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Biochemical and Haematological Parameters in Disease

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Where opportunity creates success

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Lesson Plan

At the end of this session, students should be able to:

- Describe the types of disorders that affect haemoglobin including pathogenesis and diagnosis criteria
- Describe the biochemical parameter useful in the characterisation of cardiovascular, and liver and biliary disease.
- Explain the immunological and haematological parameters reported in a full blood count

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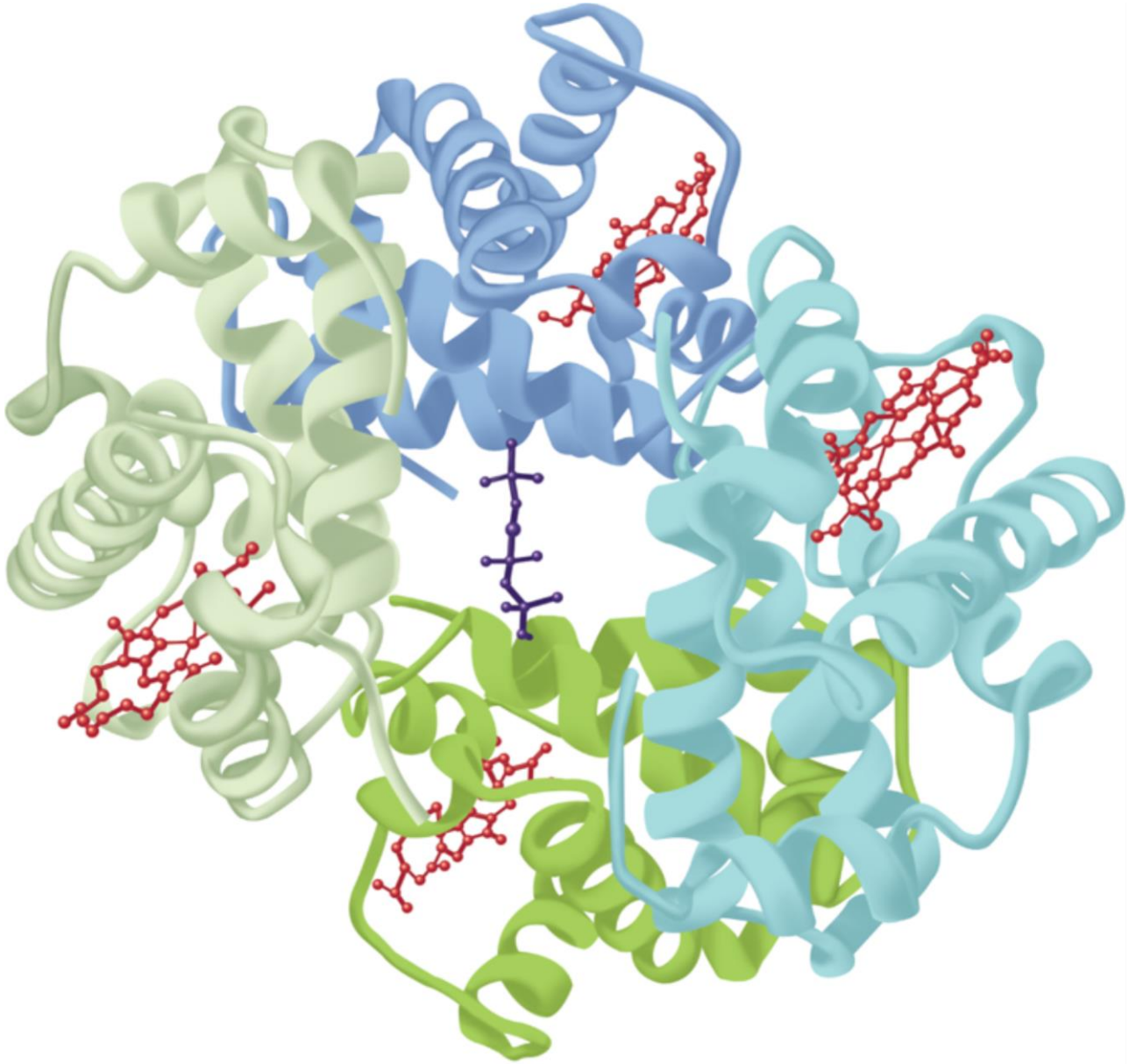
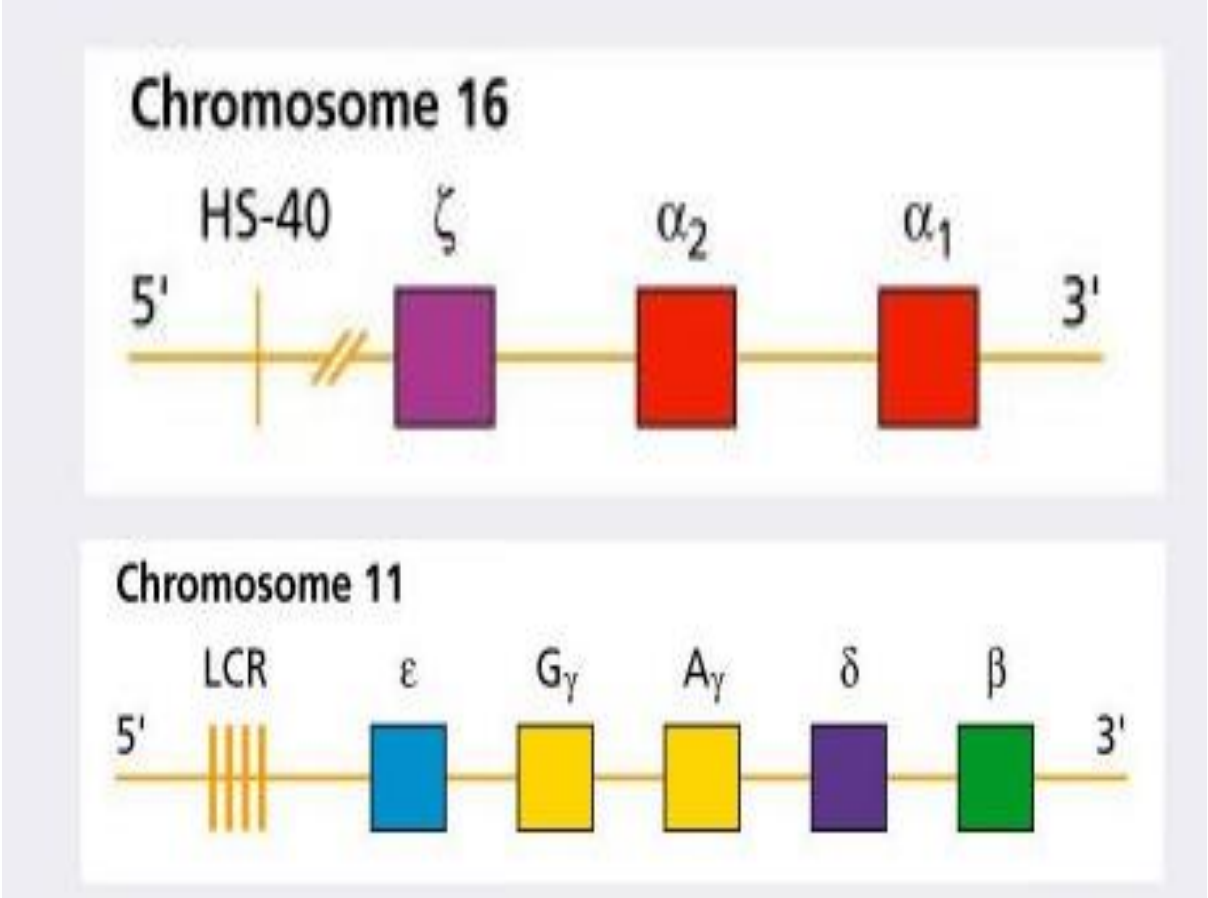
Haemoglobin



Globin Genes

Each haemoglobin molecule contains 4 globin protein chains.

In adults the predominant form is $\alpha_2\beta_2$, but there are other α-like (encoded on chromosome 16) and β-like chains (encoded on chromosome 11)



Physiological Forms of Haemoglobin

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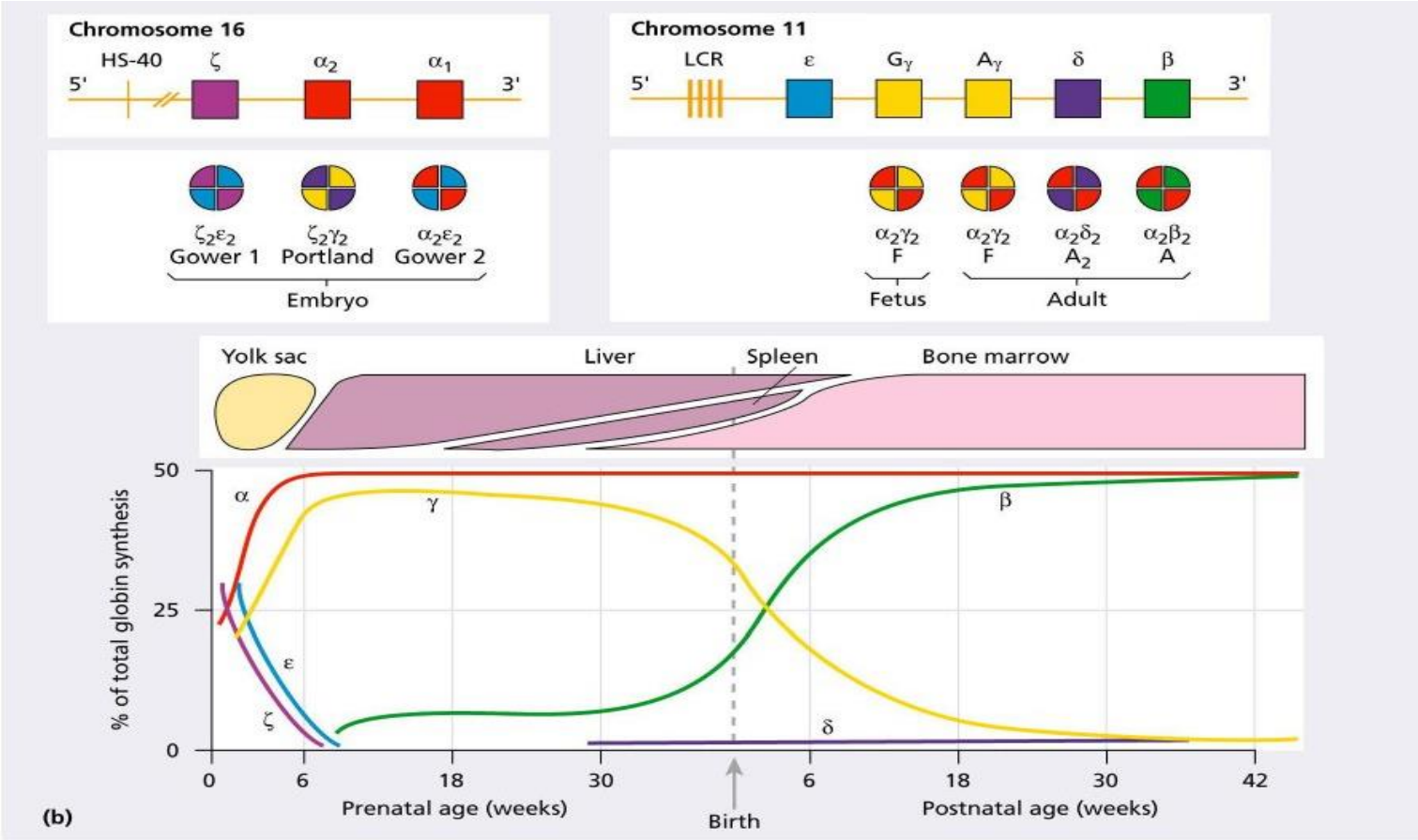
Type	Chains	Nomenclature	
Adults	$\alpha_2\beta_2$	A	
	$\alpha_2\delta_2$	A_2	
Fetal	$\alpha_2\gamma_2$	F	
Embryonic	$\alpha_2\varepsilon_2$	'Gower 2'	Present in early fetal life only
	$\zeta_2\varepsilon_2$	'Gower 1'	
	$\zeta_2\gamma_2$	Portland	

Physiological Forms of Haemoglobin

At birth, HbF ($\alpha_2\gamma_2$) is dominant, by ~20 weeks post birth is adult profile

In adult

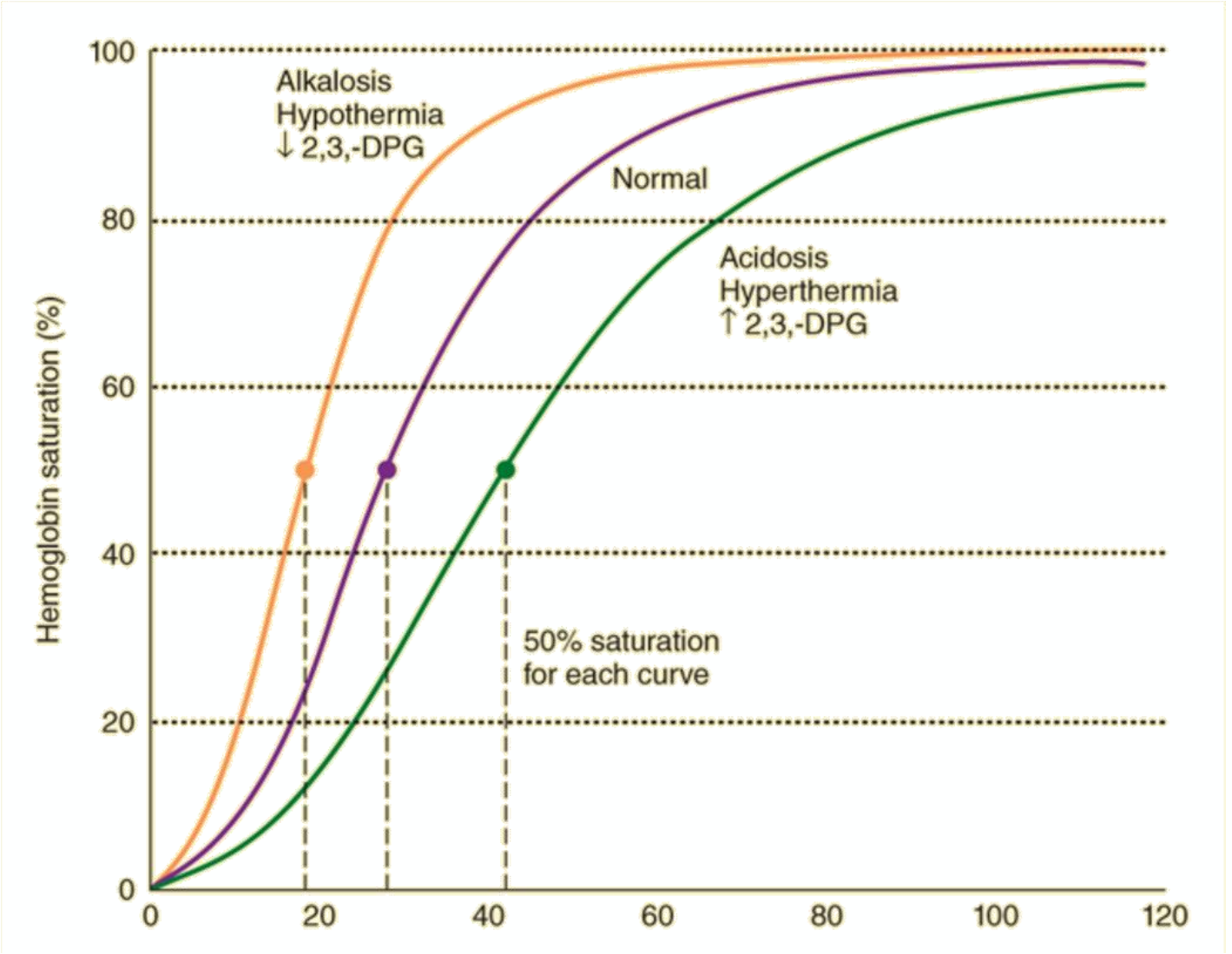
~97% HbA ($\alpha_2\beta_2$),
~2% HbA_2 ($\alpha_2\delta_2$)
< 1% HbF ($\alpha_2\gamma_2$)



Oxygen Binding

Binding of oxygen to haemoglobin is influenced by a number of factors

2,3-Bisphosphoglycerate (BPG, also known as 2,3 DPG) promotes O_2 release in the tissues

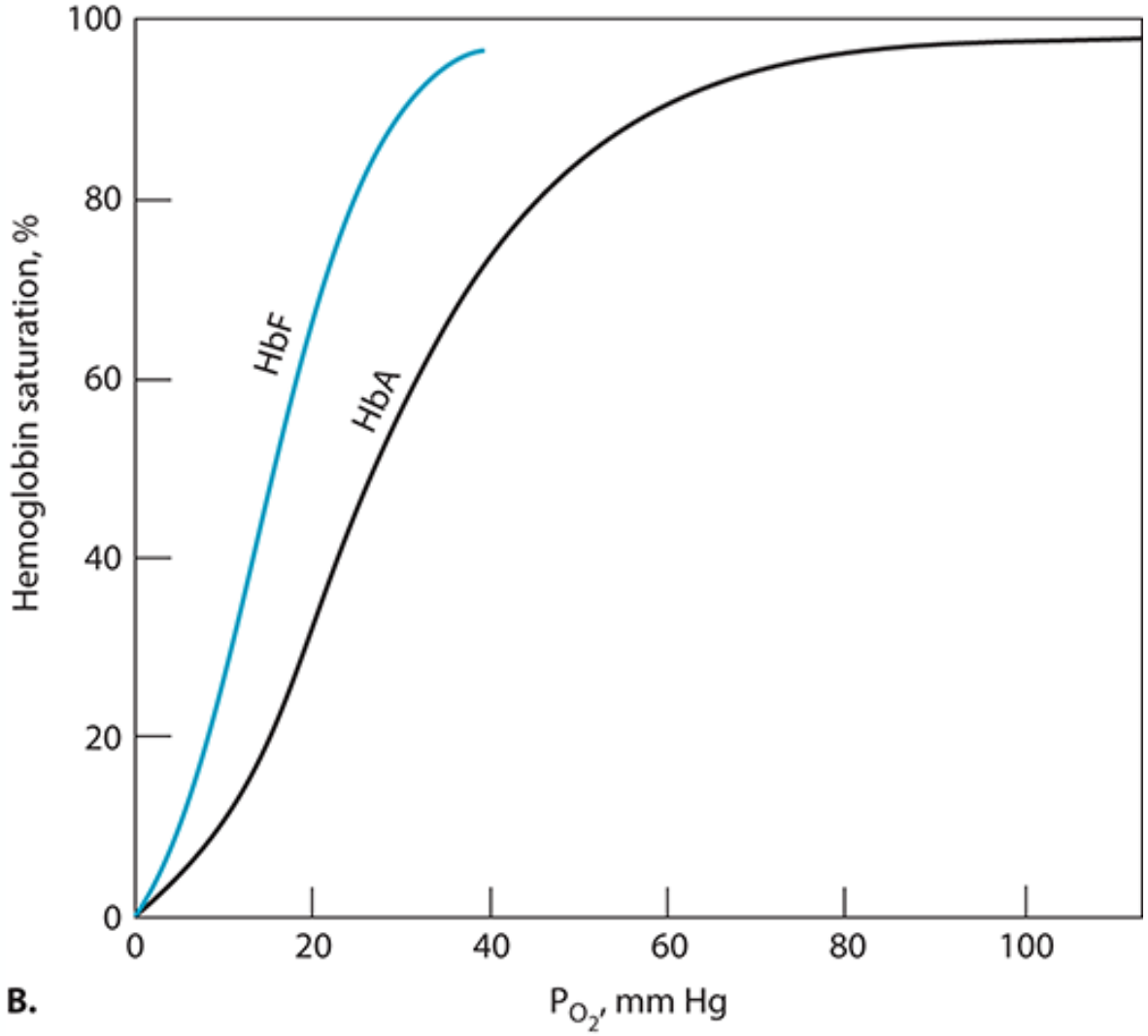


Oxygen Binding of HbF

HbF has a distinct binding curve for O_2. It allows delivery in the fetus where oxygen saturation is lower

Allow transfer of O_2 from maternal (HbA) to fetal (HbF) haemoglobin at the placenta

2,3 BPG does not influence the O_2 binding curve of HbF



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Haemoglobinopathies:

