

MEDICINE

432 Team

29 Anemia



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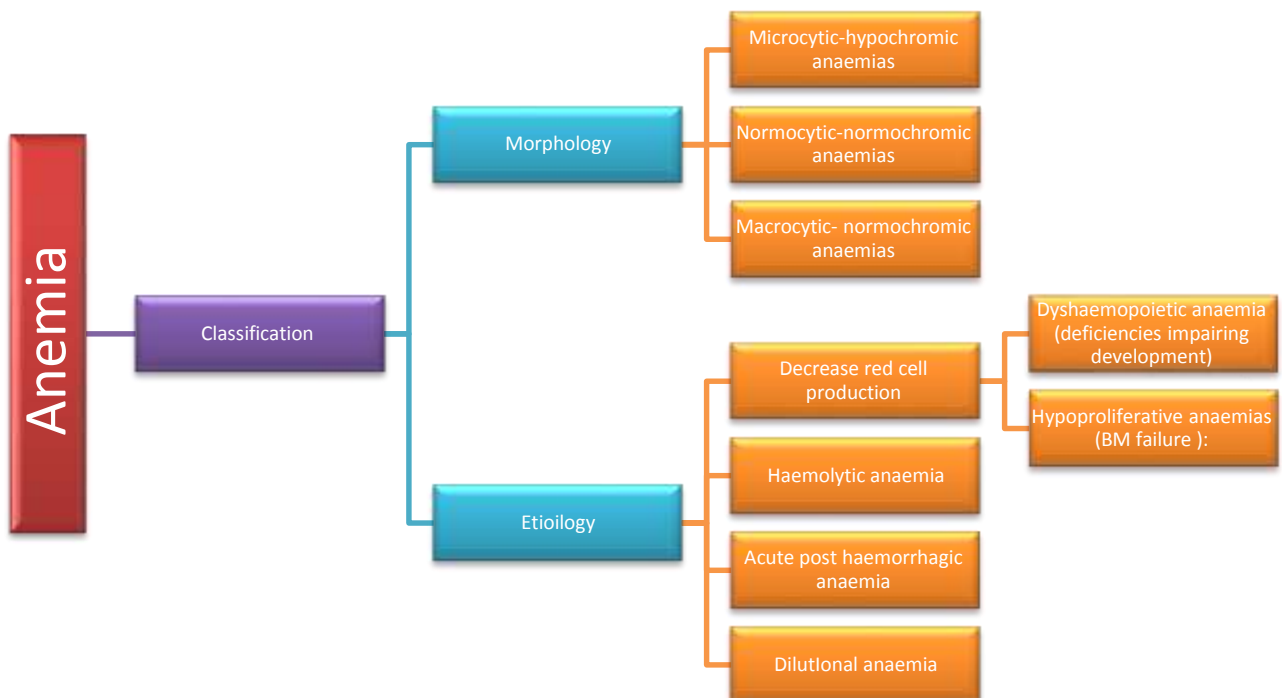
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COLOR GUIDE: • Females' Notes • Males' Notes • Important • Additional

Objectives: (not given)

General Outline



Introduction:

Blood formation:

1. Intrauterine life:

- Blood cells are formed in the liver and spleen up to the fifth months.
- After 5th month: bone marrow shares in the formation of these cells.

2. After birth:

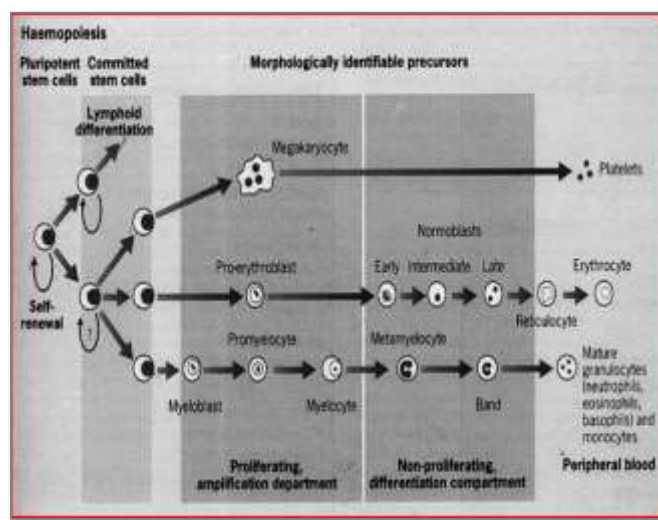
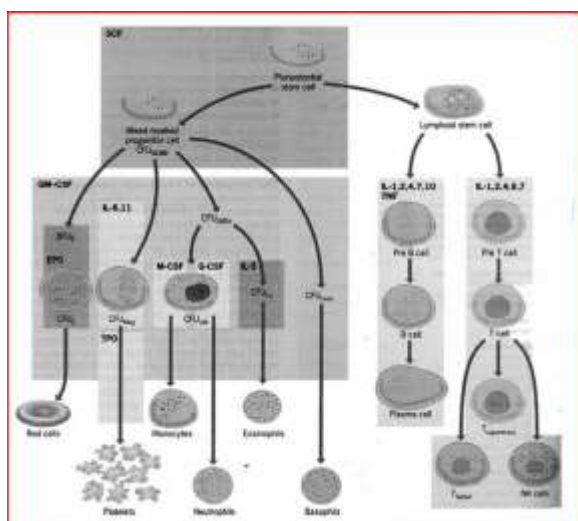
- Formation of these cells will be restricted to the bone marrow.

3. Adulthood

The active bone marrow will be restricted to axial skeleton: flat bones, vertebrae, ribs, sternum and ilia.

- Some extension to the proximal ends of long bone mainly femur.

- **Extramedullary haemopoiesis:** When demand for blood formation is increased the active red BM extends into the shafts of the long bones.
- The spleen and liver will regain their ability to produce blood elements when bone marrow is affected by some diseases.



Blood Constituents:

• Organelles (formed elements):

- RBCs
- WBCs
- Thrombocytes

- Fluid components: plasma in which the above elements are suspended and contain fibrinogen.

Erythrocytopoiesis

Normal erythrocytopoiesis depends on:

1. Healthy BM: normal stem cells and architecture.
2. Regulatory hormones: GPO, Androgen thyroxin, cortisol and ACT.
3. Nutritional elements: Protein (high biological value),
4. Minerals (Iron, copper, zinc, selenium)
5. Vitamins (B, Folic acid, vit. C)

Anaemia: Reduction of O₂ carrying capacity of the blood with inadequate O₂ supply to tissues.

- Diagnosed when there is a reduction of (RBCs number) below the reference range for the age and sex of an individual.
- More accurately is defined as reduction in the red cell mass

Classification:

A. By etiology:

1. Decreased red cell production
 - Dyshaemopoietic anaemia
 - a) Mineral deficiency: iron, zinc, selenium, copper
 - b) Vitamin deficiency: B12, folic acid, Vit C & pyridoxine
 - c) Hormonal deficiency: anemia of renal diseases, pituitary, thyroid or suprarenal deficiency.
 - d) Protein deficiency: high class
 - Hypoproliferative anaemia
 - a) Aplastic anaemia.
 - b) Myelophthisic anaemia (bone-marrow infiltration replaces haemopoietic cells leading to anemia).
 - c) Anemia of chronic diseases
2. Haemolytic anaemia:
 - Short life-span of RBCs
3. Acute post haemorrhagic anaemia:
 - Loss of RBCs
4. Mixed anaemia.
 - eg. Megaloblastosis associated with haemolysis

5. Dilutional anaemia: (relative anemia: volume is high but cell mass remains the same)
 - Pregnancy
 - Oliguric RF
 - Volume-overload

B. By Morphology

1. Microcytic-hypochromic anaemias:
 - Iron deficiency anaemia.
 - Thalassaemia.
 - Anaemia of chronic disease.
 - Sideroblastic anaemia:
 - a) Hereditary
 - b) Chronic lead poisoning.
2. Normocytic-normochromic anaemias:
 - Anaemia of chronic diseases.
 - Acute post –haemorrhagic anaemia.
 - Hemolytic anaemia.
 - Aplastic anaemia.
 - Myelophthisic anaemia.
3. Macrocytic- normochromic anaemias:
 - Megaloblastic anaemia.
 - Marked reticulocytosis.
 - Myelodysplastic syndromes.
 - Myxoedema.
 - Acquired sideroblastic anaemia.

Pathophysiology

1. Tissue Hypoxia

- Impaired functions of the tissues, the degree of impairment depends on the degree of dependency on O₂, so CVS, CNS and skeletal muscles are much affected.

2. Compensatory mechanisms

- Increased COP. **(explaining high output cardiac failure)**
- Increased O₂ delivery from HB to the tissue.
- Increased erythropoietin production with stimulation of erythropoiesis. **(Explaining the accompanying reticulocytosis)**
- Increased plasma volume.
- Redistribution of the blood from less to more vital organs.

3. Rate of blood loss:

- The rapid the rate of blood loss, the more the severe symptoms will occur especially in elderly. While the slowly falling HB allows for hemodynamic compensation with less symptom.

4. Factors related to a specific cause.

Symptoms

➤ Neurological:

- ✓ Dizziness, fainting, lack of concentration
- ✓ Blurred or diminished vision
- ✓ Headache, tinnitus
- ✓ Paraesthesia in the fingers and toes
- ✓ Insomnia, irritability.

➤ CVS:

- Angina, dyspnea, palpitation and intermittent claudication by exertion
- Heart Failure in severe cases or presence of other organic cardiac disease, it is high COP failure.

➤ Musculo skeletal:

- Easy fatigability.
- Tiredness and lassitude.

➤ GIT:

- Dyspepsia and anorexia

➤ Genital

- Loss of libido & impotence
- Menstrual abnormalities like amenorrhea.

