American Society of Hematology:

1. Minimize PRBC transfusion, do not give routine transfusion for sickle cell patients with chronic anemia or uncomplicated pain crisis without clinical indication.
2. Do not test for thrombophilia in VTE due to transient risk factors, only 3 months of anticoagulation is needed for VTE with transient risk factors.
3. Do not routinely use IVC filters
4. Do not use plasma or factor concentrates for non-emergent reversal of vitamin K antagonists
5. Do not test/treat for HIT in patients with low pretest probability of HIT,
6. Don’t treat ITP without bleeding or very low platelet count.

Stem cells
- Have the capacity for self-renewal and the ability to differentiate into functional cells
- Hematopoietic stem cell transplantation is used primarily for hematological and lymphoid cancers, but also for many other disorders

Tumors arise from malignant stem cells that continue to retain the ability for self-renewal. Cancer chemotherapy acts primarily on proliferating cells. Since stem cells (normal and malignant) are quiescent, they are insensitive to therapy.

Stem cell transplantation results in more cures and remissions than other treatments, but also causes great morbidity and mortality.
Myeloablative preparation before transplantation is done to eradicate cancer and in allogeneic transplantation, to induce the immunosuppression that permits engraftment.

Peripheral blood is a convenient source of hematopoietic stem cells and has replaced marrow for autologous and most allogeneic transplantations

Mortality is significantly lower with auto transplantation than with allotransplantation, but the absence of graft versus tumor activity in auto transplantation reduces its effectiveness.

Less than 30% of potential recipients of hematopoietic stem cells have a HLA identical sibling. Therefore, the use of unrelated donors has increased, and the rates of success have improved.

Complications of stem cell transplant: Early complications:

1. Mucositis
2. Hepatic venoocclusive disease
3. Transplantation related lung injury
4. Transplantation related infections: Due to
- Damage to the mouth, gut, and skin from preparative regimens
- Neutropenia and immunodeficiency
- Prolonged neutropenia, graft versus host disease, and the administration of corticosteroids predispose patients to fungal infection
- Cytomegalovirus pneumonia once fatal to 15% of recipients of allogeneic transplantations has become rare due to the ability to detect subclinical infection by viral PCR or antigenemia, making early treatment possible

5. Graft versus host disease
- Acute graft versus host disease damages the skin, the gut, and the liver
  - A pruritic micropapillary rash can affect the palms, soles, or the face, and may become generalized
  - Nausea, vomiting, abdominal pain, diarrhea, bloody stool, and jaundice may occur

Delayed complications

1. Chronic graft versus host disease
- Bronchiolitis, keratoconjunctivitis sicca, esophageal stricture, malabsorption, cholestasis, cytopenias, and generalized immunosuppression
2. Failure to ovulate
3. Men usually become infertile after transplantation, but younger men may recover their fertility. Semen can be cryo preserved and used later
4. Growth and development impairment
5. Secondary cancers

Most survivors of transplantation are active and healthy

**Aplastic anemia**

Aplastic anemia has **pancytopenia with bone marrow hypocellularity**. The **peripheral smear** of the patient with aplastic anemia is **normal looking, but the cells are reduced in number**.

**Etiology**
- 50% of the cases are idiopathic, specific causes are found in the other 50%

**Inherited disorders**
- Fanconi’s anemia
- Familial aplastic anemia
- Shwachman Diamond syndrome

**Other secondary causes**
- Radiation
- Drugs and chemicals
  - Chemotherapy agents
  - Benzene
- Chloramphenicol
- Chloroquine
- Non-steroidal antiinflammatory drugs
  - Phenylbutasone
  - Indomethacin
  - Ibuprofen
  - Sulindac
- Anti convulents
- Heavy metal
  - Gold
  - Penicillamine
  - Bismuth
  - Mercury
- Other agents
  - Sulfonamides
  - Antithyroid agents
  - Acetazolamide
  - Cimetidine
  - Chlorpheniramine
  - D-penicillamine

Infections
- EBV
- CMV
- Hepatitis B and C
- HIV
- Parvo virus B19
  - Transient
  - Parvovirus B19 has tropism for P antigen of RBCs

Immune diseases
- Eosinophilic fasciitis
- Graft versus host disease
- Paroxysmal nocturnal hemoglobinuria
- SLE
- Thymoma

Aplastic anemia is occasionally seen in pregnancy.

Pathology
- T-lymphocyte subsets produce local concentrations of gamma-interferon and up-regulate Fas expression on CD 34 positive cells
  - Fas antigen is a receptor of tumor necrosis factor receptor family and when activated, causes cells to undergo apoptosis
- Patients with aplastic anemia have a higher prevalence of HLA DR2

Clinical features
- The striking feature is the restriction of symptoms to the hematological system and the patient often feels and looks well
- Can appear abruptly or insidiously
- Symptoms of thrombocytopenia
  - Bleeding
- Easy bruising
- Gum and nosebleeds
- Petechiae

**Symptoms of anemia**
- Weakness
- Shortness of breath are the commonest

**Infection is an unusual symptom**

**On exam**
- Pallor
- Petechiae
- Ecchymosis
- **Lymphadenopathy and splenomegaly are not seen**


**Diagnosis**
- Peripheral smear
  - Shows paucity of cells with no abnormal cells
- Bone marrow
  - Appears dilute on the smear and may be grossly pale
  - Hematopoietic cells occupy < 25% of the marrow space
    - These cells have normal morphology

**Treatment**
- If possible, allogenic stem cell transplant (SCT) from HLA matched sibling/donor, gives long-term cure
  - Usually done for the patients under age 50
- For patients who are > age 50, and those < 50 without a donor, immunosuppressive therapy + eltrombopag is given. Eltrombopag significantly increases hematological response. Anti-thymocyte globulin + steroid + cyclosporine + eltrombopag (Immunosuppressive therapy + eltrombopag) offers good partial remission in 60% to 70% of the patients. Other growth factors (erythropoietin, G-CSF) are not useful. Transfusion support is needed.
  - Several years after immunosuppressive therapy, about 15% of patients can develop another hemolytic disorder, most commonly paroxysmal nocturnal hemoglobinuria
- Acute leukemia and myelodysplastic syndrome are the other late complications affecting the patients treated with immunosuppressive therapy
- If the patient has no HLA compatible sibling donor and experience relapse after immunosuppressive therapy then consider stem cell transplant from an unrelated donor

**To prevent pre-transplant alloimmunization, if transfusions are needed prior to transplant, do not use family members as donors.** Use leukocyte poor, irradiated blood components and use single donor platelets if at all needed.
Patients with concomitant PNH can be treated with eculizumab to reduce transfusion dependence.

**Fanconi’s anemia**
- A hereditary aplastic anemia usually seen in children
- Risk of other cancers such as AML, myelodysplasia, and solid cancers are high

**Myelodysplastic Syndromes**

A heterogenous group of clonal stem cell disorders characterized by **cytopenias associated with dysmorphic and usually cellular bone marrow**, and ineffective blood cell production.
Most common presentation is macrocytic anemia in an older patient.

**Classification of MDS, WHO**

MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS): MDS-RS + single lineage dysplasia, MDS-RS + multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable

In general, myelodysplastic syndrome is a disease of elderly. The mean age at onset is 68 years. **Main features are: Cytopenia of one or more myeloid lineages, elevated MCV, clonal karyotypic abnormalities, and varying predisposition to acute leukemic transformation.** Increase blasts in the bone marrow correlates with aggressiveness. The karyotypic abnormalities associated with myelodysplastic syndromes are: loss of all or part of chromosomes 5, 7, and 20, Trisomy 8 and in the patients with CMML there is t(5:12).

**Clinical features**
- Symptomatic anemia
- Thrombocytopenic bleeding
- Infections associated with leukopenia

At least 50% of the patients are incidentally identified on routine CBC. Other causes of marrow failure must be ruled out such as idiopathic or drug-induced aplasia, hypersplenism due to known or occult liver disease, and Vitamin B12 or folate deficiency.

**Peripheral smear**
- Red blood cells with abnormal morphological features
- Relative monocytosis
- Dysgranulopoiesis
Pelger-Huet deformity
- Nuclear atypia
- Hypogranulation

Bone marrow exam shows dysplasia of marrow progenitor cells and hypercellularity and clonal chromosomal abnormalities

**Treatment**

Treatment decisions are made based on International Prognostic Scoring System, which is based on bone marrow blast, karyotype and cytopenias. Patients are divided into low risk, intermediate risk and high risk based on their score.

Low risk patients are treated with supportive care (transfusions and growth factors) and DNA hypomethylation agents.

Transfusion dependent 5q- syndrome is treated with lenalidomide.

Intermediate to high risk patients are treated with chemotherapy and allogenic stem cell transplant if they are young enough and have good functional capacity.

- Only cure is chemotherapy, followed by allogenic stem cell transplantation
  - Can only be done for patients < age 55, with good functional status
  - Since most patients with myelodysplastic syndrome are elderly, this option is usually not possible
- Supportive care becomes the main stay of therapy
  - Red blood cells are transfused for symptomatic anemia
    - Only leuko-poor RBCs must be given
    - Secondary hemochromatosis due to repeated transfusions can be treated with desferoxamine or the newly approved oral agent, deferasirox
  - Platelets are given for bleeding episodes
  - Alloimmunization is universal, which limits the response to transfusion
  - Other treatment option is growth factor administration:
    - Erythropoietin is administered for the patients with relatively low erythropoietin levels compared to the degree of anemia
    - Erythropoietin +/- GCSF administration has been found to increase the hemoglobin and decrease the transfusion needs. Patients that respond to EPO had survival benefit, without any increase in leukemia
    - NSAIDs and aspirin are avoided in these patients

*Azacitidine (pyrimidine nucleoside)*
- The first FDA approved drug for myelodysplastic syndrome
- Given as daily subcutaneous injection for 7 days, 4 cycles of treatment
- Inhibits DNA methylation
- Has been shown to improve survival
Desitabine is also an agent that inhibits DNA methylation like azacytidine. It is given IV for 3 days, every 6 weeks.

Amifostine
- Blocks apoptosis
- Shown to improve cell count

Lenalidomide
- Shown to decrease transfusion requirement and reverse cytologic and cytogenetic abnormality in myelodysplastic syndrome associated with 5q deletion
- Approved for treatment of 5q- syndrome (used in RBC transfusion dependent 5q-syndrome)
- Has been shown to decrease dependence on transfusion
- Two thirds of the patients could stop transfusion altogether
- Also reverses chromosomal and cytological abnormalities in many patients

Patients with CMML are treated with hydroxyurea, desitabine or imatinib (in patients who have PDGF fusion gene). Younger patients may be treated with stem cell transplant. CMML has features of MDS and myeloproliferative syndrome, and it is classified as a Myelodysplastic/Myeloproliferative neoplasm. Hepatosplenomegaly, weight loss and sweating are common features. Anemia and thrombocytopenia are associated with leukocytosis, with monocytosis.
**Paroxysmal nocturnal hemoglobinuria**

- An acquired stem cells disorder. Triad: Hemolysis, pancytopenia, venous thrombosis

**Clinical features**
- Hemolytic anemia
- Venous thrombosis
- Pancytopenia
- Anemia is variable in degree
  - Usually normochromic and normocytic, unless iron deficiency is coexistent secondary to blood loss
- Granulocytopenia and thrombocytopenia are common

Hemoglobinuria is intermittent, but hemosiderinuria is constant. Venous thrombosis is common in this disorder particularly in intraabdominal veins such as hepatic, portal, or mesenteric veins. Cerebral vein thrombosis can also occur.

**PNH has an association with Aplastic anemia**

**Pathology**
- An acquired, somatic mutation in the phosphatidyl inositol glycan complementation group A gene (*the pig*-A) of the hematopoietic stem cell
  - This mutation results in a clone of cells deficient in membrane proteins, CD55 and CD59 due to the absence of glycosylphosphatidylinositol (GPI) anchor
  - Since the cells are deficient in membrane proteins, the cells become sensitive to complement mediated lysis
- This finding has resulted in a newer treatment for paroxysmal nocturnal hemoglobinuria:
  - *Eculizumab*
    - Monoclonal antibody, a compliment inhibitor
    - Has been shown to reduce intravascular hemolyses, stabilize hemoglobin level in the absence of transfusion
    - Shown to cause clinically significant quality of life improvement in a significant proportion of the patients with PNH
    - Eculizumab can be associated with life threatening meningococcal infection, and patients should be vaccinated prior to eculizumab.

**Diagnoses**
- Must be considered in patients with unexplained hemolytic anemia
  - Especially with other cytopenias, evidence of intravascular hemolysis, aplastic anemia and unusual venous thrombosis
- Hams test is a screening test
  - RBCs undergo lysis after complement activation by acidification
- Sucrose lysis test
  - Lysis by reduction in ionic strength
- Lack of CD55 and CD59 on RBC by flow cytometry is the diagnostic test for PNH
Direct Coombs test is negative in PNH because all cells that activate complement are promptly lysed.

**Treatment**
Curative therapy is allogenic stem cell transplant. This is done in young patients with severe unresponsive hemolysis or aplastic anemia. Eculizumab, a monoclonal antibody against complement C5, decreases transfusion requirement and improves quality of life. Warfarin therapy to prevent thrombosis is given to patients with a large PNH clone. Median survival for PNH is 10-15 years. Death occurs due to thrombosis or progressive pancytopenia.

**Drug-induced cytopenias**

In patients with unexplained cytopenia, review all medications. Discontinuation of suspected medications should be done. Recovery of cell counts is usually expected within one to two weeks of discontinuation of the drug. GCSF is administered if there is an active infection or if there is delay in recovery.

**Neutropenia**

Neutropenia is defined as less than 1500 neutrophils per microliter. Neutropenia can be congenital e.g. cyclic neutropenia. Cyclic neutropenia is autosomal dominant. It leads to recurrent fever due to bacterial infections and pharyngitis on a three- to six-week cycle. During the neutropenic phase, the bone marrow is hypoplastic. Patients with severe infectious episodes benefit from long-term treatment with GCSF.

Jewish and African-American patients may have familial benign neutropenia.

**Drugs associated with neutropenia**
- Phenothiazine
- Clozapine
- Trimethoprim/sulfamethoxazole
- Chloramphenicol
- Semisynthetic penicillin
- Phenytoin
- Carbamazepine
- Nonsteroidal anti-inflammatory drugs
- Gold
- Antithyroid medications
- Sulfasalazine
- Clomipramine

**Autoimmune disorders**
- Hypersplenism
- Postinfectious state
- Malnourishment
- Folate and vitamin B12 deficiency
- Myelodysplasia can also be associated with leukopenia.
Anemia

Anemia can be classified into microcytic, macrocytic, and normocytic anemia. Initial work up: H & P, review of peripheral smear, reticulocyte count (adjust for the hematocrit, corrected reticulocyte count (Retic % x patient’s hct/45), and since iron and B12 deficiencies are common, these should be checked. RDW has been found in recent studies to be of little help in the diagnosis of anemias.

Microcytic

The four major differential diagnoses
1. Iron deficiency anemia
2. Anemia of chronic disease
3. Sideroblastic anemia
4. Thalassemia

Approach
1. Peripheral smear examination may offer clues for the etiology
   a. Pencil cells are classic for iron deficiency
   b. Target cells and basophilic stippling may be seen in thalassemia

2. If microcytic anemia is found, reticulocyte count should be done
   a. A low reticulocyte count suggests iron deficiency anemia, anemia of chronic disease, or sideroblastic anemia
   b. A high reticulocyte count suggests thalassemia

In a patient with microcytic anemia, if the reticulocyte count is high, then hemoglobin electrophoresis is useful. Hemoglobin electrophoresis is abnormal with elevated levels of hemoglobin A2 or hemoglobin F in cases of beta thalassemia. Hemoglobin electrophoresis is normal in alpha thalassemia trait. Globin chain analysis may be necessary.

3. If the reticulocyte count is low in a patient with microcytic anemia, serum ferritin should be done
   a. If the ferritin level is low, it suggests iron deficiency and blood loss must be ruled out (Iron deficiency: ↓ iron, ↓ ferritin, ↑ iron binding capacity, ↓ transferrin saturation)

If the ferritin level is normal or high, then look at serum iron and iron binding capacity:

4. If the serum iron is low, and iron-binding capacity is low, then this suggests anemia of chronic disease

5. If the serum iron is normal or high, and iron binding capacity is normal, then bone marrow examination must be done to look for ringed sideroblasts, which suggest sideroblastic anemia
The most common cause of microcytic anemia is iron deficiency anemia and the most common cause of iron deficiency is blood loss. Other symptoms and signs of iron deficiency anemia include pica, cheilosis, esophageal web formation, atrophic gastritis, and nail changes (koilonychia). Causes of iron deficiency other than blood loss are infrequent. These include chronic intravascular hemolysis, iron malabsorption (celiac disease, IBD, surgical resection/bypass), partial or total gastrectomy and atransferrinemia. Hepcidin is the most important regulator of gut iron absorption.

**Iron deficiency anemia**

**Lab results**

*Peripheral smear*
- Hypochromia
- Microcytosis
- Tear drops
- Pencil forms
- Elevated platelet count

*CBC*
- Elevated RDW
- Low MCV
- Low hemoglobin
- Low RBC number
- Elevated platelets

*Iron study*
- Low serum iron
- Elevated TIBC
- Low ferritin (level > 100 rules out iron deficiency even with inflammation)
- Low percentage saturation
- Soluble serum transferrin receptor (sTfR) can distinguish iron deficiency anemia (increased) and anemia of chronic disease (normal). sTfR is also elevated in hemolysis and myelodysplasia.

*Bone marrow*
- Not necessary for diagnosis
- If done, shows absent iron stores

**Workup for blood loss is important in iron deficiency anemia.** GI blood loss is common in older men and women and menorrhagia is common in younger women. Iron replacement in the form of ferrous sulfate (cheapest), ferrous fumarate, ferrous gluconate, or polysaccharide-iron is recommended for iron deficiency anemia. 60 mg of ferrous sulphate taken every other day was found to increase iron absorption with fewer GI side effects. **Best oral iron schedule is Ferrous sulphate 325 mg every other day.** Iron deficit is calculated as: Weight (in lbs) x (desired Hg – current Hg) + 600 mg (women) and 1000 mg (men) to replace iron stores. If someone is not responding to iron replacement, the way to look for iron malabsorption is by doing **iron challenge test:** Check serum iron, then give ferrous sulphate 325 mg. Check serum iron 1 hour later, iron level should increase by 100 mcg/dL if there is no malabsorption (Consider celiac disease if there is malabsorption, if compliant). Reticulocyte count increases in 1 week,
Hematocrit increases in 2 weeks and hematocrit increases about 2 g/dL over three to four weeks after effective iron therapy. First there is normalization of hemoglobin, then MCV normalizes, then only ferritin normalizes. Iron therapy should be continued for 6 months after normalization of ferritin to replete stores. If IV or IM iron dextran is necessary, test dose is given due to risk of anaphylaxis. Iron sucrose, ferric gluconate, and ferumoxytol have much less risk of anaphylaxis. Iron sucrose is usually given in end stage renal disease patients in combination with erythropoietin.

**Anemia of chronic disease (Inflammatory block anemia)**
- Hypoproliferative disorder. It can be microcytic or normocytic
- All diseases associated with anemia of chronic disease have increased activity of cytokines TNF, IL 1 and 6, INF γ. Anemia of chronic disease can be found in inflammatory (RA, temporal arteritis, cancer) and in non-inflammatory conditions such as COPD, CHF, and DM.
- Hepcidin is a peptide synthesized in liver in response to IL-6. It reduces iron absorption and decreases release of stored iron from macrophages. Hepcidin mediates anemia of chronic disease (Hepcidin level is low in iron deficiency anemia and ineffective erythropoiesis; It is high in anemia of chronic disease and CKD)
- There is also decreased erythropoietin response to anemia, and impaired response of erythroid progenitors to erythropoietin
- Iron studies
  - Low serum iron
  - Low percentage saturation
  - Low TIBC, high Ferritin
  - Reticulocyte count is low
- Treatment
  - Treatment of the underlying condition
  - Erythropoietin may be needed (Caution: HTN, adverse CVD events, thrombosis)

Anemia due to CKD is due to low erythropoietin level and usually normocytic with low reticulocyte count. Burr cells may be seen in smear. Microcytic anemia in a CKD patient usually indicates GI blood loss. Erythropoietin treatment is recommended in CKD to maintain Hb between 11-12 g/dL. Ferritin level should be maintained > 100 ng/ml and transferrin saturation > 20% in patients receiving erythropoietin.

