MED439
KING SAUD UNIVERSITY

Megaloblastic Anemia

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

Dr's notes
Important
Extra notes
** Only in girls slide
** Only in boys slide

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Overview

In macrocytic anaemia the red cells are abnormally large (MCV >100 fL). There are several causes but they can be broadly subdivided into megaloblastic and non-megaloblastic, based on the appearance of developing erythroblasts in the bone marrow.

Macrocytic Anemia

Megaloblastic Macrocytic Anemia

- Large RBC's as a result of impaired DNA synthesis
- Etiology: **Most commonly as a result of Folate and Vitamin B12 deficiency**
- Impairment in DNA synthesis causes inability of cell replication. RNA and protein synthesis is not affected, so the cell will continue to grow and increase in size.
- Not only RBC's are affected but also leukocytes, megakaryocytes, and the intestinal epithelium
- **Presence** of hypersegmented neutrophils

Non-Megaloblastic (Normoblastic) Macrocytic Anemia

- Large RBC's with no impairment of DNA synthesis. The exact mechanisms creating large red cells in each of these conditions is not clear.
- Many possible causes like **alcoholism, liver disease, and aplastic anemia**
- **Absence** of hypersegmented neutrophils

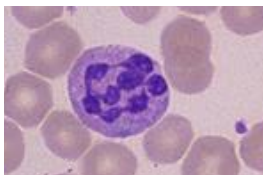
Table 5.10 Causes of macrocytosis other than megaloblastic anaemia.

Alcohol
Liver disease
Myxoedema
Myelodysplastic syndromes Antimetabolite drugs, e.g. hydroxycarbamide
Aplastic anaemia
Pregnancy
Smoking
Reticulocytosis
Myeloma and paraproteinaemia
Neonatal

Macrocytosis: term used to describe red blood cells that are larger than normal.

Hypersegmented neutrophils:

- Neutrophils with more than 5 (normal is 3 - 5 lobes)
- The presence of **hypersegmented neutrophils** is an important diagnostic feature of **megaloblastic anemias**.



Introduction

Normal Adult Red Cell Values

	Male	Females
Haemoglobin (g/L)	135-175	115-155
Haematocrit (PCV) (%)	40-52	36-48
Red cell count (x10^12/L)	4.5-6.5	3.9-5.6
Mean cell haemoglobin (pg)	27-34	
Mean cell volume (FL)	80-95	
Mean cell haemoglobin concentration (g/L)	300-350	
Reticulocyte count (x10^9/L)	25-125(1-2%) Important to distinguish between macrocytosis and megaloblastic anemia	

WBC's * Children have higher lymphocytes count	
Total	4.0 - 11.0 X 10^9/L
Neutrophils	2.5 - 7.5 x 10^9/L
Lymphocytes	1.5 - 3.5 x 10^9/L
Monocytes	0.2 - 0.8 x 10^9/L
Eosinophiles	0.04 - 0.44 x 10^9/L
Basophiles	0.01 - 0.1 x 10^9/L
Platelets	150-450 x 10^9/L

In children normal haemoglobin values are:

Newborn: 150 – 210g/L. 3 months: 95 – 125g/L. 1 year to puberty: 110 – 135g/L

Classification of Anemia

	Hypochromic Microcytic Anemia	**Normocytic Normochromic Anemia**	**Macrocytic Anemia**
MCV	<80 fL	80-95 fL	**>100 fL**
MCH	< 27 pg	>26 pg	>34 pg
Etiology	• Iron def. Anemia • Thalassemia • Anemia of chronic disease(Some cases) • Lead poisoning • Sideroblastic anemia	• After acute blood loss • Renal disease • Anemia of chronic disease (some cases) • Many hemolytic anemias • Mixed deficiencies • Bone marrow failure, e.g. post-chemotherapy, infiltration by carcinoma.	Macrocytic anaemias can be divided into those showing: • Megaloblastic erythropoiesis • Normoblastic erythropoiesis (Explained next slide)

Macrocytic Anemia

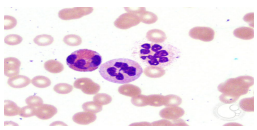
Macrocytic anemias can be divided based on the appearance of developing erythroblasts in the bone marrow into those showing:

Megaloblastic erythropoiesis نمط تكوين كرات دم تأثر من خلالها ال DNA	Normoblastic erythropoiesis نمط تكوين كرات دم طبيعي لم يتأثر ال DNA
• Describes **abnormal** red cell development characterized by a lack of synchrony (Male dr: very important) between the maturation of the red cell nucleus and its cytoplasm. • It arises as a consequence of disordered DNA synthesis and results in a macrocytic megaloblastic anemia.	• Describes the **normal** appearance of red cell maturation (Has no pathogenesis of DNA impairment), but may still be **associated with macrocytosis** in the peripheral blood.

