Anemia

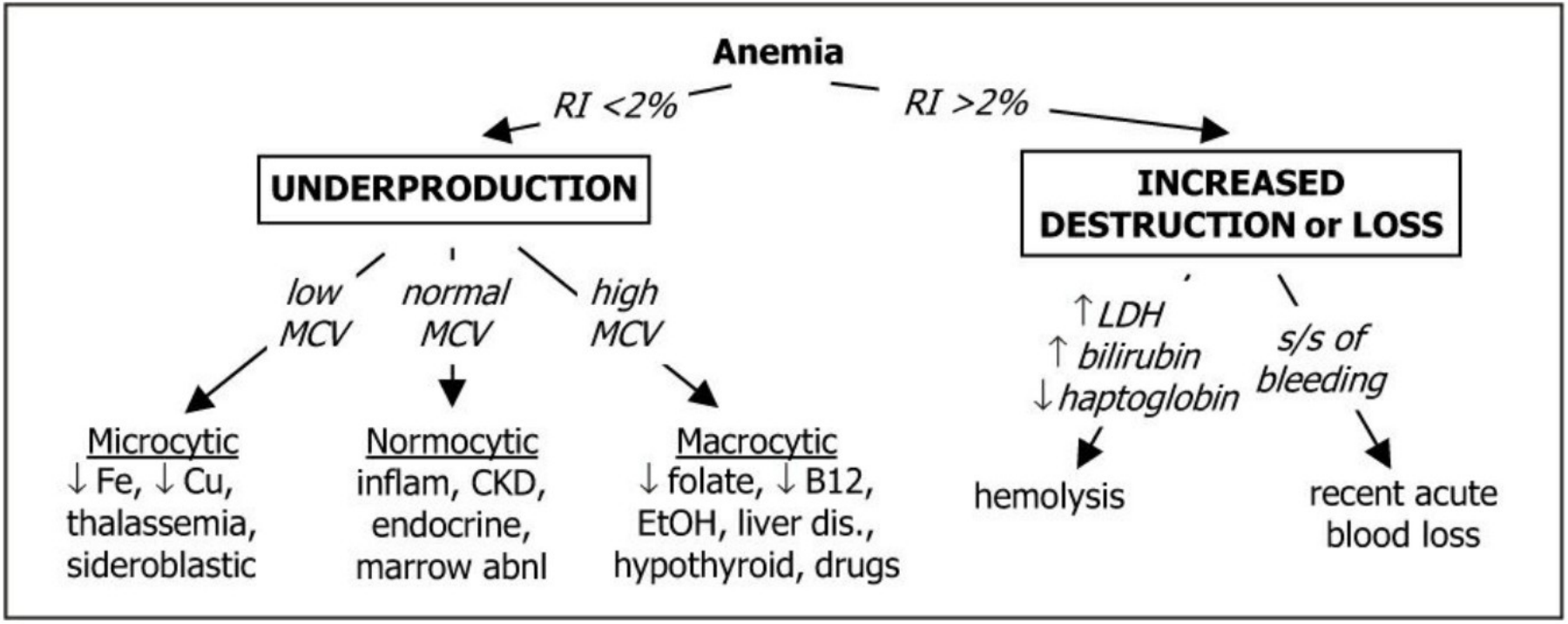
↓ *in RBC mass: Hct <41% or Hb <13.5 g/dL (men); Hct <36% or Hb <12 g/dL (women)*

