Hematology

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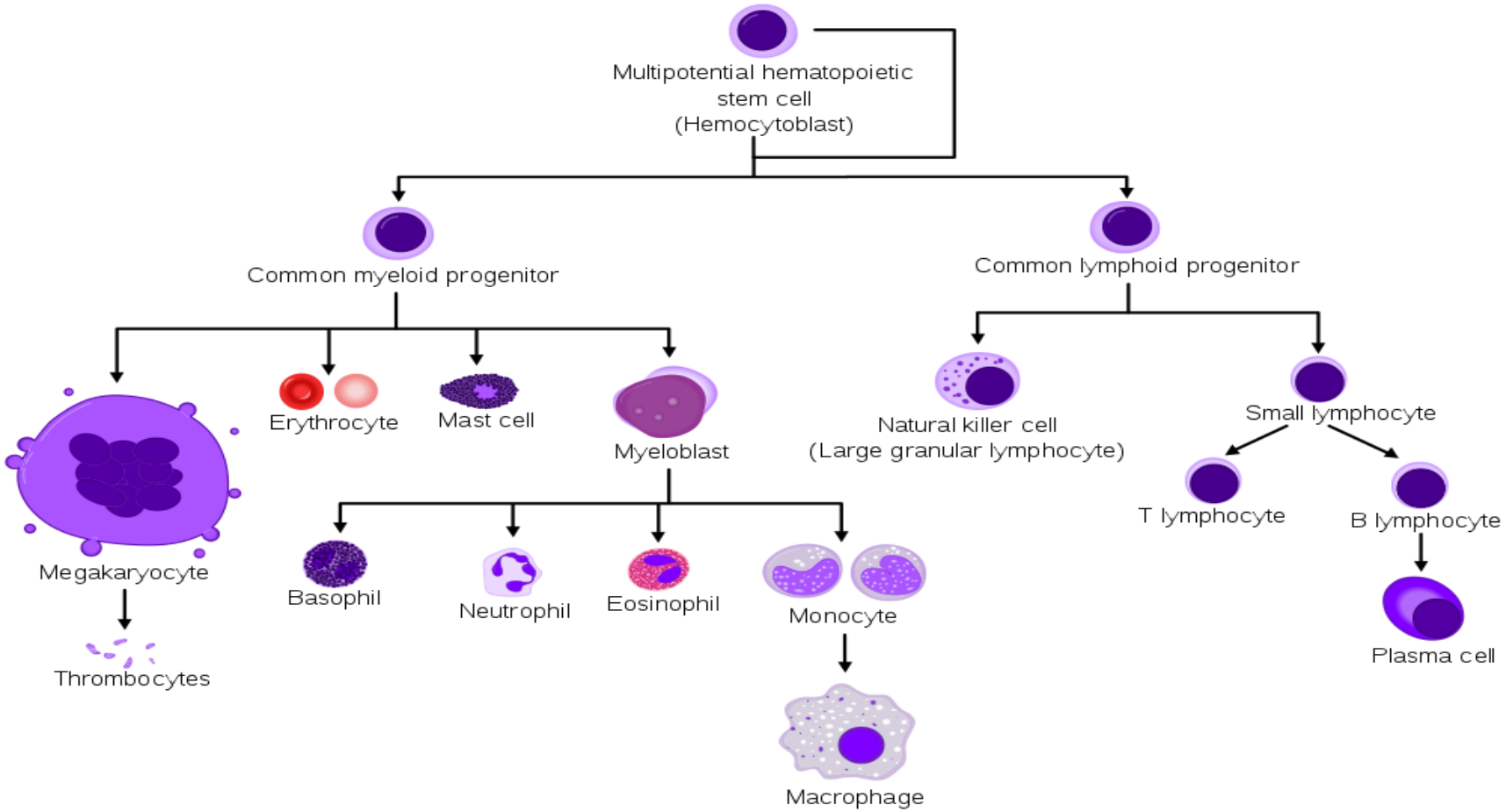
Hematopoiesis

- Hematopoiesis begins by 3 weeks of gestation with **erythropoiesis** in the yolk sac. By 2 months' gestation, the primary site of hematopoiesis migrates to the liver. By 5 to 6 months' gestation, the process shifts from the liver to the bone marrow.

- During infancy, virtually all marrow cavities are actively hematopoietic and the proportion of hematopoietic to stromal elements is quite high.

- As the child grows, hematopoiesis moves to the central bones of the body (vertebrae, sternum, ribs, and pelvis), and the marrow is gradually replaced with fat.


Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Thrombocytes
Erythrocyte
Mast cell
Myeloblast
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell

The normally high hemoglobin level of the fetus is a result of fetal erythropoietin production in the liver in response to low Po2 in utero.

- After birth, the anatomic site of EPO production shifts to the kidney.

- Embryonic hemoglobin's are produced during yolk sac erythropoiesis, then replaced by fetal hemoglobin (hemoglobin F, $\alpha 2\gamma 2$) during the hepatic phase.

- During the third trimester, gamma chain production gradually diminishes, replaced by beta chains, resulting in hemoglobin A ($\alpha 2\beta 2$).

- During the first few months of postnatal life, rapid growth, shortened RBC survival, and cessation of erythropoiesis cause a gradual decline in hemoglobin levels, with a nadir at 8 to 10 weeks of life.

- This so-called physiologic nadir is accentuated in premature infants.

- By 6 months of age in healthy infants, only trace gamma chain synthesis occurs.

- Hemoglobin is a tetramer of 4 globin chains (2 alpha (α) and 2 beta (β) polypeptide chains) with an iron-containing porphyrin ring called *heme* bound to each chain.

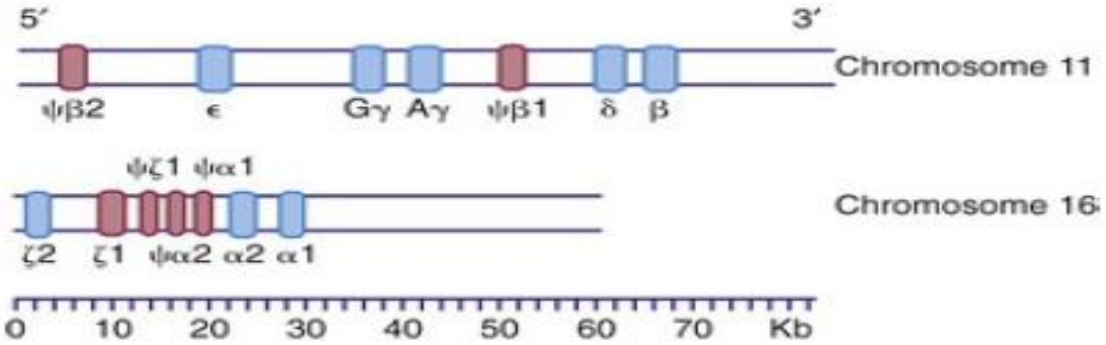
- A dynamic interaction between heme and globin gives hemoglobin its unique properties in the reversible transport of oxygen.

- The α-globin and β-globin gene clusters are located on chromosome 16 and 11, respectively.

- There are 4 α-globin genes and 2 β-globin genes.

Red Cell Life Span in the Fetus and Neonate

- The average erythrocyte life span in normal adults is approximately 120 days.
- The life span of fetal/neonatal erythrocytes was once estimated to be considerably less, with an average of 60- 90 days.



Embryonic:	Gower 1	ζ_2 ϵ_2
	Gower 2	α_2 ϵ_2
	Portland	ζ_2 γ_2
Fetal:	Hemoglobin F	α_2 γ_2
Adult:	Hemoglobin A	α_2 β_2
	Hemoglobin A_2	α_2 δ_2

FIG. 473.5 Organization of the globin genes. The *bottom line* reflects the scale in kilobases. The *upper segment* represents the β-like globin genes on chromosome 11, and the *lower segment* the α-like genes on chromosome 16. Regions of the gene that code for primary globin proteins are shown as *blue segments* , and regions that code for pseudogenes ("ψ," nonexpressed remnants) are shown as *pink segments* . The composition of embryonic, fetal, and adult hemoglobins is listed. α, Alpha; β, beta; γ, gamma; δ, delta; ε, epsilon; ζ, zeta.

Embryonic Hemoglobins

- Gower-1 and Gower-2, and Hb Portland, which has HbF-like mobility.

- In embryos up to 6 wk gestation, the Gower hemoglobins predominate but are no longer detectable by 3 month of gestation.

Fetal Hemoglobin

- By 6-8 wk gestation, HbF ($\alpha_2 \gamma_2$) is the predominant hemoglobin; at 24 week gestation it constitutes 90% of the total hemoglobin.
- HbF declines modestly in the third trimester, such that the HbF comprises 70–80% of the total hemoglobin at term.
- HbF production decreases rapidly postnatally and by 6-12 mo of age reaches adult levels of <2%.
- Hb F has a higher affinity for oxygen than adult hemoglobin

Adult Hemoglobins

- HbA constitutes 5–10% of total hemoglobin at 24 week gestation, at term, HbA averages 30% of total hemoglobin.
- By 6-12 month of age, reach adult levels of HbA (96%)
- The minor adult hemoglobin component, HbA2, at birth, <1.0% of HbA2 is detected, by 12 month of age the normal level is 2.0–3.4%.
- The normal ratio of HbA to HbA2 is about 30 : 1

Anemia

- **Definition.** Anemia is a reduction in the red blood cell (RBC) number or in the haemoglobin (Hgb) concentration to a level more than 2 standard deviations below the mean.

- The haemoglobin is **high at birth** in most newborns and then declines, reaching the **physiologic lowest point (nadir)** between **2 and 3 months of age in the term infant** and between **1 and 2 months of age in the preterm infant.**

Age	Hemoglobin (g/dL)		Hematocrit (%)		MCV (fL)		RDW (%)	
	Lower limit	Upper limit	Lower limit	Upper limit	Lower limit	Upper limit	Lower limit	Upper limit
6 months to <2 years*	11.0[¶]	13.5	31	42	73	85	12.3	15.6
2 to 6 years	11.0[¶]	13.7	34	44	75	86	12.0	14.6
6 to 12 years	11.2	14.5	35	44	78	90	11.9	13.8
12 to <18 years								
Female	11.4	14.7	36	46	80	96	11.9	14.6
Male	12.4	16.4	40	51	80	96	11.9	13.7

Terminology

- Hematocrit (HCT): Is the fractional volume of a whole blood sample occupied by RBCs, expressed as a percentage.
- Hemoglobin (HGB): This is a measure of the concentration of the RBC pigment HGB in whole blood, expressed as grams per 100 mL (dL) of whole blood.
- Mean corpuscular volume (MCV): Represents the mean value (in femtoliters [fL]) of the volume of individual RBCs in the blood sample.
- Red cell distribution width (RDW): The RDW is a quantitative measure of the variability of RBC sizes in the sample (anisocytosis).
- Mean corpuscular hemoglobin concentration (MCHC): is a calculated index (MCHC = HGB/HCT) yielding a value of grams of HGB per 100 mL of RBC.

