HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

6 - Megaloblastic and Sideroblastic Anemias

Vera Malkovska, MD, FRCPath

1

Disclosures

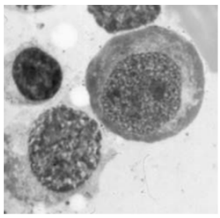
Disclosures of Financial Relationships with Relevant Commercial Interests

- None

2

Megaloblastic Anemias: Definition

Disorders of DNA synthesis that lead to nuclear-cytoplasmic maturation asynchrony and characteristic morphological changes



3

Causes of megaloblastic anemias

- Cobalamin (CBL) deficiency
- Folate deficiency
- Drugs: 5-flurouracil, azathioprine, hydroxyurea, zidovudine, anticonvulsants
- Hereditary disorders of DNA synthesis and other rare errors of metabolism: orotic aciduria, thiamine-responsive megaloblastic anemia, Lesch-Nyhan sy, intrinsic factor and cubilin defects, methyl- and adenosyl-CBL synthesis defects

4

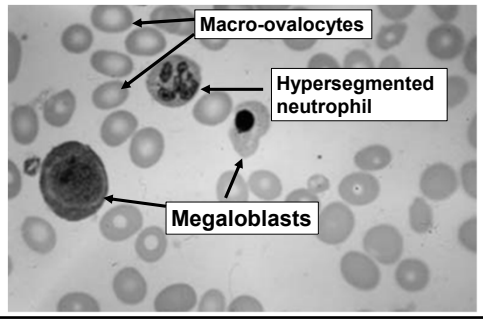
Morphology

Peripheral blood
- Macro-ovalocytes
- Hypersegmented neutrophils

Bone marrow
- Megaloblasts
- Giant metamyelocytes and bands
- Decreased and hypersegmented megakaryocytes

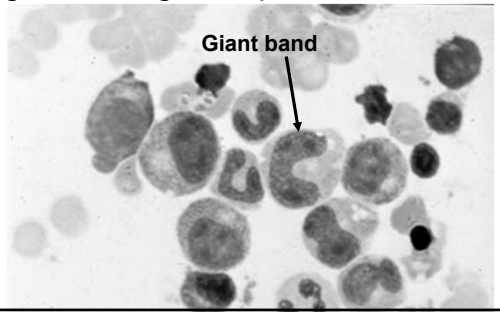
5

Bone marrow morphology



6

Megaloblastic granulopoiesis



7

Labs reflect ineffective erythropoiesis

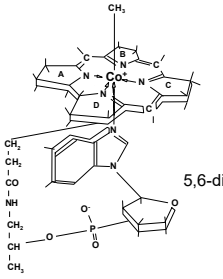
- Cytopenia in peripheral blood, ↓ retics
- ↑ MCV (masked by microcytic disorders)
- Hypercellular bone marrow
- ↑ LDH, indirect bilirubin
- ↓ Haptoglobin
- ↑ Ferritin, iron, transferrin saturation

