# Haemolytic anaemias

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# Red Cell Destruction

- Extravascular in macrophages of RES (BM, liver, spleen)
- Haem to iron and protoporphyrin
- Globin to amino acids
- Hb binds to haptoglobin and the complex is removed by the liver. Haptoglobin is produced by the liver – low levels in haemolysis and liver disease
- Pathological red cell destruction may occur intravascular

# Clinical Features

- Anaemia (unless compensated)
- Jaundice (mild)
- Splenomegaly (many types)
- Ankle ulcers (e.g. sickle cell anaemia)
- Pigment gallstones
- Bone expansion in children due to marrow expansion (e.g. frontal bossing)
- Aplastic crises (Parvo virus or folate deficiency)

# Lab Features

- Normal or reduced Hb
- Biochemistry: raised unconjugated bilirubin, low haptoglobin, increased f-stercobilinogen & u-urobilinogen, raised LDH
- Increased reticulocyte count
- Bone marrow: hyperplastic erythropoiesis
- Blood film: polychromasia, normoblasts. red cell shape changes (e.g. schistocytes, spherocytes)

# Lab Features

- Radioactive chromium labeling of red cells
  - Measures lifespan
  - Assess sites of destruction
- Intravascular haemolysis
  - Increased plasma & urine Hb
  - Methaemalbumin
  - Urine-haemosiderin

# Causes of intravascular haemolysis

- Mismatched blood transfusions
- G6PD-deficiency with oxidant stress
- Red cell fragmentation syndromes
- Some auto-immune haemolytic anaemias
- Some drug and infection induced haemolytic anaemias
- Paroxysmal Nocturnal Haemoglobinuria
- March haemoglobinuria
- Unstable haemoglobins

# Classification of haemolytic anaemia

| Hereditary | Acquired |
|---|---|
| ▪Membrane<br>Hereditary spherocytosis<br>▪Metabolism<br>G6PD deficiency<br>▪Haemoglobin<br>HbS, HbC | ▪Immune<br>Autoimmune:<br>Warm antibody type: Idiopatic/secondary<br>Cold antibody type: Idiopathic/secondary<br>Alloimmune<br>Haemolytic transfusion reaction<br>HDN<br>▪Red cell fragmentation syndromes<br>▪Infection<br>▪Chemical and physical agents<br>▪PNH |

# Inherited Haemolytic Anaemias

## Membrane Abnormalities

1. Hereditary spherocytosis

- Whites, AD (variable severity); 25% new mutation
- Deficient/abnormal RBC membrane proteins (vertical)
- Lipid bilayer unsupported causing loss of lipid/membrane passing through RES (esp. spleen) – low surface area:volume
- Spherocytes less deformable and osmotically fragile
- $K^+$ loss and dehydration

# Clinical features

- Mild anaemia, splenomegaly, mild jaundice
- Severe transfusion-dependent anaemia
- Aplastic crises
- Increased haemolysis during infection
- Gall stones (50%)
- Lower leg ulcers
- Iron overload

# Laboratory features

- Hb varies
- Micropspherocytes and polychromasia (reticulocytosis), occasional normoblasts
- MCHC increased (50%)
- Increased osmotic fragility (median corpuscular fragility –MCF) and auto-haemolysis
- Negative direct antiglobulin test
- Red cell membrane protein analysis (**SDS-PAGE**, sodium dodecyl sulphate polyacrylamide gel electrophoresis)

