeloma is next in frequency, then amyloidosis, lymphoma, others

PC Dyscrasias:
Key Pathologic Abnormalities
- Excessive Ig secretion in blood/urine
- Plasma cell accumulation + Ig deposition in organs / tissues

Serum Protein Electrophoresis
On serum protein electrophoresis (PEP), proteins run in groups / bands ("regions")
- Albumin is usually biggest peak
- γ region has "gamma-globulins" (Igs)
- narrow peak = monoclonal

Immunofixation Electrophoresis (IFE)
sIFE: like a western blot (run PEP, then use specific Abs to detect Ig isotypes)
sIFE vs PEP
- PEP tells you that there is an M-spike, for instance, but you don’t know what it is!
- PEP also less sensitive (can have normal PEP but detect on sIFE)
- MUST perform an IFE when monoclonal gammopathy suspected.

Multiple Myeloma
Multiple Myeloma: Neoplastic proliferation of a plasma cell clone, resulting in excessive production of a monoclonal Ig
- Can often evolve from MGUS

Epidemiology:
- VERY COMMON
  (1% of all cancers, 10% hematologic cancers, 4/100k incidence in US)
- Incidence ↑ with age (69/71 yo median in M/F, <5% are under 40yo, exceedingly rare in children/adolescents)
- African American > White, M>F a bit

MM: Clinical Features
- BONE PAIN (70% pts at Dx) (spine/ribs, worse with movement)
- Pathologic fractures
- Weakness/fatigue (anemia)
- Renal insufficiency (myeloma kidney / hypercalcemia)
**Classic (symptomatic) MM:**
- Single clone of plasma cell making an **M-protein** *(monoclonal or myeloma, not IgM)*
  - Usually **IgG** (52%), can be **IgA** (20%)
  - Free **light chain** (κ or λ) about 7-9% of the time: **Bence-Jones type**, indicates poor prognosis
  - Rarely others (heavy chain, etc) / biclonal
- Positive monoclonal band on sIFE tells you there’s a **plasma cell dyscrasia**; need other info to Dx MM

**Urine electrophoresis:**
- **Normal**: usually few proteins, mostly albumin but small peak
  - Glomerular disease: big band, but albumin (not filtering)
- **MM**: a huge band in monoclonal light-chain region
  - Overwhelming re-uptake in tubules (tubular proteinuria; IgGs can’t go through because they’re still filtered @ glomerulus)
  - **Bence-Jones proteinuria** (monoclonal light-chain proteinuria)
  - Urine IFE shows a monoclonal light chain band

**Bone marrow aspirate: cellular morphology & MM**
- try to demonstrate tumor (MM vs other PC dyscrasias)
- look for **morphology, PC count, PC markers by IHC**

**Normal plasma cells:**
- big ER (making lots of Ab), low number (3-6%) in BM & RES
- oval shaped, deeply **basophilic cytoplasm** with perinuclear halo
- eccentric nucleus

**Morphology in BM sample:**
- mature (look like normal PC)
- immature (large nucleoli, ↑N/C ratio, open/dispersed chromatin)
- anaplastic (prominent immunoblasts / plasmablasts from de-differentiation)

**Other features in MM:**
- **Cytoplasm:**
  - morula (Mott) cells: multiple, pale, whitish, grapelike accumulations of Ig inclusions
  - **Russell bodies**: round, refractile inclusions
    - general response to synthesis of mutant Ig (can’t exit / be degraded)
  - Crystalline rods
- **Nucleus**: **Dutcher bodies** (invagination of cytoplasmic material into nucleus)

**PC Immunophenotype**

<table>
<thead>
<tr>
<th>Benign PCs</th>
<th>Malignant PCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD38+ <em>(cell proliferation)</em></td>
<td></td>
</tr>
<tr>
<td>CD138+ <em>(anchor PCs to ECM in BM)</em></td>
<td></td>
</tr>
<tr>
<td>Polyclonal light chains (λ &amp; κ)</td>
<td><strong>Monotypic</strong> light chains (λ OR κ)</td>
</tr>
<tr>
<td>Have B-cell markers</td>
<td>Lack B-cell markers <em>(CD19 -)</em></td>
</tr>
</tbody>
</table>
Bone marrow biopsy: cellular morphology & MM

- **MONOTONY**: diffuse CD38/138+ staining (all PCs!) is diagnostic
- PCs **infiltrate** bone marrow in different patterns
  - Nodular, interstitial, mixed
  - **Diffuse** infiltration = worst prognosis
- **Solid aggregates** suggests MM; dispersed / non-aggregated suggests reactive plasmacytosis

### Bone lesions

- osteolytic “punched out” lesions
- osteoporosis with demineralization / compression fractures / vertebral collapse

### Increased serum calcium

- >10mg/dL, secondary to bone destruction, ~40% MM pts
- large disease burden
  - (lethargy/nausea/vomiting/constipation/polyuria
- Need to measure **free** serum calcium (some M-proteins bind calcium!)

