
بسم الله الرحمن الرحيم

Megaloblastic Anemia

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DEFINITION

- This is a group of anaemias in which, due to impaired DNA synthesis, the erythroblasts in the bone marrow show a characteristic abnormality – maturation of nucleus being delayed(lags behind) relative to that of cytoplasm resulting in the formation of megaloblast.

DEFINITION (Cont…)

- In megaloblast the nuclear chromatin maintains an open, stippled, lacy appearance despite normal haemoglobin formation in the cytoplasm of the erythroblasts, as they mature
- Two main deficiencies lead to megaloblastic anaemia
  - (i) Folic Acid or Folate deficiency
  - (ii) Vitamin B 12 deficiency

Causes of Macrocytosis (MCV > 95 fl)

Non Megaloblastic

Pregnancy

Neonates

Reticulocytosis

Alcoholism

Liver Disease

Myxodema

Aplastic anemia

Myelodysplastic Syndrome

Megaloblastic

Megaloblastic Anaemia is Characterized by

- Defect in nuclear replication & division
- Affects all marrow elements
- Macrocytosis in the peripheral blood
- Pancytopenia
- Megaloblasts in the bone marrow
- Ineffective hemopoiesis

VITAMIN B 12 AND FOLATE - COMPARISON

	VITAMIN B 12	FOLATE
CONTENTS IN FOOD	Vegetables – Poor Meat – Rich	Vegetables – Rich Meat – Moderate
EFFECT OF COOKING	10 -30 % loss	60 – 90 % loss
SITE OF ABSORPTION	Ileum	Duodenum & Jejunum
NEUROLIGCAL MANIFESTATIONS	An Important feature	Absent
MALNUTRITION	Unusual	Most common cause of Folate deficiency
ONSET	Rapid Onset (Takes weeks)	Slow (Takes years)

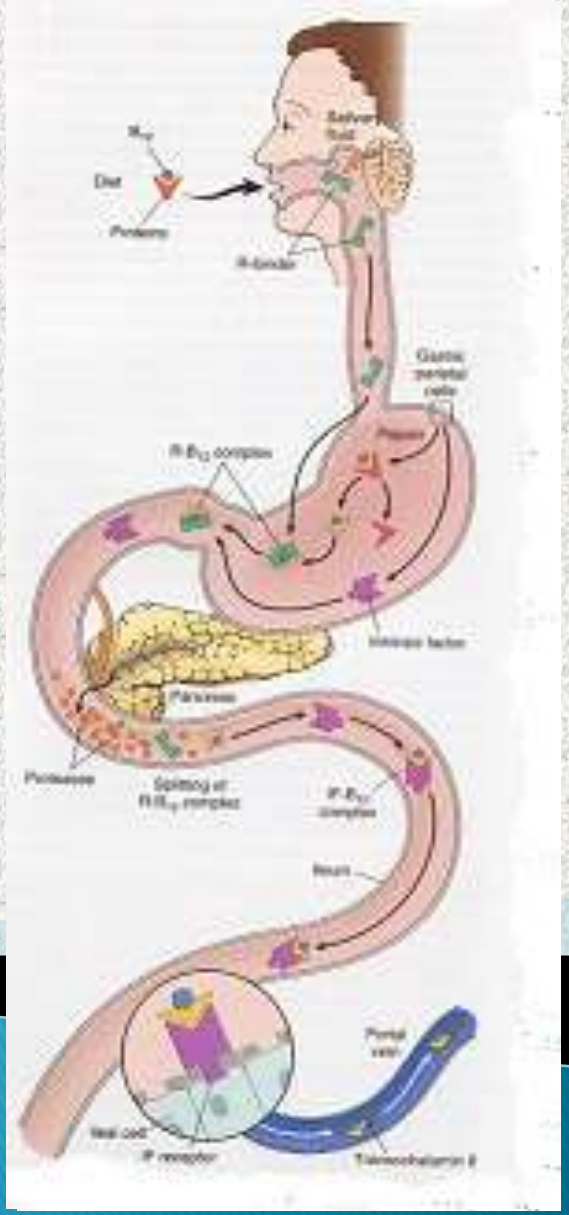
ABSORPTION OF VITAMIN B 12

Absorption of B12 requires Intrinsic Factor (IF), which is secreted by the parietal cells of the fundal mucosa of stomach along with hydrochloric acid.

Initially the vitamin is released from its protein - bound form by the action of pepsin in the acidic environment of the stomach.

The liberated vitamin is then bound to salivary vitamin B12 – binding protein called R- binder.

In the duodenum, R- vitamin B12 complexes are broken down by the action of pancreatic enzymes, and the released vitamin B12 then attaches to IF.