➤ May be polyuria.

Hematocrit and Symptoms	
Hematocrit	Expected symptoms
>30%–35%	None
25%–30%	Dyspnea (worse on exertion), fatigue
20%–25%	Lightheadedness, angina
Under 20%–25%	Syncope, chest pain

Signs

- ✓ Pallor of the skin and mucous membranes
 - The color of the skin is unreliable because it depends upon the degree of skin pigmentation and the amount of fluid in the subcutaneous tissues.
 - Best examined: palmer creases, nail bed, and mucous membranes.
- ✓ Peripheral edema:
 - Slight edema of the legs probably due to increase in the capillary permeability secondary to hypoxia.
- ✓ High COP failure.
- ✓ Fever:
 - Mild fever may occur in severe anemia but other causes should be excluded.
- ✓ Fundal changes:
 - Retinal hemorrhages of the flame shape type, exudates and rarely papilledema.
- ✓ The cardiovascular system:
 - Increased velocity with decreased viscosity of the blood in addition to capillary dilation can cause the following:
 1. Tachycardia, bounding pulse
 2. Cardiac examination: Loud HS, S3 over mitral or tricuspid area
 3. Haemic murmur: ejection systolic, heard all over the precordium
 4. Raised jugular venous pressure.
- ✓ Proteinuria and impairment of the concentrating power of kidneys due to anoxia of renal tubules.

Now that we established some of the basic principles regarding anemia, let's talk diseases.
 (Remember that just saying anemia is not establishing a diagnosis)

Microcytic Anemia

How to Answer "What Is the Most Likely Diagnosis?" for Anemia	
Feature in the history	What is the most likely diagnosis?
Blood loss (GI bleeding)	Iron deficiency
Menstruation	Iron deficiency
Cancer or chronic infection	Chronic disease
Rheumatoid arthritis	Chronic disease
Alcoholic	Sideroblastic
Asymptomatic	Thalassemia

A. IRON DEFICIENCY ANEMIA

Sources, absorption and metabolism:

- ✓ Red meat and liver, bread, eggs and green vegetables, mainly in ferric form.
- ✓ Daily minimum requirement 10-12mg of which about 1mg is absorbed.
- ✓ In the stomach the iron is released from its complex form and is reduced to ferrous form (action of gastrin and HCl).
- ✓ Iron absorption takes place in the duodenum and proximal jejunum.
- ✓ Iron absorption is under regulatory system (Apo-ferritin-Transferrin system) present in the intestinal mucosa and regulates absorption of the iron according to body requirement.
- Factors enhancing iron absorption
 - ✓ Pregnancy
 - ✓ Iron deficiency anemia
 - ✓ Increased erythropoiesis
 - ✓ Vit. C.
- Factors decrease iron absorption:
 - ✓ Excess phosphate, tannates, phytate in diet
 - ✓ Iron overload haemochromatosis
 - ✓ Decreased erythropoiesis eg aplastic anaemia
 - ✓ Malabsorption syndrome
 - ✓ Decreased HCl atrophic gastritis.
- Transport
 - ✓ There is a diurnal rhythm with higher level in the morning; iron is transported in the plasma bound to **transferrin**.
- Utilization: incorporation into Hb, myoglobin and tissue enzymes.
- Storage:
 - ✓ About two-thirds of the total body iron is in the circulation as haemoglobin.
 - ✓ Iron is stored in **reticulo-endothelial cells, hepatocytes, spleen, bone marrow and skeletal muscle about two thirds as ferritin and one-third as hemosiderin.**
- Iron loss: (0.5 mg/day). Via
 - ✓ Hair and nail growth.
 - ✓ Epithelium of the skin and mucus membrane.
 - ✓ Body excreta: stool and urine.
- Deficiency:
 - ✓ True deficiency (defective intake) Rare:
 - Prolonged starvation and famines.
 - Infancy (milk is poor in iron)

- ✓ Conditioned deficiency (normal demand and intake but there are defective absorption and utilization) Not common:
 - Ferric form cause.
 - Decreased HCL
 - Iron binder: phosphate, phytate, tannates
 - Malabsorption syndrome.
- ✓ Relative deficiency: (increased requirement) common cause.
 - Menstruating females.
 - Pregnancy, labor.
 - Growing children.
 - Convalescence from disease.
- ✓ Chronic blood loss: the commonest cause

Frank blood loss:

- Menorrhagia
- Repeated GI bleeding, hematemesis, epistaxis, and hematuria.
- Bleeding tendencies
- Repeated blood donation

Occult blood loss via GIT:

- Anckylstoma & schistomiasis
- Oozing OV, PU.
- Neoplasm.
- Inflammatory bowel disease ulcerative colitis
- TB enteritis.

Clinical features

- General manifestations of anemia
- Angular stomatitis.
- Atrophic glossitis (red, glazed, smooth tongue)
- Cheilosis (crusting of angle of mouth)
- Atrophy of the gastric mucosa
- Small splenomegaly
- Brittle nails, thinning and ridging and loss of luster.
- Koilonychia (spoon-shaped nails) in severe cases
- Brittle hair
- Features of special types:
 - a. Plummer-Vinson –syndrome:
 - Common in middle aged female

Note:

Fe KAP:
Fe (iron)/ Fatigue
Exercise intolerance
Koilonychia
Angular cheilosis
Pica/Pallor

- Postcricoid esophageal web causes dysphagia
- b. Ankylostoma infestation:
 - Perverted appetite: (pica) eating mud, stones and chalk.
 - Epigastric pain (DD= D. ulcer), altering bowel habits
 - Endemic parasites (trapezoid face)
 - The symptoms are mild and variable.
 - Investigations:
 - Stool: Ova.
 - CBC: eosinophilia.

Investigations

Unique Features and Diagnoses of Iron Studies	
Unique feature	Diagnosis
Low ferritin	Iron deficiency
High iron	Sideroblastic anemia
Normal iron studies	Thalassemia

➤ CBC:

- The red cells are **microcytic**, (MCV<80f1) and **hypochromic** (MCH < 27pg) anisocytosis and poikilocytosis (variation in size and shape, respectively). **With red cell distribution width RDW>15**
- Eosinophilia is present in cases of ankylostoma infestation.

➤ Bone Marrow:

- Absent iron store
- **Normoplastic hyperplasia** (because it is trying to compensate)
- Decreased Hb in maturely erythroblasts.

➤ Serum iron study

- Decreased serum iron (n=70-170 mg%).
- Increased total iron binding capacity (TIBC). (N=250-450mg%).
- Decreased serum **ferritin levels**.
 - Reflect iron stores.
 - The normal values are 30-300 mg/L in males and 15-200 mg/L in females and investigation of gastrointestinal tract are often required.
 - Falsely raised value in cases of acute phase reactant e.g malignancy.
- Decreased transferrin saturation (n=25-50%).

➤ Investigations for specific causes

- ✓ Stool analysis:
 - Occult blood, ankylstoma & Bilharziasis.
- ✓ GI cause:
 - Imaging: barium swallow, meal or enema.
 - Endoscopic studies: upper and lower.
 - Achlorhydria: histamine test.
- ✓ Hemostatic profile.

Case:

*A 47 year old man presents with iron deficiency anemia.
Think: colon cancer unless proven otherwise (up to 20%).
Next step: colonoscopy and endoscopy.*

Treatment: The aim of the treatment is to correct the anemia and build up iron stores

- I. Correction of underlying cause if possible. (E.g. treat cancer...)
- II. Iron replacement
 - Oral iron
 - ✓ 200 mg anhydrous ferrous sulphate three times daily.
 - ✓ The response is a rise in Hb concentration of 1 gm/dl per week.
 - ✓ Side effects nausea, abdominal pain, diarrhea or constipation.
 - Parenteral iron:
 - ✓ Indications:
 - General intolerance of oral preparation even at low dose.
 - Severe malabsorption and those who have gastrointestinal diseases.
 - Rapid iron loss.
 - ✓ Preparation
 - a. Iron sucrose (ferrosac – venofer)
 - Good safety and efficacy profile.
 - Given IM or slowly IV infusion.
 - Amp: 5ml = 100 mg.
 - b. Iron dextran (cosmofer - imferon)
 - Good efficacy but more side effects.
 - Giving IM, or IV infusion.
 - Amp: 2 ml = 100mg.
 - Side effects:
 - Local pain, staining inflammation, abscess formation

- General: hypersensitivity reaction, fever, rigor, hypotension

✓ N.B: Test dose is required prior to the use of iron therapy.

