Laboratory Tests in Anemia

Anemia is defined as a reduction in hemoglobin concentration below the level, which is expected for healthy persons of same age and sex, and in the same environment. Adequate oxygen cannot be delivered to various organs and tissues due to low oxygen carrying capacity of blood.

Anemia may occur without symptoms and may be detected incidentally during medical examination. When severe enough, clinical features due to anemia result from hypoxia such as fatigue, weakness, dizziness, fainting, and mental confusion. Pallor of skin, mucous membranes, and conjunctiva is present. Hyperdynamic circulation causes palpitations and heart murmurs, and in severe cases congestive cardiac failure can develop, particularly in elderly. Anginal pain can result from myocardial hypoxia.

CLASSIFICATION OF ANEMIAS

There are two ways of classifying anemia:

- Etiological classification
- Morphological classification

1. Etiological classification: Anemia can result from a variety of causes (Table 27.1).

Table 27.1: Etiological classification of anemia

I. Anemia due to decreased production of red blood cells
   - Nutritional deficiencies: Iron deficiency anemia, megaloblastic anemia due to deficiency of folate or vitamin B_{12}
   - Anemia of chronic disease
   - Sideroblastic anemia
   - Aplastic anemia
   - Anemia due to infiltration of bone marrow by malignant cells
   - Anemia of chronic renal failure

II. Anemia due to increased destruction of red blood cells (Hemolytic anemia)
   A. Hereditary:
      - Defect in red cell membrane: Hereditary spherocytosis
      - Defect in hemoglobin: Sickle cell disease, thalassemia, hemoglobin E disease
      - Defect in red cell enzymes: Glucose-6-phosphate dehydrogenase deficiency

   B. Acquired:
      - Autoimmune hemolytic anemia
      - Paroxysmal nocturnal hemoglobinuria
      - Hemolytic transfusion reaction
      - Hemolytic disease of newborn
      - Mechanical hemolytic anemia
      - Hypersplenism
      - Malaria

III. Anemia due to acute blood loss: Hemorrhage due to trauma, massive gastrointestinal bleeding, or child delivery.

2- Morphological classification: This classification is based on red cell size and hemoglobin content (red cell indices) in a case of anemia. This classification is given later in Table 27.16.

Table 27.16: Morphological classification of anemia

Microcytic hypochromic anemia (MCV < 80 fl; MCHC < 30 gm/dl)

- Iron deficiency anemia
- Thalassemia
- Anemia of chronic disease
- Sideroblastic anemia

Macrocytic anemia* (MCV > 100 fl; MCHC 30-35 gm/dl)

- Megaloblastic
  - Folate or vitamin B_{12} deficiency
- Non-megaloblastic
  - Alcoholism
  - Liver disease
  - Hemolytic anemia
  - Myelodysplastic syndrome
  - Hypothyroidism

Normocytic and normochromic anemia (MCV 80-100 fl; MCHC 30-35 gm/dl)

- Reticulocyte count increased
  - Recent blood loss
  - Hemolysis
- Reticulocyte count low
  - Aplastic anemia
  - Chronic renal failure
  - Anemia of chronic disease
  - Anemia due to infiltration of marrow

*Another way of classification of macrocytic anemia: (1) Round macrocytosis: alcoholism, liver disease, hypothyroidism; and (2) oval macrocytosis: folate or vitamin B_{12} deficiency, myelodysplastic syndrome. *Abbreviations*: MCV: mean cell volume; MCHC: mean cell hemoglobin concentration

ANEMIAS DUE TO DECREASED PRODUCTION OF RED BLOOD CELLS

Iron Deficiency Anemia

Iron deficiency is the most common cause of anemia worldwide. Pregnant women, women in reproductive age group, and children under 5 years of age are particularly susceptible for nutritional deficiency. In men and postmenopausal women, blood loss (especially from the gastrointestinal tract) is the main cause. Common causes are listed in Table 27.2.

Table 27.2: Causes of iron deficiency anemia

- Nutritional deficiency due to poor diet or increased requirements: infants and children (6 months-2 years), women in reproductive age group, pregnancy
- Blood loss:
  a. *Gastrointestinal*: Esophageal varices, peptic ulcer, carcinoma of stomach, hookworm infestation, colorectal carcinoma, hemorrhoids
  b. *Genitourinary tract*: Menorrhagia, hematuria
- Malabsorption: Celiac disease

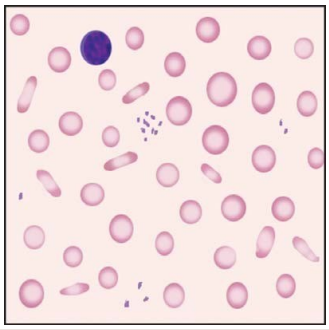


Fig. 27.1: Blood smear in iron deficiency anemia showing microcytic hypochromic red cells and "pencil" cells

Stages of Iron Deficiency

There are three stages of iron deficiency as shown in Box 27.1.

