Megaloblastic Anaemia

Aeitology and pathogenesis:

It is characterized by↓ DNA synthesis, while RNA & protein synthesis r N → nucleo-cytoplasmic asynchrony (mature cytoplasm e' defective nuclear chromatin).

Finally : cell → die (ineffective erythropoiesis)

or →x terminal division → survive as oversized cell e' short life span.

The defect in DNA synthesis occur in all proliferating tissues, e.g: BM, GIT etc…

Causes:

- B12 or abnormal metabolism
- Folate or abnormal metabolism.
- Other causes:
  - Erythroleukemia
  - Sideroblastic anaemia
  - Orotic aciduria (abn. Pyrimidine metabolism)
  - Cytotoxic drugs (interfere e' DNA synthesis)
  - Alcohol

Clinical picture:

- Most patients r detected on routine CBC by ⇧ MCV (present before any other symptoms).
- General signs & symptoms of anaemia.
- Fever, jaundice (due to ineff.erythropoiesis)
- Purpura, infections.
- Anorexia, weight loss.
- GIT symptoms : diarrhea, constipation
- Palbable spleen
- Sterility (affection of gonads)

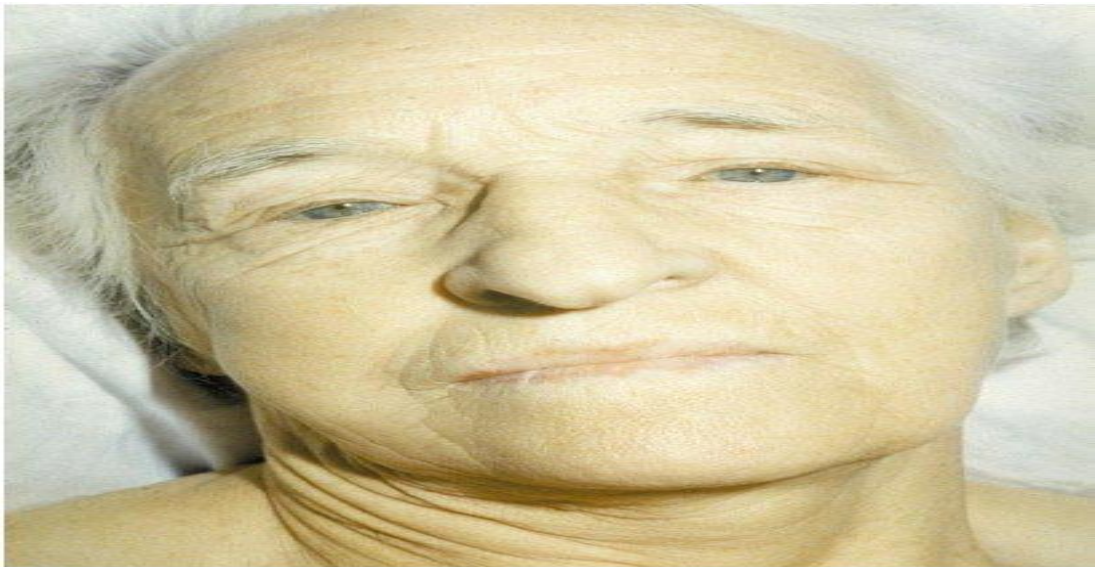
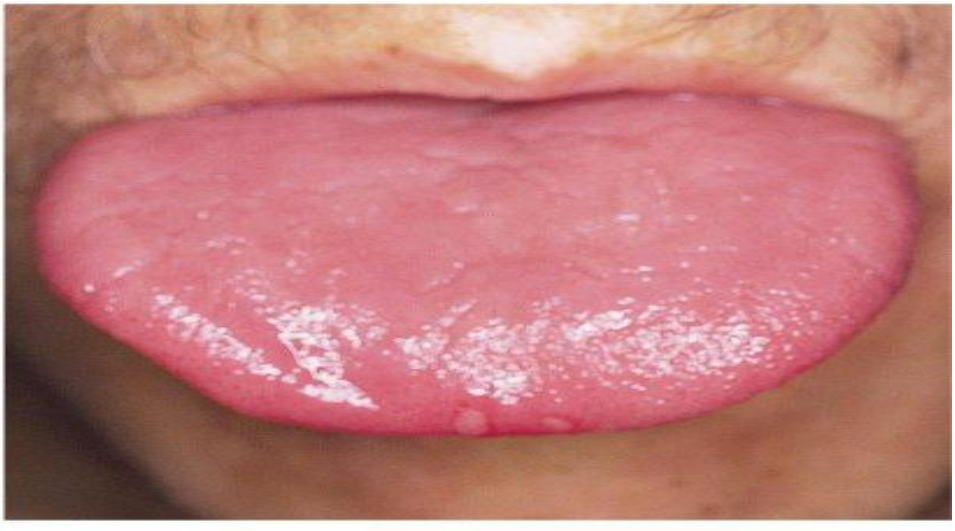


Figure 5.6 Megaloblastic anaemia: pallor and mild icterus in a patient with a haemoglobin count of 7.0 g/dL and a mean corpuscular volume of 132 fL.



