As normoblasts mature, there is gradual increase in haemoglobin production. Normoblast generally spends 5–7 days in proliferating and maturing compartment of the marrow.

After maturation in the marrow, the reticulocytes are released into the marrow sinuses and gain access to peripheral blood. It continues to mature in blood for 1 or 2 days.

Description of Erythroid Series Cells (Fig. 1.2)

Erythroblast (Normoblast)

- 14–20 µ, round shaped, nucleus round.
- Nucleus large occupies 4/5th of the cell and cytoplasm is 1/5th of the cell.
- The cytoplasm is basophilic.
- The nucleus has nucleoli (1–2).
- Dividing cell.

Basophilic erythroblast (early normoblast)

- 12–15 µ
- Round shaped
- Basophilic cytoplasm
- Nucleus—chromatin is dense
- Dividing cell
- Nucleolus (1) present.

Polychromatic erythroblast (intermediate normoblast)

- 12–14 µ
- Round shaped
- Cytoplasm is polychromatic (purplish pink)
- Pink tint is because of haemoglobin.

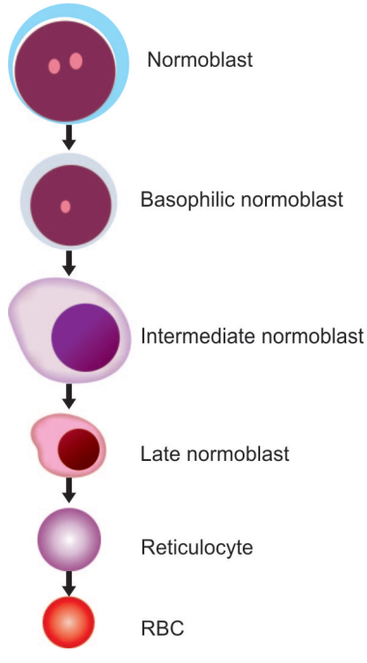

Fig. 1.2: Erythroid series cells (schematic)

- Nucleus—chromatin clumped.
- No division, cell develops by maturation.

Orthochromatic erythroblast (late normoblast)

- 12–14 µ
- Round shaped
- Cytoplasm—more pinkish because of increased content of Hb
- Nucleus small and pyknotic with blue black colour
- The cell matures into reticulocyte.

Reticulocyte

- 8 µ
- Slightly larger than normal RBCs
- Biconcave discoid shaped
- Cytoplasm—polychromatic, contains RNA material which can be stained with supravital stains.
- Matures to RBCs in 1–2 days.

Red Blood Cell

- 7.2 µ
- Biconcave, discoid shaped
- Central 1/3rd is pale, peripheral 2/3rd pinkish.

MYELOID SERIES (Fig. 1.3)

Myeloblast

- 15–20 µ, round shaped
- Nucleus—round, occupies 4/5th of the cell and cytoplasm is 1/5th of the cell.
- Nuclear chromatin less coarser than that of lymphoblast
- The cytoplasm is basophilic.
- The nucleus has 4–5 nucleoli.
- Dividing cell
- Sometimes Aner rod is found in the cytoplasm. It is purplish pink in colour.

Promyelocyte

- Nucleus—round
- Nuclear chromatin coarse
- Cytoplasm has primary granules which are dusty and purplish pink
- Nucleoli are few, 1–2 in number.
- Other features are similar to myeloblast.
- Dividing cell.

Myelocyte

- Nucleus is round.
- Nuclear chromatin still coarser, no nucleoli.
- Cytoplasm less basophilic and abundant.

- Changes in the nails—longitudinal ridging, flattening and **koilonychia** (spoon-shaped nails) or nails that are weak or brittle.
- Poor appetite
- Unusual obsessive food craving, known as pica
- **Plummer-Vinson syndrome** (Paterson-Brown-Kelly syndrome): Dysphagia due to formation of oesophageal webs, iron deficiency anaemia, glossitis and cheilitis. Spleen may be enlarged, most commonly seen in postmenopausal females.
- **Tayanc-Prasad syndrome** (growth retardation, hypogonadism, hepatosplenomegaly, zinc and iron deficiency, geophagia).

