Megaloblastic anemia

In macrocytic anemia → the red cells are abnormally large (Mean Corpuscular Volume, MCV > 95 fL)

Macrocytic anemia can be broadly subdivided into megaloblastic and non-megaloblastic, based on the appearance of developing erythroblasts in the bone marrow.

Causes of *macrocytosis* other than megaloblastic anemia:

1. Alcohol
2. Liver disease
3. Myxoedema (hypothyroidism)
4. Myelodysplastic syndrome
5. Cytotoxic drugs
6. Aplastic anemia
7. Pregnancy
8. Neonatal
9. Smoking
10. Myeloma
11. Reticulocytosis

Definition of megaloblastic anemia:

A group of anemias in which the erythroblasts in the bone marrow show a characteristic abnormality (maturation of the nucleus being delayed relative to that of the cytoplasm)

Causes of megaloblastic anemia:

1. Vit B12 Deficiency

2. Folate Deficiency

3. Abnormalities of vitamin B12 or folate Metabolism (e.g. Antifolate drugs, transcobalamin deficiency)

4. Other defects of DNA synthesis:

Congenital enzyme deficiency (orotic acidurea)
Acquired enzyme deficiency (alcohol, therapy with hydroxyurea)

Vitamin B12 (cobalamin) and folate nutrition and absorption

	Vitamin B12	Folate
Dietary source	Only food of animal origin, especially liver	Most foods, especially liver, green vegetable and yeast; destroyed by cooking
Average daily intake*	7 - 30 µg	200-250 µg
Minimum daily requirement*	1-3 µg	100-200 µg†
Body stores*	3-5 mg, mainly in the liver	8-20 mg, mainly in the liver
Time to develop deficiency in the absence of intake or absorption*	Anaemia in 2-10 years	Macrocytosis in 5 months.
Requirements for absorption	Intrinsic factor secreted by gastric parietal cells	Conversiion of polyglutamates to monoglutamates by intestinal folate conjugase
Site of absorption	Terminal ileum	Duodenum and jejunum

Vitamin B12 (cobalamin) absorption:

- B12 is combined with intrinsic factor (IF) that is synthesized by the gastric parietal cells
- The IF – B12 complex can then bind to a specific surface receptor for IF, cubulin
- Absorption Takes place at the distal (terminal) ileum where B12 is absorbed and IF is destroyed

- Vitamin B12 becomes attached to transcobalamin which delivers it to bone marrow and other tissues. (Pls refer **slide # 11** in the lecture or **fig. 4.2** in the book)

Causes of Vit B12 Deficiency:

I. Nutritional:

Especially vegans

II. Malabsorption:

A. Gastric causes:

1. Pernicious anemia
2. Congenital lack or abnormalities of IF
3. Total or partial gastrectomy

B. Intestinal causes:

1. Intestinal stagnant loop syndrome- jejunal diverticulosis, blind loop, stricture
2. Chronic tropical sprue
3. Ileal resection and crohn's disease

* Pernicious anemia:

1. Caused by autoimmune attack on the gastric mucosa leading to atrophy of the stomach (thin wall with plasma cell and lymphoid infiltrate of the lamina propria)

2. Secretion of IF is absent or almost absent

3. Affects more females with peak age of 60 y

4. May be associated with autoimmune disease including polyendocrine syndrome (myxoedema, thyrotoxicosis, addison's disease, hypoparathyroidism)

5. Increased incidence of carcinoma of stomach

6. IF Ab are specific for pernicious anemia but occur in serum of only half of the patients

Tests useful in establishing the diagnosis and cause of vitamin B12 deficiency

Investigation	Findings
Dietary assessment	No intake of animal protein in vegans.
Blood count	High MCV*
Blood film neutrophil granulocytes*	Oval macrocytes,* hypersegmentation of
Bone marrow aspirate	Megaloblasts,* giant metamyelocytes*
Serum vitamin B12	Low (also low in one-third of folate-deficient patients)
Red cell folate	Normal or low
Schilling test for B12 Absorption	Abnormal in pernicious anaemia, diseases of the terminal ileum and in the 'stagnant loop syndrome'. Abnormality improves when the test is performed with intrinsic factor only in pernicious anaemia
Barium meal and follow through	Demonstrates various lesions of the small Intestine in the 'stagnant loop syndrome' and in diseases of the terminal ileum
Assay of intrinsic factor in gastric juice.	Very low or absent in pernicious anaemia

*Also found in folate deficiency.

1. Diet history
2. B12 absorption + / - IF
3. IF, Parietal cell Ab
4. Endoscopy or barium meal and follow through

* Absorption test:

Using an oral dose of radioactive cobalt, cyanocobalamin for distinguishing malabsorption from an inadequate diet.

When the test is repeated with an active IF preparation, gastric lesions (like pernicious anaemia) can be distinguished from intestinal lesions.

Absorption is measured indirectly by the urinary excretion (Schilling) technique.