**Macrocytic Anemias**
- Important differential diagnosis
  - Vitamin B12 deficiency
    - Oval macrocytes and hypersegmented PMNs on peripheral smear
  - Folate deficiency
    - Oval macrocytes and hypersegmented PMNs on peripheral smear
  - Myelodysplastic syndrome
    - Round macrocytes and neutrophils with pseudo-Pelger-Huet anomaly
  - Liver disease
    - Round macrocytes and target cells on peripheral smear
  - Alcohol abuse
- Hypothyroidism
- Cold agglutinin disease
- Hemolysis
  - MCV is elevated due to the presence of reticulocytes
  - Peripheral smear shows round macrocytes and polychromatia along with findings of hemolytic anemia

**Approach**
1. Peripheral smear must be looked at
2. Reticulocyte count
   a. **Most important next step**
   b. If the reticulocyte count is high in a patient with macrocytic anemia, then evaluation for hemolytic anemia must be done
   c. If the reticulocyte count is low, serum levels of vitamin B12 and folate and RBC folate level must be measured

**Vitamin B12 deficiency**
- Vitamin B12 level is low and the folate level is normal
- **Testing for anti-intrinsic factor antibody and anti-parietal cell antibody to diagnose pernicious anemia is not needed, since treatment will not change.**
  - Causes of vitamin B12 deficiency:
    - Achlorhydria (partial gastrectomy, PPI, H2B)
    - Pernicious anemia (due to anti-intrinsic factor and anti-parietal cell ab against hydrogen/potassium ATP in parietal cell membrane)
    - Celiac disease
    - Bacterial overgrowth, H.pylori infection
    - Ileal disorders (IBD)
    - Vegetarians/vegans
    - Metformin

B12 level may not be accurate especially in older patients. Measuring methylmalonic acid (particularly in older patients) and homocysteine level (more sensitive indicator of tissue folate stores than serum/RBC folate levels) is preferred to diagnose B12 and folate deficiency, when vitamin B12 level is low normal but with clinical suspicion.

Cobalamin is cofactor for two enzymes:
Methionine synthase and methylmalonyl-CoA mutase.

Folate is needed for many reactions.

Deficiencies of vitamin B12 and folate lead to megaloblastic anemia, due to defective DNA synthesis. DNA synthesis requires 5,10 methylene THF polygutamate. In both vitamin B12 and folate deficiency, there is insufficient 5,10 methylene THF.
Adenosylcobalamin is the form that is required for conversion of methylmalonyl-CoA to succinyl-CoA. Lack of adenosylcobalamin leads to elevated methylmalonyl-CoA levels.

**In vitamin B12 deficiency, the levels of both methylmalonic acid and homocysteine are elevated. In contrast, in folate deficiency, only homocysteine level is elevated.** Vitamin B12 deficiency leads to ineffective erythropoiesis that results in megaloblastic anemia and hemolysis (elevated LDH, bilirubin). Neurological manifestations include dementia, psychosis, and loss of position and vibration sense (posterior column) that leads to spastic paraparesis.

When folic acid is given to treat vitamin B12 deficiency, the hematological defect may improve, but neurological side effects are not reversed.

**Vitamin B12 deficiency can be treated with high dose oral replacement therapy (1000-2000mcg/day) regardless of etiology. This has been shown to be as effective as parenteral therapy.** Reticulocyte count peaks at five days after B12 administration, and LDH and bilirubin fall. Hb normalizes in 2 months, hypersegmentation improves after 2 weeks. In workup of macrocytic anemia with low reticulocyte count, if the vitamin B12 level is normal and deficiency is ruled out, consider folate deficiency. Serum folate level may be unreliable since serum folate level can normalize after a single serving of folate rich foods such as green leafy vegetables, banana, melon etc. RBC folate level is more reliable but can be low in vitamin B12 deficiency as well. Homocysteine level is always elevated in folate deficiency and can be checked when serum folate is normal. Causes of folate deficiency include dietary deficiency, hemolytic anemia (Hb SS), psoriasis (high cell turnover), alcohol, drugs, malabsorption, or pregnancy. Daily recommended folate intake is 400 mcg/day. Deficiency is treated with 1-4 mg/day.

**In cases of macrocytic anemia with low reticulocyte count when vitamin B12 and folate deficiency has been ruled out, and liver disease/alcohol/hypothyroidism have been excluded, myelodysplastic syndrome should be considered, and bone marrow examination should be done.**

**Normocytic anemia**

**Approach**

1. Reticulocyte count
   a. If the reticulocyte count is high, this can be post hemorrhage or due to hemolytic anemia
   b. Peripheral smear must be examined for presence of microangiopathic hemolytic anemia, spherocytes or acanthocytes

2. Coombs test:
   a. Done in cases of normocytic anemia with high reticulocyte count and the smear does not show any diagnostic abnormality
   b. Positive test may suggest allo or autoantibody or drug induced hemolytic anemia
In a normocytic anemia, if the retic count is low, smear should be examined for evidence of liver disease or dimorphic cells suggestive of combined vitamin B12 and iron deficiency or sideroblastic anemia. Medical evaluation to rule out anemia of chronic disease is done. Renal and endocrine causes (e.g., hypothyroidism) should be ruled out.

Copper deficiency anemia:

Copper deficiency can occur due to poor intake of copper (anorexia, bariatric surgery) or due to excessive zinc intake (zinc supplements, zinc lozenges). Copper deficiency leads to anemia, neutropenia and neurological findings – peripheral neuropathy/sensory deficits/ataxia. Treatment is by copper replacement.

Hemolytic anemia

Hemolytic anemia may be due to abnormalities intrinsic to the RBCs or abnormalities not intrinsic to the RBCs.

Abnormalities intrinsic to RBC

Intracellular defects

- Inherited intracellular defects
  - Membrane abnormalities
    - Hereditary spherocytosis
    - Hereditary elliptocytosis
    - Hereditary stomatocytosis
    - Hereditary pyropoikilocytosis
  - Enzyme abnormalities
    - Deficiency of glycolytic enzymes
    - Deficiency of enzymes in the pentose phosphate pathway
    - Deficiency of enzymes in glutathione metabolism
  - Hemoglobinopathies
    - Sickle cell anemia
    - Thalassemias
- Acquired intracellular defects
  - Paroxysmal nocturnal hemoglobinuria
  - Lead poisoning

Extracellular defects (Abnormalities not intrinsic to RBCs)

- Defects can be Coombs positive or Coombs negative
  - Coombs positive
    - Autoimmune hemolytic anemia
    - Transfusion reaction (alloimmune)
  - Coombs negative
    - HUS
    - TTP
    - Drugs
    - Chemicals
▪ Physical agents
▪ Infection
▪ Hypersplenism

General features
▪ Anemia in association with reticulocytosis
▪ Elevated indirect bilirubin
▪ Elevated LDH
▪ Low haptoglobin

Laboratory findings of hemolysis: Hemolysis can be extravascular or intravascular
▪ Peripheral smear
  o Polychromatophilia
    ▪ Found in both extravascular and intravascular hemolysis
▪ Retic count
  o Elevated in both extravascular and intravascular
▪ Bone marrow
  o Erythroid hyperplasia in extravascular and intravascular
▪ Unconjugated bilirubin
  o Elevated in both extravascular and intravascular
▪ Haptoglobin
  o Reduced or absent in both
▪ Plasma hemoglobin
  o Extremely elevated in intravascular hemolysis
  o Usually normal in extravascular hemolysis
▪ LDH
  o Elevated in extravascular
  o Remarkably elevated in intravascular
▪ Urine
  o Bilirubin is not seen in both forms
  o Hemosiderin is seen only in intravascular
  o Urine hemoglobin is seen in severe cases of intravascular

Membrane defects
▪ RBC membrane’s viscoelastic properties make it deformable, and help them travel through capillaries
▪ Membrane proteins:
  o Spectrin
  o Actin
  o Ankyrin
  o Protein 4.1/4.2
▪ These proteins are present in cytoplasmic membrane surface
▪ There are also integral membrane proteins
  o Protein 3
▪ Any defect in membrane proteins makes RBCs susceptible to lysis
**Hereditary spherocytosis**
- Most common
- Autosomal dominant
- Molecular basis is deficient or abnormal membrane proteins

Different abnormalities exist in different kindred. The defect leads to decrease in the deformability, and splenic sequestration of RBCs. Once trapped in the spleen, the surface area of the RBCs is further reduced. The incidence is one in 5000 in USA.

**Clinical features**
- Anemia
- Spherocytosis
- Splenomegaly
- Jaundice
- Elevated erythrocyte osmotic fragility

The severity varies - patients can be asymptomatic or can have severe hemolysis. Many patients with hereditary spherocytosis have biliary tract symptoms. More than 40% of the patients develop cholelithiasis by the third decade of life. Chronic leg ulcers can also occur. Parvovirus B19 infection can lead to aplastic crisis. Folate deficiency also causes severe anemia.

**Blood smear**
- Spherocytes and polychromatophilia
  - Spherocytes have no central pallor, due to the loss of membrane surface area

**CBC**
- MCV is normal
- **Mean corpuscular hemoglobin concentration (MCHC) is elevated**
  - This is the only condition where MCHC is elevated

CBC with diff, tests for hemolysis, Coomb’s test to rule out immune mechanism, and Osmotic fragility test are initial tests. Eosin-5-maleimide (EMA) binding test has high sensitivity (93%) and specificity (98%). Acidified glycerol lysis test (AGLT) and Pink test also have high sensitivity and specificity. EMA and AGLT combined is useful for diagnosis.

**Treatment**
- Folic acid therapy is needed.
- For patients with moderate-to-severe symptomatic hereditary spherocytosis, to avoid aplastic crisis, cholelithiasis, and chronic leg ulcers, splenectomy is recommended
- It is a planned splenectomy and vaccination with pneumococcal, Haemophilus influenzae and Neisseria meningitidis are recommended prior to splenectomy. There is increased risk of sepsis and possibly increased risk of arterial and venous thrombosis post splenectomy.
Hereditary elliptocytosis is like hereditary spherocytosis, but splenectomy is contraindicated in some forms of stomatocytosis.

Enzyme defects
- The main metabolic substitute for RBCs is glucose
- Glucose is metabolized by the Embden-Meyerhof pathway and Hexose monophosphate shunt pathway

The major metabolic product of Embden-Meyerhof pathway is adenosine triphosphate (ATP). ATP is needed for sodium, potassium transport and phosphorylation, and nicotinamide adenine dinucleotide (NAD) that is needed to prevent oxidation of ferrous heme to ferric heme. Shunting of phosphoglycerides from Embden-Meyerhof pathway to form 2, 3 DPG decreases the oxygen affinity.

Hexose monophosphate shunt pathway protects the hemoglobin from oxidation by generating glutathione. The critical enzyme in this pathway is glucose 6 phosphate dehydrogenase (G6 PD). G6 PD functions to reduce NADP to NADPH, which maintains sulphhydryl groups, which are important in decreasing free radicals and peroxidases.

**G6 PD deficiency**
- X-linked disorder
- Polymorphisms of G6 PD gene lead to variants
- Males are hemizygotes and are markedly deficient in enzyme activity
- Females are heterozygotes and range from normal to deficient levels
- African-Americans with G6 PD deficiency usually have no hemolysis unless challenged by oxidant drugs, infection.

During an acute event, there is intravascular hemolysis, which causes hemoglobinurina with abdominal pain or back pain. Common drugs that precipitate hemolysis include sulfa, primaquine, dapsone, INH, rasburicase, phenazopyridine, nitrofurantoin. **It is important not to measure the enzyme activity during acute hemolysis because reticulocytes have near normal enzyme activity.** The enzyme activity must be measured after the hemolytic episode.

**The peripheral smear during a hemolytic episode shows bite cells.** Heinz’ bodies are small, round, dark erythrocyte inclusions seen with crystal violate stain. Mediterranean variant of G6PD deficiency is associated with chronic hemolysis with acute hemolytic crisis when exposed to noxious agents like fava beans (favism).

**Pyruvate kinase deficiency**
- Most common deficiency in the glycolytic pathway
- Phosphoenolpyruvate is converted to pyruvate by the enzyme pyruvate kinase generating a net gain in ATP
  - Without the synthesis of ATP, there is increased permeability of RBCs to cations
- The diagnosis is confirmed by enzyme assay
Patients with hemolysis requiring transfusion can undergo splenectomy
Patients with myelodysplastic syndrome or AML can develop an acquired pyruvate kinase deficiency

**Immune mediated hemolytic anemias**

**Etiology**

- Alloantibodies
  - Transfusion reactions
- Autoantibodies
  - Warm autoimmune hemolytic anemia (WAHA), Cold autoimmune hemolytic anemia or Cold agglutinin disease, and Paroxysmal cold hemoglobinuria
- Drugs

**Autoimmune hemolytic anemia**

- Antibodies reacting at 37 degree centigrade are called warm antibodies, causing warm autoimmune hemolytic anemia
- Antibodies reacting at 4 degree centigrade cause cold autoimmune hemolytic anemia

**WAHA (Warm Antibody hemoglobin Anemia) or Autoimmune hemolytic anemia (AIHA)**

- Most common type
- Primary when there is no underlying disease
- Secondary, if it occurs in association with
  - Connective tissue disorders
  - Lymphoproliferative disorders
  - Inflammatory bowel disease
  - HIV

**Features:** (Triad: Anemia, jaundice, splenomegaly)

- **Anemia**
- **Jaundice**
- **Dark urine**
- **Splenomegaly**
- Peripheral smear
  - Microspherocytes
  - Nucleated RBCs
  - Polychromatophilia
- Bone marrow
  - Not necessary, unless suspicion of lymphoma is present

**Direct Coombs**

- Positive for IgG and weakly positive or negative for complements
- Coombs test clinches the diagnosis of AIHA/WAHA
Mechanism: Autoantibody directed against red cell antigen. The autoantibody binds to the RBC. Once RBC is coated by antibody, it is destroyed by splenic macrophages. Macrophages recognize the Fc portion of the antibody thro’ their Fc receptor.

Treatment

- **Glucocorticoid 1 to 1.5 mg/kg of prednisone per day**
  - Prednisone interferes with macrophage Fc and C3b receptor function
  - Therefore clearance of RBC from circulation is decreased
  - Prednisone decreases the production of autoantibody
  - Prednisone decreases the affinity of autoantibodies to their target antigens

Response usually occurs within one to two weeks. Response is unlikely to occur after three or more weeks of treatment. Some cases of steroid failure are managed with high dose pulse steroid. Steroid unresponsive disease can be treated with rituximab or splenectomy.

Other second line agents include: azathioprine, cyclophosphamide, cyclosporine + mycophenolate mofetyl, and danazol.

Transfusion therapy may be needed because of anemia. Transfused RBCs have low survival, but no acute hemolysis occurs. Occasionally plasma exchange is used to stabilize patients with warm autoimmune hemolytic anemia until more definite therapy becomes effective.

**Cold autoimmune hemolytic anemia (Cold agglutinin disease)**

- Mediated by IgM autoantibodies that have specificity for Li blood antigens
- The titer of cold agglutinin is not predictive of the severity of anemia
- Usually a **chronic disease of the elderly**
- Secondary cold agglutinin disease can occur due to infections
  - Mycoplasma
  - Epstein-Barr virus infection
- Cold agglutinin disease can be secondary to lymphoproliferative disorders

**Clinical features**

- Related to small vessel occlusion
  - Acrocyanosis of ears, tip of the nose, toe, and fingers
  - Skin can assume a dusky blue hue and then turn normal upon warming
- Livedo reticularis can be seen in severe cases
- Increased hemolysis with cold exposure
  - Coombs test: **Positive for compliment and negative for IgG**
- Peripheral smear
  - Agglutination of RBCs (causes false elevation of MCV)
    - Disappears if smear is prepared at 37 degree centigrade
- Diagnosis: Clinical features + smear review + significant cold agglutinin titer + coomb’s test (positive for bound complements)

**Treatment**

- Preventive measures include warm clothing and avoiding cold exposure
For mild cases, supportive care (warm clothing) is given. For severe cases: Rituximab +/- fludarabine can be used. Steroids are not effective since they do not inhibit complement-mediated hemolysis. Splenectomy is not useful since RBCs are cleared in the liver. Chlorambucil and cyclophosphamide have been used for cold autoimmune hemolytic anemia associated with monoclonal gammopathies. For acute hemolysis, packed RBC through a blood warmer is given. Donor red cells are I positive and can be removed rapidly. Plasmapheresis may reduce the antibodies in acute severe cases.

Treatment of the underlying disease is very important.

Paroxysmal cold hemoglobinuria
- Due to an IgG cold antibody to the RBC P antigen
  - Donath-Landsteiner antibody
- Can cause acute intravascular hemolysis after viral infections
- Treatment includes transfusion and prednisone

Drug-induced hemolytic anemia

Mechanisms
1. Autoantibody: Drug may induce autoantibody production by altering antigens on RBCs leading to autoimmune hemolytic anemia
   - Examples: alpha methylldopa, levodopa, cephalosporins, procainamide, diclofenac
   - Hemolysis and spherocytosis can occur
   - Patients have IgG and complements on RBCs
   - Positive Coombs test
2. Hapten mechanism: The drug binds to the RBC membrane and an antibody then binds to the RBC drug complex
   - Example: penicillins, cephalosporin, tetracycline, carbenicillin
   - Direct Coombs test is positive for IgG only
   - Peripheral smear shows spherocytosis
3. Ternary complex: Drug-antibody complex binds to RBC
   - Examples: Amphotericin B, cephalosporin, chlorpromazine, INH, melphalan, rifampin, diclofenac, hydrochlorothiazide
4. Unknown Mechanism:
   - Examples: Chlorpromazine, melphalan, INH, acetaminophen, thiazides, ibuprofen, omeprazole, sulindac, rifampin, tricyclic antidepressants

Commonly implicated drugs causing hemolytic anemia are cephalosporins (cefotetan, ceftriaxone), penicillins (piperacillin), NSAIDs, and quinine. Interferon has also been reported to cause autoimmune hemolytic anemia.
Hemoglobinopathies

Sickle cell disease

- Hemoglobin SS has mutation in the beta-globin gene
  - The sixth amino acid in the beta chain is changed from glutamic acid to valine
- Spectrum of disorders
  - Homozygous hemoglobin SS and S trait
  - Hemoglobin SC
  - Hemoglobin S beta thalassemia
  - Sickle cell trait (*heterozygous state*)
    - Usually causes painless hematuria and isosthenuria

Diagnosis is by hemoglobin electrophoresis.

Multiple complications occur in Hb SS disease.

Acute chest syndrome

- Important complication and most common cause of death
- Presents with chest pain, tachypnea, fever, cough, and hypoxia
- Chest Xray shows diffuse infiltrates
- The frequent underlying or concomitant conditions are pulmonary infarction and pneumonia

Treatment: Simple transfusion to Hb of 10 mg/dL when Hb is > 1g/dL below baseline, supplemental oxygen, antibiotics. Exchange transfusion for severe disease (< 90% Pulse ox while on O2, respiratory distress, progressive pulmonary infiltrates, decline in Hb despite transfusion)

Other complications of Hb SS include:

1. Stroke (Needs exchange transfusion acutely, monthly prophylactic transfusions and hydroxyurea are given after stroke). Vaculopathy can lead to collateral formation (Moyamoya syndrome) which can lead to hemorrhage.
2. Pigment gallstones leading to cholecystitis
3. Pain crisis and chronic pain
4. Sequestration crisis is seen in children
5. Splenic infarction leading to functional asplenia
6. Aplastic crisis can occur due to folate deficiency (supplement), parvovirus B19 infection, drugs, or hyperhemolysis (hemolytic crisis)
7. Leg ulcers
8. Pulmonary hypertension and R heart failure (treated like primary Pulm HTN)
9. Priapism
10. Secondary hemochromatosis due to recurrent transfusions
11. Dactylitis usually occurs in children
12. Osteomyelitis
13. Recurrent infections due to functional asplenia (vaccinate for encapsulated organisms: pneumococcus, hemophilus, meningococcus)
14. Avascular necrosis of hip (SS, SC, and sickle-thal)
Sickled RBCs show enhanced adhesion to vascular endothelium by specific receptors. Their interaction with leukocytes and endothelium may be important in the development of vasoocclusive events. The presence of high levels of hemoglobin F has been found to inhibit sickling; therefore, sickle cell patients with recurrent vasoocclusive crisis can be treated with hydroxyurea to elevate the hemoglobin F level. Recently hydroxyurea has also been shown to prolong survival in Hb SS disease.

**Exchange transfusion for hemoglobin SS disease is usually done for severe acute chest syndrome with hypoxia, stroke, and acute multi-organ failure.** Simple transfusion is done for acute severe symptomatic anemia due to low reticulocytes and prior to surgery (“top up” transfusion to Hb goal 10 g/dL prior to surgery). Prophylactic transfusion is done for primary and secondary prevention of stroke. Folate therapy should be given to all patients with sickle cell disease.