Thalassaemia

Haemoglobinopathies

We will look at 2 main categories

1. Reduced (quantity) synthesis of haemoglobin e.g. α, β thalassaemia

2. Synthesis of an abnormal (quality or structure) haemoglobin e.g. Sickle Cell (HbS) or production of HbC, HbD, HbE

Haemoglobinopathies

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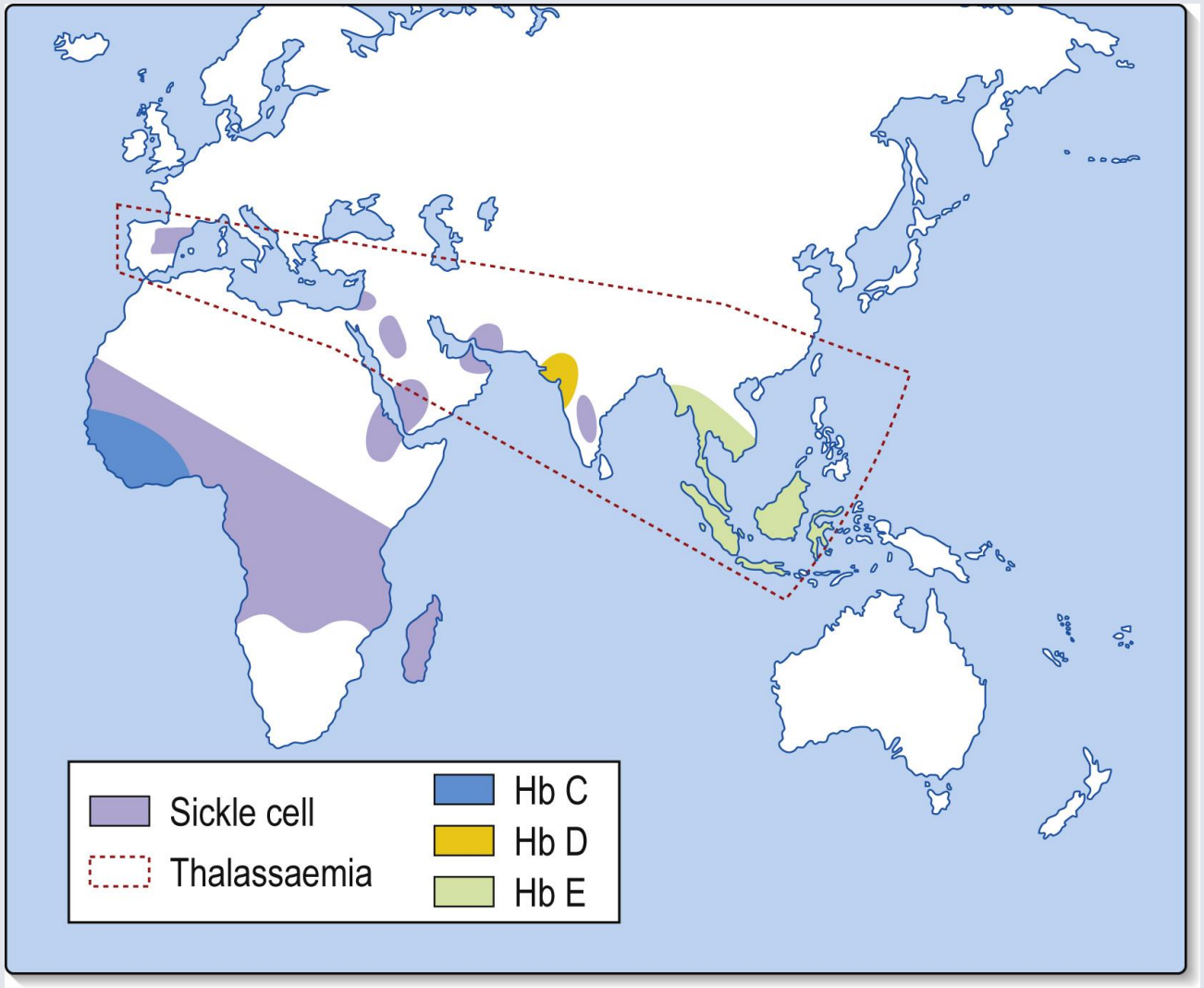
The most common genetic disorders worldwide

May provide some protection for malarial parasite



Thalassaemia

Two conditions arise from alpha or beta gene absence or mutation that leads to reduced globin production

α thalassaemia

β thalassaemia

Both conditions exist in minor and major forms.

Because α and β chain production are not balanced, the chain which is present in excess [β in α-thalassaemia and α in β-thalassaemia] precipitates leading to haemolysis of the mature RBC.

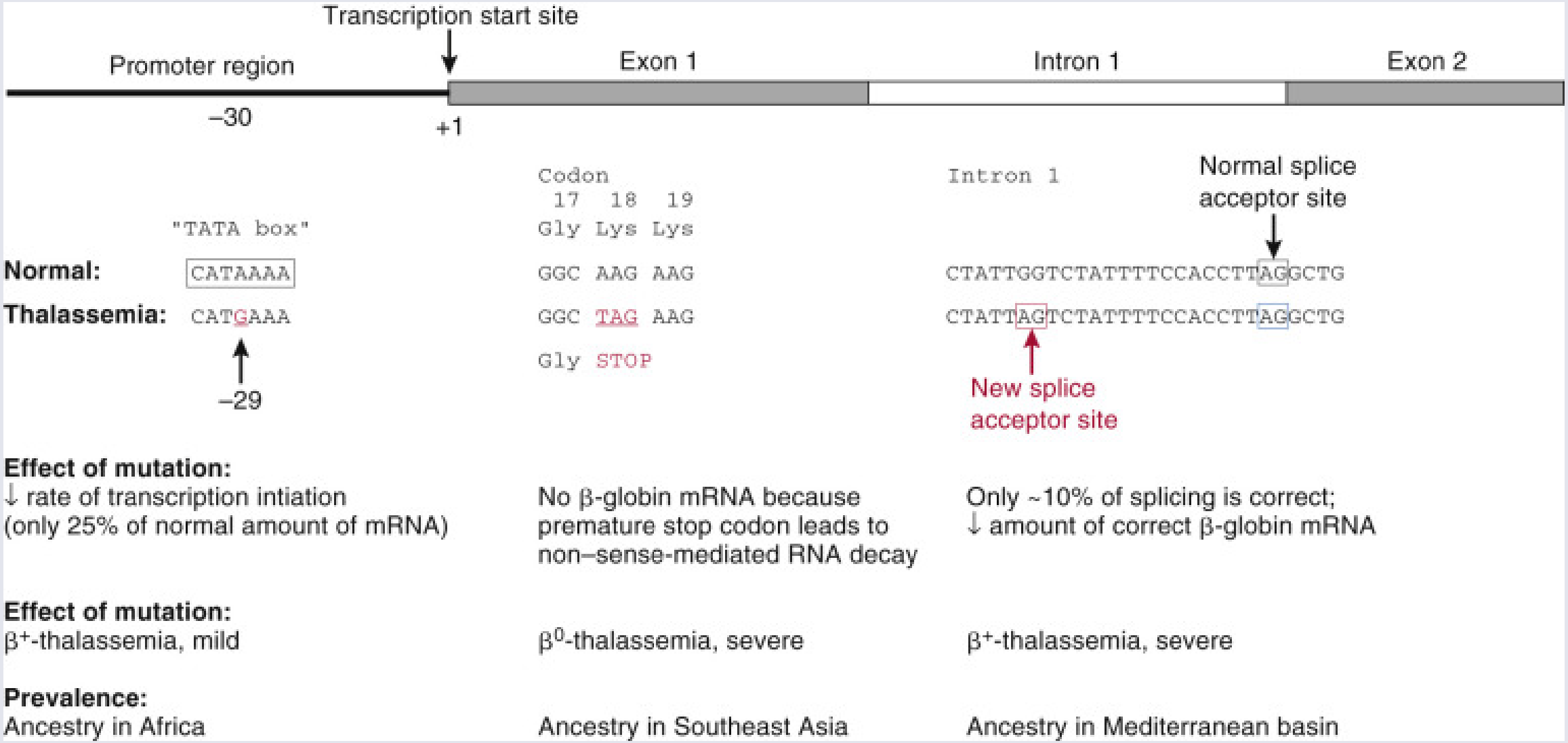
β-Thalassaemia

Mutations (>200 described) in the beta globin gene on chromosome 11 involving the promoter, coding sequences, intron-exon boundaries (splice sites), or the polyadenylation site of the β-globin gene.

Often single nucleotide substitutions or small insertions/deletions that alter the reading frame, rather than large scale deletions.

- β0: no functional β-globin is made
- β+: a small amount of normal β-globin protein is produced

β-Thalassaemia Mutations

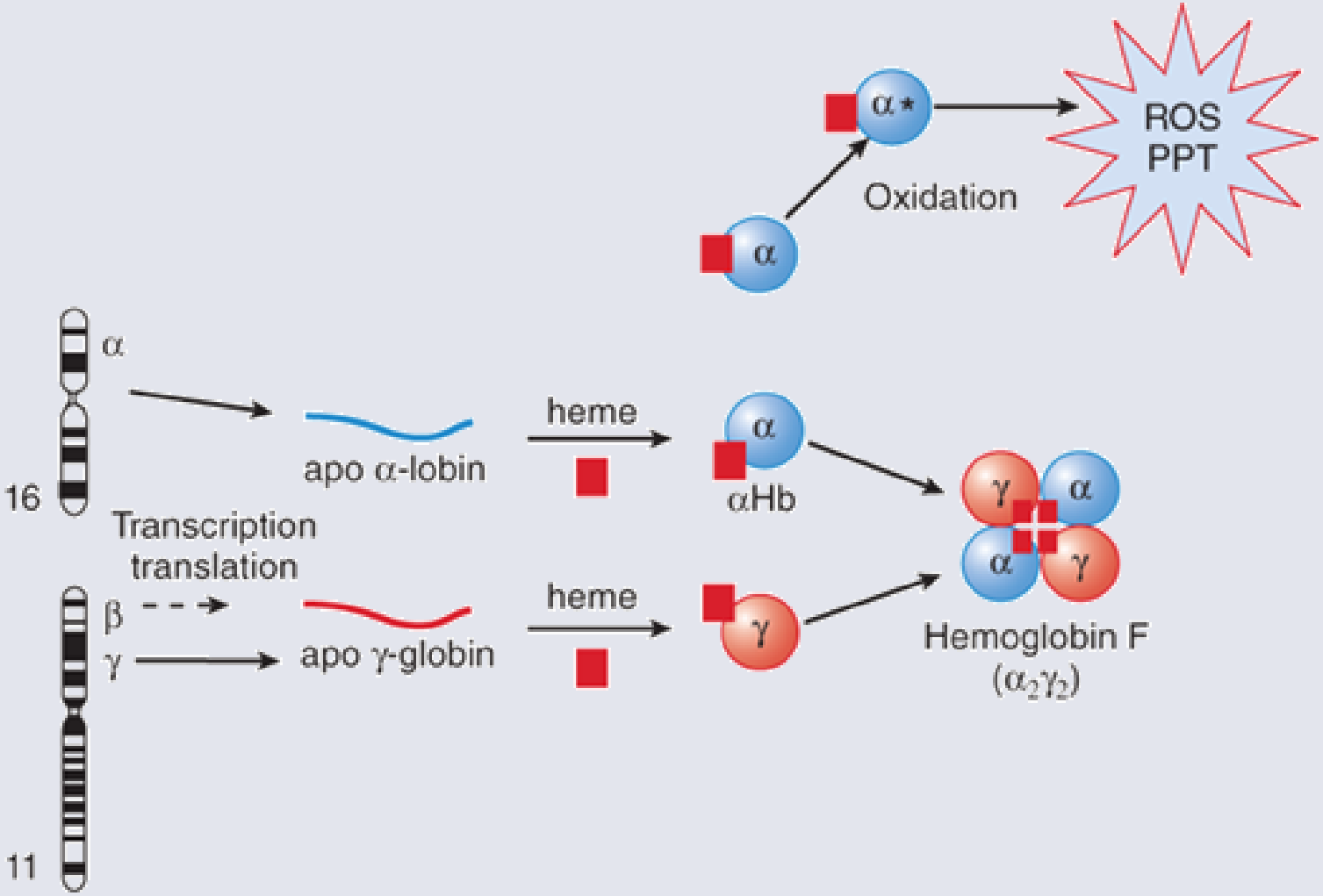


For illustration: You do not need to know the *details* of these specific mutations

β-Thalassaemia

Mutation in gene encoding β globin results in reduced β globin production

- A reduction in HbA (α2β2)
- An increase in HbF (α2γ2) and HbA_2 (α2δ2)
- Accumulation of unpaired α globin chains



Classification of β-Thalassaemia

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Heterozygous

- >100 million people globally. May be asymptomatic carrier, or have thalassaemia trait. With thalassaemia trait, mild or subclinical microcytic anaemia

Homozygous

- Range of clinical presentations
- Transfusion dependent thalassaemia (TDT; thalassaemia major). Often presents at a few months of age with progressive pallor and abdominal distension, decreased activity; hepatosplenomegaly; recurrent infections and bony abnormalities (skull). Life threatening anaemia.
- Non-transfusion dependent thalassaemia (NTDT; thalassemia intermedia). Presents in older children, symptoms are usually less pronounced, and the course is usually more insidious

β-Thalassaemia Clinical Presentation

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- Homozygous disorder
- Significant imbalance of α/β globin chains
- Severe anemia presenting early in life
- Requires lifelong RBC transfusions
- If untreated, leads to death usually in first decade

TDT

β–Thalassemia major

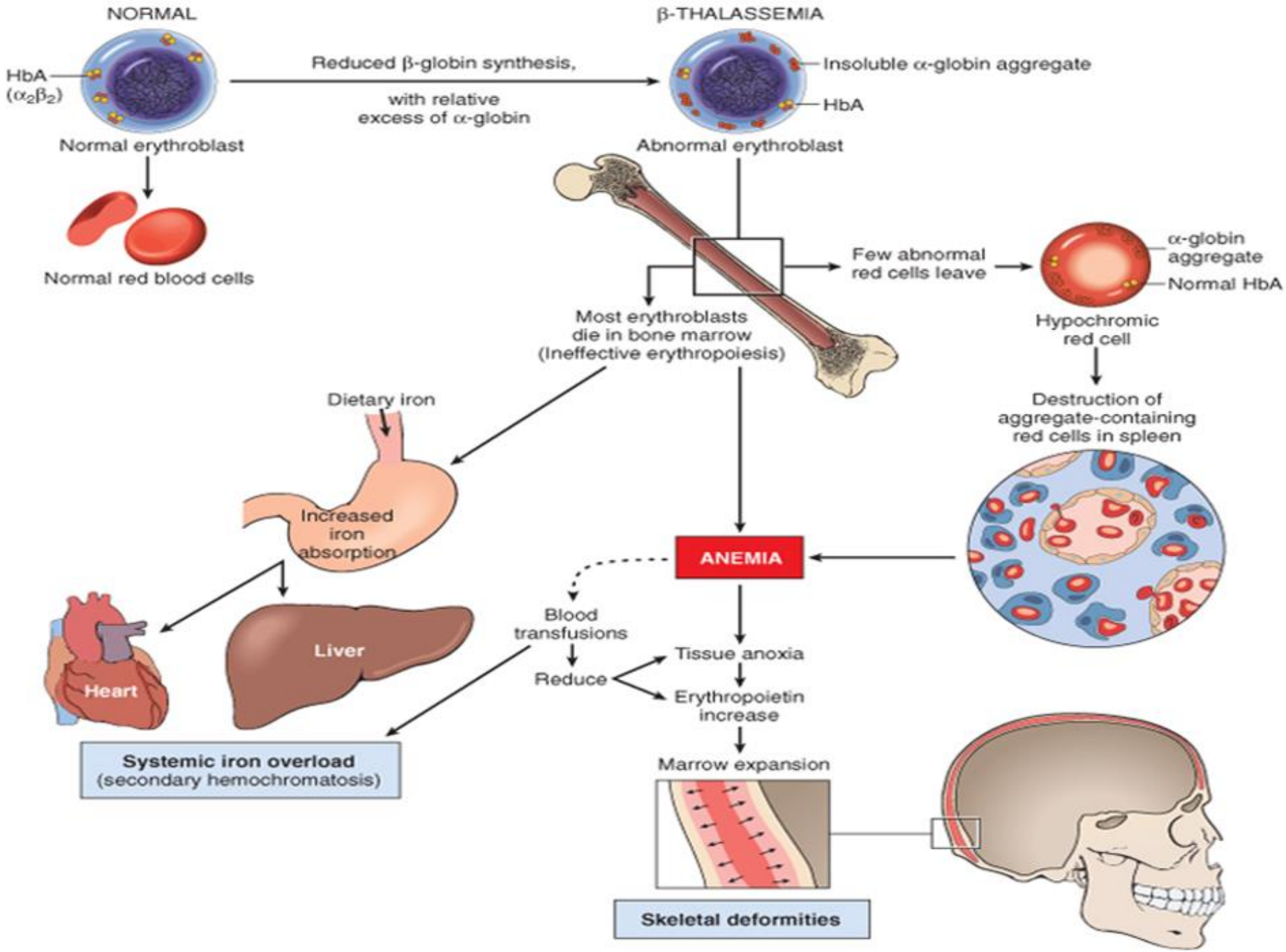
- Various genetic interactions
- Globin chain production moderately impaired
- Mild anemia, diagnosed usually in late childhood
- Occasional blood transfusions may be required

NTDT

β–Thalassemia intermedia

- Heterozygous condition
- Asymptomatic
- May require genetic counseling

β–Thalassemia minor


NORMAL
β-THALASSEMIA
HbA
($\alpha_2\beta_2$)
Reduced β-globin synthesis,
with relative
excess of α-globin
Insoluble α-globin aggregate
HbA
Normal erythroblast
Abnormal erythroblast
Normal red blood cells
Few abnormal
red cells leave
α-globin
aggregate
Normal HbA
Hypochromic
red cell
Most erythroblasts
die in bone marrow
(Ineffective erythropoiesis)
Dietary iron
Destruction of
aggregate-containing
red cells in spleen
Increased
iron
absorption
ANEMIA
Heart
Liver
Blood
transfusions
Reduce
Tissue anoxia
Erythropoietin
increase
Systemic iron overload
(secondary hemochromatosis)
Marrow expansion
Skeletal deformities

β-Thalassaemia Major

(see image on previous slide)

- Develop severe anaemia (leading to failure to thrive, pallor, swollen abdomen)
- Bone marrow expansion produces skeletal changes, including a protuberant upper jaw and cheekbones and thinning of the long bones (making them susceptible to fracture).
- Splenomegaly and infections are common, and patients with untreated β-thalassemia major often die during the first decade of life
- Transfusion help to treat the anaemia and its attendant complications but also adding to the systemic iron overload. This may lead to damage to the endocrine glands, liver, pancreas and myocardium by adolescence. The cardiomyopathy and tachyarrhythmias are the leading causes of morbidity and mortality.