Box 27.1: Stages of iron deficiency

- *Stage 1 (Iron depletion)*: Depletion of storage iron (low serum ferritin), normal transport iron (serum iron, total iron binding capacity), normal hemoglobin
- *Stage 2 (Low transport iron)*: Depletion of storage iron, low transport iron, normal hemoglobin
- *Stage 3: (Low hemoglobin production)*: Depletion of storage iron, low transport iron, low hemoglobin

Laboratory Features

1. *Low hemoglobin and low packed cell volume*
2. *Low mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration*; red cell distribution width is increased.

3. *Blood smear* shows microcytic, hypochromic red cells and pencil cells (Fig. 27.1).

4. *Serum ferritin* is less than 15 µg/dl. The best determinant of storage iron is serum ferritin. Serum ferritin >100 µg/dl rules out diagnosis of iron deficiency in most cases. Serum ferritin is a storage form of iron and is reduced only in iron deficiency anemia. If iron deficiency anemia is associated with inflammatory, neoplastic, or liver disease, its concen- tration is raised; therefore, in these conditions, estimation of serum ferritin will not be helpful for diagnosis of iron deficiency. In the presence of an inflammatory disorder, assay for soluble transferrin receptor is preferable for diagnosis as it is not affected by inflammation.

5. *Serum iron, total iron binding capacity (TIBC), and transferrin saturation*: Serum iron is a measure of iron bound to transferrin (transport protein for iron). Transferrin saturation is the ratio of serum iron to transferrin. Typically, serum iron is low, TIBC is increased, and transferrin saturation is <10% in iron deficiency anemia. Free erythrocyte protoporphyrin is increased.

6. *Increased soluble transferrin receptor (sTfR) in serum*: This assay is useful in cases with associated inflammation. The transferrin receptor is a specific receptor for transferrin-iron complex and is located on cell membranes. In iron deficiency, its level increases due to increased expression on cell membranes. However, increased levels are also found in any condition associated with increased erythropoietic activity like hemolytic anemias and myeloprolife- rative disorders.

7. *Bone marrow iron stain*: This is the gold standard for diagnosis of iron deficiency and shows lack of stainable iron in the bone marrow. The test assesses storage iron in bone marrow (confined mainly within macrophages; small amount in erythroblasts). This test is expensive, and not required for routine diagnosis.

8. *Response to oral iron therapy*: Increased reticulocyte count beginning around 3rd day and reaching maximum on 5th-10th day after starting oral iron therapy indicates optimal response. Hemoglobin rises at the rate of 0.5-1.0 gm/dl/week.

9. Iron deficiency anemia should be differentiated from other causes of microcytic hypochromic anemia such as anemia of chronic disease, thalassemia, and sideroblastic anemia (Table 27.3).

Megaloblastic Anemia

Megaloblastic anemia results from deficiency of either folate or vitamin B_{12} (Table 27.4). Folate and vitamin B_{12} are essential for synthesis of deoxyribonucleic acid(DNA); in case of deficiency, therefore, there is defective synthesis of DNA in all the proliferating cells including bone marrow cells. Vitamin B_{12} is also required for neurological functions. Anemia, mild jaundice (due to ineffective erythropoiesis or premature destruction of erythroid precursors in bone marrow), and glossitis are common to both folate and vitamin B_{12} deficiency. Neurologic features like symmetric paresthesiae especially in lower limbs, reduced vibration sense, ataxia, loss of memory, and personality change occur in vitamin B_{12} deficiency but not in folate deficiency. Untreated cases may develop subacute combined degeneration of the spinal cord. Neurological damage due to vitamin B_{12} deficiency can occur in the absence of anemia.

Table 27.3: Differential diagnosis of microcytic hypochromic anemia

Parameter	*Iron deficiency anemia*	*Anemia of chronic disease*	*β thalassemia minor*	*Sideroblastic anemia*
1. Mean cell volume	Low	Normal or low	Markedly low	Low
2. Red cells on blood smear	Microcytic hypochromic	Normocytic normochromic; rarely microcytic hypochromic	Marked anisopoikilocytosis, basophilic stippling	Dimorphic
3. Serum iron	Low	Low	Normal	Increased
4. Serum ferritin	Low	Normal or increased	Normal	Increased
5. TIBC	Increased	Low	Normal	Normal
6. Storage iron in marrow	Absent	Normal or increased	Normal	Normal
7. Hemoglobin electrophoresis	Normal	Normal	Increased HbA_2	Normal
8. Iron in erythroblasts	Absent	Present	Present	Ringed sideroblasts
9. Serum soluble transferrin receptor	Increased	Normal	Normal or increased	Normal or increased