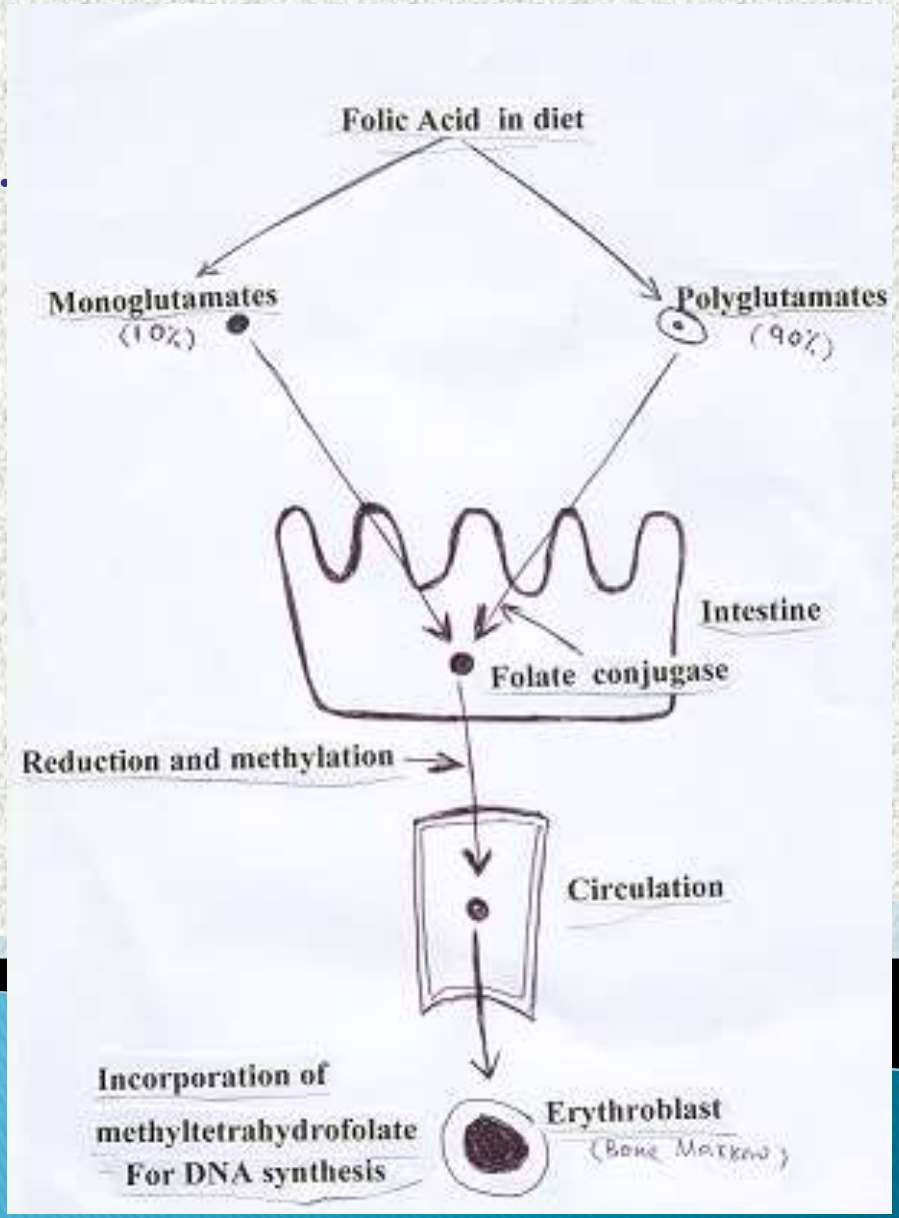
ABSORPTION OF VITAMIN B 12

In this form, IF – B12 complex is then transported to ileum, where it adheres to IF- specific receptors on the ileal cells. Vitamin B12 then transverses the plasma membrane to enter the mucosal cell.