**Interventions that improve outcome in Hb SS disease:**

Patient education
Newborn screening
Penicillin prophylaxis (particularly from newborn to age 5) and vaccination
Hydroxyurea (shown to improve survival)
Selected patients: chronic transfusion
Improved supportive care and improved care during pregnancy and surgeries.

Patients with sickle cell anemia can develop cerebrovascular accidents and proliferative retinopathy. Transcranial Doppler and fundus exam are used to screen. Prophylactic transfusion can be done for primary stroke prevention in patients with abnormal transcranial Doppler.

Sickle cell disease management guidelines were issued by NHLBI expert panel in 2014. Some of the strong recommendations in the guidelines:

**Oral penicillin prophylaxis for SCD till age 5**
**Yearly transcranial Doppler from ages 2-16**
**Long term transfusion to prevent stroke in children with abnormal transcranial Doppler**
**Immediate start of opioid pain medication for severe pain due to vasoocclusive crisis, and incentive spirometry for inpatients with vasoocclusive crisis**
**SCD with proliferative retinopathy: refer for laser photocoagulation and for ECHO to evaluate for pulmonary hypertension (dilated eye exam q1-2 years to start at age 10)**
**ACEi for microalbuminuric adults with SCD**
**Analgesia and PT for avascular necrosis**
**Hydroxyurea for adults with ≥ 3 severe vasoocclusive crises in 1 year, SCD pain interfering with day to day activities, or severe or recurrent episodes of acute chest syndrome**
**Preoperative transfusion to increase Hb to 10g/dL and a moderate strength recommendation to maintain sickle Hb level < 30% prior to next transfusion during longterm transfusion therapy**
Assessment of iron overload with a moderate strength recommendation to begin iron chelation therapy when indicated
Unvaccinated adults (age 19 or more) with SCD: PCV 13, then at least 8 weeks later PPSV 23, then PPSV 23 5 years later, and then PPSV 23 at age 65

New drugs for sickle cell disease are being developed:
1. Selectin inhibitors: Inhibitors of leucocyte adhesion
2. Non-anticoagulant heparins: Inhibits adhesive interactions through P-selectin
3. MEK/ERK kinase inhibitors that prevent RBC trapping
4. Poloxamer: Nonspecific inhibitor of adhesion to reduce vasoocclusive crisis duration
5. Approaches to downregulate inflammatory pathways
6. Drugs to induce HbF

**Crizanlizumab**, is a monoclonal antibody which binds to P-Selectin and blocks endothelium and leucocyte interaction. In a RCT, crizanlizumab, in patients with or without hydroxyurea treatment, reduced pain crises by 45% annually, and delayed time to first crisis, without serious adverse effects.

**Hemoglobin C, SC**
- Causes mild hemolytic anemia, microcytosis, target cells, and splenomegaly
- May be associated with aplastic crisis, gallstones may occur, and there is a higher incidence of retinal disease and aseptic necrosis than hemoglobin S. Hb crystals in peripheral smear (precipitated Hb C) can be seen in smear.

**Beta-thalassemia**
- Inherited disorders of beta-globin synthesis
- There is decreased hemoglobin synthesis
  - Microcytosis and ineffective erythropoiesis secondary to unbalanced synthesis of alpha and beta-globin leading to hemolysis

- There are several types
  - Severe beta-thalassemia (*Cooley’s anemia*)
    - Severe anemia
    - Growth retardation
    - Hepatosplenomegaly
    - Bone marrow expansion
    - Transfusion dependent
  - Thalassemia major and thalassemia intermedia reflects clinical heterogeneity
    - Majority of patients have transfusion dependence
    - Intermedia has no regular transfusion requirement
  - Beta-thalassemia trait or beta-thalassemia minor are asymptomatic and they show mild anemia and microcytosis, target cells in the peripheral smear. Hb electrophoresis is abnormal with increased HbA2. Beta thalassemia trait: Low MCV with normal or high RBC count, **Metzner index: MCV (fl)/RBC count (million) > 13 is iron deficiency, < 13 is thalassemia.**
Alpha-thalassemia

- There are four genes for alpha-globin production
- Hemoglobin H disease occurs with loss of three genes
- Clinical features
  - Moderate hemolytic anemia
  - Icterus
  - Splenomegaly
  - Hydrops fetalis occurs when all four genes are lost and there is death in utero
  - A silent carrier has loss of only one gene and they are hematologically normal
  - Alpha-thalassemia minor or alpha-thalassemia trait is associated with loss of two genes and these patients have mild anemia

Genetic counseling is important for couples. Patients who are of child bearing age with α or β thalassemia traits should have their spouses screened to avoid severe thalassemia in their offspring.

In the cord blood, hemoglobin Barts can be measured. Percentage of hemoglobin Barts indicates the number of alpha-genes lost. Less than 5% indicates loss of only one gene. These patients are carriers for alpha-thalassemia minor and are hematologically normal. 5-10% of Barts hemoglobin indicate alpha-thalassemia minor associated with loss of two genes. More than 10% hemoglobin Barts indicates more severe forms of alpha-thalassemia.

Hemoglobin E is an unstable beta hemoglobin chain with a presentation similar to thalassemia. It is the most common hemoglobinopathy in the world, and common in Southeast Asia. Heterozygotes are microcytic, and homozygotes are mildly anemic with microcytosis and target cells. Patients who inherit both the genes for Hb E and beta thalassemia become severely anemic and have features similar to homozygous beta thalassemia. Therefore, spouses of patients with beta thalassemia should be screened for Hb E in addition to beta thalassemia.

Secondary iron overload
Complication of chronic hemolytic anemias requiring transfusions. Iron chelation is the therapy of choice. Deferoxamine is the parenteral iron chelator, but deferasirox is now available, which is a oral iron chelator.
Anticoagulation and Fibrinolytic Pathways

Contact System
- XII
- High Molecular Weight Kininogen (HMWK)
- Prekallikrein (PK)

Tissue Factor
- VII

Factor V Leiden
(protein C resistance)

Fibrinogen
- Fibrin monomer
- Fibrin polymer
- Cross linked fibrin

Antithrombin III

↑ PTT
1. No bleeding or clotting ⇒ HMWK, PK, or factor XII deficiency
2. Some bleeding ⇒ Factor XI deficiency
3. Major bleeding ⇒ Factor VII (most common) and IX deficiency

↑ PT:
Factor VII or vitamin K deficiency and Warfarin
Prolonged PT and/or PTT, not corrected by plasma:

Specific or non-specific inhibitors

Clot solubility in 5M urea

Factor XIII deficiency

Rapid clot lysis

a2 plasmin inhibitor

*Partial Thromboplastin Time (PTT)*
- Screens the intrinsic limb of the coagulation system
- Tests for the adequacy of factors XII, high molecular weight kininogen, prekallikrein, and factors IX and VIII

*Protime (PT)*
- Screens for the extrinsic or the tissue factor dependent pathway

Both PT and PTT also evaluate the common coagulation pathway, i.e., all the reactions that occur after activation of factor X. Thrombin time (TT) evaluates the time taken for conversion of fibrinogen to fibrin.

*von Willebrand disease*
- The most common inherited bleeding disorder
  - 1 in 100 to 1 in 400 individuals in the population
- von Willebrand factor has two functions
  - Facilitates platelet adhesion
  - Acts as a carrier for factor VIII
- von Willebrand factor (vWF) is synthesized in the endothelial cells and megakaryocytes
- Except for type 3, all other types are autosomal dominant

In mild cases, bleeding occurs after surgery or trauma. In more severe cases, spontaneous epistaxis, gingival bleeding, and GI or GU bleeding can occur. (i.e., oozing bruising patient)

Three major types

**Type 1**
- The commonest form
- Autosomal dominant
- Mild-to-moderate decrease in the plasma vWF
- Factor VIII activity is decreased
- Ristocetin cofactor activity is decreased
  - Both the ristocetin tests are decreased
The spectrum of multimers detected by Agarose gel electrophoresis is normal

**Type 2 (several subtypes)**
- Normal or near normal levels of a dysfunctional protein
  - In type 2 vWD, the vWF antigen measurement is normal and factor VIII level is normal
  - The functional assays, i.e., both ristocetin tests are severely decreased and the electrophoresis is abnormal

**Type 3**
- Rare
  - 1 in 1,000,000
- Autosomal recessive
- Causes severe mucosal bleeding
- **No detectable vWF antigen or activity**

**Treatment**
- A common manifestation is menorrhagia
  - Usually controlled with oral contraceptive pills
- DDAVP
  - Can be used for type 1 disease only
  - Not useful for most type 2
  - Not effective in type 3
  - It is important to test for adequate response before surgery
  - Tachyphylaxis may occur
  - **The major side effect is hyponatremia**
    - Water restriction is advised for 24 hours after use
- Humate – P
  - An inactivated vWF containing concentrate (FDA approved)

Acquired von Willebrand disease can occur due to vWF antibodies, in patients with von Willebrand disease after multiple transfusions, or in patients with autoimmune and lymphoproliferative disorders. Acquired von Willebrand disease can also occur due to absorption of vWF to tumor surfaces (Wilms tumor, lymphoma, and Waldenstrom macroglobulinemia).

**Factor VIII deficiency**
- Called hemophilia A
- Factor VIII is synthesized in the liver
- Circulates complexed to vWF
- **X – linked**
  - Affects 1 in 10,000 males
- Bleeding occurs into soft tissues, muscles, and weightbearing joints
- Severity varies
  - If there is less than 1% of factor VIII activity
    - Severe disease that bleeds frequently
Between 1 and 5% activity
  ▪ Moderate disease, bleeding episodes are less frequent
  ▪ If the factor VIII activity is more than 5%
    ▪ Mild, bleeding is infrequent and usually only associated with trauma

Hemophilic bleeding occurs hours or days after injury, and may continue for days or weeks if untreated; this can lead to a large collection of partially clotted blood, with complications such as: compartment syndrome, venous congestion, ischemic damage to nerve, etc. Large calcified masses of blood can form, causing pseudotumors.

Patients with severe disease are usually diagnosed immediately after birth by the presence of extensive hematoma. Moderate disease manifests only when the baby begins to walk or crawl. The most feared complication of hemophilia is oropharyngeal or CNS bleeding.

Labs
  ▪ Platelet count is normal
  ▪ Bleeding time is normal
  ▪ PT is normal
  ▪ Isolated elevation of PTT (corrected by mixing study)

A male with bleeding history and elevated PTT should have specific assays done for factor VIII and factor IX.

Treatment
  ▪ Aspirin and NSAIDS must be avoided
    ▪ These agents impair the platelet function *(leading to severe hemorrhage)*
  ▪ COX-2 inhibitors can be used
  ▪ When a patient with factor VIII deficiency undergoes surgery, screen for inhibitors to factor VIII
  ▪ Mild disease *(more than 5% activity)*
    ▪ Desmopressin can be given for minor procedures
    ▪ Factor VIII (recombinant or monoclonal ab purified) is given prior to surgery and after moderate trauma
  ▪ Moderate disease *(activity is between 2 and 5%)*
    ▪ Factor VIII (recombinant or monoclonal ab purified) is given for all bleeding episodes and for surgery
  ▪ Severe disease *(level is less than 1%)*
    ▪ Factor VIII concentrate is required as prophylactic therapy when strenuous activity is anticipated

Many patients treat themselves with factor VIII concentrate on a schedule for prophylaxis &/or at the first sign of bleeding.

  ▪ Dental procedures
    ▪ Usually need a single infusion of factor VIII concentrate prior to tooth extraction
    ▪ Epsilon aminocaproic acid is given four times a day for four days thereafter
Complications of longstanding hemophilia A include hepatitis C, and > 80% of hemophilia A patients over age 20 are HCV positive. The risk of HIV currently is very low due to vigorous screening.

A major complication is formation of inhibitor to factor VIII. This is an IgG antibody. Patients must be screened for this inhibitor prior to surgery. Patients can be treated with prothrombin complex concentrate, recombinant factor VIIa or porcine factor VIII. Enzyme (monoclonal antibody that binds Factor IXa to Factor X thereby bypassing Factor VIII) has now been found to be useful in the treatment of hemophilia A with and without inhibitors.

Factor IX deficiency
- Called hemophilia B
- It affects 1 in 100,000 male
- Indistinguishable clinically from hemophilia A
- Accurate diagnosis is necessary since the treatment is different
- Factor IX concentrates (recombinant human factor IX concentrate) should be used.

Factor X deficiency
- Amyloidosis can lead to factor X deficiency, which prolongs PT and PTT

Factor XI deficiency
- Autosomal recessive
  - Usually seen in people of Jewish ancestry
  - Patients usually bleed with trauma or surgery
  - Treatment is with administration of FFP

Inherited deficiencies of factors other than VIII, IX and XI are rare. Bleeding symptoms from these rare disorders can range from asymptomatic to severe life threatening bleeding (factor XIII deficiency and factor X deficiency)

Factor XIII deficiency
- Factor XIII stabilizes the fibrin clot
- Hemorrhage, poor wound healing, and CNS bleeding can occur
- PT, PTT, and thrombin time are all normal
- Diagnosed by clot solubility in 5 M urea
- Treatment is given with FFP or purified factor XIII

Vitamin K deficiency
- Can be due to inadequate dietary intake, malabsorption, or hepatocellular disease
- Vitamin K dependent factors are factors II, VII, IX, X, protein C, and protein S
  - Factor VII has the shortest half life
    - Falls rapidly after start of coumadin or with vitamin K deficiency
  - Therefore, even with mild deficiency there is prolonged PT
  - Severe deficiency can prolong both PT and PTT
  - Treatment includes vitamin K injection and four factor prothrombin complex concentrate (PCC) for bleeding episodes. PCC lead to effective and timely reversal of warfarin with a reduction in all-cause mortality (meta-analysis) compared to FFP.
Platelet Disorders

Platelet function
- Attachment to an injured blood vessel surface (adherence)
- Attachment to other platelets (aggregation)
- Support enzymatic reactions of the coagulation cascade

Primary hemostasis
- Platelet adhesion, followed by aggregation leading to a platelet plug that is attached to the site of vessel injury constitutes primary hemostasis
- Adhesion: Platelets adhere to the blood vessel surface through vWF, anchoring the growing thrombus to the vessel wall
- Blood vessels exposed to high shear forces are dependent on vWF mediated platelet adhesion
- The receptor for vWF is platelet Gp1b/IXa complex
  - Other glycoproteins Ia, IIa, IV, and VI bind to collagen and fibronectin exposed on the endothelial surface
- vWF is the predominant means of platelet adhesion

Aggregation: Platelets attach to each other forming the initial hemostatic plug
- Aggregation requires platelet activation, in order to initiate change of shape, stimulate release of granules, and create the fibrinogen receptor
- Platelet activators
  - Adenosine diphosphate
  - Thrombin
  - Collagen
  - Epinephrine

Activators bind to platelet surface receptors leading to increased cytosolic calcium and activation of internal enzymes

Membrane Phospholipids
  - Phospholipase A

Arachidonic acid
  - COX-1 (Aspirin/NSAIDs inhibit COX-1)

PG endoperoxide - PG H2
  - Thromboxane synthase

Thromboxane A2

Thromboxane A2 diffuses from the interior of platelet and binds to its receptor on the platelet surface. It is a very potent platelet activator.
Protein kinase C pathway triggers the platelets to release granules that contain active substances and proteins important for hemostasis and blood vessel repair.
Both thromboxane A2 and the release of granules are necessary for forming large irreversible platelet aggregates.

Aggregation cannot occur until the fibrinogen receptor is formed. Protein kinase C promotes a conformational change in the platelet glycoprotein IIb/IIIa on the platelet surface to form the **platelet glycoprotein IIb/IIIa complex, and this is the receptor for fibrinogen.** This receptor belongs to the integrin family.

Fibrinogen binds to GpIIb/IIIa receptor, and the fibrinogen bridge holds platelets together. Without platelet glycoprotein IIb/IIIa receptor and fibrinogen, aggregation will not occur even if platelet is fully activated.

Platelets also support the enzymatic reactions that create the fibrin clot by contributing binding sites for coagulation factors, releasing proteins from their granules, and providing an appropriate phospholipid surface for the reactions.

Coagulation is the secondary hemostatic reaction and it supports the platelet plug created during primary hemostasis.

**Lab evaluation of platelet function**
- Platelet count
- Peripheral smear evaluation
  - Platelets are increased in number in essential thrombocytosis
  - Giant platelets are seen in Bernard-Soulier syndrome
  - Large platelets are seen in Wiskott-Aldrich Syndrome
  - Clumped platelets are seen in paraproteinemina
  - Grey platelets are seen in alpha-granule deficiency
- Platelet function is evaluated by platelet aggregation assay

Bleeding time does not show a linear response in relation to platelet number. It cannot predict the risk of bleeding during surgical procedure and it is not better than hematocrit or platelet count in predicting bleeding in patients with uremia. Bleeding time is replaced by Platelet Function Analyzer-100 (PFA-100) assay.

**Platelet aggregation studies**
These studies are difficult to do and interpret. A platelet activator (**agonist**) is added to a sample of platelet-rich plasma or whole blood. Platelet aggregates are then detected. Adenosine diphosphate, epinephrine, arachidonic acid, thromboxane A2, collagen, and thrombin all activate platelets differently. In vivo, the defect in any single pathway may prevent formation of an adequate platelet plug.

**Ristocetin assays**
Ristocetin is a lab reagent. It promotes **clumping** of **nonactivated** platelets by promoting the interaction of vWF with the platelet receptor Gp1b/IX. Ristocetin serves as the surrogate for vessel subendothelial layer. Ristocetin is used for two assays:
- Ristocetin-induced platelet agglutination study
a. **This study tests if the patient’s vWF is capable of binding to Gp Ib/IX receptor on his or her own platelets**
   b. Ristocetin is added to the patient’s blood sample
      i. If platelet clumps form, then the patient’s platelets and vWF are interacting appropriately

2. Ristocetin cofactor assay
   a. **This detects vWF activity**
   b. This is a test of vWF function in the patient’s plasma
   c. The patient’s platelets are removed from the sample and a fixed platelet preparation is added to replace the patient’s own platelets
      i. Therefore, vWF Ristocetin cofactor assay tests only the patient’s von Willebrand factor activity and does not test the patient’s platelets

All platelet tests should be interpreted with clinical correlation.

Nosebleeds, bleeding gums, and bruising (mucosal bleeding) are all suggestive of a disorder of primary hemostasis.

**Congenital disorders of platelet function**

- **Glycoprotein abnormalities**
  - Glanzmann thrombasthenia
    - Abnormal glycoprotein IIb/IIIa
  - Bernard-Soulier syndrome
    - Abnormal glycoprotein Ib/IX
  - Pseudo-von Willebrand disease
    - Due to abnormal glycoprotein Ib
  - There are several other defects in collagen binding, fibronectin binding, and platelet secretion

- **Disorders that mimic platelet dysfunction in patients with normal platelets**
  - von Willebrand disease and congenital afibrinogenemia

All platelet disorders present in a similar fashion. The clinical findings include an “oozing and bruising patient”, superficial purpura, petechiae, epistaxis, mucous membrane bleeding, and prolonged immediate bleeding.

**Glycoprotein abnormalities**

- The main ones are Glanzmann Thrombasthenia and Bernard-Soulier Syndrome
- Autosomal recessive disorders
- Clinical presentation is similar
- Glanzmann thrombasthenia
  - Mutations affecting glycoprotein IIb/IIIa
    - Platelets do not bind fibrinogen and cannot form aggregates
- Bernard-Soulier syndrome
  - Several mutations affecting platelet glycoprotein Ib/IX
This condition causes decreased platelet adhesion, since platelets cannot bind to vWF due to deficiency or dysfunction of glycoprotein Ib/IX

- Associated with modest thrombocytopenia
- **Peripheral smear shows giant platelets**
- Bleeding times can range from normal to more than 20 minutes
- Symptoms can wax and wane

Scott syndrome is a disorder of binding of coagulation factors to platelet surface.

**Treatment**

*Only definitive way to treat bleeding due to intrinsic platelet defects is to transfuse with normal platelets.* Transfusion reactions and antiplatelet antibodies complicate therapy. Prevention of bleeding must be done with good dental hygiene, hormonal control of menses, and by avoidance of antiplatelet agent. Nonspecific methods of treatment include use of topical thrombin for accessible sites, desmopressin (*this agent should be avoided in patients with pseudo-von Willebrand disease*). Cryoprecipitate may correct bleeding tendency especially in patients with granule deficiency. Antifibrinolytic agents like aminocaproic acid and tranexamic acid may decrease bleeding by preventing plasmin interference with platelet function and by stabilizing the existing thrombus.