Haematological Findings β-Thalassemia

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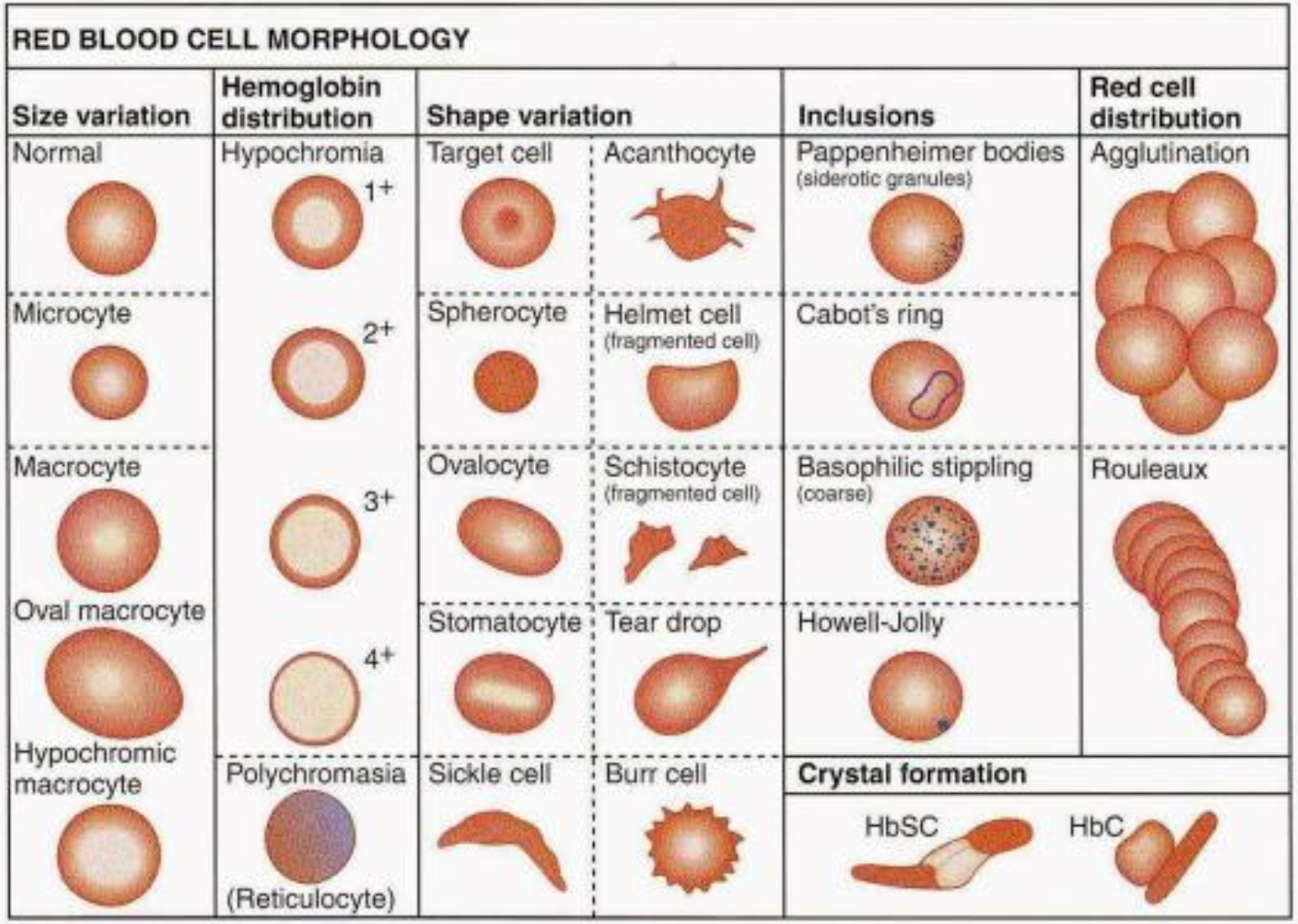
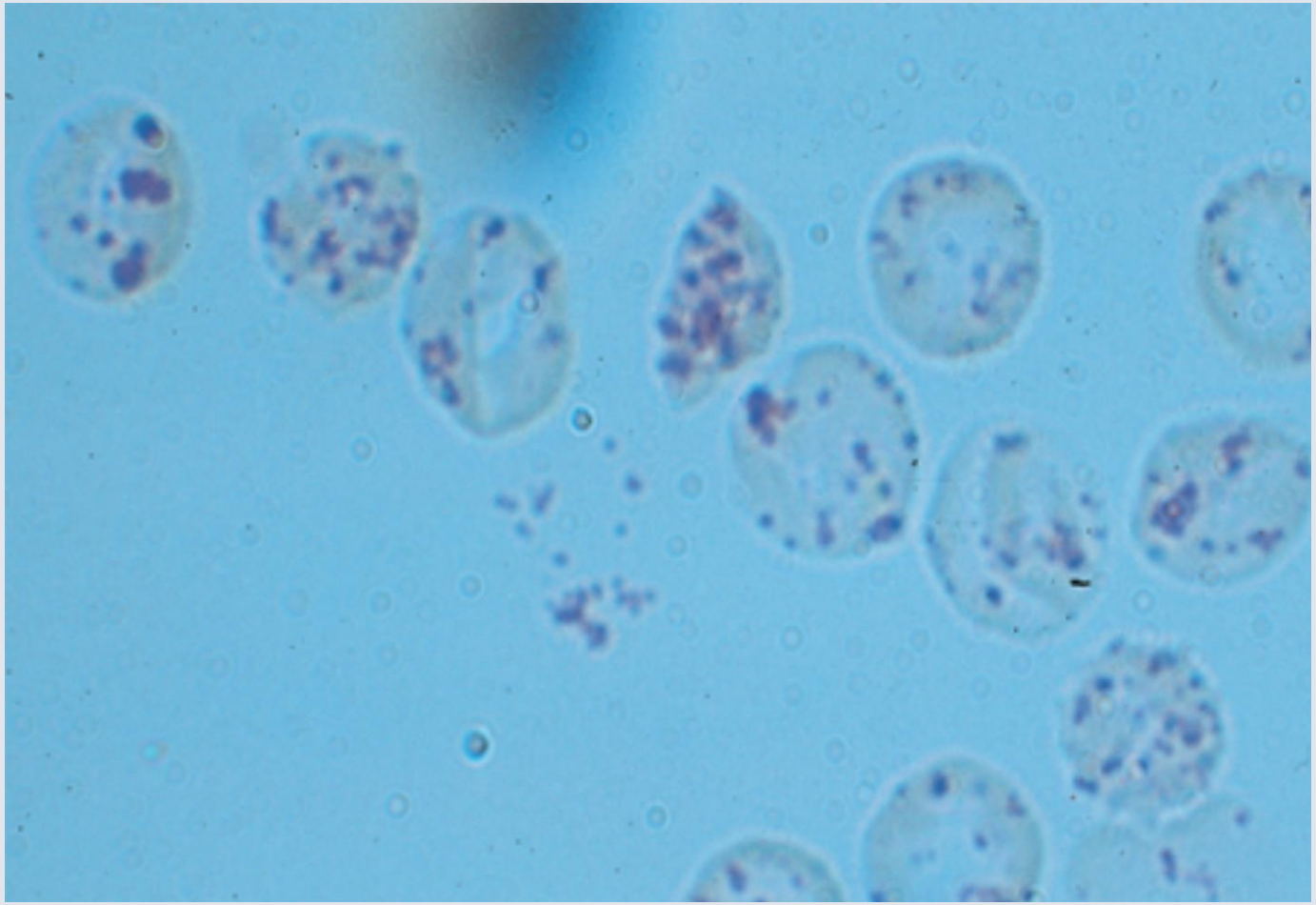
β Thalassemia minor	β Thalassemia intermedia	β Thalassemia major
Mild anaemia Microcytosis with normal RDW Hypochromia Target cells Poikilocytosis HbA_2 level high HbF level may be raised	Moderate anaemia but usually not transfusion dependent Microcytosis Hypochromia Target cells Poikilocytosis	Transfusion-dependent severe anaemia Microcytosis Hypochromia Target cells Anisopoikilocytosis Reticulocytosis Nucleated RBCs Basophilic stippling Inclusion bodies on supravital staining HbA absent or very low level HbF level high

Recap: Blood Smear



Poikilocytosis: Variation in RBC *shapes*
Anisocytosis: Variation in RBC *sizes*
Basophilic stippling: abnormal ribosomal RNA appears as blue dots

Precipitated Haemoglobin

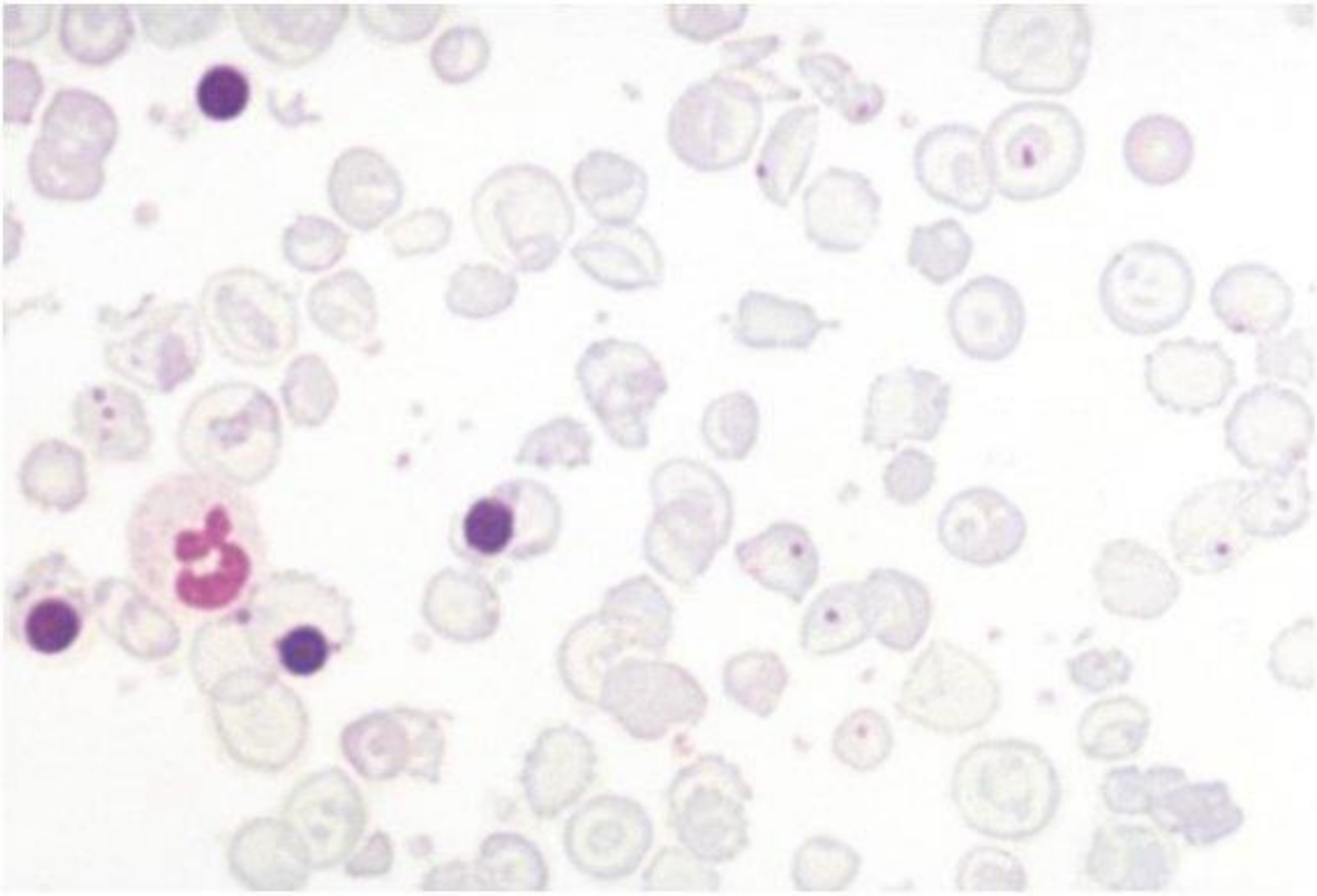


Heinz bodies

Blood mixed with hypotonic solution of dye (e.g. brilliant cresyl blue, new methylene blue crystal violet.

The stained material is **precipitates of denatured haemoglobin** within cells.

β Thalassemia Major



Microcytic,
Hypochromic,
Target cells,
Anisopoikilocytosis,
Nucleated RBCs

α-Thalassaemia - Genetics

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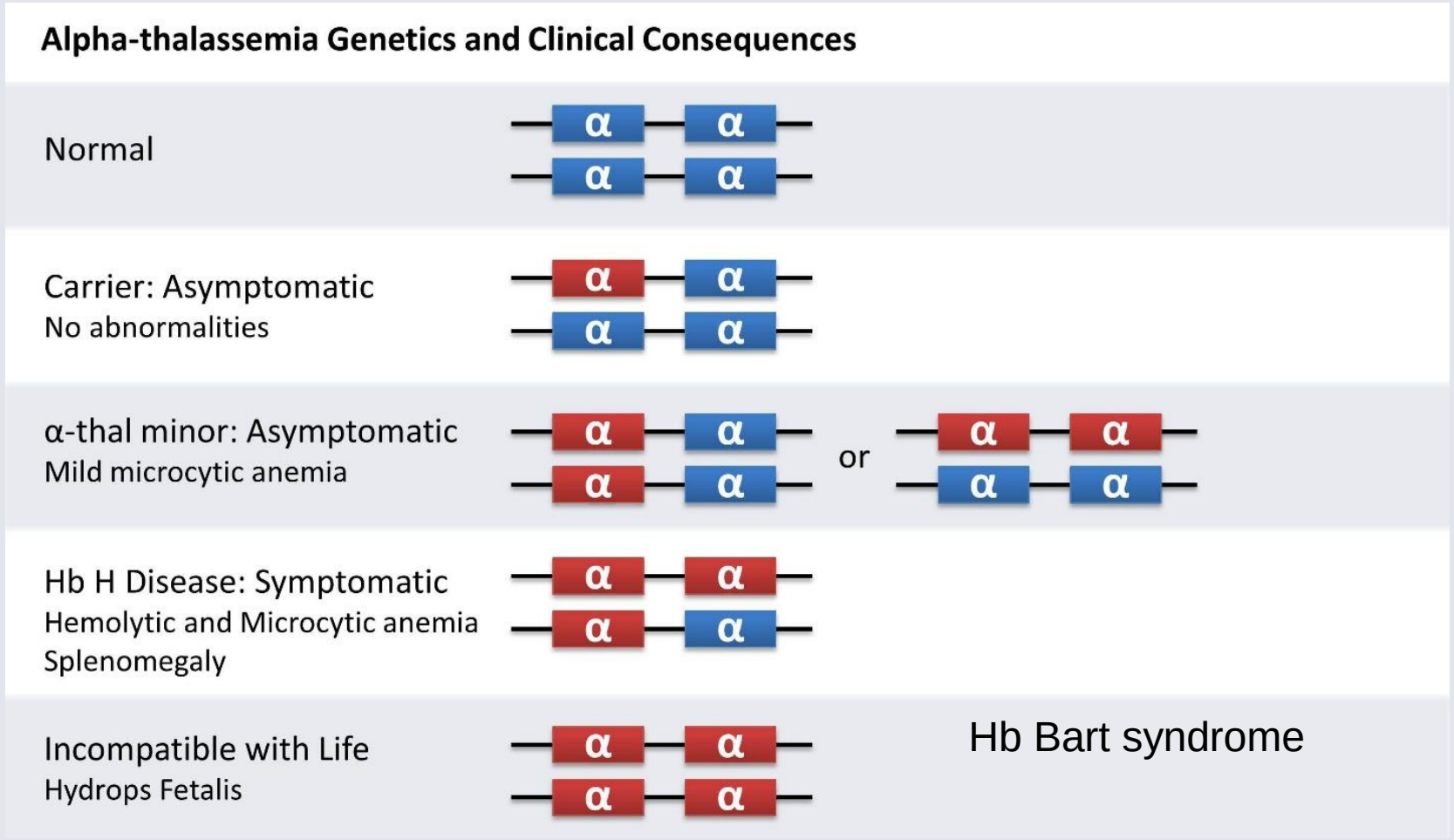

Commonly due to gene deletions

α-Thalassaemia

HbH (--/-a) Some HbA produced (25–30% normal levels). β chains accumulate and form tetramers called HbH. HbH forms inclusions and precipitates in circulating RBC. Patients with HbH disease have thalassemia intermedia characterised by moderately severe haemolytic anaemia. Survival into adult life without transfusions is common.

Hydrops Fetalis (--/--) No physiologically useful haemoglobin is produced beyond the embryonic stage. Excess γ globin forms tetramers called *Hb Barts* (γ_4), which has a very high oxygen affinity. It delivers almost no O_2 to fetal tissues, causing tissue asphyxia, oedema (hydrops fetalis), congestive heart failure, and death in utero.

Haematological Parameters α-Thalassaemia

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Parameter	
Normal	
Haemoglobin (g/L)	140 - 180
Mean Cell Volume ($x10^{15}$/L)	76 - 96
Red Cell Distribution Width (%)	11.5 - 15
Reticulocytosis	Normal
Alpha trait (mutation in 2/4 alleles)	
Haemoglobin (g/L)	100 - 140
Mean Cell Volume ($x10^{15}$/L)	60 - 80
Red Cell Distribution Width	Normal
Reticulocytosis	Normal
Alpha HbH (mutation in 3 /4 alleles)	
Haemoglobin (g/L)	20 - 80
Mean Cell Volume ($x10^{15}$/L)	60 - 70
Red Cell Distribution Width	Normal
Reticulocytosis	Mild

While you need to know the pattern, you do not need to memorise the numbers here

α-Thalassemia Blood Smear

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HbH Syndrome

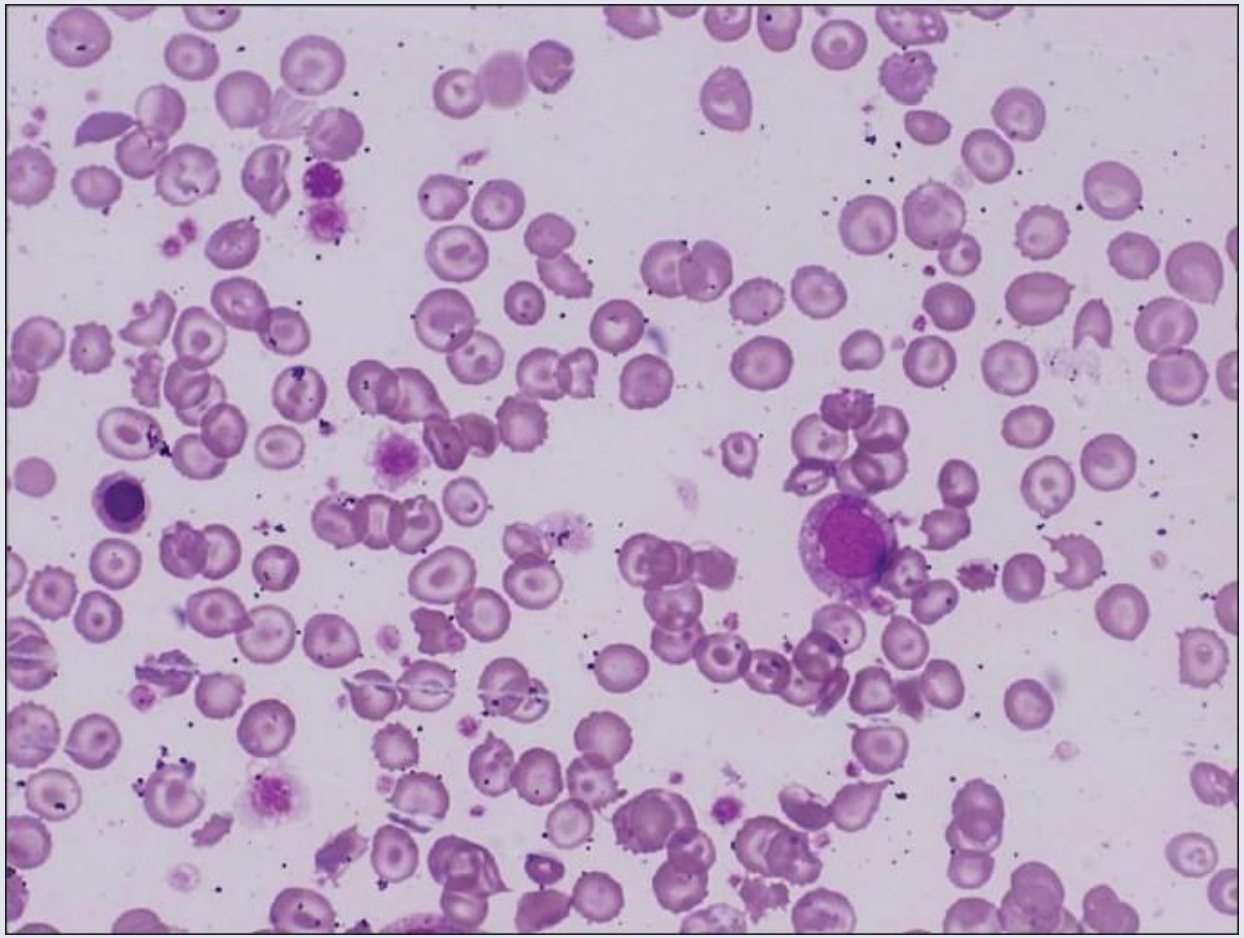
Microcytic

Hypochromic

Target Cells

Anisopoikilocytosis

Nucleated RBCs



Supravital staining would reveal Heinz bodies

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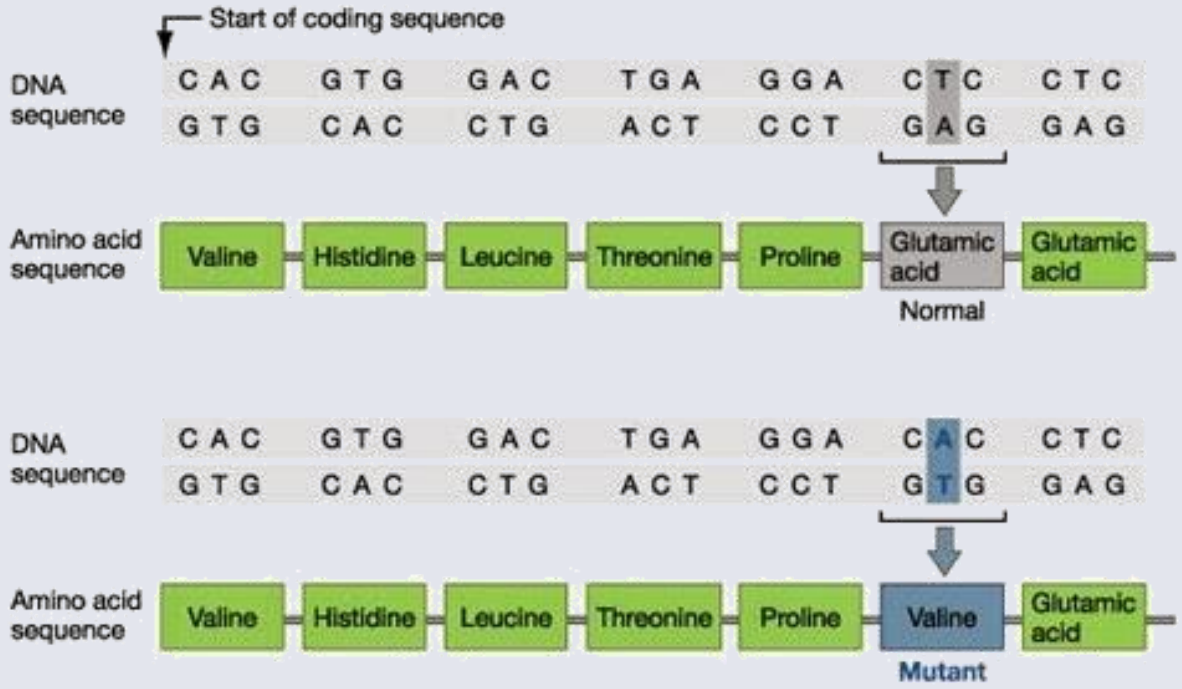
Haemoglobinopathies:

Sickle Cell Anaemia

Sickle Cell Anaemia

Sickle Cell anaemia is caused by the production of HbS due to a point mutation in β globin gene. The mutation is common in the West and Central Africa, the Mediterranean, Middle East and some parts of India



The disorder is autosomal recessive

2 copies of the mutated gene give rise to sickle cell anaemia

Individuals with one copy of the mutated gene have sickle cell trait.

