Hematology

438 teamwork

Megaloblastic anemia

this video would help you to understand the lecture

Color index:
Red: Important
Gray: notes
Blue: extra

Editing file

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

Normal Values

NORMAL WHITE CELLS (WBC) COUNT IN ADULTS	
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
Eosinophils	0.04 - 0.44 x 10^9/L
Basophil	0.01 - 0.1 x 10^9/L
Platelets	150-450 x 10^9/L

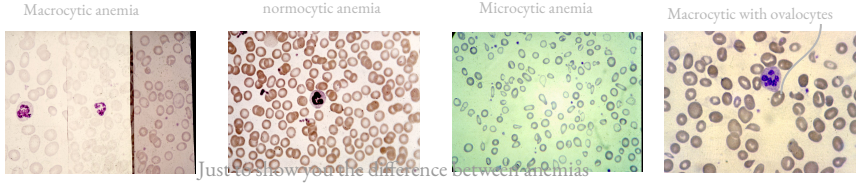
* Children have higher lymphocytes count

Normal adult red cell values		
	Male	Female
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)[1]	80 – 95	
Mean cell haemoglobin concentration (g/L)	300 – 350	
Reticulocyte count (x10^9/L)	25 – 125 (1.0 – 2%)	

*In children normal haemoglobin values are:
newborn (150–210g/L) , 3 months (95–125g/L) , 1 year to puberty (110–135g/L)
*PCV: packed cell volume.

Classification of Anemia

Microcytic, Hypochromic Anaemia	Normocytic, Normochromic Anaemia	MACROCYTIC ANAEMIA [2]
- MCV< 80 fL - MCH<27pg - Iron deficiency - Thalassaemia - Anaemia of chronic disease (some cases) - Lead poisoning such as sidroblastic anemia. - Sideroblastic anaemia (some cases)	- MCV 80 – 95 fL - MCH >26pg - Many haemolytic anaemias increased reticulocytes. - Anaemia of chronic disease (some cases) - After acute blood loss - Renal disease due to decrease in erythropoietin. - Mixed deficiencies high RDW. - Bone marrow failure, e.g. post-chemotherapy, infiltration by carcinoma, etc.	**1.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 macrocytic anaemia. **2.Normoblastic erythropoiesis** describes the normal appearance of red cell maturation - but may still be associated with a macrocytosis in the peripheral blood.



Just to show you the difference between anemias

Conditions in which Macrocytosis or hypersegmented neutrophils may occur in the absence of megaloblastic anaemia

Macrocytosis	Macrocytosis with Normoblasts	Hypersegmented Neutrophils
→ Alcohol → Liver disease (especially alcoholic)	→ Normal neonates (Physiological) → Chronic alcoholism	→ Congenital (familial) abnormality
→ Reticulocytosis (haemolysis or haemorrhage) → Aplastic anaemia or red cell aplasia	→ Myelodysplastic syndromes (MDS) → Chronic liver disease	
→ Hypothyroidism → Myelodysplasia including acquired Sideroblastic anaemia	→ Hypothyroidism	
→ myeloma and macroglobulinaemia	→ Normal pregnancy	
→ Leucoerythroblastic anaemia	→ Therapy with anticonvulsant drugs	
→ Myeloproliferative disease	→ Haemolytic anaemia	
→ Pregnancy[3]	→ Chronic lung disease (with hypoxia)	→ Iron deficiency
→ Newborn[3]	→ Hypoplastic and aplastic anaemia	
→ Chronic respiratory failure	→ Myeloma	

This slide is confusing but know that Non megaloblastic macrocytic anemia is caused by: 1-pregnancy 2-neonatal 3-reticulocytosis 4-alcohol 5-smoking 6-hypothyroidism 7-liver disease 8-some hematological conditions like: MDS, aplastic anemia and multiple myeloma

Causes of megaloblastic anaemia :

Cobalamin [4] deficiency or abnormalities of cobalamin metabolism

Folate deficiency or abnormalities of folate metabolism

causes of Folate deficiency (poor diet (e.g.,Alcoholics,elderly), increased demand(e.g.,pregnancy,cancer), folate antagonists drugs)

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)
c. Therapy with drugs interfering with synthesis of DNA (e.g. cytosine arabinoside, hydroxyurea[5], 6-mercaptopurine, azidothymidine (AZT)
d. Thiamine responsive

Therapy with **anti folate drugs** (e.g. **methotrexate** [5])