III. Transfusion therapy:

- Packed RBCs.
- Indications:
 - Severe ($\text{Hb} < 7 \text{ gm/dl}$) and symptomatizing anemia)
 - Complicated anemia: HF

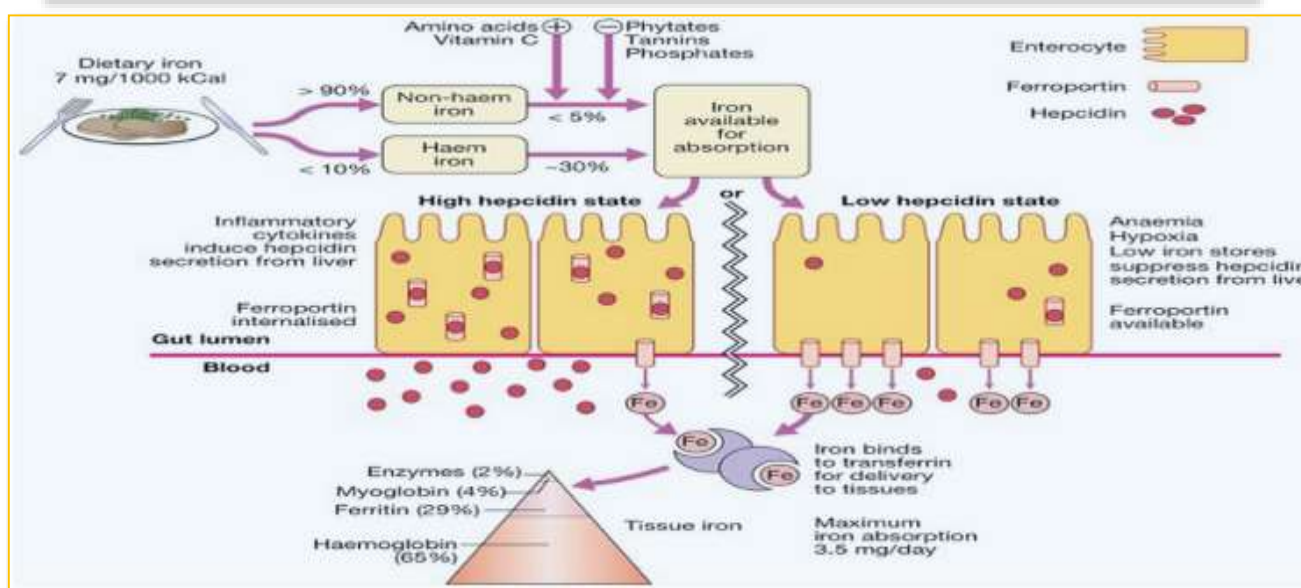
B. Anemia of chronic disease

- Type of anemia may be microcytic hypochromic or **more common normocytic normochromic**.
- Endocrinal disorders: hypothyroidism, hypocorticalism, hypogonadism in male.
- Inflammatory diseases: collagenosis, Chron's disease.
- **Rheumatological diseases.**
- Chronic infection: as TB, sarcoidosis, oestomyilitis.
- **Neoplastic** diseases.
- Malabsorption.
- Organ failure, liver and kidney

Note(s):

Pathogenesis

It has recently become clear that the key regulatory protein that accounts for the findings characteristic of ACD is hepcidin, which is produced by the liver (see Fig.). Hepcidin production is induced by pro-inflammatory cytokines, especially IL-6. Hepcidin binds to ferroportin on the membrane of iron-exporting cells, such as small intestinal enterocytes and macrophages, internalising the ferroportin and thereby inhibiting the export of iron from these cells into the blood. The iron remains trapped inside the cells in the form of ferritin, levels of which are therefore normal or high in the face of significant anemia. Inhibition or blockade of hepcidin is a potential target for treatment of this form of anemia.



Laboratory:

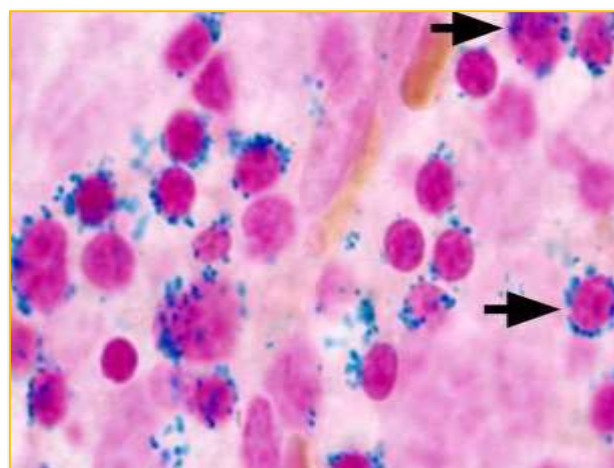
- Decreased serum iron and TIBC.
- Normal or raised serum ferritin
- Normal BM iron.

C. Sideroblastic anemia

It is a refractory microcytic hypochromic anemia characterized by the presence of sideroblasts in the bone marrow. Sideroblasts are Erythroblasts inside, which iron accumulates into the mitochondria of erythroblasts owing to disordered haem synthesis. A ring of iron granules is formed around the nucleus.

Types:

- Inherited: X-linked disease transmitted by females.
- Acquired:
 1. Primary: one of the myelodysplastic syndromes
 2. Secondary:
 - ✓ Other types of myelodysplasia
 - ✓ Myeloproliferative disorders
 - ✓ Myeloid leukemia
 - ✓ Drugs as isoniazid, alcohol, lead.



	Ferritin	Iron	TIBC	Transferrin saturation	Soluble transferrin receptor
Iron deficiency anaemia	↓	↓	↑	↓	↑
Anaemia of chronic disease	↑/Normal	↓	↓	↓	↓/Normal

(TIBC = total iron binding capacity)

Macrocytic anemia

With megaloblastosis:

Anemia in which DNA synthesis is impaired with delayed nuclear maturation. This will lead to formation of megaloblasts i.e. large cells with large immature nuclei (cellular gigantism). The cells affected are those rapidly developing: haematopoietic cells, GI mucosa, and skin thus producing symptoms related to these organs. In BM, the normoblasts are replaced by these megaloblasts. Some of these abnormal cells are destroyed in BM. (intramedullary haemolysis) Some of these erythroid megaloblasts lose their nuclei and join peripheral blood as macrocytes, irregular in shape with remnant of the nucleus. These cells are hatched and destroyed by the spleen during their circulation. Thus, anemia is multifactorial:

- Defective erythropoiesis
- Intramedullary hemolysis.
- Destruction by the spleen.
- Increased Serum bilirubin
- MCV>96 fl unless there is a coexisting cause of microcytosis.

The peripheral blood film shows macrocytes with hypersegmented polymorphs with six or more lobes in the nucleus. If severe there may be leucopenia and thrombocytopenia (deficient DNA synthesis)

Causes include:

- ❖ Vitamin B12 deficiency.
- ❖ Folic acid deficiency
- ❖ Congenital enzyme deficiencies in DNA synthesis or drugs interfering with DNA synthesis (hydroxyurea, azathioprine).
- ❖ Myelodysplasia due to dyserythropoiesis

1. Vitamin B12 deficiency

- Sources: meat, fish, eggs, and milk.
- Not destroyed by cooking.
- Daily requirement is 1-2 mg / day.
- Absorption and transport: Vit. B12 is liberated in the stomach, bound to intrinsic factor (IF) and absorbed through the terminal ileum, transported by transcobalamin I (and to lesser extent by transcobalamin II and III) to be utilized by the tissues.
- It is essential for:
 - Hematopoiesis
 - GIT mucosa integration

- Formation of myelin of nervous system.
- Storage: the average adult stores 2-3 mg in the liver, it **may take two years or more before B12 deficiency develops** as the daily loss are small (1-2mg).

Causes of deficiency:

- Dietary deficiency:
 - Poor socioeconomic status.
 - Strict **vegans** and to a lesser extent, Vegetarians.
 - Alcoholics
 - Malabsorption syndrome. (**Celiac**)
 - Ileal diseases:
 - **Terminal ileum resection**
 - TB enteritis.
 - **Crohn's disease. (Remember that the most common area to be affected by crohn's is the ileocecal area)**
 - Blind loop syndrome with bacterial over-growth. **(Can be a result of motility disorders or hypogammaglobulinaemia and easily rectified with the appropriate use of antibiotics)**
 - Infestation by fish tape worm *Diphyllobothrium latum*.
 - Gastric pathology:
 - **Pernicious anaemia.**
 - This is an organ-specific **autoimmune** disorder in which the gastric mucosa is atrophic, with loss of parietal cells causing intrinsic factor deficiency. In the absence of intrinsic factor, less than 1% of dietary vitamin B12 is absorbed.
 - Pernicious anaemia has an incidence of 25/100 000 population over the age of 40 years in developed countries, but an average age of onset of 60 years.
 - **It is more common in individuals with other auto-immune disease (Hashimoto's thyroiditis, Graves' disease, vitiligo, hypoparathyroidism or Addison's disease) or a family history of these or pernicious anaemia.**
 - **The finding of anti-intrinsic factor antibodies in the context of B12 deficiency is diagnostic of pernicious anaemia without further investigation.**
 - Antiparietal cell antibodies are present in over 90% of cases but are also present in 20% of normal females over the age of 60 years; a negative result makes pernicious anemia less likely but a positive result is not diagnostic.
 - The Schilling test, not favorable anymore.
 - Total or partial gastrectomy
 - Atrophic gastritis
 - Gastric cancer
 - Non- Addisonian pernicious anaemia

- In association with hypogammaglobulinemia, gastric atrophy, achlorhydria and absent intrinsic factor but no antibodies
- In infancy: Selective failure of IF, normal mucosa.
- Juvenile PA: failure of IF secretion with gastric atrophy rarely with antibodies
- Rare congenital enzyme deficiency.
- Lack of transcobalamin II.
- Increased demand
 - Infancy, pregnancy, lactation
 - Hemolysis and active hematopoiesis
 - Malignancy.
 - Thyrotoxicosis
- Increased loss: Hemodialysis
- Decrease of stores. Far advanced chronic liver diseases.

Clinical features

1. Hematological manifestations

- General manifestation of anemia.
- Bleeding tendencies (Thrombocytopenia)
- Increased risk of infection (Leucopenia)
- Hepatosplenomegaly.
- Color of the skin is lemon yellow owing to combination of pallor and jaundice.