Haemoglobin S (HbS)

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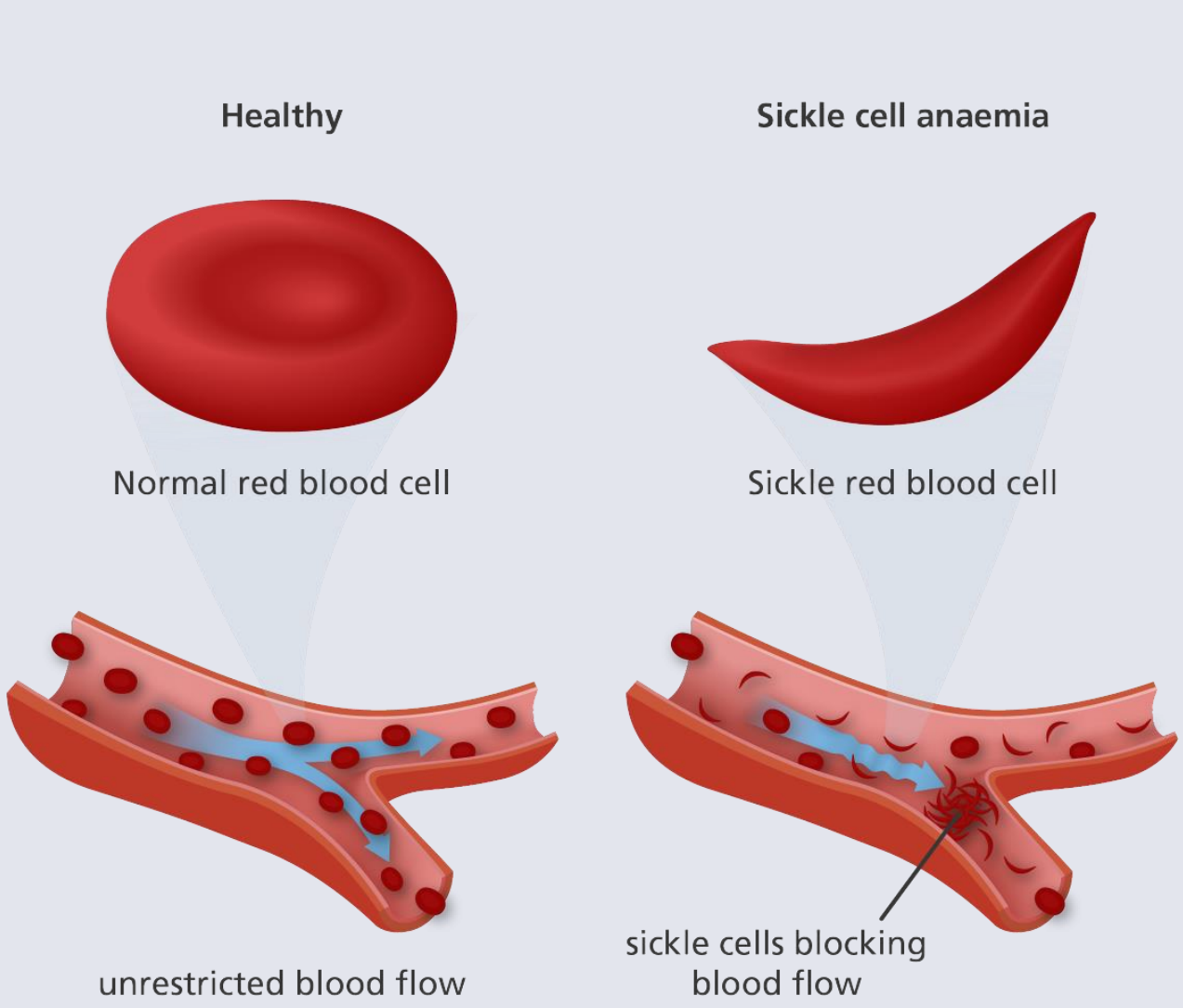
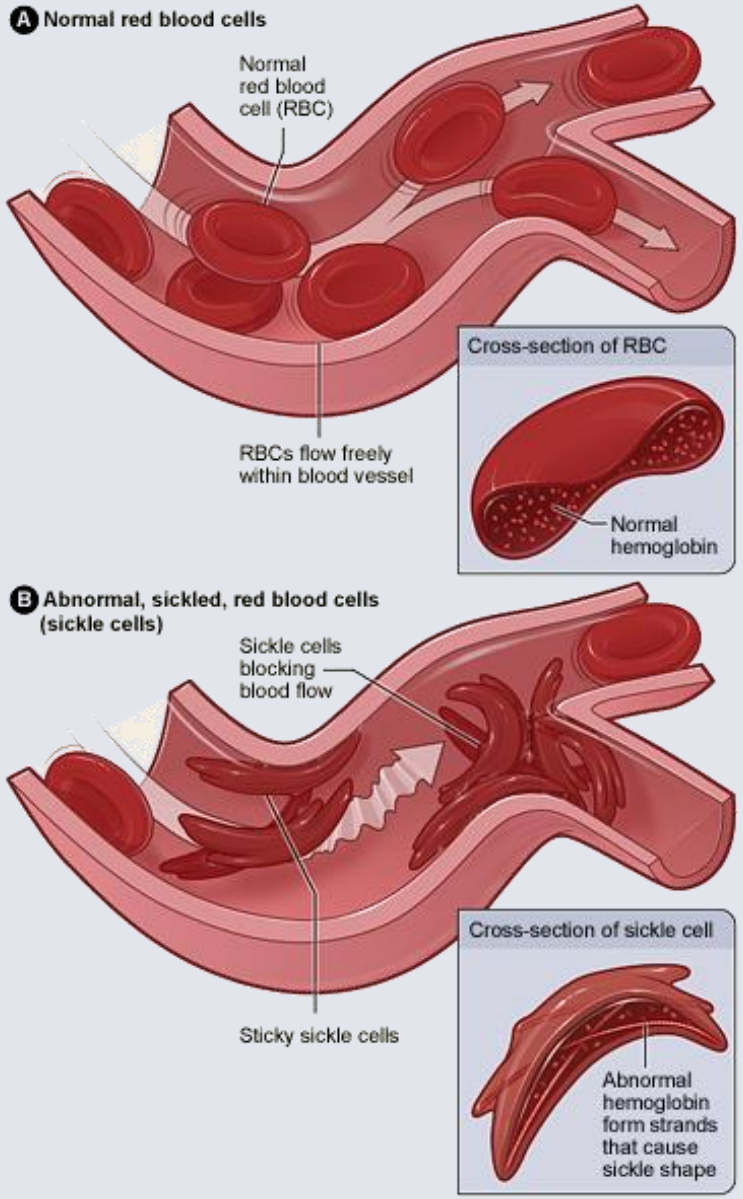
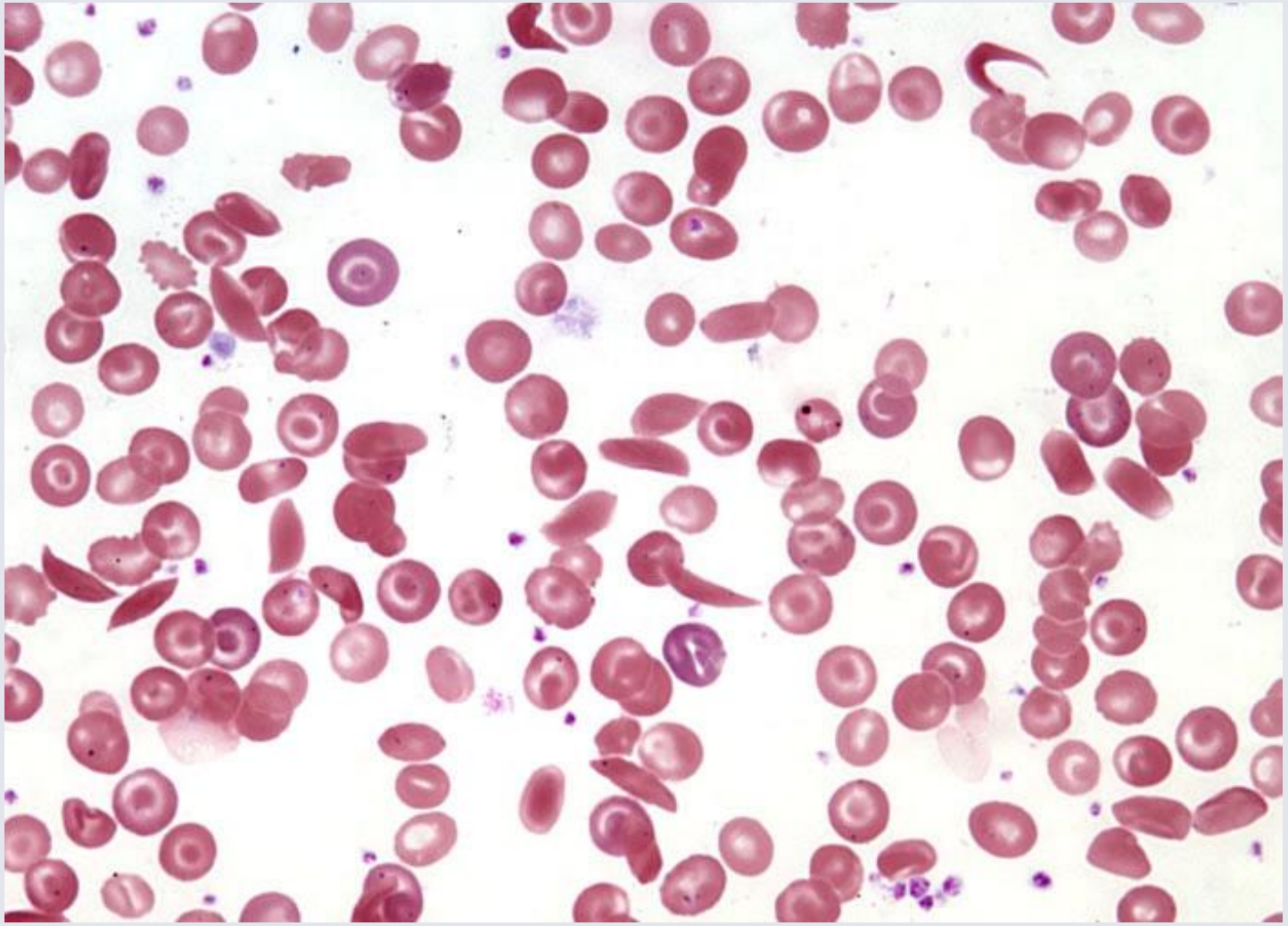




Diagnosis of Sickle Cell Anaemia



Normocytic
Normochromic
Target cells
Sickle RBC
Late normoblasts

Presentation of Sickle Cell Anaemia

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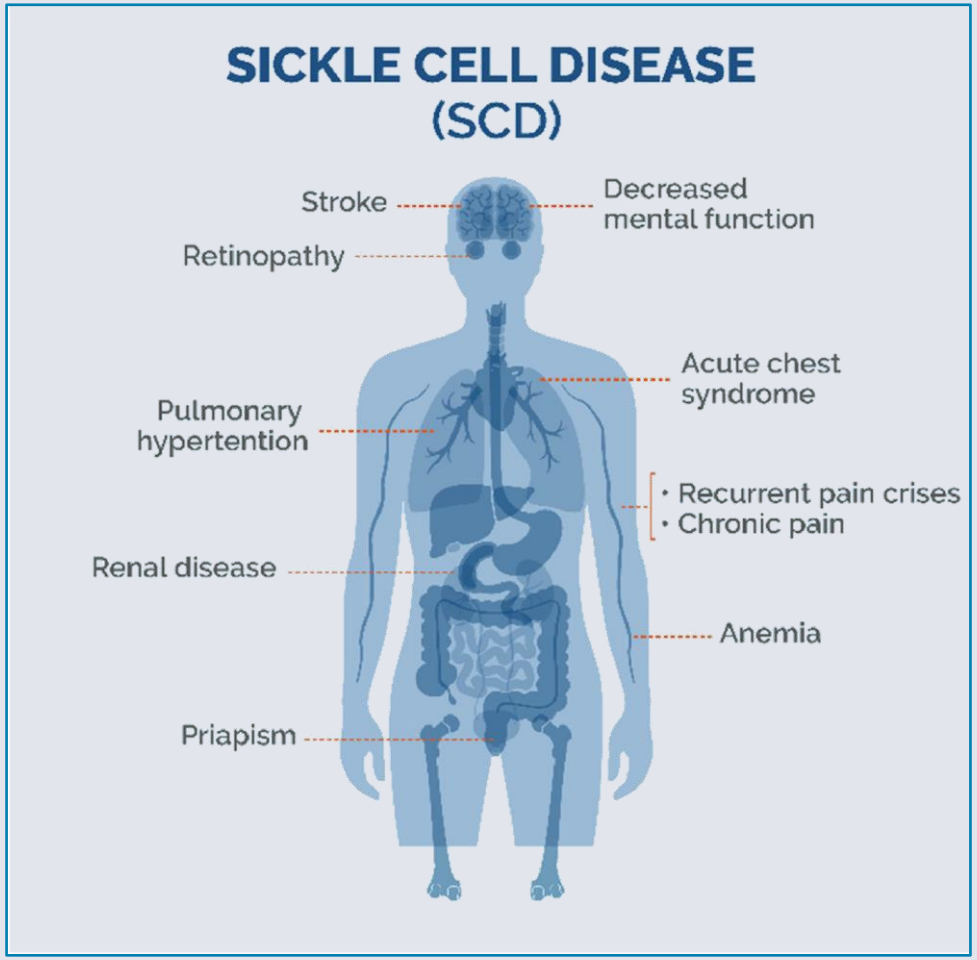
Sickle cells can obstruct blood flow and break down prematurely. SCA is associated with lifelong morbidity and reduced life expectancy.

Obstruction of small blood capillaries can cause painful crises, damage to major organs, and increased vulnerability to severe infections.

- acute pain caused by vaso-occlusion
- sporadic and highly unpredictable.
- localised to a limited area, particularly the abdomen, chest, back, or joints
- often preceded by a viral or bacterial infection

Reduced nitric oxide levels can lead to thrombosis and pulmonary hypertension



Management of Sickle Cell Anaemia

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- Prenatal screening (more on this later)

Chronic Disease

•Hydroxycarbamide if frequent crises (Increased production of HbF).

- Splenic infarction leads to hyposplenism and immunocompromise.

  Prophylaxis, in terms of antibiotics and immunization

- Febrile children risk septicaemia: consider early rescue out-patient antibiotics

- Bone marrow transplant?

- Gene Therapy

Other Haemoglobinopathies

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HbC, D and E

Point mutations in the beta chain gene.

In the homozygous state, they produce mild chronic haemolysis with splenomegaly but without the occlusive manifestations of sickle cell disease.

Found in West Africa, India and South-East Asia, respectively.

Due to their geographical distribution, the gene for HbC is often inherited with that for HbS. HbS-C disease behaves as a milder sickling disorder with a particular tendency to venous thrombosis.

Gene Therapy for Hb Disorders

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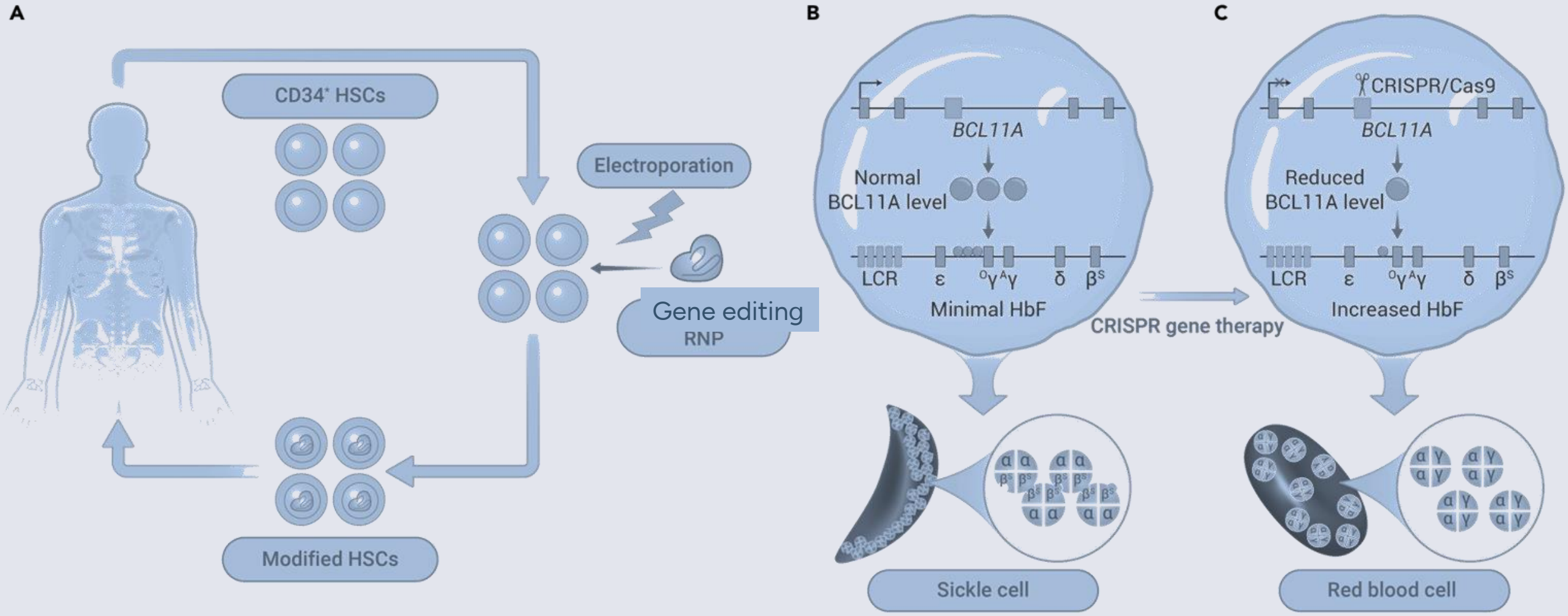


Gene Therapy

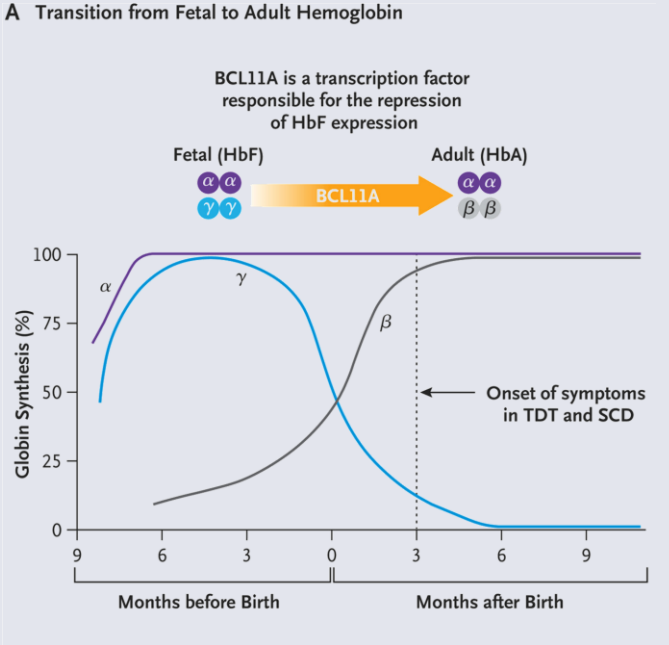
(see previous image)

Haematopoietic stem cells (HSC) are isolated from patients. Outside the body, these cells are changed via the CRISPR/Cas gene editing system before being reintroduced to the patient

BCL11A is a transcription factors that represses the expression of the γ globin gene and is involved in the transition from fetal to adult haemoglobin profile.