Suggested but poorly documented causes of megaloblastic anaemia not due to cobalamin or folate deficiency or metabolic abnormality:

a. Vitamin E deficiency
b. Lesch-Nyhan syndrome (responds to adenine)

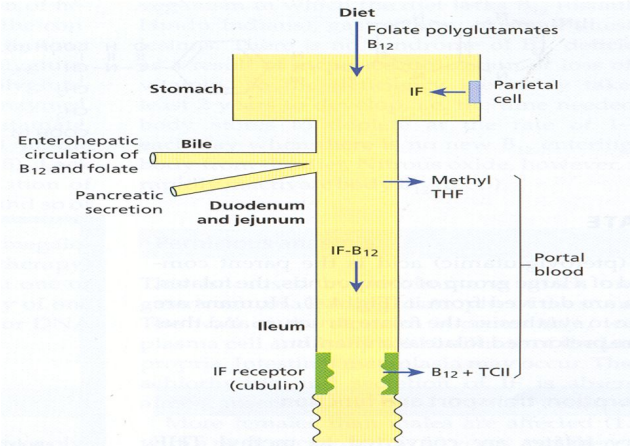
Other causes of megaloblastic anaemia :

- Abnormalities of nucleic acid synthesis
- Drug therapy
  - a. Antipurines (mercaptopurine, azathioprine)
  - b. Antipyrimidines (fluorouracil, zydovudine (AZT))
  - c. Others (hydrozyurea)
- Orotic aciduria (Inability to convert orotic acid to UMP (de novo pyrimidine synthesis pathway) because of defect in UMP synthase. Autosomal recessive)
- Uncertain aetiology (Myelodysplastic syndromes, erythroleukemia)
- Some congenital dyserythropoietic anaemias

Vitamin B_{12} and folate nutrition and absorption

	Vitamin B_{12}	Folate
Dietary source	Only food of animal origin, especially liver	Most foods, especially liver, green vegetable and yeast; destroyed by cooking
Average daily intake (in adults)	7 - 30 µg	200-250 µg
Minimum daily requirement (in adults)	1-3 µg	100-200 µg (higher during pregnancy & lactation)
Body stores (in adults)	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* (in adults)[6]	Anaemia in 2-10 years → Pathoma: Due to large hepatic stores of vitamin B12	Macrocytosis in 5 months.
Requirements for absorption	Intrinsic factor secreted by gastric parietal cells	Conversion of polyglutamates to monoglutamates by intestinal folate conjugase
Site of absorption	Terminal ileum	**Duodenum and jejunum**

Normal Sequence of B12 Absorption



➔ Essential Hematology

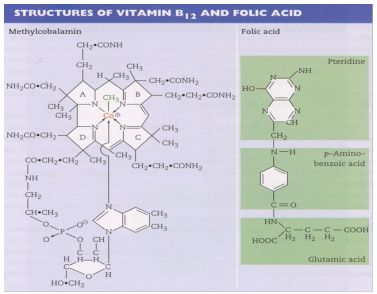
Absorption & transport of B12

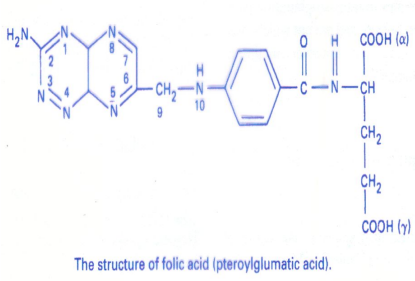
- ➔ A normal diet contains B12
- ➔ B12 is released from protein - binding in food and is combined with the glycoprotein intrinsic factor (IF) which is synthesized by the gastric parietal cells.
- ➔ The IF – B12 complex can then bind to a specific surface receptor for IF, **cubilin**, which then binds to a second protein, amnionless, which directs endocytosis of the cubilin IF – B 12 complex in the distal ileum where B12 is absorbed and IF destroyed.
- ➔ Vitamin B12 is absorbed into portal blood where it becomes attached to the plasma - binding protein transcobalamin (TC, previously called transcobalamin II) which delivers B 12 to bone marrow and other tissues.

Structure of vitamin B12 and folic acid

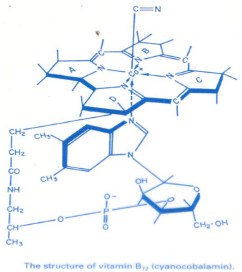
Vit B12 forms:-methylcobalamine: mostly found in blood circulation.-adenosylcobalamin: major storage form in liver.-Hydroxycobalamine (supplement).-cyanocobalamine (supplement).