**Acquired disorders of platelet function**

**Drugs**

- **Aspirin**
  - Irreversibly acetylates COX-I and COX-2
  - COX-I is seen in platelets
  - Blocks the production of thromboxane A2, a potent platelet aggregator for the life of the platelet, which is seven to ten days

- **NSAIDs**
  - Reversible effect on COX-1 and 2
  - The effect on thromboxane A2 production is dependent on the half-life of the drug

- **COX-2 inhibitors**
  - Do not affect platelet function

- **P2Y12 receptor blockers:**
  - **Clopidogrel, Prasugrel, Ticagrelor, Cangrelor**
  - P2Y12 is the ADP receptor
  - Clopidogrel and prasugrel irreversibly bind to P2Y12
  - Ticagrelor reversibly binds to P2Y12. More rapid onset of action than clopidogrel
  - Rare cases of TTP are associated with use of clopidogrel

- **Glycoprotein IIb/IIIa inhibitors**
  - **Abciximab, tirofiban and eptifibatide**

**Treatment**

- Mainly stopping the drug
- Nonspecific therapy with antifibrinolytic agents may be needed
- Transfusion of platelets is required for severe bleeding
There are other drugs with antiplatelet activity. These include certain beta-lactam antibiotics, prostacyclin, heparin, thrombolytic agents, dipyridamole, dextran, lepirudin, and alcohol.

**Uremia**
- Associated with several disorders of hemostasis
- Predominant component is platelet dysfunction:
  - Due to altered prostaglandin metabolism and abnormalities in the interaction between vWF and platelet receptor
- Mucocutaneous and GI bleeding are troublesome
- Platelets do not adhere and have decreased response to ADP, collagen and thrombin
- Nitric oxide released from damaged endothelial cells contributes to platelet dysfunction
- Washed platelets, re suspended in normal plasma, function normally
- Treatment:
  - Erythropoietin to maintain hematocrit more than 30%
  - Dialysis to control azotemia
  - Desmopressin to control bleeding
  - Cryoprecipitate to control bleeding

Antifibrinolytic agents such as epsilon aminocaproic acid may be needed and for severe bleeding, platelet transfusion is necessary. Desmopressin in dialysis patients promotes the release of vWF from the endothelial cells. **Desmopressin can be administered prior to procedures in dialysis patients.** Estrogen is useful by limiting the nitric oxide production. This is most useful for bleeding from telangiectasia.

**Dysfunction of platelets due to monoclonal and polyclonal gammopathies**
- E.g. Multiple myeloma
- Paraproteins coat the platelets and interfere with surface binding
- Treatment: Treat the underlying condition

**Mechanical platelet damage**
- Occurs secondary to hemodialysis or cardiopulmonary bypass
- Aprotinin
  - Improves platelets survival and function
  - Inactivates plasmin and limits compliment activation
  - This agent is used during cardiopulmonary bypass

**Work-up of thrombocytopenia**
- Thrombocytopenia refers to a platelet count of < 150,000/mcL
- If hemoglobin and WBC count are abnormal
  - Then bone marrow exam is needed
- If the hemoglobin and WBC count are normal
  - Peripheral smear must be examined
    - If platelets are clumped
      - CBC should be redrawn in sodium citrate or heparin
▪ If fragmented RBC
  • MAHA such as DIC and TTP is the diagnosis
▪ If RBCs are normal, and the platelets are normal or increased in size
  • Consider:
    o Drug induced thrombocytopenia (common in inpatients e.g. vanco)
    o Infection induced thrombocytopenia (common in inpatients)
    o Idiopathic immune thrombocytopenia
    o Congenital thrombocytopenia

HIT should be a strong consideration in patients exposed to heparin. HIT is the most common cause of thrombocytopenia in hospitalized patients.

**Immune thrombocytopenia (ITP)**

There are antibodies against glycoprotein IIb/IIIa and glycoprotein Ib/IX. Usually diagnosis is made in patients with low platelet count without other hematological disorders. Viral infections including hepatitis C, and HIV, connective tissue disorder such as SLE, splenomegaly, and drugs must be excluded. Either IgG or IgM antiplatelet antibodies directed against platelet membrane glycoproteins mediate reticular endothelial destruction of platelets.

Acute and self-limited form of immune thrombocytopenia occurs in children. In adults, it is usually chronic autoimmune thrombocytopenia.

**Acute immune thrombocytopenia**
▪ Occurs in children
▪ Peak age of incidence is 2-6
▪ Usually a history of viral infection, followed by acute onset of bruising, petechiae, and mucosal bleeding
▪ If thrombocytopenia persists for more than six months in spite of treatment, it is termed chronic autoimmune thrombocytopenia
▪ Viral infection evokes an immune response in acute immune thrombocytopenia

**Immune thrombocytopenia (ITP)**
▪ Initial symptoms include petechiae and mucosal bleeding
  o If bleeding is significant and recurrent, anemia can occur
▪ Physical exam is normal, except for the bleeding manifestation
▪ Evaluation: History, family history, physical exam, CBC and reticulocyte count, peripheral smear exam, quantitative immunoglobulin, bone marrow aspiration/biopsy in selected patients, blood group, direct Coomb’s, H.pylori, HCV, HIV. Other tests of potential benefit: ANA and APL antibodies, TSH and antithyroid ab, pregnancy test when indicated, viral PCR for CMV, parvovirus
▪ Peripheral smear
  o Thrombocytopenia and large platelets
▪ Bone marrow exam
  o Usually not necessary to make a diagnosis of ITP
Bone marrow exam is indicated 1. Age > 60 2. Prior to splenectomy 3. Unresponsive to treatment
- ITP is a diagnosis of exclusion: Isolated thrombocytopenia (< 100,000/µL), large platelets, no other cause identified
- Newly diagnosed ITP: < 3 months of diagnosis, Persistent ITP: 3-12 months, Chronic ITP: > 12 months, severe ITP: Bleeding at presentation and/or during treatment, or need for additional treatments
- Secondary ITP: APLA syndrome, SLE, Evans syndrome (autoimmune thrombocytopenia and autoimmune hemolytic anemia), Common variable Ig deficiency, Drugs, Infections: CMV, H.pylori, HCV, HIV, HZV, lymphoproliferative disorders, bone marrow transplant side effects, vaccination side effect

Treatment
- First line treatment for ITP is prednisone
  - Platelet count < 30,000/mcL (even if asymptomatic) or bleeding with thrombocytopenia
  - Prednisone decreases antibody levels and suppresses phagocytic activity of the spleen
    - Usually administrated as 1 mg/kg per day
    - 50% of the patients respond in three to six weeks
    - Relapse usually happens when tapered. After sustained response, taper slowly
    - Dexamethasone at a high dose of 40 mg/day for 4 days/month for 1-2 cycles lead to better overall response, complete response and time to response in an open label RCT, but with similar sustained response and less side effects when compared to traditional prednisone (can be used when patient does not want daily prednisone).

Patients without response to steroids can be treated by splenectomy (traditional therapy), or rituximab, or immunosuppression (mycophenolate mofetil), or TPO-receptor agonists (second line therapies)
- Splenectomy
  - Done for patients without response to steroid or for patients requiring long-term steroids
  - Vaccination for encapsulated organisms should be given prior to splenectomy
  - Successful in up to 70% of the patients
- IVIG
  - Effective in ITP patients who are bleeding, to elevate platelet count quickly
  - Used in patients who are bleeding, who have a spleen, who do not respond to steroid
  - Also used as pretreatment prior to platelet transfusion for major bleeding
  - Effective in 70 to 90% of the patients
  - Effect is transient and the drug is expensive
- Platelet transfusion in ITP
  - Only indicated for life-threatening/major bleeding and CNS bleeding
Platelet transfusion is always administered with steroids and IVIG

- **RhoGam (IV Anti-D)**
  - Useful for Rh-positive patients with ITP, who have not undergone splenectomy if they have bleeding episode
  - Can be used interchangeably as IVIG in Rh-positive patients who are not anemic

- **Rituximab**
  - A monoclonal anti-CD 20 antibody
  - Upto 50% response rate
  - Eliminates B cells that produce the antibody
  - Most patients have relapse, and many of these patients respond when given again.

- **Thrombopoietin (TPO) agonists (Romiplostim/Eltrombopag)**
  - Stimulates platelet production
  - Romiplostim is a thrombopoiesis stimulating peptide. It increases and maintains platelet counts in refractory ITP (approved)
  - Eltrombopag is a TPO mimetic (approved for ITP)
  - TPO agonists are the only platelet producers. All other treatments of ITP reduce platelet destruction

Some studies have suggested that Helicobacter pylori eradication may improve platelet count in ITP. Pregnancy can cause some mild thrombocytopenia called gestational thrombocytopenia. The count is never less than 70,000.

ITP causes thrombocytopenia with bleeding; TTP and HIT cause thrombocytopenia with clotting.

**Thrombotic Microangiopathies**

- **TTP (systemic)**
- **HUS (renal)**
- Chemotherapy/immunosuppression/transplant induced
- Malignancy related
- Pregnancy associated – Eclampsia/HELLP
- **DIC**

**TTP:**
Classic pentad for diagnosis of thrombotic thrombocytopenic purpura (TTP)

1. **Microangiopathic hemolytic anemia** (MAHA)
2. **Thrombocytopenia**
3. **Neurological symptoms**
4. **Fever**
5. **Renal dysfunction**

**Diagnosis**

- Clinical symptoms and signs in association with **thrombocytopenia**
- Microangiopathic hemolytic anemia in the peripheral smear showing **schistocytes**
- **Normal PT and PTT**
- **Elevated LDH**
Thrombocytopenia is associated with thrombosis rather than bleeding
No other identifiable etiology
PLASMIC score (clinical) + ADAMSTS 13 activity/inhibitor is used for diagnosis.

**Evaluation:**
- Peripheral blood smear, renal function, LDH, haptoglobin, reticulocyte count, bilirubin, Coomb’s test (DAT), PT/INR, PTT, d-dimer, fibrinogen
- ADAMTS13 activity, ADAMTS13 inhibitor titer
- Complement levels, factors H and I, Antiphospholipid antibodies (LA, anticardiolipin, β2glycoprotein ab)
- Medication/drugs review, History of Cancer and its treatment

**Pathogenesis**
- Deficiency of/or antibodies to a cleaving factor of vWF called ADAMTS13
  o ADAMTS13 deficiency leads to ultra large vWF
  o Pathologic platelet adhesion and aggregation
  o ADAMTS 13 level correlates with severity, severe deficiency is associated with severe disease. TTP usually has < 10% ADAMTS 13, HUS/atypical HUS has > 10%

**Treatment**
- **Best treatment is total plasma exchange + steroid +/- Rituximab**
  o Plasmapheresis and replacement with FFP
  o Done daily until symptoms resolve, LDH normalizes, and platelet count is ≥ 150,000/mcL for at least two days
  o Retrospective study from UK TTP registry has shown that rituximab + plasma exchange quickens recovery from TTP and decreases relapse in acquired TTP
  o **Caplacizumab (a nanobody)** which inhibits platelets/VWF interaction has been shown to reduce time for platelet count response, faster resolution of TTP and decrease recurrence.
- Adjunctive measures
  o **Steroids**
  o Antiplatelet agents
  o Rituximab (as above), bortezomib, recombinant ADAMTS13
  o Immunosuppressive agents
  o Splenectomy
    - For patients who do not respond to plasma exchange with FFP
    - Plasma exchange is continued after splenectomy
    - Sometimes done during remission to reduce relapses

**Sporadic hemolytic uremic syndrome (HUS)**
- No association with E. coli; due to deficiency of factor H
- Severe renal insufficiency is seen associated with microangiopathic hemolytic anemia. Factor H deficiency leads to immune complex mediated injury to glomeruli, and activation of platelets
- Neurological symptoms are less frequent
Prognosis is relatively poor

Treatment
- Plasma exchange, but these patients do not respond as well as TTP patients
- Eculizumab is now approved for treatment of atypical HUS.

**Verotoxin associated thrombotic microangiopathy (HUS)**
- Most commonly occurs in children younger than 5-years-old
- **Develops after infection with a verotoxin (shiga toxin) producing E. coli O157:H7 or Shigella infection**
- Outbreaks have been associated with undercooked ground beef
  - Bagged lettuce/ spinach contaminated with this E. coli has led to outbreak
- Usually preceded by gastroenteritis with abdominal pain and watery diarrhea then bloody diarrhea
- Patients have oliguria, microangiopathic hemolytic anemia, and thrombocytopenia
- 30 to 50% of the patients have neurological manifestation
- Stool culture is usually positive for E. coli
- 2/3rd of patients require temporary dialysis
- **Treatment:** Supportive (transfusion, fluid and electrolyte balance, dialysis, treatment of HTN/seizures, eculizumab is used if neurological symptoms occur) + plasma exchange

Cyclosporin and tacrolimus can cause thrombotic microangiopathy. Mitomycin C, cisplatin, cyclophosphamide, gemcitabine, and fludarabine have been associated with precipitation of thrombotic microangiopathy. Bevacizumab (Avastin) is an anti-angiogenesis monoclonal ab associated with HUS like syndrome, by inactivating VEGF (vascular endothelial cell growth factor) produced by renal epithelium. Quinine associated thrombotic microangiopathy is like sporadic HUS. These patients have good prognosis once quinine is discontinued (quinine is a component of tonic water, thrombocytopenia in a patient who drinks large amounts of gin and tonic). HIV associated TTP is like idiopathic TTP and treated with plasma exchange. Systemic vasculitis can also be associated with microangiopathic hemolytic anemia. Pregnant women have a higher incidence of TTP and HUS.

*Pregnancy and thrombotic microangiopathy*
- Preeclampsia (see nephrology)

**HELLP syndrome**
- Criteria
  - Microangiopathic Hemolysis
    - Schistocytes in the peripheral smear
    - Serum bilirubin > 1.2 mg/dL
    - Serum LDH > 600; AST > 70 U/L
  - Elevated Liver enzymes
    - AST > 70 units/L.
  - Low Platelets *(less than 100,000)*
It usually occurs between 27 and 36 weeks of gestation. 10% of the patients: < 27 weeks, 30 to 40%: at term or immediately postpartum. Patients have nausea, fatigue, epigastric, or right upper quadrant pain in association with microangiopathic hemolytic anemia and thrombocytopenia. HELLP syndrome in a patient with more than 34 weeks gestation should immediately be treated with delivery. Neonatal mortalities are up to 10 to 20% due to placental abruption, hypoxia, or prematurity. Steroids and plasma exchange may be needed for recovery postpartum. Several days after delivery is required for complete resolution of HELLP syndrome.

Acute fatty liver of pregnancy
- Usually occurs in the third trimester
- Present with nausea, vomiting, malaise, and right upper quadrant pain, in association with dyspnea and mental status changes

- Laboratory
  - Hyperbilirubinemia
  - Liver enzyme elevation indicative of cholestasis
  - Coagulopathy
    - Antithrombin III levels are markedly reduced
- Treatment is medical stabilization with urgent delivery

As discussed earlier, HUS and TTP occur more frequently during pregnancy. HUS most often occurs postpartum. Treatment is similar to non-pregnant women. Urgent delivery is not indicated.

Causes of thrombotic microangiopathy or microangiopathic hemolytic anemia (≥ 2 schistocytes/100xHPF): TTP, HUS, drug induced thrombotic microangiopathy, malignant HTN, scleroderma crisis, post stem cell transplant, DIC, catastrophic APL ab syndrome, preeclampsia, malignancy

Heparin-induced Thrombocytopenia
- All patients treated with heparin should have platelet counts monitored carefully

**Type I or Heparin associated thrombocytopenia (HAT)**
- Nonimmune mediated
- Occurs soon after initiation of heparin
- Thrombocytopenia is not severe
- Due to direct stimulation of platelet aggregation by heparin
- This is self-limited even with continued heparin use

**Type II**
- Immune mediated
- Thrombocytopenia is not severe, and it is associated with thrombosis, not bleeding
- Occurs 5 to 15 days after heparin exposure.
If a patient is pre-sensitized with heparin, it can occur as rapid onset HIT, within 24 hours, due to presence of preformed heparin: PF4 antibodies due to heparin exposure 30-100 days prior. Delayed onset HIT occurs mostly in cardiac patients, and refers to HIT occurring 10-40 days after stopping heparin.

Diagnosis:
- ≥ 50% decrease in platelet counts from the baseline even in the absence of absolute thrombocytopenia is diagnostic
- New thrombotic events, skin necrosis at site of heparin injection, and acute systemic reaction after IV heparin bolus are sequelae that can occur and support diagnosis
- The diagnosis is clinical. Laboratory tests can confirm the diagnosis
- Points are given according to 4Ts to diagnose HIT: Thrombocytopenia, Timing of thrombocytopenia, Thrombosis or other sequelae (skin necrosis/acute systemic reaction to IV bolus of heparin), Thrombocytopenia – presence or absence of other causes. Another score used in the diagnosis of HIT is HIT expert probability score.

Highest risk of HIT is among female, surgical patients given UFH. The risk of HIT is about 5% in patients receiving heparin. HIT is 5 – 10 times more common with UFH than LMWH.

Pathogeneses
- Antibodies are directed against heparin - platelet factor 4 complex
  - Binding of the immune complex (containing heparin bound platelet factor 4 and anti-heparin platelet factor 4 antibodies to platelet Fc-gamma receptors) induces platelet activation
  - This leads to release of procoagulant platelet microparticles
  - Concurrent binding of HIT-IgG to platelet factor 4 bound to endothelial cell heparin sulfate
  - Endothelial cell activation and tissue factor expression

Altogether, this leads to a hypercoagulable state. Thrombosis develops in 30% of the patients. Presence of antibodies without clinical features in not HIT. Venous thrombi, pulmonary embolism, and arterial thrombi can all occur leading to amputation, stroke, or death.

Laboratory conformation
- ELISA detects HIT IgG by measuring heparin-PF4 complexes
  - Very sensitive, specificity is lower
- Serotonin release assay (SRA) is a functional assay

A combination of clinical, heparin – PF4 ELISA, (+/- SRA) is used for definite diagnosis.
Treatment
- Heparin/LMWH must be stopped after clinical diagnosis and blood sent for heparin antibody
- Alternative anticoagulants must be started even in the absence of thrombosis. Only for patients who are at high risk of bleeding without any thrombosis, observation is an option.
  o Direct thrombin inhibitors are the alternative anticoagulants –Argatroban (FDA approved for HIT). Danaparoid is also FDA approved.
    ▪ Direct thrombin inhibitors prolong PTT (should be kept 1.5 to 2.5 times the control)
      • Argatroban is contraindicated in patients with liver disease
      • Other anticoagulants that can be used are danaparoid (FDA approved for HIT), fondaparinux, and bivalirudin (FDA approved for patients with HIT undergoing PCI). These agents are suggested per ACCP guidelines.
      • For pregnant women with HIT, ACCP guidelines recommend danaparoid. Lepirudin or fondaparinux is recommended only if danaparoid is not available.
      • The newer oral anticoagulants are emerging as management of HIT
  o Warfarin
    ▪ Not started until platelets > 150,000 and stable
    ▪ Start only 5 mg
    ▪ Continue the non-heparin anticoagulant until platelet count has reached a stable plateau, INR has reached target range, and minimum 5 days overlap has occurred between warfarin and non-heparin anticoagulant.
    ▪ In patients receiving warfarin at the time HIT is diagnosed, warfarin should be stopped, and vitamin K should be given to reverse its effect to correct protein C deficiency

For patients with confirmed or strongly suspected HIT, whether or not there is clinical evidence of thrombosis, a routine Doppler US of LE should be done.
In patients with thrombosis, warfarin is given for 3 – 6 months. In patients without thrombosis, warfarin is given for 1-3 months (3 months per ACP annual meeting expert lecture) after initial thrombin inhibitors.
Patients with history of HIT, who are HIT antibody negative and require cardiac surgery, can be given IV heparin during cardiac surgery.
In patients with acute or subacute HIT, ACCP guidelines recommend use of argatroban or danaparoid during renal replacement therapy.
For use during hemodialysis and for catheter locking, ACCP recommends regional citrate rather than heparin or LMWH for patients with history of HIT.
Avoiding unfractionated heparin (except for dialysis, cardiac surgery and some patients with ACS) has been shown to decrease the incidence of HIT.
Acquired Bleeding Disorders

Liver disease
- Liver synthesizes all the haemostatic proteins except von Willebrand factor and tPA
- Factor VIII is produced in liver sinusoidal cells and endothelial cells outside liver
- Liver clears most of the procoagulant and anticoagulant proteins
- Liver failure leads to bleeding due to decreased production of procoagulants and increased fibrinolysis
- Platelet count and function may also be affected
- Dysfibrinogenemia is common and DIC can develop in chronic liver disease
- PT, PTT and TT are prolonged depending on degree of liver damage.
- Thrombocytopenia and increased fibrin degradation products (FDP) can occur

Factor VII has the shortest half-life of 6 hours and the liver fails to maintain normal levels. More severe dysfunction causes all clotting factors to decrease and both PT and PTT can increase. Fibrinogen is decreased in fulminant hepatic failure or advanced liver disease. Fibrinogen is also decreased in associated DIC.