Not all macrocytic anemias are megaloblastoid in nature

Conditions in which Macrocytosis or hypersegmented neutrophils may occur in the <u>absence</u> of megaloblastic anemia

Macrocytosis	Macrocytosis with Normoblasts
• **Alcohol** • **Liver disease (especially alcoholic)** • **Reticulocytosis** (hemolysis or haemorrhage) • **Aplastic anaemia** or red cell aplasia • **Hypothyroidism** • Myelodysplasia including acquired Sideroblastic anaemia • Myeloma and macroglobulinaemia • Leukoerythroblastic anemia • Myeloproliferative disease • Pregnancy • Newborn • Chronic respiratory failure • Renal failure	**Skip the causes** • Chronic alcoholism • Chronic liver disease • Haemolytic anaemia • Hypoplastic and aplastic anaemia • Hypothyroidism • Myelodysplastic syndromes • Myeloma • Normal pregnancy • Normal neonates (Physiological) • Chronic lung disease (with hypoxia) • Therapy with anticonvulsant drugs

Hypersegmented neutrophils	
• Congenital (familial) abnormality • Iron deficiency	 Hypersegmented neutrophils

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

Megaloblastic Anemia

Medicosis Pathoma

Causes

Male's dr: focus in the first 3 causes and the rest is for your reading

- **Cobalamin (Vit B12) deficiency** or abnormalities of cobalamin metabolism
- **Folate deficiency** or abnormalities of folate metabolism
- Therapy with **anit-folate drugs** (e.g. **methotrexate**), interferes with folic acid metabolism
- Independent of either cobalamin or folate deficiency and refractory to cobalamin and folate therapy:
  - Orotic aciduria (responds to uridine)
  - Therapy with drugs interfering with synthesis of DNA (e.g. cytosine arabinoside, hydroxyurea, 6-mercaptopurine, azidothymidine (AZT)
  - Some cases of acute myeloid leukaemia, myelodysplasia
  - Thiamine responsive
- Suggested but poorly documented causes of megaloblastic anaemia not due to cobalamin or folate deficiency or metabolic abnormality: (not read by doctor)
  - Vitamin E deficiency
  - Lesch-Nyhan syndrome (responds to adenine)

Other Causes of Megaloblasts

Male's dr: it's above your level (not imp)

You should know: there are causes of megaloblastic anemia that are NOT associated with vitamin B12 or folate. Therefore they will not respond to treatment with usual treatment of megaloblastic anemia (giving vit b12 or folic acid)

- Abnormalities of nucleic acid synthesis
- Drug therapy:
  - Antipurines (mercaptopurine, azathioprine)
  - Antipyrimidines (fluorouracil, zydovudine (AZT))
  - Others (hydrozyurea)
- Orotic aciduria
- Myelodysplastic syndromes, erythroleukaemia
- Some congenital dyserythropoietic anaemias
- Uncertain aetiology

Megaloblastic Anemia

★ Vitamin B12 and Folate

	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 (Higher during pregnancy and lactation)
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	Anemia in **2-10 years** due to large hepatic stores of Vit B12	Macrocytosis in **5 months**, because body stores are minimal
Requirements for absorption	Intrinsic factor secreted by gastric parietal cells	Conversion of polyglutamates to monoglutamate by intestinal folate conjugase
Site of absorption	**Terminal ileum**	**Duodenum and jejunum**

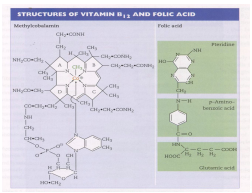
Vitamin Structure & Forms

Vit B12 forms:

- Methylcobalamin: mostly found in blood circulation
- Adenosylcobalamin: main form in tissue
- Hydroxocobalamin: supplement, main form of treatment
- cyanocobalamin: supplement

Folic acid forms:

- Tetrahydrofolic acid (THF): active form
- Methyl THF: primary form found in blood



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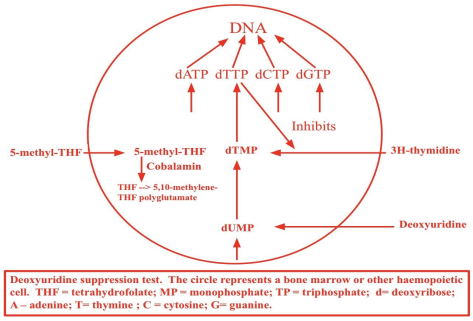
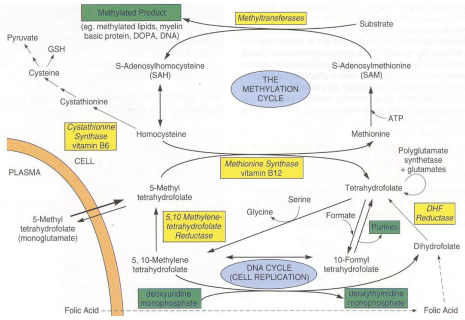
Absorption and metabolism of B12 and Folate



Explained next slide



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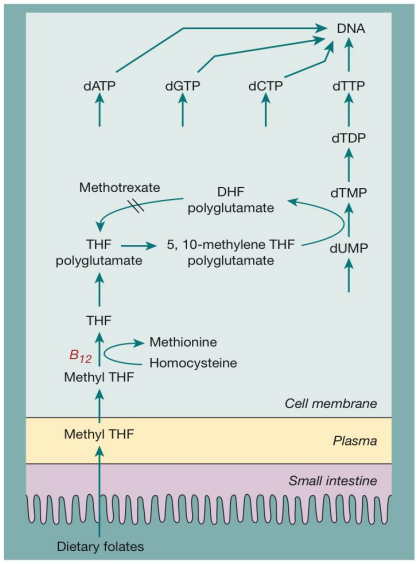
Megaloblastic Anemia

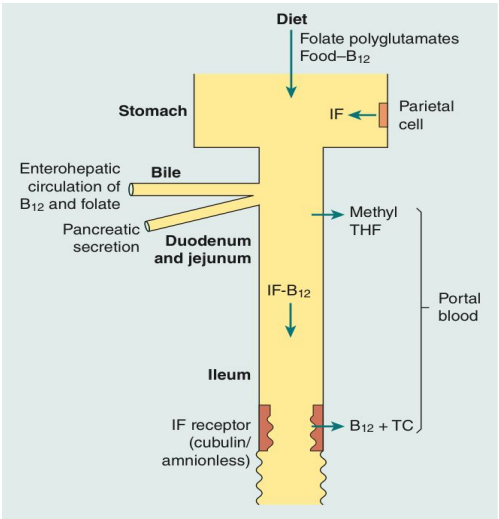
Absorption and metabolism of B12 and Folate

Folic acid

- Dietary **folate is converted to methyl tetrahydrofolate** form which circulates in plasma.
- After entering cells vitamin B12 is needed to convert:
  - **Methyl THF** to **THF** (the active form of folate)
  - Homocysteine to methionine (by transferring a methyl group).
- THF becomes **THF polyglutamate**, then becomes **5,10- methylene THF polyglutamate** which is essential for the formation of DNA building blocks (dTMP: dThymine-Monophospate).
- Once it forms DNA precursor, **5,10- methylene THF polyglutamate** becomes **DHF polyglutamate** which can be reconverted to **THF polyglutamate** again though the **enzyme: dihydrofolate reductase** (**inhibited by methotrexate** which is useful in the treatment of malignant diseases)