8

Cobalamine (CBL) deficiency

9

Structure of methyl-CBL

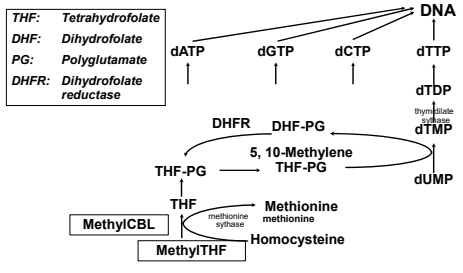


Planar corrin nucleus: 4 pyrrole rings linked to central Co+

Nucleotide: 5,6-dimethyl-benzimidazolyl

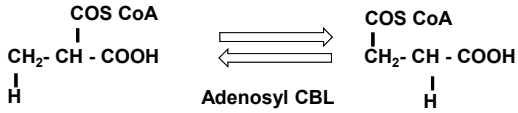
10

Biochemistry of CBL & folate deficiency



11

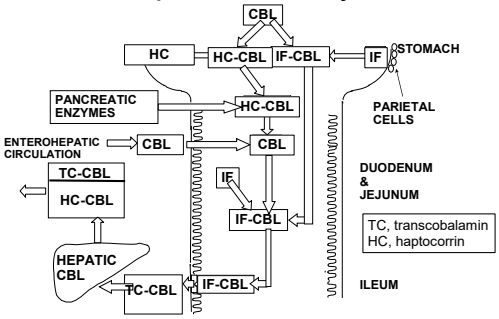
Adenosyl CBL-dependent reaction in mitochondria



Methylmalonyl CoA — Succinyl CoA

12

Absorption of dietary CBL



13

Absorption of oral CBL

	Normal	Malabsorption
Daily loss	**1 µg**	**2 µg**
Body stores	**~ 2,500 µg**	**variable**
% absorbed from single oral dose		
1 µg	**0.56 µg**	**0.01 µg**
10 µg	**1.6 µg**	**0.1 µg**
1000µg	**9.7 µg**	**7.0 µg**

Adapted form Carmel R, Blood 2008,112:2214

14

CBL retention from injection

	Normal	Malabsorption
Daily loss	**1 µg**	**2µg**
Body stores	**~ 2,500 µg**	**variable**
% absorbed from single injection of*:		
10 µg	**9.7 µg**	**same as N**
100 µg	**55 µg**	**same as N**
1000µg	**150 µg**	**same as N**

*Adapted form Chanarin I. The Megaloblastic Anemias,1979

15

Causes of clinical CBL deficiency

- Dietary deficiency (vegans)
- Inadequate proteolysis of food-CBL (gastric surgery)
- Intrinsic factor (IF) deficiency - pernicious anemia (PA)
- Blind loop sy – bacterial overgrowth
- Intestinal malabsorption (sprue, Crohn's, ileal resection)
- Nitrous oxide inhalation

16

Causes of clinical CBL deficiency

- Pancreatic insufficiency (rare)
- Fish tapeworm infection
- Use of metformin
- Use of drugs blocking stomach acid
- Breastfed infants of B12 def. mothers
- Rare genetic disorders

17

Pathophysiology of PA

- Autoimmune gastritis
- Anti-parietal cell (anti-Na+/K+ ATPase)
- Anti-IF antibodies, high gastrin
- Decreased parietal cell function:
  - Decreased IF production and CBL absorption
  - Achlorhydria
  - Decreased iron absorption

18

PA: Clinical manifestations

Hematological
- Symptoms of anemia
- Pallor, lemon yellow skin

Neuropsychiatric
- Paresthesiae
- Dementia, psychosis
- Ataxia, weakness, spasticity

Other: Glossitis, other epithelial changes

19

PA: Clinical manifestations

Associated autoimmune disorders
- Hypothyroidism, Hashimoto's
- Vitiligo, rarely hyperpigmentation
- Diabetes mellitus
- Addison's disease

Increased risk of gastric cancer and carcinoid

20

Diagnosis and management of CBL deficiency

21

Questions to answer at diagnosis

- Is this a true clinical deficiency?
- What is the underlying disorder?
- Is it reversible?
- Are there other nutrient deficiencies?
- Could this be due to a rare disorder? (nitrous oxide, genetic, metabolic or transport)

22

Accurate diagnosis impacts therapy

- Short vs long term therapy
- Parenteral vs oral
- Treatments of underlying and associated diseases
- Addressing long-term risks

23

Low B12 levels without clinical deficiency

- Lab artifact
- Folate deficiency
- Pregnancy (2^{nd},3^{rd} trimester)
- Haptocorrin (HC) deficiency
- Rare: myeloma, oral contraceptives
- Intake of megadoses of ascorbic acid

24

Clinical deficiency with normal B12 levels

- TC deficiency (with normal HC level)
- Nitrous oxide exposure
- Inborn errors of CBL metabolism
- Myeloproliferative disorder with B12 malabsorption

25

How to diagnose "true" B12 deficiency?