Gene editing disables BCL11A expression and this allows high-level expression of γ-globin, and thus HbF, which can substitute for abnormal Hb (as in HbS) or decreased levels as in beta thalassaemia

This treatment has been shown to reduce the dependency on transfusion; and to reduce vaso-occlusive episodes in SCA.



Biochemical Indicators of Disease

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Clinical Biochemistry

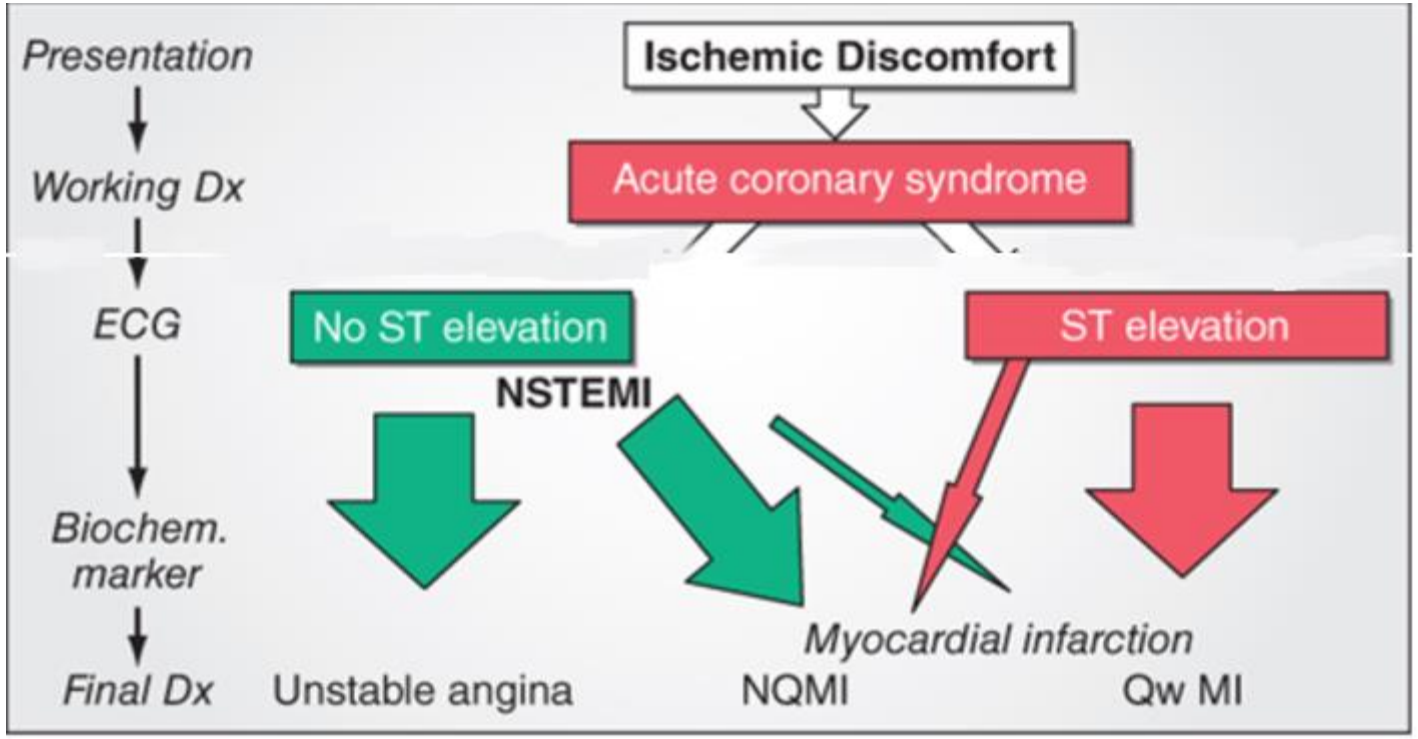
An ideal biochemical test has both high sensitivity and high specificity. Elevated blood concentrations of tissue enzymes can be a useful indicator of damage.

May be useful in diagnosis, prognosis, monitoring of response.

Two examples:

1. Cardiovascular Disease
2. Liver and Biliary Disease

Clinical Biochemistry



Acute coronary syndrome is a term used to describe a range of conditions associated with sudden, reduced blood flow to the heart.

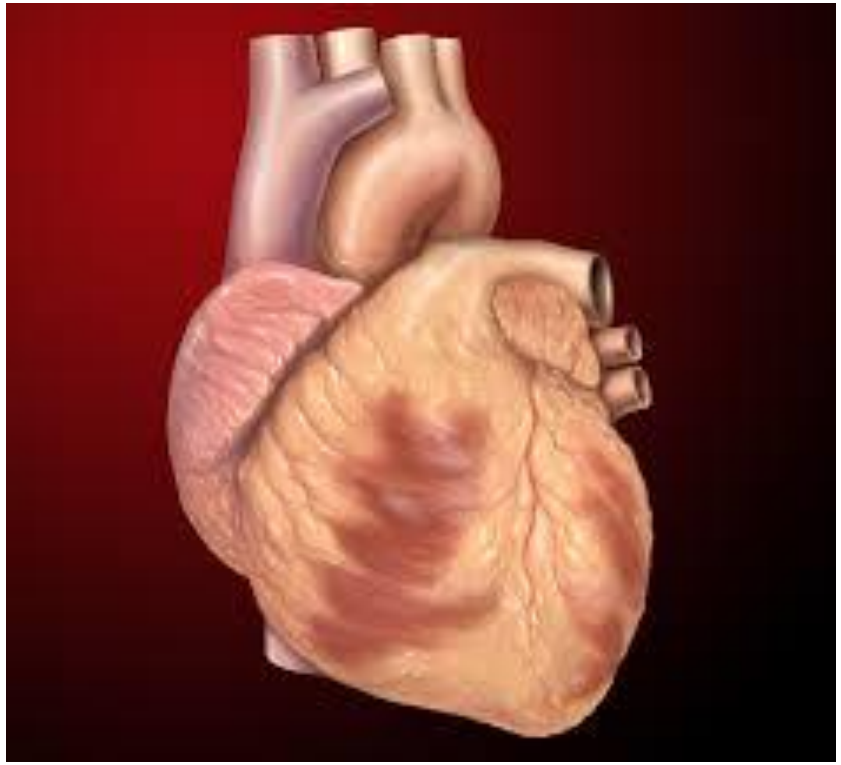
Myocardial infarction involves cell death and results in damaged or destroyed heart tissue. Intracellular enzymes released can be detected in the blood.

(Much!) More on Cardiovascular disease later

Clinical Biochemistry

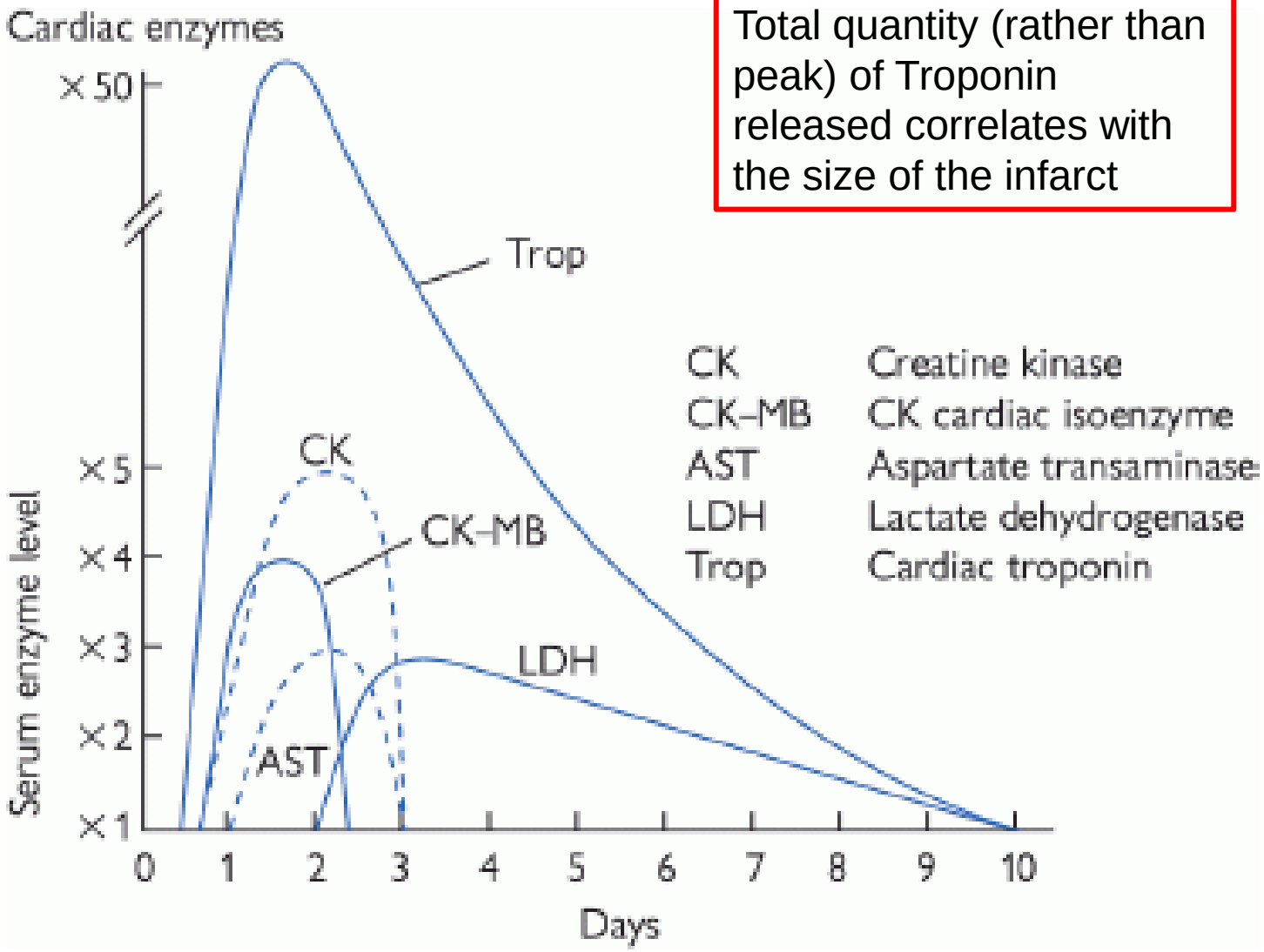
Potential Markers of Cardiac Cell Death

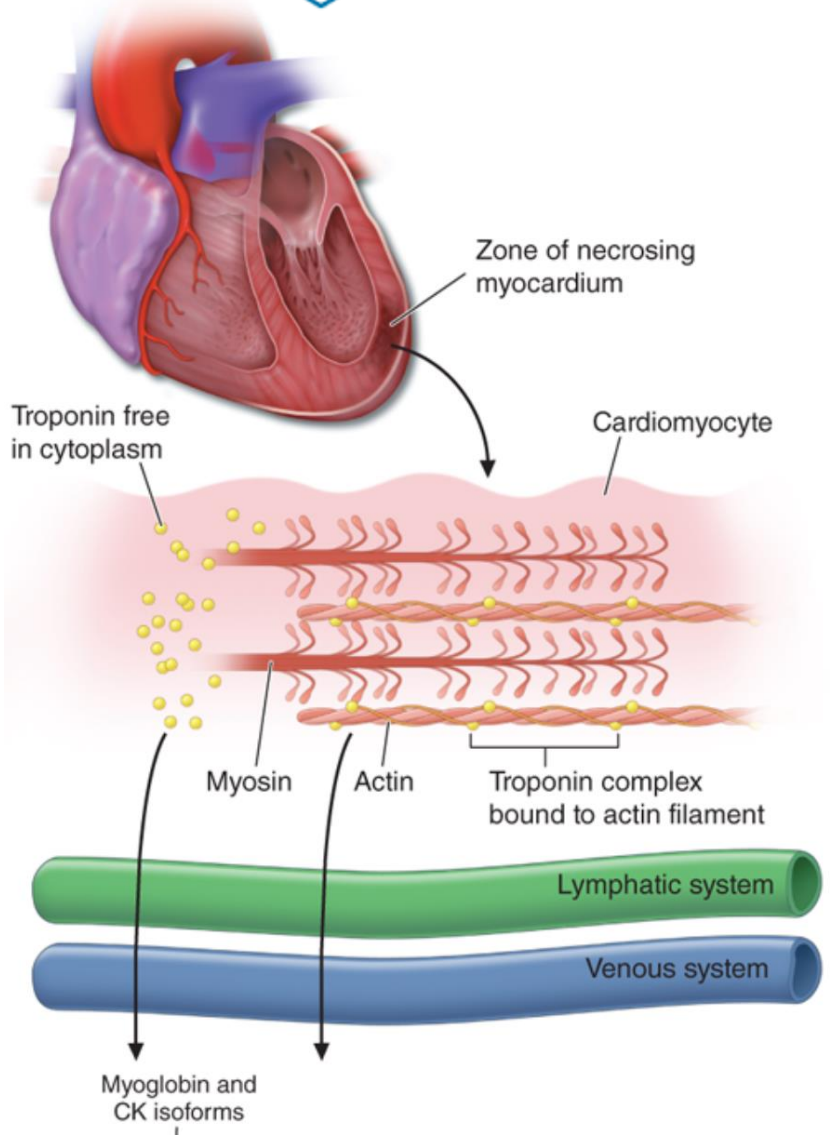
- **Troponin I and T**
- **Creatine phosphokinase (CK); CK-Mb**
- **B-type natriuretic peptide (BNP)**
- Myoglobin
- Lactic dehydrogenase (LDH)
- Aspartate aminotransferase (AST)



Biochemical Changes after MI

Total quantity (rather than peak) of Troponin released correlates with the size of the infarct

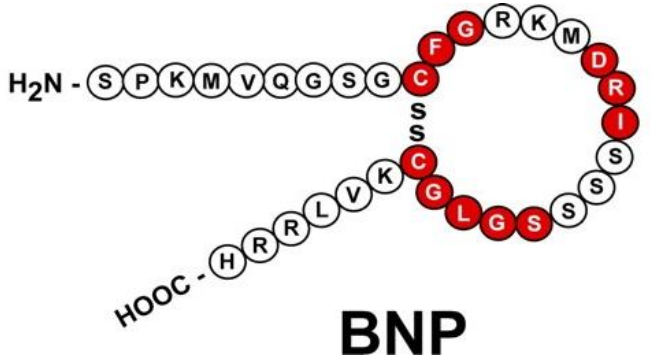




BNP in Heart Failure

HF symptoms such as dyspnoea are relatively non-specific and ECG, and x-rays are also not accurate enough to always make the correct diagnosis

- BNP is a cardiac hormone produced from ventricular muscle cells in response to ventricular dilation and pressure overload, and NT-proBNP is the inactive N-terminal fragment produced from the cleavage of proBNP
- Levels can be used in establishing prognosis or disease severity in chronic HF, achieve optimal dosing of medical therapy
- May also be useful in ACS (peaks after troponin)



BNP

BNP in Heart Failure

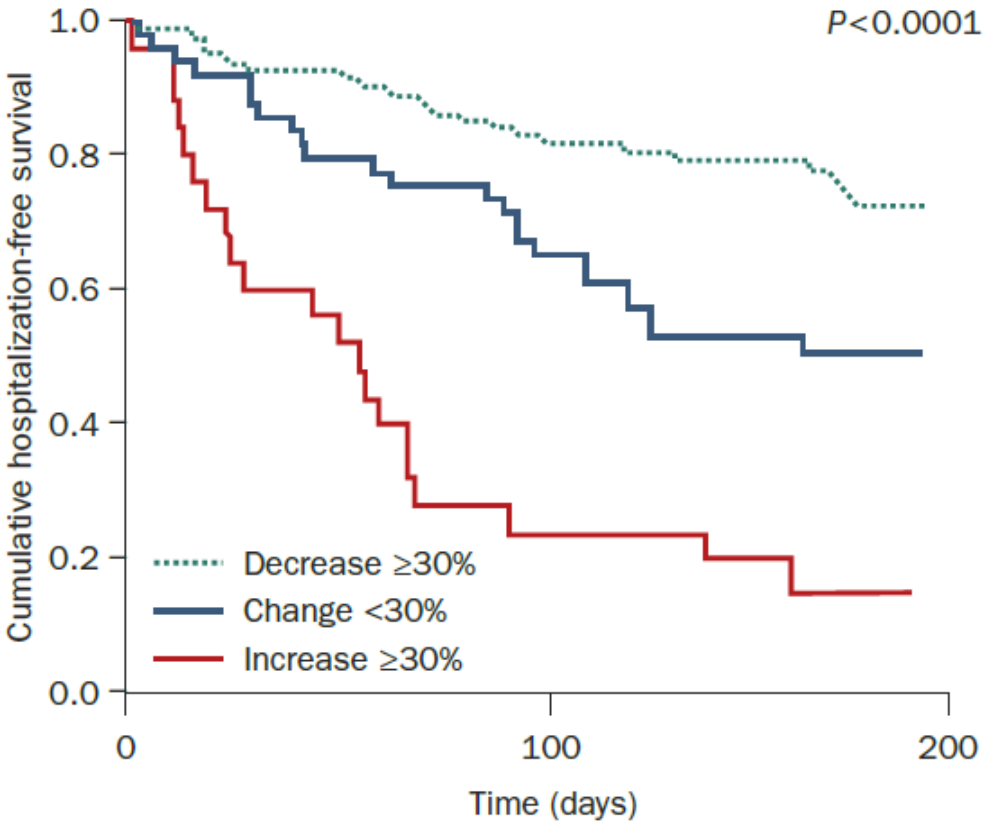


Figure 1 | Cumulative hospitalization-free survival following hospital discharge stratified by the change in NT-proBNP concentration during HF hospitalization. Change in NT-proBNP levels during hospitalization was the strongest independent predictor of death or hospital readmission. Abbreviations: HF, heart failure; NT-proBNP, N-terminal proB-type natriuretic peptide. Permission obtained from Wolters Kluwer Health © Bettencourt P. *et al.* N-terminal-pro-brain natriuretic peptide predicts outcome after hospital discharge in heart failure patients. *Circulation* **110**, 2168–2174 (2004).

