**Nutritional deficiencies and Haemolytic Anaemias**

FRACP Lectures 2010

---

**Iron Deficiency**

- Iron absorption
  - Heme iron
  - Non-heme iron
  - Enhanced by gastric acid, ascorbate (vit C), breast milk
  - Decreased by bovine milk proteins, egg white, phytates, bran, calcium, zinc, lead

---

**Iron Deficiency**

- Stages in development of iron deficiency
  - Iron depletion: low ferritin, normal Hb and indices (MCV)
  - Iron deficiency: low ferritin and indices; Hb normal
  - Iron deficiency anaemia

---

**Prevalence of iron deficiency in Australian children**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>n</th>
<th>Iron depletion%</th>
<th>Iron deficiency%</th>
<th>Anaemia%</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-23</td>
<td>182</td>
<td>18.7</td>
<td>5.4</td>
<td>1.4</td>
</tr>
<tr>
<td>24-35</td>
<td>176</td>
<td>14.2</td>
<td>3.7</td>
<td>3.0</td>
</tr>
<tr>
<td>36-47</td>
<td>148</td>
<td>6.6</td>
<td>1.7</td>
<td>0.0</td>
</tr>
<tr>
<td>48-62</td>
<td>172</td>
<td>1.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>9-62</td>
<td>678</td>
<td>10.5</td>
<td>2.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>

---

**Risk factors:**

- GIT disease
  - Coeliac disease
  - IBD
  - Cow’s milk enteropathy
  - Worm infestation
- Blood loss
  - Menstrual
  - Hereditary haemorrhagic telangiectasia
  - Urinary
  - Pulmonary
Iron Deficiency

Clinical consequences of iron deficiency
- Anaemia
- Poor growth
- Exercise intolerance
- Epithelial changes
- Immunity
- Pica

Iron Deficiency

Neurological dysfunction
- Lower scores on Bayley Scale of infant development
- Changes reversible at Hb <100 g/L even with therapy
- Impaired short term memory and reduced attention span in older children

Iron Deficiency

Laboratory evaluation of iron deficiency
- FBE:
  - Hb
  - MCV and MCH
  - RCC
  - RDW
  - Platelets
- Film:
  - Elongated cells++
  - Target cells+

Iron Deficiency

Iron studies:
- Serum iron unreliable
- Diurnal variation
- Falls in acute illness
- Transferrin
- Ferritin
  - Acute phase reactant
- Soluble transferrin receptors (a erythroid activity/inverse to iron availability): sTfR/log ferritin?
  - More useful <1 ACR >2 IDA or combined
- Bone marrow iron
- RBC Zinc protoporphyrin (elevated in both IDA/ACD)

Iron Deficiency

Therapy
- Underlying factors
- Iron supplements
  - Oral iron: 4-6 mg/kg in 2-3 divided doses per day
  - Adolescents 100-300 mg/day
  - Ascorbic acid
  - Parenteral iron
- Iron-fortified cereals/formulae after 6/12
- Irons supps for exclusively breast fed infants after 6/12
Iron Deficiency

- Causes of poor response to oral iron
  - non-compliance
  - ongoing losses
  - insufficient duration of treatment
  - high gastric pH
  - inhibitors of iron absorption
    - tannins, calcium
    - lead, aluminium
  - incorrect diagnosis
    - thalassaemia
    - anaemia of chronic disease
    - sideroblastic anaemia

Vitamin B12 Deficiency

- Cobalamin essential coenzyme for
  - synthesis of methionine from homocysteine
  - Methylcobalamin
  - requires 5-methyl-THF
  - S-adenosyl methionine is the principle methyl donor in numerous biological reactions
  - conversion of methylmalonyl CoA to succinyl CoA
  - Adenosylcobalamin
    - transported in plasma bound to transcobalamin II
    - contained only in animal products

Vitamin B12 Deficiency - Risk factors for vitamin B12 deficiency

- Maternal B12 deficiency
- pernicious anaemia
- vegetarian diet
- terminal ileal disease/gastric bypass/gastritis
- NEC
- Crohn’s disease
- Small bowel resection
- Blind loops/intestinal infections
- Pancreatic insufficiency