Typical Graphs for RBC Osmotic Fragility

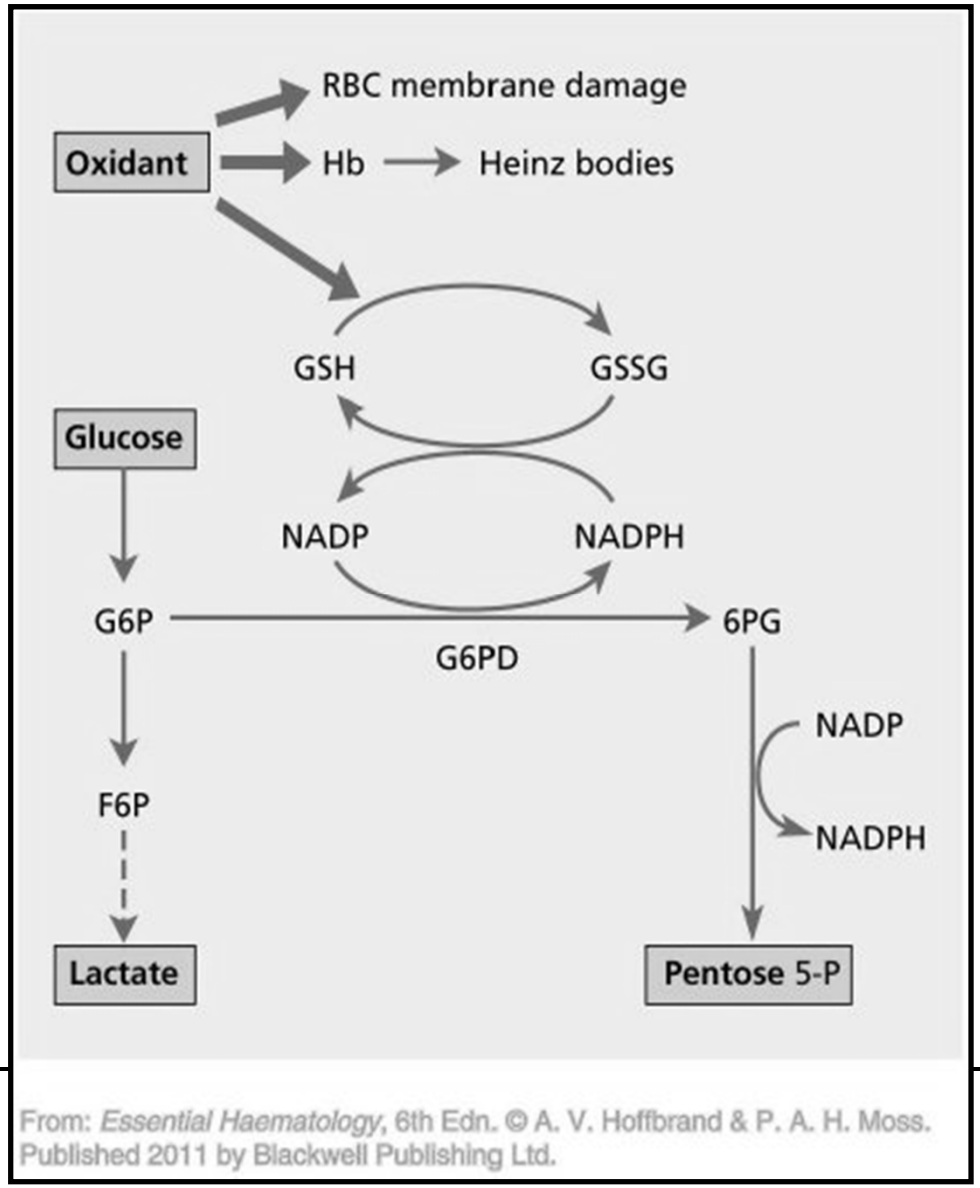
- Treatment
  - Folic acid
  - Blood transfusion
  - Splenectomy
  - Cholecystectomy

# Enzyme abnormalities

1. Glucose 6 phosphate dehydrogenase (G6PD) deficiency

- Deficiency renders cells susceptible to oxidant stress (e.g. drugs, infection, fava beans etc.)
- Inheritance is sex-linked
- Blacks, Mediterranean, middle eastern, oriental
- Protection against malaria

# Hexose monophosphate pathway



# Clinical picture

- Haemolysis 1-3 days after drug exposure or febrile illness or hours to days after ingestion of Fava beans
- Severe cases: abdominal/ back pain, dark urine
- Heinz bodies appear and Hb decreases rapidly
- Neonatal jaundice (impaired conjugation) esp. Gilbert disease
- Hereditary nonsherocytic haemolytic anaemia (chr jaundice, splenomegaly, gall stones)

