Diagnostic approach to Anemia

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Outline

- Basic hematological parameters
- Discuss types of anemia and their clinical manifestations
- Talk about the relevant diagnostic tests

Basic hematologic lab tests

- Complete blood count (CBC)
  - Amount of hemoglobin
  - Number, size, and shape of red blood cells (RBCs)
  - Number of white blood cells (WBCs) and platelets
  - +/- automated WBC differential
- Manual differential/manual peripheral smear review
  - Abnormalities that fall outside of established parameters result in manual review

Complete blood count (CBC)

- Hemoglobin (g/dL) Amount of oxygen carrying protein
- Hematocrit (%) % of blood volume occupied by RBCs
- RBC count (M/uL) # of RBCs
- MCV (fL) mean cell volume
- MCH (pg) mean cell hemoglobin
- MCHC concentration (g/dL) mean cell hemoglobin
- RDW (%) red cell distribution width
- WBC (K/uL) # of WBCs
- Platelet count (K/uL) # of platelets

Measuring RBC parameters

- Hemoglobin (Hb)
  - Measured directly as absorbance of cyanomethemoglobin
- RBC count (RBC)
  - Measured directly by impedance
- Hematocrit (Hct)
  - Measured by centrifugation; ratio of volume of RBCs to volume of whole blood
  - Can also calculate (MCV x RBC)
- MCV
  - Measured by mean height of voltage pulses in an impedance counter
  - Can also calculate (Hct / RBC)
- MCH = Hb / RBC
- MCHC = Hb / Hct

Definition of anemia

- From Greek meaning "without blood"
- Condition where capacity of blood to transport oxygen to tissues is reduced
  - Decreased hemoglobin, RBC count, and hematocrit
- Anemia is not a disease but a *manifestation* of disease
- Treatment depends on discovering underlying cause

Evaluating anemia in the lab

Basic information

- Size of red blood cells: (small/ normal/ big)
- Abnormal cells on microscopic examination
- Status of leukocytes and platelets
- Reticulocyte count (ability of marrow to respond to anemia)
- Evidence of destruction (elevated LDH, indirect bilirubin)

A practical approach to anemia

Size of RBCs

- MCV (Mean Cell Volume)

Microcytic	< 80	fl
Normocytic	80-100	fl
Macrocytic	> 100	fl

Differential diagnosis of microcytic anemia

- Iron deficiency
- Anemia of chronic disease
- Thalassemias
- Hemoglobinopathies
- Hereditary spherocytosis
- Hereditary X-linked sideroblastic anemia
- Lead poisoning (usually mild microcytosis)

Iron deficiency anemia

- Common nutritional deficiency
- Bleeding is a leading cause of iron deficiency anemia
- Iron facts
  - Body iron:
    - 80% functional (Hgb, myoglobin, cytochromes, etc.)
    - 20% storage
  - Absorption: primarily in the duodenum
  - Transferrin: transports iron in blood
  - Ferritin: storage form of iron
  - Hemosiderin: derived from ferritin, long-term storage of iron

Lab studies in iron deficiency anemia

- Microcytic, hypochromic anemia
  - Decreased MCV, MCH, & MCHC
- Iron studies
  - Low serum iron
  - High total iron binding capacity (TIBC, transferrin concentration)
  - Low % transferrin saturation
  - Low ferritin
  - Decreased bone marrow storage iron (hemosiderin)

Thalassemia

- Hemoglobin is a tetramer; with two alpha and two beta
- Due to abnormally low production of alpha or beta-globin chains: named for the chain which is decreased or absent
- + Indicates diminished, but some production of globin chain still happens: e.g. β^{+}
- 0 Indicates complete absence of production production of globin chain by gene: e.g. β^{0}

Demographics: Thalassemia

- Found most frequently in the Mediterranean, Africa, Western and Southeast Asia, India and Burma
- Distribution parallels that of Plasmodium falciparum

Distinguishing features between iron def (IDA) and thalassemia

- Mentzer index: MCV/RBC <13 favors thalassemia
- England and Fraser Index: MCV– (5 $\times$ Hemoglobin)

Distinguishing features between iron def and thalassemia

- The RBC count in thalassemia is more than 5.0 x 106/µL (5.0 x 1012/L) and in IDA is less than 5.0 x 106/µL (5.0 x 1012/L)
- MCV usually less than 70 in TT, more than 70 in IDA
- The red cell distribution width (RDW) in IDA is more than 17% and in TT is less than 17%.

Anemia of chronic disease

- Mild to moderate anemia due to increased hepcidin, leading to iron sequestration
  - Body unable to use iron stores
- Mildly microcytic or normocytic anemia
- Etiologies: chronic immune activation
  - Chronic infections
  - Collagen vascular disease
  - Malignancy

Macrocytic Anemia

- Non-megaloblastic
  - Liver disease
  - Myelodysplastic syndrome
  - Increased reticulocyte count
    - Hemorrhage
- Megaloblastic
  - Vitamin B12 deficiency
  - Folic acid deficiency

Anemia Due to Folate or Vitamin B_{12} (Cobalamin) Deficiency

- Folate and cobalamin required for DNA synthesis
- Deficiency results in megaloblastic anemia due to impaired DNA replication
  - Impaired nuclear development but abundant cytoplasm (nuclear-cytoplasmic asynchrony)
  - Large marrow progenitors
- Similar clinical features* in peripheral blood and marrow morphology in folate and cobalamin deficiency