Folic acid (Vit B12) forms:-tetrahydrofolic acid (the active form). -methyltetrahydrofolate (the primary form found in blood).

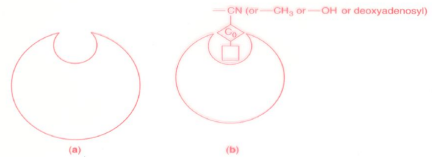




The structure of folic acid (pteroylglumatic acid).



The structure of vitamin B12 (cyanocobalamin).



(a) Intrinsic factor and (b) intrinsic factor–cobalamin complex. Intrinsic factor has been estimated to have a molecular radius of 3.6 nm, vitamin B12 0.8 nm, and the complex 3.2 nm.

Causes of Deficiency Vitamin B_{12} and folate

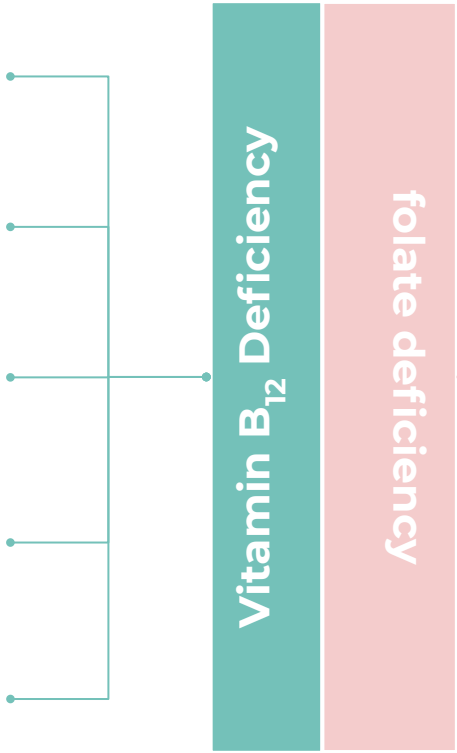
1/ Inadequate intake
2/ Veganism
3/ Inadequate secretion of intrinsic factor

4/ Pernicious anaemia
5/ Total or partial gastrectomy

6/ Congenital intrinsic factor deficiency (rare)
7/Partial gastrectomy, vagotomy, gastritis, acid-suppressing drugs, alcohol abuse

8/ Abnormal intestinal bacterial flora, multiple jejunal diverticula, small intestinal strictures
9/ Diphyllobothrium latum

10/ Malabsorption
11/ Crohn's disease, ileal resection, chronic tropical sprue



1/ Inadequate dietary intake
2/ Malabsorption
(Coeliac disease, jejunal resection, tropical sprue)

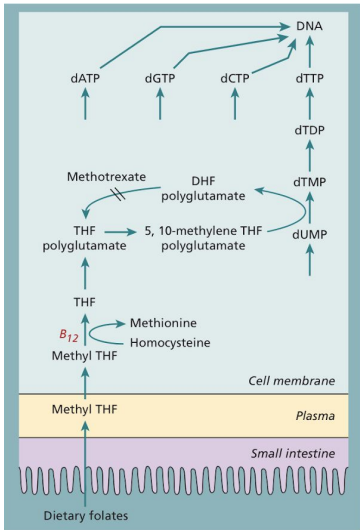
3/ Increased requirement
(Pregnancy, premature infants, chronic haemolytic anaemias, myelofibrosis, various malignant diseases)
.

4/ Increased loss
(Long-term dialysis, congestive heart failure, acute liver disease)

5/ Complex mechanism
(Anticonvulsant therapy, * ethanol abuse) *

* Only some cases with macrocytosis are folate deficient.

Folic acid & Vitamin B12 Metabolism

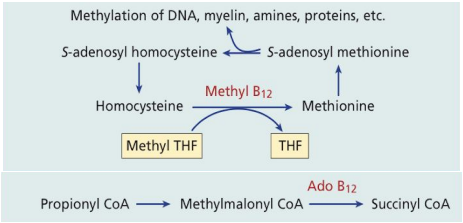


A, adenine; C, cytosine; d, deoxyribose; DHF, dihydrofolate; DP, diphosphate; G, guanine; MP, monophosphate; T, thymine; TP, triphosphate; U, uracil.