The medical history and physical examination in the child with anemia

- **Patient characteristics**
- In neonates and young infants, immune hemolytic disease (ABO and RH incompatibility), infection, and hereditary disorders are most common(Alpha Thalassemia,G6PD,HS)

- Anemia detected at 3 to 6 months of age suggests a hemoglobinopathy(Beta Thalassemia and SCD)

- Nutritional iron deficiency is an unlikely cause of anemia before the age of 6 months in term infants

- In older children, acquired causes of anemia are more likely, particularly iron deficiency anemia (dietary or due to blood loss)

Sex: Some inherited causes of anemia are X-linked (eg, G6PD deficiency and X-linked sideroblastic anemia)

Ethnicity/ancestry: Hemoglobin S and C are most commonly seen in individuals of African or Hispanic descent and Middle Eastern populations

Thalassemia syndromes are more common in individuals of Mediterranean and Southeast Asian descent

G6PD deficiency is more common among Sephardic Jewish individuals; Black individuals from sub-Saharan Africa or Brazil; African Americans; and people from Thailand, Sardinia, Greece, South China, and India

Symptoms

- Changes in urine color, scleral icterus, or jaundice suggest a hemolytic disorder

- Bloody stools, hematemesis, severe epistaxis, or severe menstrual bleeding suggest anemia from blood loss and/or iron deficiency

- Infectious symptoms (fevers, cough) suggest an infectious etiology of anemia

History of anemia

- Prior episodes of anemia suggest an inherited disorder

- Blood transfusion

- Anemia in a patient with previously documented normal CBC suggests an acquired etiology

- Hyperbilirubinemia in the newborn period suggests a hemolytic etiology; microcytosis at birth suggests chronic intrauterine blood loss or thalassemia

- **Underlying medical conditions**

- Underlying renal disease, malignancy, or inflammatory/autoimmune disorders may be associated with anemia

- **Drugs and toxin exposure**

- Anemia following exposure to oxidant drugs or fava beans suggests G6PD deficiency

- Exposure to paint, home renovations, or use of imported or glazed ceramics suggest lead toxicity

- **Family history**

- Family members with jaundice, gallstones, or splenomegaly suggests an inherited hemolytic anemia

Dietary history
•In infants and young children, iron deficiency is suggested by the following: •Introduction of unmodified cow's milk before the age of 1 year •Excessive milk intake (>24 ounces per day) •Poor intake of iron-rich foods (meats or fortified infant cereal)
Travel history
•Travel to/from areas of endemic infection suggests infectious etiology such as malaria or tuberculosis
Developmental history
•Developmental delay is associated with iron deficiency, vitamin B12/folic acid deficiency, and Fanconi anemia

Finding	Possible etiology
Skin	
Hyperpigmentation	Fanconi anemia
Petechiae, purpura	Autoimmune hemolytic anemia with thrombocytopenia, hemolytic-uremic syndrome, bone marrow aplasia, bone marrow infiltration
Jaundice	Hemolytic anemia, hepatitis, and aplastic anemia
Cavernous hemangioma	Microangiopathic hemolytic anemia
Ulcers on lower extremities	Sickle cell disease (S and C hemoglobinopathies), thalassemia
Facies	
Frontal bossing, prominence of the malar and maxillary bones	Congenital hemolytic anemias, thalassemia major, severe iron deficiency

Eyes	
Microcornea	Fanconi anemia
Tortuosity of the conjunctival and retinal vessels	Sickle cell disease (S and C hemoglobinopathies)
Microaneurysms of retinal vessels	Sickle cell disease (S and C hemoglobinopathies)
Vitreous hemorrhages	S hemoglobinopathy
Retinal hemorrhages	Chronic, severe anemia
Edema of the eyelids	Infectious mononucleosis, exudative enteropathy with iron deficiency, renal failure

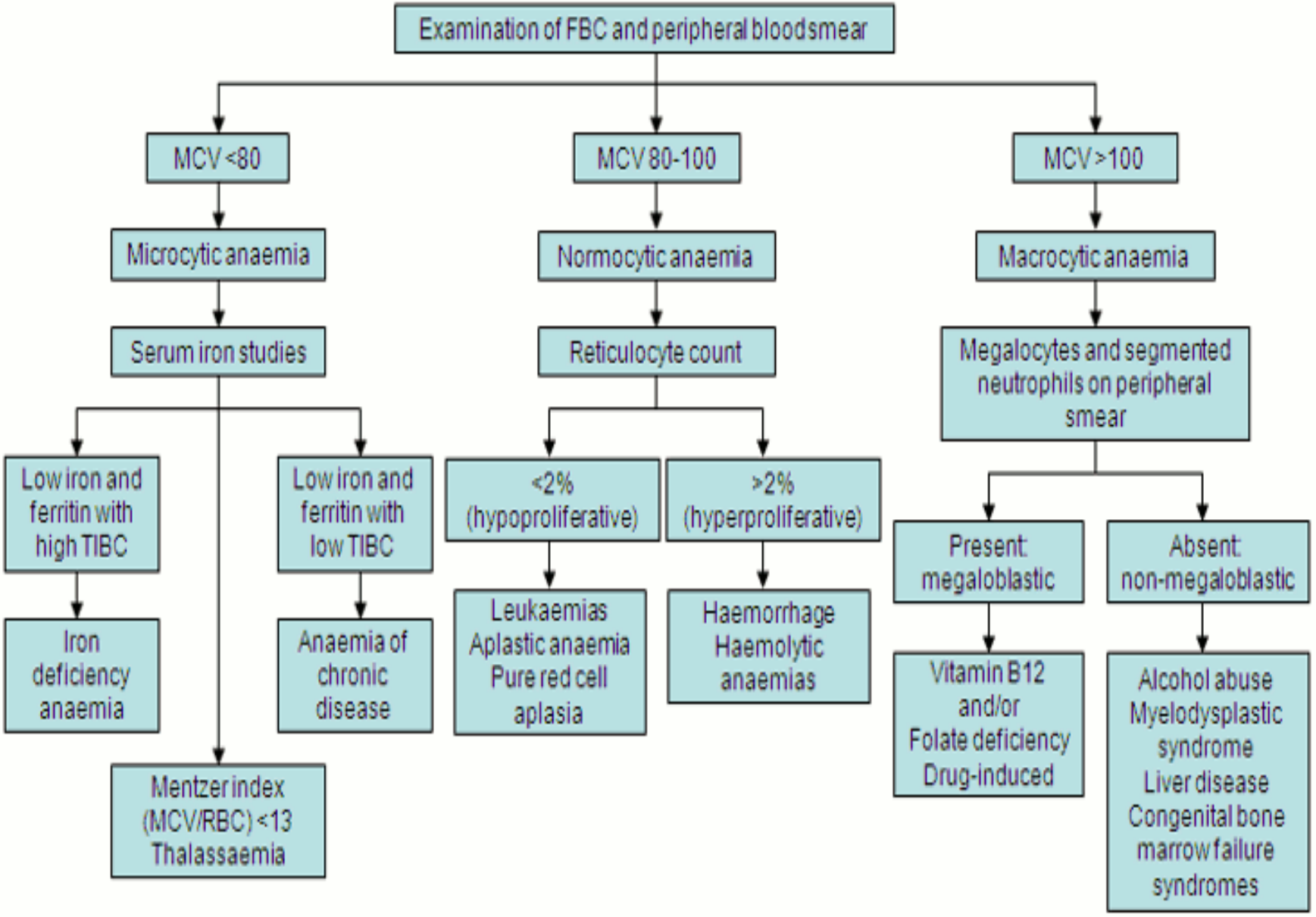
Mouth	
Glossitis	Vitamin B12 deficiency, iron deficiency
Angular stomatitis	
Hands	
Triphalangeal thumbs	Red cell aplasia DBA
Hypoplasia of the thenar eminence	Fanconi anemia
Spoon nails	Iron deficiency
Spleen and Liver	
Enlargement	Congenital hemolytic anemia, leukemia, lymphoma acute infection, portal hypertension

Table 474.1

Normal Mean and Lower Limits of Normal for Hemoglobin, Hematocrit, and Mean Corpuscular Volume

AGE (yr)	HEMOGLOBIN (g/dL)		HEMATOCRIT (%)		MEAN CORPUSCULAR VOLUME (μM^3)	
	Mean	Lower Limit	Mean	Lower Limit	Mean	Lower Limit
0.5-1.9	12.5	11.0	37	33	77	70
2-4	12.5	11.0	38	34	79	73
5-7	13.0	11.5	39	35	81	75
8-11	13.5	12.0	40	36	83	76
12-14 female	13.5	12.0	41	36	85	78
12-14 male	14.0	12.5	43	37	84	77
15-17 female	14.0	12.0	41	36	87	79
15-17 male	15.0	13.0	46	38	86	78
18-49 female	14.0	12.0	42	37	90	80
18-49 male	16.0	14.0	47	40	90	80

From Brugnara C, Oski FJ, Nathan DG: *Nathan and Oski's hematology of infancy and childhood* , ed 7, Philadelphia, 2009, Saunders, p 456.


Examination of FBC and peripheral blood smear
MCV <80
MCV 80-100
MCV >100
Microcytic anaemia
Normocytic anaemia
Macrocytic anaemia
Serum iron studies
Reticulocyte count
Megalocytes and segmented neutrophils on peripheral smear
Low iron and ferritin with high TIBC
Low iron and ferritin with low TIBC
<2% (hypoproliferative)
>2% (hyperproliferative)
Present: megaloblastic
Absent: non-megaloblastic
Iron deficiency anaemia
Anaemia of chronic disease
Leukaemias
Aplastic anaemia
Pure red cell aplasia
Haemorrhage
Haemolytic anaemias
Vitamin B12 and/or
Folate deficiency
Drug-induced
Alcohol abuse
Myelodysplastic syndrome
Liver disease
Congenital bone marrow failure syndromes
Mentzer index (MCV/RBC) <13
Thalassaemia

- **Fetal hemoglobin (Hgb F)** is a major constituent of Hgb during fetal and early postnatal life. It declines and gradually disappears by 6–9 months of age.

- Anemia is one of the **most common laboratory abnormalities** during childhood.

- Approximately 20% of all children in the United States and 80% of children in developing nations have anemia at some time during childhood.

- Classification is made on the basis of the **mean corpuscular volume (MCV)**

- **Microcytic, hypochromic anemia** (small, pale RBCs; low MCV)

- **Macrocytic anemia** (large RBCs; high MCV)

- **Normocytic, normochromic anemia** (normal RBCs in size, color, and shape; normal MCV)

Classification

- Classification based on **reticulocyte count** is also helpful.

- The reticulocyte count reflects the number of immature RBCs in the circulation

- The usual percentage of RBCs that are reticulocytes is 2%

- In the steady state, when a patient has a normal Hemoglobin level, the reticulocytes should constitute 2% of all RBCs.