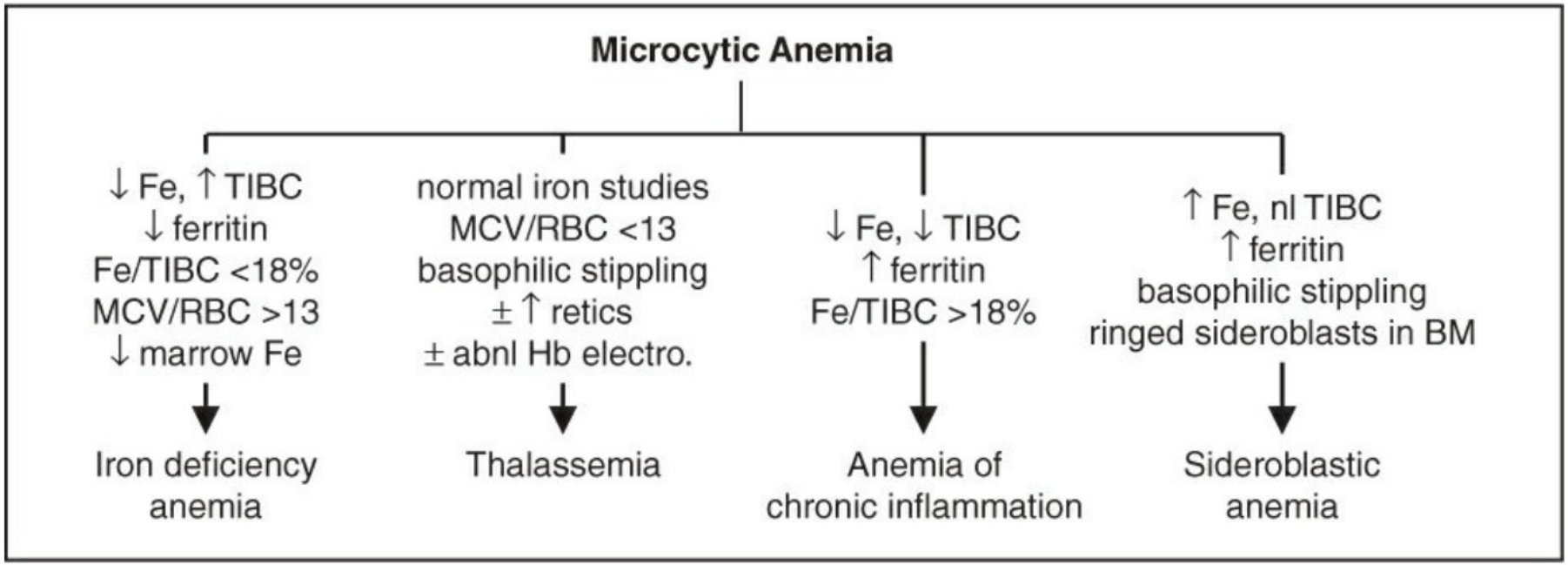
Clinical manifestations

- Symptoms: ↓ O_2 delivery → fatigue, exertional dyspnea, angina (if CAD)
- Signs: pallor (mucous membranes, palmar creases), tachycardia, orthostatic hypotension
- Other findings: jaundice (hemolysis), splenomegaly (thalassemia, neoplasm, chronic hemolysis), petechiae/purpura (bleeding disorder), glossitis (iron, folate, vitamin B_{12} defic.), koilonychia (iron defic.), neurologic abnormalities (B_{12} defic.)

Diagnostic evaluation

- History: bleeding, systemic illness, drugs, exposures, alcohol, diet (including pica), FHx
- CBC w/ diff.; RBC params incl. retics, MCV (nb, mixed disorder can → nl MCV), RDW
- Reticulocyte index (RI) = [reticulocyte count × (Pt's Hct/nl Hct)]/maturation factor
  maturation factors for a given Hct: 45% = 1, 35% = 1.5, 25% = 2, 20% = 2.5
  RI >2% → adequate marrow response; RI <2% → hypoproliferation
- Peripheral smear: select area where roughly ⅓ RBCs touch each other; ✔ RBC size, shape, inclusions (see "Appendix" & "Peripheral Smear"), WBC morphology, plt count
- Additional labs as indicated: hemolysis labs (if RI >2%, see below), iron/TIBC, ferritin, folate, B_{12}, LFTs, BUN & Cr, TFTs, Hb electrophoresis, enzyme/gene mutation screens
- Bone marrow (BM) aspirate and biopsy (bx) with cytogenetics as indicated


Anemia
RI <2%
RI >2%
UNDERPRODUCTION
INCREASED DESTRUCTION or LOSS
low MCV
normal MCV
high MCV
↑LDH
↑ bilirubin
↓haptoglobin
s/s of bleeding
Microcytic
↓ Fe, ↓ Cu, thalassemia, sideroblastic
Normocytic
inflam, CKD, endocrine, marrow abnl
Macrocytic
↓ folate, ↓ B12, EtOH, liver dis., hypothyroid, drugs
hemolysis
recent acute blood loss