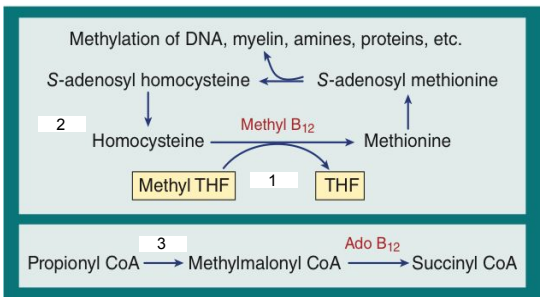


Vitamin B12



- **B12** from the food is combined with the **intrinsic factor** (IF) which is secreted from the stomach by the parietal cells.
- IF–B12 complex reaches **terminal ileum,** then binds in the **ileum** to a specific surface receptor for IF called **cubilin**.
- Cubilin directs endocytosis of the IF–B12 complex in the ileal cell so that B12 is absorbed and IF destroyed
- Vitamin B12 is absorbed into portal blood where it becomes attached to the plasma-binding protein **transcobalamin** (TC, also called transcobalamin II), which delivers B12 to bone marrow and other tissues.

Deficiency in both Folic acid and Vitamin B12 can lead to neural tube defects (discussed later in the lecture)



Vitamin B12 is a coenzyme for these biochemical reactions:

1. Conversion of methyl THF to the active form THF (By taking the methyl group), forming the main form of circulating B12 which is (methylcobalamin)
2. Conversion of Homocysteine (harmful) to methionine (By giving the methyl group).
3. Conversion of methylmalonyl coA to succinyl coA (By acting as a coenzyme (in the form Adenosylcobalamin, which is the main form in tissues)

B12 and Folate Deficiency

Megaloblastic anemia is **usually caused by deficiency of vitamin B12 or folate**. Less commonly, it can be caused by abnormalities of metabolism of these vitamins.
So what are causes their deficiency? **Be familiar with all causes.**

Causes of Vitamin B12 deficiency	Causes of Folate deficiency *Only some cases with macrocytosis are folate deficient

Malabsorption:
• Intestinal causes:
  ○ **Crohn's disease**
  ○ **Ileum resection**
  ○ **Chronic tropical sprue**
  ○ Multiple jejunal diverticula
  ○ Abnormal intestinal bacterial flora
  ○ Small intestinal strictures
• Gastric causes:
  ○ Total or partial gastrectomy
  ○ Gastritis
• Vagotomy
• **Pernicious anaemia**
males' dr: common Q they like to ask
• **Inadequate secretion of intrinsic factor**
	Malabsorption: males' dr skipped it
	• Coeliac disease
	• Jejunal resection
	• Tropical sprue
	
	Increased requirement:
	• Pregnancy
	• Premature infants
	• Chronic haemolytic anaemias
	• Myelofibrosis
	• Various malignant diseases
	
	Increased loss: males' dr skipped it
	• Long-term dialysis
	• congestive heart failure
	• acute liver disease

Nutritional: • Inadequate intake • **Veganism (vegans)**	**Nutritional:** males' dr skipped it • Inadequate intake
Others: • Diphyllobothrium latum • acid-suppressing drugs • **alcohol abuse** • Congenital intrinsic factor deficiency (rare)	**Complex mechanisms:** • Anticonvulsant therapy • ethanol abuse (only some cases with macrocytosis are folate deficient)

B12 and Folate Deficiency

Neuropathy

Neuropathy can manifest due to vit B12 or folate deficiency. It is mostly due vit B12 to deficiency. Severe B12 deficiency can cause a progressive neuropathy affecting: the peripheral sensory nerves, posterior and lateral columns of the spinal cord (subacute combined degeneration of spinal cord). Rarely optic atrophy or psychiatric symptoms are present

Cause of the neuropathy as a result of B12 deficiency is likely to be related to

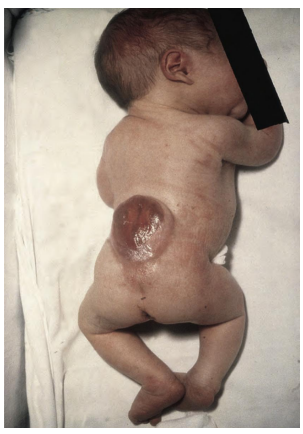
1. **Accumulation of S-adenosyl homocysteine and reduced levels of S-adenosyl methionine in nervous tissue** **males' dr very important**, Leading to defective methylation of myelin and other substrates increasing the risk of cardiovascular disease and thromboembolic events