Vitamin B12 Deficiency - Inborn errors of vitamin B12 transport and metabolism

- Transport
  - Transcobalamin I and II deficiency- AR
  - Intrinsic factor deficiency-Juvenile PA
  - Immerslund Grasbeck syndrome – abnormality in cubulin gene results in failure of absorption of B12 in terminal ileum +/- proteinuria

- Utilisation
  - Methylmalonic aciduria
  - Methylcobalamin deficiency- distinct phenotype and biochemical abnormalities
  - Other- drugs NO (-methionine synthetase), PPI, metformin

Vitamin B12 Deficiency - Clinical manifestations:

- Megaloblastic anaemia
- macrocytic anaemia +/- leucopenia/thrombocytopenia
- Neurological
  - posterior columns
  - pyramidal tracts
  - peripheral neuropathy
  - depression
  - dementia
Vitamin B12 Deficiency

- Vitamin B12 deficiency in infancy:
  - period of normal development followed by developmental delay or regression
  - macrocytosis, anaemia and marrow changes may be mild or absent
  - +/- seizures
  - may be irreversible

Laboratory evaluation:
- FBE and film
  - oval macrocytes
  - anaemia +/- other cytopenias
  - hypersegmented neutrophils
  - +/- teardrop cells
- Bone marrow
  - hypercellular; left shift
  - megaloblastic; nuclear:cytoplasmic dysynchrony
  - giant metamyelocytes
- Raised LDH, homocysteine and methylmalonic acid
- Low serum B12 (except TCII deficiency)
- Holotranscobalamin level (measures B12/TCII)

Holotranscobalamin = "active B12"
- Has replaced serum B12 as investigation of choice at RCH
- Earliest marker of B12 deficiency
- More sensitive and specific than serum B12
- More sensitive than Hcy or MMA

Therapy:
- Cyanocobalamin 1000 μg IM daily for 1 week
  weekly for 3 weeks
  3 monthly for maintenance
- Pernicious anaemia: oral B12 1000 μg/d
- Infants of B12 deficient mothers: maintenance not required once stores replete - monitor to exclude inborn error of metabolism

Folate Deficiency

- Folates widely distributed in foods
  - Adult diet
    - 1/3 from meats and fish
    - 1/3 from cereals and bread
    - 1/3 from fruit and vegetables
  - Adequate quantities in breast milk but may be inadequate in cow’s milk
  - No folate in goat’s milk

- Folates act in numerous single carbon reactions
  - Synthesis of methionine from homocysteine
  - Purine and pyrimidine metabolism
- Circulates in plasma as 5-methyl THF
- Body folate stores limited to several weeks
- Acute folate deficiency may develop in hospitalised patients
Folate Deficiency

- Risk factors for folate deficiency
  - Poor absorption
  - Coeliac disease; Crohn’s
  - Parasitic infestations
  - Inadequate stores
  - Maternal deficiency
  - Prematurity
  - Increased demand
  - Pregnancy
  - Haemolysis (thalassaemia; sickle cell disease, congenital haemolytic anaemia)

- Drugs/toxins
  - Alcohol
  - Anticonvulsants
  - Oral contraceptives
  - Methotrexate
  - Bactrim

Folate Deficiency

- Clinical associations:
  - Megaloblastic anaemia
  - Anaemic crisis in chronic haemolysis
  - Neurological
    - Depression
    - Dementia
    - Psychosis
    - Cardiovascular disease
    - Malignancy
    - GIT, cervical

- Laboratory evaluation:
  - FBE, film and BM as for B12 deficiency
  - NB acute folate deficiency not macrocytic
  - Raised LDH, homocysteine but not methylmalonic acid
  - Folate assay
    - Serum folate
    - RCF

Folate Deficiency

- Therapy
  - Exclude vitamin B12 deficiency
  - Increase dietary folate
  - Folate 100 μg/kg/day
  - Preconception folate supplements for prevention NTD
  - Fefol/FGF inadequate in pregnant women with increased folate requirements

