Megaloblastic Anemia

= Haematology 435 =

Objectives:

- To understand the mechanisms by which macrocytic anaemia may arise.
- To appreciate the signs and symptoms of macrocytic anaemia.
- To understand how macrocytic anaemia can be classified.
- To be able to know the causes of macrocytic anaemia.
- To understand the normal metabolism of vitamin B12 and folic acid, and to appreciate how megaloblastic anaemia may arise.
- To suggest some normoblastic causes of macrocytosis.

➢ **Color Codes:**

- Pink: Girls' notes. Blue: Boys' notes. Red: Important Notes. Gray: Extra notes.
- Purple: Lecture notes & Pathoma notes.

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➢ **References:**

- Girls&Boys Doctors Slides and Notes.
- Lecture notes pathology (chapter 12)
- Pathoma (chapter 5)
- Team 434 & 433.

➢ **Correction file:** (HERE)

➢ **Check Your Understanding!** (HERE)

❖ Normal adult red cell values:

	Male	Female
Haemoglobin* (g/L)	**135-175**	**115-155**
Haematocrit(PCV)(%) packed cell volume.	**40-52**	**36-48**
Red cell count (x1012/L)	**4.5-6.5**	**3.9-5.6**
Mean cell haemoglobin (pg)	**27-34**	
Mean cell volume (FL) (important)	**80-95** **Less than 80 =microcytic,more than 95 =macrocytic**	
Mean cell haemoglobin concentration g/L	**300-350**	
Reticulocyte count (x109/L)	**25-125 (1.0-2%)**	

- Note that reticulocytes are directly before the mature RBCs,these reticulocytes are normal in the CBC if the bone marrow is under stress or if there is anemia.
- Other precursors are NOT normally found in CBC!
- If you find a CBC with high lymphocytic count,you MUST ask about the age of the patient because children have NORMALLY HIGH LYMPHOCYTES in blood and why is that ? because they are more prone to infection.

❖ Types of anaemias:

Microcytic, Hypochromic Anaemia	Normocytic, Normochromic Anaemia
MCV <80 fL	MCV 80 – 95 fL
MCH<27pg	MCH>26pg
Types: • Iron deficiency. • Thalassaemia. • Anaemia of chronic disease (some cases). • Lead poisoning. • Sideroblastic anaemia (some cases)	**Types:** • Many haemolytic anaemias. • Anaemia of chronic disease (some cases). • After acute blood loss. • Renal disease. • Mixed deficiencies.(such as :both B12 and iron deficiency) • Bone marrow failure, e.g. (post-chemotherapy, infiltration by carcinoma)

❖ MACROCYTIC ANAEMIA:

Macrocytic anaemias can be divided into those showing:

Megaloblastic erythropoiesis	Normoblastic erythropoiesis
Describes *abnormal red cell* development characterized by a **lack of synchrony between the maturation of the red cell nucleus and its cytoplasm.** "Nuclear-Cytoplasmic asynchrony" It arises as a consequence of disordered DNA synthesis and results in a macrocytic anaemia.	Describes the normal appearance of red cell maturation, but may still be associated with a macrocytosis in the peripheral blood.

- Megaloblast large erythroblast.
- Normoblast normal erythroblast.

❖ Conditions in which Macrocytosis or hypersegmented neutrophils:

- **May occur in the absence of megaloblastic anaemia"**

"No apparent change in the erythroid precursors ,they have normal synchrony"

Macrocytosis	• Alcohol "Cause lipid deposition in RBC>Large cells" • Liver disease (especially alcoholic). (so due to lipid deposition also) • Reticulocytosis (haemolysis or haemorrhage). *Note:* When reticulocytes are high in the blood,this will show increased MCV because reticulocytes are larger than mature RBCs. • Aplastic anaemia or red cell aplasia. • Hypothyroidism. • Myelodysplasia including acquired Sideroblastic anaemia myeloma and macroglobulinemia. • Leucoerythroblastic anaemia. • Myeloproliferative disease. • Pregnancy & Newborn "Physiological"
Hypersegmented Neutrophils	• Renal failure. • Congenital (familial) abnormality.