Prolonged TT, normal fibrinogen and FDP level – dysfibrinogenemia
Factor VIII level is normal or elevated in liver failure. Low factor VIII – DIC
Factor V is synthesized in liver and is NOT vitamin K dependent. Therefore, low Factor V level indicates liver failure. Normal factor V and low Factor VII – vitamin K deficiency

Treatment
- Vitamin K may be given to improve hemostasis
- FFP is necessary to correct the coagulation defect in a patient with liver disease with bleeding manifestation (or) prior to surgery
- Platelets are given if count is < 10 – 20,000 or to control bleed or prior to surgery if count < 50,000

Vitamin K deficiency
- Factor II, VII, IX, X, protein C, and S are vitamin K dependent factors
- Vitamin K is required for the gamma carboxylation
- Obtained from dark green vegetables
- Deficiency can be due to low dietary intake
  - Interruption of bile flow prevents its absorption because it is a fat-soluble vitamin
  - Antibiotic therapy can decrease the intestinal bacteria and reduce the intestinal source of vitamin K
  - Warfarin administration antagonizes vitamin K

Vitamin K deficiency initially affects factor VII and elevates the protime. As other factors decline, both protime and partial thromboplastin time can increase.
Antibodies to clotting factors

Acquired inhibitors are immune mediated antibodies to clotting factors. **Factor VIII antibody is most common.** Acquired inhibitors are usually seen after age 60. These can be primary or associated with diseases like lymphoma, autoimmune disease and pregnancy. Patients present with bleeding, and elevated PTT. Mixing study does not correct the PTT after 2 hours incubation. Patients with low titer antibody can be treated with factor VIII concentrate. High titer antibody requires treatment with recombinant human Factor VIIa concentrate or prothrombin complex, since these can activate factor X independent of factor VIII. Rituximab and steroids have been used to suppress antibody production.

Antibodies to factor II and V can be induced by exposure to fibrin sealants such and fibrin glue used in surgery. Factor II autoantibody can be present in antiphospholipid syndrome. The clue is elevation of both protime and partial thromboplastin time in patients with lupus anticoagulant.

**DIC**
- DIC can be seen in:
  - Sepsis
  - Trauma, burns, rhabdomyolysis
  - Malignancy (*solid tumors*)
  - Fulminant hepatic failure/advanced cirrhosis
  - Promyelocytic leukemia
  - Abruptio placentae, amniotic fluid embolism, dead fetus syndrome
  - Drugs: aprotinin, warfarin, amphetamine
  - Vascular abnormalities - Aortic aneurysm, Giant hemangioma
  - Toxins and venoms
  - Shock, ARDS, massive transfusion
  - Acute hemolytic transfusion reaction, GVHD, transplant rejection

Damaged tissues release tissue factor and along with factor VIIa, thrombin is generated. Antithrombin removes the thrombin initially. Once antithrombin is consumed, thrombin is unopposed. Protein C anticoagulant system is suppressed due to cytokine induced down regulation of thrombomodulin. Therefore, the initial phase of DIC is prothrombotic. There is extensive intravascular fibrin formation due to increased production and decreased clearance of thrombin.

Thrombosis of end-organ vessels leads to ischemia and organ hypoxia. As DIC progresses, bleeding occurs. Platelets and fibrinogen are consumed. Factors V and VIII are degraded. Fibrin stimulates tPA and there is fibrinolysis. Thrombi are lysed and hemorrhage occurs.

The phase that is clinically apparent is the bleeding phase. Diagnosis is suspected only at this bleeding phase. Initial ischemic phase is usually clinically silent. Antithrombin levels
decline early in DIC and this may identify patients in the thrombotic phase, but anti-thrombin III assay turn around time is too slow to be clinically useful. Laboratory tests for DIC include PT, PTT, TT, and D-dimer levels. Platelet count, and analysis of peripheral smear is also done. Serial testing may be necessary to confirm diagnosis. **Fibrinogen is an acute phase reactant and has a long half-life, levels may be misleadingly normal in early DIC.**

**PT/PTT: prolonged**  
**Platelet count:** < 100,000 or rapidly declining  
**Smear: Schistocytes**  
**Elevated FDP level (D-dimer) – Most sensitive test for DIC**

**Treatment**  
The most important part is treating the underlying condition and replacement therapy (FFP, cryoprecipitate, platelets).

*Workup of isolated elevation of PT or PTT*  
- Once a patient has been found to have prolonged PT and/or PTT, mixing study is done  
- Correction of the prolonged PT and PTT by mixing study indicates factor deficiency  
  - If the normal plasma does not correct the prolonged PT and PTT, then specific or nonspecific inhibitors are present (or) there is lupus anticoagulant.  
- A patient with factor inhibitor has clinically a bleeding disorder and a patient with lupus anticoagulant, clinically has a clotting disorder.

Mixing study: Corrects PT, or PTT. Clinical: bleeding => Factor deficiency  
Mixing study: Does not correct PT, PTT. Clinical: bleeding => Factor inhibitor  
Mixing study: Does not correct PT, PTT. Clinical: clotting => Lupus anticoagulant
Hypercoagulable states:

When to perform a hypercoagulable workup?

- Unprovoked VTE < age 50, with positive family history in 1st degree relatives: If planning to stop anticoagulation treatment, consider testing for hereditary thrombophilia.
- Consider testing for antiphospholipid antibodies in unprovoked VTE if planning to stop anticoagulation
- Cerebral venous thrombosis
- Hepatic/portal/mesenteric vein thrombosis (here rule out myeloproliferative syndromes and PNH)
- Second and third trimester pregnancy loss
- Neonatal purpura fulminans

It is also reasonable to do hypercoagulable work-up in patients with VTE associated with OCP, HRT, and pregnancy. Thrombophilia testing may be useful in women with family history of inherited thrombophilias/VTE prior to pregnancy or estrogen use. Women with FH of VTE have an increased risk of estrogen associated VTE.

ACCP guidelines did not use presence of a hereditary hypercoagulable state as a major factor to guide duration of anticoagulation.

**Thrombophilia testing should not be done in provoked VTE**

**Inherited thrombophilias:**

**Major disorders:**
- Factor V Leiden
- Prothrombin 20210A gene mutation
- Protein C deficiency
- Protein S deficiency
- Antithrombin III deficiency

**Minor disorders:**
- MTHFR mutation (there is high homocysteine level)
- Elevated Factor VIII
- Dysfibrinogenemias (bleeding and thrombosis can occur)
- Elevated vWF
- PAI-1 gene 4G/5G polymorphism

**Acquired thrombophilias:**
- Malignancies
- Antiphospholipid antibody syndrome (both arterial and venous thrombosis)
- HIT
- Nephrotic syndrome, liver disease
- Autoimmune disorders (SLE, IBD)
- Increased estrogen (OCP, tamoxifen, HRT, pregnancy)
- Myeloproliferative disorders and PNH (splanchnic thrombosis/VTE/MI/CVA)
- Endocrinopathies (DM, Cushing’s)

**Factor V Leiden:**
- Most common inherited thrombophilia (5% Caucasians, < 1% Blacks/Asians)
- Clinical manifestations: VTE, DVT > PE, Cerebral vein thrombosis and superficial vein thrombosis. Probable relation to increased risk of MI.
- VTE risk: There is a strong association with estrogen. Heterozygous: 4-8 x normal, homozygous 80 x normal
- Testing: Molecular testing for factor V leiden

**Prothrombin gene G 20210A mutation:**
- Second most common inherited thrombophilia (1-5% American/European Jews, ≤ 0.2% Blacks/Asians)
- Clinical manifestations: VTE, cerebral vein thrombosis, stroke in young patients
- VTE risk: Heterozygous: 2-4 x normal, homozygous: variable
- Testing: Molecular testing for G 20210A mutation

**Antithrombin III deficiency:**
- Rare disorder, 0.2 – 0.4% of all patients
- Clinical manifestations: VTE, heparin resistance
- VTE risk: High risk
- Testing: Antithrombin III antigen and activity

**Protein C deficiency:**
- Rare disorder, 0.2 – 0.5% of all patients
- Clinical manifestation: VTE, neonatal purpura fulminans, warfarin skin necrosis
- VTE risk: 7 x normal
- Testing: Protein C antigen and activity

**Protein S deficiency:**
- Rare disorder, 0.1% of all patients
- Clinical manifestation: VTE, levels decrease during pregnancy
- VTE risk: 7 x normal
- Testing: Protein S total and free antigen, protein S activity

The 5 inherited thrombophilias are low in prevalence in the general population and among patients with VTE. Testing of antigen levels is affected during acute VTE and anticoagulation, but mutation testing will not be affected.

**Risk of recurrent VTE is low in patients with provoked VTE and thrombophilia testing should not be performed in provoked VTE.**

**Thrombophilia testing in unprovoked VTE:** There are no clear cut consensus recommendations about the role of thrombophilia testing in unprovoked VTE, and
thrombophilia testing usually does not influence duration of anticoagulation in most patients with unprovoked VTE. Exceptions: Compound heterozygosity for 2 thrombophilias, Antithrombin III deficiency, homozygous factor V Leiden, and antiphospholipid antibody syndrome. Clinical risk assessment tools such as d-dimer, DASH score, and Vienna prediction model and risk/benefit assessment influences anticoagulation duration more than presence or absence of thrombophilies.

Malignancy associated thrombosis
- Underlying malignancy can be detected in up to 14% of patients who present with idiopathic venous thrombosis
- Occult neoplasm may become clinically evident within six to twelve months of DVT
- In a patient with idiopathic venous thrombosis, one should make sure age appropriate cancer screening is done and it is important to work-up signs and symptoms that may suggest a malignancy.
- Recombinant erythropoeitin and darbopoeitin given to treat chemotherapy associated anemia in cancer patients have been found to increase VTE and mortality

Anticoagulants

Anticoagulant that activate antithrombin:

1. Unfractionated heparin: IV
   It increases antithrombin’s ability to inactivate thrombin and factor Xa. It is reversible with protamine sulfate. aPTT is used for monitoring.

Anticoagulants that inactivate Factor Xa

1. Low Molecular Weight Heparin: SC
   LMWH is prepared from heparin by depolymerization chemically or enzymatically. It inactivates factor Xa more than antithrombin activation. Factor Xa level is used for Monitoring in special circumstances (morbid obesity, renal dysfunction, pregnancy)

2. Fondaparinux: SC
   Fondaparinux is a pentasaccharide which is similar to the antithrombin binding site of heparin. It only inhibits factor Xa. Factor Xa is used for monitoring. It cannot be reversed with protamine sulfate. Approved for prophylaxis of VTE (hip/knee replacements, hip fracture, other surgical/medical conditions) and treatment of acute DVT and PE.

Vitamin K antagonists:

Warfarin: Vitamin K dependent coagulation factors II, VII, IX, X and anticoagulation factors protein C and S need gamma corboxylation by vitamin K for their function. Warfarin blocks this step. Response to warfarin can vary by genotype (CYP2C9 and VKORC1). Warfarin can be reversed with vitamin K or prothrombin concentrates.
**Direct thrombin inhibitors:**

1. Argatroban: It is a direct thrombin inhibitor. It is cleared by the liver. Given IV and Monitored by aPTT. It cannot be reversed. Used for HIT.

2. Bivalirudin: It is a direct thrombin inhibitor that is inactivated by thrombin. Used for PTCA and HIT with PTCA, administered IV.

   It is FDA approved for: Non-valvular atrial fibrillation at 150 mg BID (renal dose adjustment needed), treatment of DVT and PE after 5-10 days of parenteral anticoagulant, prevention of recurrent DVT and PE, and for prevention of DVT and PE after hip replacement. If bleeding occurs, stop drug, give activated charcoal if within 2 hours, consider HD (dialyzable), consider Factor VIIa/prothrombin complex. **IDARUCIZUMAB** is a monoclonal antibody now approved, which reverses the anticoagulant effect of dabigatran in patients with serious bleeding or patients who need emergency surgery or intervention.

**Oral Factor Xa inhibitors:**

1. Rivaroxaban: Direct factor Xa inhibitor. Orally administered.
   Rivaroxaban is FDA approved for: Reduction of stroke and systemic embolization in non-valvular A. fibrillation, treatment of DVT and PE, reducing risk of recurrence of DVT/PE, prevention of DVT/PE in patients undergoing hip and knee replacement surgery, and in combination with aspirin to reduce the risk of major cardiovascular events (CV deaths, MI, stroke) in patients with CAD or PAD. Clearance: 66% renal and 34% hepatic.
   Its level is affected by P-glycoprotein and CYP3A4 inhibitors/inducers.
   Drugs that potentiate: Itraconazole, PIs, erythromycin, quinidine, amiodarone, CCB
   Drugs that inhibit: Carbamezepine, phenytoin, phenobarb, rifampin, St. John’s wort
   Dose: A.fib: 20 mg daily (15 mg daily if Cr cl 15-50), VTE treatment: 15 mg q12 Hours for 21 days, then 20 mg daily, VTE prophylaxis (THA/TKA) 10 mg daily. Reduction of major CV events: 2.5 mg BID + aspirin 75-100 mg/day. It should be avoided in moderate and severe liver impairment.

   It is approved for non-valvular atrial fibrillation, treatment and prevention of DVT/PE and VTE prophylaxis in THA/TKA.
   Dose: A.fib: 5 mg q12 hours, 2.5 mg q12 hours if any 2 are present: Age ≥ 80, Weight ≤ 60 kg, creatinine 1.5-2.5 mg/dL.
   THA/TKA: 2.5 mg q 12 hours.
Treatment of DVT/PE: 10 mg BID for 7 days, 5 mg BID for 6 months
Prevention of DVT/PE after initial treatment: 2.5 mg BID
It is contraindicated in severe liver disease. Limited experience only in moderate liver disease. Clearance: renal 25%, Liver 75%. It also has drug-drug interactions like rivaroxaban.

3. Edoxaban: Oral direct factor Xa inhibitor

Approved for reduction of stroke and systemic embolism in non-valvular A.fibrillation and for the treatment of DVT/PE after 5-10 days of parenteral anticoagulation. Dose 60 mg daily. Edoxaban is not recommended in patients with creatinine clearance > 95 ml/min, and for creatinine clearance < 50 ml/min, dose is 30 mg/day. Do not give for Cr Clearance < 15 ml/min

4. Betrixaban: Oral direct factor Xa inhibitor

Approved for VTE prophylaxis for adult inpatients at risk of VTE. Starting dose of 160 mg and then 80 mg daily. Avoid ion liver impairment and dose reduce in renal impairment.

For the newer anticoagulants, always check for drug interactions, assess renal function and liver function.

New Factor Xa inhibitor reversal agent for rivaroxaban and apixaban:

Andexanet: A factor Xa decoy that has high affinity for factor xa inhibitors – approved for reversal of rivaroxaban and apixaban. Adverse events include arterial and venous thromboembolic events, including MI, stroke, cardiac arrest and sudden deaths. It should be used only for life threatening or uncontrolled bleeding. Most common adverse events were UTI and pneumonia, and infusion related reactions.

Management of oral anticoagulant related bleeding (dabigatran, rivaroxaban, apixaban):

Determine time of last dose, renal function, CBC. Stat coagulation assessment – Dabigatran: dilute thrombin time or ecarin clotting time (aPTT is alternate option) FXa inhibitors: Anti-Factor Xa assay, (PT is alternate option). If bleeding is mild: Delay or discontinue next dose and consider choice of oral anticoagulant and dosing. If bleeding is major but not life threatening: Supportive measures (compression, endoscopic hemostasis for GI bleed, surgical hemostasis, fluid and PRBC/platelet transfusion if applicable, consider tranexamic acid. For dabigatran: Consider idarucizumab or hemodialysis if idarucizumab unavailable. Life threatening bleed: Dabigatran: Idarucizumab. FXa inhibitors: Andexanet. If not available, consider prothrombin complex concentrate.
For urgent procedures to be done < 24 hours of newer oral anticoagulants: Assess coagulation (as above). If coagulation test is normal, no reversal agent is needed. If coagulation tests are abnormal, then for dabigatran: Idarucizumab. For factor Xa inhibitors: prothrombin complex concentrate, andexanet has not been studied in non-bleeding patients.

**Myeloproliferative disorders**

Myeloproliferative disorders are clonal stem cell disorders. They overproduce one or more formed elements of the blood without significant dysplasia, and have predilection to extramedullary hematopoiesis, myelofibrosis and acute leukemia.

WHO Classification of chronic Myeloproliferative neoplasms:

- **Chronic myelogenous leukemia** (Ph chromosome or bcr-abl positive)
- Chronic neutrophilic leukemia t(15:19)
- Chronic eosinophilic leukemia, NOS (deletion or translocation involving PDGFRα gene)
- **Polycythemia vera** (JAK2 mutation V617F)
- **Primary myelofibrosis** (with extramedullary hematopoiesis) (JAK2 mutation)
- **Essential thrombocytosis** (JAK2 mutation)
- Mastocytosis
- Chronic myeloproliferative disease, unclassified

**Chronic myelogenous leukemia (CML)**

- Clonal expansion of hematopoietic stem cells with a reciprocal translocation between Chromosomes 9 and 22
  - **Fusion of the BCR gene on chromosome 22q11 with an ABL gene located in chromosome 9q34 (Philadelphia chromosome)**
  - BCR-ABL gene codes for a 210-kilodalton protein which functions as tyrosine kinase that activates other kinases that prevent apoptosis

**Chronic phase**

- Neutrophilia of mostly mature granulocytes
- Eosinophilia
- Basophilia
- Splenomegaly

CML is the only disorder associated with a low leukocyte alkaline phosphatase level (LAP score), in the presence of leukocytosis

Chronic phase patients are usually asymptomatic or may have symptoms of fatigue, malaise, weight loss, and symptoms of splenomegaly, which include early satiety and left upper quadrant pain.

**Accelerated phase**

- Increasing constitutional symptoms
- Worsening Splenomegaly
- Elevation in the leukocyte count with a left shift and blast forms
- Increasing anemia
- Increasing blood or marrow blasts of 10 to 20%
- Blood or marrow basophils increased to more than 20%
- Platelet counts fall

Blast crisis is acute leukemia derived from any lineage (AML or ALL).

CML progresses on an average of three to four years. Occasionally, patients present in blast crisis, without a previous diagnosis of chronic phase. These patients may be the Philadelphia positive AML patients.

Peripheral smear in chronic phase:

Increased WBC count with various degrees of immaturity of the granulocytic series. **Basophilia is characteristic.** Platelets are usually high at diagnosis.

**Diagnosis is by demonstration of BCR-ABL by cytogenetic analysis or molecular techniques.**

Prognosis is assessed by using the Sokol Index or Hasford system. Sokol index is based on spleen size, platelet count, age, and cytogenetic clonal evolution. Hasford is based on spleen size, age, platelet count, and circulating blasts, and percentage of eosinophils and basophils.

**Treatment**

The proven curative treatment, allogeneic transplantation has significant toxicity. Imatinib has excellent 5-year outcome. **Imatinib mesylate is the first line therapy in chronic phase CML,** with stem cell transplant reserved for accelerated phase, blast phase or resistance to imatinib.

- **Stem cell transplant:**
  Only cure is allogeneic stem cell transplant following high dose chemo and radiation therapy. Since the advent of Imatinib, allogeneic stem cell transplant is done for high-risk patients or accelerated/blastic phase or failure of Imatinib or progression on Imatinib.

- **Imatinib mesylate**
  - An inhibitor of BCR-ABL tyrosine kinase. Hematological remission rate: 98%, Complete cytogenetic remission rate: 76%
  - Progression free survival at 5 years in patients who achieve complete cytogenetic remission on Imatinib is 98%
  - Imatinib is given orally. It can cause fluid retention, diarrhea, nausea, muscle cramps, myelosuppression and skin rash.
  - Some patients develop re-emergence of clone with resistance to Imatinib mesylate due to mutations or amplification of BCR-ABL
New targeted drugs are available to treat these patients, who are resistant to Imatinib as alternate to SCT – Dasatinib and Nilotinib are approved for imatinib resistant CML. Both nilotinib and dasatinib have been found to be superior to imatinib as initial agents for treatment of chronic phase CML in phase III trials. These agents are now approved for first line therapy in newly diagnosed CML. Ponatinib is a new tyrosine kinase inhibitor, that has been shown to be effective against mutated bcr-abl, that is resistant to all other tyrosine kinase inhibitors in phase I trial.

**Polycythemia Vera (PV):** Clonal stem cell disorder with unregulated production of RBCs, WBCs and platelets that are morphologically normal.