- Inborn errors of folate metabolism and transport
  - MTHFR deficiency
    - No megaloblastic anaemia
    - Neurological abnormalities and developmental delay
    - Homocysteinaemia and hypomethioninaemia
  - Rx with folate, MTHF, B12, pyridoxine, carnitine and betain
  - Hereditary folate malabsorption- AR
    - Megaloblastic anaemia, FTT and CNS abnormalities
    - May require parenteral folate
Folate Deficiency
- Inborn errors of folate metabolism and transport
- Thermolabile variant of MTHF reductase
- 10% population homozygous deficiency
- Mild homocysteinaemia
- Increased arterial thrombosis
- Venous thrombosis
- No clinical expression in childhood

Classification of Haemolysis
- Pathologic
  - Intrinsic
    - Abnormal haemoglobin
    - Red cell enzyme deficiencies
    - Red cell membrane disorders
  - Extrinsic
    - DIC
    - Drug-induced
    - Mechanical
    - Immune-mediated

Classification of Haemolysis
- Morphologic
  - Spherocytic-spherocytes, acanthocytes
  - Oxidative-bite and blister cells
  - Microangiopathic-fragments
  - Other-spurr cells, bizarre pyknocytes

Classification of Haemolysis
- Clinical
  - Sick versus well child
  - Congenital versus acquired
  - Associated with other abnormalities
    - Coagulopathy
    - Thrombocytopenia
    - Neurological abnormalities

Laboratory evaluation of Haemolysis
- Screening
  - FBE and film
  - Reticulocyte count
  - Blood group and Ab screen
  - Coomb's test
  - Biochemistry
    - Bilirubin
    - LDH
    - Haptoglobin

Laboratory evaluation of Haemolysis
- Further investigations
  - Flow cytometry for eosin-5 maleimide staining
  - Hb instability
  - Hb electrophoresis
  - RBC enzyme assays
    - G-6-PD
    - Pyruvate kinase
    - Others
Immune-mediated Haemolysis

- Primary AIHA
  - Warm - IgG
  - Cold - IgM

- Secondary AIHA
  - Systemic autoimmune disease eg SLE
  - Immune deficiency
  - Infections
  - Drugs
  - Malignancy esp lymphoma

- Secondary AIHA
  - Systemic autoimmune disease eg SLE
  - Immune deficiency
  - Infections
  - Drugs
  - Malignancy esp lymphoma

- Drug-associated AIHA
  - penicillins
  - cephalosporins
  - alpha methyldopa
  - quinidine/quinine
  - isoniazid
  - rifampicin

- Drug-associated AIHA
  - penicillins
  - cephalosporins
  - alpha methyldopa
  - quinidine/quinine
  - isoniazid
  - rifampicin

- Investigations
  - DCT
  - Warm - IgG and complement
  - Cold – complement only
  - Blood film
    - polychromasia
    - spherocytosis (warm Ab)
    - agglutination (cold Ab)
  - Haemophagocytosis (PCH)
    - Donath-Landsteiner Ab for PCH
    - Serology

- Investigations
  - DCT
  - Warm - IgG and complement
  - Cold – complement only
  - Blood film
    - polychromasia
    - spherocytosis (warm Ab)
    - agglutination (cold Ab)
  - Haemophagocytosis (PCH)
    - Donath-Landsteiner Ab for PCH
    - Serology

- Therapy:
  - Underlying disease
  - Infection-associated haemolysis usually self-limiting
  - Minimise transfusions
  - Immunosuppression
    - More effective for IgG vs IgM
    - steroids; second line agents
    - IVIG
  - Splenectomy - curative in 60-80%
  - Monoclonal anti-CD20 antibody

- Therapy:
  - IgM mediated cold haemolysis
    - warm extremities
    - plasma exchange
    - monoclonal Ab to CD20

- Therapy:
  - IgM mediated cold haemolysis
    - warm extremities
    - plasma exchange
    - monoclonal Ab to CD20
Red Cell Fragmentation Syndromes

- Endothelial Damage
  - Haemolytic-uraemic syndrome/TTP
  - Haemangioma (Kasabach-Merritt syndrome)
  - Autoimmune disorders eg. SLE
- Trauma
  - Extracorporeal circulation- ECMO
  - Cardiac malformations/prostheses- VAD