### Renal insufficiency

- creatinine >2mg/dL, many causative mechanisms
- **Myeloma kidney**: large, waxy casts in DCT, most often with λ light chains. precipitates of monoclonal light chains with giant cells around it → dilation → atrophy of tubule, impairs nephron
- **Hypercalcemia**: Calcium deposits w/ lgG in tubules → hypercalciuria → osmotic diuresis (water follows) volume depletion → kidney failure
- **Deposition of light chains (κ)** in glomeruli (usually minimal proteinuria)
- **Primary amyloidosis**: deposit insoluble light chains in kidney; present with nephritic syndrome, minimal proteinuria
- Increased serum IL-6, nephrotoxic medicines, etc.

### Recurrent bacterial infections

- Neoplastic cells destroy normal BM lineages: lots of Ig but monoclonal & useless; other cell types decrease too
- GNRs & GPC

### Anemia

- **Normocytic normochromic anemia** (like anemia of chronic disease)
- **Growth** of PC in BM as well as cytokines released inhibit erythroid development
- Aggravated by expansion of blood volume, hyperviscosity → rouleaux formation (pile-of-coins aggregation of RBC

### Hyperviscosity Syndrome (HVS)

- Blood flow to tissues/organs impaired due to change in blood itself
- Viscosity is resistance of blood to flow; ↑ with abnormalities in RBC (sickle cell, etc) or in serum lgs (waldenstrom MG/MM)
- Uncommon in MM (IgA when it does happen); more common in Waldenstrom macroglobulinemia

### Amyloidosis

- Deposition of insoluble fibrillar proteins; 3% MM pts have overt amyloidosis, 30% do but Asx; adds to morbidity & decreases survival
  - Ig Light chains (amyloid L, AL)
  - Serum amyloid A – an acute-phase reactant (amyloid A, AA)
- **Manifestations**: carpal tunnel syndrome (flatten thenar eminence), generalized edema (nephritic syndrome)
**MGUS**

Monoclonal Gammopathy of Unknown Significance
- No single test can tell MGUS from MM: need several
- Common, increases with age (1% 50+yo, 3% 70+yo)

Can **PROGRESS TO MULTIPLE MYELOMA OR OTHER DISORDERS**
(1% per year risk total)
- 46x RR for Waldenstrom Macroglobulinemia
- 25x RR for MM
- Plasmacytoma, primary amyloidosis, CLL too

**Risk factors** for progression: high serum M-protein, > 10% PCs in BM, IgM or IgA MGUS are worse

**Waldenstrom Macroglobulinemia**

*He didn’t talk about it, but it sure seems to come up a lot on House*

A.k.a. **lymphoplasmacytic lymphoma**
- Clonal disorder of small lymphocytes that mature to PCs making IgM

**Epidemiology**
- Rarer than MM (1% all heme malignancies)
- whites > blacks, older adults (median age 63yo @ Dx)

**Secondary lymphoid organs** affected (LN + spleen)
- bone marrow too

**Morphology**: large heterogeneity in malignant clone morphology
- All are IgM+, most have B-cell markers (CD19+, CD20+, CD22+)
- 25% have CLL makers (CD5, CD23+)

**Sx from too many monoclonal IgMs:**
- **Hyperviscosity** syndrome
  - IgM is more prone: high MW, all intravascular
  - Bleeding (nose/mouth/retina, blurred vision, neuro abnormalities)
  - **Fundoscopic exam is best**: see retinal hemorrhage, papilledema, sausage-shape veins

- **Cryoglobulinemia**
  - Igs that precipitate when chilled (20% pts with WM)
  - **Type 1**: precipitate at low temperature:
    - affects ends of fingers, etc. (see aggregates of Ig on slides)
    - Raynaud’s phenomenon, acrocyanosis, skin ulcerations
  - **Type 2**: precipitates at low temp and has rheumatoid factor activity
    - IgM binds Fc region of IgG to form immune complex
    - Immune complexes precipitate in vessels
    - Sx of systemic vasculitis: recurrent purpura of lower extremities

- **Cold agglutinin** hemolytic anemia
  - Ab (usually IgM) against RBC Ags; bind at low temperature, fix C' → intravascular hemolysis
  - 25-31 C (skin & distal extremities' microvascular affected)
  - Acute: infectious mononucleosis / M. pneumonia

**MGUS: Diagnosis of exclusion:**
- No evidence of end-organ damage (bone lesions, renal failure, hematopoietic suppression)
- No evidence of other B-cell proliferative disorders
- Serum M-protein < 3g/dL and stable
- BM clonal plasma cells <10%

**WM: Clinical Picture**
- Nonspecific (fatigue, weakness, anorexia)
- LAD + hepatosplenomegaly
- Bence-Jones proteinuria in 40%
- Sx from too many monoclonal IgMs:
  - Hyperviscosity syndrome
  - Cryoglobulinemia
  - Cold agglutinin hemolytic anemia
  - Peripheral neuropathy
• Chronic: WM/CLL associated