**WHO criteria (3 major + 1 minor or first 2 majors + the 1 minor)**

1. **Hemoglobin** > 16.5 g/dL in men, > 16.0 g/dL in women or hematocrit > 49% in men and > 48% in women or other evidence of increased RBC volume

2. **Bone marrow biopsy showing hypercellularity with trilinear growth (erythroid, granulocytic and megakaryocytic proliferation) with pleomorphic mature megakaryocytes**

3. **JAK2V617 or JAK2exon 12**

**Minor Criteria:** low erythropoietin level below ref range

Normal red cell mass + decreased plasma volume: Diuretics, androgens, smoking, pheochromocytoma

O2 saturation < 93%: Hypoxic erythrocytosis.

Erythrocytosis with high erythropoietin level: renal disease, tumors (cerebellar hemangioblastoma, renal cell carcinoma, hepatocellular carcinoma, pheochromocytoma, uterine leiomyoma), high affinity hemoglobin, VHL mutation

Aquagenic pruritus is a symptom seen in PV, but not in secondary erythrocytosis

Erythrocytosis: First step, repeat CBC in 4 weeks. If persistently elevated, measure serum erythropoietin level. If Epo level is low, check JAK2V617F mutation or JAK2exon 12 mutation.

The JAK2 mutation can also be found in patients with essential thrombocytosis and myelofibrosis but the most common association is with PV (97%).

**Clinical features/complications of PV**

- **Neurological:**
  - Vertigo/tinnitus
  - TIA (due to thrombocytosis)
  - Headache, ocular migraine (due to thrombocytosis)
  - Visual disturbance
- Organomegaly, pulmonary hypertension (due to extramedullary hematopoiesis and elevated RBC mass)
- Thrombosis, hypertension and hemorrhage (due to high RBC mass and decreased vWF multimers)
- Hyperuricemia, gout, uric acid stones (due to high cell turnover)
- Erythromelalgia (due to thrombocytosis)
- Pruritus, PUD (due to inflammatory cytokines)
- Myelofibrosis
- Acute leukemia (due to treatment or clonal evolution)

Natural history of PV: Asymptomatic→erythrocytosis→quiescent or spent phase→Postpolycythemic myeloid metaplasia with myelofibrosis→acute leukemia

**Gaisbock syndrome**
- Syndrome of relative erythrocytosis due to an inherently reduced plasma volume

Surreptitious use of erythropoietin or testosterone by athletes and muscle builders can lead to polycythemia.

Peripheral smear of PV shows microcytic erythrocytosis. Other differential diagnosis for microcytic erythrocytosis include beta-thalassemia trait and hypoxic erythrocytosis.

10% to 20% of the patients ultimately show signs of marrow fibrosis, a spent phase like myelofibrosis. Leukemic transformation is rare with a risk of only 1% to 2%.

**Management**

Phlebotomy, aspirin 81 mg/day + hydroxyurea for patients at risk for thrombosis. Splenectomy is done for patients with painful splenomegaly or recurrent infarctions of spleen. Currently **Ruxolitinib, a JAK 2 inhibitor is approved for patients who have low response or intolerance to hydroxyurea**. Ruxolitinib reduced spleen size and decreased need for splenectomy. **Ruxolitinib abrupt withdrawal can resemble septic shock due to cytokine storm, don’t hold it without Hematology consult.**

**Essential thrombocytosis (ET)**
- **The most common myeloproliferative disorder**
- Thrombocytosis in the absence of reactive thrombocytosis (due to iron deficiency, underlying inflammation/infection, malignancy, splenectomy, rebound after correction of vitamin B12/folate deficiency and post ethanol abuse, surgery, hemorrhage, hemolysis, familial etc.)
- **Splenomegaly is seen in less than 50% of the patients unlike other myeloproliferative disorders**
- **WHO Criteria for diagnosis:** (All 4 major criteria or First 3 major + the minor)
  Major Criteria:
  1. Platelet count ≥ 450,000/mcL
2. Bone marrow biopsy: Megakaryocytic proliferation with large mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift of granulopoiesis or erythropoiesis and very rarely an increase in reticulin fibers

3. Not meeting WHO criteria for bcr-abl CML, Polycythemia vera, primary myelofibrosis, MDS, or other myeloid neoplasms

4. Positive JAK2, CALR, or MPL mutation

Minor Criterion: Presence of a clonal marker or absence of reactive thrombocytosis

Complications:
- Thrombosis: Stroke, ACS, PAD, digital gangrene, DVT
- Microvascular ischemia: Migraine, erythromelalgia, TIA
- Hemorrhage due to acquired von Willebrand disease
- Acute leukemic transformation

Laboratory
- Peripheral smear
  - Thrombocytosis with giant platelets and megakaryocytic fragments
- Bone marrow
  - Remarkable proliferation of atypical megakaryocytes and varying degrees of marrow fibrosis
- Cytogenetic evaluation
  - Normal
  - Usually done to rule out CML and 5q- syndrome
  - JAK2 V617F mutation may be present (50%)
- Diagnostic criteria: WHO 2008
  - Persistent Platelet count > 450,000/mcL
  - Bone Marrow: Increased number of enlarged matured megakaryocytes and no significant increase in granulopoiesis or erythropoiesis
  - Not meeting WHO criteria for PV, CML, MDS, PMF, or other myeloid neoplasm
  - JAK 2, CALR or MPL mutation
  - Another clonal marker or no reactive cause of thrombocytosis (minor criterion)

If massive splenomegaly is present, then consider another myeloproliferative disorder as this is not typical for ET.

There is a bimodal age distribution for ET, it occurs in women in the ages of 20s and 30s. It can also occur in the patient’s over the age of 60. Young patients with ET have prolonged asymptomatic survival. The risk of stroke, ischemic event, and miscarriage is increased.

High risk ET: H/O thrombosis (any age) and/or age > 60 with JAK 2 V617F mutation
Intermediate risk ET: Age > 60, No JAK 2 mutation, no h/o thrombosis
Low risk ET: Age ≤ 60 with JAK 2 mutation and no h/o thrombosis
Very low risk ET: Age ≤ 60, no JAK 2 mutation, no h/o thrombosis

**Treatment**

- Asymptomatic young patients without history of thrombosis and with a platelets < 1.5 million are observed without treatment
- Vasoocclusive symptoms due to platelet aggregates such as migraine headache, burning and painful dysesthesias, ischemic changes of the hands or feet and erythromelalgia respond to aspirin (check for ristocetin cofactor activity prior to aspirin, patients may have acquired vWD, if acquired vWD present, then no aspirin).
- High risk ET with h/o thrombosis: Hydroxyurea + anticoagulation (venous thrombosis) and/or aspirin (arterial thrombosis). Age > 60 with JAK 2 mutation, but without thrombosis: hydroxyurea + aspirin. Goal platelet count: ≤ 400,000/microL
- Patients with life threatening symptoms like MI, stroke, limb ischemia can be treated with rapid reduction of platelet count by platelethperesis and hydroxyurea
- Acquired qualitative von Willebrand disease can coexist with ET

Agents available for specific treatment of ET include hydroxyurea, interferon and anagrelide. An RCT compared treatment with hydroxyurea + aspirin versus anagrelide + aspirin. The study found that compared to hydroxyurea and aspirin, treatment with anagrelide and aspirin was associated with increased risk of arterial thrombosis, serious hemorrhage, and transformation to myelofibrosis, but decreased rate of venous thromboembolism. Anagrelide patients also withdrew more from the study. Platelet count control was same in both groups. **Therefore, hydroxyurea with aspirin is first line in the treatment of ET, in patients > age 60 (? > age 40).** Patients < age 60 may be treated with Interferon or anagrelide unless they are contraindicated due to CVD risk factors or TIAs. The concern with hydroxyurea is secondary cancer associated with long-term treatment. In ET, before the patient undergoes elective surgery platelet count should be dropped to under 400,000. Leukemic transformation in essential thrombocytosis is very rare. Anagrelide is contraindicated in pregnancy and in patients wanting to get pregnant. Pregnant patients are treated with IFN-α.

**Primary Myelofibrosis**

- Chronic idiopathic myelofibrosis (agnogenic myeloid metaplasia) is usually seen in older individuals
- Median age of diagnosis is 60
- Marked marrow fibrosis and extra medullary hemopoiesis occur. There is **massive hepatosplenomegaly**.
- Complications: Bone marrow failure, portal and pulmonary HTN, thromboembolism.
- **Peripheral smear:** leukoerythroblastic blood picture with anemia, many teardrop cells, nucleated RBCs, and promyelocytes and myelocytes in the peripheral blood (**features of extramedullary hematopoiesis**)
- Autoimmune abnormalities can occur (RF, positive coomb’s test, ANA) and CD34 cells are remarkably increased in circulation early in the disease
- Cure is only possible with allogenic bone marrow transplant (**only done for young patients**)
WHO Criteria for diagnosis of overt PMF: (All 3 major + at least 1 minor)

Major Criteria:
1. Megakaryocytic proliferation and atypia + either reticulin &/or collagen fibrosis
2. Not meeting criteria ET, PV, bcr-abl CML, MDS or myeloid neoplasms
3. Positive JAK2, CALR, or MPL mutation or if mutations not seen, positive clonal marker or absence of reactive myelofibrosis

Minor Criteria: At least 1, confirmed x 2
1. Anemia not due to another condition
2. Leukocytosis $\geq 11,000$ /mcL
3. Palpable splenomegaly
4. Elevated LDH
5. Leucoerythroblastosis

Prognostic factors:

- Age $\geq 65$
- Anemia (hemoglobin <10 g/dL).
- Constitutional symptoms: fever, night sweats, or weight loss.
- Leukocytosis (white blood cell count $>25 \times 10^9$/L).
- Circulating blasts of at least 1%.

Prognostic scoring is based on the above factors. Median survival is 3.5-5.5 years. Patients without any of the above poor prognostic features have a median survival > 10 years. **Asymptomatic low-risk patients should be followed with a watchful waiting approach.** Development of symptomatic anemia, significant leukocytosis, night sweats, weight loss, fever, or symptomatic splenomegaly indicates need for treatment.

- **Symptomatic therapy is given for older patients:** hydroxyurea for increased counts, Erythropoietin, danazol, prednisone, thalidomide/lenalidomide (if 5q- present), or pomalidomide may be used for anemia (patients on thalidomide derivatives need prophylaxis for thrombosis) IFNα for splenomegaly/marrow fibrosis, RBC and platelet transfusion if there is anemia or thrombocytopenia may be needed. Patients can become transfusion dependent and develop secondary hemochromatosis. Massive splenomegaly with hypersplenism may need splenic radiation. Splenectomy is associated with high mortality and morbidity.

- **Ruxolitinib, an oral JAK inhibitor, reduces splenomegaly, improves survival, and relieves constitutional symptoms in primary myelofibrosis, independent of JAK mutation status, and it is approved for intermediate to high risk primary myelofibrosis.**
Transfusion medicine

Introduction
- Major blood groups (A, B, AB, and O)
  - Type A people have anti-B isoagglutinin
  - Type B people have anti-A isoagglutinin
  - Type AB individuals have no isoagglutinin (universal recipient)
  - Type O individuals have both anti-A and anti-B isoagglutinin (universal donor)

Bombay phenotype: Oh. Can only get blood from other hh donors

A and B antigens are secreted by the cells and are in circulation. Non-secretors have increased risk of various infections.

Rh system
- Rh (+) = presence of D antigen
- Rh (-) = absence of D antigen
- D antigen is a potent alloantigen
- Exposure of Rh-negative people to even small amounts of Rh-positive cells (e.g. Rh+ fetus to Rh- mother) leads to anti-D alloantibody. Rh-negative women of childbearing age who are exposed to Rh-positive RBCs should receive anti-D (Rhogam) to prevent sensitization.

Other systems of antigens include Lewis, Kell, Kidd, Duffy, I/I, P, MNSsU.

Duffy
- Important as the receptor for plasmodium vivax

Kidd
- Important in delayed hemolytic reaction

(I/I)
- Important in cold autoimmune hemolytic anemia

P
- The receptor for parvovirus B19 and E coli

Blood typing
- Forward typing determines A, B, O, and Rh phenotype of recipient’s RBC
- Reverse typing detects the isoagglutinins

Screening
- Done for identifying alloantibody
- Identifies antibodies directed against other RBC antigens
- Cross matching
  - Ordered when there is a high probability that the patient will be transfused
  - Should be non reactive
  - Reserves the blood for the patient
Blood products
- Packed RBC: Each unit increases Hb by 1g/dL in the absence of bleeding.
  - Packed RBCs are filtered to decrease the leukocyte count
    - Filtration decreases post transfusion fever, CMV infection, and alloimmunization
  - Washing of the RBCs removes plasma
    - Plasma may cause allergic reaction, including anaphylaxis

Platelets
- For invasive procedures/active bleeding, platelet count ≥ 50,000/mcL is the target
- For CNS bleeding or surgery, platelet count ≥ 100,000/mcL is target
- Prophylactic platelet transfusion is given when platelets are < 10,000/µL
- Can be from pooled donor, single donor, or HLA matched donor
- Administration is calculated as 1 unit per 10 kg body weight. Platelets are issued in pools of 6 units.
  - Each unit of platelet increases the platelet count by 10,000 per micro liter
- Patients who may need multiple platelet transfusions should received single donor transfusion
- Refractoriness to platelet transfusion is managed by giving single donor, ABO-matched or if HLA antibodies are found, HLA matched platelets.

FFP
- Has all the coagulation factors and plasma proteins
  - Fibrinogen, antithrombin, albumin, and protein C and S
- Uses:
  - Rapid reversal of warfarin (active bleeding, CNS bleeding)
  - Bleeding due to factor deficiency – DIC, liver disease
  - Treatment of TTP
  - Coagulopathy after multiple unit PRBC transfusion
- FFP is acellular
  - No risk of CMV, but TRALI, allergy, fever, anaphylaxis can occur
- In IgA deficient patient IgA deficient FFP is recommended
- Average unit of FFP raises coagulation factor levels by 5%
- Dose: 10-15 ml/Kg body weight

Cryoprecipitate
- Good source of fibrinogen, factors VIII, XIII, fibronectin, and von Willebrand factor
- Supplies fibrinogen to volume sensitive patients. Indications: Replacement for consumptive coaguloapthies and hypofibrinogenemia – DIC, massive bleeding and Obstetrical syndromes
- Also supplies von Willebrand factor to patients with type 2 or 3 von Willebrand disease
- Can be used in the place of factor VIII, if factor VIII concentrate is not available
Adverse reactions to transfusion

Acute hemolytic transfusion reaction
- Preformed antibodies lyse the donor RBCs
- Majority of the cases is due to A, B, and O incompatibility
- Patients develop hypotension, tachycardia, tachypnea, fever, chills, hemoglobinemia, hemoglobinuria, chest and/or flank pain and discomfort at the infusion site
- Once it is recognized transfusion must be immediately stopped
- Post transfusion blood sample and untransfused blood should be sent to the blood bank
- Fluids, pressor support, and mannitol are given to prevent acute renal failure
- DIC may occur
- Protime, partial thromboplastin time and platelet count must be monitored
- Almost always it is due to human error

Delayed hemolytic reaction
- Not completely preventable
- Occurs in patients previously sensitized to RBC alloantigen who have negative alloantibody screen in cross match due to low antibody level
- After transfusion, due to an anamnestic response, there is elevation in the level of alloantibody, which binds the donor RBC
- Post transfusion direct Coombs test is positive, since donor RBCs are coated with antibody or complement, and are cleared by the RES
- Kidd antigen (Jk\(a\), Jk\(b\)) is responsible (delayed appearance of anti Jk\(a\))

Febrile non-hemolytic reaction
- Chills and rigors, and more than one-degree centigrade increase in temperature is febrile non-hemolytic reaction
- Anti WBC IgG antibodies directed against donor WBCs and HLA antigens
- Premedication with acetaminophen and transfusion with leuko-poor blood can be used to avoid febrile non-hemolytic reaction

Allergic reaction
- Urticarial reaction (urticarial, itching, wheezing) following transfusion
- Due to plasma proteins
- Treatment is to hold the transfusion temporarily and administration of Benadryl
- Prevention of allergic reaction can be done by premedication with Benadryl and by washing the packed RBCs

Anaphylactoid reaction, more severe allergic reaction
- Anaphylactic reaction can occur in IgA deficient recipient receiving IgA containing blood (screen for anti IgA ab in recipients with IgA deficiency). Plasma proteins may mediate a more severe allergic reaction, particularly FFP. Acute treatment: SC epinephrine + prednisone. Prevention: Prophylactic prednisone 50 mg q6 for 24 hours before and after transfusion + 50 mg Benadryl PO and ranitidine 300 mg before transfusion.
Graft-versus-host disease (GVHD)
- Mediated by donor T lymphocytes that recognize host HLA antigen as foreign and mount an immune response
- Fever, rash, diarrhea, liver function abnormalities, marrow aplasia and pancytopenia occur
- Highly resistant to treatment and uniformly fatal
- Prevention requires gamma irradiation of cellular components

Patients at risk are:
- Lymphoma
- Fetuses
- Immunocompromised patients
- Receiving blood from a blood relative
- Post stem cell transplant

Discourage donation from family members. Patients at risk should receive irradiated blood products.

Transfusion related acute lung injury (TRALI)
- Occurs due to transfusion of plasma that has high titer anti HLA or anti neutrophil antibodies that bind recipient’s leukocytes.
- Occurs < 6 hours of plasma containing blood product, usually 1-2 hours after transfusion.
- Leucocytes aggregate in pulmonary vasculature leading to mediator release and increased capillary permeability
- End result is respiratory compromise and ARDS
- Treatment is supportive
- Donors are usually multiparous women (avoid plasma from multiparous women)
- TRALI is the most common cause of death due to transfusion (5% mortality)
- TRALI is acute lung injury or ARDS within 6 hours of transfusion. It is characterized by dyspnea, hypoxia, and bilateral patchy infiltrates on CXR. TRALI is usually associated with hypotension, hypertension if occurs is transient. O2 and mechanical ventilation +/- pressor is needed.

Transfusion associated circulatory Overload (TACO)
- TACO is the development of acute pulmonary edema with transfusion. TACO begins towards the end of transfusion or within 6 hours. Headache is a common feature. TACO presents with dyspnea, hypoxia, and tachycardia. TACO is usually associated with hypertension. Rapid improvement with diuresis.

Post transfusion purpura
- Occurs due to delayed thrombocytopenia secondary to platelet specific antibodies in the recipient
- Treatment is IVIG or plasmapheresis

Alloimmunization
- Can occur to antigens on cellular components or plasma proteins
Other complications
- Fluid overload
- Hypothermia
- Hyperkalemia (neonates and CKD patients are at risk)
- Hypocalcemia (due to citrate) from multiple rapid transfusions
- Iron overload (each unit PRBC has 200 – 250 mg iron)
- Immunosuppression
- Hypotension can occur in the patients taking ACE inhibitors because bradykinin in the transfused blood is not degraded

Blood is screened for:
1. Hepatitis C with hepatitis C antibody and hepatitis C RNA by NAT. The risk of transmission is < 1 in 1 million units.
2. HIV with HIV-1 p24 antigen, and HIV RNA using NAT, and HIV 2 antibody. The risk is < one in 2 million.
3. The risk of hepatitis B transmission is 1/280,000.
4. Blood is also screened for CMV, HTLV-I and HTLV-II, parvovirus B19 and West Nile virus by NAT (Nucleic acid amplification test), treponema pallidum, Zika

Yersinia, pseudomonas, serratia, acenetobacter, and eschericia can grow at 1 degree to 6 degree centigrade. Blood is stored at 4 degree centigrade. Platelets though are stored at room temperature and can contain skin contaminants. Malaria, babesiosis, Chagas disease, Lyme disease, and Creutzfeldt-Jakob Disease can be all be transmitted by transfusion.

Blood transfusion is one of the five “overuse” interventions. Blood transfusion is now considered an ‘undesirable outcome’. Single unit transfusion and reassessment of patients between transfusions is desirable.

AABB Clinical Practice Guidelines:
5. Adhere to a restrictive transfusion strategy of Hb 7-8 g/dL in hospitalized stable patients
6. In patients with pre-existing CVD, consider transfusion for patients with symptoms or Hb < 8 g/dL. There is no recommendation for or against liberal vs restrictive transfusion strategy in patients with ACS
7. Transfusion decision should be made using symptoms + hemoglobin concentration

Most medical society guidelines recommend Hb threshold of 7-8 g/dL. A recent RCT studying transfusion strategy in acute UGI bleed found a higher 6 week survival in the restrictive strategy cohort. The 5 key studies of blood transfusion requirement in 5 important clinical settings: Critical Care: TRICC trial Hb 7 Vs 10, Cardiothoracic surgery: TRACS trial Hb 8 Vs 10, Repair of hip fracture: FOCUS trial Hb 8 Vs 10, Acute UGI bleed: Hb 7 vs 9, all supported restrictive strategy, and Symptomatic coronary Artery Disease - Pilot Study for transfusion in ACS, by Carson et al had trend towards liberal strategy in 30 day survival. There is no difference in outcome by transfusing PRBC stored short-term (13 +/- 7.6 days) or long-term (23.6 +/- 8.9 days)
Prophylactic platelet transfusion is recommended by most practice guidelines for a threshold of < 10,000 platelet/mcL.