- Disseminated intravascular coagulation
  - Sepsis
  - Deficiency of natural anticoagulants (Purpura fulminans)
  - Malignancy
  - T-activation
  - Necrotising enterocolitis
  - Drugs eg. Cyclosporin, chemotherapy

Haemolytic-uraemic Syndrome

- Age 6 months-5 years in 90%
- Preceding diarrhoeal illness
  - E coli 0157:H7
  - Shigella
  - Salmonella
- Familial/relapsing forms
  - Anaemia, thrombocytopenia and renal impairment +/- fever and CNS disturbance

Haemolytic Uraemic syndrome

- Investigations
  - Microangiopathic haemolytic anaemia
  - Thrombocytopenia
  - Leucocytosis
  - Coags normal or only mildly abnormal
  - D-Dimers normal or only mildly increased
- Treatment
  - supportive
  - Plasma infusion or exchange for atypical HUS and TTP
  - Avoid platelet transfusions

TTP (Thrombotic Thrombocytopenic Purpura)

- Red cell fragmentation/haemolysis, low plt, renal dysfunction, neurological dysfunction, fever
- Deficiencies of Metalloprotease (ADAMTS 13) - enzyme responsible for breakdown of ULVWM, excess multimers cause intravascular fibrin linkage, fibrin strands mechanical red cell destruction with thrombosis
- Acquired causes: infection (HIV/pneumo), drugs (quinine, chemotherapy, cyclosporin, ticlopidine/clopidogrel), post BMT, pregnancy
- Inherited deficiency of ADAMTS 13, AR, Rx prophylactic FFP
- Acute Rx: plasma exchange- cryodeplete FFP (removed ULVWM)
- Significant mortality and morbidity: 50% fatality
- Congenital Form- Upshaw Schulman syndrome

Hereditary Spherocytosis

- Mechanism
  - deficiency of spectrin, ankyrin, band 3 or protein 4.2
  - Affects vertical stability of red cell membrane
  - membrane blebs 2nd to poor membrane attachment
  - spherocytes formed in spleen
- Increased Na+ flux across membrane
- Activation of K+-Cl- cotransporter
- Neonatal jaundice or anaemia
- Haemolysis, splenomegaly and anaemia
- 75% AD, 25% AR/spontaneous mutations
Red Cell Membrane Abnormalities

- Hereditary Spherocytosis
  - Variable numbers of spherocytes
  - Acanthocytes
  - Pincer cells
  - Raised MCHC

- Hereditary Elliptocytosis
  - Autosomal dominant
  - Mutation of α or β spectrin or protein 4.1
  - No spectrin tetramers formed
  - Membrane instability and fragmentation
  - Linkage to Rh and Duffy phenotype
  - Variable phenotype
  - Silent
  - Mild haemolysis
  - Severe haemolysis

Red Cell Enzyme Deficiencies

- G-6-PD deficiency
  - X-linked
    - hemizygous male
    - heterozygous female
  - homozygous female
  - Multiple mutations
  - Africa, Mediterranean, Middle East, SE Asia
  - Reduced production of NADPH and ability to reduce oxidant compounds

- G-6-PD deficiency
  - Osmotic fragility and autohaemolysis normal
  - Screening tests
    - Decolourisation assays
  - May miss heterozygotes
  - G-6-PD assay
  - False negatives with brisk haemolysis
Red Cell Enzyme Deficiencies

- **G-6-PD deficiency**
  - Neonatal jaundice
  - Usually male
  - Onset G2-3
  - Variable severity
  - Morphology usually non-specific
  - Jaundice > anaemia
  - ?Role of neonatal liver function and exogenous oxidant agents

Red Cell Enzyme Deficiencies

- **G-6-PD deficiency: Acute Haemolysis**
  - Exposure to exogenous oxidant or infection
  - Fever, abdo pain, pallor, dark urine and jaundice
  - Precipitous fall in Hb
  - Self-limiting

Red Cell Enzyme Deficiencies

- **G-6-PD deficiency: Chronic non-spherocytic haemolytic anaemia**
  - Chronic anaemia
  - Reticulocytosis
  - +/- Macrocytosis
  - Extravascular haemolysis
  - Acute exacerbations with oxidant stress