• Peripheral neuropathy
  o Very debilitating
  o Chronic, sensorimotor, distal + symmetric
    ▪ (chronic with period of relative stability between progressions)
  o Monoclonal IgM against CHO on myelin-associated glycoprotein (MAG)
    ▪ Can detect MAG Ag with ELISA, in CSF, etc.
Leukemia

**Leukemia**: malignant, clonal proliferation of white blood cells with resultant accumulation in blood, bone marrow, sometimes other tissues

- not a single disease (family); different leukemias have different prognoses

**Low quality hematopoietic stem cell** is target in leukemic transformation:

- As normal cells mature / differentiate, phenotype changes: leukemic cells are often caricatures of those changes
- **Different** leukemias: **degree** of differentiation maintained (acute vs chronic) & **direction** (myeloid vs lymphoid)

**Classification** of leukemia

- **Traditional**: phenotypic properties (morphology, clinical behavior, expression of lineage features)
  - Acute vs chronic
  - Lymphoid vs myeloid
- **Genetic** classification increasing in use

### Chronic myelogenous leukemia

A.k.a. chronic myeloid / chronic granulocytic leukemia

- See a range of maturing myeloid cells (whole spectrum – look at chart); **high** WBC (leukocytosis); all **granulocytes** (granulocytosis): both PMNs and granulocytes at different stages of maturation
- Megakaryocytes are **SMALL** (micromegakaryocytes)

**Natural History** (accelerated / blast crisis are “advanced” phases)

1. **Chronic phase**: indolent; 5-6 years
2. **Accelerated phase**: 6-9mo
3. **Blast crisis**: 3-6mo median survival
   - 90% die w/o Tx
   - Can be **either lymphoid** or myeloid (stem cell is target!)

**Philadelphia chromosome** t(9,22) → **bcr-abl tyrosine kinase**

- Causative abnormality of CML; constitutively active
- **REQUIRED** for CML diagnosis
- **Imatinib** is specific inhibitor; 2nd/3rd gen too (standard of care)

CML is a **chronic myeloproliferative neoplasm**

- Stem cell neoplasm but have **mature elements** predominating
- Often **panmyelosis** (different types predominate in different dzs)
- Diseases of **adults**, generally **indolent**, subclassified in lots of ways, **JAK2 kinase** mutations are common in most of them

---

**CML: Fast Facts**

- Tumor of **adults** characterized by marked granulocytosis with **myeloid** cells of all differentiation stages seen in blood.
- Hypercellular marrow with myeloid preponderance and increased (micro)megakaryocytes.
- Defined by bcr-abl translocation (Philadelphia chromosome); can treat with imatinib (Gleevec).
- **Splenomegaly** common, due to infiltration of red pulp by neoplastic myeloid cells; other organ involvement can be seen less often.
- **Chronic course** lasts 4-6 years on average, followed by accelerated phase (6-9mo), then blast crisis (acute leukemia) usually **resistant to therapy**.
**Chronic Lymphocytic Leukemia**

A.k.a. “chronic lymphoid leukemia”

**Neoplasm of “mature” B-cells** but pts display B-cell dysfunction (monoclonal!)
- Mature-appearing lymphocytes in peripheral blood
- **NUMBER, NOT CYTOLOGIC APPEARANCE** is indication of neoplasia
- Hypercellular bone marrow biopsy with lots of lymphocytes

**Epidemiology:** Common, elderly, males

**Natural History:** Chronic course; **transformation** to aggressive disease possible
- Spleen & LN involvement is common

**Complications:**
- Cytopenias (from hypersplenism / sequestration, auto-Ab production & immune destruction, marrow replacement)
- Infections (from neutropenia / hypogammaglobulinemia)
- Transformation to aggressive lymphoma possible

**Chronic lymphoproliferative disorder:** any indolent proliferation of morphologically mature lymphocytes; CLL is one specific variety of these.

---

**The Chronic Leukemias:** “General Thoughts”

- **Easy** to tell CML vs CLL: mature lymphocytes vs granulocytes
  - Easiest way to DDx CML vs CLL? **Blood smear**!
- **Hard** to distinguish from benign conditions (look normal)
  - Easiest way to DDx CML vs reactive granulocytosis? **PCR**!
- Additional studies: FISH, flow cytometry, cytogenetics for Dx

**Pathogenesis:** proliferation of cells with mature phenotype → **diseases of cellular accumulation**
- Usually **not** proliferation advantage but upregulated anti-apoptotic machinery / survival
- Additional genetic changes (more hits) → **transformation** (more proliferation advantage!)