TIBC: Total iron binding capacity

Table 27.4: Causes of megaloblastic anemia

Mechanism	*Folate deficiency*	*Vitamin B_{12} deficiency*
Inadequate intake	Poor diet, chronic alcoholism	Strict vegetarians
Inadequate absorption	Malabsorption syndromes like celiac disease, tropical sprue; Crohn's disease	Pernicious anemia*, gastrectomy, resection of ileum, Crohn's disease, fish tapeworm infestation
Increased demand	Pregnancy, chronic hemolysis, neoplasia	–

*Autoimmune disorder characterized by gastric atrophy and loss of production of intrinsic factor in stomach that is necessary for absorption of vitamin B_{12}

Laboratory Features

- Macrocytic anemia (mean cell volume >100 fl in adults). Elevation of mean cell volume is an early sign and precedes the onset of anemia. Pancytopenia is common.

- Blood smear shows oval macrocytosis, basophilic stippling, Howell-Jolly bodies, and hyperseg- mentation of neutrophils (>5% of neutrophils showing 5 or more lobes) (Fig. 27.2).
- Reticulocyte count is normal or low.
- Bone marrow shows megaloblasts, erythroid hyperplasia, and giant metamyelocytes and bands (Fig. 27.3).
- Vitamin assays: See Table 27.5.

Identification of cause It is necessary to distinguish between folate and vitamin B12 deficiency and to determine the cause for appropriate treatment.
(Table 27.5, and Fig. 27.4). Treatment only with folate in vitamin B12 deficiency can worsen the neurological abnormalities. In vitamin B_{12} deficiency, test for vitamin B_{12} absorption (Schilling test) can be carried out. In this test, urinary excretion of oral dose of radio-labeled vitamin B12 is compared with the excretion of oral dose of radiolabeled vitamin B_{12} bound to intrinsic factor. In pernicious anemia, deficient absorption is corrected with addition of intrinsic factor.

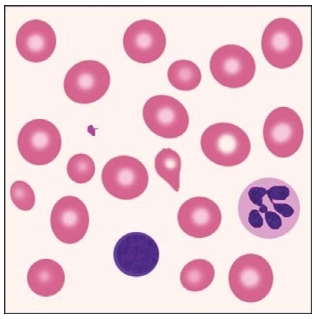


Fig. 27.2: Blood smear in megaloblastic anemia showing oval macrocytes and a hypersegmented neutrophil

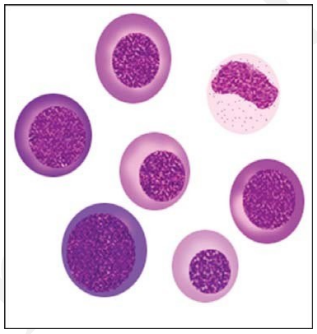


Fig. 27.3: Bone marrow smear showing megaloblasts and a giant metamyelocyte

Table 27.5: Differences between folate and vitamin B_{12} deficiency

Parameter	*Folate deficiency*	*Vitamin B_{12} deficiency*
1. Usual mechanism of deficiency	Inadequate intake	Inadequate absorption
2. Neurologic features	Absent	May be present
3. Serum vitamin B_{12}	Normal	Low
4. Serum folate	Low	Normal or increased
5. Red cell folate	Low	Low
6. Serum homocysteine	Increased	Increased
7. Serum methylmalonic acid	Normal	Increased
8. Therapeutic trial	Optimal response to folate	Optimal response to vitamin B_{12}

Anemia of Chronic Disease

Anemia of chronic disease is the most common form of anemia amongst hospitalized patients. The three disease categories associated with anemia of chronic disease are chronic infection, inflammation, and malignancy (Table 27.6). There is a block in release of storage iron from macrophages for erythropoiesis that is mediated by inflammatory cytokines.

Anemia is mild (9-10 gm/dl) and non-progressive. Clinical features reflect underlying disease. The main differential diagnosis is iron deficiency anemia.

Table 27.6: Diseases associated with anemia of chronic disease

1. **Chronic infections:** Tuberculosis, urinary tract infection, bronchiectasis, osteomyelitis, subacute bacterial endocarditis
2. **Chronic inflammation:** Rheumatoid arthritis, systemic lupus erythematosus.
3. **Malignancy**

Laboratory Features

- Normocytic normochromic anemia (70% cases) or microcytic hypochromic anemia (30% cases).
- Decreased serum iron, decreased total iron binding capacity, and normal or raised serum ferritin. Serum transferrin receptor level is normal.
- Increased marrow storage iron
- Erythrocyte sedimentation rate is increased out of proportion to the degree of anemia.