# Laboratory features

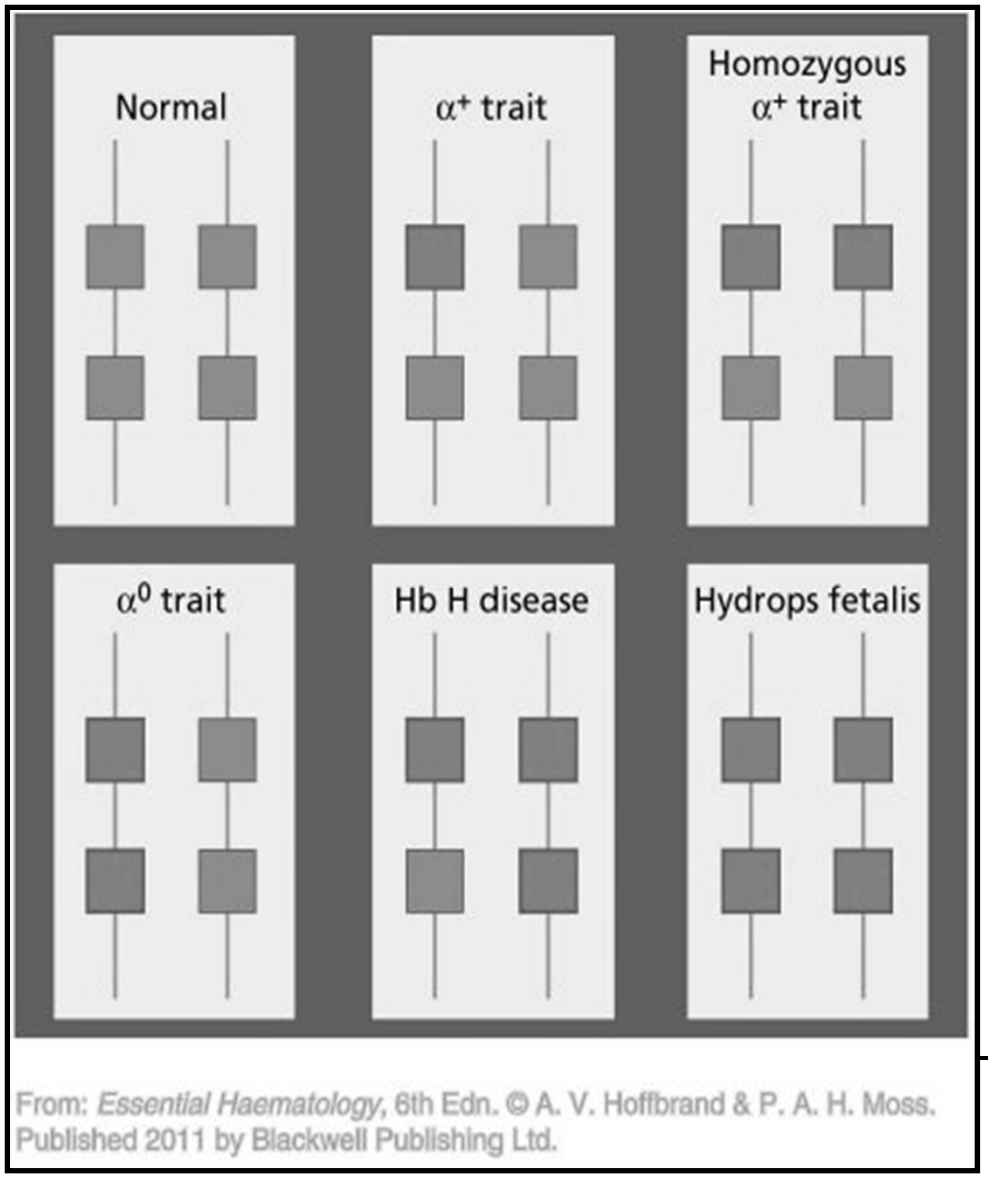
- Normal blood film between crises
- Bite / blister cells and polychromasia with acute intravascular haemolysis. Heinz bodies on supravital staining
- Screening test (fluorescent spot test) for generation of NADPH in steady state
- Enzyme assays and DNA analysis

- Management
  - Stop offending drug, treat infection, transfuse red cells, consider splenectomy

# 1. Thalassaemia

- Commonest single gene disorders
- Mediteranean region hence thalassaemia (greek Θαλασσα, meaning ‘the sea’)
- Autosomal recessive inheritance
- α or β depending on reduced synthesis of α or β globin chains