- B12 should not be checked without clinical indications
- Clinical suspicion of deficiency should be investigated regardless of B12 levels
- Reassay marginally abnormal levels
- Confirm deficiency by 2 biomarkers
- Monitored trial of parenteral B12

26

Tests for the diagnosis of CBL and folate deficiency

Test	CBL deficiency	Folate deficiency
Serum CBL	↓	N or ↓
Serum folate*	N or ↑	↓
Red cell folate	↓ or N	↓
MMA*	↑	N
Plasma homocysteine	↑	↑
Deoxyuridine Suppression test*	Abnl.	Abnl.

*Best discriminators of CBL vs folate def.

27

Other tests

- Anti-IF antibody
  - Very high specificity for PA
  - False positive within 2 days of CBL injection
- Parietal cell antibody
  - Positive in 80-90% of PA patient
  - Low specificity
- Increased serum gastrin in ~ 90% of PA
  - Low specificity

CBL absorption test ("Schilling test") is unavailable

28

Suggested therapy

Drug	Hydroxycobalamin or cyanocobalamin
Route	IM
Dose	1,000 µg
Regimen	8-10 x over 2 months, then monthly life-long

Route and schedule are less important than close monitoring!

29

Oral maintenance therapy

PROS
- Easy and painless
- Cheap

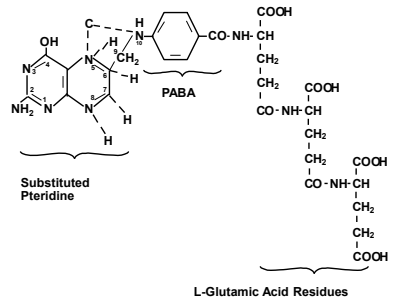
CONS
- Variable absorption with food
- Compliance problems
- Monitoring essential
- Long term experience limited

30

Folate deficiency

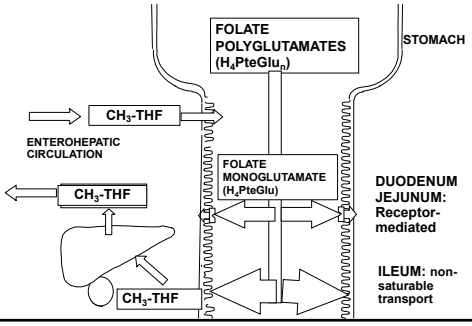
31

Structure of folates



32

Absorption of Dietary Folates



33

Folate in diet

- Destroyed by cooking
- Food folate less bioavailable than folic acid
- Absorption 50-80% of dietary amount
- Enterohepatic recycling 100 µg/day
- All grain products supplemented in the US
- Recommended dietary intake 400 µg/day

34

Causes of folate deficiency

- Increased requirement (pregnancy, rapid growth, hemolytic anemia, skin disorders)
- Dietary – rare in U.S.
- Intestinal malabsorption (celiac disease)
- Defective utilization (methotrexate, trimethoprim)
- Anticonvulsants (decreased absorption)
- Rare genetic disorders

35

Serum vs red cell (RBC) folate

Serum Folate	RBC Folate
• Unstable in vitro	• Better reflect body stores
• May normalize after one hospital meal	• Low in 60% CBL deficiency
• Increased with hemolysis	• Increased with hemolysis

36

Treament of folate deficiency

Drug	Folic acid
Route	Oral
Dose	1-5mg daily x 4 months

Maintenance if cause persists

Prophylaxis: Pregnancy, prematurity, chronic hemolysis, dialysis

CBL deficiency must be ruled out before folic acid therapy!