Microcytic Anemia
↓ Fe, ↑ TIBC
↓ ferritin
Fe/TIBC <18%
MCV/RBC >13
↓ marrow Fe
Iron deficiency anemia
normal iron studies
MCV/RBC <13
basophilic stippling
± ↑ retics
± abnl Hb electro.
Thalassemia
↓ Fe, ↓ TIBC
↑ ferritin
Fe/TIBC >18%
Anemia of chronic inflammation
↑ Fe, nl TIBC
↑ ferritin
basophilic stippling
ringed sideroblasts in BM
Sideroblastic anemia

Iron deficiency (*NEJM* 2015;372:1832; *Lancet* 2016;387:907)

- ↓ marrow iron & depleted body iron stores → ↓ heme synthesis → microcytosis → anemia
- Special clinical manifestations: angular cheilosis, atrophic glossitis, pica (consumption of nonnutritive substances such as ice, clay), koilonychia (nail spooning)
  Plummer-Vinson syndrome (iron deficiency anemia, esophageal web & atrophic glossitis)
- Etiologies: chronic bleeding (GI—incl. cancer, menstrual, parasites, NSAIDs, etc.), ↓ supply (malnutrition; ↓ absorp. due to celiac sprue, Crohn's, ↑ gastric pH, subtotal gastrectomy), ↑ demand (preg; *Blood* 2017;129:940). Iron-refractory iron-defic. anemia (IRIDA; rare genetic disorder due to hepcidin dysregulation; *Nat Genet* 2008;40:569).
- Diagnosis (eval ideally before Rx): ↓ Fe, ↑ TIBC, ↓ ferritin (esp. <15), ↓ transferrin sat (Fe/TIBC; esp. <15%), ↑ soluble transferrin receptor; ↑ plt. Unless hx c/w other etiology, *initiate workup for GIB*, incl. *H. pylori* serology. ? Celiac labs (anti-TTG, antigliadin, anti-endomysial Abs). Cytogenetics & molecular testing as indicated.
- Treatment: oral Fe tid (~6 wks to correct anemia; ~6 mo to replete Fe stores; nb, oral Fe does not give ⊕ Hemoccult). In excessive/persistent GI losses or dialysis, cancer, CHF, or prior to Epo Rx, *IV iron* (Fe-sucrose, -gluconate, -dextran) should be considered.

Thalassemias (*Lancet* 2018;391:155)

- ↓ synthesis of α- or β-globin chains of Hb → ≠ subunits → destruction of RBCs and erythroid precursors; ∴ anemia from hemolysis *and* ineffective erythropoiesis
- α-thalassemia (*NEJM* 2014;371:1908): deletions in α-globin gene complex (nl 4 α genes), seen w/ Southeast Asian, Mediterranean, African, Middle East ancestry
  3 α → α-thal-2 trait = silent carrier; 2 α → α-thal-1 trait or α-thal minor = mild anemia
  1 α → HbH (β_4) disease = severe anemia, hemolysis, and splenomegaly
  0 α genes → Hb Barts (γ_4) = intrauterine hypoxia and hydrops fetalis
- β-thalassemia: mutations in β-globin gene → absent or ↓ gene product seen w/ Mediterranean (espec. Greek or Italian), African, or Asian ancestry
  1 mutated β gene → thal minor (or trait) = mild anemia (no transfusions)

  2 mutated β genes → thal intermedia (occasional transfusions) or thal major (= Cooley's anemia; transfusion dependent) depending on severity of mutations

- Special clinical manifestations: chipmunk facies, pathologic fractures, hepatosplenomegaly (due to extramedullary hematopoiesis), high-output CHF, bilirubin gallstones, Fe overload
- Dx: MCV <70, normal Fe, ferritin, MCV/RBC count <13 [Mentzer Index, 60% Se, 98% Sp; (*Ann Hem* 2007;86:486)], ± ↑ retics, basophilic stippling; Hb electrophoresis: ↑ HbA_2 ($\alpha_2\delta_2$) in β-thal; *normal* pattern in α-thal trait, ∴ PCR or supravital stain for dx
- Treatment: folate; transfusions + Fe chelator [either deferoxamine (IV) or deferasirox (PO)]; ? splenectomy if ≥50% ↑ in transfusions; consider allo-HSCT in children w/ severe β-thal; gene therapy in development (*NEJM* 2018;378:1479)