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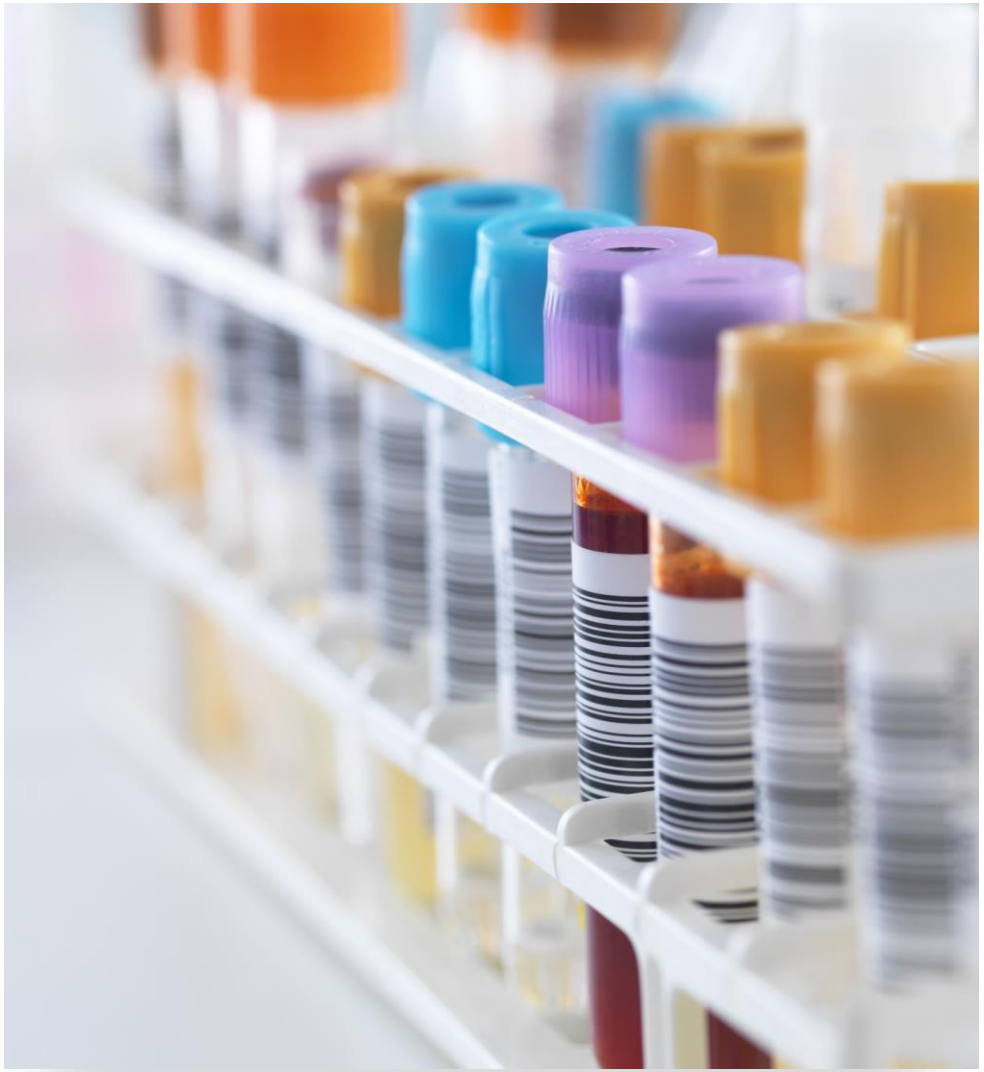
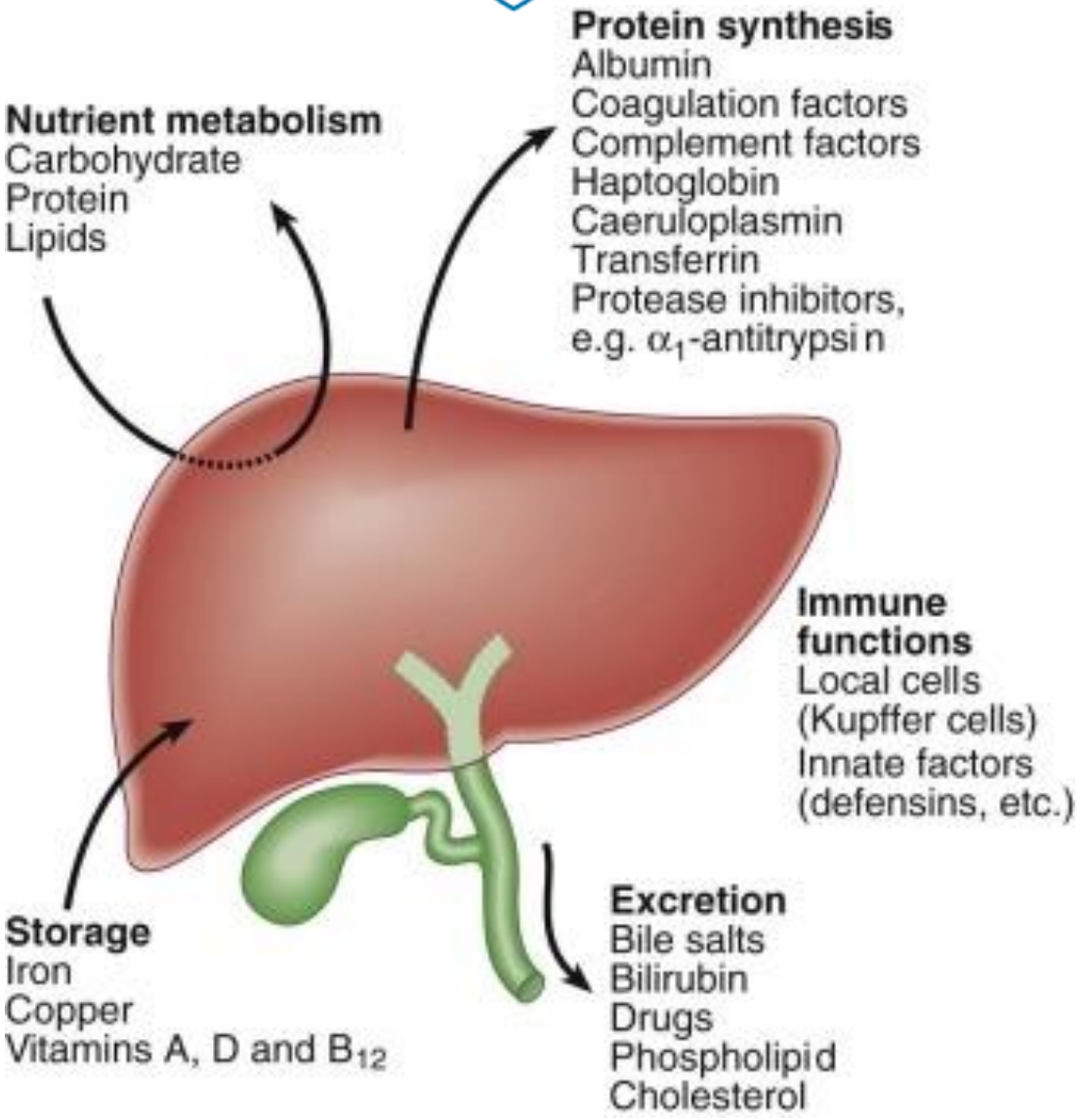
Liver and Biliary Disease



Liver Function

Wide range of functions in the liver

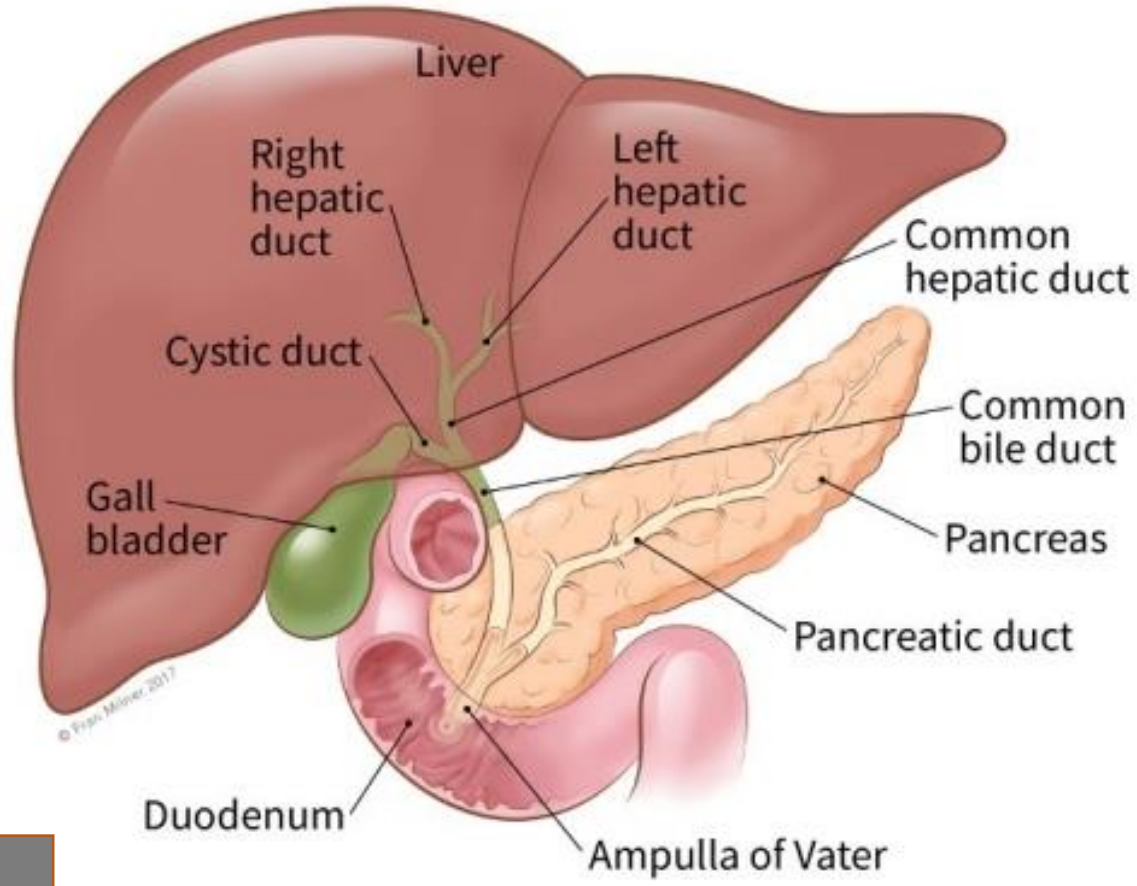
- Liver function test (LFTs) do not attempt to measure each of these functions, rather detect signs of liver disorder.
- No one test is highly specific for liver disorders – used and interpreted in combination
- Used to implicate liver damage, help identify disease, get measure of severity, track treatment response.



Liver and Biliary Disease

Detection of:

- Hepatocyte damage leading to release of intracellular contents
- Reduced liver synthetic function
- Deficiency in excretion/secretion of bile (cholestasis)



More on liver diseases later

Serum Enzymes

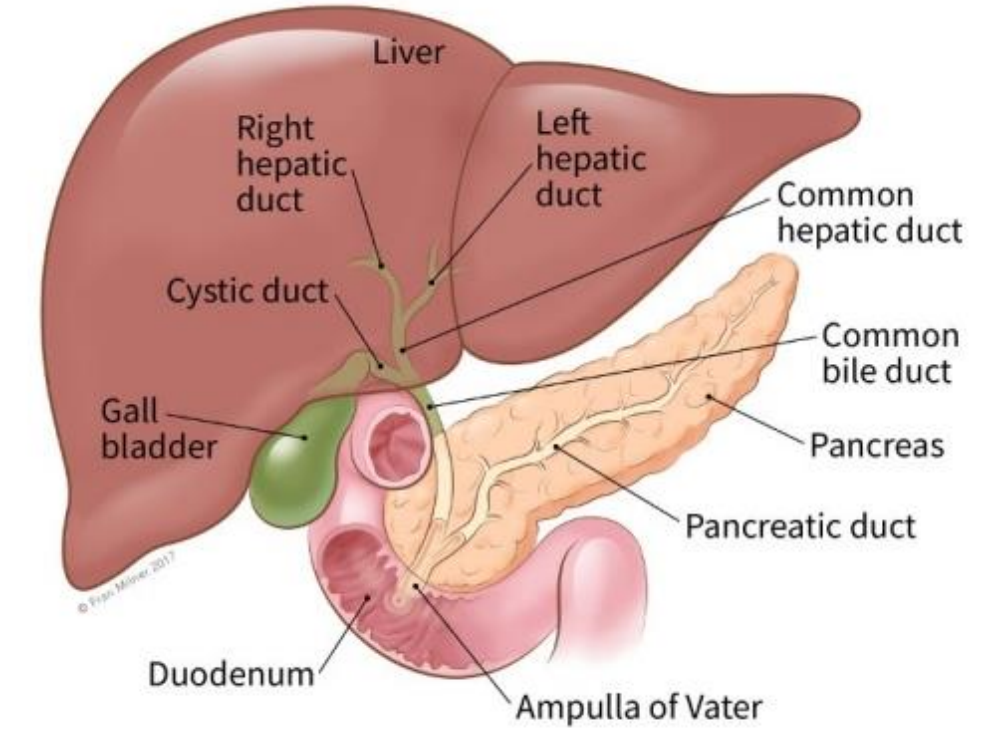
- Alanine aminotransferase (**ALT**) and aspartate aminotransferase (**AST**) are present in hepatocytes. Hepatocyte damage causes increased levels in blood. AST also present in cardiac and skeletal muscle as well as other tissues.
- Liver damage may reduce **serum albumin** concentrations although its long half life means it is less useful in acute liver disease. In contrast clotting factors have a short half life so reductions, leading to increased in prothrombin time (PT), are an indication of acute liver damage. Clotting will also be reduced in a Vitamin K deficiency due to obstructive jaundice (or other reason) as several clotting factors require Vit K.
- Cholestasis (reduction in bile secretion or flow) leads to increased in **alkaline phosphatase** (also present with bone growth and pregnancy) and γ-glutamyl transpeptidase (**GGT**) levels in the blood.

Aminotransferases

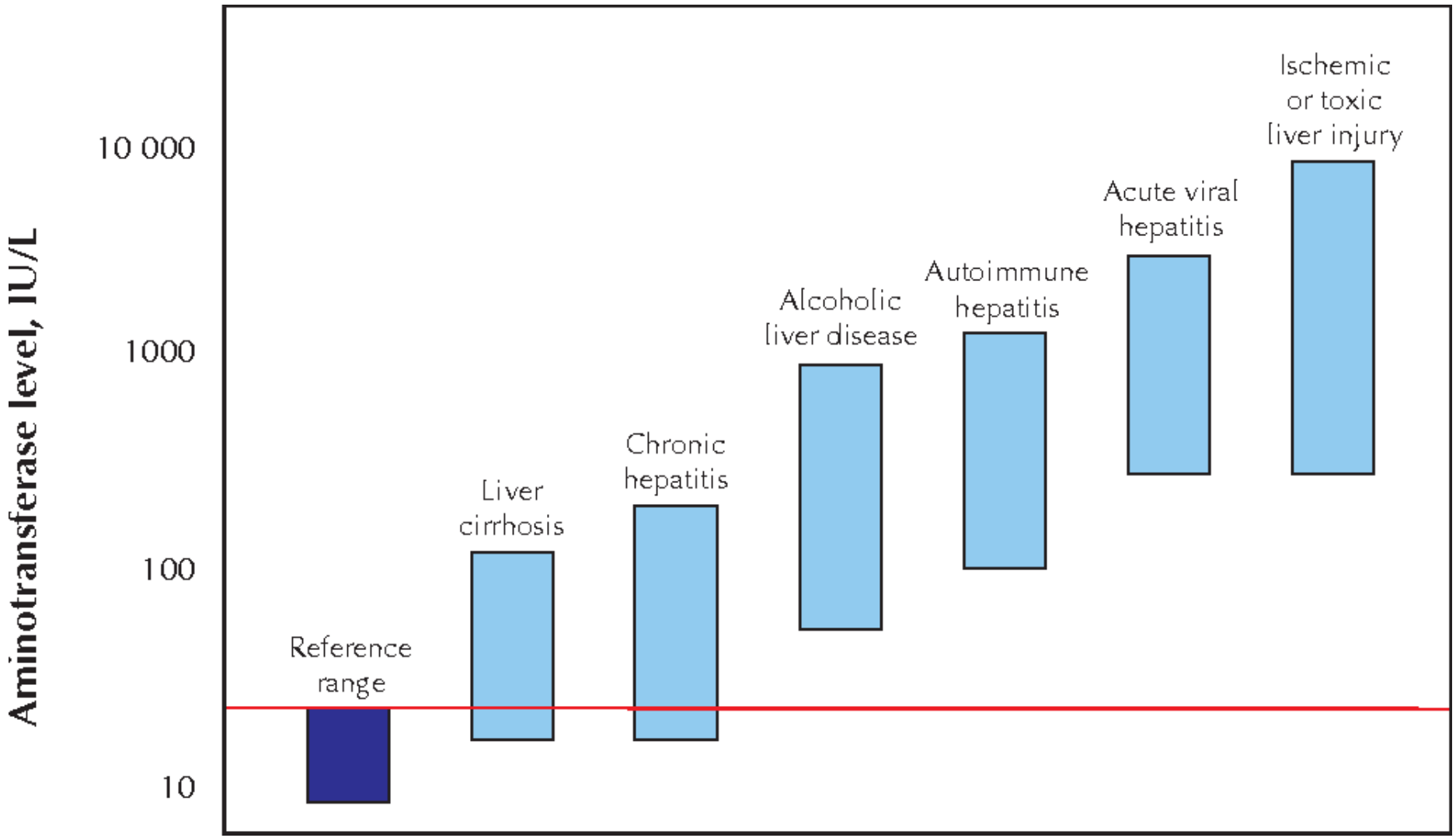
Sensitive indicators of acute hepatic injury

- Up to 300 U/L are non specific and may be found in any liver disorder
- High >1000U/L typically extensive hepatocellular injury e.g. viral hepatitis, ischemic liver injury, toxin/drug-induce injury

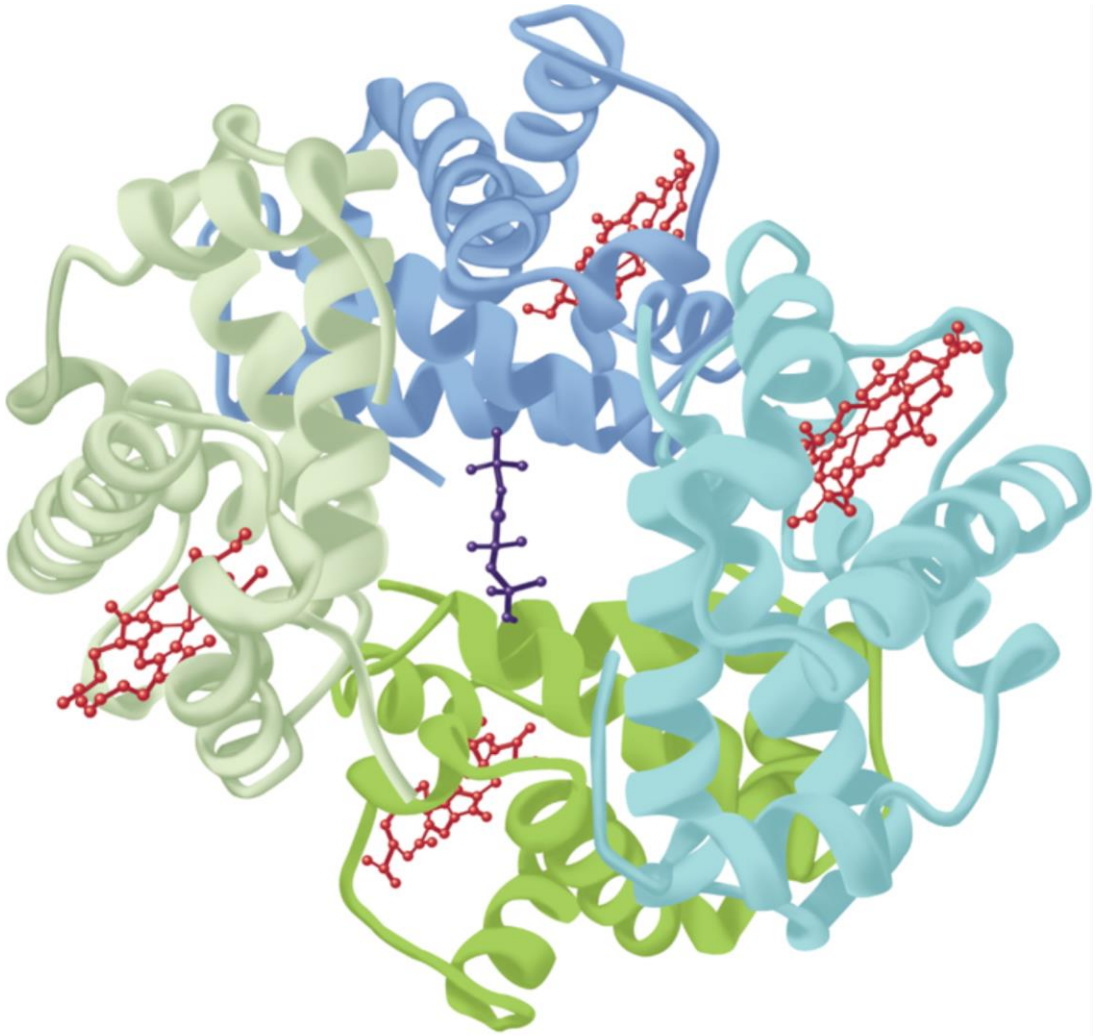
In health and in many disorders ALT > AST. AST is both a cytosolic and mitochondrial protein – **alcohol** damage that causes mitochondrial damage raises AST relative to ALT