Sideroblastic Anemia

In sideroblastic anemia, heme synthesis is deficient. There is a mitochondrial defect that leads to the failure of incorporation of iron into heme. Iron accumulates in mitochondria that surround the nucleus of erythroblasts forming ringed sideroblasts. Sideroblastic anemia is characterized by:

- Dimorphic anemia (i.e. blood smear shows dual population of cells: normocytic normochromic, and microcytic hypochromic) (Fig. 27.5).
- Ringed sideroblasts in bone marrow (see Fig. 27.5).
- Sideroblastic anemia may be hereditary (X-linked) or acquired. Most **cases are acquired and causes include:**

(i) drugs: isoniazid, chloramphenicol, cytotoxic drugs,
(ii) alcoholism, (iii) lead poisoning, (iv) myelodysplastic syndrome, and (v) acute myeloid leukemia.

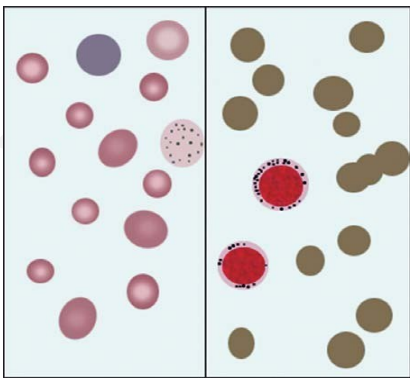


Fig. 27.5: Blood smear (on left) in sideroblastic anemia showing dimorphic red cells, basophilic stippling, and a polychromatic red cell. Bone marrow smear stained with iron stain (on right) shows ringed sideroblasts

Aplastic Anemia

Aplastic anemia is characterized by pancytopenia in peripheral blood (reduction of red cells, leukocytes, and platelets in peripheral blood) and decreased cellularity in bone marrow. Aplastic anemia can occur at any age and clinical presentation is related to

pancytopenia (anemia, risk of infections, and bleeding manifestations). Organomegaly is typically absent.

Causes of aplastic anemia are listed in Table 27.7.

Investigations in a Suspected Case of Aplastic Anemia

- *Tests for confirmation of aplastic anemia*: Complete blood count (to demonstrate pancytopenia, low hemoglobin, and low reticulocyte count) and bone marrow examination (to demonstrate depletion of hema- topoietic precursors and hypocellularity).

Table 27.7: Causes of aplastic anemia

Acquired
Idiopathic Drugs: *Idiosyncratic:* antibacterials (chloramphenicol, sulfonamides), nonsteroidal anti-inflammatory drugs (phenylbutazone, indomethacin, piroxicam, diclofenac), antithyroid drugs, furosemide, phenothiazines, allopurinol, oral antidiabetics, *Dose-related:* Cytotoxic drugs
Chemicals (e.g. benzene) or radiation
Infections: Hepatitis; Epstain Barr virus, Mycobacteria
Paroxysmal nocturnal hemoglobinuria Systemic lupus erythematosus
Graft vs. host disease
Inherited
Fanconi's anemia, Dyskeratosis congenita

HEREDITARY DISORDERS OF HEMOGLOBIN

Hereditary disorders of hemoglobin result either from
(i) reduced synthesis of one globin polypeptide chain, leading to chain imbalance, or (ii) synthesis of a polypeptide chain with abnormal structure (Table 27.8).

Thalassemias

The thalassemias are inherited disorders characterized by reduced or absent synthesis of α or β globin polypeptide chains.

Classification

There are two main forms of thalassemias:

- α thalassemias (deficient synthesis of α globin chains).
- β thalassemias (deficient synthesis of β globin chains).

Distribution

- *α thalassemias*: Southeast Asia, Eastern Mediterranean region, Middle East.
- *β thalassemias*: Mediterranean region, Africa, Middle East, India, Pakistan, Southeast Asia.

β thalassemias: Normally, there are two β globin genes, one on each member of chromosome 11 (genotype β/β). β thalassemias result from a single base substitution (point mutation) in β globin gene. More than 150 mutations of β globin gene have been reported to cause β thalassemia. There are two main forms of β thalassemias β^0 and β^+. β^0 thalassemia is characterized by complete absence of β chain synthesis, while in β^+ thalassemia β chain synthesis is reduced.

There are three clinical forms of β thalassemias: thalassemia major, thalassemia intermedia, and thalassemia minor.

1. *β thalassemia major (Homozygous β thalassemia, Cooley's anemia):* β thalassemia major results when both the β globin genes are defective, e. g. genotype $\beta^{\circ}/\beta^{\circ}$ or β°/β^{+}. It is characterized by severe anemia, and requires regular blood transfusion therapy for survival. Clinical features of β thalassemia major are severe hemolytic anemia that develops around 6 months of age, jaundice and progressive splenomegaly, severe growth retardation and delayed development, skeletal defor- mities (frontal bossing, malar prominence), susceptibility to infections and to folate deficiency, and transfusion- dependence for survival.

   Characteristic laboratory features are:
   - Severe anemia (hemoglobin < 7.0 gm/dl).
   - Blood smear shows marked variation in size and shape of red cells, red cell fragments, hypochromia, target cells, basophilic stippling, and nucleated red cells (Fig. 27.6).
   - Reticulocytosis (5-15%).
   - Low mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration.
   - Markedly elevated hemoglobin F on electrophoresis (Fig. 27.28, later).