From: *Essential Haematology*, 6th Edn. © A. V. Hoffbrand & P. A. H. Moss.

Figure 5.7 Megaloblastic anaemia: glossitis – the tongue is beefy-red and painful.

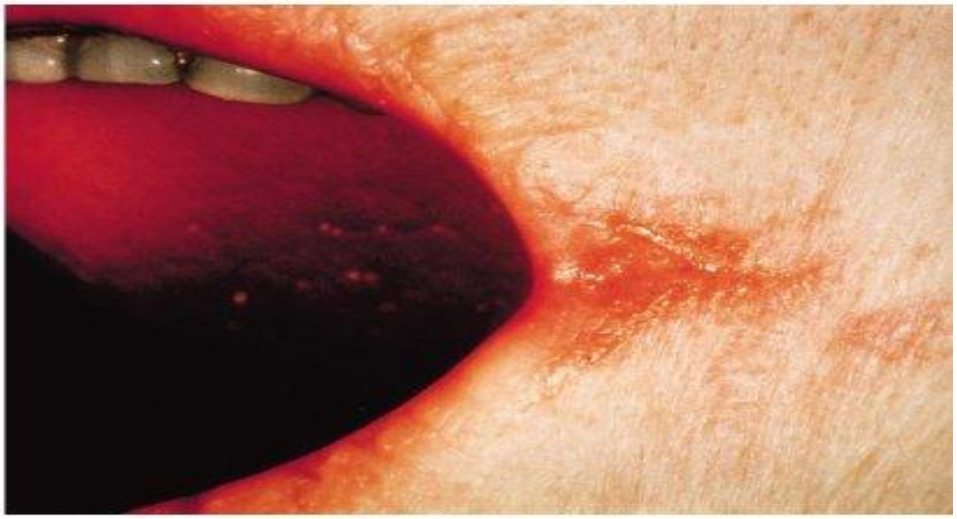


Figure 5.8 Megaloblastic anaemia: angular cheilosis (stomatitis).

Neurological symptoms:

A- ↓B12 (↓50 ng/L):

- bilateral peripheral neuritis.
- degeneration of posterior cord → deep sensory loss.
- degeneration of pyramidal tracts→affection of motor muscles.
- optic atrophy, retinal haemorrhage .
- mental abnormalities.
- visual impairment due to: optic atrophy
  retinal hge
  retrobulbular neuritis

B- ↓Folates:

- mental changes
- slowness
- Dementia

All megaloblastic signs & symptoms r reversible by ttt Except CNS manifestations, as CNS cs r not regenerating cs, so its defect is permanent.

Diagnosis:

- careful history taking & clinical examination.
- lab investigations.

<u>Lab investigations</u>:

1-CBC:

RBCs :

- oval macrocytes, anisocytosis, poikilocytosis
- ↑ MCV (↑100 fl) unless combined e' Fe def

Dimorphic cs (macro & micro & MCV is nearly normal).

WBCs:

- leucopenia
- hypersegmented neutrophils
- giant staff & juvenile
- leucoerythroplastic picture:
  immature RBCs (normoblasts)
  immature WBCs (juv & myelo)

Platelets: ↓

Reticulocytes:

mild ↑ in relation to anaemia.

2-Bone marrow : is a must in megaloblastic an Hypercellular e' dyserythropoietic changes

In severe anaemia:

- BM : hypercellular
- M/E ratio: N or ↓
- ↑primitive cells

Erythroid series:

- Megaloblasts: larger than normoblasts, normoblasts e' stippled chromatin (open, fine lacy appearance of nucleus) & cytoplasm is fully haemoglobinzed.
- Intercytoplasmic or internuclear bridges
- ↑ mitotic figures
- Basophilic stippling & Howel Jolly bodies (remnants of DNA)

Myeloid series:

Giant staff & juvenile

Hypersegmentation of nucleus of neutrophils.