-**Folic acid circulates in plasma in the form of methyltetrahydrofolate after the dietary intake.**
-when it goes to cell membrane in order to function it needs to be converted to tetrahydrofolate (THF) by methylation of vit B12.
-then vit B12 gives the methyl group to homocysteine (which is harmful) to become methionine (not harmful).
-THF becomes THF polyglutamate then becomes **5,10- methylene THF polyglutamate** which is essential for the formation of DNA precursors mainly (**thymin**).
-when methylene THF polyglutamate forms the DNA precursor it becomes DHF polyglutamate which has the ability to form THF polyglutamate again.
- as we mentioned antifolate drugs (methotrexate) it will inhibit the conversion of DHF polyglutamate to THF polyglutamate.

→ **Essential Hematology**

Folates are needed in a variety of biochemical reactions in the body involving:
1- Amino acid interconversions (e.g. homocysteine conversion to methionine) and serine to glycine.
2- synthesis of purine precursors of DNA.



Ado B 12 , deoxyadenosylcobalamin; CoA, coenzyme A; THF, tetrahydrofolate.

Vit B12 is important for these 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} for methyl transferase enzyme).

PERNICIOUS ANAEMIA

- Severe megaloblastic anaemia due to autoimmune attack on the gastric mucosa leading to atrophy.
- **More common in elderly female patients** than males (1.6:1) at the age of 60 and above
- More common in Northern European and tends to be in families
- **The mucosa become thin with plasma cells and lymphoid infiltration of the lamina propria.**
- Intestinal metaplasia may occur
- 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).
- **It may be associated with autoimmune diseases including the autoimmune polyendocrine syndrome**
- Progressive neuropathy is a common feature
- Achlorhydria and absent secretion of intrinsic factor (IF).
- Raised serum gastrin levels.
- Absent serum vitamin B12 level or almost absent level.

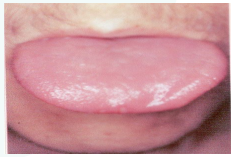
Clinical Features of Megaloblastic Anaemia – Progressive symptoms and signs of anaemia

01:

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

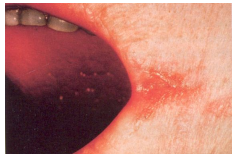
02:

Mild jaundice [8], glossitis, stomatitis, angular cheilosis



03:

Purpura, melanin pigmentation [2]



04:

Infections

Neuropathy due to Vit B12 and folate deficiency

Progressive neuropathy affecting

01 The peripheral sensory nerves [7]

02 Optic atrophy

03 Posterior and lateral columns of the spinal cord (subacute combined degeneration of the cord)

04 Accumulation of S-adenosyl homocysteine and reduced level of S-adenosyl methionine in nervous tissue resulting in defective methylation of myelin and other substrates.

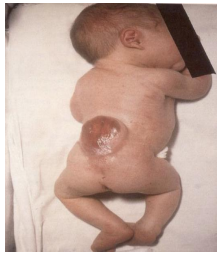
05 Neuropathy is mostly due to vitamin B12 deficiency

06 Psychiatric symptoms

Pathoma

- Vitamin B12 a cofactor for the conversion of methylmalonic acid to succinyl CoA (important in fatty acid metabolism).
- Vitamin B12 deficiency results in increased levels of methylmalonic acid,which impairs spinal cord myelinization.

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 folat and high serum is homocysteine in the parents and fetus with NTD.

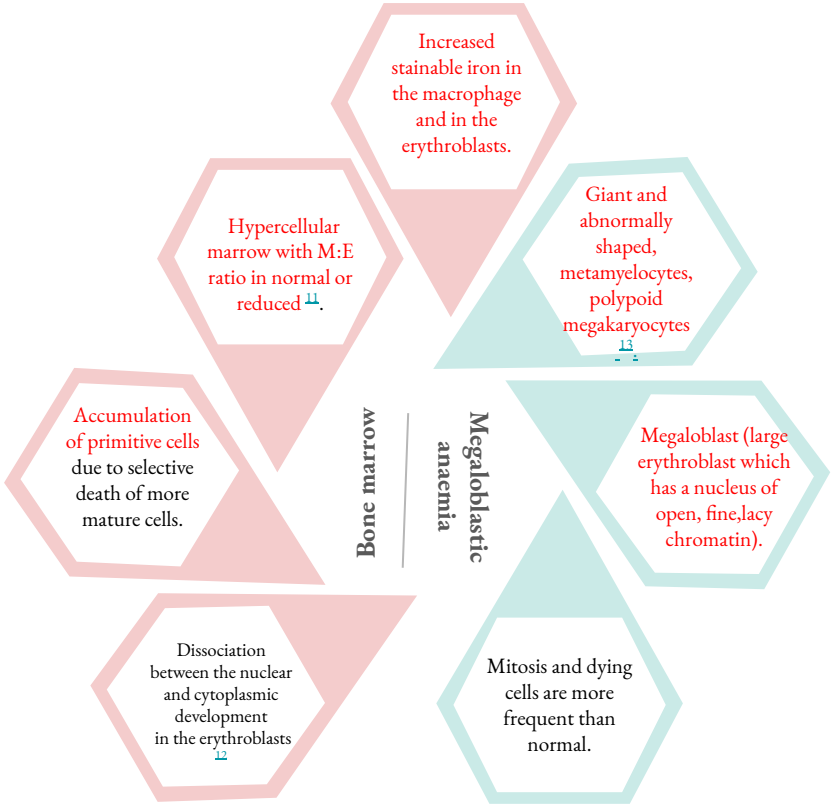
Cleft palate and hair lip.