2. β thalassemia *intermedia:* This results from homozygous inheritance of mild β^{+} thalassemia (e.g. genotype β^{+}/β^{+}). It is characterized by moderate degree of anemia which does not require regular blood transfusion therapy. Worsening of anemia occurs during infections and pregnancy. Thalassemia intermedia presents at a later age (2-5 years) than thalassemia major. These patients have chronic hemolytic anemia, splenomegaly, and skeletal changes.

Laboratory features are:
- Moderate degree of anemia (hemoglobin 7-10 gm/dl).
- Blood smear shows less severe abnormalities than thalassemia major
- Reticulocytosis (5-10%)
- Varible elevation of hemoglobin F on electrophoresis

3. *β thalassemia minor (thalassemia trait, asymptomatic thalassemia):* Usually individuals having one normal and one abnormal β globin gene has β thalassemia minor (e.g.β/β^{+} or β/β°). In this heterozygous carrier state, anemia is either mild or absent. This condition is often detected during the course of routine hematological investigations.

Laboratory features are:
- Hemoglobin slightly low or normal (> 10 gm/dl)
- Low mean cell volume and mean cell hemoglobin; normal mean cell hemoglobin concentration
- Blood smear shows microcytic hypochromic red cells, target cells, and basophilic stippling (Fig. 27.7)
- Normal serum ferritin

- Hemoglobin electrophoresis shows elevated hemoglobin A2 (> 3.5%); this is a diagnostic test for β thalassemia minor.

α thalassemias: Normally, there are four α globin genes, two on each member of chromosome 16 (genotype αα/αα). α thalassemias usually results from gene deletions. Since α chains are present in both fetal and adult hemoglobin, α thalassemia manifests in both fetal and adult lives. In α° thalassemia, both α globin genes on one chromosome are lost, while in α^{+} thalassemia, one α globin gene of the pair is lost.

There are three clinical forms of α thalassemia: Hb Bart's hydrops fetalis syndrome (--/--), Hb H disease (--/-α), and α thalassemia carrier state (-α/αα, -α/-α, or --/αα) (Fig. 27.8).

Hb Bart's hydrops fetalis syndrome is the most severe form and infants are either stillborn prematurely or die soon after birth. They have severe anemia, generalized edema, and hepatosplenomegaly. Mothers of such infants usually have toxemia of pregnancy. Blood smear shows marked variation in size and shape of red cells, microcytosis, hypochromia, and nucleated red cells. Hemoglobin electrophoresis reveals predominance of **Hb Bart's and absence of Hb A.**

Patients with HbH disease have moderate anemia (hemoglobin 7-10 gm/dl), jaundice, and hepatosplenomegaly. Typical laboratory features are microcytic hypochromic red cells, Hb H inclusions in red cells, and variable amount of HbH on electrophoresis.

α thalassemia carriers are asymptomatic. **Microcytic hypochromic red cells may or may not be present**. **Hemoglobin electrophoresis is normal**. Determining ethnic origin, family studies, globin chain analysis, or genetic analysis may be needed for definitive diagnosis

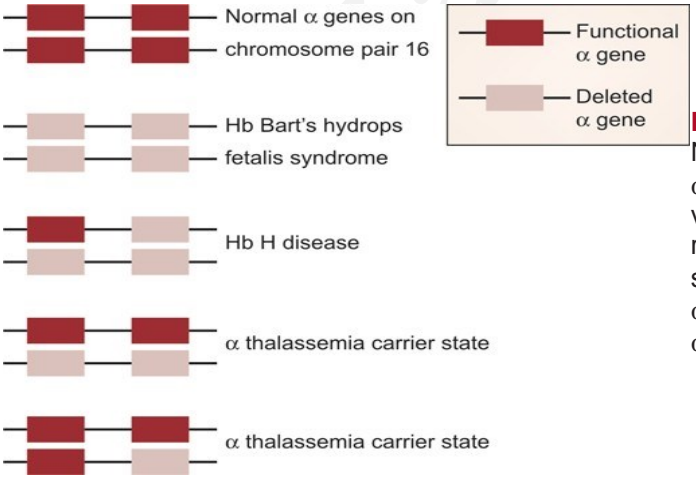


Fig. 27.8: Genotypes in α thalassemias. Normal genotype is represented as αα/αα. The effective genotypes of various thalassemia syndromes are represented as: Hb Bart's hydrops fetalis syndrome: --/--; Hb H disease: α-/--; and α thalassemia carrier state: αα/-- or αα/α-

Sickle Cell Disorders

Sickle cell disorders are characterized by the presence of **hemoglobin S** (HbS) in red cells due to a point mutation **A---T** in the 6 th codon of β globin gene, which results in substitution of valine for glutamic acid at position 6 of β polypeptide chain

(β^{6glu}--^{val}). Upon deoxygenation, HbS polymerizes in red cells leading to the formation of sickle-shaped red cells; such red cells are rigid, occlude the microvasculature, and cause painful vaso-occlusive crises. Sickle cells adhere to vascular endothelium due to increased expression of adhesion molecules. Deformed red cells are removed from the circulation and destroyed prematurely, leading to chronic hemolytic anemia. Disease manifests in individuals with homozygous sickle cell disease; heterozygotes are usually asymptomatic.