Megakaryocytes:

May be enlarged e' hyperpolyoidy (no of nuclei)

Iron stain:

↑ iron in RES & in developing megaloblasts

↑ sideroblasts. Both r due to ineff. erythropoiesis

3-Ineffective Haematopoiesis:

- ↑ Indirect bilirubin
- ↑ Urobilinogen
- ↑ Stercobilinogen
- ↑ LDH ↑ CO
- ↓ Haptoglobin, ↓ haemopexin
- ↑ Serum iron
- ↑ Lysosomes (ineffective granulopoiesis)
- +ve urine haemosedrin
- **+ve Schumm's test** (detection of metalbumin)

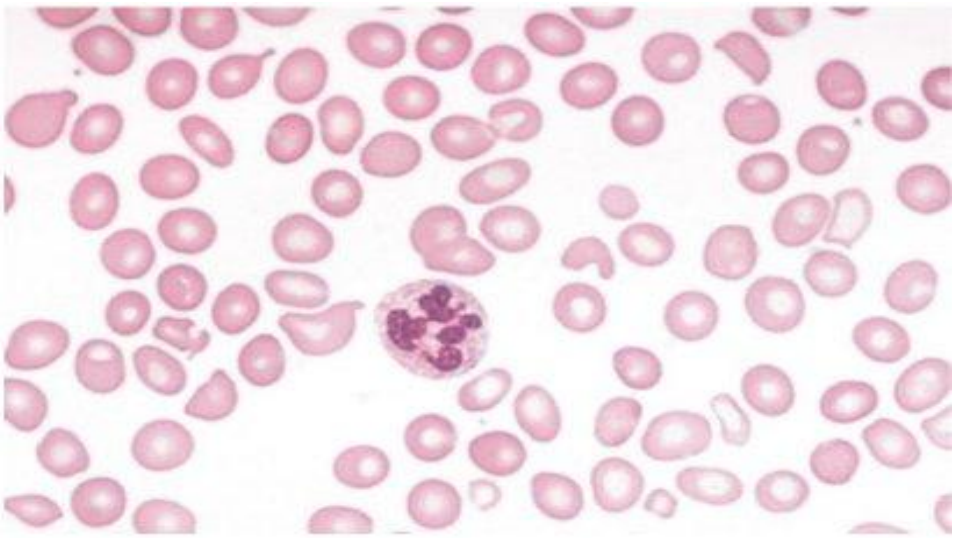
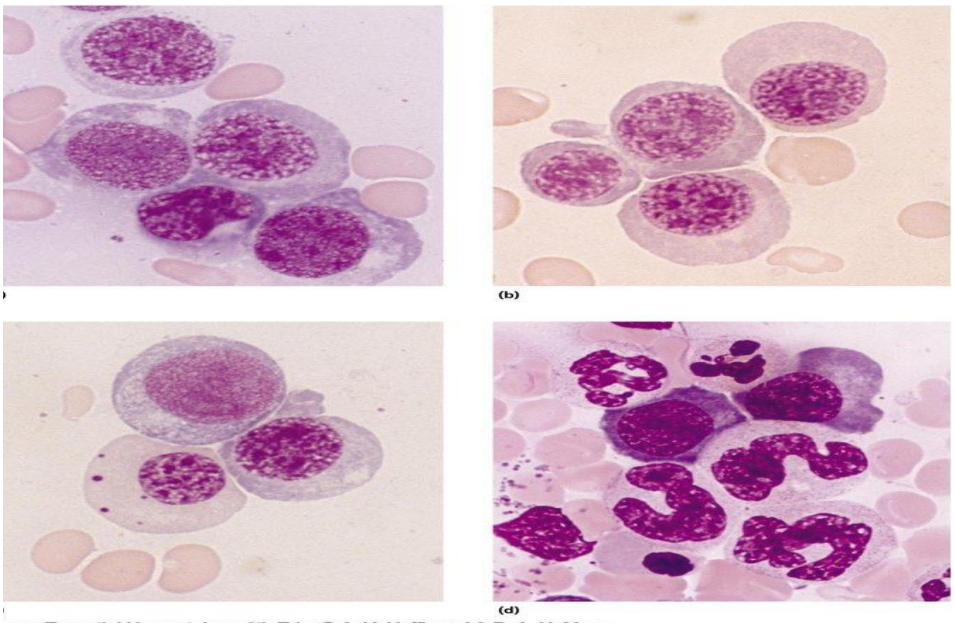


Figure 5.11 Megaloblastic anaemia: peripheral blood film showing oval macrocytes.



gure 5.12 Megaloblastic changes in the bone marrow in a patient with severe megaloblastic anaemia. **–c)** Erythroblasts showing fine, open stippled (primitive) appearance of the nuclear chromatin even in late lls (pale cytoplasm with some haemoglobin formation). **(d)** Abnormal giant metamyelocytes and band forms.