2. ↑ Methylmalonic acid, **How?**
   - Vitamin B12 a cofactor for the conversion of methylmalonic acid to succinyl CoA (important in fatty resulting in defective acid metabolism).
   - Vitamin B12 deficiency results in increased levels of methylmalonic acid, which impairs spinal cord myelinization.
   - Damage results in a condition known as **subacute combined degeneration of spinal cord**

Neural tube defect (NTD)

Folate or Vit B12 deficiency in the mother predisposes the fetus to neural tube defect (anencephaly, spina bifida or encephalocoele). The lower the maternal serum or red cell folate or serum B12 levels, the higher the incidence of NTDs.

- The exact mechanism is uncertain but is thought to be related to **build-up of homocysteine and S-adenosyl homocysteine** in the fetus which impair methylation of various proteins and lipids.



- A mutation (677C → T) in the enzyme 5,10-methylene tetrahydrofolate reductase (5,10-MTHFR), which reduces 5,10-MTHF to methyl-THF, results in higher serum homocysteine, low serum folate, and low red cell folate in both the parents and fetus with NTD.

- Cleft palate and hair lip are birth defects that may occur due to those vitamin deficiencies.

Pernicious Anemia (PA)

Definition

Severe megaloblastic anemia caused by vit B12 deficiency

Etiology

- **Autoimmune attack** of autoantibodies against intrinsic factor and/ or gastric parietal cells of the gastric mucosa leading to atrophy.
- Helicobacter pylori infection may initiate autoimmune gastritis (PA) which present in:
  - Younger age as iron deficiency anaemia
  - Elderly as pernicious anaemia

Epidemiology

Tends to be in families

More common in elderly female patients than males (1.6:1) at the age of 60 and above

More common in Northern European

Pathophysiology

Male dr: Pernicious anemia is not caused by a problem in the uptake (not a cubilin receptor problem) of vitamin B12, it's caused by a **problem in the secretion of Intrinsic factor. Q: Is pernicious anemia caused by a defective problem in the uptake of vitamin b12? The answer is no.** It's a problem in secretion of intrinsic factor

- The mucosa become thin with plasma cells and lymphoid infiltration of the lamina propria.
- Intestinal metaplasia may occur, Increased incidence of gastric carcinoma in (2-3% of pernicious anaemia patients).
- It may be associated with autoimmune diseases including the autoimmune poly-endocrine syndrome.
- Achlorhydria is present and **absent secretion of intrinsic factor (IF)**

Findings

Absent serum vitamin B12 level or almost absent level

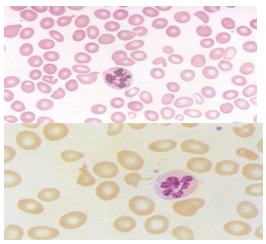
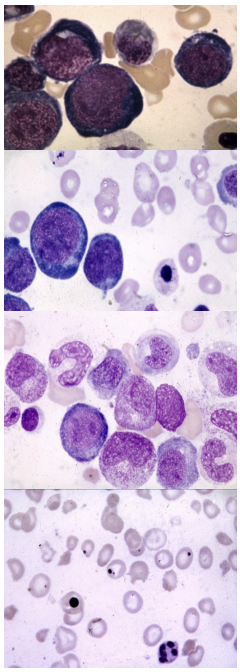
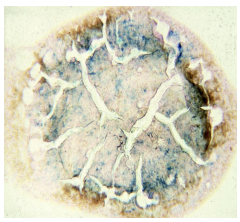
males' dr: it is the main finding