---

**Acute leukemias**

- Proliferation of **immature cells** (“blasts”) with arrest in maturation: cells aren’t “growing up”
- Lymphoid & myeloid blasts **look alike** (much harder to tell ALL vs AML with just blood smear)

**Pathogenesis:** translocation → **altered transcription factor** (block normal differentiation)
- More mutations → **proliferative advantage**

---

**Acute Myeloid Leukemia**

**High WBC** with **blasts**; decrease in mature elements of all lineages

**Any organ** can be involved (diffuse or tumor mass);
- complications of AML are important in organ pathology
- **Kidney:** hemorrhage from thrombocytopenia + WBC infiltrating, for example

**Subclassification:** AML is **not** a single disease
- **FAB** is traditional; not good for prognosis
- **More recent**: better genetic understanding

**De novo**: specific translocations; abnormal transcription factors

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**Acute Promyelocytic Leukemia**

- Specific AML subtype
- Proliferation of **immature** but **differentiated** cells with **distinct granules**
- **t(5:17)** translocation (PML – retinoic acid receptor) → **PML-RARα** fusion protein
- Responds to **retinoic acid** Tx

---

**CLL: FAST FACTS**

- **Commonest** of all leukemias (at least in West), seen in **older adults** (M > F).
- **Peripheral blood lymphocytosis**, often to **very high levels**, with proliferation of **mature** lymphocytes in blood, bone marrow and other lymphoid tissues.
- Tumor is a **clonal proliferation** of **CD5+** B cells (good for Dx)
- Biologically similar or identical to one form of non-Hodgkin’s lymphoma, so called **small lymphocytic lymphoma**.
- Sx in CLL pts from **anemia**, **thrombocytopenia** and **neutropenia**; ± hypogammaglobulinemia and evidence of autoimmunity.
- Disease usually has an **indolent** course, (many years), but is **incurable** with conventional chemotherapy
After myelodysplastic syndrome: WORSE prognosis

- Bone marrow failure state characterized by clonal abnormality of stem cell
- Ineffective hematopoiesis: hypercellular marrow but pancytopenia
- Characteristic cytogenetic abnormalities: deletions/losses (chr 5/7), not translocations like others

Natural history:
- rapidly fatal if not treated; remission in 2/3 but relapses common
  - genetic lesions affect prognosis (MDS-related worse, certain translocations (APL t(15:17)) better
  - Death: infections & bleeding
    - Cytopenia from therapy too!
    - Complications of therapy are like complications of disease

Acute Lymphoid Leukemia
A.k.a. acute lymphoblastic leukemia

- Childhood (80% leukemias);
  - adults too (20% adult acute leukemias) – worse prognosis
- Same presenting characteristics as AML; different Tx / prognosis

Subclassification:
- immunologic properties (B-precursor = 75-85%, T-precursor = 15-25%)
- genetics: lots of prognostic importance (esp. children)

Outlook: remission rates high but relapses common (especially adults)
- 3/4 children but less than 1/3 adults can be cured

Prognostic factors in ALL
- WBC (high is bad)
- Age (adults do poorly; among children <1yr or >10yr do worse)
- Cytogenetics: children are also more likely to have good cytogenetics
  - Can have Ph chromosome! Bad prognosis
  - Good: hyperdiploidy - t(12:21)

Acute Leukemias: General Thoughts

<table>
<thead>
<tr>
<th></th>
<th>ALL</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Nothing specific</td>
<td>Granules</td>
</tr>
<tr>
<td>Enzymology</td>
<td>TdT</td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>Immunology</td>
<td>B or T surface markers</td>
<td>Myeloid surface markers</td>
</tr>
</tbody>
</table>

TDT: nuclear enzyme from early lymphoid development; detect with immunofluorescence or flow cytometry; characteristic but not specific for ALL
<table>
<thead>
<tr>
<th><strong>Acute Leukemias</strong></th>
<th><strong>Chronic Leukemias</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Immature cells</td>
<td>• Mature cells</td>
</tr>
<tr>
<td>• Translocations involving transcription factor leads to maturation arrest</td>
<td>• Defective apoptosis leads to survival advantage</td>
</tr>
<tr>
<td>• Aggressive course but may respond to Rx</td>
<td>• Indolent course but generally incurable</td>
</tr>
<tr>
<td>• Treatment depends on classification</td>
<td>• May transform to aggressive disease</td>
</tr>
</tbody>
</table>
Lymph Nodes & Hodgkin’s Lymphoma

The normal lymph node

- Small (<1.5cm), round/reniform organ usually in chains/groups; solid/homogenous parenchyma
- Normally non-palpable

**Structure**

**Capsule** & invaginating trabeculae; **hilum** for entry/exit of lymphatics

**Parenchyma:** 4 compartments

- **Cortex:** **Follicles** (B-cells)
  1. **Mantle zones** (small, round lymphocytes, recruiting ground for germinal centers)
  2. **Germinal centers:** where Ag is presented, rearrangement / mutation of Ig genes happens; determination of who has best match of BCR to Ag presented by APC
     - centrocytes/centroblasts
     - macrophages, dendritic cells
     - lots of **division** (mitotic figures)