- In most anemias, reticulocyte counts should rise. A **low reticulocyte count indicates bone marrow failure or diminished hematopoiesis.**

Clinical Features of Anemia

- **Mild**
- Pallor (noted especially on skin and on mucous membranes)
- **Moderate**
- Weakness and fatigue
- Decreased exercise tolerance
- Irritability
- Tachycardia
- Tachypnea
- Anorexia
- Systolic heart murmur

Clinical Features of Anemia

- **Severe**
- Congestive heart failure
- Cardiac dilation
- Shortness of breath
- Hepatosplenomegaly
- Spoon-shaped nails

Microcytic, hypochromic anemias

- Iron-deficiency anemia
- β-or alpha thalassemia minor
- Anemia of chronic disease
- Sideroblastic Anemia
- Lead poisoning

Iron-deficiency anemia

- Is the **most common blood disease during infancy and childhood.**

- The majority of cases are caused by **inadequate iron intake.**
- **Nutritional iron deficiency** is most common in two age groups.
- **Nine to twenty-four months of age:** owing to inadequate intake and inadequate iron stores (which are typically depleted by 4–6 months of age).

Iron-deficiency anemia

- The typical toddler's diet consists of large quantities of iron-poor cow's milk.

- Iron rich foods (e.g., iron-fortified cereal, meats, legumes) or iron supplementation is therefore recommended beginning at 4–6 months of age to prevent anemia.

Iron-deficiency anemia

- **Adolescent girls:** owing to poor diet, rapid growth, and loss of iron in **menstrual blood**

- **Occult blood loss** :
- Polyps, Meckel diverticulum, (IBD), PUD,celiac disease, and the early ingestion of whole cow's milk before 1 year of age.

Clinical Manifestations

Iron deficiency has nonhematologic systemic effects.

Both iron deficiency and iron-deficiency anemia are associated with impaired neurocognitive function in infancy.

Iron-deficiency anemia is also associated with later, possibly irreversible, cognitive defects.

Other nonhematologic consequences of iron deficiency include pica , the desire to ingest nonnutritive substances, and pagophagia , the desire to ingest ice.

Differential Diagnosis

The most common alternative causes of microcytic anemia are α- or β-thalassemia and other hemoglobinopathies, including hemoglobin's E and C.

A presumptive diagnosis of iron-deficiency anemia is most often made by a complete blood count (CBC) demonstrating a microcytic anemia with a high RDW, reduced RBC count, normal WBC count, and normal or elevated platelet count.

An increase in hemoglobin ≥1 g/dL after 1 month of iron therapy is usually the most practical means to establish the diagnosis.

Children 6 months to <5 years: Ferritin <12 micrograms/L

children 5 to <12 years: Ferritin <15 micrograms/L

Laboratory findings

- Because iron stores disappear first, an **early finding of iron-deficiency anemia** is **low serum ferritin.**

- Ferritin is also an acute-phase reactant, it may be increased in infection, disease states, and stress, therefore appearing normal.

- As **serum iron decreases, iron-binding capacity increases**
- **Low RBC count and high RDW**

- Other findings include **a normal or decreased reticulocyte count and thrombocytosis.**

Management

- **Elemental iron** (4–6 mg/kg/day) is prescribed orally for mild to moderate anemia for 3-4 months
- IV Iron in special situations
- **Dietary counselling**
- RBC transfusion may be required for severe anemia hemoglobin values less than 4 g/dL
- **Further evaluation to rule out other causes of anemia is necessary in patients with anemia unresponsive to iron**.
- Compliance , wrong dose, concomitant blood loss, and malabsorption

Table 482.4

Responses to Iron Therapy in Iron-Deficiency Anemia

TIME AFTER IRON ADMINISTRATION	RESPONSE
12-24 hr	Replacement of intracellular iron enzymes; subjective improvement; decreased irritability; increased appetite; increased serum iron
36-48 hr	Initial bone marrow response; erythroid hyperplasia
48-72 hr	Reticulocytosis, peaking at 5-7 days
4-30 days	Increase in hemoglobin level; increase in mean corpuscular volume; increase in ferritin
1-3 mo	Repletion of stores

β-Thalassemia minor

- **Mild asymptomatic hypochromic, microcytic anemia**
- **Elevated hemoglobin A2 > 3.5**
- Patients with thalassemia minor have normal to elevated RBC counts as opposed to iron deficiency in which the RBC count is low to normal.
- **Mentzer index = MCV/RBC**
  less than 13 = Thalassemia minor
  more than 13 = iron deficiency anemia
- **Normal RDW**
- **Alpha Thalassemia minor have same features as B thalassemia minor but diagnosis is made by genetic test**

Sideroblastic anemia

- Is a group of anemias characterized by the presence of **ring sideroblasts** in the bone marrow.

- Ring sideroblasts result from the accumulation of iron in the mitochondria of RBC precursors.

- Sideroblastic anemia may be inherited or may be acquired as a result of drugs or toxins (e.g., isoniazid, alcohol, lead poisoning, chloramphenicol).

Hypochromic microcytic anemia

- **Lead poisoning : Basophilic stippling on blood film**
- **Anemia of chronic diseases**, such as malignancy, infections, and kidney disease
- Usually **Low TIBC and high ferritin**

Table 482.2

Laboratory Studies Differentiating the Most Common Microcytic Anemias

STUDY	IRON-DEFICIENCY ANEMIA	α- OR β-THALASSEMIA	ANEMIA OF CHRONIC DISEASE
Hemoglobin	Decreased	Decreased	Decreased
MCV	Decreased	Decreased	Normal-decreased
RDW	Increased	Normal or minimally increased	Normal-increased
RBC	Decreased	Normal-increased	Normal-decreased
Serum ferritin	Decreased	Normal	Increased
Total Fe binding capacity	Increased	Normal	Decreased
Transferrin saturation	Decreased	Normal	Decreased
FEP	Increased	Normal	Increased
Soluable transferrin receptor	Increased	Normal	Normal
Reticulocyte hemoglobin concentration	Decreased	Normal	Normal-decreased

FEP, Free erythrocyte protoporphyrin; MCV, mean corpuscular volume; RBC, red blood cell count; RDW, red cell distribution width.

Adapated from Zimmermann MB, Hurrell RF: Nutritional iron deficiency, *Lancet* 370:511–520, 2007.

Normocytic, Normochromic Anemias

- These anemias are characterized by normal size (normal MCV) and shape of the RBCs.

- **Common causes** include hemolytic anemias (premature destruction of RBCs), some RBC aplasias, and sickle cell (SS) anemia.

Normocytic, Normochromic Anemia

- **Reticulocyte count** may be used to differentiate among the disorders

- **Low reticulocyte count** reflects bone marrow suppression or failure and can be seen with RBC aplasias, viral suppression, medication effect, and pancytopenia associated with aplastic anemia.

- **High reticulocyte count** reflects high bone marrow production of RBCs as seen in hemolytic anemias, recent acute hemorrhage

Hemolytic anemias

Intrinsic RBC defects that cause hemolysis include :

- **RBC membrane disorders** include hereditary spherocytosis and hereditary elliptocytosis
- **RBC enzyme disorders** include glucose-6-phosphate dehydrogenase (G6PD) deficiency and pyruvate kinase (PK) deficiency.
- **Hemoglobinopathies** include Thalassemia and sickle cell anemia

Intrinsic red blood cell defects
Hemoglobinopathies (eg, sickle cell disease, thalassemias)
Membrane defects (eg, hereditary spherocytosis, elliptocytosis)
Enzyme deficiencies (eg, G6PD, pyruvate kinase deficiencies)
Extrinsic hemolytic processes
•Autoimmune hemolytic anemia (AIHA) Warm-reactive •Cold agglutinin disease •Paroxysmal cold hemoglobinuria
Hypersplenism

Extrinsic hemolytic processes
Systemic disease •Infection (eg, malaria, *Clostridium perfringens)* •Liver disease •Renal disease
Drugs and toxins*
•Microangiopathies •Hemolytic uremic syndrome (HUS) •Thrombotic thrombocytopenic purpura (congenital or acquired) •Disseminated intravascular coagulation (DIC)
Mechanical damage (eg, artificial heart valves, Kasabach-Merritt phenomena)
Wilson disease

Hereditary spherocytosis

- **Most common inherited abnormality of the RBC membrane**
- Northern European
- large spectrum of phenotypes, from asymptomatic to transfusion-dependent starting in infancy.

- There is a deficiency or abnormality of the structural RBC membrane protein **spectrin,** causing the RBC to assume its spherical shape.

- Inheritance is usually **autosomal dominant.**

Hereditary spherocytosis

- Infants may present with jaundice and anemia.
- By 2–3 years of age, patients develop pallor, weakness, and **splenomegaly**, as spherocytes are trapped in the spleen and destroyed.
- Other complications include **aplastic crises,** most commonly associated with parvovirus B19 infection, and **pigmentary gallstones.**

Hereditary spherocytosis

- **Laboratory findings of hemolysis:**
- **Elevated reticulocyte count, indirect hyperbilirubinemia, high LDH, and low Haptoglobin**
- **Spherocytes** on blood smear, increased **MCHC** (mean corpuscular hemoglobin concentration)
- **Abnormal RBC fragility with osmotic fragility studies.**
- **Flow cytometry using the eosin-5'-maleimide (EMA) binding test**

Hereditary spherocytosis

Management

- Treatment includes transfusions
- Folic acid
- Splenectomy cures the disorder :Is generally delayed until after 5 years of age.

Hereditary elliptocytosis

- **Autosomal dominant** defect in the structure of **spectrin**
- Clinical features are more variable than in hereditary spherocytosis.

- The **majority of patients are asymptomatic,** although 10% have jaundice at birth, and later may develop splenomegaly and gallstones.

- Treatment includes **splenectomy** for patients with severe chronic hemolysis.

Glucose-6-phosphate dehydrogenase deficiency

- **(G6PD) is the most common RBC enzymatic defect.** It may occur as an **acute** hemolytic disease, induced by infection or medications, or as a **chronic** hemolytic disease.

- **X linked disease**

Triggers of hemolysis

- Infection
- Fava beans
- Drugs (e.g., sulpha, salicylates, antimalarials)
- Naphthalene balls
- henna

Glucose-6-phosphate dehydrogenase deficiency

- Clinically evident jaundice, dark urine resulting from bilirubin pigments, hemoglobinuria when hemolysis is intravascular, and decreased haptoglobin levels are common during hemolytic episodes

- Transfusions are indicated when significant cardiovascular compromise is present. Maintaining hydration and urine alkalization protects the kidneys against damage from precipitated free hemoglobin.

PK deficiency

- Is an **autosomal recessive** disorder
- **Clinical features** include pallor, jaundice, and splenomegaly.
- Kernicterus has been reported in neonates.
- **Laboratory findings** include varying degrees of anemia and a blood smear showing **polychromatic RBCs.**
- **Diagnosis** is by finding decreased PK activity in the RBCs.
- **Management** includes transfusions and splenectomy for severe disease.