# The genetics of α-thalassaemia

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- Normal (αα/αα); silent carrier (α-/αα)
- α -Thal trait (α-/α-) or (--/αα). Low MCV, MCH
- HbH disease (--/α-). Hypochrome, anaemia, splenomegaly HbH
- Hydrops fetalis (--/--)

# Phenotype

- B thalassaemia ($\beta^o/\beta^+$)
  - Minor: symptomless carrier state
  - Intermedia: anaemia and splenomegaly but does not require regular transfusions
  - Major: severe, transfusion dependent

# Clinical features

- Anaemia (3-6 months), milder cases later ≤ 4 years
- Failure to thrive, intercurrent infection, mild jaundice
- HSM, expansion of bones
- Iron overload

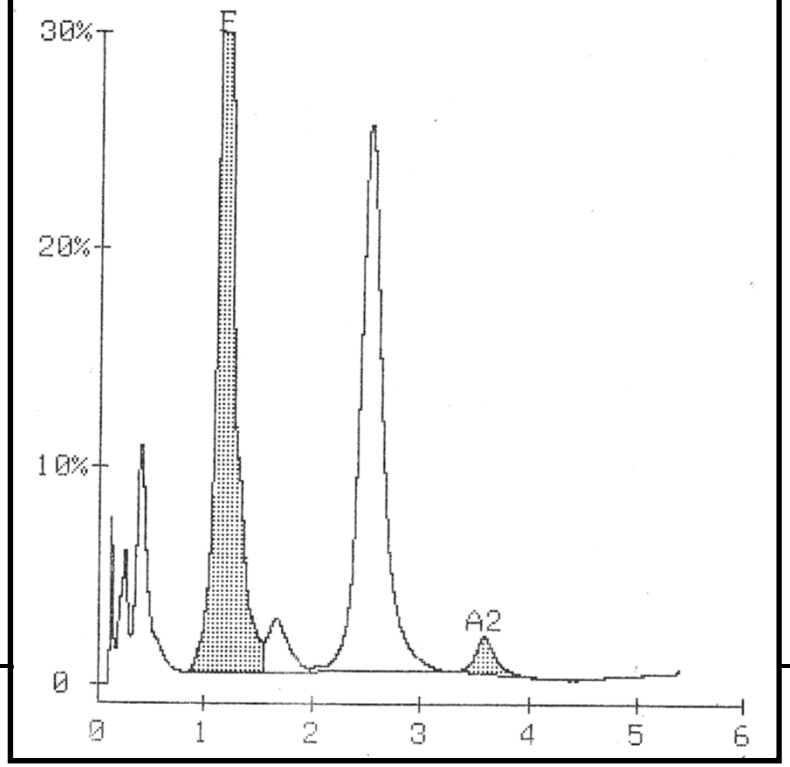
# Laboratory features

- Blood film: Hypochromic microcytic anaemia, target cells and erythroblasts
- Raised RCC >5.5 X $10^{12}$/l
- HPLC
- Hb electrophoresis:
  - B thalassaemia maj: HbF 10-90%, HbA absent if $\beta^o$ thal.
  - B thalassaemia min: HbA2 > 3,5%
- Hypercellular bone marrow (erythroid hyperplasia)
- Globin chain synthesis studies
- DNA analysis shows specific mutations

# Haemoglobin electrophoresis/ HPLC

| ANALYTE ID | % | TIME | AREA |
|---|---|---|---|
| F | 56.0 | 1.18 | 648377 |
| P3 | 3.3 | 1.67 | 37314 |
| Ao | 36.6 | 2.52 | 410546 |
| A2 | 2.3 | 3.58 | 24921 |
| | TOTAL AREA | | 1121158 |

F 56.0% A2 2.3%



# Management

- Maintain Hb > 9 – 10 g/dl
- Iron chelation therapy
- Immunise: hep B
- Splenectomy
- Bone marrow transplant

# Acquired Haemolytic Anaemias

Autoimmune haemolytic anaemia (AIHA)

Warm-type

- Red cells coated with IgG alone or complement
- 37$^{o}$C
- Taken up by the RE macrophages
- Part of coated membrane is lost $\rightarrow$ progressively more spherical