Sideroblastic anemia

- Defective heme biosynthesis within RBC precursors
- Etiologies: hereditary/X-linked (*ALAS2* mutations), idiopathic, MDS-RARS, reversible (alcohol, lead, isoniazid, chloramphenicol, copper deficiency, hypothermia)
- Special clinical manifestations: hepatosplenomegaly, iron overload syndromes
- Dx: social, work & TB hx; can be micro-, normo-, or macrocytic; variable populations of hypochromic RBCs; ↑ Fe, nl TIBC, ↑ ferritin, basophilic stippling, RBC Pappenheimer bodies (Fe-containing inclusions), ring sideroblasts (w/ iron-laden mitochondria) in BM
- Treatment: treat reversible causes; trial of pyridoxine, supportive transfusions for severe anemia with chelation therapy; high-dose pyridoxine for some hereditary cases

NORMOCYTIC ANEMIAS

Anemia of chronic inflammation (ACI; *NEJM* 2012;366:4)

- ↓ RBC production due to impaired iron utilization and functional iron deficiency from ↑ hepcidin; cytokines (IL-6, TNF-α) cause ↓ Epo responsiveness/production
- Etiologies: autoimmune disorders, chronic infection, inflammation, HIV, malignancy
- Dx: ↓ Fe, ↓ TIBC (usually normal or low transferrin sat), ± ↑ ferritin; usually normochromic, normocytic (~70% of cases) but can be microcytic if prolonged
- Coexisting iron deficiency common. Dx clues include ↓ serum ferritin levels, absence of iron staining on BM bx, ⊕ response to a trial of oral iron and/or ↑ soluble transferrin receptor/ferritin index (*Am J Clin Pathol* 2012;138:642).
- Treatment: treat underlying disease ± iron and/or erythropoiesis-stimulating agent (ESA; eg, Epo). Iron if ferritin <100 or Fe/TIBC <20%. Consider ESA if Epo <500. Avoid ESA in cancer if treatment goal is cure (*Lancet* 2009;373:1532). Transfuse PRBCs only if symptomatic & insufficient time to wait for response to Epo or underlying disease Rx.

Pure red cell aplasia

- Destructive antibodies or lymphocytes → ineffective erythropoiesis
- Associated with thymoma, CLL and parvovirus infection, autoimmunity, drugs
- Diagnostic studies: lack of erythroid precursors on BM bx, other lines normal
- Treatment: thymectomy if thymus enlarged; IVIg if parvovirus and immunosuppressed (*Clin Infect Dis* 2013;56:968); immuno-suppression/chemoRx if CLL or idiopathic; supportive care w/ PRBC transfusions; ? erythropoietin receptor agonist if due to antierythropoietin Ab (*NEJM* 2009;361:1848) consider hematopoietic cell transplantation.

MACROCYTIC ANEMIAS

includes megaloblastic and nonmegaloblastic causes

Megaloblastic anemia

- Impaired DNA synthesis → cytoplasm matures faster than nucleus → ineffective erythropoiesis and macrocytosis; due to folate or B_{12} deficiency; also in MDS
- ✔ folate and vitamin B_{12}; ↑ LDH & indirect bilirubin (due to ineffective erythropoiesis)
- Smear: neutrophil hypersegmentation, macro-ovalocytes, anisocytosis, poikilocytosis

Folate deficiency

- Folate present in leafy green vegetables and fruit; total body stores sufficient for 2–3 mo
- Etiologies: malnutrition (alcoholics, anorectics, elderly), ↓ absorption (sprue), impaired metabolism (methotrexate, pyrimethamine, trimethoprim; *NEJM* 2015;373:1649), ↑ requirement (chronic hemolytic anemia, pregnancy, malignancy, dialysis)
- Diagnosis: ↓ folate; ↓ RBC folate, ↑ homocyst. but nl methylmalonic acid (unlike B_{12} defic.)
- Treatment: folate 1–5 mg PO qd for 1–4 mo or until complete hematologic recovery; *critical to r/o B_{12} deficiency first (see below)*

Vitamin B_{12} deficiency (*NEJM* 2013;368:149)