2. GIT manifestations

- Atrophic glossitis.
- Angular stomatitis.
- Gastric atrophy dyspepsia, anorexia nausea, vomiting.
- Intestinal atrophy diarrhea and malabsorption
- Stomach cancer on top of atrophic gastritis

3. Neurological manifestations (not seen in folate deficiency).

- **Peripheral neuropathy**
 - Glove and stocking paraesthesiae
 - Loss of ankle reflexes
- **Subacute combined degeneration of the cord (SCD)**
 - Pyramidal tract affection (UMNL) → spastic paraplegia.
 - Posterior column affection → loss of deep sensation, vibration, proprioception.
- **Dementia**
- Optic atrophy
- Psychosis (rare)
- Autonomic neuropathy

Causes of vitamin B12 deficiency:

VITAMIN B:

*Vegan
Ileal resection
Tapeworm
Autoimmune (PA)
Megaloblastic anemia
Inflammation of terminal ileum
Nitrous oxide
Bacterial overgrowth*

Investigations (you could probably guess those by now☺)

1. CBC

- RBCs:
 - **Macrocytic normochromic anemia.**
 - Poikilocytosis and anisocytosis
 - Howell-Jolly bodies may be present
- WBCs:
 - Moderate Leucopenia.
 - **Shift to the right** (hypersegmented neutrophils).
- Platelets
 - **Moderate thrombocytopenia.**
 - Giant platelets
- Reticulocytes are decreased but increased by treatment with vit. B12

2. Bone marrow:

- Show **megaloblastic erythropoiesis**. The most characteristic is dissociation between nuclear & cytoplasmic development in erythroblasts with the nucleus maintaining a primitive appearance despite maturation and haemoglobinization of the cytoplasm.
- Erythroid hyperplasia, maturation defect in erythropoiesis, giant metamyelocytes, atypical megakaryocytes with hypersegmented nuclei.

3. Estimation of serum B12 level

- **Low** using radioisotope dilution or immunological assays.

4. Biochemistry

- Serum iron is high, more than 175-mcg/100 ml.
- Increased serum indirect bilirubin reflects mild hemolysis and ineffective erythropoiesis.
- Increased serum lactic dehydrogenase (LDH) reflecting ineffective megaloblastic erythropoiesis.

5. Immunology. (Imp. For diagnosis of pernicious anemia).

- Parietal cells antibodies and gastrin receptor antibodies, present in 70% of patients. (Not specific)
- Intrinsic factor antibodies type I blocking (more specific) and type II precipitating antibodies.

6. Other investigations

- Gastric biopsy: proximal 2/3 of stomach is atrophic.
- Augmented histamine test: achlorhydria.
- Schilling test for pernicious anemia.
- **High homocystine (also seen in folate deficiency)**
- **High methylmalonate in urine.**

Treatment

- Treatment of the underlying cause.
- Replacement therapy.
 - Initial treatment is often as the 1000 µg B12 IM/day for 2 weeks then in a dosage of 1000 µg twice/week till correction of anemia. Maintenance therapy 1000 mg IM every 3 months.
- **Neurological damage can be precipitated by treating incorrectly with folic acid.**
- Transfusion therapy by packed RBCs in the following conditions
 - Severe anemia (Hb < 7 gm/dl)
 - Heart failure
 - Marked symptoms.

Note(s):

The reticulocyte count will peak by the 5th–10th day after starting replacement therapy. The haemoglobin will rise by 10 g/L every week until normalised. The response of the marrow is associated with a fall in plasma potassium levels and rapid depletion of iron stores. If an initial response is not maintained and the blood film is dimorphic (i.e. shows a mixture of microcytic and macrocytic cells), the patient may need additional iron therapy. A sensory neuropathy may take 6–12 months to correct; long-standing neurological damage may not improve.

2. Folate deficiency

- Folates are produced by plants and bacteria, hence dietary leafy vegetables (spinach, broccoli, and lettuce), nuts, yeast, fruits (bananas, melons) and animal protein (liver, kidney) are a rich source.
- Minimum daily requirements 100-200 ug.
- An average Western diet contains more than the minimum daily requirement but excess cooking destroys folates.
- Most dietary folate is present as polyglutamates; these are converted to monoglutamate in the upper small bowel (mostly in the jejunum) and actively transported into plasma.
- Plasma folate is loosely bound to plasma proteins such as albumin and there is an entero-hepatic circulation.
- Total body stores of folate are small and deficiency can occur in a matter of weeks.
- It is essential for DNA synthesis and cell maturation.
- Its deficiency leads to nuclear maturation defect of blood cells and GIT mucosal cells.

Causes of deficiency:

1. Decreased intake
 - Particularly in infancy, **old age**, poor social conditions, starvation, **alcohol** excess and in **psychiatric patients**.
2. Increased demand
 - Physiological: **pregnancy**, lactation, prematurity
 - Pathological:
 - Hematological disease with excess red cells production e.g **hemolysis**.
 - Malignant disease with increased cell turnover
 - Inflammatory diseases e.g. rheumatoid arthritis.
 - Metabolic disease e.g. homocystinuria (rare congenital defect in the conversion of homocysteine to cystathion folate).
3. Decreased absorption
 - Small bowel disease especially **celiac disease**.
4. Drugs affecting folate metabolism.
 - Anticonvulsants (**e.g. phenytoin may inhibit intestinal conjugase inhibiting conversion of polyglutamates into monoglutamates**), methotrexate (**cytotoxic**), pyrimethamine and trimethoprim (**inhibit the enzyme responsible for the conversion of dihydrofolate into tetrahydrofolate**) and oral contraceptives.
5. Increased loss
 - Plasma folate is loosely bound to plasma proteins such as albumin; thus, Hemodialysis or peritoneal dialysis can cause loss.

Note:

Pregnant MAN!!
Pregnancy and lactation
Malabsorption (celiac and crohn's)
Alcoholism
Nutritional (tea and toast elderly diet)

Clinical features

- ❖ May occur at any age, degrees of folate deficiency are very common and mild deficiency states are frequently unrecognized.
- ❖ The clinical picture varies according to possible underlying causes.
- ❖ The onset may be insidious or rapid as when negative folate balance is precipitated by e.g infection
- ❖ **Anemia** and sometimes-slight jaundice.
- ❖ Glossitis.
- ❖ **No defined neurological changes.**

Investigations

- Essentially as in vit B12 deficiency.

- Howell Jolly bodies and target cells in the blood film would suggest splenic atrophy (coeliac disease)
- Bone marrow shows megaloblastic changes.
- Reduced folate levels: (N= 2.5-25mg/ml) **serum** levels are labile (a single folate rich meal could normalize the lvls even in a truly deficient person) and **red cell levels** are better reflection of tissue folate by radioisotope dilution or immunological methods.

Treatment

- Folic acid therapy is contraindicated if there is any suspicion of B12 deficiency (Neurological changes may be precipitated).
- Folic acid: 5mg daily is more than adequate.
- Prophylactic folic acid is also given in chronic hematological disorders where there is rapid cell turnover 5mg each week. **As in sickle cell anemia.**
- Prophylactic folic acid (400ug daily) is recommended in pregnancy to reduce the risk of megaloblastosis and fetal neural tube defects. **Pregnancy-induced folate deficiency is the most common cause of megaloblastosis worldwide and is more likely in the context of twin pregnancies, multiparity and hyperemesis gravidarum.**

Without megaloblastosis:

- A raised MCV with macrocytosis on the peripheral blood film can occur with a normablastic BM. In all these conditions, the level of Vit B12 and folate are normal.
- The exact mechanisms are uncertain, but it seems there is increased lipid deposition in the red cell membrane.
- Physiological: pregnancy and newborn.
- Pathological
 - Alcohol excess
 - Liver disease
 - Reticulocytosis
 - Hypothyroidism
 - Some hematological disorders (e.g aplastic anaemia, sideroblastic anemia)
 - Drugs (e.g. cytotoxic as azathioprine). **Also, don't forget anti-retroviral treatment (ART)**
 - Agglutinated red cell measured on red counters.
 - Cold agglutinins due to autoagglutination of red cells, MCV decreases to normal with warming of the sample to 37C.

Causes of macrocytosis in general:

Feed Them:

Folate and vitamin B12 deficiency

Ethanol and liver disease

Drugs (ART, methotrexate)

Thyroid (hypo)

Hemolysis (reticulocytes are large cells)

Myelodysplastic syndrome

Case:

A 38 year old with HIV on HAART presents with macrocytosis.

Think: ART

Hemolytic Anemia

- The hemolytic anemias are a group of diseases in which red cell life span is shortened.
- Pathophysiology:
- Hemolysis of RBC can occur either.
 - Intravascular i.e. within the circulation.
 - Extravascular i.e. by phagocytes in RES in the liver, bone, spleen.
- Bone marrow compensatory reactions:
 - Erythroid hyperplasia in BM, can increase erythropoiesis several times, so that anemia may not develop till RBCs life span is less than 20 days
 - **Reticuocytosis** is a hallmark.
 - Slight macrocytosis in the peripheral blood
- Consequences:
 - Low Hb.
 - Excess hemolysis increases bilirubin (**unconjugated**) since the liver can increase its capacity several times; the jaundice is mild if there's any at all.
 - The intravascular liberated Hb is bound to haptoglobin. Hb-haptoglobin is rapidly cleared from the circulation by RES, and plasma level of haptoglobin is reduced. In cases of excessive **IV haemolysis, haptoglobin is depleted.**
 - **Haemoglobinuria and dark urine.**
 - Extramedullary erythropoiesis will cause specific bone changes.

Causes:

1. CORPUSCULAR CAUSES:

- ***Congenital Abnormalities***
 - I. Membrane defects
 - Hereditary spherocytosis
 - Hereditary elliptocytosis
 - Hereditary stomatocytosis
 - II. Haemoglobinopathies
 - Sickle cell anemia
 - Thalassemia
 - III. Enzymopathies
 - Abnormal aerobic glycolysis e.g. G6PD deficiency
 - Abnormal anaerobic glycolysis e.g. pyruvate kinase deficiency
 - Non glycolytic enzymopathies
- ***Acquired Abnormalities***
 - Paroxysmal nocturnal haemoglobinuria (**abnormal membrane**)
 - Vitamin E deficiency.