4-Specific tests for B12:

- therapeutic tests
- serum B12
- methyl malonic acid execration test
- deoxy uridine suppresion of thymidine uptake test
- tests to detect the cause of B12
- tests for vit B12 absorption.

5- Specific tests for folate:

- therapuetic tests
- deoxy uridine suppresion of thymidine uptake test
- serum folates
- red cell folate
- FIGLU

6- Cytogenetics:

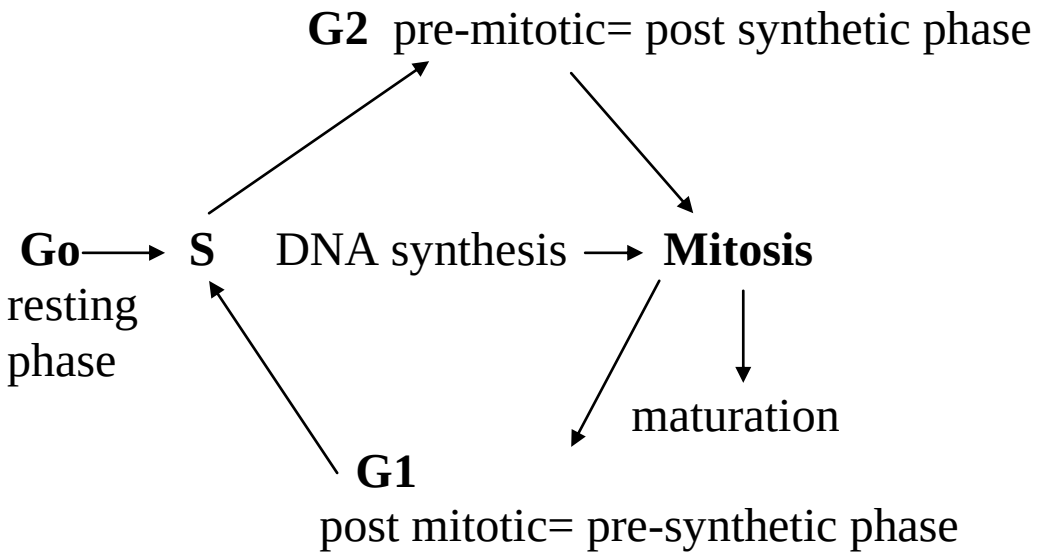
chromosomes show:

- random breaks
- spreading of centromere
- exaggeration of 2ry constrictions

due to:

Replication of chromosomal DNA in the presence of reduced concentration of one of the basis → random breaks & spreading of centromere → as thymidine is incomplete → exaggeration of 2nd chromosomal constriction → death in S phase → Ineffective (haemopoiesis). erythropoiesis

Cell Cycle:



D.D:

Other causes of macrocytosis & hypersegmentation:

Macrocytosis:

- Alcohol
- Liver disease
- ↑ Retics
- Aplastic anaemia
- 1ry acquired sideroblastic an.
- Myxoedema
- MDS
- Pregnancy
- Newborn

N.B:

In these situations, BM shows normoblasts rather than megaloblasts.

Macrocytes in normoblastic anaemia r round rather than oval in megaloblastic an.

Hypersegmentation:

- Renal failure
- Congenital abnormality

Tests for B12 & folate

Vit B12	Folate
1-Therapeutic test: Patient is placed on a diet low in B12 & folate for 1 week before starting ttt, then give:	
1 ug IM/d	100 ug orally/d if malabsorption is suspected give parental. In case of B12 def., it responds to higher dose 400ug/d

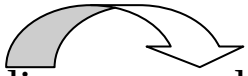
Response:

- After 2 days ⟶ normoblastic picture return N
- After 3 days ⟶ reticulocytosis (↑on 3rd day peak on 6th day)
- After 7 days → platelets →N
- ↑ Hb by 1 gm/dl/week
- After 14 days → leucopoiesis →N

Poor response:

- Combined causes (B12/folate def± iron def.)
- Complicated megaloblastic anaemia
- Severe folate def