Note:- High MCV recorded when cold agglutinins or paraproteins are present.

❖ Macrocytosis with Normoblasts:

- Normal neonates (Physiological)
- Chronic alcoholism.*
- Myelodysplastic syndromes*
- Chronic liver disease*
- Therapy with anticonvulsant drugs*

*Some patients show B12- and folate-independent megaloblastic erythropoiesis.

❖ Causes of megaloblastic anaemia:

- **Cobalamin deficiency** or abnormalities of cobalamin metabolism.
- **Folate deficiency** or abnormalities of folate metabolism.
- Therapy with antifolate drugs (e.g. **methotrexate**)
- Independent of either cobalamin or folate deficiency and refractory to cobalamin and folate therapy:-

A- Some cases of acute myeloid leukaemia, myelodysplasia.

B- **Orotic aciduria** (responds to uridine)

- **Abnormalities of nucleic acid synthesis.**
- Myelodysplastic syndromes "Some patients show normoblastic erythropoiesis", erythroleukemia Some congenital dyserythropoietic anaemias.

❖ Vitamin B12 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	200-250µg
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	Conversion of polyglutamates to mono-glutamates by intestinal folate conjugase
Site of absorption	**Terminal ileum**	**Duodenum and jejunum Like iron**

* In adults. Note: Higher during pregnancy and lactation.

General notes:

- It is known that b12 requires intrinsic factor that transports it to the terminal ileum where the absorption of B12 occurs by transcobalamin 2.
- In order to absorb folic acid ,it must be converted from folate polyglutmate to methyl tetrahydrofolate, which then enters the portal circulation.

❖ Causes of Vitamin B12 Deficiency:

- **Inadequate intake**, **Veganism**, lacto vegetarianism (some cases).
- Inadequate secretion of intrinsic factor.
- **Pernicious anaemia.**
- Total or partial **gastrectomy**
- **Malabsorption.(**Crohn's disease, ileal resection, chronic tropical sprue)
- B12 malabsorption with proteinuria (**Imerslund-Grasbeck syndrome**)

❖ Causes of folate deficiency:

- **Inadequate dietary intake.**
- **Malabsorption:** Coeliac disease, jejunal resection, tropical sprue.
- **Increased requirement:**Pregnancy, premature infants, chronic haemolytic anaemias, myelofibrosis, various malignant diseases
- **Increased loss:** Long-term dialysis (THESE PEOPLE COMMONLY HAVE FOLATE DEFICIENCY), congestive heart failure, acute liver disease
- **Complex mechanism:** Anticonvulsant therapy, ethanol abuse "Only some cases with macrocytosis are folate deficient"

❖ PERNICIOUS ANAEMIA:

Severe megaloblastic anaemia due to **autoimmune attack on the gastric mucosa leading to atrophy.**

- More common in elderly female patients. And in Northern European and tends to be in families.
- Majority of the patients had H.pylori Or IDA when they were child then over 60Y-O develope PA .

Pathogenesis:

- The mucosa become thin with plasma cells and lymphoid infiltration of the lamina propria.
- Intestinal metaplasia may occur.
- It maybe associated with autoimmune diseases including the *(autoimmune polyendocrine syndrome)*

Findings:

- Achlorhydria ? "Combination of Vit.b12 and IF need acidic environment" and absent secretion of intrinsic factor (IF).
- **Progressive neuropathy is a common feature .**
- Absent serum vitamin B12 level or almost absent level.
- Raised serum gastrin levels.(an important characteristic of pernicious anemia)
- Helicobacter pylori infection may be the cause which present in younger age as iron deficiency anaemia and in the elderly as pernicious anaemia.
- **Increased incidence of gastric carcinoma** in (2-3%of pernicious anaemia patients). (MCQ!!!)

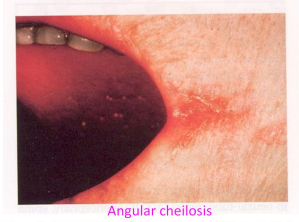
Histology:

- Heavy infiltration of lamina propria with plasma cell & lymphocytes.
- Gastric atrophy in patients with pernicious anemia.