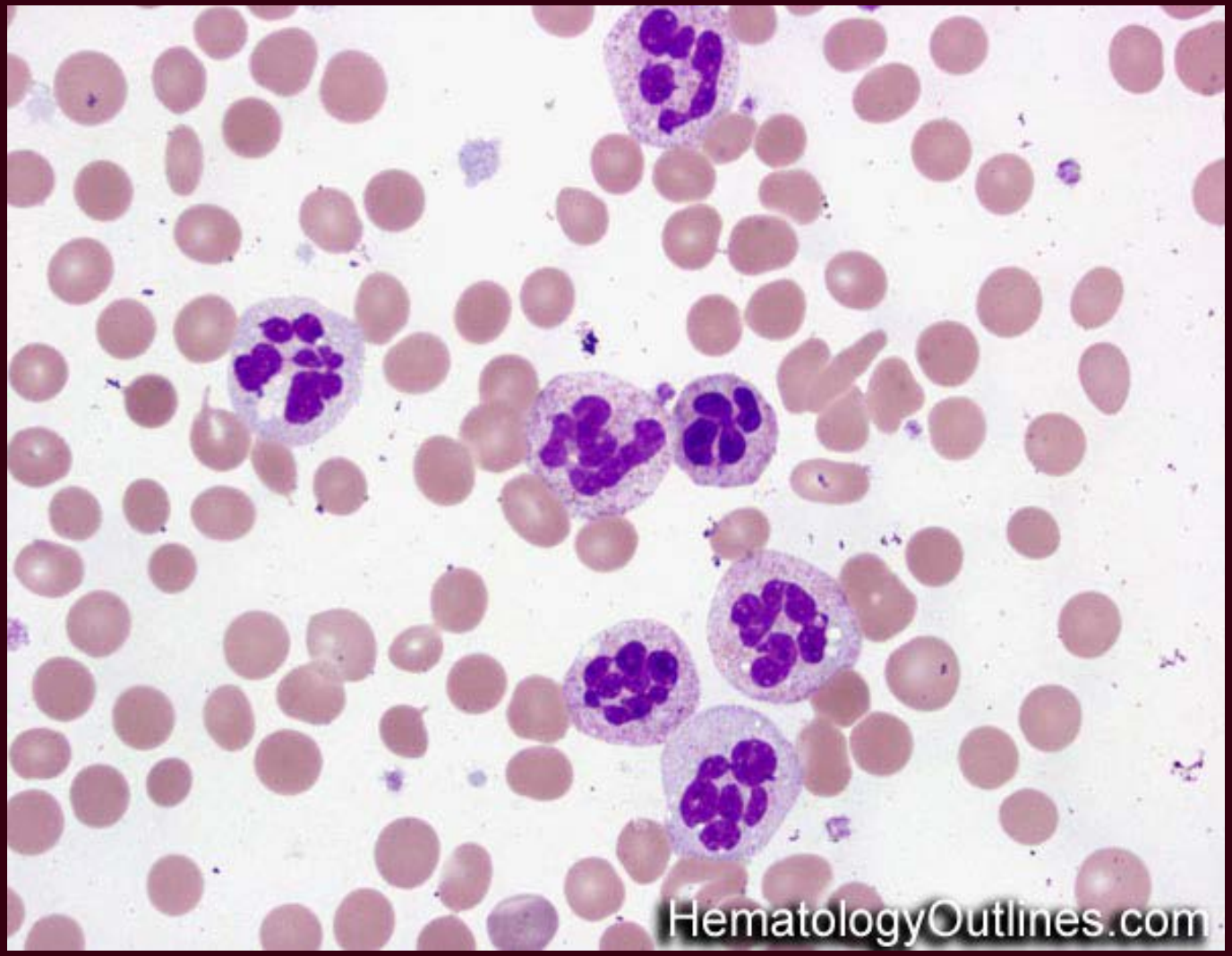
* Exception: Neurologic abnormalities in B_{12} deficiency

Folate and Cobalamin Deficiency

Clinical and Laboratory Findings

- Non-specific signs and symptoms of anemia
- Macrocytic anemia
- Relatively low reticulocyte count
- Hypersegmentation of neutrophils
- Mild thrombocytopenia and/or neutropenia
- Megaloblastic changes in marrow
- Neurological findings (B_{12} deficiency only): loss of position sense, ataxia, psychomotor retardation, seizures