So you must start ttt e' higher doses B12+folic acid may response

Vit B12	Folate
2- Deoxy uridine suppression test	
In N BM: 5-10 methyl THF $\xrightarrow{B12}$ DHF  deoxy uridine → thymidine So it suppress uptake of radioactive thymidine < 10% **In B12 or folate ↓** there is↑ radioactive uptake of thymidine due to block of conversion of DU to thymidine	
corrected by	
B12, folic acid, folinic acid But not by methyl THF as It requires B12 to enter cell	any folate but not B12
3- serum B12	**3- serum folate**
a-microbiological assay	
Lactobacillus leishmanii (B12 is imp.for its growth)	lactobacillus casii (it feeds on folate)
b-radio isotope dilution assay	
N level: 160-925 ng/L **Border line:** 100-160ng/L **Megaloblastic an.:** <100 ng/L 50 ng/L ↓ CNS changes	3-5 ug/L 3-6 ug/L

B12	**Folate**
Raised serum B12: ▸ Recent therapy ▸ ↑ TCI ▸ Liver or kidney disease ▸ Bacterial contamination	**Raised serum folate:** ▸ Severe B12 def (trap) ↑ serum , ↓ red cell folate ▸ Liver or kidney disease ▸ Bacterial contamination ▸ Stagnant loop syndrome ▸ Haemolysed sample **False low level:** ▸ In patients taking drugs inhibiting L casii growth: as antibiotics (methotrexate trimethopreum)
4- Methyl malonic acid (MMA) excretion: MMA ↓ MMA reductase B12 Succinyl CoA In megaloblastic an., MMA → urine **N excretion**: 0-4 mg/d Test can be more sensitive by overloading the patient e' **Valine** (10gm) ↑ MMA in urine > 4mg/d Test is +ve in: B12 def or MMA reductase def	**4-Formi minoglutamic acid excretion (FIGLU):** FIGLU folate → glutamic a ↓ Urine (if x folate) **Normally:** -ve Overloading dose e' **Histadine:** ↑ FIGLU in urine in: **+ve:** folate def. 2/3 B12 def. **False +ve:** liver dis malignancy **False –ve:** prgnancy kwashiorkor
5-serum MMA: ↑ serum MMA in serum	

Vit B12	Folate
6- Detect cause of B12: History diet, drugs, operation, clinical B12 absorption must be tested Measurement of IF in: Gastric juice after maximal saturation e' pentagastrin	**5-Detect stores (red cell folate):** **A-microbiologically** **B-radioisotopic assay:** Most of blood folate r inside RBCs (10-20 times serum folate) **N** =160-460 ug/L packed RBCs ↓ in :severe folate def. 2/3 meg an due to B12 **False N:** recent transfusion ↑ retics **N.B:** this test can't diff between folate & ↓ B12

6-7- serum homocysteine level:

5 M THF ——B12——→ THF

homocystiene methionine

It is ↑ in:

- B12 def
- folate def

But also in:

- renal disease
- alcoholism

8- Tests for B12 absorption:

- Urinary excretion **(Schilling test)**
- Faecal excretion (measurement of radioactivity in urine or faeces).
- Hepatic uptake
- Plasma radioactivity
- Whole body counting

Principle of all methods:

- Oral dose of radioactive B12 labelled by 57 Co

(1ug) is given orally alone

- If there is malabsorption, repeat e' IF:
- **If corrected**: pernicious an.
- **If not corrected**: it is malabsorption: repeat the test+ antibiotics

<u>Schilling test:</u> (D.D gastric from intestinal causes)

- Oral dose of radioactive B12 (1 ug)+ parental large dose (1 mg) of unlabelled B12 (for saturation of TCII)
- **Normally:** up to 10% of radioactive dose appears in urine.
- **In pernicious an.:** only 5-7% appears in urine (corrected by IF).
- **In intestinal causes:** low dose appears & is not corrected by IF, but corrected by antibiotics.
- If not corrected by any means, so the defect is in **ileal receptors**.

Pernicious Anaemia

Def:

- Autoimmune disease
- Resulting in severe lack of IF due to gastric atrophy
- Resulting from immune destruction of acid & pepsin secreting portion of gastric mucosa.

