Anaemia is defined as a reduction in the total red cell circulating mass below normal limits. This is difficult to measure, so instead diagnosis is usually based on a reduction in haematocrit and haemoglobin concentration of the blood below normal levels.

Anaemia may be caused by too few RBCs or too little haemoglobin in the cells.

Types of anaemia

Anaemias can be defined as intrinsic (inherent defects due to leukaemia or autoimmune disease) or extrinsic (drug-induced for example).

- **Haemolytic anaemia** → excess destruction of RBCs which exceeds regenerative capacity
  - Immunohaemolytic → autoantibodies target RBCs for destruction
  - Genetic haemolytic → gene defects that render RBCs more likely to be destroyed prematurely
  - Trauma → increased RBC breakdown due to cardiac valves etc.

- **Non-haemolytic anaemia**
  - Blood loss → acute or chronic
  - Diminished erythropoiesis → vitamin deficiencies or bone marrow failure

How to reach a diagnosis

- **Red cell size** → normocytic, microcytic, macrocytic
- **Degree of nuclear material** → normochromic, hyperchromic, hypochromic, polychromatic (suggests level and speed of erythropoiesis)
- **Red cell shape** → biconcave discs, spherical, pencil, crescent

Microcytic hypochromic → disorders of haemoglobin synthesis
Macrocytic → impaired maturation of erythroid precursors
Normocytic normochromic → many causes, red cell shape helps determine which one
HAEMOLYTIC ANAEMIA

Haemolytic anaemias are associated with shortened RBC lifespan, elevated EPO levels and compensatory increases in erythropoiesis and accumulation of haemoglobin products as a result of increased haemolysis.

There are different types of haemolysis:

- Extravascular haemolysis
  - Red cell deformity causes them to sequester in the spleen and get phagocytosed by splenic macrophages
  - Clinical features → splenomegaly and jaundice
- Intravascular haemolysis
  - Red cells damaged by mechanical injury, complement fixation, parasites or toxins cause their degradation within the vasculature
  - Clinical features → haemoglobinuria (free Hb in urine), haemoglobinemia (excess Hb in plasma), haemosiderinuria (haemosiderin in urine)

Epidemiology

Haemolytic anaemias account for only 5% of all anaemias and are not specific to any race. They most often present in middle aged and older individuals and are uncommon before the age of 30. Autoimmune haemolytic anaemias are more likely to affect women than men.

Warm Type Immunohaemolytic anaemia

- Rare → 1:100,000 affected
- Most common form of immunohaemolytic anaemia

Due to a breakdown in tolerance, IgG (and less commonly IgA) autoantibodies are produced, mainly against Rhesus antigen on RBCs. IgGs (which have maximum binding capacity at 37°C) coat RBCs and bind to Fc receptors on phagocytes leading to partial phagocytosis. This occurs mainly in the spleen, because splenic macrophages have the best affinity for the Fc region of immunoglobulins.

Repeated partial phagocytosis of RBCs gradually destroys red cell membranes, converting them from biconcave discs to spherocytes, as they assume the smallest diameter for their volume. Cell membrane destruction renders RBCs less deformable and their spherical shape prevents them from squeezing through splenic sinusoids. This results in red cell sequestration within the spleen and phagocytosis by resident macrophages.

Signs & Symptoms

- Fatigue
  - Increased RBC haemolysis → exceeds regenerative capacity of bone marrow → reduced RBCs in circulation → reduced oxygen transport to tissues
- Breathlessness on exertion
  - Fall in haematocrit → decreased resistance to blood flow → increased venous return → increased cardiac output → on exercise, heart cannot pump much greater quantities of blood needed for oxygen demand by tissues
- Syncope (fainting)
  - Reduced RBC in circulation → reduced oxygen supply to tissues and brain
• Tachycardia
  o Reduced oxygen supply to tissues → low pO\(_2\) sensed by peripheral chemoreceptors → activates cardiovascular control centre in medulla → increased sympathetic tone → increased HR (in an attempt to circulate reduced oxygen around the body)

• Pallor
  o Lack of circulating RBCs to tissues

• Jaundice
  o Increased red cell lysis → increased production of unconjugated bilirubin from haem by macrophages → too much bilirubin to be conjugated in hepatocytes → excess spills into the blood → accumulation of unconjugated bilirubin in skin → jaundice