A systematic review and meta-analysis by Murad et al on the effect of plasma transfusion on morbidity and mortality concluded that very low-quality evidence suggests that plasma transfusion in the setting of massive transfusion for trauma patients may be associated with a reduction in the risk of death and multiorgan failure, and a survival benefit was not demonstrated in most other transfusion populations. The author also concluded that a single retrospective study showed reduction in mortality associated with plasma transfusion in anticoagulated adults with ICB.

**Lymphoma & Myeloma**

**Lymphoma**
Lymphoid malignancies are named according to WHO classification. WHO classifies lymphoid malignancies into 3 main groups: B cell, T cell and Hodgkin’s disease.

**B Cell:**
- Precursor B lymphoblastic leukemia/lymphoma (ALL extremely aggressive)
- Chronic lymphocytic leukemia/small lymphocytic lymphoma (indolent)
- B cell prolymphocytic leukemia
- Lymphoplasmacytic lymphoma
- Splenic marginal zone B cell lymphoma (indolent)
- **Hairy cell leukemia**
- Plasma cell myeloma/plasmacytoma
- Extranodal marginal zone B cell lymphoma (MALT)(indolent)
- **Mantle cell lymphoma** (aggressive)
- Follicular lymphoma (indolent)
- Nodal marginal zone B cell lymphoma (indolent)
- Diffuse large B cell lymphoma (most common aggressive type NHL)
- Burkitt’s lymphoma (extremely aggressive)

AIDS related lymphomas are also very aggressive.

**T cell:**
- Precursor T cell ALL
- T cell promyelocytic leukemia
- T cell granular lymphocytic leukemia
- **Adult T cell lymphoma/leukemia (HTLV 1)**
- Extranodal NK/T cell lymphoma, nasal type
- Enteropathic type T cell lymphoma
- Hepatosplenic T cell lymphoma
- Subcutaneous panniculitis like T cell lymphoma
- **Mycosis fungoides/Sezary syndrome**
- Anaplastic large cell lymphoma
- Peripheral T cell lymphoma
Angioimmunoblastic T cell lymphoma
Anaplastic large cell lymphoma

**Hodgkin’s disease:**
Nodular lymphocytic predominant Hodgkin’s
Classic Hodgkin’s: Nodular sclerosis, Lymphocyte rich, Lymphocyte depletion,
and Mixed cellularity.

Non Hodgkin’s lymphoma makes up 63% of all lymphoid malignancies, followed by Plasma cell disorders, CLL, Hodgkin’s disease, and ALL.

Infectious agents and lymphoma:

H. pylori: Gastric MALT lymphoma
HTLV1: Adult T cell leu...kg.
Hepatitis C: Lymphoplasmocytic lymphoma
HHV8: Multicentric Castleman’s disease and primary effusion lymphoma
EBV: Burkitt’s lymphoma, post transplant lymphoma, primary CNS lymphoma,
Extranodal NK/T cell lymphoma nasal type, Hodgkin’s
HIV: Diffuse large B cell lymphoma, Burkitt’s lymphoma

Patients treated for Hodgkin’s lymphoma can develop NHL.

When lymphoma is suspected due to progressively enlarging lymphnode or persistent lymph node (> 2 weeks), then biopsy should be obtained rather than FNA. Once lymphoma is confirmed by pathology, flow cytometry, cytogenetics, and staging work up is done.

NHL work-up:

- Detailed H & P with special attention to B symptoms.
- Labs: CBC, comprehensive metabolic panel including liver and renal function tests, HIV, hepatitis serologies, LDH, uric acid, and β2 microglobulin level. Consider PPD.
- Invasive tests: Bone marrow biopsy. LP is done for Burkitt’s, lymphoblastic type or if bone marrow is positive. LP is also done if there is testicular, eye or sinus involvement.
- Imaging: CT neck, chest, abdomen and pelvis, MUGA scan for EF, and PET scan.

Staging is based on AnnArbor staging system.

**Ann Arbor Staging**
Ann Arbor staging is used for both Non-Hodgkin’s and Hodgkin’s lymphoma. Hodgkin’s lymphoma spreads contiguously from one lymph node region to another, but non-Hodgkin’s lymphoma spreads hematogenously. Therefore, Ann Arbor staging applies poorly to Non-Hodgkin’s lymphoma.
Stage I: Involvement of 1 lymph node site or 1 extralymphatic site
Stage II: 2 or more lymph node regions or extralymphatic sites on the same side of the diaphragm
Stage III: Lymph node regions on both sides of the diaphragm (can include spleen, localized extranodal disease, or both)
Stage IV: Disseminated extranodal sites with or without lymphnodes

A refers to no B symptoms
B symptoms:
- Fever
- Night sweats and
- Weight loss

International prognostic index for Large Cell Non-Hodgkin’s lymphoma
This is based on five clinical risk factors,
1. Age more than or equal to 60
2. Elevated LDH
3. Performance status ≤ 70 in the Karnofsky scale, or ≥ 2 in ECOG scale
4. Ann Arbor stages III or IV
5. More than one site of extranodal involvement

Patients are assigned a number for each risk factor they have. Patients are also grouped differently based upon the type of lymphoma.

For a patient with diffuse large B cell lymphoma, presence of 0-1 risk factor indicates low risk. The five-year survival is 73%. With two risk factors, this is considered low to intermediate risk and the five-year survival is 51%. In the presence of three risk factors, this is considered intermediate to high risk, the five-year survival is 43%, and a patient with diffuse large B cell lymphoma with four or five risk factors is a high-risk patient with a five-year survival only 26%.

**Diffuse large B cell lymphoma**
- Most common type of non-Hodgkin’s lymphoma
- Aggressive
- Diagnosis
  - Review of adequate biopsy and proof of B cell immunophenotype
  - Mutations involving BCL2 gene on chromosome 3q27 can be associated
- Can be primary lymph node disease or extranodal such as involvement of gastrointestinal system or bone marrow

**Treatment**
- R-CHOP (Rituximab + CHOP)
  - CHOP
    - Cyclophosphamide
    - Doxorubicin
    - Vincristine
- Prednisone
  - If the patient relapses or if there is no response, then the patient is given high-dose salvage therapy with stem cell transplantation.
  - T-cell diffuse large cell NHL is treated with CHOP, without R. Prognosis is worse.

**Mantle Cell lymphoma:**
- Aggressive lymphoma, incurable, usually advanced at diagnosis
- It is CD5 positive, and has t(11:14), and overexpresses cyclin D1
- R-CHOP treatment leads to short disease free survival, median survival < 3 years
- Patients with localized disease and low growth rate survive > 6 years
- Mantle cell lymphoma has a separate prognostic index to determine course of therapy due to its variable course.
- New chemotherapies, such as the aggressive R-HYPERCVAD is now the standard treatment and recurrent disease is treated with allogenic stem cell transplant

**Follicular lymphoma**
- Most common indolent-type non-Hodgkin’s lymphoma, second most common NHL
- Associated with long survival (10 – 12 years)
- Usually advanced at presentation with bone marrow involvement, and incurable
- Derived from germinal center B cells
- Associated with t(14:18)
  - The BCL 2 gene from chromosome 18 into the immunoglobulin heavy chain region on chromosome 14 (which prevents apoptosis)
  - It has CD 20 and CD 10.
- Can transform to diffuse large B cell lymphoma at later stages
- In asymptomatic patients due to the prolonged survival, watchful waiting is an option
- For advanced stages, single agent rituximab, combination chemo + rituximab, radioimmunoconjugates are all options, and relapse is treated with stem cell transplant in young patients.
- Early stage localized disease can be treated with radiation & rituximab for cure

**Burkitt's lymphoma**
- Rare in USA
- t(8:14) is seen in classic Burkitt's
  - Can also be associated with t(2:18) or t(8:22)
- Is endemic in Africa and is seen in children
- In the United States, it is sporadic. Immunodeficiency associated Burlitt’s is seen with HIV
- If diagnosed, CSF exam is always done
- Treatment is done within 48 hours of diagnosis with aggressive ALL like regimens with CNS treatment
- Curable

**Lymphoblastic lymphoma**
- Extremely aggressive and treated as ALL. It is curable.
**Extra nodal marginal zone B cell lymphoma (MALT)**
- Gastric MALT lymphoma is H. pylori associated
- Localized mild forms are treated by eradication of H. pylori
- Transformation to diffuse large B cell lymphoma can occur
- Salivary gland MALT lymphoma can be associated with Sjogren’s syndrome
- Thyroid gland MALT lymphoma can be associated with Hashimoto’s disease

Chemotherapy refractory B-Cell malignancies (refractory diffuse large B cell lymphoma, indolent lymphoma, chronic lymphocytic leukemia) have been shown to respond to **CAR-T cells** (anti CD-19 chimeric antigen receptor T cells). CAR-T cells control T cell antigen recognition, remove need for HLA specificity, enhance proliferation and potentiate effector function.

**Mycosis fungoides**
- A cutaneous T cell lymphoma
- Typical patient is a male African-American, > 50 years old
- Skin lesions start as a patch, becomes a plaque, and then becomes a tumor-like elevation (early stage)
- When the patient presents with erythoderma, this is associated with circulating tumor cells, and this is called Sézary syndrome
- Extracutaneous involvevemnt occurs in advanced disease
- Early stage can be observed, when cutaneous symptoms develop, topical steroid/retinoid, psoralen + PUVA with IFN-α, and advanced disease is treated with CHOP
- Suberoylanilide hydroxamic acid has been recently approved by FDA for treatment of cutaneous T cell lymphoma.

(Differential diagnosis of diffuse erythroderma
- Psoriasis
- Mycosis fungoides with Sezary syndrome
- Severe allergic reaction (e.g., drugs)
- TSS)

**Adult T-cell lymphoma/leukemia**
- Associated with HTLV-1 infection
- Transmission can be transplacental, through blood transfusion, or sexual transmission
- HTLV-1 infection can cause spastic paraparesis with a short latency of less than three years
- Adult T-cell lymphoma/leukemia has a long latency of up to 55 years
- **This lymphoma is characterized by CD-4 positive cells with a flower-shaped nucleus**
- Very aggressive
- Associated with lymphadenopathy, hepatosplenomegaly, skin infiltration, hypercalcemia, lytic bone lesions, and elevated LDH
- Treated with combination chemotherapy and true complete remission is unusual
- Median survival is 7 months
**Peripheral T-cell lymphomas**
Anaplastic Large T/Null cell lymphoma: Type of NHL. CD 30, t(2;5). Aggressive lymphoma in young males. Treated with NHL treatment.

Peripheral T-cell lymphomas: CD4+ or CD8+, or both CD4+ and CD8+
1. Angioimmunoblastic lymphoma is a common subtype. Generalized lymphadenopathy, fever, weight loss, skin rash, and polyclonal hypergammaglobulinemia can occur.


**Hodgkin’s disease**
There are two types
1. Nodular lymphocytic predominance
2. Classic Hodgkin’s
   - Nodular sclerosis
   - Lymphocyte-rich classic Hodgkin’s
   - Mixed cellularity
   - Lymphocyte depletion

**Nodular lymphocytic predominance**
- Predominance of small lymphocytes and rare Reed-Steinberg cells
- Nodular lymphocytic predominant lymphoma with localized involvement is treated with radiation
- Disseminated disease is treated just as classic Hodgkin’s disease

**Classic Hodgkin’s disease**
- Most patients present with palpable nontender lymphadenopathy
- Common sites are the neck, axilla, or supraclavicular lymph nodes
- Staging is done by the Ann Arbor system
- Older patients can present as fever of unknown origin

Other unusual manifestations
- Unexplained itching
- Erythema nodosum
- Sudden onset ichthyosis
- **Paraneoplastic cerebellar degeneration**
- **Minimal change nephrotic syndrome**
- Immune hemolytic anemia
- Thrombocytopenia
- Hypercalcemia (*occasional*)
- Pain in the lymph nodes after alcohol intake

In the United States, nodular sclerosis Hodgkin’s disease is the most common. In HIV-infected individuals, Lymphocyte depletion and mixed cellularity types are commonly seen.
Staging evaluation

- Careful history and physical (attention to B symptoms and HIV risk factors)
- Complete blood count
- Erythrocyte sedimentation rate
- Liver enzymes and liver function
- Other serum chemistries including kidney function and LDH
- CT scan of the chest, abdomen, pelvis, bone marrow biopsy, and PET scan

Once the staging evaluation is complete and the patient is classified according to Ann Arbor staging, treatment is decided. All stages are generally treated initially with chemotherapy. Localized non bulky disease (IA and IIA) are given brief chemotherapy followed by local radiation. If there is more extensive disease or B symptoms, then the patient is treated with combination chemotherapy.

- ABVD is the most popular regimen used in USA
  - Doxorubicin, Bleomycin, Vincristine, and Dacarbazine
  - For lymphocyte predominant type, rituximab is added
- Other regimens are Stanford V and BEACOPP
- Hodgkin’s lymphoma has a high cure rate
- Late complications are now the most important feature that internists need to follow
- Important late complications include second malignancies and cardiac injury

Long-term complications of survivors of lymphoma:

NHL survivors:

Patients who received radiation can develop sarcomas, breast cancer, and mesothelioma. There is increased risk of cardiotoxicity (CHF) and infertility. Patients should be advised to quit smoking and lead a healthy lifestyle. Annual skin exam can help with early detection of skin cancer. Age appropriate cancer screening should be done.

Hodgkin’s survivors:

Acute leukemia can occur within the first 10 years after therapy. Acute leukemia occurs more commonly with the MOPP regimen than ABVD. Myelodysplastic syndrome can also occur. Cancers after Hodgkin’s therapy usually occur more than 10 years after treatment. Cancers occur more commonly with radiation therapy than with chemotherapy. Cancers that can occur within the radiation field include soft tissue sarcomas, melanoma, head and neck cancer, lung cancer, breast cancer, GI cancer, and urogenital cancer depending upon the radiation field. Thoracic radiation therapy is associated with increased risk of breast cancer. ACS recommends that women who had mantle radiation between ages 10-30 should undergo yearly mammogram + MRI 8-10 years after radiation, and not before age 25.
Thoracic radiotherapy also accelerates coronary artery disease. Adriamycin increases risk of CHF. Patients should be advised to stop smoking, and their lipids must be treated aggressively. Other complications of thoracic radiotherapy include hypothyroidism (annual TSH monitoring) and thyroid cancer. Lhermitte's syndrome refers to electric shock-like sensation going into the lower extremity with flexion of the neck *(also associated with MS)*. This can happen after radiation for Hodgkin’s lymphoma. Infertility after treatment is age related. Most young patients recover fertility. ABVD increases the chance to retain fertility compared to MOPP regimen. There is increased incidence of NHL after treatment of Hodgkin’s disease.

Prognostic factors for Hodgkin’s lymphoma are: age ≥ 45, male sex, stage IV, leucocytosis (>15K), lymphopenia (< 600/mcL), hypoalbuminemia (<4 g/dL) and anemia (Hb < 10.5 g/dL)

**Monoclonal gammopathies – new definitions have been issued by MM working group**

Monoclonal gammopathies can complicate hematological neoplasms, solid tumors, cirrhosis, sarcoidosis, parasitic diseases, Gaucher’s disease, pyoderma gangrenosum, rheumatoid arthritis, Sjögren’s syndrome, myasthenia gravis, and cold agglutinin disease. Some patients have a plasma cell disorder such as myeloma or solitary plasmacytoma.

**POEMS syndrome**

This denotes polyneuropathy, monoclonal gammopathy, + any 1 major criteria (sclerotic bone lesion, Castleman’s disease, increased level of VEGFA) and any one of the minor criteria (organomegaly, extravascular volume overload, endocrinopathy, papilledema, thrombocytosis/polycythemia and skin changes).

**MGUS – IgM, non-IgM and light chains MGUS (new definitions)**

- Isolated asymptomatic monoclonal gammopathy
- In about 5% of the population older than 70 years
- When first identified, the following should be done: a bone marrow exam to enumerate the plasma cells, quantitation of monoclonal gammopathy in the serum and urine, and skeletal survey to rule out multiple myeloma
- Who should be tested for monoclonal gammopathy? Unexplained hypercalcemia/renal failure/proteinuria/normocytic anemia/lymphadenopathy, abnormal bone lesions, recurrent bacterial infections

**Non IgM MGUS:**

1. Serum non IgM monoclonal protein < 3g/dL
2. Clonal BM plasma cells < 10%
3. Absence of CRAB damage or amyloidosis

1%/year progression to multiple myeloma, solitary plasmacytoma or Ig related amyloidosis

**IgM MGUS:**

1. Serum IgM monoclonal protein < 3g/dL
2. BM lymphoplasmocytic infiltration < 10%
3. No evidence of anemia, constitutional symptoms, hyperviscosity, hepatosplenomegaly, or other end organ damage attributable to the lymphoproliferative disorder
1-5%/year progression to Waldenstroms or Ig related amyloidosis

**Light chain MGUS:**
1. Abnormal free light chain ratio (< 0.26 or > 1.65)
2. Increased kappa free light chain in patients with ratio > 1.65 and increased lambda free light chain in patients with ratio < 0.26
3. No Ig heavy chain expression on immunofixation
4. Absence of CRAB or amyloidosis attributable to the plasma cell disorder
5. Clonal BM plasma cells < 10%
6. Urine monoclonal protein < 500 mg/24 hours
0-3% progression/year to light chain myeloma, Ig light chain amyloidosis.

Initial work up of MGUS: Mayo Clinic Model

MGUS: Low risk (< 1.5 g/dL IgG type, normal free light chain ratio or IgM < 1.5 g/dL or light chain MGUS with FLC ratio < 8): If low risk is uncomplicated (no unexplained symptoms or lab features): Bone marrow and skeletal survey may be deferred. If there are unexplained symptoms or lab features, or intermediate to high risk: Bone marrow biopsy/skeletal survey

Follow up of MGUS: Mayo Clinic Risk Stratification Model

MGUS: Follow up in 6 months (All patients).
1. If stable MGUS level, risk stratify (as above). Low risk: No further MGUS follow up. Intermediate or high risk: Check MGUS every year and f/u CBC, Calcium, Creatinine, SPEP, Free light chain
2. Possible MGUS progression: Work up for lymphoplasmacytic malignancies. If negative, F/U like intermediate to high risk as above. If malignancy is diagnosed, evaluate and treat.

**Multiple Myeloma**
Criteria: **International Myeloma working Group – new criteria**

1. Bone marrow clonal plasma cells ≥ 10% or biopsy proven bony or extramedullary plasmacytoma
   AND
2. 1 of the following myeloma defining events: CRAB damage attributable to the plasma cell disorder
   a. Serum calcium > 1 mg/dL above upper limit of normal or > 11 mg/dL
   b. Renal insufficiency, Creatinine Clearance < 40 ml/min
   c. Anemia, Hgb > 2g/dL lower than lower limit of normal or < 10 g/dL
   d. Bone lesions ≥ 1 osteolytic lesion on skeletal survey, CT or PET-CT
OR

3. Presence of any 1 of the biomarkers of malignancy regardless of CRAB:
   a. Clonal plasma cells > 60% on bone marrow exam
   b. Serum involved/uninvolved free light chain ratio ≥ 100 provided the involved light chain’s absolute value is ≥ 100 mg/L
   c. ≥ 1 focal bone lesion in MRI ≥ 5mm in size

So, if CRAB is not there, look for SLiM CRAB (S: 60% plasma cells, Li: Light chain ratio, M: MRI for bone lesion)

The incidence of myeloma is 4.3 cases per 100,000. In African-Americans, it is 9.6 per 100,000. The median age at diagnosis is 70 years and the median survival is 2 to 2.5 years. The 5-year survival is only 25%.

Smoldering myeloma is now defined as serum monoclonal protein IgG or IgM ≥ 3g/dL or urine monoclonal protein ≥ 500 mg/24 hours and/or clonal bone marrow plasma cells 10-60% and no myeloma defining events or amyloidosis. It needs frequent monitoring without treatment outside of a clinical trial. One small clinical trial has shown better outcomes in high risk smoldering myeloma with lenalidomide/dexamethasone treatment.