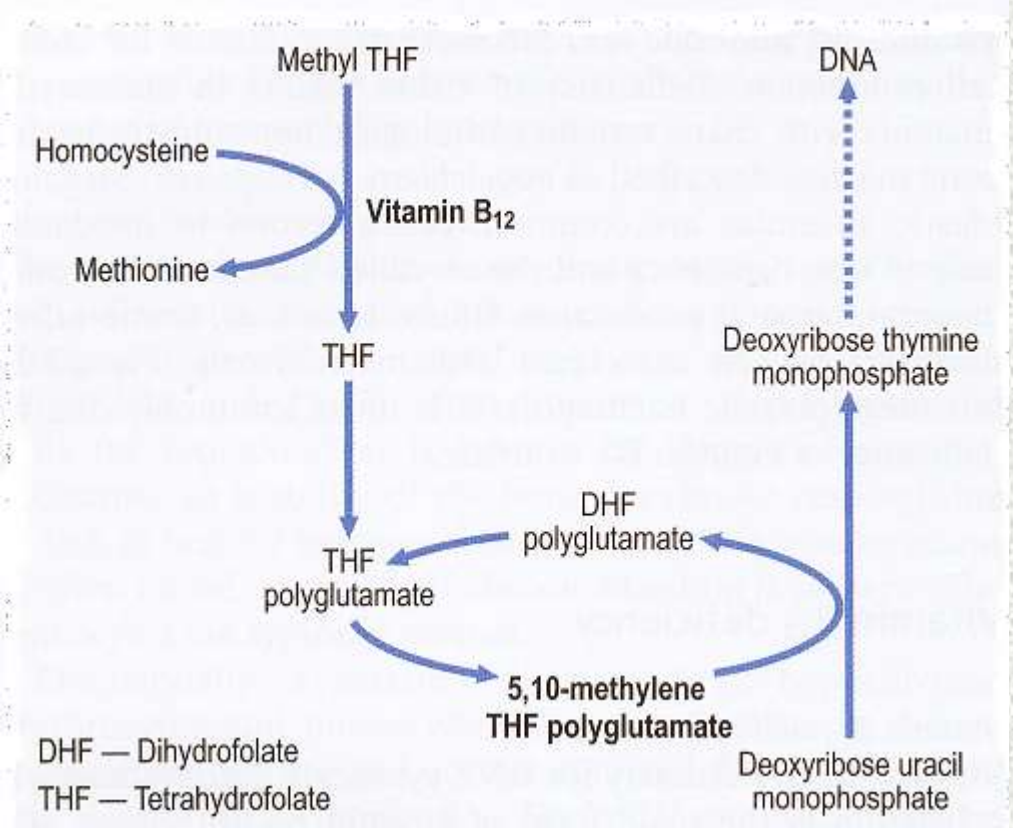
It is picked up from the cell by a plasma protein , Transcobalamin II, which is capable of delivering it to liver and other cells of the body, Particularly cells of the bone marrow where it is incorporated in the nuclei for DNA synthesis.

ABSORPTION OF FOLIC ACID

- Folic acid in the diet is in two form Monoglutamates and Polyglutamates.
- More than 90% is Polyglutamates .
- Intestinal conjugases split Polyglutamates into Monoglutamates that are readily absorbed in the proximal jejunum. During intestinal absorption they are modified so that only 5- methyltetrahydrofolate enters the circulation as the normal transport form of Folate.



PATHOGENESIS OF HOW B12 AND FOLATE DEFICIENCY PRODUCE MEGALOBLASTIC ANAEMIA



Lack of Vitamin B12 or Folate causes slowing of DNA synthesis in developing erythroblasts with an accumulation of cells in premitotic phase of cell cycle. The neutropeina and thrombocytopenia also appears to result from ineffective and abnormal precursor cells in the marrow due to impaired DNA synthesis

CAUSES OF MEGALOBLASTIC ANAEMIA

- Folate Deficiency
- Vitamin B 12 Deficiency
- Def. of both Folate and B 12
- Drugs (Anti folate drugs ,e.g.
- Methotraxate
- Pyramethamine

CAUSES OF VITAMIN B12 DEFICIENCY

1. Decreased intake of Vitamin B12:

Nutritional deficiency (Vegetarians)

2. Impaired absorption of Vitamin B12:

(i) Pernicious Anaemia (Lack of Intrinsic Factor; Antibodies against Intrinsic Factor)

(ii) Gastrectomy(No release of Intrinsic Factor)

3. Intestinal Causes:

(i) Lesions of small intestine

(ii) Coeliac Disease

(iii) Tropical Sprue

(iv) Fish Tapeworm(Diphylobothium Latum) infestation

CAUSES OF FOALTE DEFICIENCY

1. **Decreased intake of Folic Acid:**
   - Nutritional Deficiency

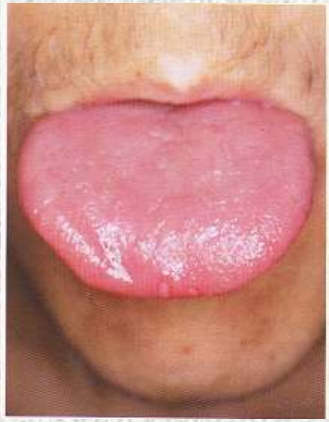
2. **Impaired Absorption of Folic Acid:**
   - (i)Coeliac Disease
   - (ii)Tropical Sprue

3. **Increased demand:**
   - (i) Pregnancy
   - (ii) Haemolytic Anaemias
   - (iii) Myeloproliferative disorders
   - (iv) Carcinoma
   - (v) Inflammatory disorders
   - (vi) Skin diseases

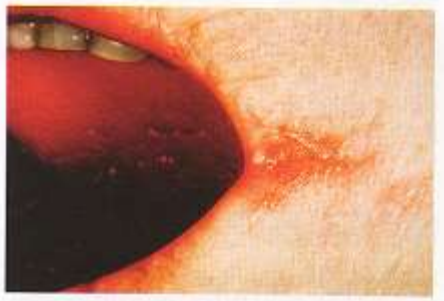
4. **Drugs:**
   - Dihydrofolate reductase inhibitors