Table 27.10: Sickle cell disorders

Disorder	*Genotype*
1. Sickle cell anemia	β^S/β^S
2. Sickle cell trait	β^S/β
3. Double heterozygous states	
• Hemoglobin S-C disease	β^S/β^C
• Hemoglobin S-D disease	β^S/β^D
• Sickle cell-β thalassemia	β^S/β^0

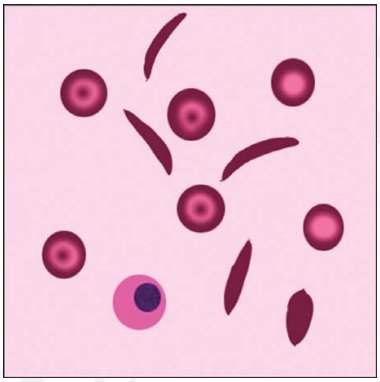


Fig. 27.9: Blood smear in sickle cell anemia.

Manifestations include chronic hemolytic anemia, vaso-occlusive crises (acute episodes of severe pain in chest, abdomen, back, or extremities), aplastic crisis (sudden aggravation of anemia due to infection by parvovirus), hemolytic crisis (exacerbation of hemolysis due to infection or associated glucose-6-phosphate dehydrogenase deficiency), splenic sequestration crisis (sudden enlargement of spleen due to pooling of blood leading to circulatory failure), risk of infections (especially *Streptococcus pneumonia, Hemophilus influenzae, Neisseria meningitidis, Escherichia coli)* and retarded growth and development.

In sickle cell anemia, blood smear shows sickled red cells and target cells (Fig. 27.9), solubility test is positive, and hemoglobin electrophoresis shows mostly HbS with no HbA. In sickle cell trait, blood smear is normal or shows target cells, solubility test is positive, and hemoglobin electrophoresis shows **HbA (60%) and HbS (40%).**

Although blood smear shows sickle cells in sickle cell anemia, and both slide test and solubility test are positive in sickle cell disorders, hemoglobin electrophoresis is essential for diagnosis (Fig. 27.28, later). This is to differentiate between sickle cell trait and sickle cell anemia, and for detection of double heterozygous states like sickle cell-β thalassemia or sickle cell-HbD disease

Hemoglobin D

Blood smear shows hypochromia, microcytosis, and target cells. On hemoglobin electrophoresis at alkaline pH, hemoglobin D co-migrates with HbS. Slide test for sickling, however, is negative in Hb D disease.

Table 27.11: Hemoglobin electrophoresis patterns in sickle cell disorders

Disorder	*Hemoglobin electrophoresis*	*Parents' phenotype*
1. Sickle cell anemia	SF	Both parents: AS
2. Sickle cell trait	AS	One parent: AA; Other parent: AS
3. Sickle cell-β^0 thalassemia	SF; A_2 increased	One parent: AS; Other parent: β thalassemia minor
4. Sickle cell-β^+ thalassemia	SAF; A_2 increased	One parent: AS; Other parent: β thalassemia minor

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked disorder characterized by reduced activity of G6PD enzyme in red cells and occurrence of hemolysis on exposure to oxidant stress. Numerous (>400) biochemical variants of G6PD enzyme have been reported due to point mutations or deletions in the gene. **G6PD deficiency leads to inability of red cells to remove toxic hydrogen peroxide (H2O2), an oxidative metabolite**. **Accumulated H2O2 leads to oxidation of hemoglobin with subsequent denaturation and precipitation of globin chains**. Precipitated globin form inclusions attached to red cell membrane (Heinz bodies). Red cells containing Heinz bodies are destroyed in spleen (extravascular hemolysis). Oxidant damage also causes peroxidation of membrane lipids of red cells leading to intravascular hemolysis.

G6PD deficiency is usually asymptomatic. It can cause (i) neonatal jaundice, (ii) acute hemolytic anemia on exposure to oxidant stress (Table 27.12), or (iii) chronic hemolytic anemia.

Examination of blood smear during hemolytic episode shows polychromasia, fragmented red cells, spherocytes, bite cells (red cells having bitten out margins) and half-ghost cells (one half of the red cell is empty and the other half is filled with hemoglobin) (Fig. 27.11). Biochemical abnormalities include increased serum bilirubin, hemoglobinemia, and hemoglobinuria (due to intravascular hemolysis). Heinz bodies can be demonstrated by methyl violet staining. The screening tests used for G6PD deficiency are fluorescent spot test, methemoglobin reduction test, and dye decolorization test.