2. EXTRACORPUSCULAR CAUSES:

- i. Immune mechanisms
 - ❖ Alloantibodies:
 - Incompatible blood transfusion
 - Hemolytic disease of the newborn
 - After allogeneic BM or organ transplantation
 - ❖ Autoantibodies:
 1. **Warm antibodies** (react at 37° C and do not cause agglutination. Type of AB= IgG).
 - Primary: Idiopathic
 - Secondary:
 - CLL, Lymphoma, SLE and rheumatoid disease
 - Drugs e.g methyl dopa.
 2. **Cold antibodies** (react at 32°C (best at 4°C) and usually agglutinates and hemolyse red cells. Type of AB = IgM).
 - Cold haemoagglutinin disease
 - This affects elderly patients and may be associated with an underlying low-grade B cell lymphoma. It causes a low-grade intravascular haemolysis with cold, painful and often blue fingers, toes, ears or nose (so-called acrocyanosis). The latter is due to red cell agglutination in the small vessels in these colder exposed areas.
 - Primary: Idiopathic
 - Secondary (mycoplasma pneumonia infection, infectious mononucleosis and lymphomas).
 - Paroxysmal cold haemoglobinuria
 - Idiopathic
 - Secondary (some viral infections congenital & tertiary syphilis).
 - ❖ Immunochemical mechanisms: drug induced hemolytic anemia.
- ii. Non-immune mechanism:
 - A. Mechanical injury
 - Cardiac haemolytic anaemia (valve prosthesis).
 - Microangiopathic haemolytic anaemias.
 - March haemoglobinuria.
 - B. Chemicals and Drugs
 - Snake venom
 - Lead
 - Naphthaline
 - Phenacetin
 - Sulpha drugs
 - Infections
 - Clostridium perfringens (formerly known Clostridium welchii) septicemia (Secondary to septic abortion)

- Malarial infestation (Black water fever) (named for its associated hemoglobinuria)
- Metabolic causes
 - Abetalipoproteinemia leads to cell membrane abnormality and eventually Acanthocytosis.
 - Liver porphyria
 - Spurr cell anaemia
 - Zieve's syndrome
 - Zieve's syndrome is an acute metabolic condition that can occur during withdrawal from prolonged alcohol abuse. The underlying cause is liver delipidization.
 - Wilson's disease
 - Severe hypophosphataemia
- Secondary to systemic disease:
 - Renal and liver failure
 - Burns.
- Hypersplenism

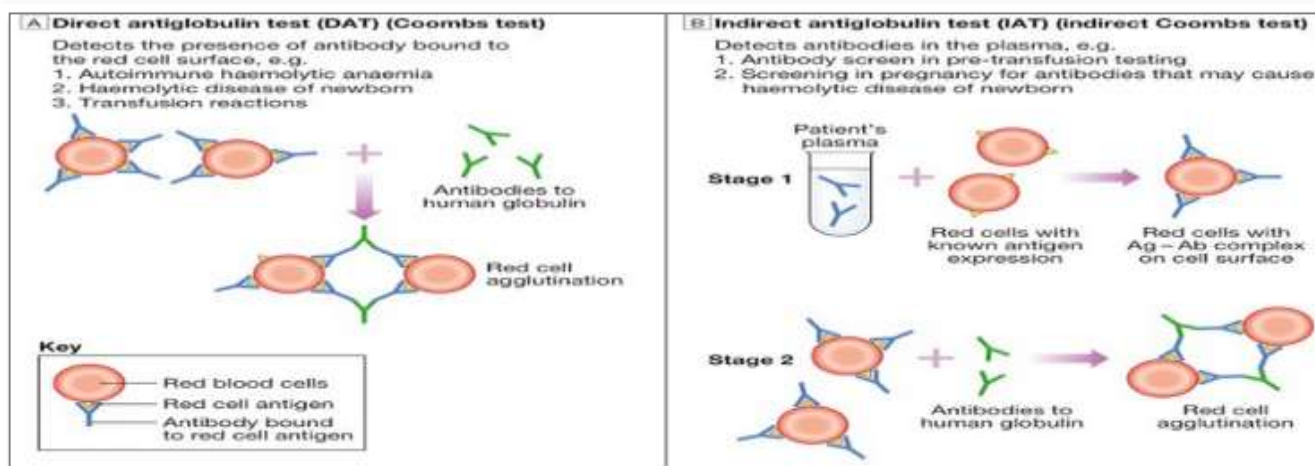
Note(s):

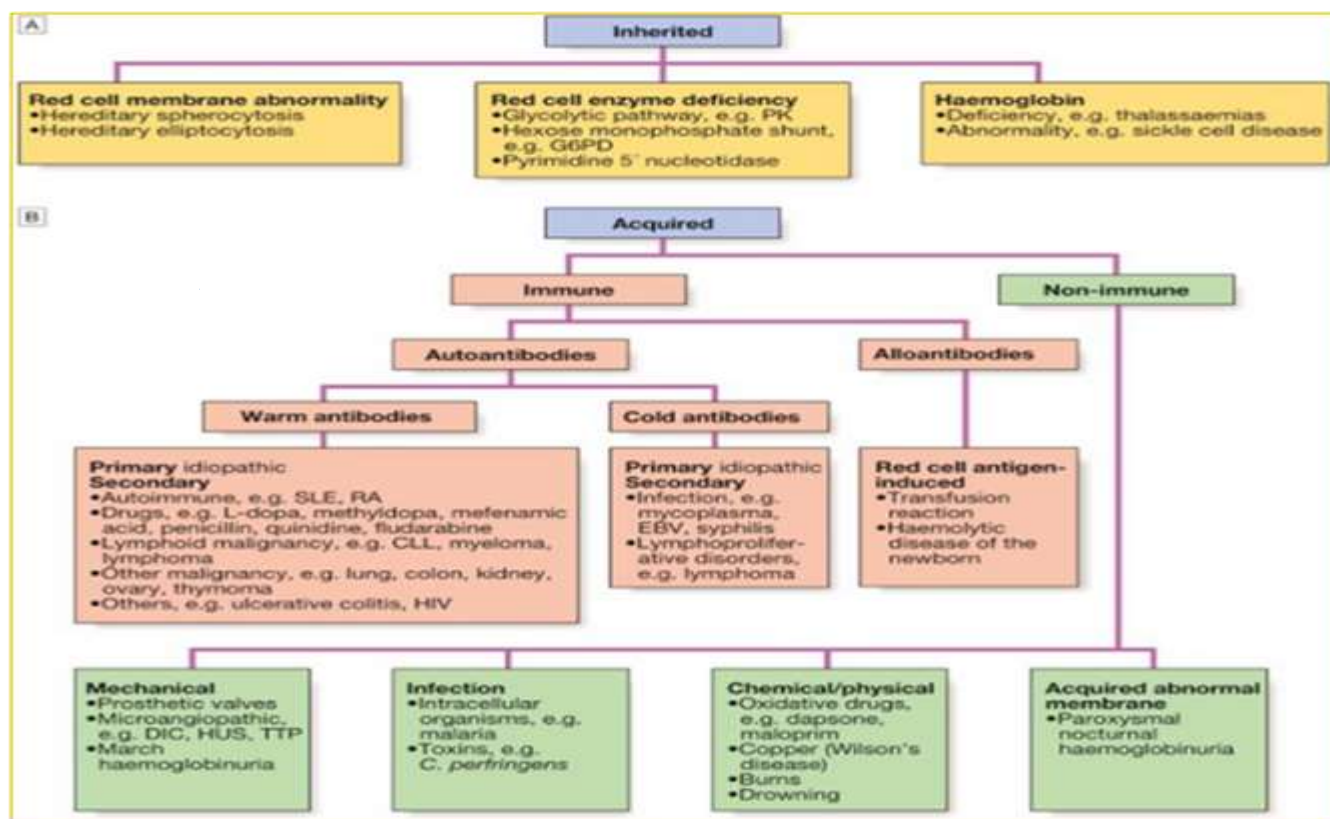
Antibody-coated red cells lose membrane to macrophages in the spleen and hence spherocytes are present in the blood. The optimum temperature at which the antibody is active (thermal specificity) is used to classify immune haemolysis:

Warm antibodies bind best at 37°C and account for 80% of cases. The majorities are IgG and often react against Rhesus antigens.

Cold antibodies bind best at 4°C but can bind up to 37°C in some cases. They are usually IgM and bind complement. To be clinically relevant, they must act within the range of normal body temperatures. They account for the other 20% of cases.

The patient's red cells are mixed with Coombs reagent, which contains antibodies against human IgG/M/complement. If the red cells have been coated by antibody in vivo, the Coombs reagent will induce their agglutination and this can be detected visually. The relevant antibody can be eluted from the red cell surface and tested against a panel of typed red cells to determine against which red cell antigen it is directed. The most common specificity is Rhesus and most often anti-e; this is helpful when choosing blood to cross-match. The direct Coombs test can be negative in the presence of brisk haemolysis. A positive test requires about 200 antibody molecules to attach to each red cell; with a very avid complement-fixing antibody, haemolysis may occur at lower levels of antibody-binding. The standard Coombs reagent will miss IgA or IgE antibodies. Around 10% of all warm autoimmune haemolytic anaemias are Coombs test-negative.