• Dark urine
  o Increased red cell lysis and bilirubin production → Increased bilirubin conjugation in hepatocytes → conjugated bilirubin excreted into GI tract and converted to colourless urobilinogen → more gets reabsorbed into blood and filtered out into renal tubules where it’s oxidised to yellow urobilin → increased urobilin production, darker yellow urine
  o Increased intravascular haemolysis → large amounts of free Hb released into blood → bound by haptoglobin that prevents Hb clearance from kidneys → depletion of haptoglobin → free Hb oxidised to methemoglobin → renal PCT reabsorbs most Hb and methemoglobin but some passes out into urine → urine becomes red/brown

• Splenomegaly
  o Increased red cell lysis by splenic macrophages → macrophage hyperplasia
  o Sequestration of non-deformable spherocytes in splenic cords

Investigations & RBC Morphology

• Haematocrit
  o Will be low, due to excessive haemolysis which exceeds regenerative capacity

• Low haemoglobin
  o Excessive RBC haemolysis → exceeds regenerative capacity of bone marrow → normocytic anaemia but reduced haematocrit therefore less Hb

• Marginally high WBC
  o Increased rate of haematopoiesis to compensate for reduced RBC number → low oxygen tension stimulates increased sympathetic tone & inflammation triggers cytokine release → stimulates release of growth inducers (GM-CSF etc) and cytokines → increased haematopoiesis
  o Could also be due to infection, but less likely if only marginally high

• Very high platelets
  o Increased rate of haematopoiesis to compensate for reduced RBC number → low oxygen tension stimulates increased sympathetic tone & inflammation triggers cytokine release → stimulates release of growth inducers (GM-CSF etc) and cytokines → increased haematopoiesis

• Blood film
- Leukoerythroblastic → presence of immature red cells (normoblasts) and white cells (myelocytes and promyelocytes) → due to increased amount and rate of haematopoiesis → immature cells released into circulation early
- Polychromatic cells → different coloured cells as immature erythroblasts (reticulocytes) are “rushed” through maturation and released into circulation immaturely, with varying amounts of nuclear material → indicates increased erythropoiesis is occurring
- Spherocytes → destruction of RBC membranes by phagocytosis due to opsonisation by autoantibodies → cells assume spherical shape to attain the smallest diameter for their volume → indicates red cell damage
- Normoblastic cells → normal cell production, just excessive destruction
  - **Positive direct antibody test (particularly with IgG and C3d)**
    - An anti-antibody will agglutinate with autoantibody or complement bound to the surface of the patient’s RBCs → positive tests indicates the presence of IgG and C3d → indicates immunohaemolytic anaemia
- **High unconjugated bilirubin**
  - Increased haemolysis → increased unconjugated bilirubin produced
  - Conjugated bilirubin should stay normal, as the hepatocytes will conjugate it as a steady rate
- **Normal LFTs**
  - Rules out liver disease as a cause of jaundice
- **Normal thyroid function tests**
  - Rules out other autoimmune diseases as cause of tachycardia etc.
### Cold Type Immunohaemolytic Anaemia

- Very rare $\rightarrow$ 1:1,000,000 affected

A breakdown in tolerance leads to the production of IgM autoantibodies against polysaccharide I antigens on RBCs. IgM antibodies are produced following T cell independent B cell activation. They have maximum binding capacity at temperatures below 37°C and tend to form immune complexes in the peripheries, such as the fingers, toes and ears. IgM tends to bind antigen with lower affinity, so requires a lower temperature to have an effect here.

IgM fixes complement really well and when cells leave the peripheries to enter warmer regions, IgM detaches and leaves complement bound to the RBCs. Kupffer cells have a high affinity for C3b, so extravascular haemolysis tends to occur in the liver.

Cold type can occur transiently following infection with EBV, cytomegalovirus and influenza virus.

