

Notes & Notes

For MRCP part 1 & 11

By

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Basic sciences

Biochemistry & metabolism

Updated

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Chapter 14 Biochemistry & metabolism

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Anion gap (AG)

Renal tubular acidosis causes a normal anion gap

- The anion gap allows for the differentiation of 2 groups of metabolic acidosis.
 1. **Metabolic acidosis with a high AG** is associated with the **addition of** endogenously or exogenously **generated acids**.
 2. **Metabolic acidosis with a normal AG** is associated with the **loss of HCO₃ or the failure to excrete H⁺ from the body**.
- The anion gap is calculated by: (sodium + potassium) - (bicarbonate + chloride)
- A normal anion gap is **8-14 mmol/L**
- It is useful to consider in patients with a metabolic acidosis:
 - ⇒ **Causes of a normal anion gap or hyperchloraemic metabolic acidosis**
 - gastrointestinal bicarbonate loss: diarrhoea, uretero-sigmoidostomy, fistula
 - **renal tubular acidosis**
 - ⇒ **Causes of a raised anion gap metabolic acidosis**

<ul style="list-style-type: none"> ▪ lactate: shock, hypoxia ▪ ketones: diabetic ketoacidosis, alcohol 	<ul style="list-style-type: none"> ▪ urate: renal failure ▪ acid poisoning: salicylates, methanol
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mnemonic of high anion gap acidosis:

- DR. MAPLES: D-DKA; R-renal; M-methanol; A-alcoholic ketoacidosis; P-paraldehyde, phenformin; L-lactic (ie, CO, HCN); E-ethylene glycol; S-salicylates

Remember the mnemonic MUDPILES → high anion gap acidosis

- M Methanol
- U Uremia
- D Diabetic ketoacidosis
- P Paraldehyde
- I Infection
- L Lactic acidosis
- E Ethylene glycol
- S Salicylates

- Metabolic acidosis associated with bladder reconstruction (e.g: for carcinoma of the bladder).**
- Hyperchloraemic metabolic acidosis is a documented complication of neobladder formation. However, it usually improves with time and is mild.
 - Severe and persistent metabolic acidosis may manifest when patients undergo further surgery for other reasons, as is the case in this patient.
 - Neobladder formation following radical cystectomy or cystoprostatectomy is becoming increasingly more common, and medical staff treating patients with neobladders should recognise and **treat metabolic acidosis with intravenous fluids and bicarbonate**.

Metabolic alkalosis

Pathophysiology

- Metabolic alkalosis may be caused by a **loss of hydrogen ions (H⁺) or a gain of bicarbonate (HCO₃⁻)**.
- It is due mainly to problems of the **kidney or gastrointestinal tract**.
- The initial disturbance of metabolic alkalosis is an increased HCO₃⁻ concentration, followed by a compensatory response of increased Pco₂.
- All renal tubular defects result in metabolic alkalosis, except for Fanconi syndrome.

ABG picture

- pH : Elevated
- PCO₂: Expected compensatory response: ↑
- HCO₃: Elevated

Compensation mechanism

- Hypoventilation is an immediate compensatory response to metabolic alkalosis.
- ↑ Arterial and CSF pH (with ↑ HCO₃⁻) → ↓ stimulation of the medullary chemoreceptors → ↓ respiratory rate and/or tidal volume (hypoventilation) → ↑ CO₂ retention → ↑ PCO₂

Causes

- | | |
|---|---|
| <ul style="list-style-type: none"> Vomiting / aspiration (e.g. peptic ulcer leading to pyloric stenosis, nasogastric suction) Diuretics Liquorice, carbenoxolone Hypokalaemia Bulimia nervosa | <ul style="list-style-type: none"> Primary hyperaldosteronism <ul style="list-style-type: none"> ⇒ Liddle syndrome ⇒ Con syndrome Cushing's syndrome Bartter's syndrome Gitelman syndrome Congenital adrenal hyperplasia |
|---|---|