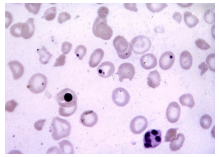
Haematological findings in Megaloblastic Anaemia

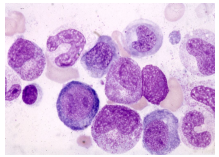
Peripheral Blood:

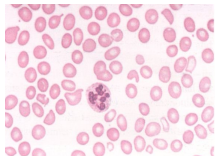
- Macrocytic anaemia, oval macrocytes, anisocytosis, poikilocytosis high MCV.
- Dimorphic anaemia when it is associated with iron deficiency or with thalassaemia trait.
- Leucopenia and thrombocytopenia
- Hypersegmented neutrophils [10].

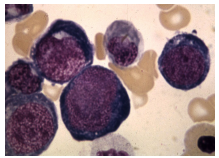
Bone Marrow:

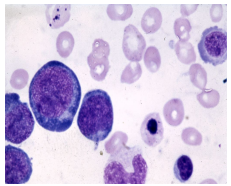


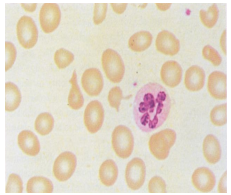












Other laboratory abnormalities

01 Ineffective haemopoiesis. (Intramedullary cell death by apoptosis) associated with increased serum indirect bilirubin.

02 ↑ urobillinogen and faecal stercobillinogen.

03 ↑ LDH[14] ↑ **serum iron** ↑ **blood carbon monoxide.**

04 ↑ serum lysozyme

05 ↓ reduced haptoglobins [15]

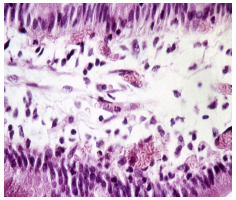
06 Positive urine haemosiderin.

Treatment of megaloblastic anaemia [16]

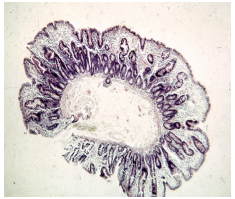
	Vitamin B12 deficiency	Folate deficiency
Compound	Hydroxocobalamin	Folic acid
Route	Intramuscular	Oral
Dose	1000 µg	5mg
Initial dose	6X1000 µg over 2-3 weeks	Daily for 4 months
Maintenance	1000 µg every 3 months	Depends on underlying disease; life-long therapy may be needed in chronic inherited haemolytic anaemia, myelofibrosis, renal dialysis
Prophylactic	Total gastrectomy Ileal resection	Pregnancy, severe haemolytic anaemias, dialysis, prematurity

Histopathology

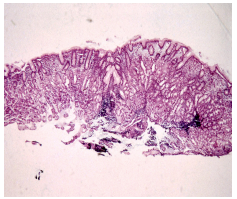
Normal histology of small intestinal mucosa



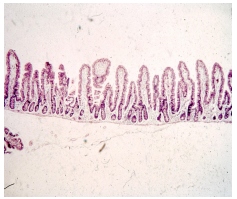
Flattering of small intestinal mucosa in malabsorption syndrome