### Signs & Symptoms

- Raynaud syndrome like symptoms $\rightarrow$ painful cyanosis of peripheries in the cold $\rightarrow$ pentavalent IgMs are bulky and form bulky immune complexes in tiny vessels within the peripheries $\rightarrow$ restricts blood flow $\rightarrow$ ischaemia $\rightarrow$ build-up of lactate and other metabolites due to anaerobic respiration $\rightarrow$ pain

### Investigations & RBC Morphology

- Normocytic, polychromatric anaemia with spherocytes due to red cell phagocytosis
- Low Hb, low haematocrit
- Positive Coombs test for IgM and breakdown products of C3b (C3d)

### Drug induced haemolytic anaemias

- **Hapten mechanism**
  - Ie. penicillin, cephalosporin, warm type
  - IgGs recognise RBC-drug complex
  - Abs no longer produced after removal of drug
- **Autoimmune mechanism**
  - Ie. methyldopa
  - IgG may recognise drug alone or RBC alone
  - Abs still produced against RBC after removal of drug
- **Immune complex mechanism**
  - Ie. quinine
  - IgM recognise drug or drug-RBC complex

Carbimazole has been linked to the development of drug-dependent antibodies against certain cell types and in one study caused neutropaenia, thrombocytopenia and anaemia due to antibody production against rhesus antigens on RBCs. These findings are very rare though, which is why the drug is still commonly used in the treatment of hyperthyroidism.
Management & Treatment of immunohaemolytic anaemias

- **Transfusion** → only if necessary as will also be susceptible to haemolysis. Consider splenectomy as the enlarged spleen will be exhibit hypersplenism and degrade erythrocytes at a faster rate, even in the absence of autoantibodies.

- **Folate** → helps increase red cell production. Vitamin B12 stores can last for 4 years, whilst folate stores may not last so long. Folate is involved in the folate cycle which contributes to the formation of thymidylate from dUMP via thymidylate synthase. Thymine is needed for DNA synthesis, which is important in rapidly diving cells such as RBCs.

- **Prednisolone** → suppresses the inflammatory response that is leading to excessive haemolysis. Beware of side effects. Prednisolone no longer needed after splenectomy.

- **Rituximab** → monoclonal antibody that targets B cells. Can be used in severe cases to target self-reactive B cells, thereby preventing production of autoantibodies.

Differential Diagnosis

- Liver disease?
  - Jaundice may indicate this
  - Check for nails, hepatomegaly, spider naevi, bruising etc
  - Check LFTs
  - Conjugated bilirubin should be normal if not liver disease as hepatocytes are functioning normally

- Lymphoma?

- Chronic bleed?

- Vitamin deficiency?

- Genetic defect of RBCs?

- RBC trauma → RBC damage through narrowed vasculature in atherosclerosis? etc

Long Term Effects

Warm type haemolytic anaemia is usually chronic. In acute cases such as autoimmune haemolytic anaemia, transfusion may be required followed by a splenectomy, in order to prevent the blood being destroyed by the overactive spleen. Splenectomy increases the risk of infection. Patients are advised to have regular vaccinations, wear a pendant to notify healthcare professionals they are asplenic and take lifelong antibiotics (amoxicillin or erythromycin if allergic), or carry antibiotics with them in case of an infection.

Consider the effects of prednisolone therapy, along with having to take lifelong antibiotics following splenectomy. Patients may be at risk of harbouring antibiotic resistant microbes which not respond to antibiotic therapy after a while. Patients are advised to take care with regards to overseas travel due to their compromised immunity.

Additional Points

- Direct and indirect Coombs test (direct antiglobulin test)
  - The direct test is used to determine whether RBC-binding antibody (IgG) or complement (C3) is present on RBC membranes. The patient’s RBCs are incubated with antibodies to human IgG and C3. If IgG or C3 is bound to RBC membranes, agglutination occurs → positive result
The indirect test is used to detect IgG antibodies against RBCs in a patient’s serum. The patient’s serum is incubated with reagent RBCs, then antibodies to human IgG or human anti-IgG is added.

Drug induced haemolytic anaemia

- In immunohaemolytic anaemias, complement is activated via the classical pathway. IgM is better at fixing complement because it is pentavalent and can therefore bring more C1 molecules together

GENETIC HAEMOLYTIC ANAEMIA

Hereditary spherocytosis

- Diverse mutations of spectrin and Ankyrin (proteins of RBC membrane) affect stability of the red cell membrane
- Frameshifts or premature stop codons create non-functional proteins
- The unstable membrane sheds fragments as the cell ages → loss of membrane relative to cytoplasm forces cell to adopt spherical shape
- Reduced cell deformability → sequestration in splenic cords → increased phagocytosis
- Clinical features → splenomegaly, jaundice