- B_{12} present only in foods of animal origin; total body stores sufficient for 2–3 y
- Binds to intrinsic factor (IF) secreted by gastric parietal cells; absorbed in terminal ileum
- Etiologies: malnutrition (alcoholics, vegans), pernicious anemia (PA, autoimmune disease against gastric parietal cells, a/w polyglandular endocrine insufficiency and ↑ risk of gastric carcinoma), other causes of ↓ absorption (gastrectomy, sprue, Crohn's disease), ↑ competition (intestinal bacterial overgrowth, fish tapeworm)
- Clinical manifestations: neurologic changes (subacute combined degeneration) affecting peripheral nerves, posterior and lateral columns of the spinal cord and cortex → numbness, paresthesias, ↓ vibratory and positional sense, ataxia, dementia
- Dx: ↓ B_{12}; ↑ homocysteine and methylmalonic acid; anti-IF Ab; Schilling test; ↑ gastrin in

PA

- Treatment: 1 mg B_{12} IM qd × 7 d → q wk × 4–8 wk → q month for life

  neurologic abnormalities are reversible if treated w/in 6 mo

  folate can reverse *hematologic* abnormalities of B_{12} deficiency but not *neurologic* changes (and can lead to "steal" of B_{12} stores → worsening of neuro complications)

  oral supplementation (2 mg qd) appears feasible as well (*Cochrane Rev* CD004655) even w/o IF

Nonmegaloblastic macrocytic anemias

- Liver disease: often macrocytic, may see target cells, or spur cell anemia w/ hemolysis
- Alcoholism: BM suppression & macrocytosis independent of folate/B_{12} defic. or cirrhosis
- Reticulocytosis
- Other causes: hypothyroidism; MDS; meds that impair DNA synthesis (zidovudine, 5-FU, hydroxyurea, Ara-C); hereditary orotic aciduria; Lesch-Nyhan syndrome

HEMOLYTIC ANEMIAS

Causes of Hemolytic Anemia by Mechanism (*Lancet* 2000;355:1169 & 1260)			
Location	**Mechanism**	**Examples**	**Mode**
Intrinsic	Enzyme deficiency	*G6PD* deficiency	**Hereditary**
	Hemoglobinopathies	Sickle cell anemia, thalassemia	
	Membrane abnormalities	Hereditary spherocytosis	
		PNH, spur cell anemia in liver disease	**Acquired**
Extrinsic	Immune-mediated	Autoimmune; drug-induced, tx rxn	
	Traumatic	MAHA; prostheses (valves, TIPS)	
	Direct infections, toxins	Malaria, babesiosis; snake & spider venoms; Wilson's; hypotonic infusions	
	Entrapment	Hypersplenism	

Diagnostic evaluation

- ↑ reticulocyte count (RI >2%), ↑ LDH, ↓ haptoglobin (83% Se, 96% Sp), ↑ indirect bili
- Autoimmune hemolysis: Coombs' test = direct antiglobulin test (DAT) → ⊕ if agglutination occurs when antisera against Ig or C3 are applied to patient RBCs
- Intravascular: ↑↑ LDH, ↓↓ haptoglobin; hemoglobinemia, hemoglobinuria, hemosiderinuria
- Extravascular: splenomegaly
- Family h/o anemia; personal or family h/o cholelithiasis

Glucose-6-phosphate dehydrogenase (G6PD) deficiency (*Lancet* 2008;371:64)

- X-linked defect of metabolism (*G6PD* mutations) w/ ↑ susceptibility to oxidative damage
- Most common in ♂ of African or Mediterranean descent (malaria-endemic areas)
- Hemolysis precipitated by drugs (sulfonamides, dapsone, nitrofurantoin, rasburicase, primaquine, doxorubicin, methylene blue), infxn, DKA, foods (favism, *NEJM* 2018;378:60)
- Diagnosis: smear may show RBC Heinz bodies (oxidized Hb) that result in bite cells once removed by spleen; ↓ G6PD levels (*may be normal after acute hemolysis* because older RBCs have already lysed and young RBCs may still have near-normal levels)

Sickle cell anemia (*NEJM* 2017;376:1561 & *Lancet* 2017;390:311)