α-Thalassemia and β-thalassemia syndromes

- Thalassemia is a group of inherited anemias characterized by **defective synthesis of one of the Hemoglobin chains**

- **Normally,** the major **Hemoglobin** in RBCs is hemoglobin A1, a tetramer of two α- chains and two β-chains.

- HgbA2 and Hemoglobin F may also be present in small amounts.

- **α-Thalassemia** results from **defective α-globin chain synthesis,** and **β- thalassemia** results from **defective β-globin chain synthesis.**

- Both types of thalassemia result in hemolysis that leads to increased bone marrow activity.

- As marrow activity increases, the marrow spaces enlarge, increasing the size of bones in the face, skull, and other bones if severe and untreated.

α-Thalassemia

- Is the result of deletions of the **α-globin chain** and occurs predominantly in Southeast Asians.

- **Silent carrier.** One α-globin gene is deleted. Patients have **no anemia** and are **asymptomatic.**

- **α-Thalassemia minor.** Two α-globin genes are deleted. Patients have **mild microcytic anemia** which is diagnosed by **gene testing (PCR)**

α-THALASSEMIA		
Silent carrier	α-/αα	Normal complete blood count
α-Thalassemia trait	αα/- - (α-thalassemia 1) *or* α -/α - (α-thalassemia 2)	Mild microcytic anemia
Hemoglobin H	α -/- -	Microcytic anemia and mild hemolysis; not transfusion-dependent
Hydrops fetalis	- -/- -	Severe anemia, intrauterine anasarca from congestive heart failure; death in

α-Thalassemia

- **Hemoglobin H disease.** Three α-globin genes are deleted. Patients have **moderate to severe anemia at birth**

- **Fetal hydrops.** Four α-globin genes are deleted.

- Only Hemoglobin Bart's are formed, and in utero, this causes profound anemia , congestive heart failure (CHF), and death if not identified early enough for intrauterine transfusion to occur.

β-Thalassemia

- Is the result of mutations of the **β-globin chain.** Because there are only two β-globin genes in each cell, there are only two states:

- **β-Thalassemia major** (Cooley anemia) may be caused by either **total absence of the β-globin chains or deficient β-globin chain production.**

- β-Thalassemia major occurs predominantly among patients of Mediterranean background.

DISORDER	GENOTYPIC ABNORMALITY	CLINICAL PHENOTYPE
β-THALASSEMIA		
Thalassemia major (Cooley's anemia)	Homozygous β0-thalassemia	Severe hemolysis, ineffective erythropoiesis, transfusion dependency, hepatosplenomegaly, iron overload
Thalassemia intermedia	Compound heterozygous β0- and β+-thalassemia	Moderate hemolysis, splenomegaly, moderately severe anemia, but not transfusion-dependent; main life- threatening complication is iron overload
Thalassemia minor	Heterozygous β0- and β+-thalassemia	Microcytosis, mild anemia

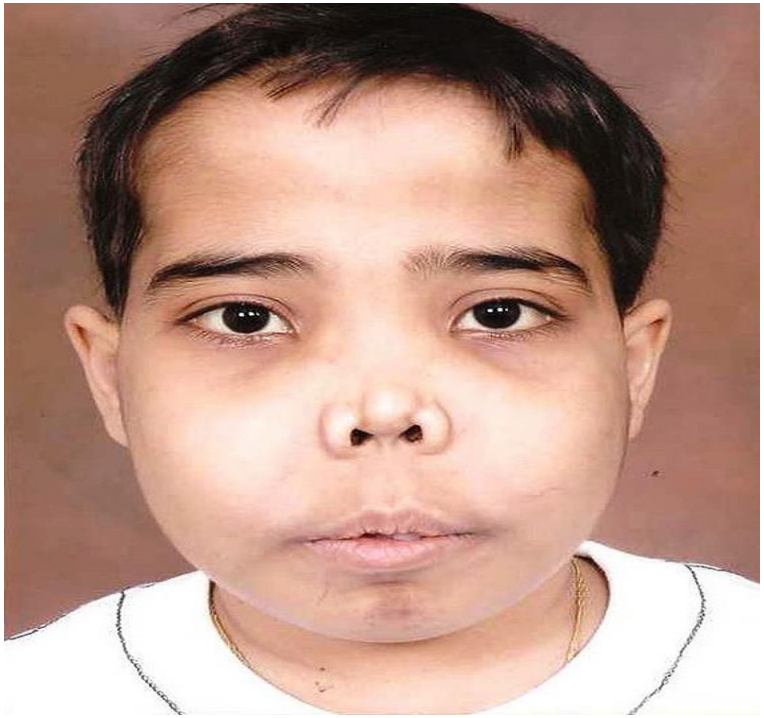
Clinical features

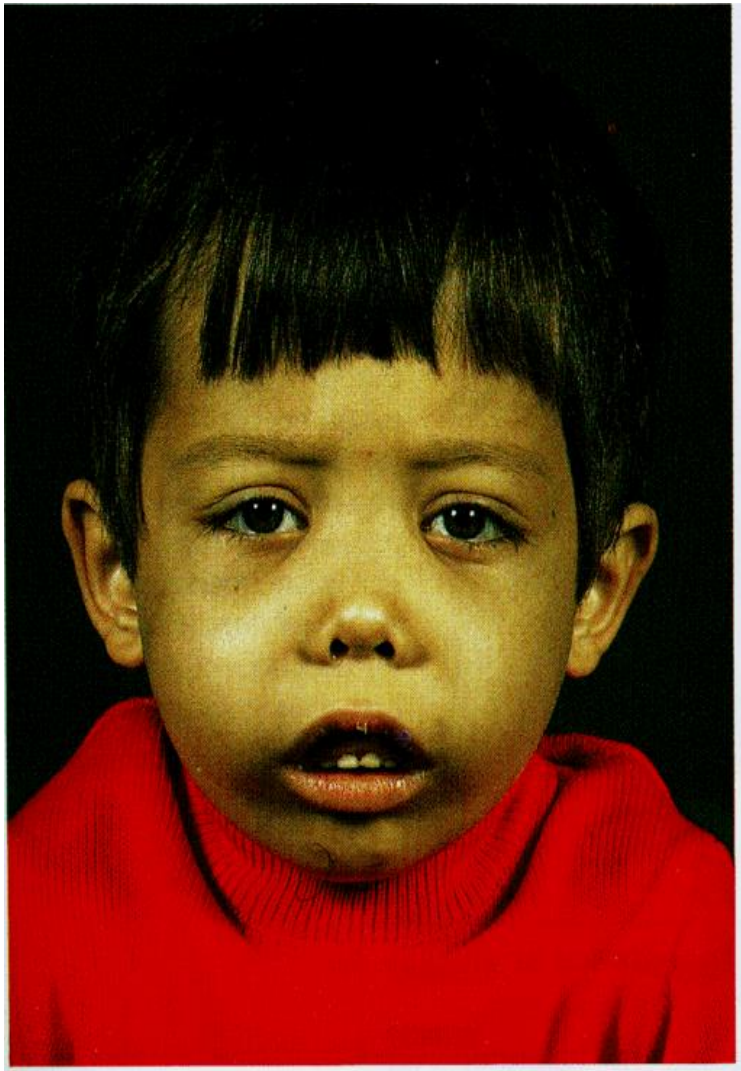
- **Profound hemolytic anemia** in infancy (6 month of age)
- If untreated, **bone marrow hyperplasia** in sites that result in a characteristic **“thalassemia facies”** (frontal bossing, maxillary hyperplasia with prominent cheekbones (chipmunk face)

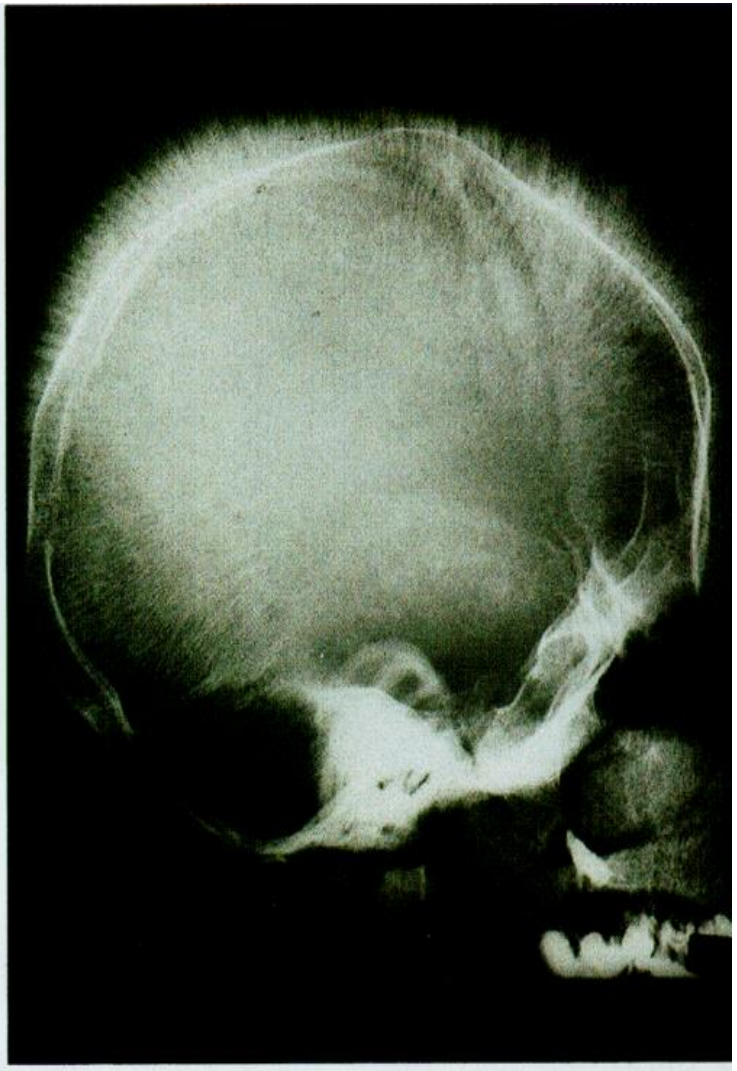
- **Extramedullary Hematopoiesis**

- **Hepatosplenomegaly**

- Delayed growth and puberty may also be present







Laboratory findings

- **Severe hypochromia and microcytosis, elevated reticulocyte count**
- Elevated unconjugated bilirubin, serum iron, and lactate dehydrogenase (LDH)
- Plain skull X-ray shows the classical '**hair on end**' appearance.
- Electrophoresis demonstrates low or absent Hemoglobin A and elevated Hemoglobin F.