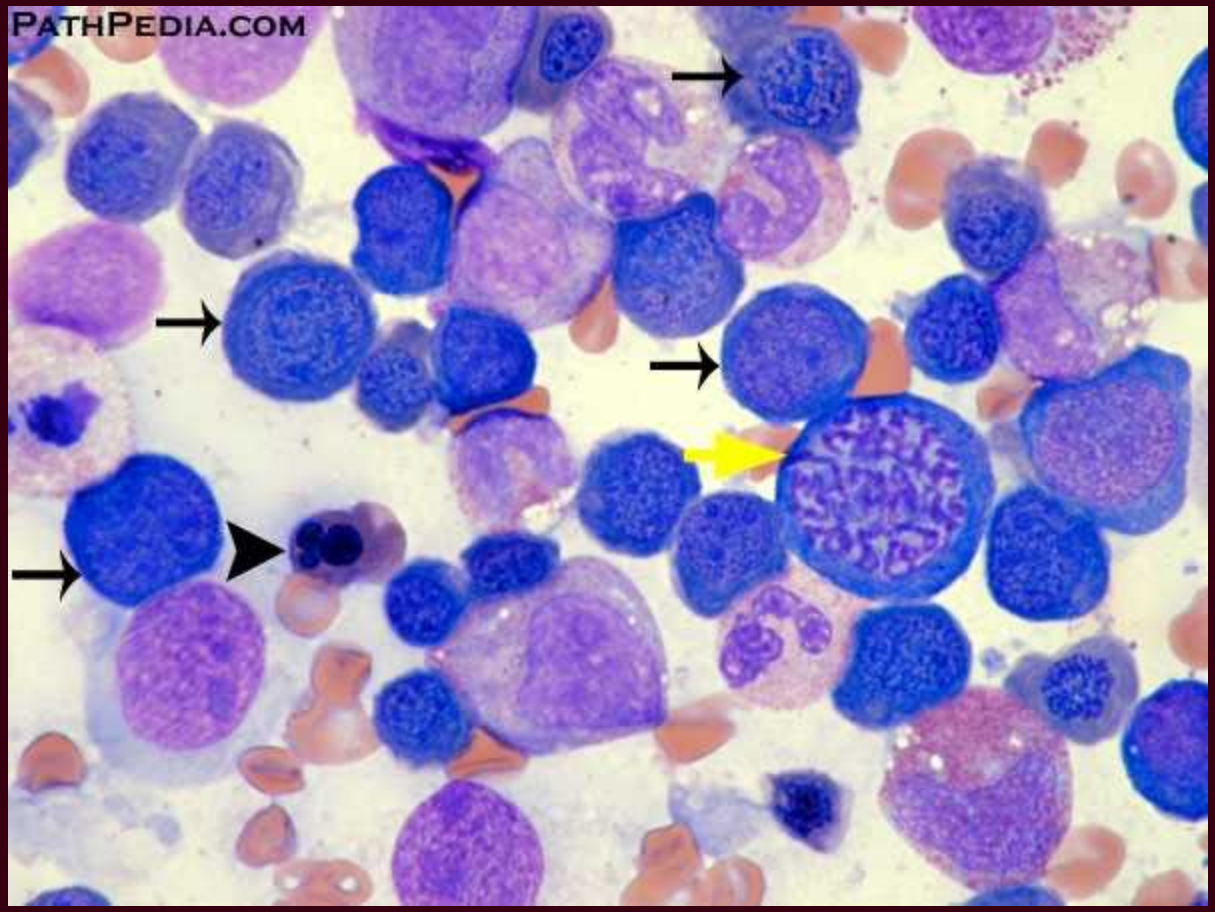
Hypersegmented neutrophils



Peripheral smear of a Megaloblastic Anemia Patient

http://hematologyoutlines.com/atlas_topics/137.html

Bone Marrow Picture of Megaloblastic Anemia



Small black arrows (erythroid hyperplasia),Yellow arrow(megaloblastic chage) and Arrohead shows the dysplasia

http://www.pathpedia.com/education/eatlas/histopathology/bone_marrow/megaloblastic_anemia.aspx

Normocytic Anemia

Differential Diagnosis of Normocytic Anemia

Increased reticulocytes

- Hemolytic anemia
- Post-hemorrhagic anemia

Decreased reticulocytes

- Anemia of chronic disorders
- Endocrine disease
- Renal disease
- Liver disease
- Hypoplastic anemia
- Marrow infiltration
  - Leukemia
  - Myeloma
  - Myelofibrosis
  - Metastases
- Myelodysplastic Syndrome

Evaluation of Normocytic Anemia

- PB smear, reticulocyte count
- Screen for liver, endocrine, renal disease
- Iron studies
- Bone marrow biopsy