37

Key points about folate deficiency

- Rare in the US with adequate diet and normal GI system
- Occurs with malabsorption, malnutrition, rapid cell turnover
- Cytopenia develops rapidly
- Elevated homocysteine but not MMA
- Associated with neural tube defects
- Neurol. manifestation very rare in adults
- Folic acid can worsen neurol. deficit in CBL deficiency

38

Sideroblastic anemias

39

Sideroblastic anemias (SA)

- Congenital or acquired, benign or malignant
- Limited to erythroid defect or syndromic
- Ring sideroblasts are diagnostic, caused by iron accumulation in mitochondria
- Ineffective erythropoiesis
- Iron overload is common

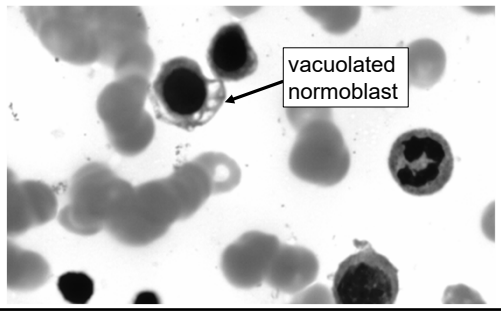
40

Sideroblasts: Definitions

- Normal sideroblasts
  1-4 siderotic granules in cytoplasm
  Correlate with TIBC saturation
- Sideroblasts in SA:
  ≥ 5 granules, coarse, form rings, reflect accumulation of mitochondrial ferritin

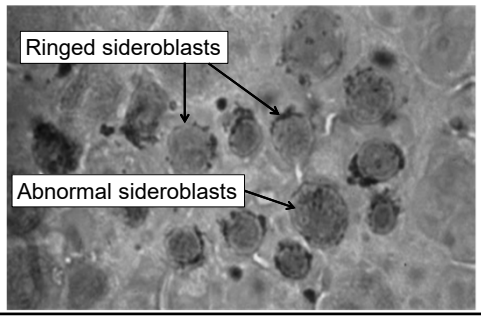
41

Bone marrow in SA



42

Bone marrow: Prussian blue stain



43

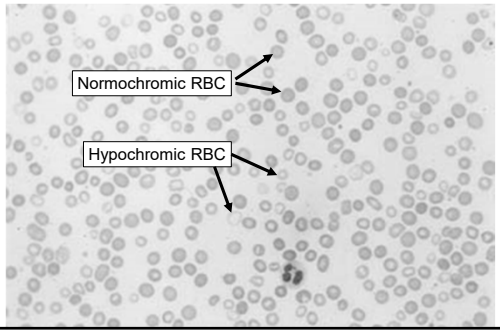
Laboratory findings

Signs of ineffective erythropoiesis

- Micro-, normo-,macrocytic anemia or dimorphic RBC
- Low reticulocytes
- Erythroid hyperplasia in bone marrow
- ↑ LDH, indirect bilirubin
- ↓ Haptoglobin

44

Dimorphic red cells in SA



45

Laboratory findings

Signs of defective iron utilization

- Hypochromic RBC
- ↑ ferritin, iron, transferrin saturation
- ↑ marrow iron stores
- ↑ liver Fe content
- Iron overload before transfusions

46

1. Congenital SA

- Erythroid only
  - Usually microcytic
  - Iron overload
  - Can present in adults
- Syndromic
  - Dominated by neuromuscular, metabolic and other defects
  - Macro- or normocytic
  - Rare, diagnosed in infancy or childhood

47

Erythroid only SA

- Heme synthesis defects
  - X-linked SA (XLSA) *
  - SLC25A38 deficiency *
  - Erythropoietic protoporphyria*
- Iron-sulfur cluster generation defects
  - GLRX5 deficiency
  - HPSA9 deficiency
  - HSCB deficiency