Management

- **Lifelong transfusions**
- **Chelation therapy** to avoid iron overload and often **splenectomy.**
- **Bone marrow transplant** is curative and is the therapy of choice.
- **Gene therapy.**

Complications

- **Hemochromatosis** (iron accumulation within the heart, liver, lungs, pancreas, and skin) is a major complication and is caused by increased iron absorption from the intestine and from iron in transfused RBCs.

- **Chelation of iron** with the intravenous agent **deferoxamine** and/or the oral agent **deferasirox** promotes iron excretion and may help prevent or delay hemochromatosis.

SS hemoglobinopathies

- SS disease occurs in 1 in 800 black newborns in the United States

- **SS disease** is caused by a **single amino acid substitution** of **valine for glutamic acid** on the **number 6 position of the β-globin chain** of Hemoglobin.

- The mutation results in polymerization of Hgb within the RBC membrane when the RBC is exposed to low oxygen or acidosis.

- **Distorted RBC shape** (sickled) that leads to decreased RBC life span **(hemolysis)** and **occlusion of small vessels,** resulting in distal ischemia, infarction, and organ dysfunction.

- Clinical characteristics are not generally present until protective Hgb F declines (by 6 months of age).

- Clinical episodes are often termed crises because they occur suddenly.

- **Anemia**
- Chronic, onset 3–4 month of age; may require folate therapy for chronic hemolysis; hemoglobin usually 6-10 g/dL

- **Aplastic crisis**
- Parvovirus infection, reticulocytopenia; acute and reversible; may need transfusion

- **Sequestration crisis**
- Massive splenomegaly (may involve liver), shock; treat with transfusion

- **Hemolytic crisis**
- May be associated with G6PD deficiency

- **Dactylitis**
- Hand-foot swelling in early infancy

- **Painful crisis**
- Microvascular painful vasoocclusive infarcts of muscle, bone, bone marrow, lung, intestines

- **Cerebrovascular accidents**
- Large and small vessel occlusion → thrombosis/bleeding (stroke); requires chronic transfusion

- **Acute chest syndrome**
- Infection, asthma, atelectasis, infarction, fat emboli, severe hypoxemia, infiltrate, dyspnea, absent breath sounds

- **Chronic lung disease**
- Pulmonary fibrosis, restrictive lung disease, cor pulmonale, pulmonary hypertension

- **Priapism**
- Causes eventual impotence; treated with transfusion, oxygen, or corpora cavernosa-to- spongiosa shunt

- **Ocular** :Retinopathy

- **Gallbladder disease**
- Bilirubin stones; cholecystitis

- **Renal**
- Hematuria, papillary necrosis, renal concentrating defect; nephropathy

- **Cardiomyopathy**
- Heart failure

- **Skeletal**
- Osteonecrosis (avascular) of femoral or humeral head

- **Leg ulceration**
- Seen in older patients

Infections

Functional asplenia, defects in properdin system; pneumococcal bacteremia, meningitis, and arthritis; deafness from meningitis; *Salmonella* and *Staphylococcus aureus* osteomyelitis; severe *Mycoplasma* pneumonia

Growth failure, delayed puberty

Psychological problems

Narcotic addiction (rare), dependence unusual; chronic illness, chronic pain syndrome

- **Usual Laboratory Findings in Sickle Cell Anemia**

- **Red blood cell life span** 10–50 days
- **Hemoglobin** 6–9 g/dL
- **Reticulocyte count** 5–15%
- **Indirect Bilirubin** Increased
- **Blood smear** Sickled cells, target cells, Howell–Jolly bodies
- **Bone marrow** Erythroid hyperplasia

		PERCENT HEMOGLOBIN					
GENOTYPE	**CLINICAL CONDITION**	**Hb A**	**Hb S**	**Hb A2**	**Hb F**	**Hb C**	**OTHER FINDING(S)**
SA	Sickle cell trait	55–60	40–45	2–3	–	–	Usually asymptomatic
SS	Sickle cell anemia	0	85–95	2–3	5–15	–	Clinically severe anemia; Hb F heterogeneous in distribution

Management

- **Historically, infection was the leading cause of death due to impaired splenic function.**

- Patients are at risk for infection with **encapsulated bacteria** (Haemophilus influenzae type b, Streptococcus pneumoniae, Salmonella,Neisseria meningitidis).

- **Fever** in any patient with SS disease is managed with **urgent** assessment and appropriate cultures (blood and urine), chest radiograph to rule out pneumonia, and parenteral antibiotics until bacterial infection can be safely excluded.

Preventive care

- **Hydroxyurea,** a chemotherapeutic agent that increases Hgb F, has been shown to decrease the incidence of Vasoocclusive crises.
- **Daily oral penicillin prophylaxis** till age of 5 .
- **Folic acid**
- **Routine immunizations**

  Yearly influenza vaccination.

  Pneumococcal vaccine at 2 years of age.

  Meningococcal vaccine.

- **Bone marrow transplant is curative and is considered for children with severe manifestations.**

Defects extrinsic to the RBC that cause hemolysis

- **Autoimmune hemolytic anemia (AIHA)** occurs when **antibodies are misdirected against the RBCs.**

- **Primary AIHA** is generally **idiopathic** in which no underlying disease is identified. Viral infections and occasionally drugs may be causal in some patients.

- **Secondary AIHA** is associated with an **underlying disease process,** such as lymphoma, systemic lupus erythematosus (SLE), or immunodeficiency

Clinical features

- **Fulminant acute-type AIHA** occurs in infants and young children and is preceded by a respiratory infection.
- Presenting features include the acute onset of pallor, jaundice, hemoglobinuria, and splenomegaly.
- **Complete recovery is expected.**

- **Prolonged-type AIHA** is characterized by a protracted course and high mortality. Underlying disease is frequently present.

- **Laboratory findings.** Studies show severe anemia, spherocytes on blood smear, prominent reticulocytosis , indirect bilirubin , and high LDH .

- A **direct Coombs test is positive** (detects coating of antibodies on the surface of RBCs or complement).

- Warm type AIHA : IgG antibodies (lymphoma, systemic lupus erythematosus (SLE), or immunodeficiency

- Cold type AIHA :IgM cold agglutinin antibodies(mycoplasma pneumonia and EBV)

Management

- **Transfusions** provides only transient benefit , and usually given in life threatening anemia
- Difficulty of finding compatible blood
- **Corticosteroids**
- **Rituximab**
- **Splenectomy**

Alloimmune Hemolytic Anemia

- Occurs when antibodies from someone else are directed at the patients' RBCs

- **Rh hemolytic disease** occurs when the mother, who has no Rh antigen (maternal Rh negative), produces antibodies to the Rh antigen on her fetus's RBCs (fetal Rh positive).

- In **subsequent pregnancies,** antibodies pass from the mother to the fetus causing hemolysis that presents as severe jaundice (which can lead to kernicterus), anemia, hepatosplenomegaly, and **hydrops fetalis.**

- A direct Coombs test is **strongly positive.**

Alloimmune Hemolytic Anemia

- **ABO hemolytic disease** occurs when the mother is blood group O and her fetus is blood group A, B, or AB.
- A direct Coombs test is **weakly positive.**
- Of note, **ABO disease can occur in the first pregnancy, unlike Rh hemolytic disease.**
- **Management.** Treatment may include phototherapy for mild to moderate jaundice and exchange transfusion for severe jaundice.

Microangiopathic hemolytic anemia

- This form of anemia results from mechanical damage to RBCs caused by passage through an injured vascular endothelium.

- Causes include severe hypertension , **hemolytic uremic syndrome** (HUS),TTP, artificial heart valves, a giant hemangioma, and disseminated intravascular coagulation.

Microangiopathic hemolytic anemia

- Signs and symptoms are those characteristic of anemia and thrombocytopenia.

- Studies show RBC fragmentation seen as “burr” cells and **Schistocytes,** along wit thrombocytopenia.

- **Management.** Therapy includes supportive care and treatment of the underlying cause.

Macrocytic anemias

- These anemias are characterized by **large RBCs** with **MCV > 95.** The two major causes in children are folic acid and vitamin B12 deficiencies

- Rare causes of macrocytic anemia include **bone marrow failure syndromes (Fanconi and DBA)**

- hypothyroidism , and alcoholic liver disease .

Folic acid deficiency

- **Decreased folic acid intake** (i.e., from a diet lacking uncooked fresh fruits and vegetables or from **exclusive feedings with goat's milk**

- **Decreased intestinal absorption of folic acid** from diseases affecting the small intestine, such as celiac disease, chronic infectious enteritis, Crohn disease, or medications, such as anticonvulsants and oral contraceptives).

Folic acid deficiency

- In addition to the characteristic signs and symptoms of anemia, patients may have **failure to thrive, chronic diarrhea, and irritability.**

- **Diagnosis.** Documentation of **low serum folic acid** is diagnostic.

- **Management.** Treatment includes **dietary folic acid** and identification and treatment of the underlying cause.

Vitamin B12 deficiency

- **Normal physiology.** To be absorbed, dietary vitamin B12 must first combine with a glycoprotein (**intrinsic factor**) secreted by the gastric parietal cells.
- Absorption then occurs in the terminal ileum.
- Causes include **inadequate dietary intake** (e.g., from a strict vegetarian [vegan] diet)
- Inherited **inability to secrete intrinsic factor** (juvenile pernicious anemia)
- or an **inability to absorb vitamin B12** (e.g., Crohns disease, short gut syndrome).

Vitamin B12 deficiency

- Anorexia, **smooth red tongue,** and **neurologic manifestations** (such as ataxia, hyporeflexia, and positive Babinski responses).

- **Diagnosis.** Documentation of **low serum vitamin B12 level** is diagnostic.

- **Pancytopenia and hypersegmented neutrophils**

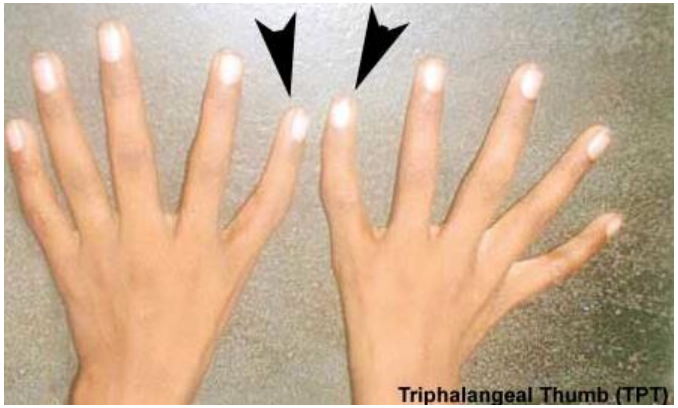
- **Management.** Treatment is by **monthly intramuscular vitamin B12 injections.**

Macrocytic anemias :RBC aplasias

- A group of congenital or acquired blood disorders characterized by anemia, reticulocytopenia, and a paucity of RBC precursors in the bone marrow.
- Three most common disorders occurring in childhood
- **Congenital pure red cell aplasia (Diamond–Blackfan anemia)**
- **Transient erythroblastopenia of childhood(TEC)**
- **Parvovirus B19–associated RBC aplasia**

Diamond-Blackfan Anemia

- The primary defects are in the erythroid progenitor cells(pure red cell aplasia) in an otherwise normally cellular bone marrow.
- Recognized in the 1st year of life, profound anemia usually becomes evident by 2– 6 month of age.
- Up to 50% of affected individuals have additional, extra hematopoietic anomalies.
- Congenital anomalies, including short stature, craniofacial deformities, triphalangeal thumbs.
- Dominantly inherited.