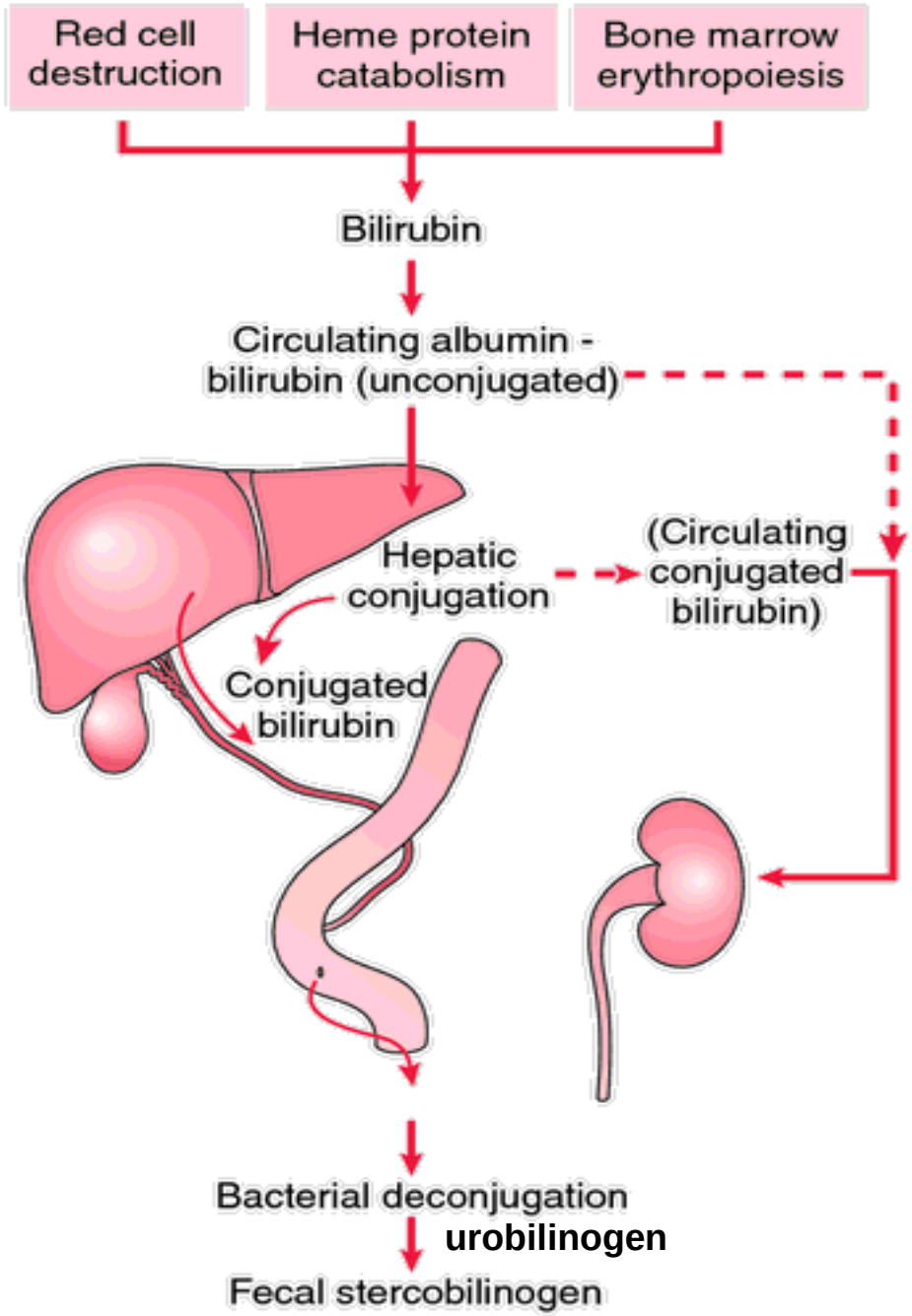


Aminotransferases



Haem Metabolism

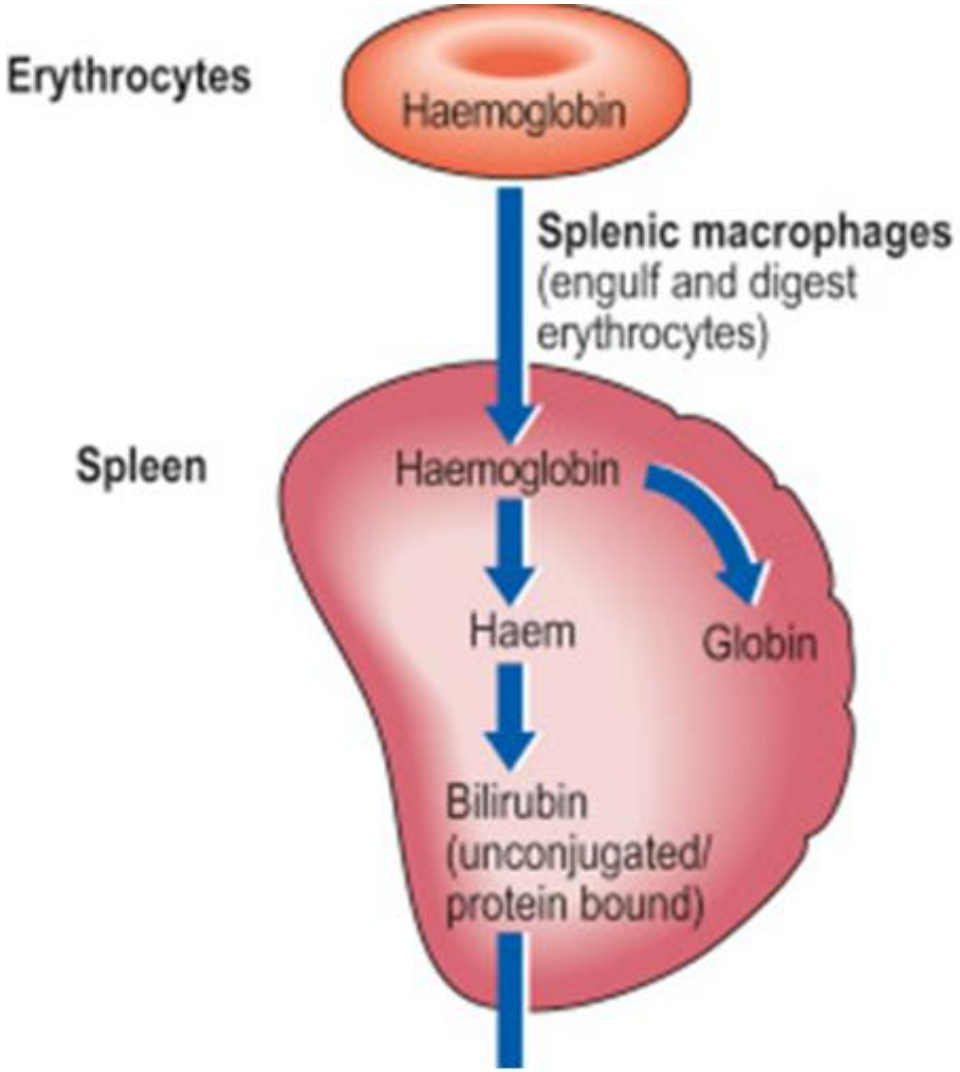




Bilirubin

In the metabolism of haemoglobin, the haem group is separated from the globin protein. The iron is recycled, and the haem is metabolised to biliverdin and then to bilirubin.

Bilirubin travels in the blood in association with albumin **(unconjugated,** not water soluble) and is taken up by hepatocytes.

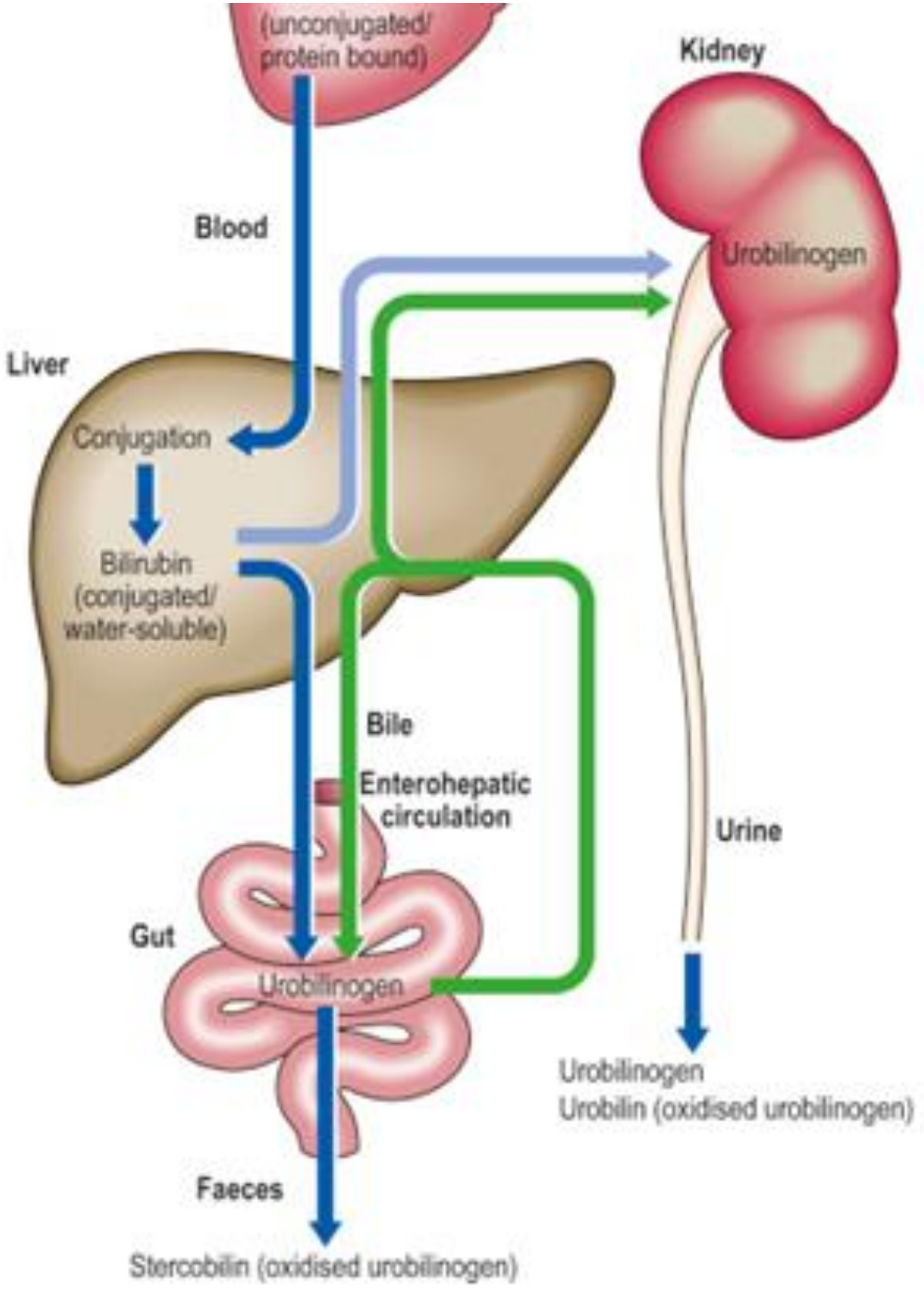


Bilirubin

In the liver it is conjugated with one or two glucuronic acid moieties by a specific UDP-glucuronosyltransferase (**conjugated**, water soluble).

Conjugated bilirubin then excreted into the bile ducts, and into the small intestine. The action of bacterial enzymes generates urobilinogen and fecal stercobilinogen.

Conjugated bilirubin and urobilinogen can be excreted by the kidneys.



Measuring Bilirubin

Total bilirubin = conjugated (direct) + unconjugated (indirect)

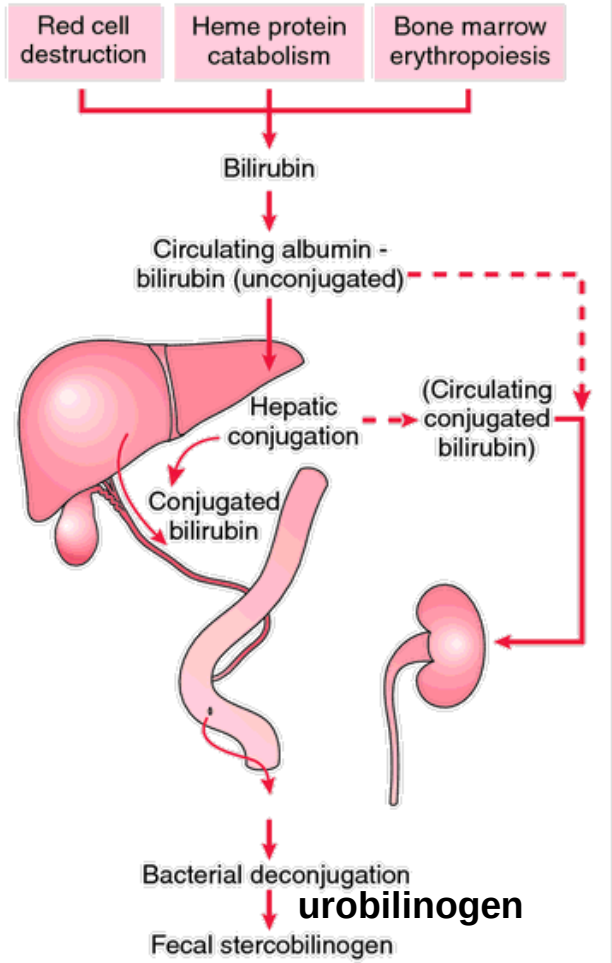
Hyperbiliruninaemia

Increased in unconjugated bilirubin alone are unlikely to be due to liver

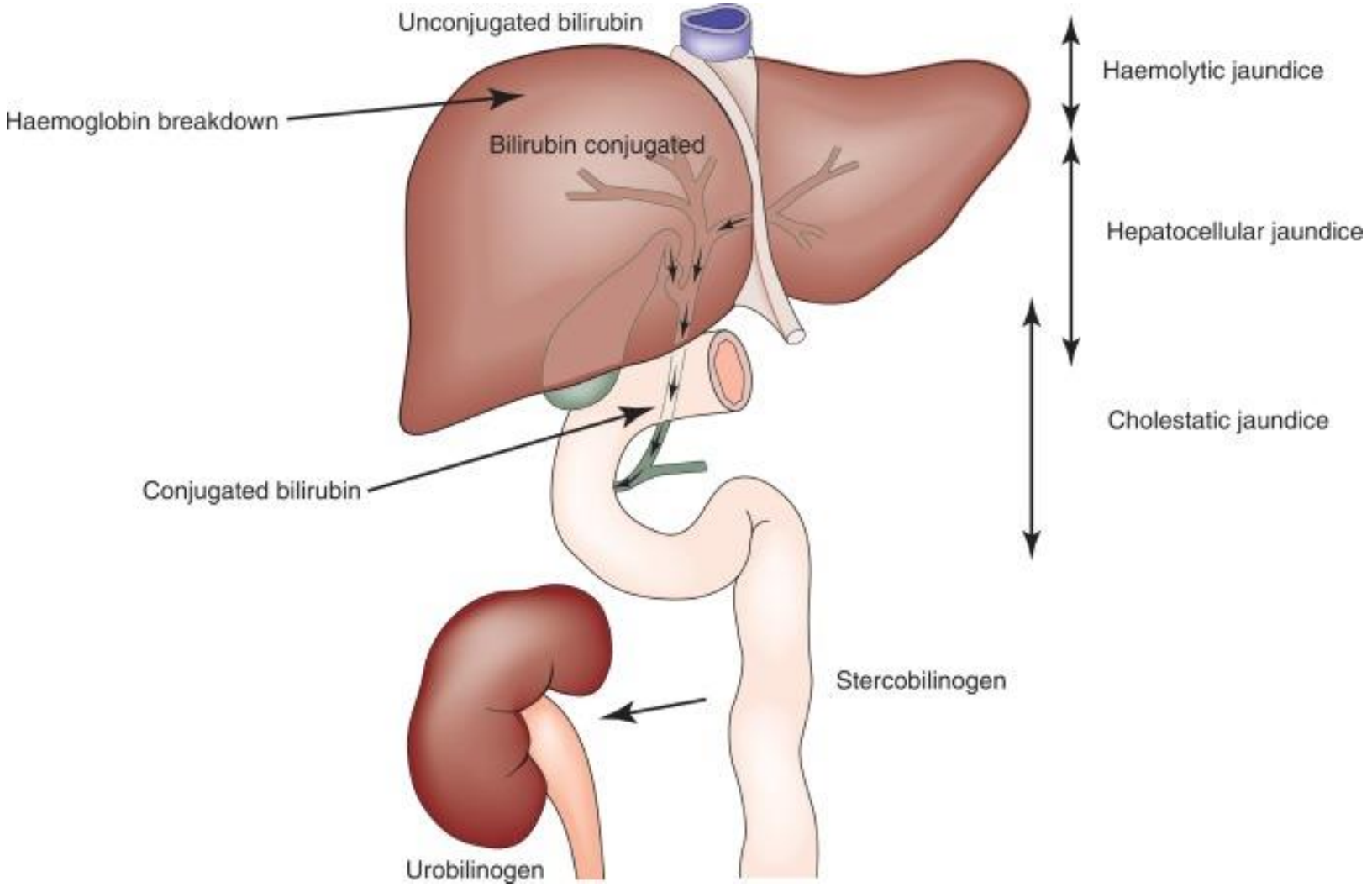
Increases in both unconjugated and conjugated/ conjugated:

- may be due to liver damage
- may be due to biliary disease

Only conjugated bilirubin will be excreted by the kidneys so bilirubinuria implies high conjugated bilirubin and liver/biliary disease