Hemolytic Anemia

- Inherited hemolytic anemia
- Acquired hemolytic anemia

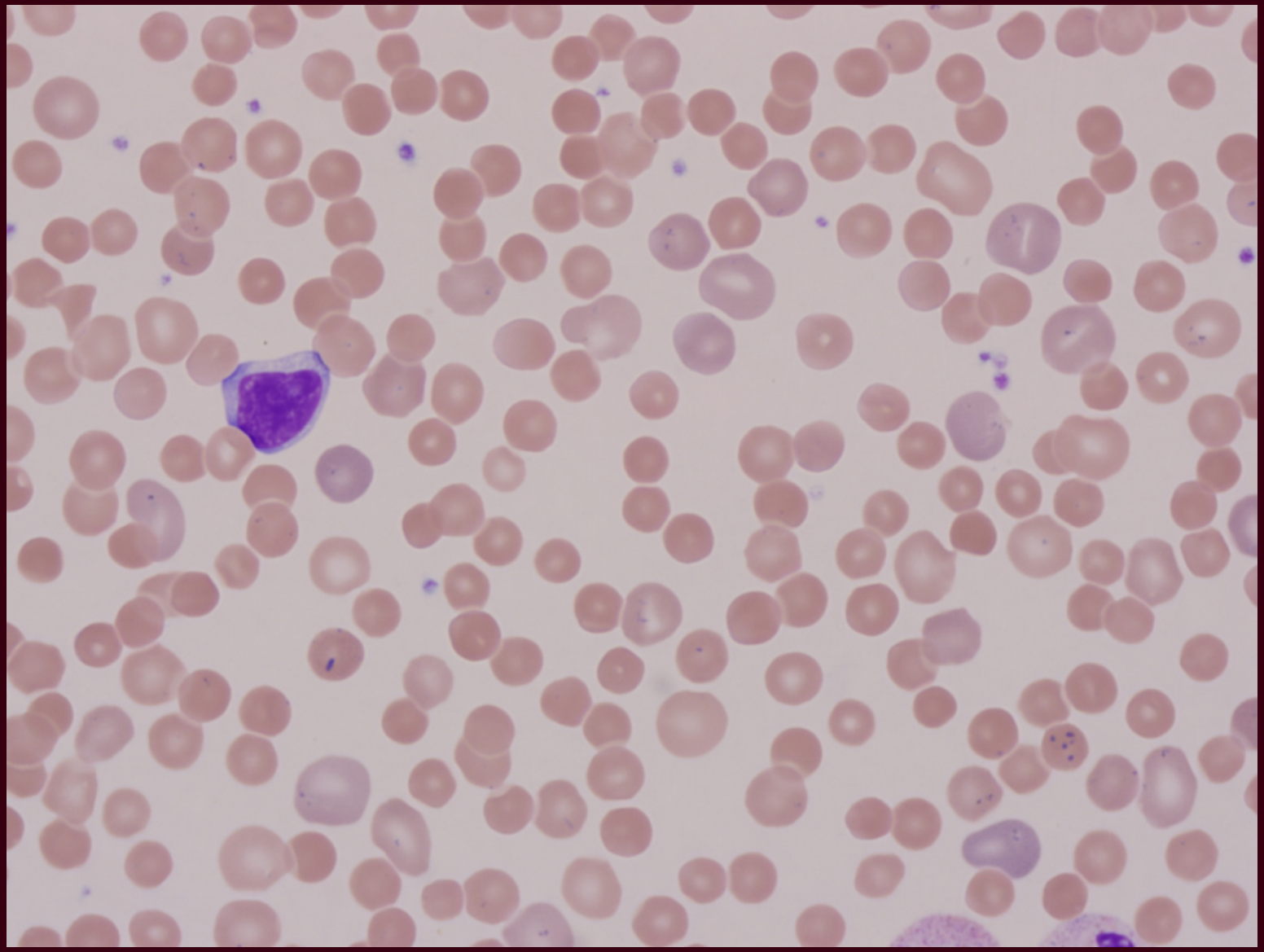
Hemolytic Anemia

- Inherited hemolytic anemia
  - Membrane defects (eg. hereditary spherocytosis)
  - Globin defects (eg. Sickle cell anemia)
  - Metabolic disorders
    - Glucose-6 phosphate deficiency
    - Pyrimidine 5′-nucleotidase deficiency (basophilic stippling).