Triphalangeal Thumb (TPT)

Laboratory Findings

- Hematologic features are macrocytic anemia, with reticulocytopenia.
- RBC enzyme patterns are similar to those of a “fetal” RBC population, elevated fetal hemoglobin (HbF).
- Erythrocyte adenosine deaminase (eADA) activity is increased in most patients with DBA, a finding that helps distinguish congenital RBC aplasia from acquired transient erythroblastopenia of childhood (TEC).
- Bone marrow erythrocyte precursors are greatly reduced in most patients; other marrow elements are usually normal.

Treatment

Corticosteroids are a mainstay of therapy, and approximately 80% of patients initially respond.

Chronic red cell transfusions are required in approximately 35% of patients.

Hematopoietic stem cell transplantation (HSCT) can be curative.

Prognosis

DBA has been identified as a *cancer predisposition syndrome* because of the higher risk of myelodysplastic syndrome, acute myeloid leukemia, colon carcinoma, osteogenic sarcoma, and female genital cancers.

Patients are at risk for iron overload related endocrine abnormalities (diabetes, hypogonadism), especially if transfused.

Transient Erythroblastopenia of Childhood

- TEC is the most common *acquired red cell aplasia* occurring in children.
- This syndrome of severe, transient hypoplastic anemia occurs mainly in previously healthy children between 6 month and 3 year of age.
- TEC often follows a viral illness.

- Temporary suppression of erythropoiesis results in reticulocytopenia and moderate to severe normocytic anemia.
- MCV and HbF are normal for age.
- RBC adenosine deaminase level is normal in TEC

- Virtually all children recover within 1-2 mo.
- *Corticosteroid therapy is of no value in this disorder.*
- RBC transfusions may be necessary for severe anemia.
- Any child with presumed TEC who requires >1 transfusion should be reevaluated for another possible diagnosis.

Red Cell Aplasia Associated With Parvovirus B19 Infection

- Parvovirus B19 is a common infectious agent that causes erythema infectiosum (fifth disease).

- It is also the best-documented viral cause of RBC aplasia in patients with chronic hemolysis .

- This virus is particularly infective and cytotoxic to marrow erythroid progenitor cells.

- The virus does not cause significant anemia in immunocompetent individuals with normal RBC life span.

- The RBC life span is much shorter in patients with hemolysis secondary to conditions such as hereditary spherocytosis, immune hemolytic anemia, or sickle cell disease.

- In these children, a brief cessation of erythropoiesis can cause severe anemia, a condition known as an aplastic crisis .

When a definitive diagnosis is required, the workup should include serum parvovirus IgM and IgG titers and, if needed, viral detection using polymerase chain reaction (PCR) techniques.

In chronically infected patients the disease may be treated with high doses of intravenous immune globulin

Macrocytic anemia
Pancytopenia and Aplastic Anemia

- Pancytopenia is defined as low white blood cells (WBCs), RBCs, and platelets and implies **bone marrow failure.**

- **Pancytopenia** may be **congenital or acquired.**

- **Congenital aplastic anemia** is also known as **Fanconi anemia.**

Fanconi Anemia

- Inheritance is **autosomal recessive.**

- The main pathology is a DNA repair defect, particularly increased chromosome breakage so diagnosis relies on chromosome breakage studies in addition to specific mutation analysis

- Onset of bone marrow failure occurs at a **mean age of 7 years.**

- Typical presentation is with ecchymosis and petechiae, anemia and recurrent infections

Fanconi Anemia

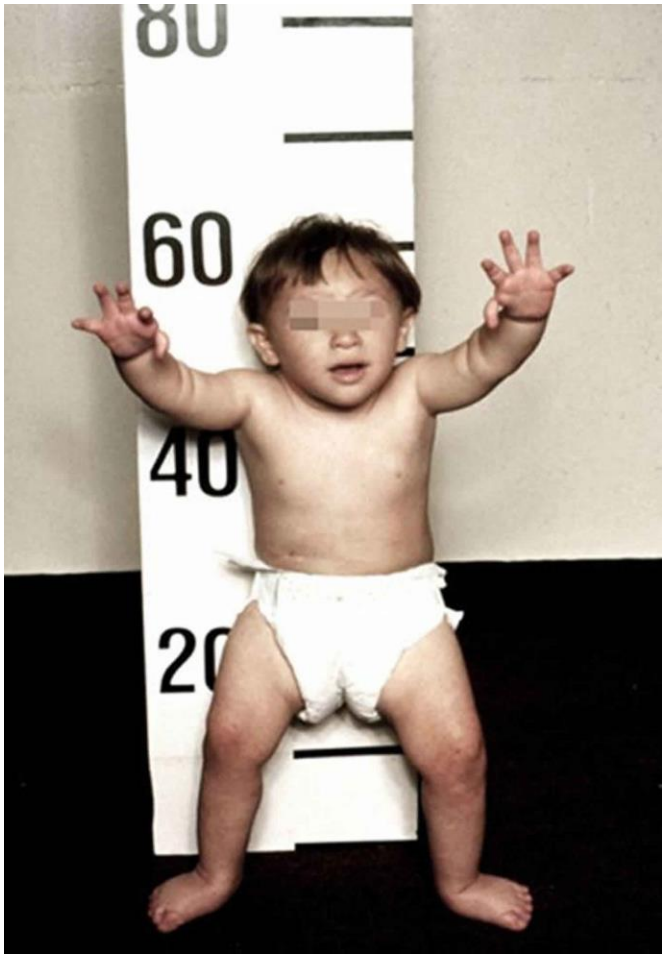
- **Skeletal abnormalities,** which include **short stature** in almost all *patients, and* **absence or hypoplasia of the thumb and radius**

- **Kidney** – Abnormal, ectopic, horseshoe, hypoplastic, or absent kidney; hydronephrosis

- **Skin** – Generalized hyperpigmentation; hypopigmented areas; large freckles, café-au-lait spots

- **Gonads** – undescended/absent testes, hypospadias

- **Head** – Microcephaly or hydrocephaly; birdlike face, mid-face hypoplasia
- **Eyes** – Microphthalmia, ptosis, epicanthal folds
- **Cardiopulmonary anomalies**
- **Gastrointestinal anomalies** – Atresias, imperforate anus, tracheoesophageal fistula, malrotation
- Patients are predisposed to many cancers, commonly **AML, squamous cell carcinoma**, brain tumors, Wilms tumor and others.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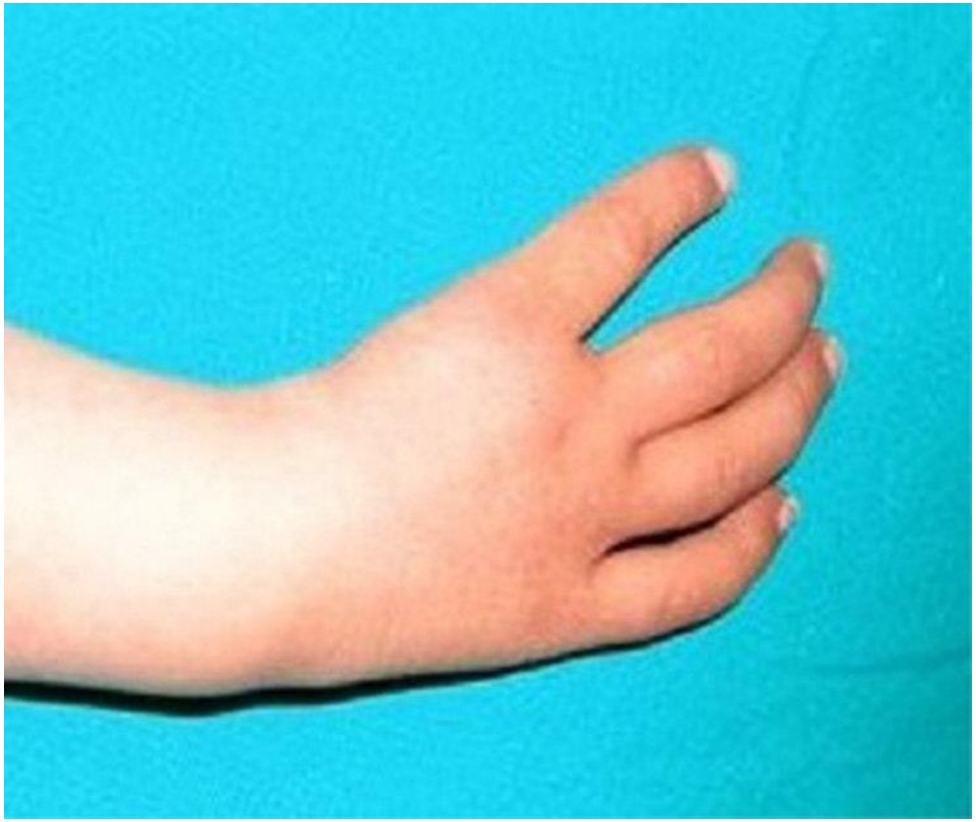


Fanconi anemia

- Studies show pancytopenia, RBC macrocytosis, low reticulocyte count, elevated Hgb F, and bone marrow hypocellularity(less than 30% cellularity)
- Chromosome breakage test (**DEB or Mitomycin**)
- Treatment includes transfusions of RBCs and platelets
- Bone marrow transplant
- Immunosuppressive therapy (e.g., corticosteroids, cyclosporin) may also help.

Acquired aplastic anemia

- Causes include **drugs** (sulfonamides, anticonvulsants, chloramphenicol), **infections** (HIV, EBV, CMV, hepatitis), **chemicals,** and **radiation.**

- These all may damage bone marrow stem cells directly or may induce autoimmune destruction.

- **Acquired aplastic anemia is most often idiopathic.**

Acquired aplastic anemia

- Signs and symptoms include bruising, petechiae, pallor, and fatigue, or serious infection as a result of neutropenia.

- Studies show pancytopenia, low reticulocyte count, and hypocellular bone marrow. (less than 30% cellularity)

- Treatment includes identifying and stopping the causative agent, transfusions as needed, bone marrow transplant, and immunosuppressive therapy.