- Recessive β-globin mutation → structurally abnl hemoglobin (HbS). ~8% African Americans heterozygotes ("sickle trait"; usually w/o sx); ~1/400 homozygotes (sickle cell disease).
- ↓ O_2 → HbS polymerizes → RBC sickles, ↓ RBC deformability → hemolysis & microvascular occlusion due to endothelial activ. & PMN adhesion (*Blood* 2013;122:3892)
- Anemia: chronic hemolysis ± acute aplastic (parvo. B19) or splenic sequestration crises
- Vaso-occlusion & infarction: acute chest syndrome & stroke (high mortality), pulmonary HTN, painful crises, splenic sequestration, renal papillary necrosis, aseptic necrosis, dactylitis (hand-foot syndrome), priapism
- Infection: splenic infarction → overwhelming infection by encapsulated organisms; infarcted bone → osteomyelitis (*Salmonella*, *Staph. aureus*), can be life threatening
- Diagnosis: sickle-shaped RBCs and Howell-Jolly bodies on smear; Hb electrophoresis
- Treatment: hydroxyurea, folic acid; ? L-glutamine to prevent pain crises (*NEJM* 2018;379:226)
- Vaccines: pneumo, meningo, H flu, HBV
- Voxelotor (Hbs polymerization inhib) ↑ hemolysis to ↑ Hb (*NEJM* 2019;epub)
- Pain & vaso-occlusive crises: analgesia (consider PCA), IVF, transfusion if sx & Hgb < baseline; crizanlizumab (anti-P-selectin; *NEJM* 2017;376:429)
- Acute chest: O_2, abx, IVF, exchange transfusion
- TIA/stroke: often exchange transfusion (goal Hgb 10) ± thrombolytics
- Gene therapy in development (*NEJM* 2017;376:848)

Hereditary spherocytosis (HS) (*Lancet* 2008;372:1411)

- Defect in a cytoskeletal protein of RBC membrane → membrane loss mutations in ankyrin, α- and β-spectrin, band 3, and pallidin have been identified
- Most common in N. European populations (1/5000 births); ⊕ FHx (75% of Pts)

- Anemia, jaundice (mostly neonates), splenomegaly, pigmented gallstones
- Diagnosis: spherocytes on smear, ⊕ osmotic fragility test (~80% Se), ↓ eosin-5-maleimide (EMA) binding (93% Se; 99% Sp; *Haemat* 2012;97:516), acidified glycerol lysis test (Se 95%)
- Treatment: folate, transfusions, splenectomy for moderate and severe HS (balance w/ ↑ risk of future thrombosis and infection; *J Thromb Haemost* 2008;6:1289)

Autoimmune hemolytic anemia (AIHA)

- Acquired, antibody-mediated RBC destruction
- Warm AIHA: IgG Abs opsonize RBCs *at body temp* → removal by spleen
  Etiologies: idiopathic, lymphoproliferative (CLL, NHL), autoimmune (SLE), drugs, HIV, Babesiosis (*NEJM* 2017;376:939)
- Cold AIHA: IgM Ab binds to RBCs *at temp <37°C* → complement fixation → intravascular hemolysis and acrocyanosis on exposure to cold
  Etiologies: idiopathic, lymphoprolif. disorders (eg, Waldenström's; monoclonal), *Mycoplasma pneumoniae* infxn and infectious mononucleosis (polyclonal)
- Diagnosis: spherocytes on smear, ⊕ Coombs'; ✔ cold agglutinin titer, splenomegaly
- Treatment (*Blood* 2017;129:2971): treat underlying disease
  Warm AIHA: corticosteroids ± splenectomy, IVIg, cytotoxic agents, rituximab
  Cold AIHA: avoid cold; steroids ineffective; rituximab (*Blood* 2004;103:2925)

Drug-induced hemolytic anemia

- Acquired, Ab-mediated, RBC destruction precipitated by a med. Abx: ceph., sulfa drugs, rifampin, ribavirin. CV: methyldopa, procainamide, quinidine, thiazides. TCAs, phenothiazines, NSAIDs, sulfonylureas, MTX, 5-FU, rasburicase (G6PD defic.)
- Diagnosis: Coombs' usually negative, ↑ LDH; Treatment: discontinue offending agent

Microangiopathic hemolytic anemia (*MAHA*; *NEJM* 2014;371:654)

- Intra-arteriolar fibrin damages RBCs → acquired intravascular hemolysis
- Etiologies: hemolytic-uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), disseminated intravascular coagulation (DIC), malignancy, malignant HTN, eclampsia/HELLP, mech. cardiac valves, infected vascular prostheses
- Diagnosis: schistocytes ± thrombocytopenia ± abnormalities a/w specific disorders (eg, ↑ PT in DIC, ↑ Cr in HUS, ↑ LFTs in HELLP)
- Rx underlying dx; urgent plasma exchange w/ TTP (replace low ADAMTS13)