- **Paracortex:** **T-cell** zone
  1. Small, round lymphocytes (complex mix, especially T-cells)
  2. Immunoblasts + plasma cells, epithelioid histiocytes, small blood vessels

- **Sinuses:** Termination of lymphatics; pale-pink staining
  1. Histiocytes (Mϕ) + endothelial lining

- **Medullary cords:** big mix of cells
  1. Small, round lymphocytes, immunoblasts, PCs, Mϕ

**Immunophenotypes:** Normal LN

<table>
<thead>
<tr>
<th>Follicular B-cells</th>
<th>Paracortical T-cells</th>
<th>Sinus Histiocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CD45+ (common leukocyte antigen)</td>
<td>CD3+, either CD4+ or CD8+</td>
<td>CD68+</td>
</tr>
</tbody>
</table>
**Reactive Lymphadenopathy (LAD)**

**Non-neoplastic enlargement** (response to variety of Ag stimuli)
- **Normal compartments** can enlarge & proliferate, usually just 1 of compartments
  - *e.g. lymphoid hyperplasia*
- **Focal distortion** of LN architecture from inflammatory response (acute or chronic) often post-infection
  - *e.g. lymphadenitis*

**Lymphoid Hyperplasia**
Can be **follicular, paracortical, sinus**, or **mixed** hyperplasia (most is mixed, very rarely just medullary)

<table>
<thead>
<tr>
<th>Picture</th>
<th>FOLLCULAR</th>
<th>PARACORTICAL</th>
<th>SINUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
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</tr>
</tbody>
</table>

**Preferential stimulation of...**
- **B-cell compartment (follicles)**
- **T-cell compartment (paracortex)**
- **Histiocytic compartment (sinuses)**

**LN enlarges because**
- Abnormal proliferation of 2+ follicles in GCs
- Abnormal expansion of interfollicular zone
- Distention of subcapsular / intraparenchymal sinuses by benign histiocytes

**Location**
- Confined within LN capsule

**Histology**
- **Follicular**
  - Crowded follicles, vary in shape/size
  - Mantle zones well defined
  - GC often polarized
  - Polymorphous cytology in follicles (not homologous like malignancy)
- **Paracortical**
  - Expanded interfollicular zone
  - Cytology polymorphic (MIX of cells: small lymphs, immunoblasts, PCs, grans, dendritic cells)
  - Can have vascular proliferation
- **Sinus**
  - Sinuses engorged with histiocytes (bland, uniform in size/shape, pale, lots of cytoplasm)
  - Phagocytosis of other hematologic elements / foreign substances

**Etiology & DDx**
- Non-specific reactive follicular hyperplasia is #1 (*HIV-associated adenopathy, toxo, RA, Sjogren’s, 2° syphilis, etc*)
- **MUST DDx from follicular lymphoma** (architecture, polymorphous cytology instead of monomorphous, bcl-2 not expressed unlike FL)
- Non-specific reactive follicular hyperplasia is #1
  - infectious mononucleosis too (or other viral adenitis)
- **MUST DDx from T-cell lymphoma**
  - architecture, polymorphous cytology instead of monomorphous, TCR gene rearrangement studies
- Non-specific reactive follicular hyperplasia is #1
  - Usually idiopathic; can see during malignancies or with prostheses.
  - **MUST DDx from malignancy** (especially those that travel & end up in sinuses of LNs: metastatic carcinoma / melanoma, ALCL, Langerhans’ cell histiocytosis)

**Other**
- CD20+
- CD3+ & either CD4+ or CD8+
- Can be associated with non-hematolymphoid malignancy

**Lymphadenitis (focal lesions)**

**Necrotizing**: focal distortion of LN architecture by prominent **necrotic** inflammation
- +/- suppuration; mostly from bacteria, *ddx: SLE adenitis, Kawasaki’s, others*
- See pale/pink dead areas (histiocytes, lymphs, etc)

**Granulomatous**: focal or diffuse distortion of LN architecture by prominent **granulomatous** inflammation
- +/- suppuration, *ddx: cat-scratch, TB, non-caseating sarcoidosis*
- Serpiginous formation often; necrotic debris in center

**reactive** LNs are **tender** and usually **mobile**
**malignant** LNs are often “fixed” / stuck to surrounding structures
**Hodgkin Lymphoma**

**Malignant lymphoma** characterized *clinically* by dissemination along contiguous LN groups and *pathologically* by:

1. Presence of Reed-Sternberg (RS) cells and/or RS variants
2. Proper immunoreactive background (abundant eosinophils, PCs, small lymphs)

Note: need both for diagnosis, almost always nodal-based

Only 1% of tumor is tumor cell & rest is reactive

**Epidemiology:** 30% all lymphomas; bimodal age distribution (15-35yo and >50)

- M:F 60:40, Caucasians 2X incidence vs non-whites

**Staging** (important for outcome)

1. 1 LN or group
2. 1+ LN/group on same side of diaphragm
3. LN groups on both sides of diaphragm
4. Extranodular involvement (spleen/liver/BM)