Approach to a patient with iron deficiency anaemia, investigations and grading of iron stores in bone marrow are shown in Tables 2.5, 2.6 and 2.7; Figs 2.2 and 2.3.

Table 2.5 Approach to a patient with iron deficiency anaemia

History

Females in reproductive period: Menorrhagia, pregnancies—number and frequency, miscarriages, iron deficient diet, GI blood loss, hematuria, epistaxis, haemoptysis, GI surgery, aspirin ingestion

Males and post-menopausal females: Iron deficient diet, haematemesis, melaena or per rectal bleeding (GI blood loss due to haemorrhoids, oesophageal varices, bleeding due to GI malignancies), haematuria, epistaxis, haemoptysis, GI surgery, aspirin ingestion

Infants and children: Dietary history regarding supplemental feeding, prematurity, multiple births, iron deficiency in mother, GI disturbances, blood loss of any cause

Physical and systemic examination

Examination of any mass, rectal examination, pelvic examination in females, telangiectasias of face and mouth

Relevant investigations commonly required

- Examination of faeces for occult blood and hookworm
- Urine microscopy for haematuria
- GI endoscopy or barium swallow study: Peptic ulcer, hiatus hernia
- Ca stomach, oesophageal varices, Meckel's diverticulum
- Barium swallow studies of oesophageal varices in a cirrhotic patient show multiple serpiginous filling defects of lower one-third of the oesophagus.

Colonoscopy: Carcinoma colon, caecum, ulcerative colitis, diverticula, angiodysplasia

Sigmoidoscopy: Carcinoma rectum, ulcerative colitis

Relevant investigations occasionally required

- Chest X-ray and bronchoscopy (haemoptysis)
- Cystoscopy (haematuria)
- Liver function tests (cirrhosis)

Table 2.6 Blood picture, bone marrow and biochemical findings in iron deficiency anaemia

1. Complete blood count
- Low haemoglobin
- Low haematocrit
- Reduced RBC count

2. RBC indices
- Low MCV
- Low MCH
- Low or normal MCHC
- Increased RDW

3. Peripheral smear

RBCs: RBCs show anisocytosis and poikilocytosis

Majority of the RBCs are microcytic hypochromic, ring/pessary type cells, pencil-shaped cells, target cells, polychromatic cells are present

WBCs: Count and distribution normal

Platelets: Count and morphology normal

4. Bone marrow examination
- Depleted iron stores (Perls' stain)
- Erythroid series—erythroid hyperplasia, micronormoblastic reaction
- Granulopoiesis—normal
- Megakaryopoiesis—normal

5. Iron studies
- Serum Iron: ↓
- Serum ferritin ↓ in general, values less than 10 µg/L are indicative of iron deficiency
- TIBC: ↑, TIBC is 1/3rd saturated under normal conditions
- Plasma transferrin ↑
- Transferrin saturation: ↓ (normal 6–33%), <5% definitely indicates iron deficiency
- Transferrin receptor: ↑ free erythrocyte protoporphyrin

6. Stool examination: Hookworm infestation

Normal values:
- **Serum iron:** Male—27–138 µg/dL, female—33–102 µg/dL
- **Serum ferritin:** Male—29–248 µg/L, female—10–150 µg/dL
- **TIBC:** Male—174–351 µg/dL, female—194–372 µg/dL
- **Plasma transferrin:** Male—194–348 µg/dL, female—181–416 µg/dL
- **Free erythrocyte protoporphyrin:** 17–27 µg/dL
- **Transferrin saturation:** 6–33%

Differential diagnosis for microcytic anaemias
- Iron deficiency anaemia
- Thalassaemia, HbC, HbE, etc.
- Sideroblastic anaemia
- Lead poisoning
- Anaemia of chronic diseases (sometimes)

Normal Values

1. Serum cobalamin levels—200–900 ng/l, <100 ng/l in megaloblastic anaemia due to vitamin B_{12} deficiency.
2. Serum methylmalonic acid >0.4 µmol/l.
3. Serum folate levels up to 5.0 µg/l, <3 µg/l in megaloblastic anaemia due to folate deficiency.
4. Homocysteine levels: Males—14–15 µmol/l; females—12–14 µmol/l.
5. Red cell folate levels >160 µg/l.