Raised serum gastrin levels

Progressive neuropathy is common

Megaloblastic Anemia

Hematological Findings

Tissue	Findings	Microscope
Peripheral Blood	1. Macrocytic anaemia, oval macrocytes, anisocytosis, poikilocytosis, high MCV. 2. Dimorphic anemia when it is associated with iron deficiency or with thalassaemia trait. **3. Hypersegmented neutrophils** **4. Leukopenia and thrombocytopenia** **Pancytopenia is associated with megaloblastic anemia -** reduction of all lineages of blood cells due to ineffective erythropoiesis	
Bone Marrow	1. **Hypercellular marrow** with M:E ratio in normal or reduced due to inc. erythroid production. 2. **Accumulation of primitive cells** due to selective death of more mature cells. 3. Megaloblast (large erythroblast which has a nucleus of open, fine, lacy chromatin). 4. Dissociation between the nuclear and cytoplasmic development in the erythroblasts. 5. Mitosis and dying cells are more frequent than normal due to ineffective hematopoiesis 6. **Giant and abnormally shaped, metamyelocytes, polypoid megakaryocytes**. 7. Increased stainable iron in the macrophage and in the erythroblasts.	
Other laboratory abnormalities **males' dr : Just read it**	1. Chromosomal abnormalities 2. **Ineffective hematopoiesis** (intramedullary cell death by apoptosis) associated with increased serum indirect bilirubin. 3. **↑LDH,** lactate dehydrogenase is an abundant enzyme is in red blood cells and functions as a marker for hemolysis (or turn-over of cells) 4. **↓ haptoglobins,** large amounts bind to free hemoglobin causing reduced serum level (marker of hemolysis) **5. ↑serum iron, ↑blood carbon monoxide** **6. ↑serum lysozyme** **7. ↑urobillinogen and faecal stercobillinogen** 8. Positive schumm's test 9. Positive urine hemosiderin	

Megaloblastic Anemia

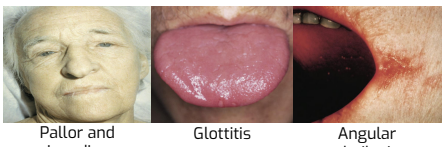
Clinical features

Progressive signs and symptoms of anemia:

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

Purpura, melanin pigmentations.

Mild **jaundice**, **glossitis**, stomatitis, **angular cheilosis**



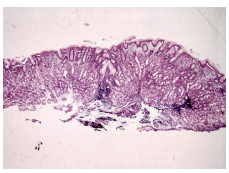
Pallor and jaundice | Glottitis | Angular cheilosis

Infections,
susceptibility due to ineffective erythropoiesis

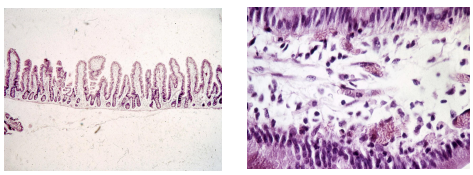
Treatment

In case of -	1) Vitamin B12 Deficiency	2) Folate Deficiency
Compound	Hydroxocobalamin	Folic acid
Route	Intramuscular	Oral
Dose	1000µg	5mg
Initial dose	6X1000 µg over 2-3 weeks	Daily for 4 months
Maintenance	1000µg 3 months	Depends on underlying disease; life-long therapy may be needed in chronic inherited hemolytic anemia, dialysis, myelofibrosis
Prophylactic Who should be given prophylactic treatment? Those with the following diseases	Total gastrectomy Ileal resection	Pregnancy, severe hemolytic anemias, dialysis, prematurity

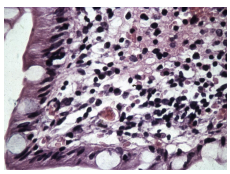
Histopathology



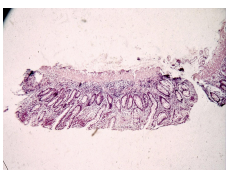
Normal Gastric mucosa



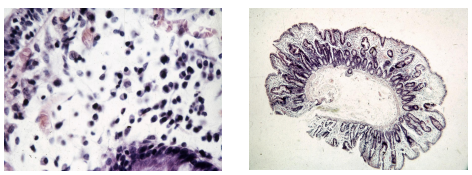
Normal small intestinal mucosa



Heavy infiltration of lamina propria with plasma cells and lymphocytes in patients with pernicious Anemia



Gastric atrophy in patients with pernicious Anemia



Histopathology of small intestinal mucosa in malabsorption syndrome

Summary

- Megaloblastic erythropoiesis describes abnormal red cell development characterized by a lack of synchrony between the maturation of the red cell nucleus and its cytoplasm. It arises as a consequence of disordered DNA synthesis and results in a macrocytic megaloblastic anemia.
- It can be caused by vitamin B12 or folate deficiency or antifolate drugs (ex. methotrexate)
- Clinical features include: jaundice, **glottitis**, **angular cheilosis**, purpura