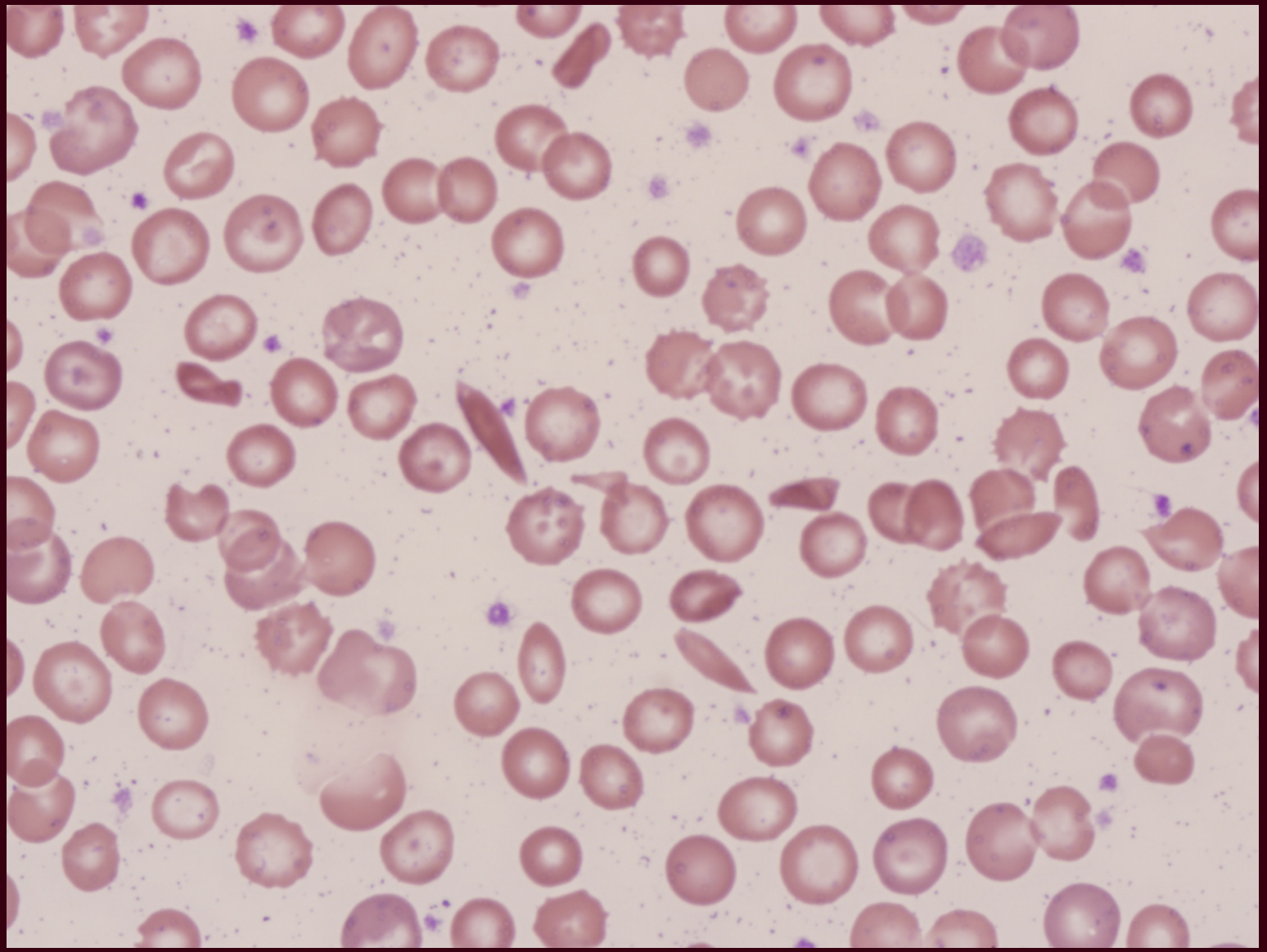
Hereditary Spherocytosis

- Most common hereditary hemolytic anemia
- Spherocytes--microcytic, abnormal osmotic fragility
- May have broad RDW, but normal to low MCV and usually increased MCHC, depending on the degree of reticulocytosis
- Autosomal dominant inheritance (75%)
- Mutations in various structural membrane proteins
- "Cured" by splenectomy

Hereditary Spherocytosis



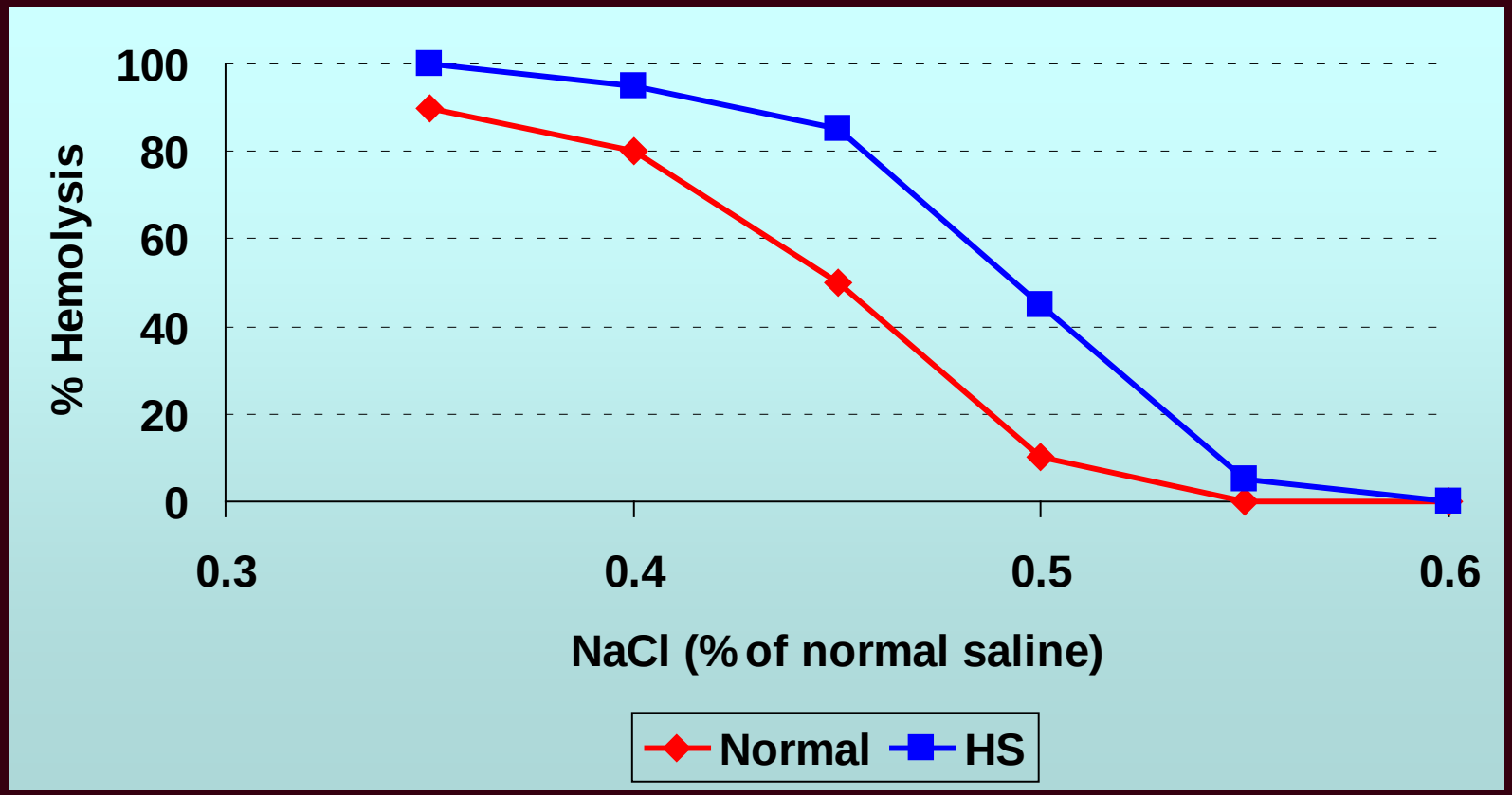
Sickle Cell Anemia



Diagnosis of HS

- Peripheral blood smear- Spherocytes
- Osmotic fragility
  - a laboratory test used in the diagnosis of HS, is sensitive but not specific. The test measures the *in vitro* lysis of RBCs suspended in solutions of decreasing osmolarity. Spherocytes are characterized by membrane loss and less redundancy to withstand

Osmotic Fragility Test



Diagnosis Discontinued!