# Warm-type AIHA

- Idiopathic
  - 30%
  - Any age (infancy, early childhood, 3rd decade, > 5th decade)
  - Evans syndrome
  - AIHA of infancy and childhood
- Secondary
  - SLE, other autoimmune diseases
  - CLL, lymphomas
  - Drugs(e.g. fludarabine, methyldopa)
  - Malignancies
  - Viral infections

# Clinical and Lab features

- Pallor or jaundice
- Splenomegaly (2-3 cm under costal margin)
- Blood film: microspherocytes, polychromasia, ± normoblasts
- Direct antiglobulin test positive
- IgG or IgG and complement

# Treatment

- Corticosteroids (1-2mg/kg/d)
- Cytotoxic immunosuppresive drugs
  - Azathioprine (1.5-2 mg/kg/d)
  - Cyclophosphamide (1.5-2mg/kg/d)
- Cyclosporin (5mg/kg/d in 2 divided doses)
- IVIG (0.4mg/kg/d X 4-5 days)
- Splenectomy
- Blood transfusion

# Cold-type AIHA

- Idiopathic cold haemagglutinin disease
  - Chronic course in older people
  - Acrocyanosis in cold weather
  - Spontaneous agglutination of red cells
  - DAT shows C3d
- Secondary
  - Infections: mycoplasma pneumonia, infectious mononucleosis
  - lymphoma
- Paroxysmal cold haemoglobinuria

# Cold-type AIHA

- Ab whether monoclonal (ICHD) or polyclonal (infection) attaches to membrane in cooler peripheral circulation
- Usually IgM and binds best at 4$^{o}$C
- Fixes complement and extra- and intravascular haemolysis can occur
- Spherocytes less marked, agglutinated red cells

# Treatment

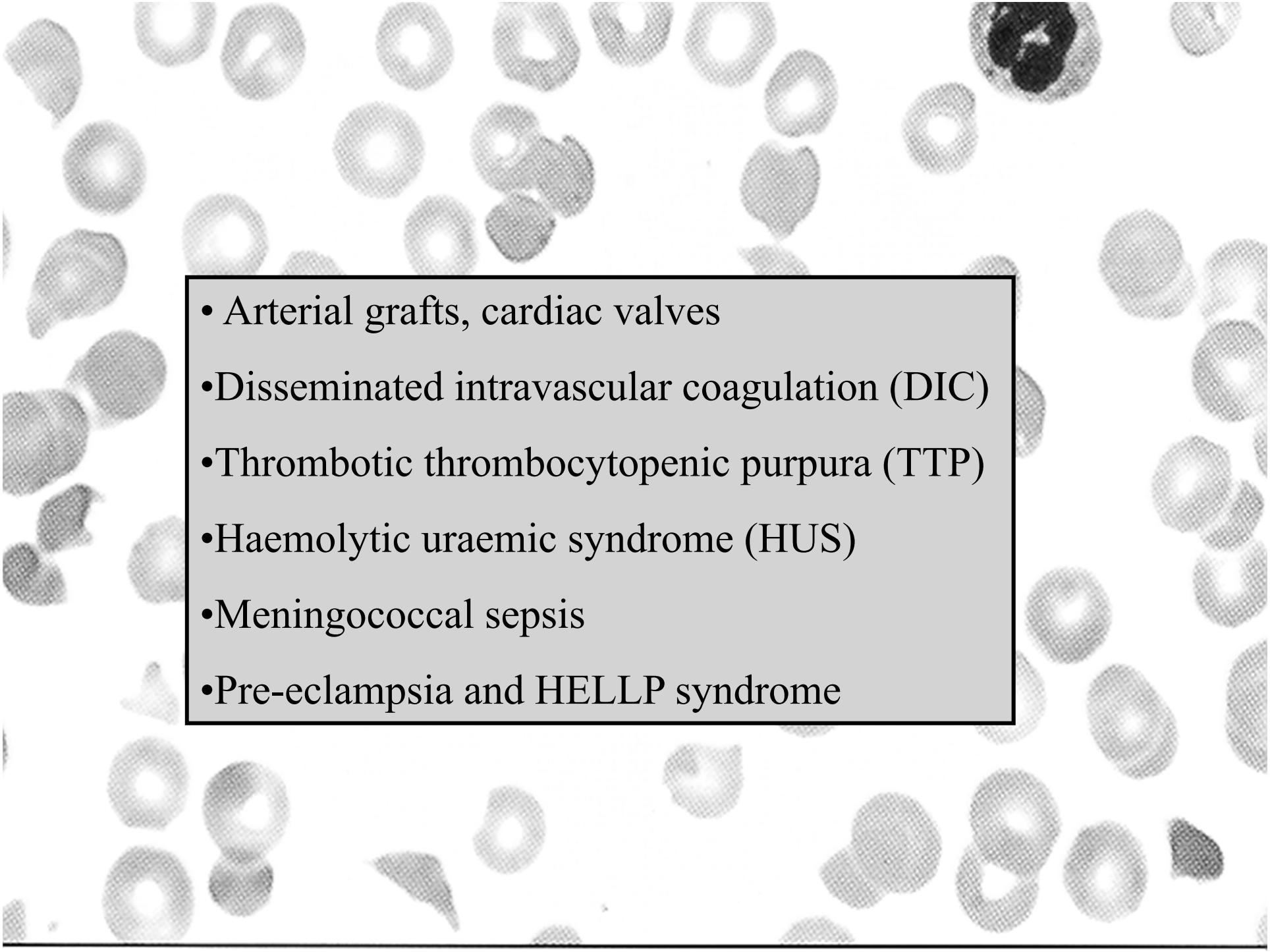
- General: management is difficult, avoid exposure to cold, folic acid supplements
- Alkylating agents: Chlorambucil intermittent or continuous
- Corticosteroids and splenectomy are rarely of use
- Blood transfusion and plasma exchange