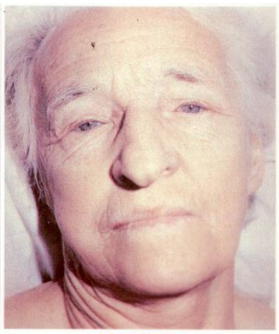
❖ Clinical Features of Megaloblastic Anaemia:

Progressive symptoms and signs of anaemia:

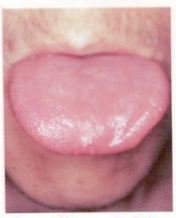
- Weakness, anorexia, weight loss, diarrhoea or constipation, tiredness, shortness of breath, angina of effort, heart failure.



Angular cheilosis
More in Vit B12 deficiency than in folate deficiency



Jaundice



Red swollen painful tongue, with beef-like appearance

- Mild jaundice, glossitis, stomatitis, angular cheilitis.
- Purpura, melanin pigmentations.
- Infections (↓ DNA synthesis causes ↓ WBCs & pancytopenia)

❖ 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) "Mostly weakness in the Lower limbs" associated with Vit.b12 mainly
- **Optic** atrophy.
- **Psychiatric** symptoms. "Most common in folate deficiency"

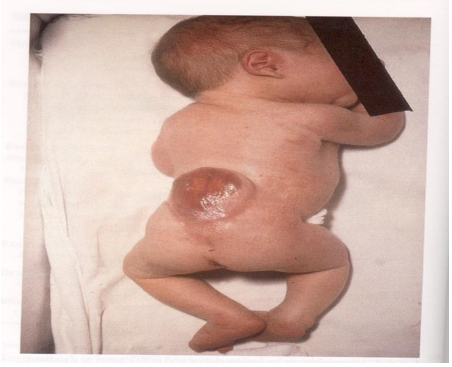
Pathogenesis:

The neuropathy is likely due to accumulation of **S-adenosyl homocysteine** and reduced level of **S-adenosylmethionine** 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.



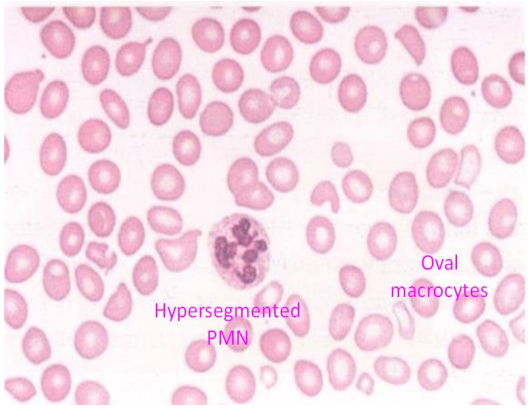
Pathogenesis:

- This result in **buildup of homocysteine and S-adenosyl homocysteine** in the fetus which impair methylation of various proteins and lipids.
- Polymorphism in the enzyme (5,10 mrthylene tetrahydrofolate reductase "5,10-MTHFR)
- Cleft palate and hair lip[1]. Due to high levels of homocysteine.

[1] A **cleft lip**, sometimes referred to as a **harelip**, is an opening in the upper **lip** that can extend into the base of the nostril.

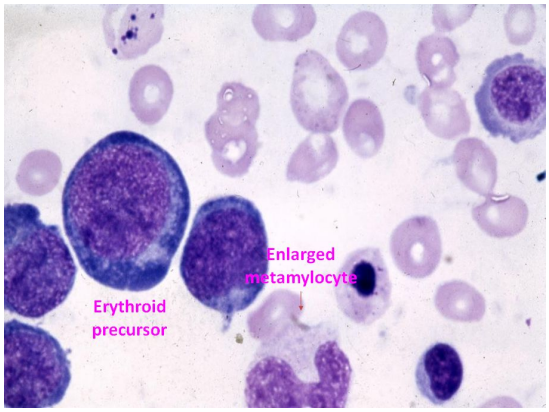
❖ Haematological findings in Megaloblastic Anaemia:

Peripheral Blood:



- Macrocytic anaemia (high MCV), oval macrocytes, anisocytosis ", poikilocytosis.
- *Dimorphic anaemia* when it is associated with **iron deficiency or with thalassaemia trait**.
- Hypersegmented neutrophils.
- Leucopenia and thrombocytopenia

Bone Marrow:



- **Hypercellular marrow (erythroid hyperplasia)** with M:E ratio in normal or reduced.
- Accumulation of primitive cells due to selective death of more mature cells.
- Megaloblast (large erythroblast which has a nucleus of 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:

- Chromosomal abnormalities
- **Ineffective haemopoiesis.** (Intramedullary cell death by apoptosis) associated with increased serum **indirect bilirubin.**
- ↑ **urobillinogen and faecal stercobillinogen.**
- ↑ LDH, ↑ serum iron, ↑ blood carbon monoxide.
- ↑ serum lysozyme
- ↓ reduced haptoglobins
- Positive schumm's test[2]
- Positive urine haemosiderin.

❖ Treatment of megaloblastic anaemia :

	Vitamin B12 deficiency	Folate deficiency
Compound	**Hydroxocobalamin**	**Folic acid**
Route, Dose	Intramuscular, 1000 µg	Oral, 5mg
Initial dose	6X1000 µg over 2-3 weeks	Daily for 4 months
Prophylactic	Total gastrectomy,Ileal resection	Pregnancy, severe haemolytic anaemias, dialysis, prematurity.

-The Dr. focused on this ENTIRE table.

[2] The **Schumm test** *(shoom)* is a blood test that uses spectroscopy to determine significant levels of methemalbumin in the blood. A positive test result occurs when the haptoglobin binding capacity of the blood is saturated, leading to heme released from cell free hemoglobin to bind to albumin. A positive result could indicate intravascular hemolysis.

(Pathoma Bridge) EXTRA YOU CAN SKIP IT!

BASIC PRINCIPLES:

- **Anemia with MCV > 100 µm, most commonly due to folate or vitamin Bl2 deficiency**
- **Folate and vitamin 812 are necessary for synthesis of DNA precursors.**

I. Folate circulates in the serum as methyltetrahydrofolate (methyl THF); removal of the methyl group allows for participation in the synthesis of DNA precursors.
2. Methyl group is transferred to vitamin Bl2 (cobalamin).
3. Vitamin B12 then transfers it to homocysteine, producing methionine.

- **Lack of folate or vitamin Bl 2 impairs synthesis of DNA precursors.**

1. Impaired division and enlargement of RBC precursors leads to megaloblastic anemia.
2. Impaired division of granulocytic precursors leads to hypersegmented neutrophils.
3. Megaloblastic change is also seen in rapidly-dividing (e.g., intestinal) epithelial cells.

- **Other causes of macrocytic anemia (without megaloblastic change) include alcoholism, liver disease, and drugs.**

FOLATE DEFICIENCY

- Dietary folate is obtained from green vegetables and some fruits.
- Absorbed in the jejunum.
- Folate deficiency develops within months, as body stores are minimal.
- Causes include **poor diet** (alcoholics and elderly) + **increased demand** (pregnancy, cancer, and hemolytic anemia)+**folate antagonists** (methotrexate,which inhibits dihydrofolate reductase).
- *Clinical and laboratory findings include:*

l. Macrocytic RBCs and hypersegmented neutrophils.
2. Glossitis
3. ↓ serum folate
4 . ↑serum homocysteine (↑ risk for thrombosis)
5. Normal methylmalonic acid.

VITAMIN B12 DEFICIENCY

- Dietary vitamin Bl2 is complexed to animal-derived proteins.

l. Salivary gland en:qmes (amylase) liberate vitamin Bl2, which is then bound by R-binder (also from the salivary gland) and carried through the stomach.
2. Pancreatic proteases in the duodenum detach vitamin Bl2 from R-binder.
3. Vitamin B12 binds intrinsic factor (made by gastric parietal cells) in the small bowel; the intrinsic factor-vitamin B12 complex is absorbed in the ileum.