Clinical features

- General features of anemia
- General features of haemolytic anaemia
 - **Mild jaundice**
 - **Hepatosplenomegaly:**
 - Common in chronic hemolysis **except in cases of sickle cell anemia where there's self-splenectomy (repeated infarctions)**
 - **Biliary obstruction and /or gall bladder stones.**
 - Pigment stones: extra hepatic obstruction.
 - Viscid bile: intrahepatic obstruction.
 - NB. In this situation jaundice becomes, mixed (hemolytic + obstructive) and abdominal pain may be present.
 - **Leg ulcer (common with sickle cell)**
 - Chronic leg ulcer surrounded by pigmentation cause by extra vascular iron deposition. Ulcers develop on the malleoli.
 - Crises:

A. Haemolytic crisis

- Fever, rigors: pyrogens release from RBCs death and subsequent inflammatory response.
- Generalized bone pain

- Acute abdominal pain, backache, may be vomiting.
- Aggravation of anaemia (pallor), increased jaundice, and dark urine.
 - Investigations:
 - Dark urine: haemoglobinuria.
 - Increased serum bilirubin
 - Reticulocytosis
 - BM erythroid hyperplasia.
 - Cause may be infection

B. Aplastic crisis

- Inability of BM to replace the destructed RBCs
- Severe anaemia without jaundice.
- **Reticulocytopenia.**
- BM erythroid hypoplasia.
- Cause: viral infection especially **parvovirus B19** and HBV.

C. Sequestration crisis

- Trapping and pooling of RBCs in the spleen (**mostly in children**) and liver (**in the adult**).
- **Can also cause sickle chest syndrome. The most common cause of death in SCA.**
- **Can cause priapism in male SCA patients.**

D. Megaloblastic crisis

- Anaemia severe without jaundice.
- Decreased reticulocytic count
- Macrocytic anaemia.
- BM: megaloblastic
- **Cause: folic acid deficiency.**

E. **Vaso-occlusive crisis** (Thrombotic phenomenon)

- Only in cases of **sickle cell anemia**.
- This is the most common crisis with SCA.
- Plugging of small vessels in the bone produces **acute severe bone pain**.

- This affects areas of active marrow: the hands and feet in children (so-called dactylitis) or the femora, humeri, ribs, pelvis and vertebrae in adults.
- Patients usually have a systemic response with tachycardia, sweating and a fever.
- Complications of hemolytic anemia:
 - Hemolytic crisis:
 - Aplastic crisis:
 - Folate deficiency caused by increased BM requirement
 - Gall stones
 - In sickle cell anaemia: signs and symptoms of thrombotic phenomena.

Investigations

Diagnostic Tests

The best **initial** test is a **peripheral smear**. Sickle cell trait (AS disease) does **not** give sickled cells. The most **accurate** test is the hemoglobin **electrophoresis**.

1. CBC

- Normocytic normochromic anemia with reticulocytosis
- Microcytic anaemia: thalassaemia, spherocytosis.
- Macrocytic + reticulocytosis: megaloblastic crisis
- Reticulocytopenia: aplastic crisis.
- Morphological evidence of RBC damage: as red cell fragment, cell containing malarial parasites.
- Morphology of RBCs: eg spherocytes & sickle cells
- WBC and thrombocytes are normal but may increase due to bone marrow stimulation.

2. Short life span of RBCs:

- Measured by radioactive chromium⁵¹ labeled RBCs

3. Bone marrow

- Hypercellular normoblastic marrow.

- Hypocellular in a plastic crisis.
 - Megaloblastic in folate deficiency.
4. Increased serum LDH
 5. Increased serum bilirubin, fecal stercobilinogen and urinary urobilinogen.
 6. In intravascular haemolysis vs. extravascular the following parameters are presents.
 - Haemoglobinuria and haemosiderinuria
 - Reduced plasma haptoglobin and haemopexin
 - Presence of methaemalbumin in plasma.

Hallmarks of haemolysis	
<ul style="list-style-type: none"> • ↓Haemoglobin • ↑Unconjugated bilirubin • ↑Lactate dehydrogenase 	<ul style="list-style-type: none"> • ↑Reticulocytes • ↑Urinary urobilinogen
Additional features of intravascular haemolysis	
<ul style="list-style-type: none"> • ↓Haptoglobin • ↑Methaemalbumin 	<ul style="list-style-type: none"> • Positive urinary haemosiderin • Haemoglobinuria

7. Tests for the cause
 - RBC morphology.
 - Osmotic fragility.
 - Hb electrophoresis.
 - Estimation of G6PD.
 - Coomb's test.

Now, let's take a closer look at some of those diseases

1. Hereditary spherocytosis

Hereditary Spherocytosis

Etiology

This is a defect in the **cytoskeleton** of the red cell leading to an abnormal round shape and loss of the normal flexibility characteristic of the biconcave disc that allows red cells to bend in the spleen.

“What Is the Most Likely Diagnosis?”

- Recurrent episodes of hemolysis
- Intermittent jaundice
- Splenomegaly

- Family history of anemia or hemolysis
- Bilirubin gallstones

Diagnostic Tests

- **Low MCV**
- **Increased mean corpuscular hemoglobin concentration (MCHC)**
- **Negative Coombs test**

The most accurate test is **osmotic fragility**. When cells are placed in a slightly hypotonic solution, the increased swelling of the cells leads to hemolysis.

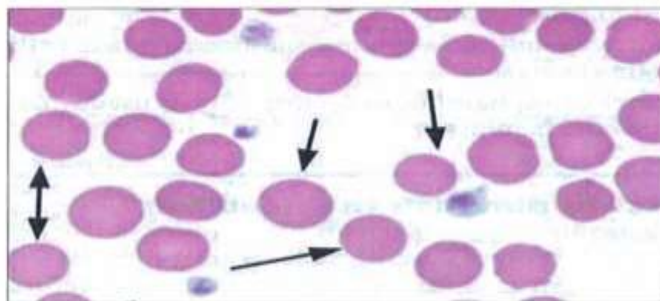


Figure 7.4: Spherocytes lose the central pallor of normal red cells. The MCHC is elevated. *Source: Alireza Eghtedar, MD.*

Treatment

1. Chronic **folic acid** replacement supports red cell production.
2. **Splenectomy** stops the hemolysis but does not eliminate the spherocytes.

- A hereditary autosomal dominant disorder in which the corpuscular membrane is abnormally less deformable and more permeable to sodium. This is due to a deficiency of the protein spectrin (**most commonly β -spectrin or ankyrin**) in the corpuscular membrane, which causes water absorption and rupture of red blood cells.

Clinical features

- Symptoms usually appear during the first decade of life, but may be delayed. The disease can present with Patients over 65 years and is often discovered on routine blood work.
- Common features of haemolytic anaemia .
- Slight or moderate enlargement of the spleen.
- Pigment biliary stones in long standing cases.
- Chronic leg ulcers.

Investigations

1. CBC
 - Spherocytosis, red cells are spherical instead of being biconcave.
 - Reticulocytosis.
 - Anemia of moderate degree.
2. Osmotic fragility
 - Is characteristically increased, hemolysis usually begins at sodium chloride concentration of 0.6 % or even higher
3. Direct coomb's test is negative, excluding an autoimmune cause of spherocytosis and hemolysis.

Treatment

- The principal form of treatment is splenectomy, although this should not be performed unless clinically indicated because of anemia.
- Splenectomy lengthens the life span of redcells, correct anaemia, prevents haemochromatosis, but does not affect the character of red cells.
- **Folic acid prophylaxis, 5 mg daily, should be given for life**
- **Acute, severe haemolytic crises require transfusion support, but blood must be cross-matched carefully and transfused slowly as haemolytic transfusion reactions may occur.**

2. Elliptocytosis

- This has similar clinical and laboratory features to hereditary spherocytosis except for the appearance of the blood film, but is usually clinically milder and don't need Tx.. Occasionally patients require splenectomy. **The management plans for severe cases or cases of neonatal hemolysis is exactly the same as in hereditary spherocytosis.**

3. Paroxysmal nocturnal hemoglobinuria

- The only corpuscular cause of hemolysis, which is acquired.
- The exact etiology is unknown.

- The underlying defect is an inability of PNH cells to make glycosyl-phosphatidylinositol (GPI) which anchors surface proteins such as decay accelerating factor (DAF) membrane inhibitor of reactive lysis (MIRL) to cell membranes.
- DAF and MIRL and other proteins are involved in complement degradation and in their absence the hemolytic action of complement is not regulated.
- It results in an increased sensitivity of RBCs Membrane to be damaged by the complement system. **(Involvement of the complement system causes intravascular hemolysis)**

Clinical features

- Features of hemolytic anemia
- Hemolysis occurs mainly after sleep by night leading to anemia and haemoglobinuria the next morning with the characteristic red-brown color.
- Abnormal platelets may lead to thrombosis
- Abnormal WBCs increase risk of infection
- Abdominal pain due to visceral ischemia caused by micro thrombi.
- Complications:
 - **Thrombosis.** (Clots may develop in common sites (deep vein thrombosis of the leg and resultant pulmonary embolism when these clots break off and enter the lungs), but in PNH blood clots may also form in more unusual sites: the hepatic vein (causing Budd-Chiari syndrome), the portal vein of the liver (causing portal vein thrombosis), the superior or inferior mesenteric vein (causing mesenteric ischemia) and veins of the skin. Cerebral venous thrombosis, an uncommon form of stroke, is more common in those with PNH)
 - Aplastic anemia.
 - Acute myeloid leukemia.