** Reported in more than 40 families*

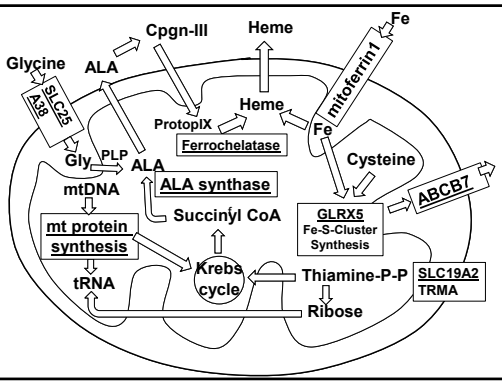
48

Syndromic SA

- Mitochondrial protein synthesis defects
  - Pearson marrow pancreas syndrome (PMPS) *
  - Myopathy with lactic acidosis SA (MLASA) *
  - SA with immunodeficiency, fever, developmental delay (SIFD)*
- Mitochondrial respiratory protein gene mutations
  - Thiamine-responsive megaloblastic anemia (TRMA)*
  - Other rare defects

** Reported in more than 40 families*

49



50

X-linked hereditary SA (XLSA)

- Most common congenital SA
- Mutations in ALA-synthase 2 (ALAS2) - 1st step in heme synthesis
- Mostly males < 40yrs
- About a quarter are females
- Microcytic hypochromic, Hb >7g/dL
- Iron overload

51

XLSA in females

- Present in mid to late adulthood
- Normocytic or macrocytic
- Dimorphic RBC
- Associated with skewed X-inactivation
- Can be mistaken for MDS!

52

Management of XLSA

- Pyridoxine 50-100 mg/day, lower dose for maintenance
- Iron chelation
- Small volume phlebotomy if possible
- Transfusions rarely needed
- Splenectomy is contraindicated in hereditary SA

53

2. Acquired clonal/neoplastic SA*

- Myelodysplastic syndromes with ring sideroblasts (MDS-RS) with single linage dysplasia (MDS-RS-SLD)¶
- MDS-RS with multilinage dysplasia (MDS-RS-MLD)¶

¶Associated with SF3B1 mutation

** The 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia*

54

MDS-RS-SLD (former RARS)

- Normo- or macrocytic, dimorphic RBC
- ≥15%/≥ 5%* of erythroblasts are RS, at all stages of maturation
- Favorable prognosis c/w other MDS
- Responsive to G-CSF with Epo
- Pyridoxin-refractory

**if SF3B1 mutation is present*

55

3. Reversible acquired SA

- Alcohol
- Drugs (INH, chloramphenicol, linezolid)
- Copper deficiency:
  - Malnourished infants
  - Patients on parenteral nutrition
  - Excess dietary zinc intake
- Hypothermia

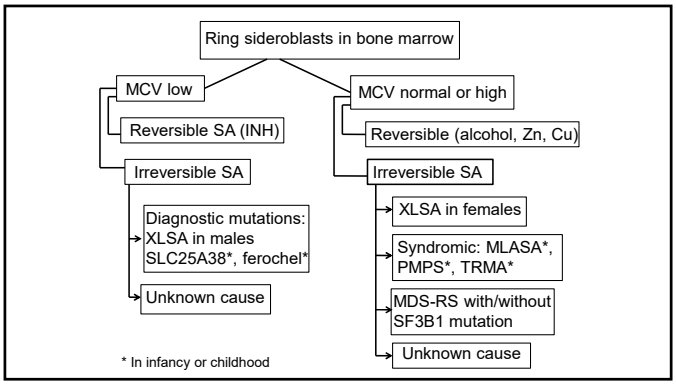
Anemia corrects upon removal of precipitating cause

56

Copper deficiency

- Micro- to macrocytic a. (MCV 70-116)
- ± neutropenia
- Hypocellular marrow
- Vacuolated erythroid and myeloid precursors ± ring sideroblasts
- Ataxia, myelopathy, sensory neuropathy
- Can be mistaken for B12 deficiency!

57



58

Thank you

59