Hypersplenism

- Stasis/trapping in spleen → Mφ attack & remodeling of RBC → spherocytosis → hemolysis

Causes of Splenomegaly	
Etiology	Comments*
RES hyperplasia	Hemolytic anemia, sickle cell disease, thalassemia major
Immune hyperplasia	Infxn [HIV, EBV, CMV, TB, malaria, kala azar ("black water fever" from visceral leishmaniasis), *Mycobacterium avium* complex], autoimmune disorders (SLE, RA w/ Felty's syndrome), sarcoidosis, serum sickness
Congestion	Cirrhosis, CHF, portal/splenic vein thrombosis, schistosomiasis
Infiltration (nonmalignant)	Lysosomal storage disorders (Gaucher's, Niemann-Pick), glycogen storage diseases, histiocytosis X, splenic cysts
Neoplasm	MPN (CML, PMF, PV, ET), CMML, leukemia, lymphoma (NHL, HL, hairy cell leukemia, CLL, PLL, WM), T-LGL, myeloma, amyloid

RES = reticuloendothelial system; *boldface = causes of massive splenomegaly.

PANCYTOPENIA

Etiologies

- Hypocellular bone marrow (nl cellularity ~100 – age): aplastic anemia, hypoplastic MDS
- Cellular bone marrow: MDS, aleukemic leukemia, PNH, severe megaloblastic anemia
- Marrow replacement (myelophthisis): myelofibrosis, metastatic solid tumors, granulomas
- Systemic diseases: hypersplenism, sepsis, alcohol, toxins

Clinical manifestations

- Anemia → fatigue
- Neutropenia → recurrent infections
- Thrombocytopenia → mucosal bleeding & easy bruisability

Aplastic anemia = stem cell failure (*NEJM* 2015;373:35)

- Epidemiology: 2–5 cases/10^6/y; biphasic (major peak in adolescents, 2nd peak in elderly)
- Diagnosis: pancytopenia w/ ↓ retics, BM bx w/ cytogenetics showing hypocellularity
- Etiologies: idiopathic ($^1/_2$ – $^2/_3$ of cases)

  Stem cell destruction: radiation, chemotherapy, chemicals (eg, benzene)

  Idiosyncratic med rxn (eg, chloramphenicol, NSAIDs, sulfa drugs, gold, carbamazepine, antithyroid)

  Viruses (HHV-6, HIV, EBV, parvovirus B19); post-viral hepatic failure (not Hep A/B/C)

  Immune disorders (SLE, GVHD post-HSCT, thymoma)

  PNH (see below); Fanconi's anemia (congenital disorder w/ pancytopenia, macrocytic anemia, ↑ risk of MDS, AML, & SCC of head & neck, and multiple physical anomalies)

  Shortened telomeres: seen w/ telomerase (*TERT, TERC*) mut. (10% of aplastic anemia), dyskeratosis congenita/DKC1 mut; a/w IPF, cirrhosis (*NEJM* 2009;361:2353)

  Somatic mutations: PNH clones in ~50% of aplastic anemia (*Haematologica* 2010;95:1075)

Paroxysmal nocturnal hemoglobinuria (PNH) (*Blood* 2009;113:6522)

- Acquired clonal stem cell disorder = inactivating somatic mutation of *PIG-A* gene → deficiency of GPI-anchor for CD55 & CD59 (inhib of complement) → complement-mediated RBC lysis, plt aggreg., & hypercoagulability
- Clinical: intravascular hemolytic anemia, hypercoagulability (venous > arterial; esp. intraabdominal, cerebral), smooth muscle dystonias, deficient hematopoiesis (cytopenias); a/w aplastic anemia, MDS and evolution to AML
- Dx: flow cytometry (↓ CD55 & CD59) on RBCs and granulocytes; urine hemosiderosis
- Treatment: supportive care (iron, folate, transfusions); consider anticoagulation
  allogeneic HSCT for hypoplasia or severe thrombosis
  eculizumab (Ab inactivates terminal complement C5s): ↓ hemolysis, improves QoL & stabilizes Hb levels (*NEJM* 2004;350:552 & 2006;355:1233; *Lancet* 2009;373:759); effective in pregnancy (*NEJM* 2015;373:1032); must have meningococcal vaccination

End of lecture