Incidence:

- F: M = 1.6: 1
- Old age> 60 ys (10% < 40 ys)
- Familial
- Northern Europe

More common in :

- Relatives
- Subjects e' autoimmune dis.
- Those e' premature hair grey, blue eyes, vitiligo, blood gp A
- Associated e' HLA-3 in some cases

Diagnosis:

- C/P
- Megaloblastic an.
- Lack of IF (detected by Schilling test)
- Tests to detect IF antibodies
- Gastric endoscopy

Gastric secretion studies:

1- Intrinsic factor:

Measured by its vit B12 binding

I unit of IF binds 1 mg of vit B12

After maximal stimulation:

N : M : secrets 2000 units in 1 hr

F: secrets ½ amount (1000 units)

In pernicious an. : only 250 units is secreted.

2- Hydrochloric acid (HCL):

Resting juice ph > 7 & doesn't fall more than 1 ph/unit after max stimulation by histamin.

In pernicious an.:

↓HCL (alkaline ph)

3- Gastrin:

↓volume of gastric secretion & pepsin

↑serum gastrin (↑in blood, ↓in gastric juice in stomach).

Gastric biopsy:

- Shows gastric atrophy e' loss of glandular elements & replacement of mucous cells
- Inflammatory cs infiltrate (lymph & plasma cs)
- May be intestinal metaplasia
- **Immune phenomena**:
- Apart from atrophy of gastric mucosa, there is evidence of immune mechanism in pathogenesis of pernicious an.
- **1-Antibodies to gastric antigens:**

IF Abs

- 2 types both r Ig G
- Can cross placenta

→temporary ↓IF in NB

- Found in serum & gastric j of 80% of P.A
- ↑CD4/CD 8 lymph

parietal cell Abs

-found in 90% of cases

-not diagnostic as it is

-present in:

60% of cases of simple atrophic gastritis

thyroid dis, D.M, addisson, myxedema, ch. active hepatitis

IF antibodies

A-Blocking Abs (Type I)

- Found in 55% of patients
- Prevent combination of IF to B12
- Found in other A.I dis.:

  myxedema

  thyrotoxicosis

  relatives of pernicious an.

B-Binding Abs (Type II)

- Found in 35% of patients
- Prevent attachment of B12/IF complex to ileal mucosa (ileal R)
- Rarely found in other diseases than P.A, so it is **Diagnostic**

2- Associated e' other autoimmune diseases:

3- Response to steroid therapy:

- improves gastric mucosal atrophy (HCL, gastric mucosa, IF) improves.

4-Hypo gamma globulinaemia : or **Ig A deficiency:**

- ↓ 40 ys
- e' intestinal malabsorption
- gastric lesion but e' out plasma cell infiltration
- history of recurrent infection

5- ↑ CD 4/CD8 ratio

Prognosis:

- F: after ttt: get better
- M after ttt: bad prognosis due to high incidence of Ca stomach

Classification:

1-Adult type:

see incidence

Cause:

atrophy of gastric mucosa → absence of IF

immune mechanism

2- Childhood type:

1ry Type	2ry Type (more common)
▸ In older children ▸ Resembles adults ▸ IF Abs r present But no parietal cell Abs ▸ Gastric atrophy ▸ Achlorhydria ▸ 50% of cases r ass. e' endocrinopathies (Addison or myxedema)	▸ In 1st years of life (after consuming B12 stores acquired from mother) ▸ No IF Abs ▸ No parietal cell Abs ▸ No gastric atrophy ▸ Normal HCL (but no IF) ▸ AR

General management of megaloblastic anaemia:

1st find whether B12 or folate is deficient & ttt accordingly
In severely ill patient, give both vits in large doses.
Platelet concentrate if there is ↓platelets.
K+ if there is hypokalamia.

ttt of vit B12 def.:
if there is a cause, ttt cause
A- Oral B12 only in case of ↓intake
In malabsorption, high doses 500-1000 ug/d
B- Parental B12 therapy:

- To replenish stores, 6 injections each 1000 ug IM (hydroxy cobalamine), given at 3-7 days intervals then,
- Maintained by 1000 ug IM every 3 months
- According to etiology, it may be temporary or permanent

Criteria to start therapy:

- Border line level of vit B12.
- Well established anaemia or neuropathy
- Haematological findings (megaloblastic changes).

ttt of folate deficiency:

- No need for injection
- Only oral dose of 5-15 mg folic a/d for 4 months (when all folate deficient RBCs r eliminated) & replaced by new folate rich RBCs.

N.B:
Before giving folate, B12 def. must be excluded, otherwise B12 neuropathy may occur (because folates utilizes B12 in its metabolism → exaggerates its deficiency.

Folic acid must be given prophylactic ally in :

- Pregnancy
- Prematurity
- Haemolytic anaemia
- Dialysis