	Vitamin B12 Deficiency	Folate Deficiency
Found in	Only food of animal origin	green vegetable and yeast
Time to develop deficiency in the absence of intake or absorption	Anemia in 2-10 years	Macrocytosis in 5 months.
Absorption	Requires **intrinsic factor** secreted by gastric parietal cells, Cubilin, transcobalamin II. TC II carries Vit B12 to portal blood	Conversion of polyglutamates to monoglutamates by intestinal folate conjugase
Site of absorption	**Terminal ileum**	**Duodenum and jejunum**
Cause of deficiency	(Malabsorption) • Total or partial gastrectomy • Inadequate secretion of intrinsic factor • **Pernicious anaemia** (Nutritional) • Veganism (Others) • Alcohol abuse	• Pregnancy • Infants • Inadequate dietary intake • Malabsorption • Increased requirement
Manifestation of deficiency	**Neuropathy** can manifest due to vit B12 or folate deficiency. Cause of the neuropathy as a result of **B12 deficiency** is likely to be related to **Accumulation of S-adenosyl homocysteine and reduced levels of S-adenosyl methionine in nervous tissue.**	
Hematological findings in peripheral blood	• **Macrocytic** anemia, oval macrocytes, anisocytosis,Poikilocytosis • Hypersegmented neutrophils, leukopenia, and thrombocytopenia	
Hematological findings in bone marrow	• Giant and abnormally shaped, metamyelocytes, polyploid megakaryocytes • **Megaloblast** (large erythroblast which has a nucleus of open, fine, lacy chromatin). • Increased stainable iron in the macrophage and in the erythroblasts. • **Hypercellular marrow** with M:E ratio in normal or reduced. • **Accumulation of primitive cells** due to selective death of more mature cells.	
Treatment	Hydroxocobalamin, IM	Folic acid, oral

Pernicious Anemia	**Q: Is pernicious anemia caused by a defective problem in the uptake of vitB12? No. It's a problem in secretion of intrinsic factor** • Severe megaloblastic anaemia due to **autoimmune attack** on the gastric mucosa leading to atrophy and **absence of intrinsic factor (IF) secretion** • **Absent serum vitamin B12** • Raised serum Gastrin

Quiz

Q1) Macrocytosis with normoblastic cells in absent of megaloblastic anemia:							
A	Myelodysplastic syndromes (MDS)	B	Sideroblastic anemia	C	Hemolytic anemia	D	Pernicious anemia
Q2) Accumulation of ….. will lead to spina bifida due to vit B12 and folate deficiency:							
A	S-Adenosyl cysteine	B	S-Adenosyl cobalamin	C	S-Adenosyl homocysteine	D	S-adenosyl methionine
Q3) In pernicious anemia there is <u>increased</u> level of:							
A	IF	B	HCl	C	Vitamin B12	D	Gastrin
Q4) Neuropathy due to Vit B12 and folate deficiency is most likely due to:							
A	↑S-adenosyl homocysteine and ↓S-adenosyl methionine	B	↓S-adenosyl homocysteine and ↑S-adenosyl methionine	C	↑S-adenosyl methionine only	D	↓S-adenosyl homocysteine only
Q5) One of the Hematological features in megalblastic anaemia is:							
A	Hypersegmented neutrophils	B	Hypercellular marrow	C	Accumulation of primitive cells	D	All of them
Q6) Which one of the following, when deficient, takes a longer time for the patient to develop megaloblastic anemia?							
A	Vitamin B12	B	Vitamin A	C	Vitamin C	D	Folate
Q7) Which of the following is the site of absorption for folate?							
A	Jejunum	B	Terminal Ileum	C	Duodenum	D	Duodenum and jejunum
Q8) which of the following is NOT a hematological finding of megaloblastic anemia?							
A	Hypersegmented neutrophils	B	Leukopenia	C	Thrombocytopenia	D	Golf-ball appearance upon supravital staining
Q9) Pernicious anemia will cause megaloblastic anemia due to:							
A	Autoimmune attack on SMCs	B	Accumulation of folate	C	Autoimmune attack on gastric mucosa	D	Accumulation of vit B12

Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9
A	C	D	A	D	A	D	D	C

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