- Flow cytometry
  - Greater than 95% sensitive and specific for HS
  - Labels patients RBCs with EMA (eosin-5-maleimide)
  - EMA binds specifically with band 3 protein
  - EMA binding is affected by all sorts of membrane protein abnormalities, not just band 3 deficiency

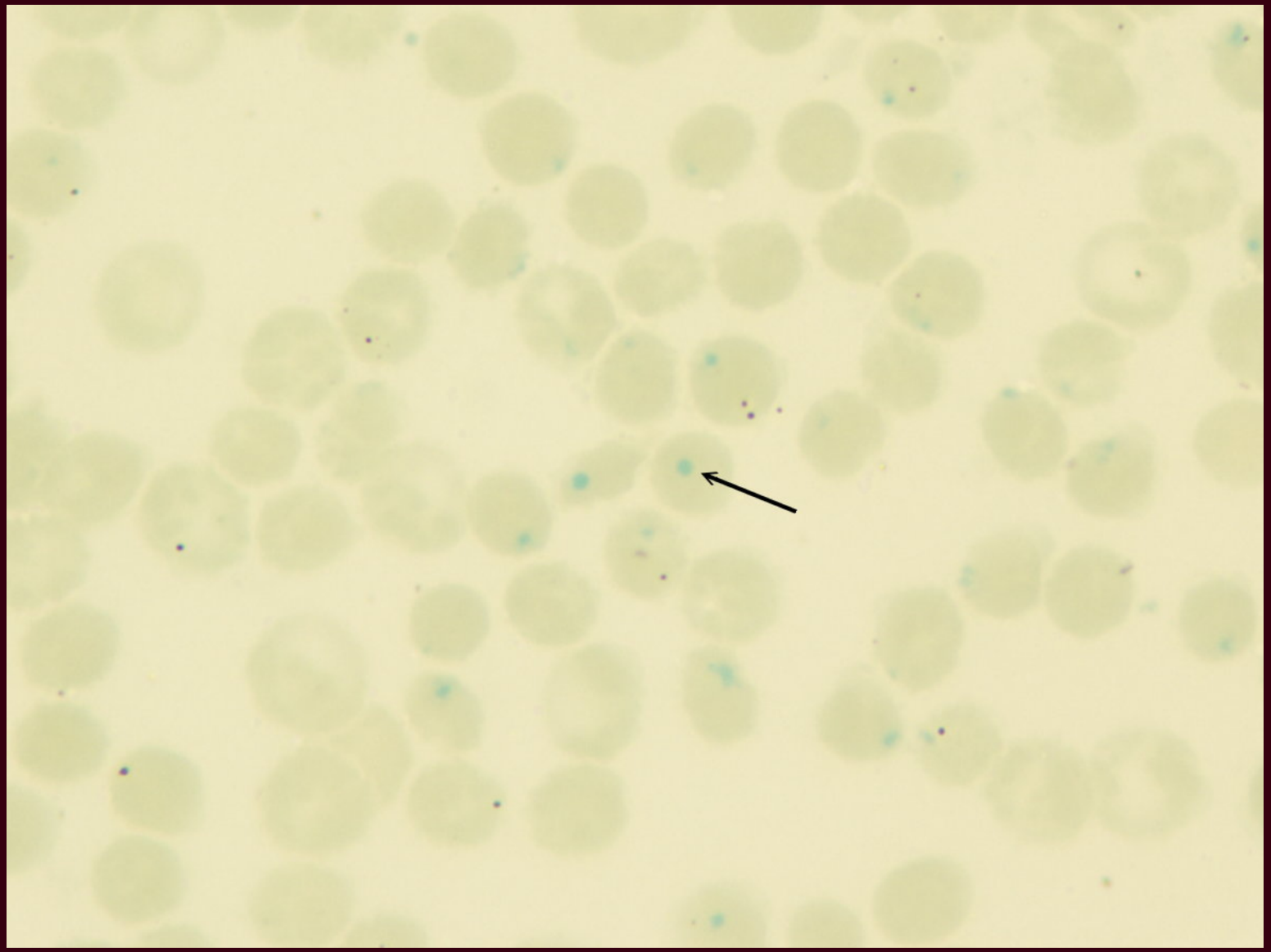
Glucose-6-Phosphate Deficiency (G6PD)

- Catalyzes the initial step in the pentose phosphate pathway
- X-linked; more than 300 variants identified
- 130,000,000 probably carry a mutant gene
  - Up to 20-30% of Africans
  - Up to 35% in Sardinia
  - Also seen in Asians