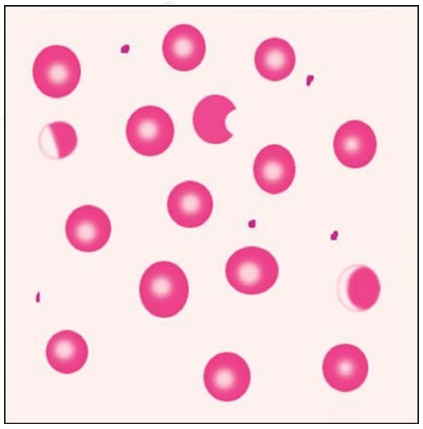


Fig. 27.11: Blood smear during acute hemolysis in glucose 6 phosphate dehydrogenase deficiency showing a bite cell and hemi-ghost cells

Table 27.12: Causes of hemolysis in glucose-6-phosphate dehydrogenase deficiency

1. Bacterial and viral infections
2. Drugs:
   - Antimalarials: Primaquine, pamaquine
   - Antibacterials: Sulfonamides, nalidixic acid, nitrofurantoin, dapsone
   - Antipyretics and analgesics
3. Chemicals: Naphthalene balls

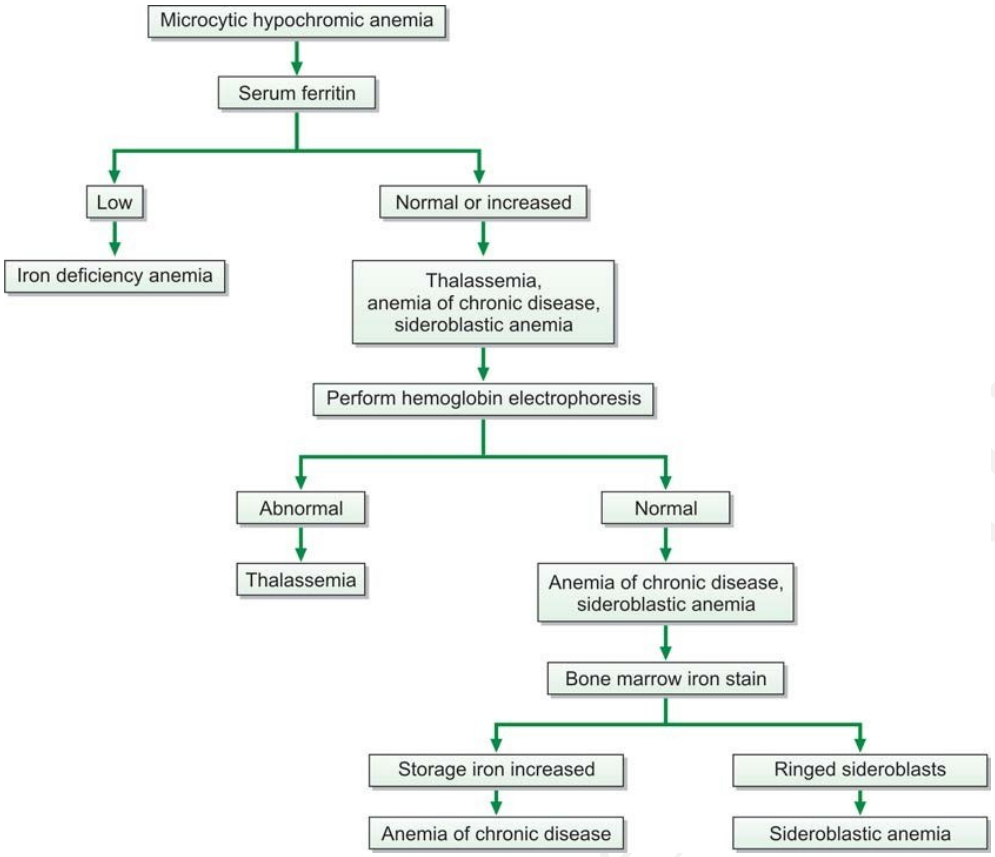


Fig. 27.20: Evaluation of microcytic hypochromic anemia

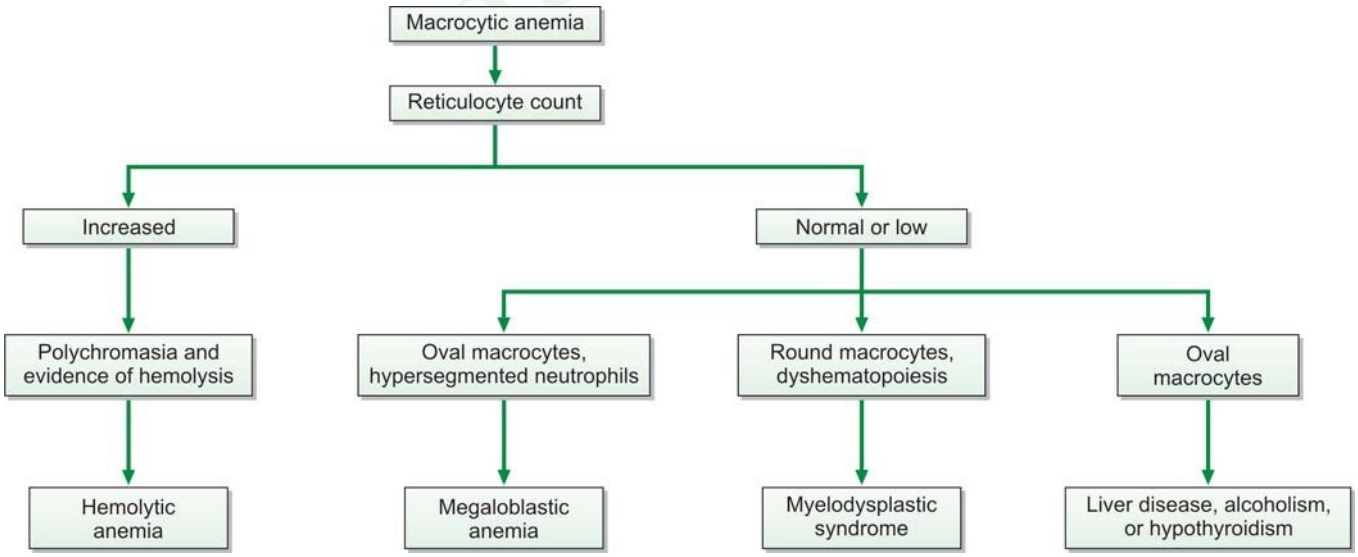
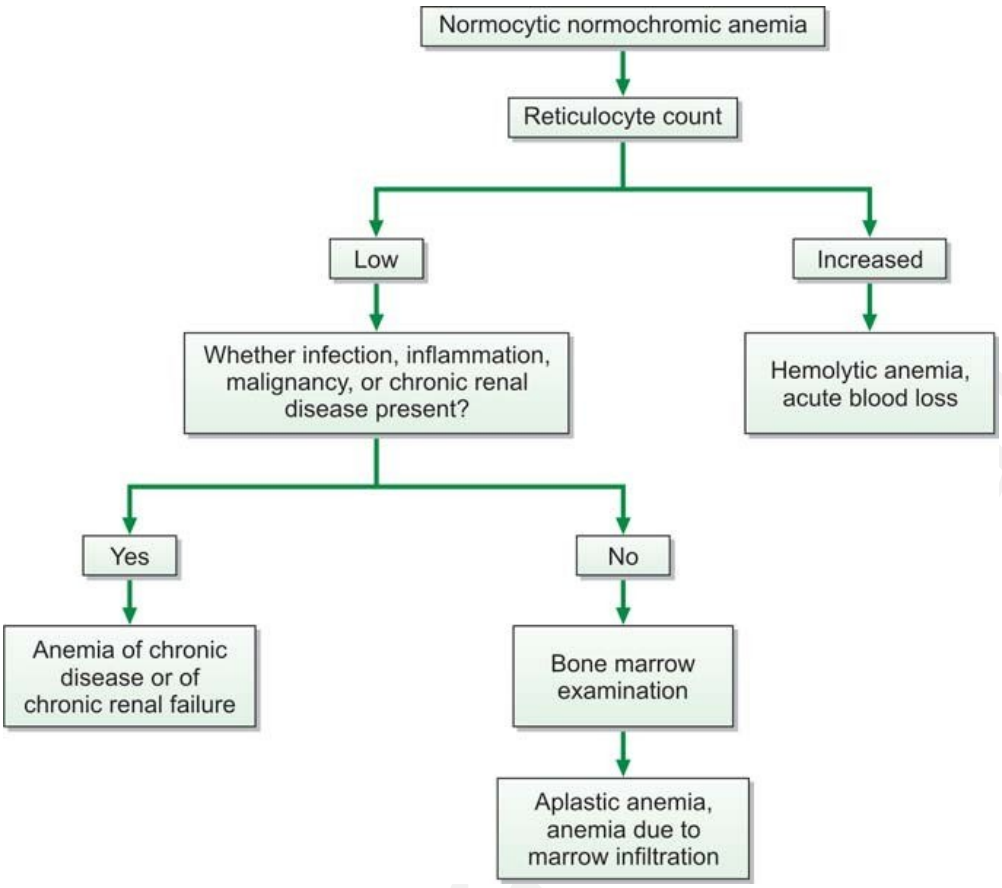


Fig. 27.21: Evaluation of macrocytic anemia



ig. 27.22: Evaluation of normocytic normochromic anemia