Also A/B & E

- B: has symptom, worse prognosis; A w/o Sx
- E (extralymphatic by direct extension)

**Clinical course**

1. Non-tender, firm adenopathy
2. Indolent; dramatic improvement with chemo advances
3. “B” sx = worse outcomes
4. Tumor burden (stage) is most important prognostic variable
   a. but generally patients do pretty well no matter what stage
   b. 5yr survival: 90% for IA/IIA; 80% even for III/IV

**Reed-Sternberg Cells**

- Large lymphoid cell, classically *binucleated*, prominent eosinophilic nucleoli
  - “Owl’s Eye”, many other variants as well
- **ESSENTIAL** for Hodgkin Lymphoma Dx (although only a small % of tumor)
- Ig rearrangements via PCR → B-cell origin but only a few are CD20+

---

**Hodgkin Lymphoma: subclassification**

<table>
<thead>
<tr>
<th>Classical Hodgkin Lymphoma</th>
<th>Lymphocyte-predominant Hodgkin Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of all HL</td>
<td>95%</td>
</tr>
<tr>
<td>Demographics</td>
<td>Bimodal age curve</td>
</tr>
<tr>
<td>Presentation</td>
<td>More than ½ with stage I/II dz</td>
</tr>
<tr>
<td>B-symptoms</td>
<td>40%</td>
</tr>
<tr>
<td>Other</td>
<td>EBV probably plays a role</td>
</tr>
<tr>
<td>Histologic subtypes</td>
<td>Nodular sclerosis</td>
</tr>
<tr>
<td></td>
<td>Mixed cellularity</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte depleted or rich</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td>RS cells: CD15+, CD30+ is classic</td>
</tr>
<tr>
<td></td>
<td>EBV+/-, CD20+/-, CD45- (no CLA!)</td>
</tr>
<tr>
<td></td>
<td>CD15-, CD30-, EBV-</td>
</tr>
<tr>
<td></td>
<td>CD45+, CD20+</td>
</tr>
</tbody>
</table>
**Histiologic subtypes of Classical Hodgkin Lymphoma**

*Morphology not as important as staging for prognosis*

### Nodular Sclerosis:
- Big nodules bound by sclerosis (scar tissue, fibrous bands)
- Bulk of cells are small lymphs with RS too
- **Mediastinal** involvement; matted group of big LNs
- Most common in **young women**

### Mixed cellularity:
- No sclerotic bands (dx of exclusion); R-S on background of immunoreactive cells

### Lymphocyte-depleted:
- Could also be called “tumor-cell abundant” – there are tons of RS cells

### Lymphocyte rich:
- Tumor cell poor: few RS cells

The line between LPHL and classic HL can be blurred as well (similar presentations and can sometimes **convert**)

---

**Comparison: HL vs NHL**

<table>
<thead>
<tr>
<th></th>
<th>Hodgkin Lymphoma</th>
<th>Non-Hodgkin Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spread</strong></td>
<td>Contiguous</td>
<td>Noncontiguous</td>
</tr>
<tr>
<td><strong>Extranodal?</strong></td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Waldeyer’s ring and tonsil involvement</strong></td>
<td>Rare</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Localized disease (low stage)</strong></td>
<td>Common</td>
<td>Less common</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td>Distinct age peaks, including young adults</td>
<td>Generally <strong>older except for Burkitt and lymphoblastic (pediatric)</strong></td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
<td>Excellent</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Cell of origin</strong></td>
<td>B cell</td>
<td>B or T cell</td>
</tr>
</tbody>
</table>
Non-Hodgkin Lymphoma

**Lymphoma**: malignant clonal proliferation of lymphoid cells in LNs and other lymphoid tissue

- Not a single disease; lots of different entities; difference from leukemia often mostly semantic

**Epidemiology of NHL**: 55-60k cases/yr

- incidence has increased 50% in last 25 yrs!
- One of fastest growing cancers!
- *HIV* associated with ↑risk NHL; doesn’t fully explain increase in incidence

**Classification**: very complex; morphology + molecular pathogenesis

**NHL Classification for Dummies**:

1. Remember the “acute” vs “chronic” classification for leukemias? The target was always a low-quality HSC but *how far it could differentiate* was what determined acute vs chronic.
   - a. These are all **lymphocytic**: so not that myeloid side of stuff (think ALL/CLL in tissues)

2. With lymphoma, there’s an added wrinkle: lymphocytes can **mature & activate** even farther
   - a. e.g. CTLs from T-cell precursor, plasma cells from B-cells, etc.
   - b. Adds a new area of classification