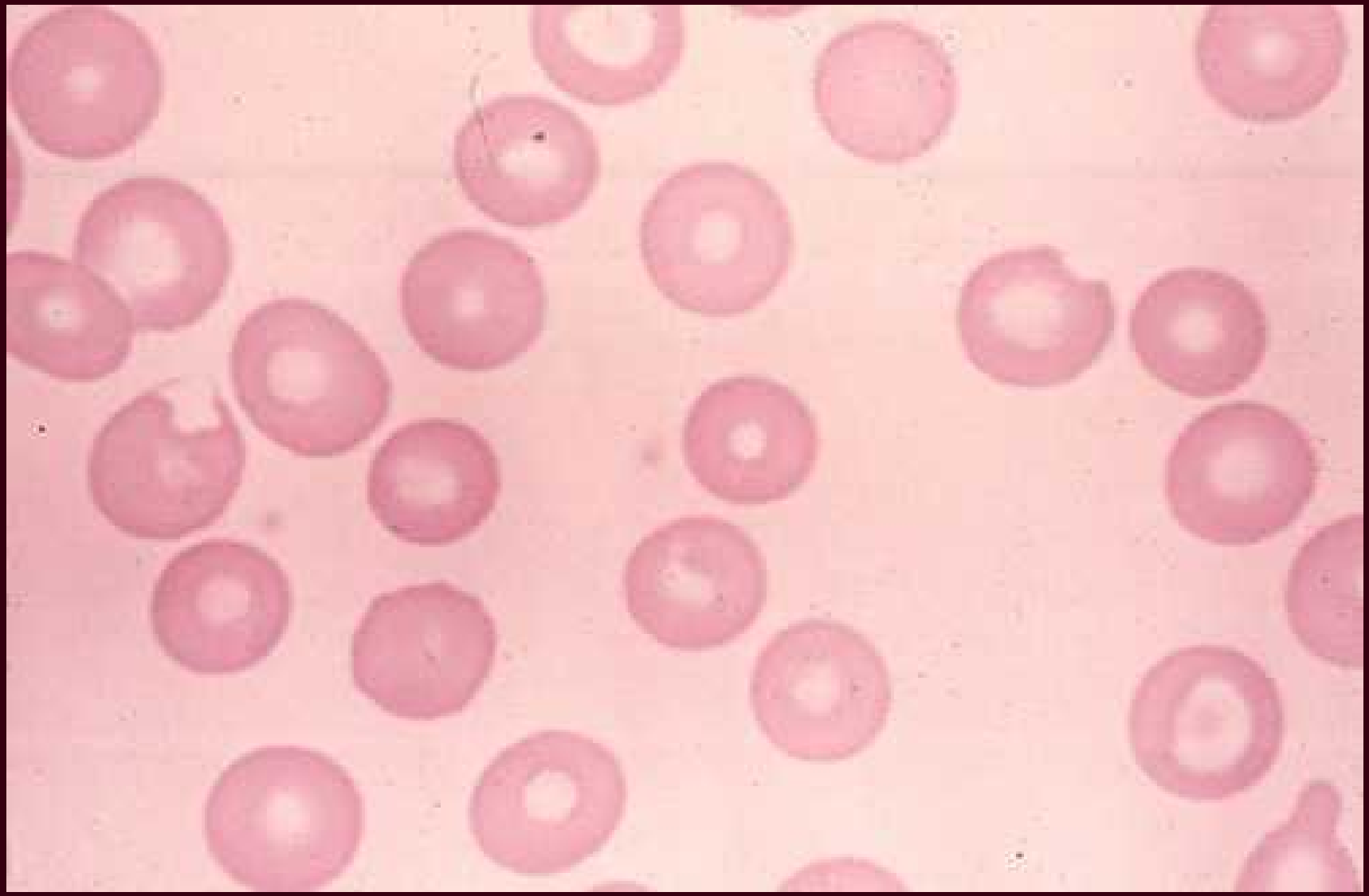
Glucose-6-Phosphate Deficiency (G6PD)

- Two types of hemolytic anemia
  - Acute, acquired hemolytic anemia
    - Associated with exposure to primaquine, sulfa drugs
  - Chronic mild hemolytic anemia (common in Africans, Caucasians, as compared to Mediterranean's)

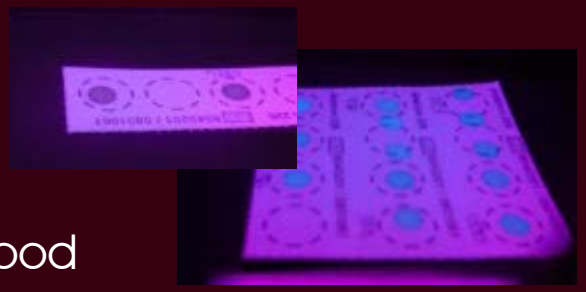
G6PD—Heinz Bodies



G6PD—Bite Cells



Tests for G6PD deficiency



- Fluorescent spot test
  - Quick and cheap
  - Detects generation of NADPH from NADP+
  - G6P and NADP added to a drop of patient blood
  - Blood spot fluoresces at 340 nm if NADPH is generated
  - Only detects severe G6PD deficiency (enzyme levels below 30%)
- Enzyme activity assay (spectrophotometric assay)
- *Caveats for non-PCR-based tests:
  - don't perform right after a hemolytic episode or blood transfusion!
  - May not detect heterozygous females or mild deficiencies
- Mutation testing by PCR
  - Useful in families with known mutation
  - Prenatal diagnosis
  - Also useful in targeted screening of populations with high frequency of common mutations, such as G6PD A^- in Africans & African-Americans

Hemolytic Anemia

- Acquired hemolytic anemia
  - Immune mediated
  - Microangiopathic hemolytic anemia
  - Associated with infections
  - Paroxysmal nocturnal hemoglobinuria