CLINICAL FEATURES OF MEGALOBLASTIC ANAEMIA

- The onset is usually insidious with gradually progressive symptoms and signs of anaemia. The patient may be mildly jaundiced (lemon yellow tint) due to the excess breakdown of haemoglobin resulting from ineffective erythropoiesis in the bone marrow
- Glossitis , sore tongue and stomatiitis
- Mild symptoms of malabsorption with loss of weight may be present due to epithelial changes
- Lethargy, breathlessness and other generalized signs and symptoms of anaemia may be present



Glossitis: tongue is beefy red and painful



CLINCAL MANIFESTATIONS DUE TO VITMAIN B 12 DEFICIENCY

Vitamin B12 is required for two important reactions in the body:

Vitamin B 12

(i) Homocysteine → Methionine

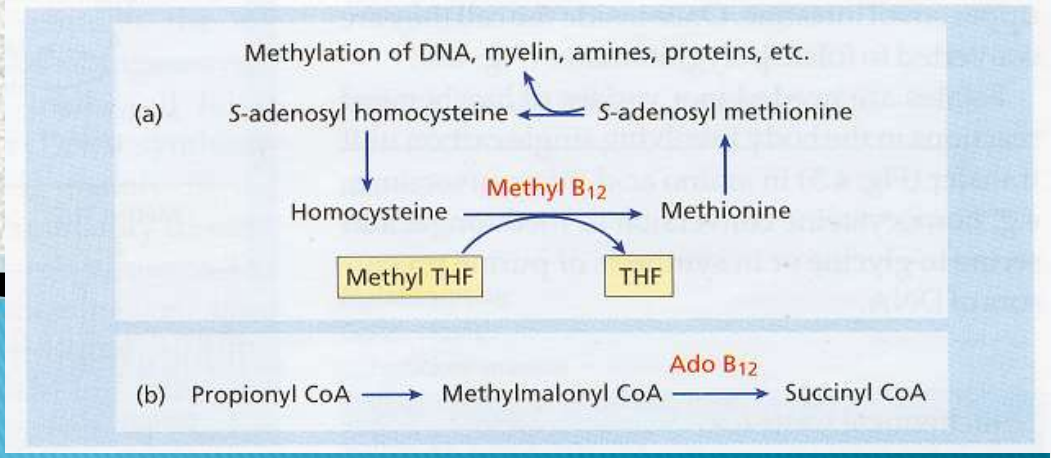
Vitamin B 12

(ii) Methylmelonyl CoA → Succinyl CoA

Absence of B12 then leads to two important consequences:

(i) Impaired methylation leads to defects in myelinatioin of neural tissues

(ii) Accumulation of Homcysteine has toxic effects on cardiovascular tissue and neural tube



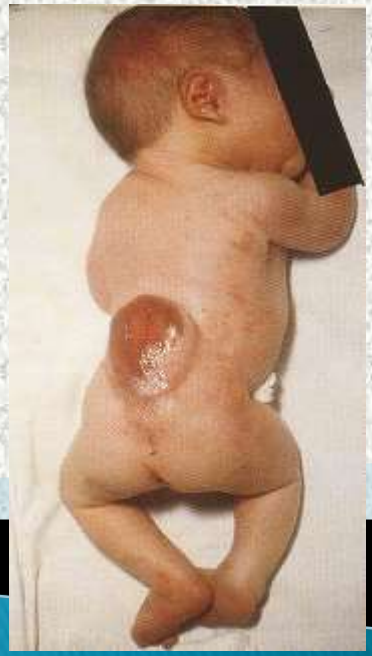
VITMAIN B 12 NEUROPATHY (SUBACUTE COMBINED DEGENERATION OF SPINAL CORD:

- progressive neuropathy- peripheral sensory, and posterior columns
- symmetrical and affects the lower limbs more than the upper limbs.
-Tingling in the feet, difficulty in walking- fall over in the dark.
-Rarely optic atrophy or psychiatric symptoms
- (Megaloblastic Madness)

Demyelination of the dorsal and dorsolateral columns

NUERAL TUBE DEFECT:

The accumulated Homocysteine -toxic substance – damage to neural tissue.
Supplementation of maternal diet with folic acid during Pregnancy reduces the incidence of neural tube defect By 75%



A baby with neural tube defect (spina bifida)

CARDIOVASCULAR DAMAGE:

Raised Homocysteine damages cardiac and peripheral and cerebral vascular tissue leading to myocardial Infarction, peripheral and cerebral vascular disease and venous thrombosis

Pernicious anemia

- Autoimmune Disease
- Anti-Parietal cell Abs: Gastric atrophy leading to achlorhydria and lack of IF
- Anti-IF Abs
  - Binding Abs
  - Blocking Abs