3. Comparison table:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Predominant cell type</th>
<th>Behavior</th>
<th>Analogous to ____ leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>High grade</td>
<td>Immature precursor</td>
<td>Aggressive</td>
<td>“Acute”</td>
</tr>
<tr>
<td>Low grade</td>
<td>Mature differentiated</td>
<td>Indolent</td>
<td>“Chronic”</td>
</tr>
<tr>
<td>High grade</td>
<td>Activated lymphocytes</td>
<td>Aggressive</td>
<td>None</td>
</tr>
</tbody>
</table>

**Low grade lymphomas**

**General characteristics**:

- derived from **mature** lymphocytes (usually small, condensed chromatin)
- low proliferation rate; *mitoses* rare
- “Survival tumor” / “tumor of accumulation” - resistant to apoptosis
- Rare in children; **incurable** but have prolonged clinical course (treat/remit/relapse)

**Follicular lymphoma**

**Morphology**:

- **nodules**, recapitulation of normal follicles but **not** confined to cortex
  - effacement of normal LN architecture

**Clinical features**:

- 30-40% NHL in **adults**; most common 40s-50s, almost never in kids
- Usually **Asx** with **generalized LAD**
- **Common**: spleen (small nodules in white pulp) + BM (paratrabecular aggregates) involved
- **Indolent** course; may respond to chemotherapy but **incurable**

**Immunophenotype**: **CD20+** (B-cell tumor; B-cells live in follicles!)
Grades determined by cytological differences

- (B-cells in GC are differentiating all the time; at what stage does the follicular lymphoma B-cell stop differentiating?)
- Grade 1: Small cleaved cell (earlier stage of lymphocyte; small, irregular cells)
- Grade 2: “Mixed” (mixture of larger cells; differentiate further)
- Both: predominance of certain cell types; don’t see mitosis or high mitotic rate that a normal LN has

Pathogenesis:

- t(14:18): Bcl-2 oncogene (18) behind IgH (heavy chain, chr 14) promoter (obviously a good one for B-cells in GC)
  - Can stain for bcl-2: if strong throughout, probably follicular lymphoma!
  - Prevents apoptosis (survival advantage); normal B-cells shut-off bcl-2

**Small lymphocytic lymphoma (SLL/CLL)**

SLL is tissue equivalent of CLL (SLL/CLL is diagnostic term): 2 manifestations of same disease

- Difference entirely semantic / based on differing clinical presentation
- More diffuse replacement of LN

**Mantle cell lymphoma**

- Specific translocation: IgH – cyclin D1 (strong promoter; strong oncogene)
- NOT INDOLENT and NOT CURABLE (worst of both worlds)

**MALT lymphomas**

- Lymphoma of mucosa-associated lymphoid tissue
- Indolent in nature

**Indolent T-cell Lymphomas**

- REALLY RARE (almost never occur)

**High Grade Lymphomas**

- Derived from immature / activated lymphocytes
  - medium to very large cells
  - dispersed/open chromatin, often prominent nucleoli
  - Highly proliferative: lots of mitoses
- Occur in all age groups
- Systemic Sx are common
- Rapidly progressive if untreated but some pts can be cured with chemo

**Lymphoblastic Lymphoma**

Prototype for high grade lymphoma related to precursor cells

Tissue equivalent of ALL

- Lymphomatous more common with precursor T-cells than precursor B-cells

T-cells \( \rightarrow \) thymus \( \rightarrow \) mediastinal mass is common presentation

**Epidemiology:**

- Relatively common in childhood (less in adults)
- Not a single entity; many but not all have oncogene translocated to TCR gene

**Morphology:**

- diffuse replacement of LN
- medium cells with dispersed chromatin & lots of mitoses
**Diffuse Large B-cell Lymphoma**

*Prototype* for high-grade lymphoma related to *activated* cells

**Epidemiology:**
- common, 30-40% adult lymphomas, also occurs in children
- *not a single disease entity* (family)

**Morphology:**
- diffuse replacement of LN architecture
- large cells with open chromatin, abundant cytoplasm, prominent nucleoli & lots of mitoses
  - “vesicular nuclei” – look paler, like little vesicles

**Disease:** Can occur *de novo* (better prognosis) or as *transformation* from underlying INDOLENT B-cell lymphoma
- Localized or disseminated
- Can be extranodal but BM/blood are rare (why there aren’t “large cell leukemias”)
  - Solid masses in extranodal sites / spleen
- 50% pts can be *cured* with chemotherapy; prognosis depends on extent (stage) at dx

---

**Burkitt Lymphoma**

Another high-grade lymphoma related to *activated* cells
- Derived from *most proliferative* GC cell (very active → very active neoplasm!)