Glucose-6-phosphate dehydrogenase deficiency

- This enzyme reduces NADP to NADPH which regenerates reduced glutathione
- Reduced glutathione protects against oxidative stress within RBCs
- GP6D variants undergo misfolding and more susceptible to proteolytic degradation. Mature red cells don’t produce new enzymes so as they lose more G6PD they get more vulnerable to oxidative stress
- Increased loss of G6PD → increased vulnerability to oxidative damage → high levels of oxidants denatures globin chains forming Heinz bodies → macrophages partially phagocytose the Heinz bodies, damaging RBC membranes → forms spherocytes which get trapped in spleen → increased haemolysis in spleen
- Can be caused by genetic defects, oxidant drugs or oxidant-generating foods

Sickle cell disease

- Point mutation in beta-globin (one of 4 globin chains) → polymerisation of deoxygenated Hb → Hb polymers protrude from red cell wall → red cell distortion → sequester in spleen and phagocytosed
- Distorted red cells also cause microvascular obstruction and ischaemic tissue damage

Thalassaemia

- Inherited mutation → decreased synthesis of alpha and beta globin chains in Hb → red cells are underhaemoglobinised, hypochromic and microcytic → subnormal oxygen transport capacity → diminished red cell survival → anaemia, tissue hypoxia and red cell haemolysis
TRAUMA INDUCED HAEMOLYTIC ANAEMIA

Trauma to erythrocytes can be caused by:

- Marathon running, karate chopping, bongo drumming!
- Artificial cardiac valves → shear forces from turbulent flow can damage red cells

NON HAEMOLYTIC ANAEMIA

Blood loss

- Acute → trauma
- Chronic → GI bleed, peptic ulcer etc

Anaemias caused by diminished erythropoiesis

Vitamin deficiencies can cause impaired haemoglobin or DNA synthesis which can lead to the production of abnormal red cells.

- Vitamin B12 deficiency
  - Can be due to pernicious anaemia or ileal resection (impaired absorption) or decreased intake
  - Pernicious anaemia associated with other AIDs
  - Vitamin B12 required for DNA synthesis → important for rapidly dividing RBCs
  - Vitamin B12 injections can be given

- Folate deficiency
  - Due to decreased uptake (alcoholism, poor diet), impaired absorption, increased requirement (pregnancy), drugs (methotrexate)
  - Folate needed for thymine production → important for rapidly dividing RBCs
  - Oral folate can be given

- Iron deficiency
  - Due to poor diet, chronic or acute bleeds, competitors for iron transport, resection of iron-absorbing parts of GI tract, increased pH which converts iron into less-absorbable state (PPIs, antacids), plant metabolites that chelate iron (tannins), increased requirement (pregnancy, growing children)
  - Oral iron can be given, but can cause GI upset

Bone marrow failure can also cause anaemia.

- Aplastic anaemia
  - Chronic primary haematopoietic failure and pancytopenia (anaemia, neutropenia, thrombocytopenia)
  - Acquired causes include idiopathic, acquired stem cell defects and immune mediated causes
  - Other causes include chemical and physical agents (viral infection – EBV, varicella zoster) whole body irradiation
SUMMARY

Haemolytic anaemia

- Spherocytes: Normocytic, normochromic (possible polychromatic), leukoerythroblastic, spherocytes
- Pallor, tachycardia, jaundice, dark urine, possibly bilirubin gallstones
- Splenomegaly if extravascular haemolysis
- Haemoglobinuria (Hb in urine) and haemoglobinemia (Hb in blood) if intravascular haemolysis
- Positive Coombs test if autoimmune related → consider whether there’s a family history of AID, what medication patient is taking, any recent infection? Etc.

Iron deficiency anaemia

- Hypochromic, microcytic
- Low serum iron and ferritin
- Glossitis (atrophy of tongue), koilonychia, alopecia, pallor, angular stomatitis
- Negative Coombs test

Vitamin B12/folate deficiency anaemia

- Macrocytic cells (impaired DNA synthesis leads to production of abnormally large cells)
- Polychromatic cells (increased erythropoiesis)
- Vitamin B12 deficiency → weakness, glossitis, weight loss, tachycardia, constipation, nausea, angular stomatitis, cognitive impairment, dementia and depression
- Folate deficiency → glossitis, angular stomatitis, weight loss, nausea, cognitive impairment, dementia and depression