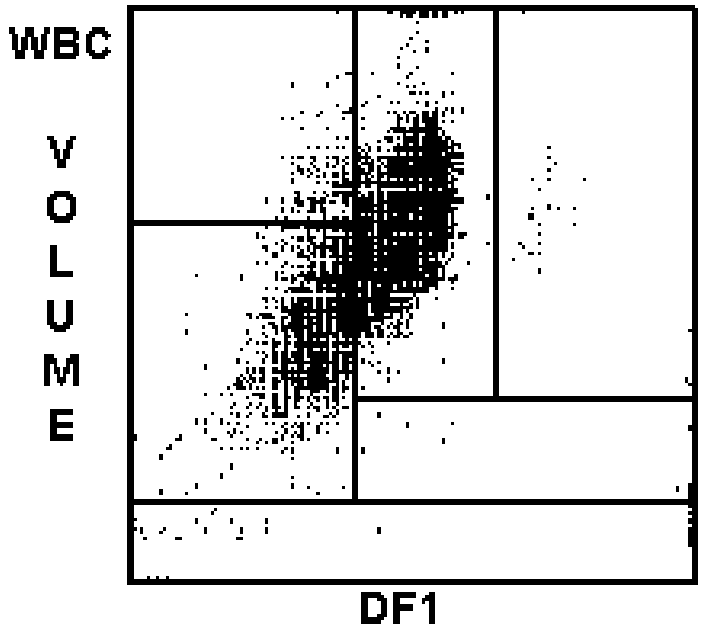
Manifestations

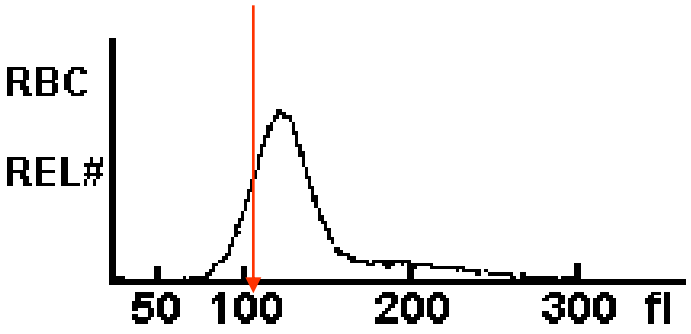
- Pancytopenia **(Ineffective Hemopoiesis)**
- Pallor
- Infections
- Mild jaundice (Icteric)
  (Ineffective Erythropoiesis)
- Bleeding tendency
- Features of underlying conditions (Diarrhea etc)

Lab Diagnosis

- Hb, RBC, PCV decreased
- TLC decreased (Neutrophils)
- Platelets decreased
- MCV increased
- MCH, MCHC normal
- Retics decreased

Macrocytic Anemia (Meg.):