Microbiological Assay in Vitamin B_{12} Deficiency Anaemia

Two micro-organisms ***Euglena gracilis*** and ***Lactobacillus leichmani*** are vitamin B_{12} dependent organisms and vitamin B_{12} in the serum is determined by comparing the growth of the organisms.

Microbiological Assay in Folic Acid Deficiency Anaemia

The folate activity can be assessed by methyl tetrahydrofolate. This compound is microbiologically active for ***Lactobacillus casei*** which is used for assay.

Stool Examination for Parasite

In vitamin B_{12} deficiency: Stool examination for proglottids of fish tapeworm *D. latum.* (Rare in India)

Diagnosis of megaloblastic anaemia

1. Oval macrocytes in peripheral smear
2. Hypersegmented neutrophils
3. Megaloblastic hypercellular marrow
4. Response to vitamin B_{12}/folate therapy

Other causes of macrocytic anaemia

1. Alcoholism
2. Hepatic causes
3. Hypothyroidism
4. Increased reticulocyte count—haemolysis
5. Drugs

HAEMOLYTIC ANAEMIAS

Definition

Haemolytic anaemia (HA) results from premature destruction of erythrocytes. The normal red cell lifespan is 120 days. In haemolytic anaemia, the lifespan of RBCs is shortened by varying degrees and in many cases they survive for only a few days.

Patient may not always be anaemic because of bone marrow compensation.

Anaemia in haemolytic anaemia develops due to:

- Reduced lifespan
- Aplastic crisis
- Haemolytic crisis

Classification of haemolytic anaemia is given in Table 2.11.

Clinical Features

- Pallor
- Intermittent jaundice
- Splenomegaly
- Gallstones—in chronic forms
- Crisis—aplastic, haemolytic
- Ankle ulcers

Table 2.11 Classification of haemolytic anaemia (HA)

HA due to intrinsic (intracorpuscular) abnormalities

CONGENITAL

Membrane abnormalities

- Membrane skeleton proteins: Spherocytosis, elliptocytosis
- Membrane lipids: Abetalipoproteinaemia

Disorders of haemoglobin synthesis

- Deficient globin synthesis: Thalassaemia syndromes
- Structurally abnormal globin synthesis (haemoglobinopathies): Sickle cell anaemia, unstable haemoglobins
- Double heterozygous disorders: Sickle cell beta thalassaemia

Enzyme deficiencies

- Glycolytic enzymes: Pyruvate kinase, hexokinase, enzymes of hexose monophosphate shunt: glucose-6-phosphate dehydrogenase, glutathione synthetase

ACQUIRED

Membrane defect: Paroxysmal nocturnal haemoglobinuria

HA due to extracorpuscular abnormalities

ACQUIRED

Immune mechanisms

- Antibody mediated: Warm antibodies/cold antibodies
- Transfusion reactions: Incompatible blood transfusion
- Erythroblastosis fetalis (haemolytic disease of the newborn)
- Autoantibodies: Idiopathic (primary), drug-associated, systemic lupus erythematosus

Non-immune mechanisms

Mechanical trauma to red cells

- Microangiopathic haemolytic anaemias: Thrombotic thrombocytopenic purpura, disseminated intravascular coagulation
- Prosthetic heart valves
- March haemoglobinuria