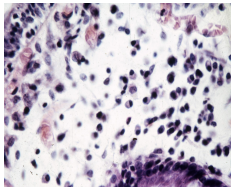


Normal gastric mucosa

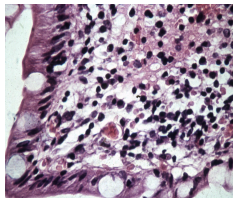


Normal small intestinal mucosa

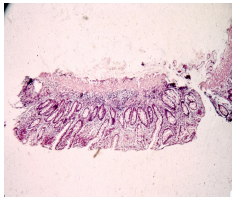




Histopathology of small intestinal mucosa in malabsorption syndrome



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



Gastric atrophy in patients with pernicious Anemia

1-when it's more than 98 you are dealing with macrocytic red cell.
2-**Macrocytic anemia is a type of anemia that causes unusually large red blood cells. Like other types of anemia, macrocytic anemia means that the red blood cells also have low hemoglobin.** it's divided into two main categories Depending on the appearance of the developing erythroblast in bone marrow:
A-megaloblastic: mainly due to problems in absorption or metabolism of **Folate or vit B12 which are very imp in DNA synthesis.**
B-non megaloblastic (normoblastic)
3-these two are physiological conditions.
4- the other name of vit B12.
5-used to treat cancer.
6-remember FOLATE DEFICIENCY DEVELOPS FASTER.
7-mainly lower limbs are affected.
8-There is an increased breakdown of RBC, what we call it ineffective haematopoiesis, like within the bone marrow the bone marrow is producing red cells but its not suitable to do the proper function, so there is an increased turnover which will give you the picture of mild jaundice
9-Folic acid and B12 are associated with some melanin pigmentation.
10- **6 and above lobes** is considered hypersegmented, it could be present in other conditions (not specific). 11-Myeloid : erythroid ratio (reduced duo to increased erythroid production)
12-Cytoplasm is trying to show maturation to attract Hb For more mature RBC, while you can observe the nucleus which is still retaining the open chromatin, it is also typical for reticulocytosis. (normally an RBC have NO nucleus)
13-A metamyelocyte is a cell in the BM undergoing granulopoiesis (we have 3 types of metamyelocytes *neutrophilic*basophilic*eosinophilic).
14-Lactate dehydrogenase is an enzyme, LDH is abundant in red blood cells and can function as a marker for hemolysis.

Dr. Notes

15-Haptoglobin is primarily produced in the liver and is functionally important for binding free hemoglobin from lysed red cells in vivo, preventing its toxic effects. Because haptoglobin levels become depleted in the presence of large amounts of free hemoglobin, decreased haptoglobin is a marker of hemolysis.
16-Prior to treatment make sure which Vit is deficient, cuz sometimes if the patient have vit b12 def and you give him folate it might aggravate the neuropathy associations

-Macrocytes in megaloblastic anemia tend to be oval with associated hypersegmented neutrophils and megaloblastic erythroid progenitors.
-In non-megaloblastic macrocytic anemias the macrocytes are round. There are many possible etiologies, which may be intrinsic to the marrow (e.g. myelodysplasia) or due to extrinsic causes (e.g. liver disease, hypothyroidism, drug therapy).

———————————

1-To differentiate between normocytic normochromic and anemia caused by mixed deficiencies from CBC, we look for RDW which will be very high in anemia of mixed deficiencies but normal in normocytic normochromic anemia
2- Folate deficiency can even cause pancytopenia
3-Folate supplements can cause masking of B12 deficiency and the patient may present later with neuropathy

Quiz

Key answers:
1-B 2-A 3-B 4- C 5-B 6-A

1- which one of the following will cause macrocytic anemia?

A. Folate accumulation
B. Vitamin B12 deficiency
C. Iron deficiency
D. Iron accumulation

2-Macrocytosis with normoblastic cells in absent of megaloblastic anemia; ***(from dr.notes)***

A. Myelodysplastic syndromes (MDS)
B. Newborns
C. Leukoerythroblastic anemia
D. Pernicious anemia

Dr note: could be any condition

3- Pernicious anemia will cause megaloblastic anemia due to;

A. Accumulation of vit B12
B. Autoimmune attack on gastric mucosa
C. Autoimmune attack on SMCs
D. Accumulation of vit folate

4- Accumulation of ..... will lead spina bifida due to vit B12 and folate deficiency ***(from dr.notes)***

A. S-Adenosyl cysteine
B. S-Adenosyl cobalamin
C. S-Adenosyl homocysteine
D. S-adenosyl methionine

5-What is the only source of vitamin B12?

A. Yeast
B. Animal
C. Green vegetables
D. None

6-In pernicious anemia there is increased level of:

A. Gastrin
B. Vitamin B12
C. HCl
D. IF

THANKS

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