Red Cell Enzyme Deficiencies

- **Pyruvate Kinase deficiency**
  - Haemolysis due to abnormalities of enzymes of glycolytic pathway rare; 90% of cases due to PK deficiency
  - Impaired formation of ATP
  - Autosomal recessive or compound heterozygosity
  - Worldwide distribution esp. Northern Europe

Red Cell Enzyme Deficiencies

- **Pyruvate Kinase deficiency**
  - Clinical
  - Neonatal jaundice
  - Chronic haemolytic anaemia + splenomegaly
  - Diagnosis
    - Osmotic fragility normal or decreased
    - Autohaemolysis normal or increased with added glucose
    - Increased red cell 2,3 DPG
    - PK assay: false normal with reticulocytosis, leucocyte contamination or variant mutations
Red Cell Enzyme Abnormalities

- Pyruvate Kinase deficiency
  - Morphology: often non-specific
  - Anisopoikilocytosis
  - Polychromasia
  - Whiskered spherocytes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>% Normal &amp; Hb Enzyme Abn.</th>
<th>Inheritance</th>
<th>Clinical Features</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate Kinase</td>
<td>2-3 AR</td>
<td>AR</td>
<td>Moderate CNSHA</td>
<td>Prominent stippling</td>
</tr>
<tr>
<td>Glucose phosphate isomerase</td>
<td>3-5 AR</td>
<td>AR</td>
<td>Moderate CNSHA</td>
<td>Neuromuscular dysfunction in some cases</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>&lt;1 AR</td>
<td>AR</td>
<td>Mild CNSHA + myopathy +/− myoglobinuria</td>
<td>Dense spiculated cells in some cases</td>
</tr>
<tr>
<td>Aldolase</td>
<td>&lt;1 AR</td>
<td>AR</td>
<td>Mild CNSHA +/− neurological deficits</td>
<td>Dense spiculated cells in small numbers</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>&lt;1 AR/Frame AD</td>
<td>AR</td>
<td>Mild CNSHA</td>
<td>Dense spiculated cells in some cases</td>
</tr>
<tr>
<td>Threonine phosphate deaminase</td>
<td>&lt;1 AR</td>
<td>AR</td>
<td>Mild CNSHA + myoglobinuria +/− myoglobinuria</td>
<td>Dense spiculated cells in some cases</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>&lt;1 X-linked</td>
<td>X-linked</td>
<td>Mild CNSHA, neurological and cardiac abnormalities</td>
<td>Dense spiculated cells in some cases</td>
</tr>
<tr>
<td>Adenosine deaminase excess</td>
<td>&lt;1 AD</td>
<td>AD</td>
<td>Mild CNSHA</td>
<td>Dense spiculated cells in some cases</td>
</tr>
</tbody>
</table>

Non-G-6-PD related Oxidative Haemolysis

- Neonatal Oxidative Haemolysis ("Neonatal Pyknocytosis")
  - Multifactorial: impaired response to oxidant injury
  - Altered hexose monophosphate shunt
  - Decreased glutathione peroxidase
  - Decreased superoxide dismutase
  - Increased in premature neonates
  - Consider maternal drug exposure e.g. lignocaine, antibiotics
  - External factors: napthalene, circumcision

Non-G-6-PD related Oxidative Haemolysis

- Neonatal Oxidative Haemolysis
  - Bite cells
  - Blister cells
  - Spherocytes
  - Fragmentation
  - Osmotic fragility variable
  - G-6-PD normal or increased

Non-G-6-PD related Oxidative Haemolysis

- Drug-induced Oxidative Haemolysis
  - Dapsone
  - Sulphas
  - Nitrofurantoin
  - Nitrofurathione
  - Methylene blue
  - Unstable haemoglobins

Approach to the Well Neonate with Persistent Haemolysis

- FBE and film
- Blood group/DAT
- G-6-PD assay
- Observe

G-6-PD deficiency
- Neonatal oxidative haemolysis
- Hereditary Spherocytosis
- Observes

Non-G-6-PD related Oxidative Haemolysis

Drug-induced Oxidative Haemolysis

- Dapsone
- Sulphas
- Nitrofurantoin
- Nitrofurathione
- Methylene blue
- Unstable haemoglobins