# Drug induced immune haemolytic anaemia

- Ab against drug-red cell membrane complex. Drug acts as hapten (penicillin)
- Ab against drug-plasma protein complex $\rightarrow$ subsequent deposition of immune complexes on red cells (quinidine, rifampicin)
- Stimulation of auto Ab against red cells (methyldopa, fludarabine)

# Red cell fragmentation syndromes

- Physical damage to red cells on abnormal surfaces or fibrin strands deposited in small vessels
- Termed microangiopatic haemolytic anaemia (TTP/HUS, pre-eclampsia, meningococcal septicaemia, adeno Ca)
- Haemolysis intra and extravascular
- Fragmented red cells



- Arterial grafts, cardiac valves
- Disseminated intravascular coagulation (DIC)
- Thrombotic thrombocytopenic purpura (TTP)
- Haemolytic uraemic syndrome (HUS)
- Meningococcal sepsis
- Pre-eclampsia and HELLP syndrome

# Infections

- Direct damage to red cells (malaria)
- Toxin production (clostridium)
- Oxidant stress (G6PD- deficiency)
- MAHA (meningococcal septicaemia)
- Antibody formation (infectious mononucleosis)
- Extravascular destruction (malaria)

# Chemical and physical agents

- Certain drugs → oxidative intravascular haemolysis (dapsoon, salazopyrin)
- Wilson's disease → acute haemolysis due to high levels of copper in the blood
- Chemical poisoning with lead, arsine
- Severe burns damages red cells

# Paroxysmal nocturnal haemoglobinuria

- Clonal disorder, acquired mutation of X-chromosome gene coding for phosphatidyl inositol glycan A (PIG-A)
- Result = Glucosyl Phosphatidyl Inositol (GPI)-linked proteins are absent from the red cell e.g:

  Decay-activating factor (DAF, CD55)

  Membrane inhibitor of reactive lysis (MIRL, CD59)

# PNH – general features

- Red cells are sensitive to lysis with complement causing chronic intravascular haemolysis
- Haemosiderinuria leads to iron deficiency
- Recurrent venous thrombosis and infections are prevalent
- The marrow may be hypoplastic with neutropenia and thrombocytopenia

# Diagnosis

- Positive acid lysis (Ham's) test
- Absence of CD55 and CD59 antigens

# Management

- Supportive (folic acid, iron if deficient, blood transfusions, short course steroids, oral anticoagulation)
- BM transplant and immunosuppressive R

# References


- Mehta A, Hoffbrand V. Haematology at a glance. 3rd Edition. Blackwell Science. 2009

- Hoffbrand V, Pettit J, Moss P. Essential haematology. 6th Edition. Blackwell Science. 2011