**Epidemiology:** children > adults; two forms
- Endemic: Africa, rapidly growing jaw tumor, EBV-associated
- Sporadic: *not* EBV-associated, often intestinal

**Morphology:**
- Diffuse replacement of LN; *high proliferation rate* (lots of mitoses)
- Uniform, medium-sized cells with fine chromatin / nucleoli
- “starry sky” pattern (Mϕ phagocytosing debris – lots of turnover)
- Morphologically similar to *lymphoblastic lymphoma* (distinguish with immunophenotyping)

**Disease:** *medical emergency* (highly proliferative) but can *cure* with chemotherapy
- Often leukemic (“burkitt lymphoma/leukemia”)

**Pathogenesis:**
- t(8:14): *myc* (powerful oncogene, chr 8) behind *Ig* promoter (chr 14)
  - gives *growth advantage*
  - actually lack *bcl-2*! High rate of cell death = good for treatment, but worry about *tumor lysis syndrome*

---

**T-cell lymphomas**

- Much less common than B-cell lymphomas
- “*peripheral T-cell lymphoma*” is generic name (peripheral, not thymic)
- Do *worse* than B-cell lymphomas (lower cure rates) – tons of different entities

*Sample question: lymphoblastic lymphoma* is most likely (vs follicular, mantle cell, SLL/CLL) to be *cured* with chemo
Ancillary studies

Lymphomas are really complex; morphology has a limited roll → use ancillary studies

Immunophenotyping

- Flow cytometry (suspensions) or IHC (tissue specimens)
- Lymphoma: B-cell tumors are clonal → LIGHT CHAIN RESTRICTION
  - (only κ or λ on B-cells, not normal mixture of both like you’d see in hyperplasia)
- Ag-expression patterns for subclassification of low-grade B-cell lymphomas (see table)

<table>
<thead>
<tr>
<th></th>
<th>CD20</th>
<th>CD10</th>
<th>CD5</th>
<th>CD23</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular lymphoma</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLL</td>
<td>+ dim</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>TdT+</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Cyclin D1+</td>
</tr>
<tr>
<td>MALT lymphoma</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Molecular biology:

- Can demonstrate clonality via Ig / TCR rearrangement
- Some types (FL, mantle, Burkitt) have specific molecular abnormalities that can be detected at gene / mRNA / protein level

NHL Summary

- NHL represents a relatively common family of diseases
- As a first approximation, low grade and high grade lymphomas represent two broad groups
  - Low grade: “Survival” tumors with indolent course but ultimately incurable
  - High grade: “Proliferative” tumors with aggressive course, sometimes curable
- Specific entities among lymphomas have characteristic morphologies, pathogenesis and clinical behavior, and in some cases require specific therapy
- Diagnosis and classification of lymphomas is based on morphologic examination, supplemented by immunophenotypic and genetic information
<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>Morphology</th>
<th>Phenotype (CD)</th>
<th>Genetics</th>
<th>Clinical Presentation</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| **Small Lymphocytic** (=SLL/CLL) | • Small cells, monotonous  
• Mature chromatin, not invasive | Other | 20 5 10 23 | • Older adults  
• Indolent but present with progressed disease  
• LAD + peripheral blood involvement (involving BM)  
• Anemia | • Indolent, incurable |
| **Follicular** | • Loss of LN architecture  
• Monotonous appearance  
• Angular centrocytes  
• Neoplastic follicles | + + + | t(14:18) (IgH-bcl2) | • Middle aged / older adults  
• Asymptomatic (LAD)  
• Frequently involves bone marrow | |
| **Mantle Cell** | • Small cells  
• Diffuse (more often) or kind of nodular (but not follicular) in infiltrate | Cyclin D1 bright | t(11:14) IgH-Cyclin D1 | • Adults  
• Asymptomatic or anemic (BM involvement)  
• LAD, can be GI | • Not indolent  
• Not curable (although it’s a low-grade lymphoma) |
| **MALT** | • Small, outside of mantle zones  
• Leaves germinal centers & spreads away to involve epithelial structures (lymphoepithelial lesions)  
• Monocytoid | + - - | Non-specific | • Extranodal; GI Sx, in mucosa  
• Area radiation as Tx  
• Can respond to abx (H. pylori) | |
| **Diffuse Large B-Cell** | • Large B-cells, open chromatin, lots of mitosis, visible nucleoli  
• Can be destructive (diffuse, spreading) | + | Non-specific | • Fever, wt loss, night sweats  
• Can have nodal or extranodal masses (usually find on PE / img) | • Aggressive  
• Needs chemotherapy  
• Potentially curable |
| **Lymphoblastic** (=ALL) | • Intermediate size  
• Blasts  
• Increased mitoses | Pre-T-cells! 3+ 5+ 7+ | Non-specific (TCR Translocns) | • Mediastinal mass +/- blood | • Aggressive  
• Treatable |
| **Burkitt’s** | • Increased proliferation  
• “Starry sky” appearance  
• Intermediate size | B-cell phenotype + + | t(8:14) IgH - myc | • Endemic (EBV positive, jaw) vs Western (often intestinal)  
• Rapidly spreading (emergency)  
• Can be leukemic (+/- blood) | • Very aggressive (tumor lysis, early danger)  
• Need Tx right away  
• Potentially curable |