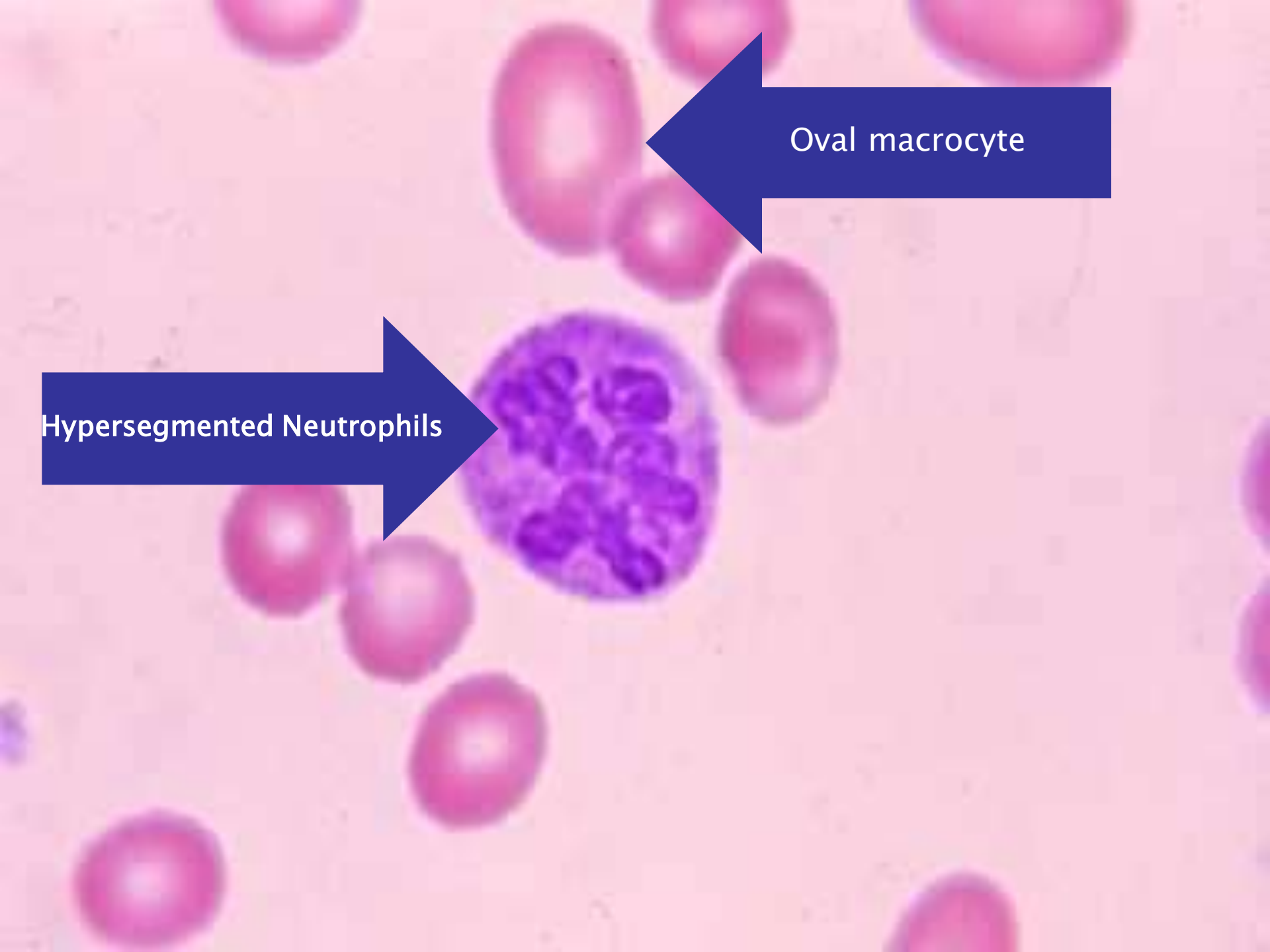




WBC	12.1	H			
	%			#	
NE	71.1		H	8.5	H
LY	15.9		L	1.9	
MO	3.3			0.5	
EO	0.5		L	0.1	
BA	8.7		H	1.1	H
RBC	2.69	L			
HGB	10.6	L			
HCT	31.6	L			
MCV	117.6	H			
MCH	39.6	H			
MCHC	33.7				
RDW	14.1				
PLT	578	H			
MPV	7.2	L			