Complete Blood Count

- **Look at 3 cell lines:**
- Hemoglobin
- Platelets
- WBC

- **BM Biopsy indications:**

- If more than one cell line affected or pancytopenia think of BM pathology (infiltration or aplastic anemia)

- Low retic count

- Macrocytic anemia

Hematological problems are either BM production failure or peripheral destruction:

- In anemia, Retic counts differentiate between them.
- Low retics indicates BM failure

- In thrombocytopenia, Mean Platelet Volume(MPV) or (platelets size) is helpful

- High MPV indicates peripheral destruction

Bone Marrow Failure syndromes

- Single line: early presentation 1-2 months of age
- Erythroid precursors: **DBA**
- Megakaryocyte precursors : congenital megakaryocytic thrombocytopenia **(CAMPT)**
- All lines: late presentation 5-7 year of age
- **Fanconi Anemia**

Peripheral destruction

3 main mechanisms:

(1) Autoimmune disorders:

Single line:

Hemoglobin: AIHA

Platelets : ITP

Multiple lines:

AIHA and Thrombocytopenia: **Evans syndrome**

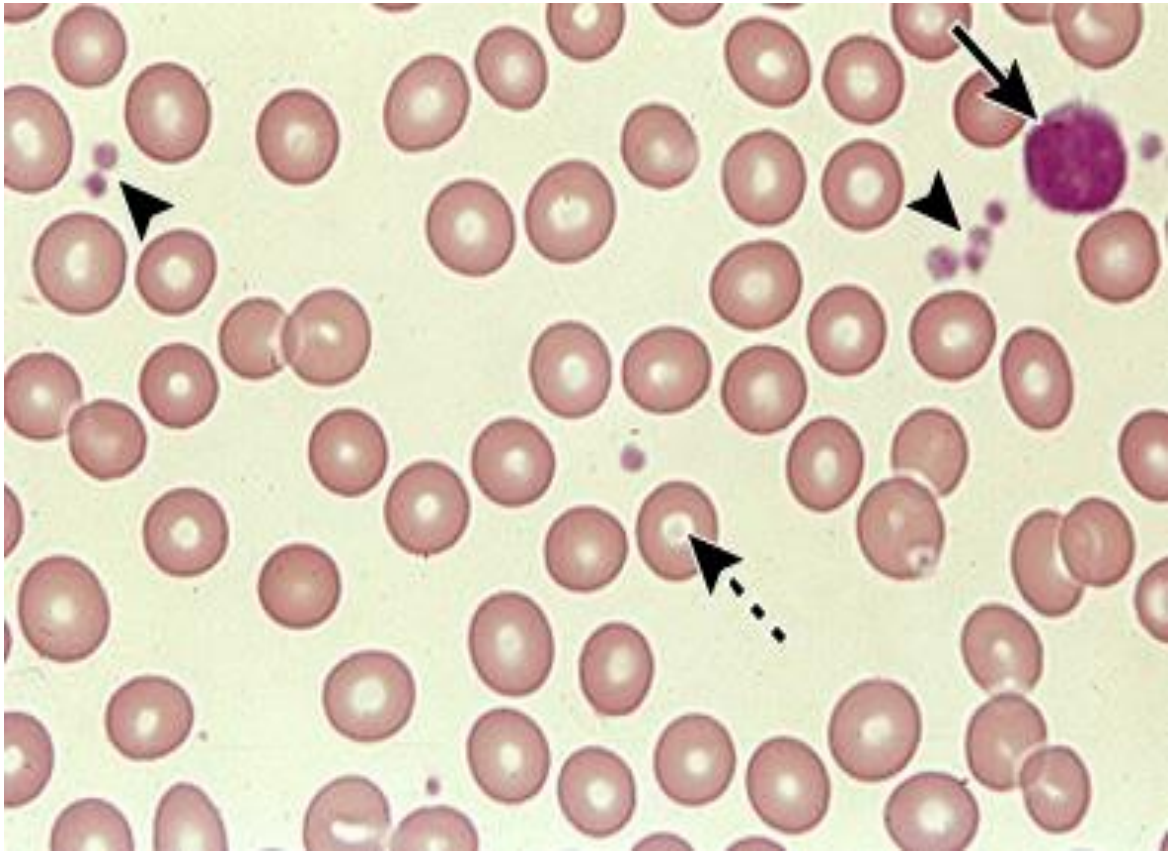
Peripheral destruction

(2) Mechanical (MAHA)