New International Staging system for myeloma

This system utilizes serum β2 microglobulin level and albumin to classify patients into 3 stages.

Stage I: β2 microglobulin < 3.5 mg/L and serum albumin ≥ 3.5 g/dL, no high risk cytogenetics, normal LDH
Stage II: Not stage I or III
Stage III: β2 microglobulin ≥ 5.5 mg/L AND high risk cytogenetics OR elevated LDH

60% of the patients with multiple myeloma have monoclonal protein that is IgG, 20% have IgA, and 10% have only light chain. Prognosis in multiple myeloma is based on performance status, renal function, stage and tumor biology (High risk: 17p-, t(14:16), t(14:20), t(4:14), standard risk: hyperdiploidy, t(11:14), t(6:14)

Peripheral smear: Rouleaux formation

- Plasma cell (seen in bone marrow)
  - A large oval cell with pale blue cytoplasm, an eccentric nucleus with cartwheel appearance of nucleolus, and a perinuclear halo

Clinical features
- Lytic bone lesions and hypercalcemia
o Osteoclasts respond to Osteoclast Activating Factor (OAF) made by myeloma cells. OAF activity is mediated by many cytokines - tumor necrosis factor, IL-1, IL-6, VEGF etc

- Renal failure
- Anemia
- Infection with encapsulated organisms
- Easy bleeding
- Neurological symptoms

**Skeletal survey is recommended with plain x-rays (do not order bone scan) due to tumor necrosis factor mediated lytic bone lesions without any osteoblastic response.** This leads to punched out lesions. This can lead to fractures and nerve compression.

Susceptibility to bacterial infections is due to diffuse hypogammaglobulinemia and inhibition of neutrophil migration. Pneumococcus, S. aureus, and Klebsiella are common in the lung and E. coli is common in the urinary tract.

Renal insufficiency is due to the elevated calcium, toxicity from light chains. Proximal RTA/Fanconi syndrome, nephrotic syndrome due to amyloid fibril deposition can occur.

Anemia is due to myelophthisis, and decreased RBC production due to inhibitory factors.

Easy bleeding is due to interference with clotting factors and platelet dysfunction due to abnormal coating of platelets.

Neurological symptoms can be associated with multiple myeloma due to the hyperviscosity. This leads to headache, dizziness, and vertigo.

Other features may be associated with cryoglobulins, amyloidosis, calcium and nerve root or cord compression.

**Proteinuria with dipstick negative for protein, low anion gap, proximal RTA with Fanconi syndrome (clue: urine dipstick positive for glucose, while the serum glucose is normal), anemia/renal failure/back pain combination, hypercalcemia, cord compression, and pathological fractures should all raise suspicion for multiple myeloma.**

**Treatment**

- Based on patients’ risk category and if SCT is needed
- Transplant candidates:
  o Lenalidomide/dexamethasone or Bortezomib based induction chemotherapy for 4 cycles followed by stem cell transplant (avoids melphalan)
  o High response rate without compromising collection of stem cells for autologous transplant

- Non transplant candidates
  o Melphalan/prednisone/thalidomide
Bortezomib/melphalan/prednisone
- Lenalidomide/dexamethasone

- Drugs active against multiple myeloma: Bortezomib, thalidomide, lenalidomide, liposomal doxorubicin. New approved drugs: Carfilzomib, Pomalidomide, Panobinostat, Ixazomib, Daratumumab (Anti CD-38 ab – found to be effective as a single agent in heavily pretreated and refractory myeloma), Elotuzumab.

Hypercalcemia is treated by bisphosphonate infusions. Infections must be treated aggressively. Vaccinations are given to protect the patient. The patient must also be hydrated adequately to prevent renal failure. Contrast studies are avoided if possible. Erythropoietin is administered for anemia. Local radiation therapy can be given for pain.

Causes of death

Progressive myeloma, renal failure, sepsis, therapy related acute leukemia or myelodisplasia. 25% die of illnesses related to their age like MI, stroke, DM, COPD.

Waldenstrom's macroglobulinemia
- Associated with monoclonal IgM > 2 g/dL
- Bone marrow ≥ 10% plasmacytoid lymphocyte infiltration
- Bone lesions and hypercalcemia are usually absent
- **Hyperviscosity is very common**
- Hyperviscosity present with:
  - Mental status changes
  - Paresis
  - Headache
  - Dizziness
  - Fundus exam shows vascular segmentation and dilatation of retinal veins
- Plasmapheresis is the treatment for hyperviscosity. The IgM is intravascular. Therefore, plasmapheresis can correct the hyperviscosity.

Normocytic normochromic anemia, rouleaux formation and positive coomb’s test are common. The monoclonal IgM can also act as cryoglobulin (Raynaud’s and vascular symptoms precipitated by cold).

Waldenstrom's macroglobulinemia does not have renal failure, since there are no light chains in the urine. Treatments for Waldenstrom's macroglobulinemia is fludarabine based chemotherapy given along with Rituximab.

Solitary plasmacytoma
- Isolated tumor mass of clonal plasma cells affecting the bone or extramedullary lesion with normal bone marrow, normal skeletal survey and MRI or CT of the spine and pelvis except for the solitary primary lesion and absence of CRAB damage. Solitary plasmacytoma with minimal bone marrow involvement is the diagnosis if the bone
marrow clonal plasma cells are positive but < 10%. (If bone marrow clonal plasma cell percentage exceeds 10%, it will become multiple myeloma)

- Treated with local radiation therapy
- Extramedullary plasmacytoma can affect any place, but usually the sinuses and the nose
  - Treated with radiation therapy

MRI, CT scan or PET-CT of whole body or spine/pelvis is now needed to define smoldering myeloma, solitary plasmacytoma or solitary plasmacytoma with minimal marrow involvement.

AL Amyloidosis can occur in patients with monoclonal gammopathy (see Rheumatology)

**ECOG performance status**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS 0</td>
<td>Without symptoms</td>
</tr>
<tr>
<td>PS 1</td>
<td>Mild symptoms not requiring treatment</td>
</tr>
<tr>
<td>PS 2</td>
<td>Symptoms requiring some treatment</td>
</tr>
<tr>
<td>PS 3</td>
<td>Disabling symptoms, but ambulatory more than 50% of the day</td>
</tr>
<tr>
<td>PS 4</td>
<td>Ambulatory less than 50% of the day</td>
</tr>
</tbody>
</table>

**Karnofsky performance scale**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Normal</td>
</tr>
<tr>
<td>90</td>
<td>Able to carry on normal activity with minor signs and symptoms of the disease</td>
</tr>
<tr>
<td>80</td>
<td>Normal activity with effort but has some signs and symptoms of the disease</td>
</tr>
<tr>
<td>70</td>
<td>Patient can care for self, but unable to carry on normal activity or do active work</td>
</tr>
<tr>
<td>60</td>
<td>Requires occasional assistance, but is able to care for most needs</td>
</tr>
<tr>
<td>50</td>
<td>Requires considerable assistance and frequent medical care</td>
</tr>
<tr>
<td>40</td>
<td>Disabled, needs special care and assistance</td>
</tr>
<tr>
<td>30</td>
<td>Severely disabled, hospitalization indicated although death is not imminent</td>
</tr>
<tr>
<td>20</td>
<td>Very sick, hospitalization is necessary and active supportive treatment is necessary</td>
</tr>
<tr>
<td>10</td>
<td>Moribund, fatal process progressing rapidly</td>
</tr>
<tr>
<td>0</td>
<td>Dead</td>
</tr>
</tbody>
</table>
Acute Leukemias (AML and ALL)

Classification - AML

French, American, and British (FAB)
- Original morphological classification
- Relied on cytochemistry and used ≥ 30% blasts as cutoff.

World Health Organization (WHO)
- Modified the FAB classification by reducing the number of blasts required for a diagnosis (≥ 20%) and uses immunophenotype, clinical features, cytogenetic and molecular abnormalities in addition to morphology

Some of the important AML:
- Acute promyelocytic leukemia associated with translocation 15 to 17 t(15:17)
  This has a very good prognosis (85% cure rate)
- Core-binding factor leukemias
  - Translocation 8: 21
  - Inversion INV (16) - acute myelomonocytic leukemia with eosinophilia
    These have good prognosis (50% cure)
- Secondary leukemias and myelodysplastic syndromes

Patients with leukemia present with features of anemia (fatigue), thrombocytopenia (easy bruising, gum bleeding) and neutropenia (infection). There is leucocytosis and peripheral smear shows blasts. Bone marrow aspiration and biopsy, flow cytometry, cytogenetics, serum chemistries, coagulation studies, CXR, EKG, MUGA for EF (if needed) are done. Management of initial complications may include management of bleeding due to DIC/thrombocytopenia, neutropenic fever etc. When patients are stabilized, IV hydration and allopurinol is given to prevent tumor lysis syndrome and leukemia therapy is started.

Acute promyelocytic leukemia t(15:17) (APL)

- Translocation of promyelocyte leukemia oncogene (PML oncogene) on chromosome 15 to the retinoic acid receptor alpha (RAR alpha gene on chromosome 17)
  - This results in a Novel Chimeric Fusion Protein that binds DNA and interferes with differentiation of hematopoetic cells
  - The affected leukemic cell’s maturation is arrested in the blast or promyelocytic stage
    - Apoptosis is inhibited and leukemic blasts accumulate

All-trans retinoic acid (ATRA) binds to PML/RAR alpha and induces unbinding of the co-repressors and allows for subsequent differentiation and apoptosis of the leukemic clone.
Labs
- Smear
  - Blasts display numerous Auer rods and show at least some evidence of early differentiation such as cytoplasmic granules
  - Express CD-34, CD-33, HLA-DR and CD-13
- Pathognomonic feature is translocation 15 to 17 by cytogenetic analysis
- Unusual coagulopathy

Treatment of APL DIC
- Intensive transfusion support including platelets, cryoprecipitate, and FFP
- ATRA reduces the hemorrhagic complication by differentiating blasts into neutrophils

Treatment of APL is different from that applied to other types of AML.

ATRA (All trans retinoic acid, tretinoin)
- ATRA alone can result in more than 80% remission
  - But these remissions are not long lasting and relapses are usually ATRA resistant
  - Therefore, combination chemo is given with ATRA
  - Retinoic acid induces terminal differentiation of tumor cells and their adhesion to vascular endothelium in the lung can produce respiratory compromise – the retinoic acid syndrome

Retinoic acid syndrome
- Occurs within first three weeks of therapy
- Patients presented with fever, dyspnea, chest pain, pulmonary infiltrates, hypoxia, pleural, and pericardial effusions
- Treatment for life threatening retinoic acid syndrome is stopping the retinoic acid, chemotherapy, and steroids. Therefore ATRA is given with chemotherapy to prevent this complication.

Treatment of APL, therefore, consists of administering retinoic acid along with anthracycline-based chemotherapy for remission. Arsenic trioxide gives up to 85% response in patients refractory to tretinoin. Once complete remission is achieved, post remission therapy (consolidation therapy) is given – intensive cytarabine based chemotherapy or SCT

Treatment of AML
At diagnosis, there may be situations requiring urgent management:
- Neutropenic fever
- Bleeding related to thrombocytopenia (which needs platelet transfusion)
- DIC
- Leukostasis associated with a blast count more than 100,000 (which requires leukapheresis and hydroxyurea)
Routine platelet transfusion is usually given when the platelets are less than 10,000. Once the patient is stabilized from the above conditions, antileukemic therapy is begun. Rapid pretreatment evaluation is needed.

Treatment for newly diagnosed AML is the same, except for acute promyelocytic leukemia.
- Starts with induction therapy with cytarabine plus anthracycline with or without etoposide
  - If the patient achieves complete remission, the patient is risk stratified
    - Patients with high risk karyotypes or normal karyotype with high risk features who achieve complete remission are candidates for allogenic stem cell transplant if they have a HLA compatible donor
    - If they do not have a HLA compatible donor, then patients are treated with three to four cycles of high dose cytarabine or other novel approaches
  - If the patient with complete remission has a low risk karyotype such as t(8:21) or inv (16), then they receive three to four cycles of high dose cytarabine, and they become candidates for stem cell transplant only if they relapse after the high-dose cytarabine treatment
  - If they are not candidates for stem cell transplant and they do not have a HLA compatible donor, then these patients are referred for investigational therapy

*Tumor lysis syndrome*
- A result of overwhelming release of tumor cell contents into the blood stream
- Most commonly associated with treatment of high-grade lymphomas and leukemias

Clinical features include
- Elevated uric acid (*can lead to renal complication*)
- Acidosis
- Hyperkalemia (*can lead to cardiac arrhythmia*)
- Hyperphosphatemia (*can lead to acute renal failure*)
- Hypocalcemia
  - Muscle cramps, cardiac arrhythmia, and tetany

Prevention is the key for tumor lysis syndrome. Allopurinol, adequate hydration, and alkalization are required prior to start of chemotherapy. Rasburicase is used, if uric acid level is still elevated. Established tumor lysis syndrome may need hemodialysis.

Neutropenic fever is discussed in Oncology.

*Secondary leukemia/myelodysplastic syndrome*
- Patients are characterized by the international prognostic scoring system
  - Uses cytopenias, age, percentage of blasts, and cytogenetics to classify patients into prognostic groups
- Higher-risk myelodysplastic syndrome tends to transform into acute leukemia
Secondary leukemia
- Characterized by cytogenetic abnormalities often involving chromosome 5 and 7
- Difficult to induce into remission and the survival is short
- Associated with previous exposure to alkylating agents

Another unique secondary leukemia is associated with 11q23 translocation. Prior exposure to epipodophyllotoxins such as etoposide is associated with increased risk of leukemias that harbor 11q23. Unlike leukemias that are induced by alkylating agents such as melphalan, these leukemias are not preceded by MDS. Survival is short and transplant is necessary for cure.

**Acute lymphocytic leukemia (ALL)**

ALL - Classification:
- L1, L2 (Precursor B-cell ALL) - most common ALL, t(9;22), t(4;11) t(1;19)
- L1, L2 (T-cell ALL) - better prognosis in adults
- L3 (B-cell ALL) - Burkitt’s Leukemia, t(8;14)

ALL in children is about 80% curable. In adults, the outcome is poorer secondary to adverse clonal cytogenetic abnormality such as t(9:22) and t(4:11).

CSF analysis for CNS involvement is indicated for all ALL.

**Treatment**
- Typically based on vincristine and prednisone along with an anthracycline with one or more: L-asparaginase, Cytarabine, Methotrexate, Cyclophosphamide
- Complete remission is achieved in 70-90% of the patients
- Intensification/post induction therapy is commonly used
- Prophylactic CNS treatment (*intrathecal chemo with or without irradiation*) is part of the treatment

Indications for allogenic stem cell transplant
- High risk cytogenetic abnormalities in first remission
**Chronic lymphocytic leukemia (CLL)**

- Most common adult leukemia in the United States
- Median age at diagnosis is 70
- Neoplastic cells are normal appearing small lymphocytes derived from **CD5 positive** B-cell subset
- 10% of the patients have **trisomy 12** (*CD5 is seen in CLL and mantle cell lymphoma*)

Two staging systems, the RAI system and the Binet system assesses the tumor burden.

**RAI**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Findings</th>
<th>Median survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAI 0</td>
<td>Lymphocytosis only</td>
<td>14+ years</td>
</tr>
<tr>
<td>RAI I</td>
<td>Lymphocytosis and lymphadenopathy</td>
<td>9 years</td>
</tr>
<tr>
<td>RAI II</td>
<td>Lymphocytosis, hepatosplenomegaly and/or splenomegaly</td>
<td>7.5 years</td>
</tr>
<tr>
<td>RAI III</td>
<td>Any of the above plus anemia (<em>Hgb &lt; 11 g/dL</em>)</td>
<td>2.5 years</td>
</tr>
<tr>
<td>RAI IV</td>
<td>Any of the above plus thrombocytopenia (<em>plts &lt; 100,000</em>)</td>
<td>2.5 years</td>
</tr>
</tbody>
</table>

**Binet system**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Findings</th>
<th>Median survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No anemia, no thrombocytopenia, and involvement of less than three lymph node areas</td>
<td>14 years</td>
</tr>
<tr>
<td>B</td>
<td>No anemia, no thrombocytopenia, and involvement of more than three lymph node areas</td>
<td>5 years</td>
</tr>
<tr>
<td>C</td>
<td>Anemia (<em>Hgb &lt; 10 g/dL</em>) or thrombocytopenia (<em>plts &lt; 100,000</em>)</td>
<td>2.5 years</td>
</tr>
</tbody>
</table>

Other factors influencing prognosis include increased β2 microalbumin, lymphocyte doubling time, increased expression of soluble CD23 and karyotypic abnormalities.

CLL patients can be assessed for immunoglobulin variable region heavy-chain gene mutation status (unmutated is bad), cytogenetic abnormalities (trisomy 12q, deletion of 13q and normal karyotype are good), and Z-chain associated protein kinase 70 expression. Patients at early stage CLL can be classified if they are at high-risk for progression based on these three tests.

Criteria for treatment:
1. Symptoms due to enlarged lymph nodes
2. B symptoms
3. Anemia or thrombocytopenia
4. Hepatosplenomegaly

In a patient with CLL, if the mechanism of anemia or thrombocytopenia is autoimmune rather than replacement of bone marrow precursors, then these manifestations may not
adversely affect the prognosis. Therapy should be directed at the mechanism of cytopenia e.g. steroids for autoimmunity. This restores normal hematopoiesis.

Autoimmune hemolytic anemia is seen in up to 35% of the patients with CLL. Autoimmune thrombocytopenia is seen in about 2% of the patients with CLL. Hypogammaglobulinemia is seen in 20 to 60% of the patients with CLL. If these patients get recurrent bacterial infections, further infections can be prevented by monthly infusion of IVIG.

Eventually, when therapy is indicated for CLL, fludarabine + cyclophosphamide is the treatment of choice, for patients who are physically fit to receive chemotherapy. Fludarabine + cyclophosphamide + rituximab significantly improves survival in patients with CLL who do not have deletion of chromosome 17p. In frail, difficult to treat patients with CLL relapse, rituximab + idelalisib has been shown to improve overall and progression free survival.

In 10% of the patients CLL can transform to prolymphocytic leukemia or large cell lymphoma which is treated with R-CHOP and probably stem cell transplant.

Patients with CLL are at increased risk of infections and non-hematological cancers, particularly skin cancer. They should have age appropriate cancer screening, yearly skin exam and be up to date with vaccinations.

**Most common cause of death**
- Infection related to disease progression
- Treatment complications
- Bleeding and second malignancies
- Other lymphoid malignancies

One common scenario is an asymptomatic patient with more than 5000 small lymphocytes per microliter of blood, on routine labs. These are asymptomatic patients with lymphocytosis who are usually assessed for indicators of high-risk for progression, but in general these patients have a median survival of more than 14 years and do not require therapy and can simply be followed up.

**Hairy cell leukemia**
- Uncommon B-cell malignancy
- Median age of diagnosis is 50
- Male to female ratio is 4:1
- Present with
  - Pancytopenia with Splenomegaly
  - Relative or absolute lymphocytosis
  - The lymphocytes in the peripheral smear have hairy cytoplasmic projections
- Bone marrow
  - Aspirate is commonly dry
  - Biopsy shows increased reticulin, collagen, and fibrosis
Also hypercellular with diffuse infiltrates of hairy cells

Unique features:
- Neutropenia, monocytopenia, and cellular as well as antibody mediated cellular cytotoxicity impairment
  - Therefore, these patients can have unusual infections
    - Atypical mycobacterial (MAI) infections and fungal infections
  - Vasculitic syndromes can also occur
- Treatment: IFN α, pentostatin, cladribine (preferred agent). Majority have CR with cladribine, with long-term disease free survival.

**Erythropoietin use in cancer patients:**

Erythropoietin has to found to be associated with increased mortality, serious cardiovascular events, thromboembolic events, and stroke. It also increases mortality and increases the risk of tumor progression or recurrence. Erythropoietin can be used for the treatment of anemia in cancer patients who are on myelosuppressive chemotherapy if the anemia is due to the myelosuppressive chemotherapy, the malignancy is non-hematological, other causes of anemia like hemolysis, blood loss etc. are ruled out and if the hemoglobin is < 10 g/dL. Furthermore, the myelosuppressive chemotherapy should be palliative and not curative, and it should be used only during the course of myelosuppressive chemotherapy per FDA labeling.

Approved uses for erythropoietin:
1. Anemia due to renal failure
2. Anemia due to cancer chemotherapy (see above)
3. Anemia due to treatment of Hepatitis C
4. Anemia due to treatment of HIV
5. Anemia due to MDS

Hemoglobin should not increase > 11 g/dL in renal failure patients and not > 12 g/dL in cancer patients. Erythropoietin should not be started unless Hb is < 10 g/dL.