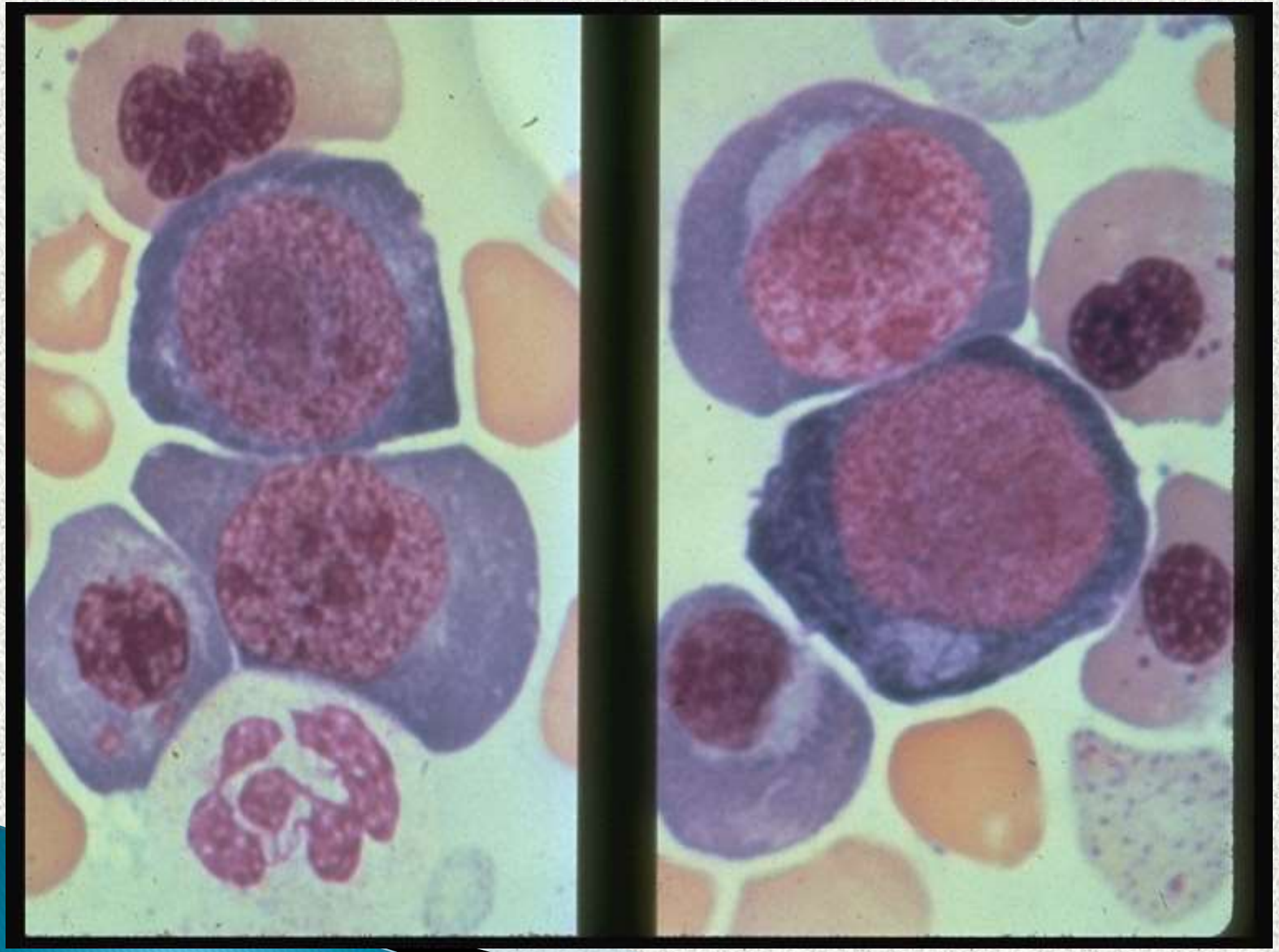
Peripheral Blood film

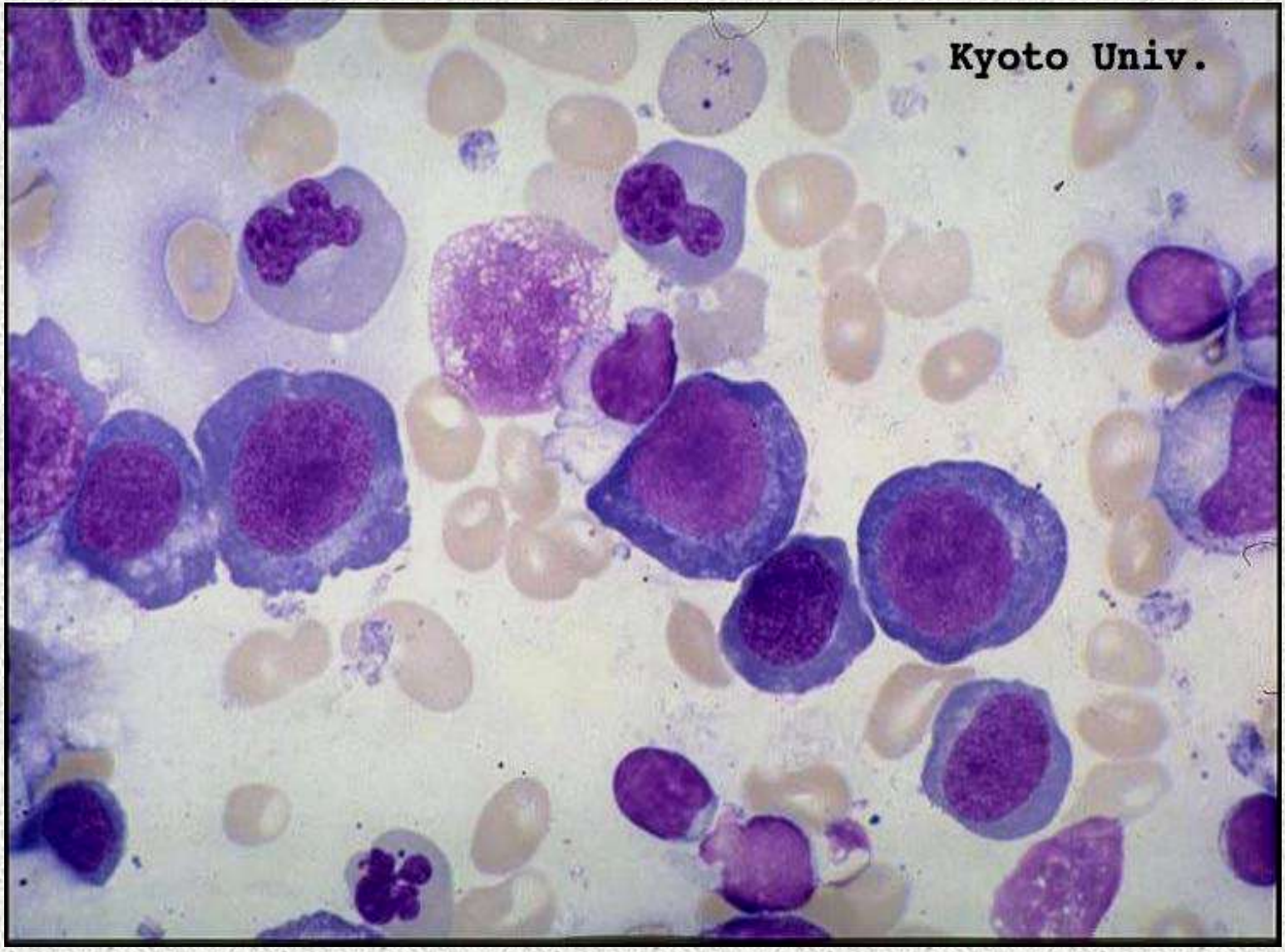
- Macrocytosis
- Macro-ovalocytosis
- Anisocytosis
- Howell Jolly Bodies
- Basophilic stippling
- Hypersegmented neutrophils
- Platelet: Decreased on smear
- Retics: N/Low


Oval macrocyte
Hypersegmented Neutrophils

Bone marrow aspiration

- Hypercellular smear
- Erythropoiesis is hyperplastic and megaloblastic
- Myelopoiesis is hyperplastic and shows left shift.
- Giant myelocytes and metamyelocytes are seen
- Megakaryocytes are decreased with left shift




Kyoto Univ.