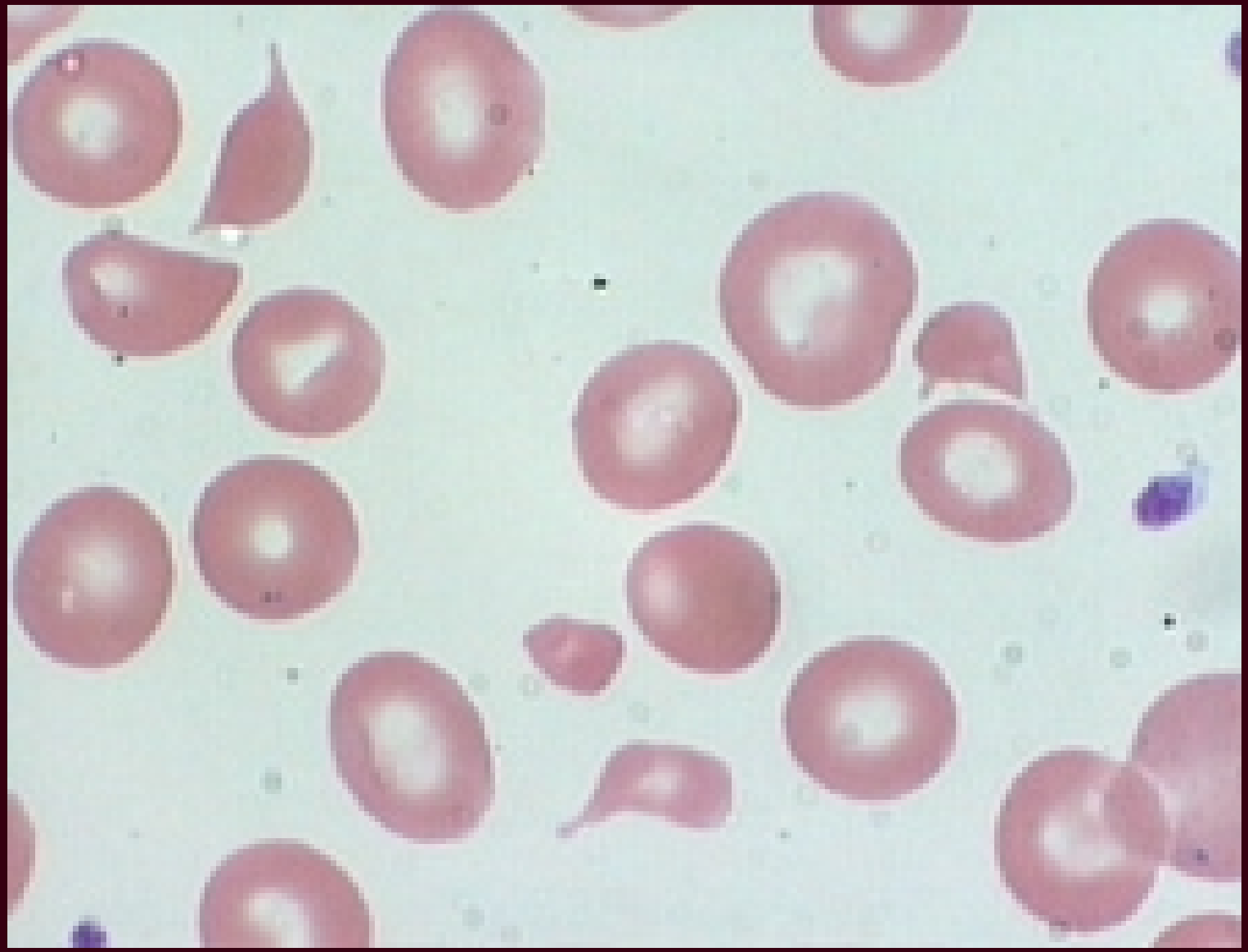
Immune-mediated Hemolytic Anemia

- Premature destruction of red blood cells due to acquired antibodies directed against red cell antigens
- Direct Coombs test: detection of immunoglobulin and/or complement molecules on the surface of red blood cells.
- Indirect Coombs test: incubates normal red blood cells with patient serum, searching for unbound red cell antibody in the patient serum.

Microangiopathic Hemolytic Anemia

- Characterized by red blood cell fragments in the peripheral blood (schistocytes)
- Differential Diagnosis:
  - Thrombotic thrombocytopenic purpura
  - HUS (renal, shiga toxin, E. coli O157)
  - Disseminated carcinoma (Mucinous adenoca)
  - DIC
  - Malignant hypertension
  - Giant hemangiomas
  - March hemoglobinuria
  - Drugs (mitomycin-C)

Microangiopathic Hemolytic Anemia



(www.dcss.cs.amedd.army.mil)

Microangiopathic Hemolytic Anemia

- March hemoglobinuria
  - Disorder of transient hemoglobinemia or hemoglobinuria due to forceful contact of body with hard surface
  - Reported in
    - Prolonged marches
    - Competitive running
    - Conga drumming
    - Karate
    - Jack hammer operators

Hemolytic Anemia Associated With Infection

- Clostridium sepsis: may be severe, overwhelming
  - Urgent identification and treatment is necessary
  - Lecithinase
- Malaria (Blackwater fever)
- Bartonella bacilliformis
- Babesia microcoti
- Trypanosomiasis
- Mycoplasma pneumonia (IgM against I antigen)
- Infectious mononucleosis (IgM against i antigen)

Paroxysmal Nocturnal Hemoglobinuria

- PNH characterized by pallor (anemia), dark urine at night, venous thrombi (especially large vessels)
- Acquired clonal disorder, mutation in the PIGA gene
- Defective synthesis of GPI-linked proteins
- Detected by flow cytometry done on both RBC and WBC initially
- Treatment with eculizumab—monoclonal Ab against C5
- Related to aplastic anemia and MDS

Questions!!!

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