Investigations

- General investigation of hemolytic anemia (shows features of **intravascular** hemolysis).
- Activation of complement to cause hemolysis.
 - Ham's test hemolysis by adding acid to the blood. (they lyse more readily)
 - **Flow cytometry is the gold standard.**

Treatment

- Symptomatic
 - Blood transfusion for patients with severe anemia
 - Long term anticoagulation may be necessary for patients with recurrent thrombotic episodes
- Prednisone.
- BMT.
- **Recently, the anti-complement C5 monoclonal antibody eculizumab was shown to be effective in reducing haemolysis.**

Haemoglobinopathies

- Normal hemoglobin consists of:
 - Globin: protein, which is formed from 4 polypeptide chains. Two alpha and two non-alpha globin chains. **Alpha globin chains are produced throughout life, including in the fetus, so severe mutations may cause intrauterine death. Production of non-alpha chains varies with age; fetal haemoglobin (HbF- $\alpha\alpha/\gamma\gamma$) has two gamma chains, while the predominant adult haemoglobin (HbA- $\alpha\alpha/\beta\beta$) has two beta chains. Thus, disorders affecting the beta chains do not present until after 6 months of age. A constant small amount of haemoglobin A2 (HbA2- $\alpha\alpha/\delta\delta$, usually less than 2%) is made from birth.**
 - Haem: iron-protoporphyrin complex
- Normal electrophoresis pattern in an adult:
 - Hemoglobin A (96%): α_2/β_2 (normal hemoglobin)
 - Hemoglobin F (3%): α_2/γ_2 (normal till 6 months of age)
 - Hemoglobin A2: α_2/δ_2 .
- Haemoglobinopathies are clinical syndromes resulting from abnormalities in the structure of globin molecule or with a reduction in the rate of production of one or other of the globin chains.
- There are three main categories:
 - Structural variant of Hb: e.s HbS.
 - Failure to synthesize Ht: e.g thalassemia.
 - Failure to switch from fetal Hb (HbF) to adult Hb (HbA) : Hereditary persistence of Fetal Hb (HPFH).

1. Sickle cell anemia

- A hereditary autosomal recessive disorder characterized by the presence of HbS (**formed by substitution of valine for glutamic acid in the sixth position of the β -hemoglobin chain**), which on exposure to hypoxia, the deoxygenated Hbs forms insoluble aggregates that distort the erythrocytes and increase their rigidity causing sickling deformation and subsequent fragmentation. In addition, the unyielding elongate crescentic erythrocytes form aggregates that block terminal arterioles, capillaries and veins, resulting in tissue infarctions and perivascular oedema in the involved organs (vascular occlusion)

Chronic disease manifestations:

Skeletal: aseptic necrosis of femoral head and salmonella osteomyelitis.

Biliary disease: pigmented gallstones (high bilirubin)

Renal chronic hematuria (renal papillary necrosis)

Liver disease: viral hepatitis (transfusion) and secondary hemochromatosis.

Pulmonary: local infection and vascular occlusion (acute chest syndrome), pulmonary hypertension.

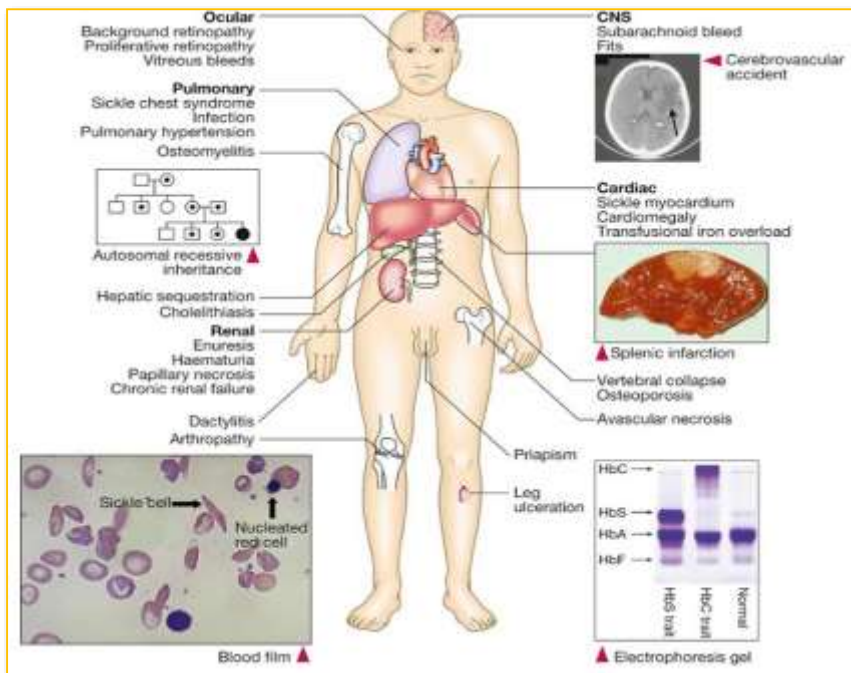
Heart: enlarged, flow murmur, MI.

Immune: functional splenism increase chance of sepsis with encapsulated organisms (strep. Pneumonia, H. influenza and salmonella osteomyelitis)

Eye: ischemic retinopathy.

Neuro: increased risk for TIA and CVAs.

- Symptoms vary from a mild asymptomatic disorder to severe haemolytic anaemia and recurrent severe painful crises.



Signs of SCA:

SICKLE:

Splenomegaly/Sludging
Infection
Cholelithiasis/acute Chest syndrome
Kidney (hematuria, papillary necrosis)
Liver congestion/Leg ulcer
Eye changes

- People with sickle cell trait (heterozygous sickling disorder) have no symptoms unless extreme circumstances cause anoxia such as flying in a non-pressurized aircraft or problems with anaesthesia.
- Sickle cell **trait protects against plasmodium falciparum malaria. (Controversy)**
 - 60% Hb A and 40% Hb S
 - The blood count and film are normal
 - The diagnosis is made by a positive sickle test or by Hb electrophoresis.

Investigations:

- Features of hemolytic anemia.
- CBC
 - The level of Hb is in the range 6-8gm/dl with high reticulocytic count 10-20%. (Blood films show features of hyposplenism)
 - Sickling of red cells on a blood film can be induced in the presence of sodium metabisulphite, which cause hypoxia.
- **Hb electrophoresis:**
 - There is no Hb A, 80-85% Hb S and 2-20% HbF .
 - The parents of the affected child will show features of sickle cell trait.

Treatment

1. Begin with oxygen/hydration/analgesia.
2. If fever or a white cell count higher than usual is present, then antibiotics are given. Use ceftriaxone, levofloxacin, or moxifloxacin.
3. Folic acid replacement is necessary on a chronic basis.
4. Give pneumococcal vaccination because of autosplenectomy.
5. Hydroxyurea prevents recurrences of sickle cell crises by increasing hemoglobin F.

IMPORTANT NOTES FROM EXTERNAL RESOURCES

Notes

Davidson

Management of SCA

All patients with sickle-cell disease should receive prophylaxis with daily folic acid, and penicillin V to protect against pneumococcal infection, which may be lethal in the presence of hyposplenism. These patients should be vaccinated against pneumococcus, meningococcus, Haemophilus influenzae B, hepatitis B and seasonal influenza.

Vaso-occlusive crises are managed by aggressive rehydration, oxygen therapy, adequate analgesia (which often requires opiates) and antibiotics. Transfusion should be with fully genotyped blood wherever possible. Simple top-up transfusion may be used in a sequestration or aplastic crisis. A regular transfusion programme to suppress HbS production and maintain the HbS level below 30% may be indicated in patients with recurrent severe complications, such as cerebro-vascular accidents in children or chest syndromes in adults. Exchange transfusion, in which a patient is simultaneously venesected and transfused to replace HbS with HbA, may be used in life-threatening crises or to prepare patients for surgery.

A high HbF level inhibits polymerisation of HbS and reduces sickling. Patients with sickle-cell disease and high HbF levels have a mild clinical course with few crises. Some agents are able to increase synthesis of HbF and this has been used to reduce the frequency of severe crises. The oral cytotoxic agent hydroxycarbamide has been shown to have clinical benefit with acceptable side effects in children and adults who have recurrent severe crises.

Relatively few allogeneic stem cell transplants from HLA-matched siblings have been performed but this procedure appears to be potentially curative

Case:

A young African American man with microcytic hypochromic anemia is found to have a high ferritin and iron with normal RDW.

Think: SCA

Next step: Hb electrophoresis.

Indications for EXCHANGE transfusion in SCA:

Stroke/TIA

Acute chest syndrome

Priapism

Third term pregnancy

Intractable vaso-occlusive crises.

2-The Thalassemias:

The Thalassaemias are anemias originally found in people living on the shores of the Mediterranean, now known to affect people throughout the world. The defective synthesis of globin genes in thalassaemia leads to imbalanced globin chain production leading to precipitation of globin chains within the red cell precursors and resulting in ineffective erythropoiesis. Precipitation of globin chains in mature red cell leads to haemolysis. **There are two α genes and one β gene on each chromosome, making a total of four α genes and two β genes.** In alpha-thalassaemia, disruption of one or both alleles on **chromosome 16** may occur, with production of some or no alpha globin chains. In beta-thalassaemia, defective production usually results from disabling point mutations causing no (β^0) or reduced (β^-) beta chain production.

i. α -thalassemia:

- In contrast to β -thalassaemia it is caused by gene deletions.
- If all four genes are absent ($_/_$) there is no α -chain synthesis and Hb Barts (γ_4) is present. Hb. Barts cannot carry oxygen and is incompatible with life and infants are either stillborn or die very shortly after birth. (hydrops fetalis)
- If three genes are deleted ($_/_ \alpha$) there is moderate anaemia and splenomegaly (Hb H disease). Hb A, Hb Barts and Hb H (B4) are present, Hb A2 is normal or reduced
- If two genes are deleted ($_/\alpha\alpha$)(α -thalassaemia trait), there is microcytosis with or without mild anaemia. ($\alpha\alpha/_$) **thalassemia trait is more common in Asians. ($\alpha/_ \alpha$) is more common in Africans)**
- If only one gene is deleted ($\alpha\alpha/\alpha _$), the patient is usually asymptomatic.

ii. β -thalassemia:

- There is defective production of beta-chain with excess production of Hb F and Hb A2.
- Types
 1. β -thalassaemia major (Cooley's anemia)
 - Homozygous (2 abnormal gene)
 - Hb F > 80-90%
 - The onset is during the first year of life (3-6 month)
 - The children fail to thrive, (stunted growth) mongoloid faces, bone deformities.
 - Feature of hemolytic anaemia, jaundice and huge splenomegaly
 - Extramedullary haematopoiesis: that soon causes hepatosplenomegaly, bone expansion giving rise to classical thalassaemic facies (mongoloid facies).
 - Feature of iron overload: liver cirrhosis, bronze skin, and cardiomegaly.
 - The disease is more common among Mediterraneans.
 - Positive family history.
 - Usually recurrent bacterial infection.
 2. β -thalassaemia intermedia Heterozygous (only one abnormal gene)
 - Both Hb F (10%) and Hb A2 (10%).
 - Presented in adults by mild Microcytic hypochromic anemia and splenomegaly.
 - Commonly symptomatic and require treatment.