	Dose of labeled B12 given alone	**Dose of labeled B12 given with IF**
Vegan	Normal	Normal
pernicious anemia Or gastrectomy	Low	Normal
Ileal Lesion	low	low
Intestinal blind loop syndrome	Low	Low

Causes of folate deficiency:

1. Inadequate dietary intake

2. Malabsorption

   Coeliac disease, jejunal resection, tropical sprue

3. Increased requirement

   Pregnancy, premature infants, chronic haemolytic anaemias, myelofibrosis, various malignant diseases

4. Increased loss

   Long-term dialysis, congestive heart failure, acute liver disease

Transport and function of folate: (Pls refer slide **# 25** in the lecture or **fig. 4.5** in the book)

1. Dietary folate are converted to methyl THF during absorption through the upper small intestine

2 .Once inside the cell, they are converted to folate polyglutamates

3. folates are needed for homocystine convrersion to methionine and in the synthesis of purine precursors of DNA

Function of Vitamin B12: (Pls refer slide **# 28** in the lecture or **fig. 4.3** in the book)

VitaminB12 is coenzyme for two biochemical reactions in the body:

1. As methyl B12, it is a cofactor for methionine synthase, the enzyme responsible for methylation of homocystine to methionine using methylTHF as methyl donor

2. as (adoB12) assists in conversion of Methylmalonyl coenzyme A (Co A) to Succinyl Co A.

Biochemical bases of megaloblastic anemia:

1.Folate deficiency cause megaloblastic anemia by inhibiting the synthesis of dTMP, rate limiting step in DNA synthesis and this reaction needs 5,10 methylene THF polyglutamate as coenzyme.

2. Lack of B12 prevents the demethylation of methyl THF thus depriving cells of THF

3. The folate polyglutamate become oxidized from THF to DHF and regeneration of active THF requires DHF reductase enzyme. Methotraxate inhibits this enzyme and therefore inhibits DNA synthesis

Clinical Features of Megaloblastic Anaemia –Progressive symptoms and signs of anaemia:

1. Weakness, anorexia, weight loss, diarrhoea or constipation, tiredness, shortness of breath, angina of effort, heart failure

2. Mild jaundice, glossitis, stomatitis, angular cheilosis.

3. Purpura, melanin pigmentations.

4. Infections

Effects of vit B12 or folate deficiency:

1. Megaloblastic Anaemia

2. Neuropthy (for vit B12 only)

3. Sterility (reduced fertility)

4. Neural tube defect in the fetus related to

5. Cardiovasular disease

6. Rarely reversible melanin skin pigmentation

Neuropathy due to Vit B12 and folate deficiency:

Progressive neuropathy affecting

- The peripheral sensory nerves
- Posterior and lateral columns of the spinal cord (subacute combined degeneration of the cord)
- Optic atrophy
- Psychiatric symptoms
- The neuropathy is likely due to accumulation of S-adenosyl homocysteine and reduced level of S-adenosyl methionine in nervous tissue resulting in defective methylation of myelin and other substrates.
- Neuropathy is mostly due to vitamin B12 deficiency.

Neural tube defect (NTD):

- (Anencephaly, spina bifida or encephalocoele) in the fetus due to folate or Vit B12 deficiency in the mother. This result in build-up of homocysteine and S-adenosyl homocysteine in the fetus which impair methylation of various proteins and lipids.

- Polymorphism in the enzyme 5,10 methylene tetrahydrofolate reductase (5,10-MTHFR). This mutation (677 C◊T) in the MTHFR gene results in low serum and red cell folate and high serum homocysteine in the parents and fetus with NTD.

- Cleft palate and hair lip.

I. Haematological findings in Megaloblastic Anaemia:

Peripheral Blood:

- Macrocytic anaemia, oval macrocytes, anisocytosis, poikilocytosis high MCV.
- Hypersegmented neutrophils.
- Leucopenia and thrombocytopenia (especially in severe anemic patients)
- Retic count is low

Bone Marrow:

- Hypercellular marrow with M:E ratio in normal or reduced.
- Accumulation of primitive cells due to selective death of more mature cells.
- Megaloblast (large erythroblast which show failure of nuclear maturation maintaining open, fine, lacy chromatin).
- Dissociation between the nuclear and cytoplasmic development in the erythroblasts.
- Mitosis and dying cells are more frequent than normal.
- Giant and abnormally shaped, metamyelocytes, polypoid megakaryocytes.
- Increased stainable iron in the macrophage and in the erythroblasts.

Other laboratory abnormalities:

- Ineffective haemopoiesis. (Intramedullary cell death by apoptosis) associated with increased serum indirect bilirubin.
- ↑ urobillinogen and faecal stercobillinogen.
- ↑ LDH ↑ serum iron ↑ blood carbon monoxide.
- ↓ reduced haptoglobins
- Positive urine haemosiderin.

Treatment of megaloblatic anaemia:

	Vitamin B12 deficiency	Folate deficiency
Compound	Hydroxocobalamin	Folic acid
Route	Intramuscular	Oral
Maintenance	1000 µg every 3 months	Depends on underlying disease; life long therapy may be needed in chronic inherited haemolytic anaemia, myelofibrosis, renal dialysis
Prophylactic	Total gastrectomy Ileal resection	Pregnancy, severe haemolytic anaemias, dialysis, prematurity