Megaloblast

- Larger than the normoblast
- Nucleus lags behind in maturation
  (Nuclear: Cytoplasmic asynchrony/ Dissociation)
- Open chromatin (Sieve-like pattern)
- Cytoplasmic maturation is normal

Biochemical Tests

- Serum B12 levels (160-925 ng/l)
- Serum folate levels (3-15µg/l)
- Red cell folate levels (160-640µg/l)
- Bilirubin (slightly increased)
- LDH (Increased)

Special Tests

- Schilling's test (in Pernicious anemia)
- Anti-IF Abs
- Anti-Parietal cell Abs

MEGALOBLASTIC ANEMIA

Diagnosis /Therapy

- **Draw levels at first suspicion of problem, BEFORE ANY THERAPY**
- **Once levels drawn, begin treatment with both B_{12} and folate**
- **Once levels are back, can stop therapy**
- **Transfusions to be avoided unless hemodynamic compromise is present, or patient having angina**

TREATMENT OF MEGALOBLASTIC ANAEMIA

	VITAMIN B12 DEFICIENCY	FOLATE DEFICIENCY
Compound	Hydroxycobalamin	Folic Acid
Route	Intramuscular	Oral
Daily Dose	100 ug	5 mg
Initial Dose	6 X 1000ug over 2-3 weeks	Daily for 4 months
Maintenance	1000 ug every 3 months	Depends on underlying disease ; life long therapy may be needed in chronic haemolytic anaemias , myelofibrosis and renal dialysis
Prophylactic	-Total Gastrectomy - Ileal Resection	Pregnancy; Severe haemolytic anameias; dialysis; prematurity

MEGALOBLASTIC ANEMIA

Summary

- Deficiency in folate or B_{12}
- Macrocytic anemia; ± other cytopenias
- Slowly developing anemia, usually well compensated
- Response to therapy rapid and dramatic
- Treatment essential to avoid other complications

- 1. A 41 year woman presents with increasing fatigue lethargy and muscle weakness. Her CBC reveals Pancytopenia. Peripheral blood examination shows Macrocytosis with hyper segmented Neutrophils. Which test will help in diagnosis in this case?
- A. Serum iron level
- B. Hb Electrophoresis
- C. Immunophenotyping
- D. Serum Folate levels
- E. ESR

- 2. A Patient with severe anaemia has peripheral blood smear with oval macrocytes, hyper segmented neutrophils, and decreased platelets. The most likely cause of the anaemia is
- A. A Red cell membrane protein defect.
- B. An amino acid substitution in the β-globin chain.
- C. Iron deficiency anaemia
- D. Vitamin B12 or Folate deficiency

- 3. The patient in question 2 is found to be a severely malnourished alcoholic. The most likely cause of this disorder is
- E. Aberrant intestinal bacterial flora.
- A. Crohn disease
- F. Fish tapeworm infestation
- G. Folate deficiency
- H. Pernicious anaemia.

- 4. A 23-year old woman with epilepsy who is desirous of becoming pregnant is found to be anemic. Examination of a peripheral blood smear reveals oval macrocytes and hyper segmented neutrophils. Neurologic examination is entirely normal. Which of the following is the most likely cause of the anaemia?
- A. Alloantibodies directed against fetal red cell antigens
- B. Bone marrow Aplasia
- C. Coating of red cells by IgG “warm” auto antibodies
- D. Folate deficiency
- E. Neoplastic replacement of the bone marrow

- 5. A 45 year old woman presents with anaemia and fatigability. She has mild jaundice on clinical examination. Blood CP shows a raised MCV of 105fl and leucopenia. Peripheral smear shows macro ovalocytes and hyper segmented neutrophils. Schilling test for absorption of orally administered cobalamin is positive. What is a gastric biopsy from this patient likely to show:
- A. H. Pylori infection
- B. Chronic Non specific Gastritis
- C. Peptic Ulceration
- D. Hypertrophic gastritis
- E. Atrophy of fundal glands with intestinalization

- 1. An 80 Years old man presented with off and on diarrhea. On examination there is mild jaundice without Organomegaly. Blood Cp shows Pancytopenia with Macrocytic red cell morphology (MCV : 115fl)
- A. What is the likely diagnosis? (1)
- B. Enlist causes of Macrocytosis (2)
- C. How will you investigate this patient in lab. (2)

- 2. Briefly Describe
- A. Pathogenesis of Megaloblastic anaemia (Illustrate) (2.5)
- B. Lab Diagnosis of Megaloblastic anaemia (2.5)

THANK YOU