Genotype	HbA	HbA ₂	HbF (%)	Other Hemoglobins
Normal β/β	97	2.5 – 3.2	<1	None
Thalassaemia major	0 Present	1.0 – 5.9	>94	Free α-chains Free α-chains None
Thalassaemia intermedia	Present	5.4 – 10.0	30 – 73	None
Thalassaemia minor	>90	3.5 – 8.0	1 – 2	None
Thalassaemia minima β ^{silent} /β	97	<3.2	<1	None

3. β-Thalassaemia minor (trait)

- This common carrier state is asymptomatic, anaemia is mild or absent, the red cells are hypochromic and microcytic with a low MCV and MCH.
- DD: Iron deficiency anemia. Must be excluded.
- Rarely requires treatment.

Electrophoresis Findings	
Alpha thalassemia	Beta thalassemia
One gene deleted: normal	Increased hemoglobin F and A ₂
Two genes deleted: mild anemia, normal electrophoresis	N/A
Three genes deleted: moderate anemia with hemoglobin H, which are beta-4 tetrads; increased reticulocytes	Beta thalassemia intermedia <ul style="list-style-type: none"> • Normal hemoglobin F • No transfusion dependence
Four genes deleted: gamma-4 tetrads or hemoglobin Bart; CHF causes death in utero	N/A

Extra

	Major	Intermedia	Minor	Minima
Hypochromia	++++	+++	++	+
Microcytosis	+++	++	+	0
Target cells	10 – 35%	++	+	±
Basophilic stippling	++	+	+	0, +
Reticulocytes (%)	5 – 15	3 – 10	2 – 5	1 – 2
Nucleated red cells	+++	+, 0	0	0

±, Little or no abnormality; +, mild abnormality; +++, prominent abnormality

Clinical syndrome	Genotype	Clinical features	Notes
β -thalassemia major	Homozygous (β^0/β^0 , β^+/ β^+) occasionally (β^0/β^+)	Manifest postnatally, growth retardation 2ry hemochromatosis (death at the 2 nd to 3 rd decade of life)	Reduction or absence of HbA, increased HbF. HbA2 normal or increased
β -thalassemia intermedia	Variable (β^0/β^+), (β^+/ β^+), (β^0/β), (β^+/β)	Not as severe as β -thalassemia major, doesn't require transfusion under normal circumstances	Similar to major yet less profound
β -thalassemia minor (also called β -thalassemia trait)	Heterozygous (β^0/β), (β^+/β)	Normal life span. Often asymptomatic, mild hypochromic, microcytic anemia (must exclude iron deficiency anemia)	Reduced HbA. Increased HbA2.
α -thalassemia silent carrier	-/ α , α/α	asymptomatic	
α -thalassemia trait	-/-, α/α (asian) -/ α , -/ α (african)	Normal life span. Often asymptomatic, mild hypochromic, microcytic anemia (must exclude iron deficiency anemia)	
HbH disease	-/-, -/ α	Not as severe as β -thalassemia major, doesn't require transfusion under normal circumstances	Detection of β_4 tetramers by electrophoresis
Hydrops fetalis	-/-, -/-	Lethal in utero	

Enzymopathies

1. Glucose-6- phosphate dehydrogenase deficiency:

“What Is the Most Likely Diagnosis?”

Look for African American or Mediterranean **men** with **sudden anemia** and jaundice who have a **normal-sized spleen** with an **infection** or are using one of the drugs previously listed.

Diagnostic Tests

The best initial test is **for Heinz bodies and bite cells**. The G6PD level will be **normal** after a hemolytic event. The most accurate test is the G6PD level after waiting 1 to 2 months after an acute episode of hemolysis.

- The enzyme glucose-6-phosphate dehydrogenase (G6PD) is pivotal in the hexose monophosphate shunt pathway. **(It reduces nicotinamide adenine dinucleotide phosphate (NADPH) while oxidizing glucose-6- phosphate. It is the only source of NADPH in red cells and as NADPH is needed for the production of reduced glutathione, a deficiency renders the red cell susceptible to oxidative stress)**
- In the absence of G6PD, irreversible oxidative denaturation of Hb occurs which becomes attached to red cell membrane to form Heinz bodies. Red cells containing such inclusion bodies become destroyed in the liver and spleen. **Resulting also in bite cells**
- Agents which may cause haemolysis in (G6PD) deficiency
 - Infections and other acute illnesses e.g diabetic ketoacidosis
 - Fava beans.
 - Drugs
 1. Sulfonamides e.g co-trimoxazole
 2. Ant. Bacterial e.g interferons, chloramphenicol
 3. Analgesics e.g aspirin, acetamenophin
 4. Anti-malarial e.g primaquine and quinine
- **Rapidly** developing intravascular haemolysis with haemoglobinuria.
- Investigations:
 - Between crises the blood count is normal
 - Direct enzyme assay.
 - During crisis, the blood film may show contracted and fragmented cells “bite” cells and blister” cells which have Red Heinz bodies removed by the spleen.
 - Haemoglobinaemia and haemoglobinuria in the acute stage.

ABCs of G6PD deficiency:

Antimalarials
Bactrim/Bite cells
Ciprofloxacin
DKA
InfEction
Fava beans

Treatment

Nothing reverses the hemolysis. Avoid oxidant stress.

- Withdrawal of the drug or toxic substance
- Blood transfusion is lifesaving in severe cases.

Case:

A 31 year old Italian male with back pain, dark urine, jaundice, and anemia after 2 days of ciprofloxacin.

Think: G6PD deficiency.

Next step: check peripheral smear looking for "bite cells", transfuse if severe anemia and check renal function.

2. Pyruvate kinase deficiency:

- This is inherited as an autosomal recessive, the affected patients being homozygous or less common heterozygous. The red cells become rigid as a result of reduced adenosine triphosphate (ATP) formation.
- Direct enzyme assay is needed to make the diagnosis.

EXTRA



24.40 Management of the splenectomised patient

- Vaccinate with pneumococcal, *Haemophilus influenzae* type B, meningococcal group C and influenza vaccines at least 2–3 wks before elective splenectomy. Vaccination should be given after emergency surgery but may be less effective
- Pneumococcal re-immunisation should be given at least 5-yearly and influenza annually. Vaccination status must be documented
- Life-long prophylactic penicillin V 500 mg twice daily is recommended. In penicillin-allergic patients, consider a macrolide
- Patients should be educated regarding the risks of infection and methods of prophylaxis
- A card or bracelet should be carried to alert health professionals to the risk of overwhelming sepsis
- In septicaemia, patients should be resuscitated and given IV antibiotics to cover pneumococcus, *Haemophilus* and meningococcus, according to local resistance patterns
- The risk of cerebral malaria is increased in the event of infection
- Animal bites should be promptly treated with local disinfection and antibiotics, to prevent serious soft tissue infection and septicaemia

Questions

- 1) All of the following may cause microcytic anemia, EXCEPT?
- a) IRON deficiency.
 - b) Vitamin B12 deficiency.
 - c) Chronic disease.
 - d) Thalassemia.
- 2) Female pt developed anemia. The following is her blood film. schistocytes were shown what is the most likely cause?

- a) Microangiopathic hemolytic anemia
- b) Sickle cell disease
- c) Thalassemia
- d) G6PD deficiency.



3) A 73-year-old man comes to the office with fatigue that has become progressively worse over the last several months. He is also short of breath when he walks up one flight of stairs. He drinks 4 vodka martinis a day. He complains of numbness and tingling in his feet. On physical examination he has decreased sensation of his feet. His hematocrit is 28% and his MCV is 114 fl (elevated).

What is the most appropriate next step in management?

- a) Vitamin B¹² level
- b) Folate level
- c) Peripheral blood smear
- d) Schilling test
- e) Methylmalonic acid level

4) A 73-year-old woman comes with decreased position and vibratory sensation of the lower extremities, a hematocrit of 28%, MCV of 114 fl, and hypersegmented neutrophils. Her B₁₂ level is decreased, but near the borderline of normal.

What is the most appropriate next step in the management of this patient?

- a) Methylmalonic acid level
- b) Anti-intrinsic factor antibodies
- c) Anti-parietal cell antibodies
- d) Schillings test
- e) Folate level
- f) Homocysteine level

5) Which of the following is a complication of B₁₂ or folate replacement?

- a. Seizures
- b. Hemolysis
- c. Hypokalemia
- d. Hyperkalemia
- e. Diarrhea

6) Which of the following can be found on smear in sickle cell disease?

- a) Basophilic stippling
- b) Howell-Jolly bodies
- c) Bite cells
- d) Schistocytes
- e) Morulae

7) A 43-year-old man with sickle cell disease is admitted with an acute pain crisis. His only routine medication is folic acid. His hematocrit on admission is 34%. On the third hospital day, the hematocrit drops to 22%.

What is the best initial test?

- a) Reticulocyte count
- b) Peripheral smear
- c) Folate level
- d) Parvovirus B-19 IgM level
- e) Bone marrow

432 Medicine Team Leaders

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Answers:

1: b

2: a

3:c

4:A

5:c

6:b

7:a