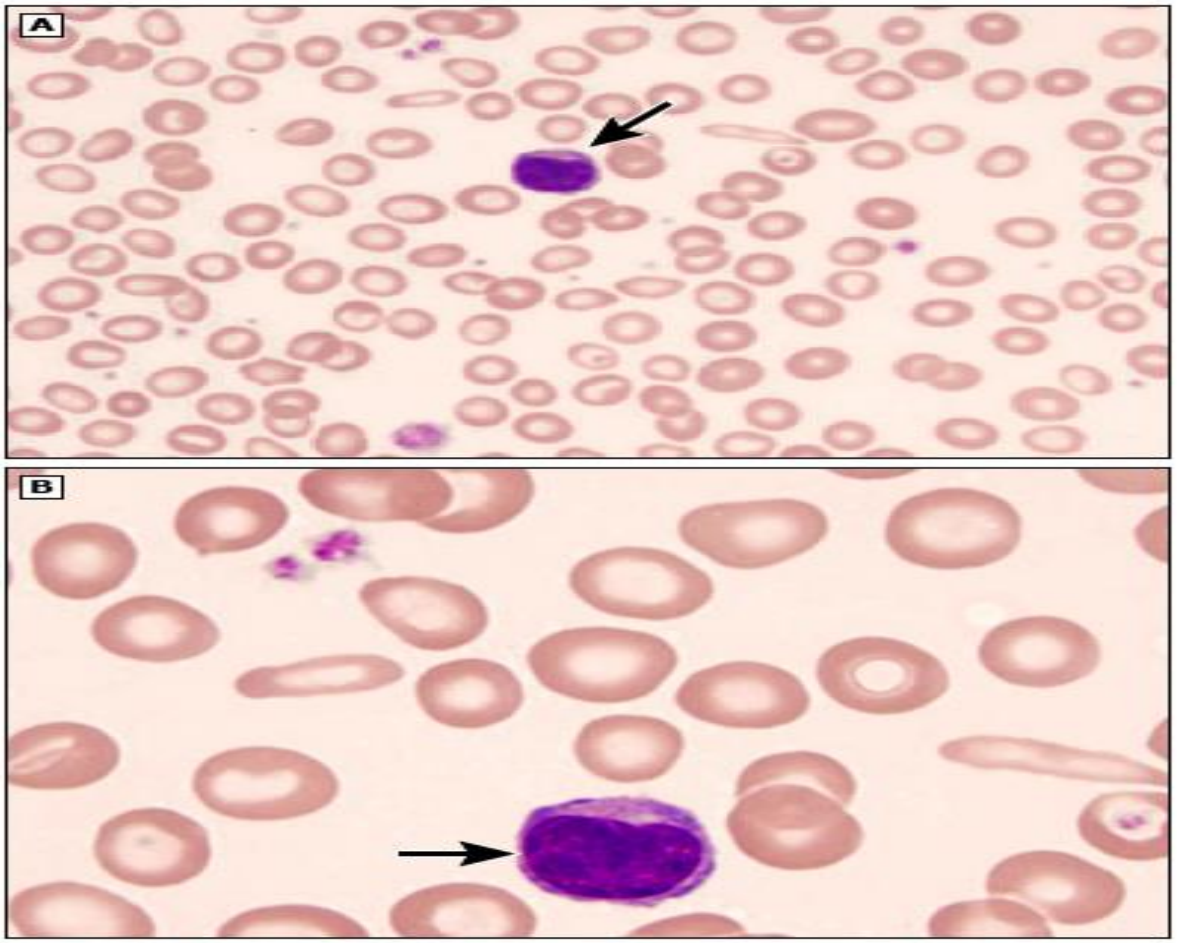
HUS, TTP, DIC, hemangiomas

(3)Hypersplenism

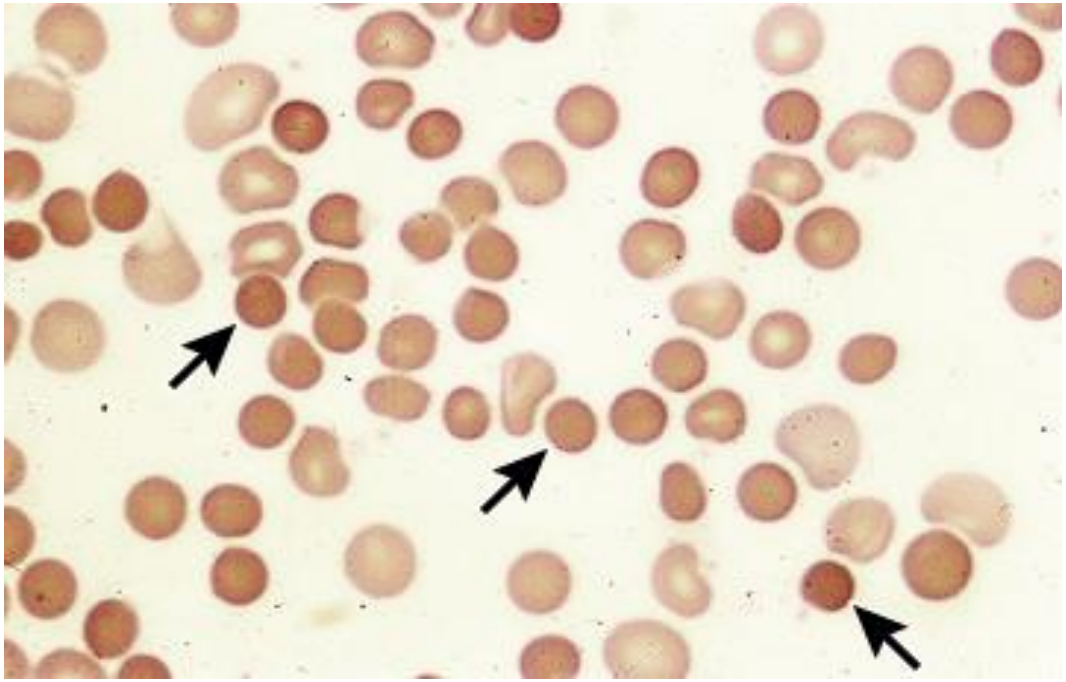
Normal



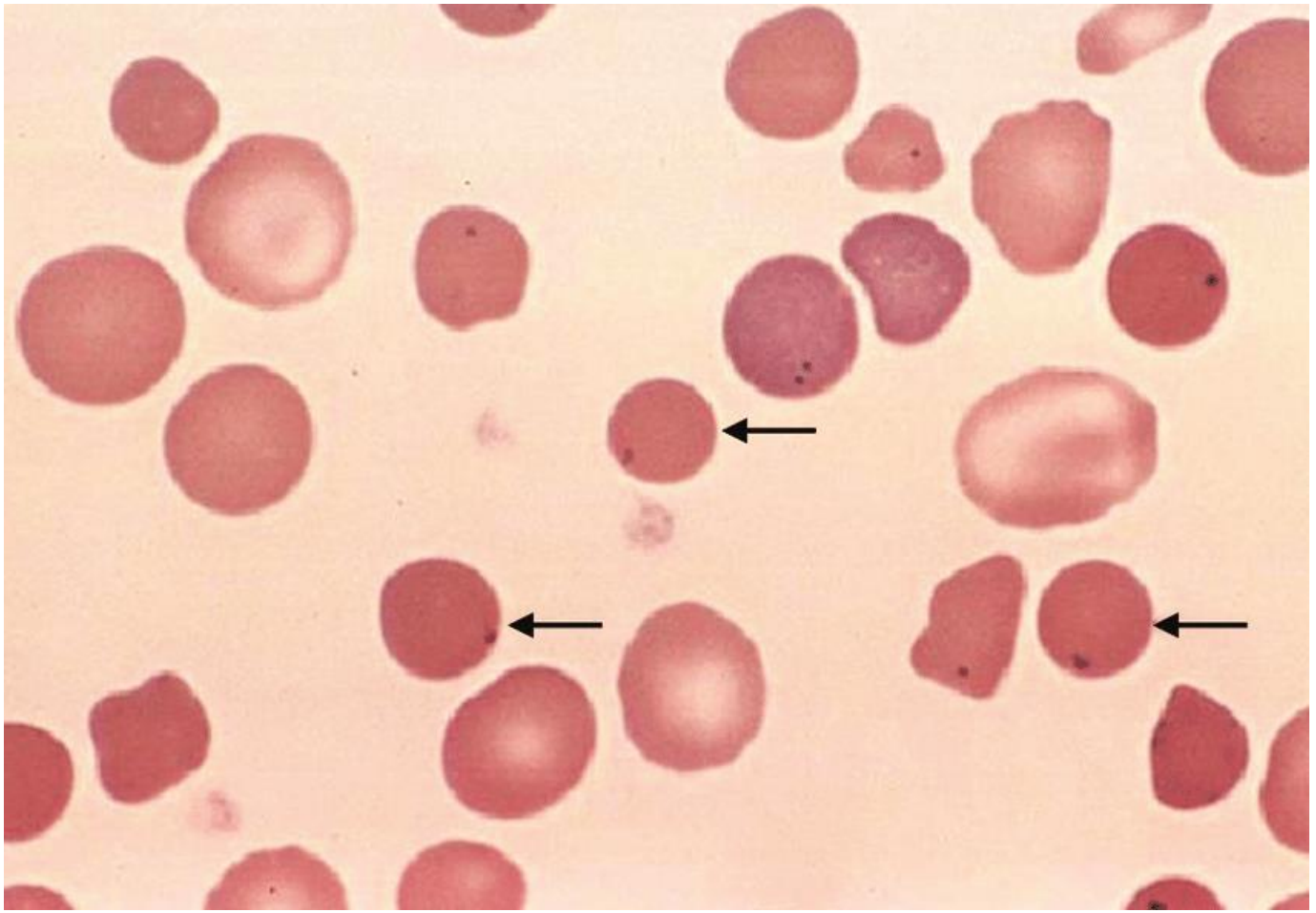
IDA



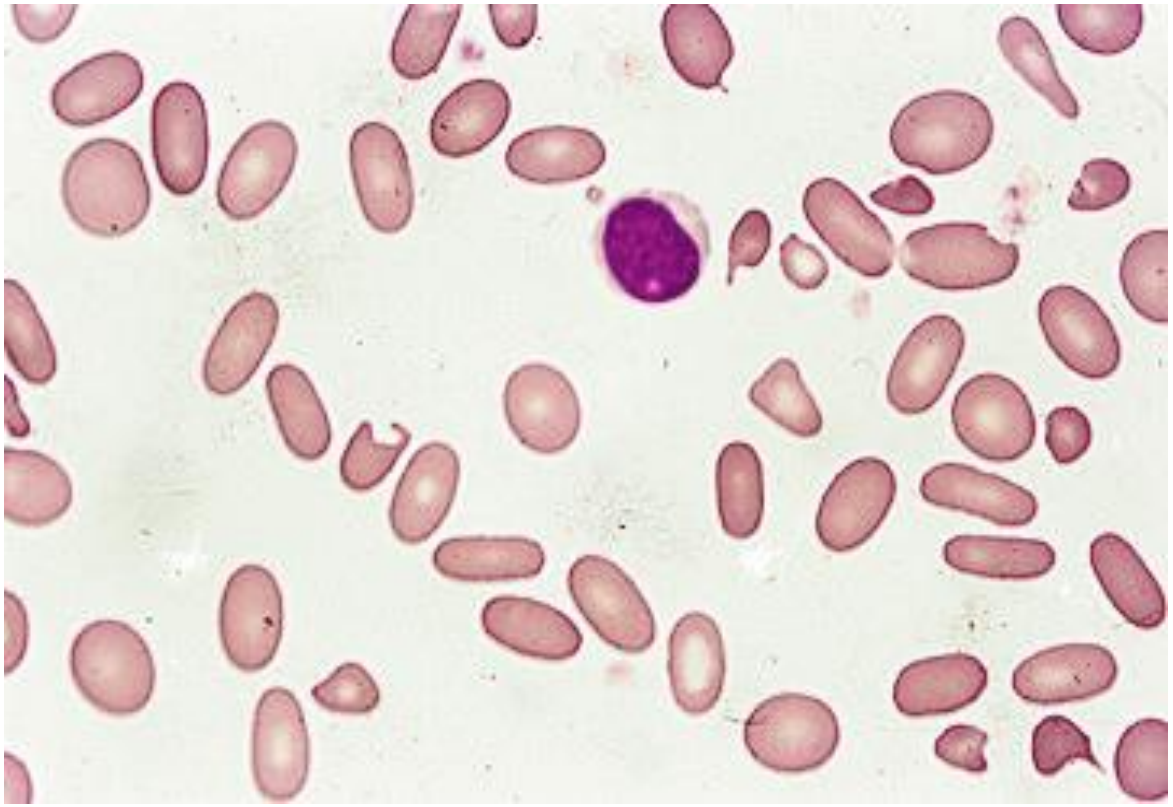
Spherocytosis



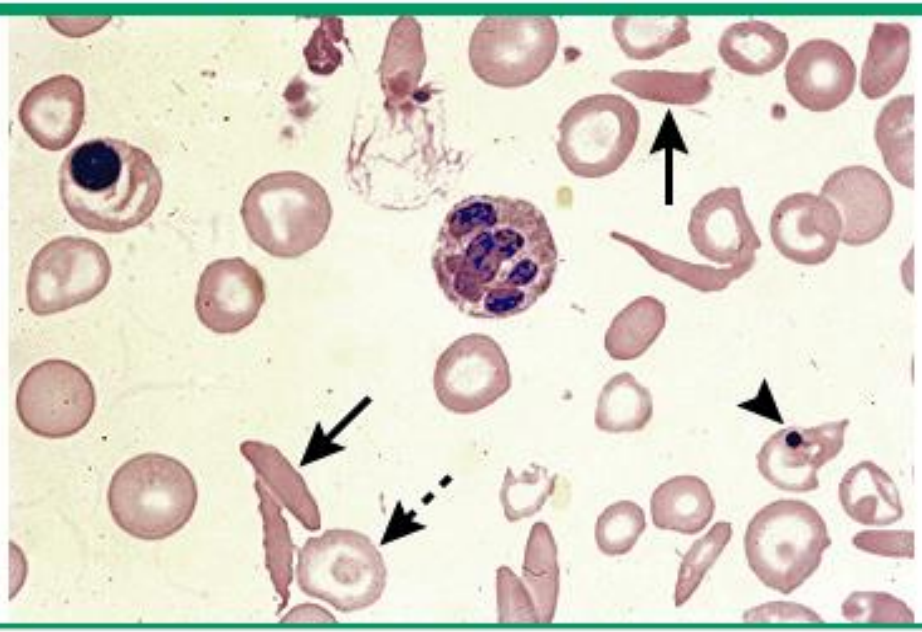
Spherocytosis



Elliptocytosis



Peripheral blood smear in sickle cell anemia



Peripheral blood smear from a patient with sickle cell anemia. This smear shows multiple sickle cells (arrows). There are also findings consistent with functional asplenia, including a nucleated red blood cell (upper left), a red blood cell containing a Howell-Jolly body (arrowhead), and target cells (dashed arrow).

Courtesy of Carola von Kapff, SH (ASCP).

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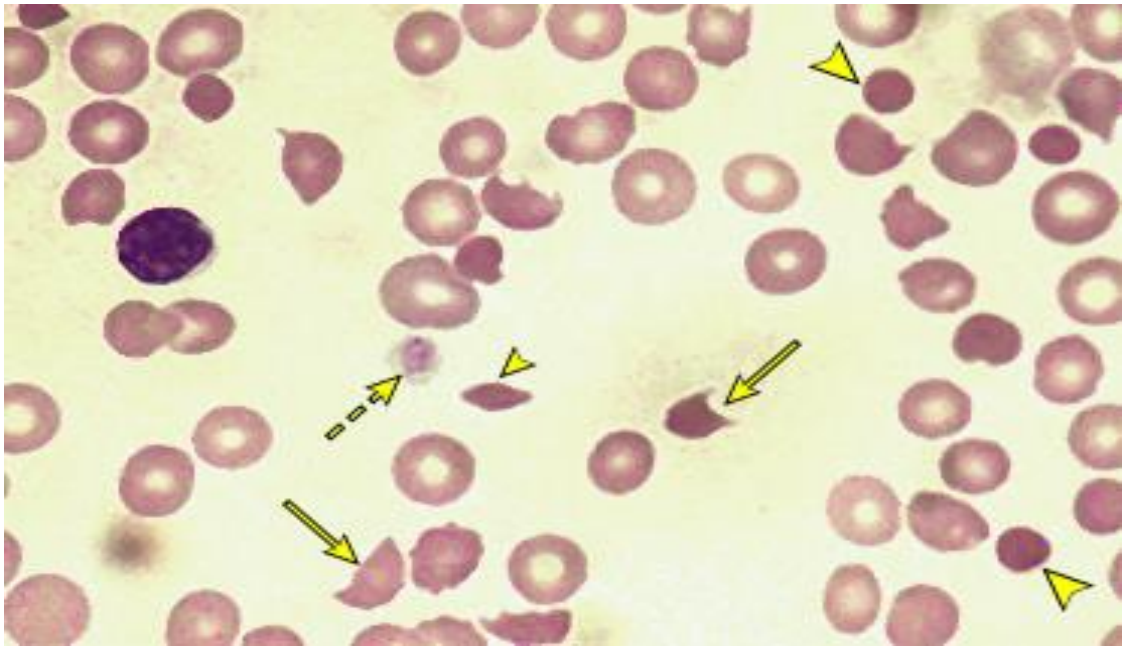
Schistocytes



Hypesegmented neutrophils