- Vitamin Bl2 deficiency is less common than folate deficiency and takes years to develop? due to large hepatic stores of vitamin Bl2.
- Pernicious anemia is the most common cause of vitamin Bl2 deficiency.

(Autoimmune destruction of parietal cells (body of stomach) leads to intrinsic factor deficiency)

- Other causes of vitamin Bl2 deficiency include pancreatic insufficiency and damage to the **terminal** ileum (Crohn disease or Diphyllobothrium latum [fish tapeworm]),dietary deficiency is rare, except in vegans.

- *Clinical and laboratory findings include:*

1. Macrocytic RBCs with hypersegmented neutrophils.
2. Glossitis.
3. Subacute combined degeneration of the spinal cord.

- Vitamin Bl2 is a cofactor for the conversion of methylmalonic acid to succinyl CoA (important in fatty acid metabolism).
- Vitamin Bl2 deficiency results in increased levels of methyl malonic acid, which impairs spinal cord myelinization.
- Damage results in poor proprioception and vibratory sensation (posterior column) and spastic paresis (lateral corticospinal tract).

4. ↓ serum vitamin B12.
5. ↑ serum homocysteine (similar to folate deficiency), which increases risk for thrombosis.
6. ↑ methylmalonic acid (unlike folate deficiency)

(Lecture Notes summary)

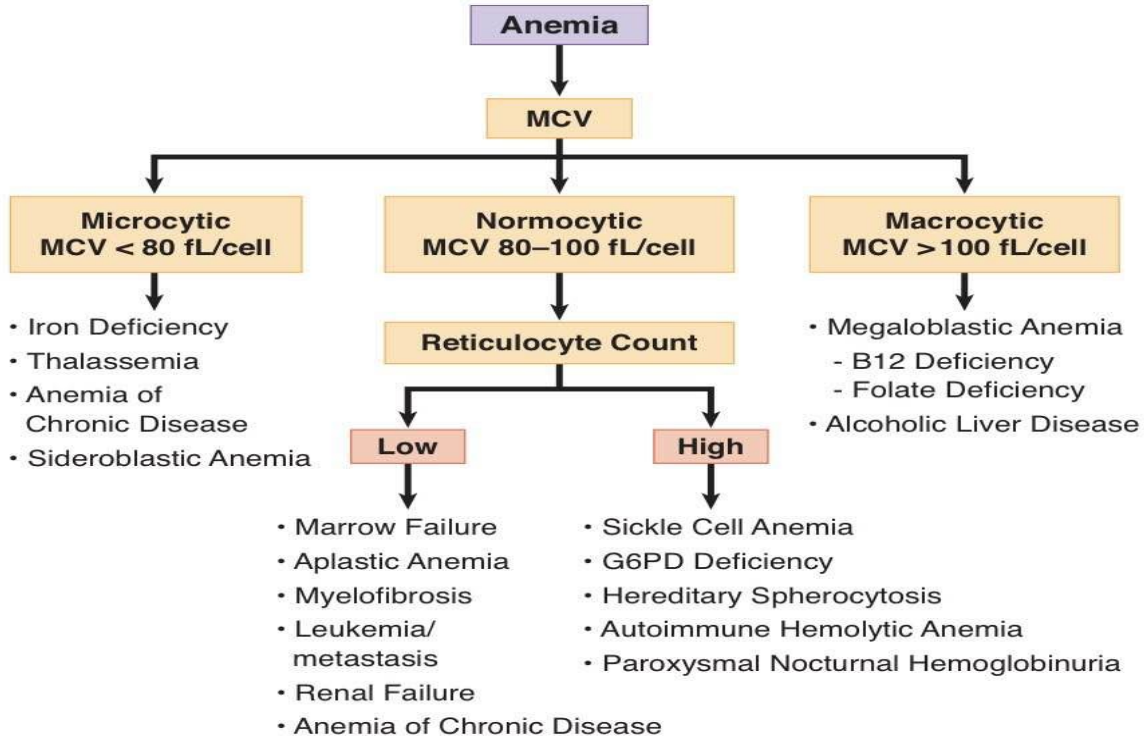


Figure 11-1. Classification of Anemias Based on MCV