Miscellaneous causes

- Infections: Malaria
- Burns
- Lead poisoning

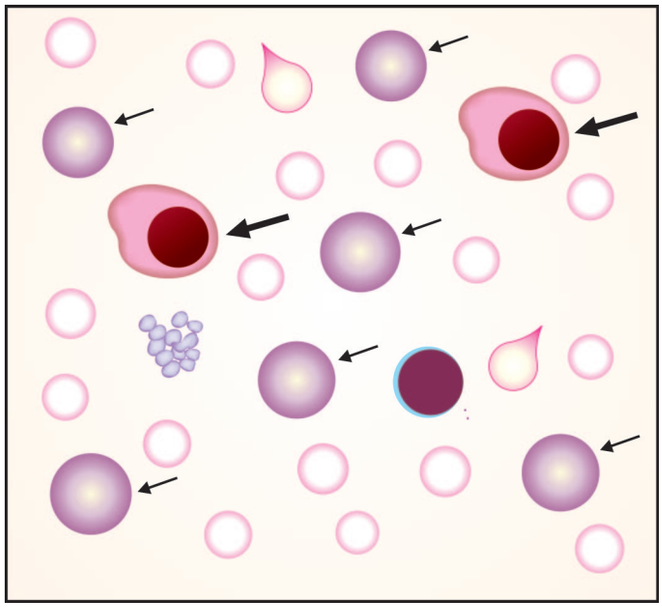

Fig. 2.16: Peripheral smear in thalassaemia major (thick arrow—nucleated RBCs, thin arrow—polychromatophilic cells)

- Increased serum uric acid
- Normal free RBC protoporphyrin
- Necked eye single test tube red cell osmotic fragility (NESTROF) test **positive in thalassaemia minor**
- Serum hepcidin: Reduced

Bone Marrow

- Normoblastic erythroid hyperplasia
- Increased macrophages
- Inclusion bodies in normoblast—methyl violet
- Prussian blue stain—abundance of iron

Lab findings—Hb electrophoresis: The following procedures can be done.

- Citrate agar electrophoresis at alkaline or acid pH
- Capillary electrophoresis
- Automated high performance liquid chromatography
- Isoelectric focusing
- Globin chain electrophoresis

HEREDITARY SPHEROCYTOSIS

Hereditary spherocytosis (HS) has autosomal dominant inheritance. Primarily, the red cells have membrane skeletal disorder of vertical protein interaction. There is defective or absent spectrin molecules, protein 4.2, ankyrin and band 3 protein. Most common amongst these are deficiency of spectrin and ankyrin.

Mechanism

In hereditary spherocytosis, the common defect is spectrin deficiency and spectrin lacks the ability to attach to protein 4.1. Functionally, it is associated with increased permeability to sodium, which passively enters and actively sent out of the cells. This requires ATP. Hypoxia, glucose deprivation and reduced deformity of the cells are the main problems in these cells than the normal ones. These RBCs are delayed in the splenic sinusoids for longer time, with loss of cell membrane with sphering and more rigidity.

Laboratory Findings

1. Moderate/mild/no anaemia
2. Reticulocytosis (5–20%)
3. Nucleated RBCs
4. The peripheral blood smear shows characteristic **microspherocytes**, which appear small, dark, round with no central pallor and decreased diameter
5. Polychromasia
6. Normal/decreased MCV
7. Increased MCHC—hyperhaemoglobin
8. Hyperbilirubinaemia
9. Negative antiglobulin test
10. Increased osmotic fragility (OF)
11. Mild cases with incubation, OF is increased. Blood incubated for 24 hrs at 37°C. Normal RBCs also show increased fragility on incubation—due to swelling. HS cells lose membranes more readily than normal RBCs when incubated. This test has increased sensitivity and is the most reliable diagnostic test for HS.
12. Autohaemolysis
13. Cryohaemolysis

Other tests:

1. Glycerol lysis test
2. Flow cytometry analysis of red cell labelling with eosin-5′-maleimide: Lower intensity of red cells in HS cells than red cells in other HA. This has high specificity and sensitivity.

Autohaemolysis test: This is a screening test for HA with membrane and enzyme defects.

It measures spontaneous haemolysis of blood which is incubated at 37°C for 48 hrs. Blood is incubated at 37°C for 24–48 hrs. After 24 hrs, thoroughly mix the contents by gently swirling. After 48 hrs, estimate PCV, Hb and estimate spontaneous lysis by colourimetry or spectrometry at 540 nm. Normal—0.2 to 2% lysis is seen at 48 hrs. With added glucose—0 to 0.9% lysis is seen at 48 hrs.