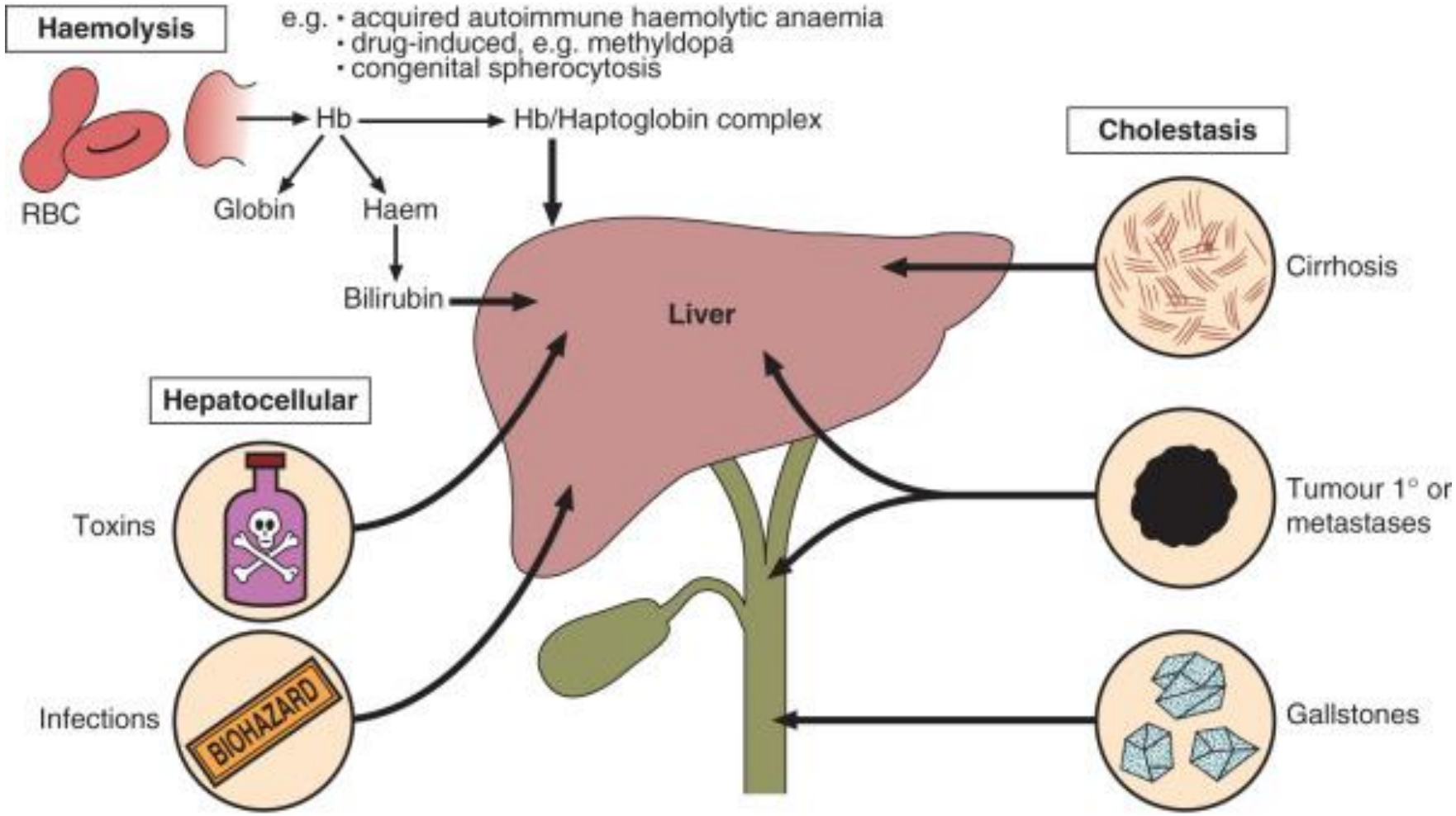


Types of Jaundice



Jaundice (icterus) is a yellowish or greenish pigmentation of the skin and whites of the eyes due to high bilirubin levels

Causes of Jaundice



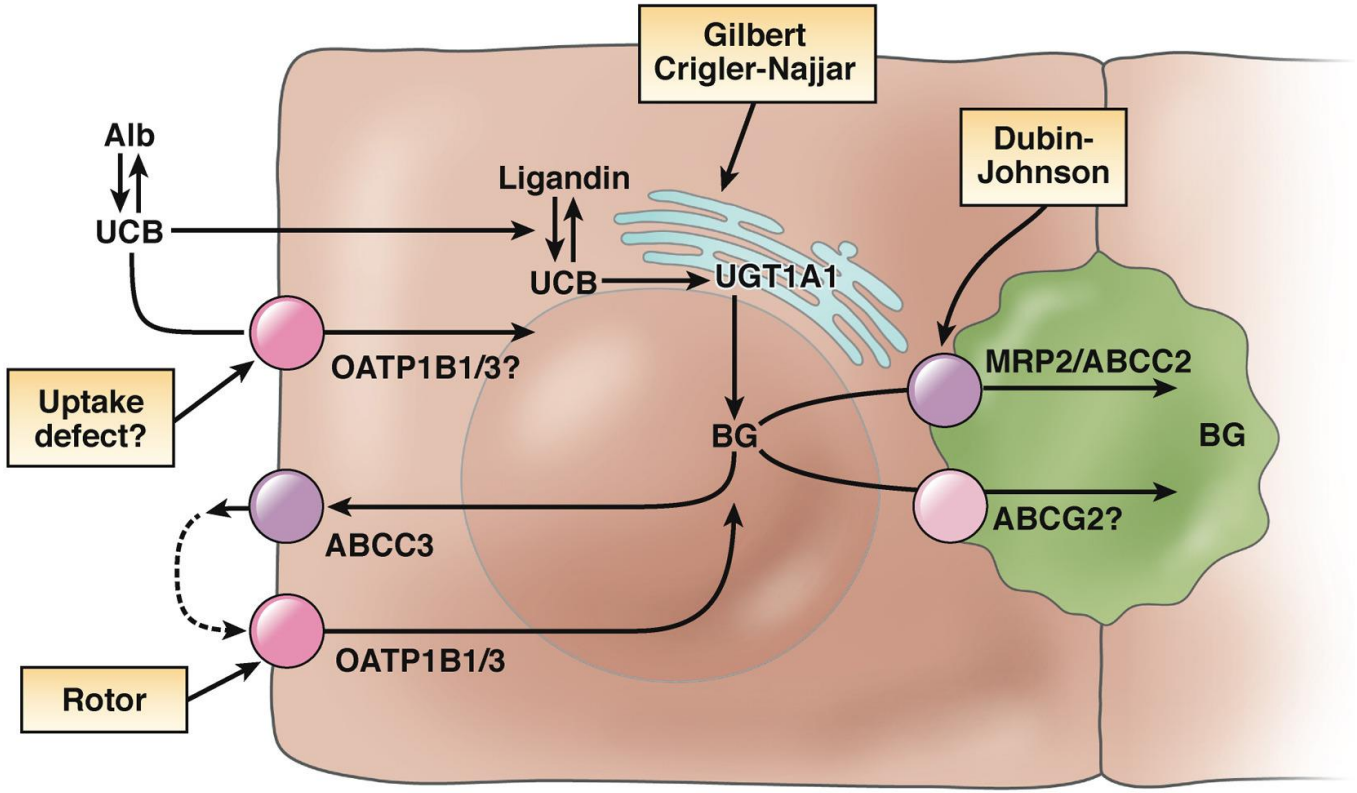
Hereditary Disorders of Bilirubin Metabolism

Rotor syndrome – defect of bilirubin uptake

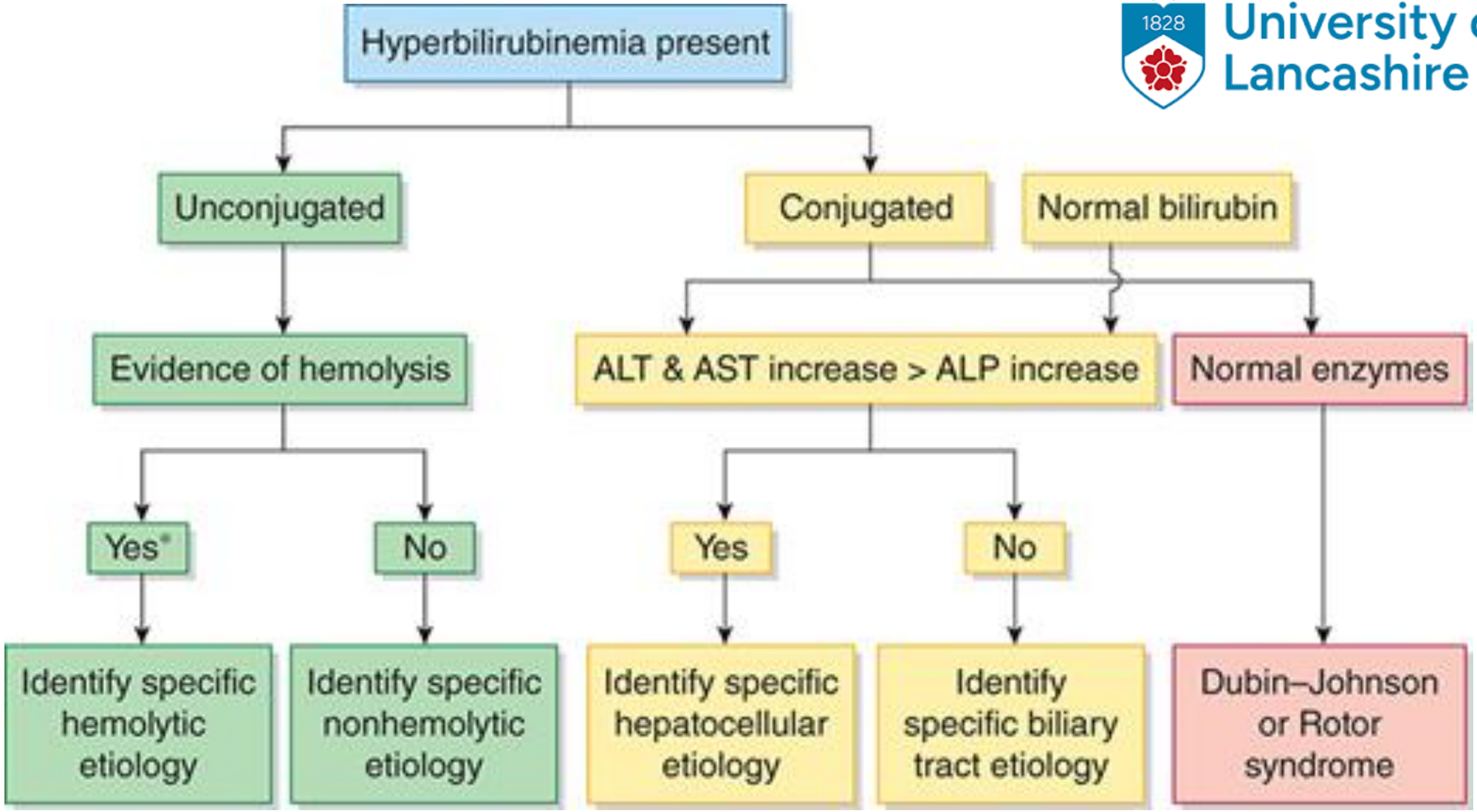
Gilbert syndrome Impaired conjugation of bilirubin due to reduced bilirubin UDP glucuronosyltransferase activity. Results in mild unconjugated hyperbilirubinemia. Affects 3–7% of the population.

Crigler-Najjar Syndrome – defect in bilirubin conjugation

Dubin-Johnson syndrome - Defective canalicular secretion



UCB, unconjugated bilirubin;
Alb, albumin;
BG, bilirubin glucuronide


Hyperbilirubinemia present
Unconjugated
Conjugated
Normal bilirubin
Evidence of hemolysis
ALT & AST increase > ALP increase
Normal enzymes
Yes*
No
Yes
No
Identify specific hemolytic etiology
Identify specific nonhemolytic etiology
Identify specific hepatocellular etiology
Identify specific biliary tract etiology
Dubin–Johnson or Rotor syndrome

Liver Disorders I

- **Haemolysis**

Increased bilirubin (unconjugated), ALT/AST moderately increased, ALP normal, albumin and PT normal

- **Acute hepatocellular necrosis (viral, drug, toxin, acute heart failure)**

Mixed hyperbilirubinaemia; ALT and AST increased; ALT>AST; ALP high, albumin normal, PT prolonged

- **Chronic hepatocellular disorders**

Mixed hyperbilirubinaemia; mild AST, ALT elevation, ALP moderately increased. Albumin decreased, PT prolonged

Liver Disorders II

- **Alcoholic hepatitis cirrhosis**

Mixed hyperbilirubinaemia; Raised ALT, AST; AST:ALT>2; ALP high. Albumin decreased, PT prolonged

- **Intra- and extrahepatic cholestasis**

Mixed hyperbilirubinaemia; Slight elevation of ALT, AST; ALP increased Albumin normal or slightly decreased; PT prolonged (this is due to Vit K)

- **Infiltrative (tumour, granuloma)**

Bilirubin normal, ALT, AST normal or slightly raised; ALP high; albumin and PT normal

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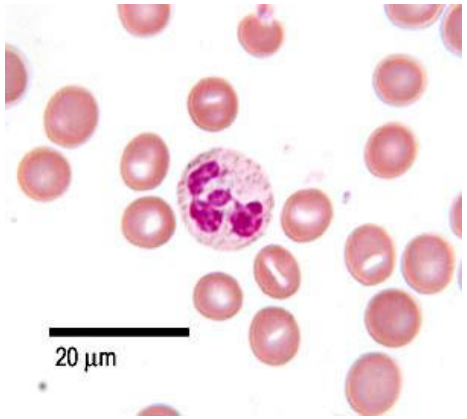
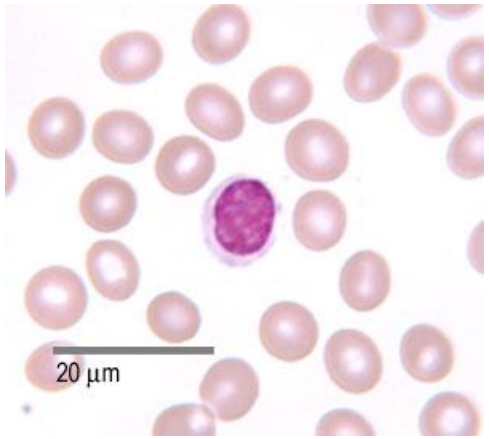
Summary of liver function tests

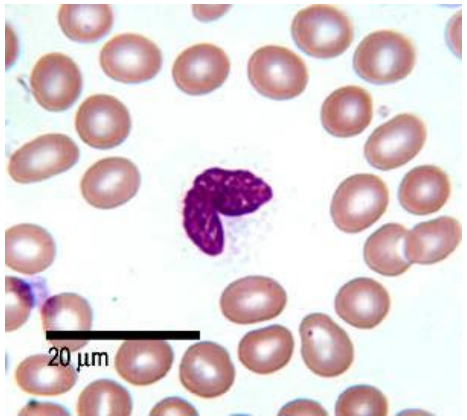
ENTITY MEASURED	LAB TEST	SIGNIFICANCE OF TEST
Measure of synthetic liver function	Prothrombin time (PT)	Clotting factors are manufactured by hepatocytes. PT is not typically prolonged until severe liver damage occurs.
	Serum albumin	Albumin is manufactured by hepatocytes. Severe liver damage can result in a decrease in serum albumin.
Measure of liver damage/ injury	Aspartate transaminase (AST) Alanine transaminase (ALT)	Damage to hepatocytes causes leakage of aminotransferases, causing elevation of serum AST/ALT. ALT is usually more elevated than AST in viral infections, whereas AST is usually twice as great as ALT in patients with liver damage secondary to alcohol abuse.
Measure of liver and biliary tree damage/ injury	Alkaline phosphatase (ALP)	Elevated with damage to the bile duct and liver. Produced in the liver, bile duct, bone, kidney, and placenta; relatively nonspecific for liver injury
	Gamma-glutamyl transferase (GGT)	Elevated with damage to bile duct/liver; more specific marker than ALP. May also be elevated secondary to alcohol abuse (marker of mitochondrial damage)
Measure of function clearance of the liver	Bilirubin	Elevated unconjugated and conjugated bilirubin can result from hepatocyte damage due to decreased ability of the liver to conjugate bilirubin and congestion of the canaliculi, obstructing movement of bilirubin into the gut lumen.

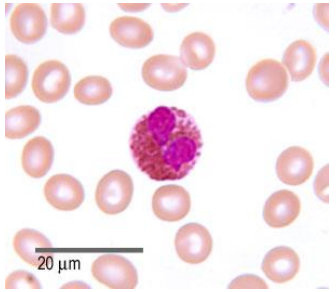
Immunological Indicators

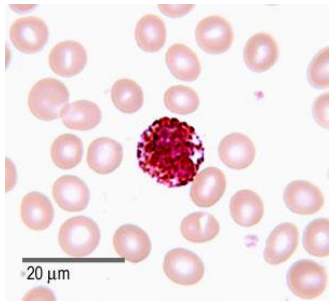
Parameter
Haemoglobin (g/L)
White Blood Cells (x10^9/L)
Platelets (x10^9/L)
Mean Cell Volume (x10^{15}/L)
Packed Cell Volume/haematocrit
Red Blood Cells (x10^{12}/L)
Mean Cell Haemoglobin (pg)
Mean Cell Haemoglobin concentration (g/L)
Red Cell Distribution Width
Neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils











	Neutrophils (60-70%)	Lymphocytes (20-50%)	Monocytes (2-10%)	Eosinophils (1-6%)	Basophils (<1%)
Increased	Bacterial infections, Trauma, surgery, burns, haemorrhage, Inflammation, infarction, polymyalgia, poly arteritis nodosa (PAN), Myeloproliferative disorders. Marked increase in leukaemias, Disseminated malignancy	Viral infections, toxoplasmosis; whooping cough; Brucellosis, Chronic lymphatic leukaemia	Acute and chronic infection; malignant disease – acute myeloid leukaemia/ Hodgkin's disease; myelodysplasia	Allergy; parasitic infections (helminths); malignant disease (lymphomas and eosinophilic leukaemia); during the convalescent phase of any infection	Viral infections; chronic myeloid leukaemia; systemic mastocytosis; haemolysis; polycythaemia rubra vera
Decreased	Viral infections, brucellosis, typhoid, TB, Folate/B12 deficiency Chemotherapy	Systemic Lupus Erythematosis; legionnaire's disease; HIV infection; post-chemo/radio-therapy	aplastic anaemia		

Do not need to memorise details now – some elements will come up again later

Immunoglobulins

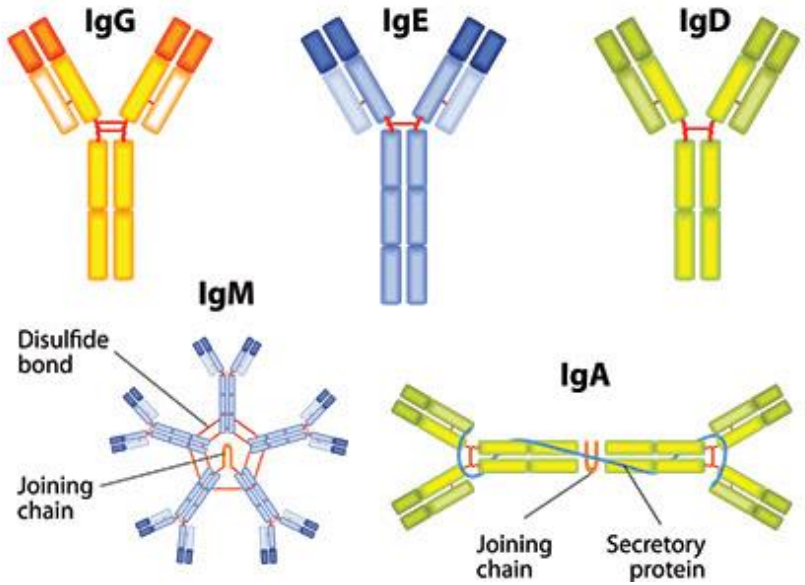
Measuring Antibody Levels

Increased Production

- Non-specific (Rheumatoid arthritis, lupus, autoimmune liver disease)
- Infection
- Myeloma (monoclonal)

Decreased Production

- Immunodeficiency



Immunoglobulins

Presence of autoantibodies in autoimmune diseases

Presence of anti-pathogen antibodies

- Post-exposure e.g. as evidence of exposure, to measure protection levels
- Post-vaccination to measure protection levels

Presence of allergen-specific IgE in allergy

Transplantation

- To explain, or predict, rejection

MBBS Learning Objectives

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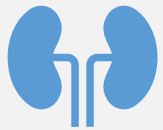
Describe the types of haemoglobin abnormalities and the pathophysiological mechanisms responsible for disease development

Recognise the role of haematological parameters for the differential diagnosis and disease management of clinical conditions initiated by haemoglobinopathies

Describe the indication of CV diseases by clinical biochemistry parameters



Describe the use of biochemical parameters for the diagnosis and management of clinical conditions originated from liver and biliary diseases

Additional Reading

Kumar and Clarke Clinical Medicine (Available via Clinical Key)

- Chapter 14: Liver Disease
- Chapter 23 Cardiovascular Disease.

Pathophysiology of blood disorders (Available via AccessMedicine)

- Chapter 8 Thalassaemia
- Chapter 9 Sickle Cell Anaemia.

BMJ Best Practice

- Alpha and Beta thalassaemia;
- Sickle cell anaemia