Mechanism of metabolic alkalosis

- The main mechanisms of metabolic alkalosis in the setting of vomiting are increased H⁺ excretion in the distal tubule and increased bicarbonate reabsorption in the proximal tubule.**
 - ⇒ ECF depletion (vomiting, diuretics) → Na⁺ and Cl⁻ loss → activation of renin-angiotensin II-aldosterone (RAA) system → ↑ aldosterone → reabsorption of Na⁺ in exchange for H⁺ in the distal convoluted tubule
- In hypokalaemia, K⁺ shift from cells to ECF, alkalosis is caused by shift of H⁺ into cells to maintain neutrality

A patient with liver cirrhosis develops metabolic alkalosis. What is the most likely pathological mechanism? → **Reduced urea synthesis**

A patient in the intensive care unit following liver transplant surgery has a metabolic alkalosis.

What is the most likely cause?

- ⇒ **Diuretic-induced volume depletion**
 - Cirrhosis → hypoalbuminaemia → low colloid osmotic pressure → Relative volume depletion → ↑ aldosterone, (which is not adequately metabolised by an impaired liver).
 - Furosemide use in the post-operative period further exacerbates alkalosis driven by hyperaldosteronism .**

Aetiology of metabolic alkalosis

Mechanism	Causes
Chloride-responsive metabolic alkalosis (urine chloride < 25 mEq/L)	<ul style="list-style-type: none"> ⌚ Gastrointestinal losses: due to vomiting, nasogastric suction, or diarrhea ⌚ Renal losses: due to loop or thiazide diuretics ⌚ Cystic fibrosis
Chloride-resistant metabolic alkalosis (urine chloride > 40 mEq/L)	<ul style="list-style-type: none"> ⌚ Severe magnesium deficiency ⌚ Extreme hypercalcemia, hypokalemia ⌚ High alkali load (e.g., due to antacid use, alkalinization therapy) ⌚ Loop or thiazide diuretics ⌚ Other (less common causes) <ul style="list-style-type: none"> ▪ Associated with low or normal blood pressure <ul style="list-style-type: none"> ☞ Bartter syndrome ☞ Gitelman syndrome ▪ Associated with high blood pressure <ul style="list-style-type: none"> ☞ Hyperaldosteronism ☞ Cushing syndrome ☞ Liddle syndrome ☞ Licorice ingestion ▪ Ingestions or drugs (Laxative abuse, ampicillin, penicillin) ▪ Recovery from starvation ▪ Hypoalbuminemia

Prognosis

- when the pH is greater than 7.65 → mortality rate is 80%

Treatment

- Chloride-responsive metabolic alkalosis
 - ⇒ Start isotonic saline to increase urinary bicarbonate excretion and correct extracellular volume loss
- Chloride-resistant metabolic alkalosis
 - ⇒ Consider bicarbonate excess as a potential cause and administer acetazolamide.
 - ⇒ Acetazolamide is a diuretic used to alkalinize the urine or treat metabolic alkalosis as it inhibits carbonic anhydrase.

Respiratory acidosis

Causes

Mechanism	Causes
Acute respiratory acidosis	<ul style="list-style-type: none"> ⇒ Acute lung disease (e.g., pneumonia, pulmonary edema) ⇒ Acute exacerbation of chronic obstructive airway disease (e.g., COPD, asthma) ⇒ CNS depression due to: <ul style="list-style-type: none"> ▪ Head trauma ▪ Postictal state ▪ Drug toxicity (e.g., from opiates, barbiturates, benzodiazepines) ▪ Central sleep apnea
Chronic respiratory acidosis	<ul style="list-style-type: none"> ⇒ Airway obstruction (e.g., COPD, asthma) ⇒ Respiratory muscle weakness, e.g., due to: <ul style="list-style-type: none"> ▪ Myasthenia gravis ▪ ALS ▪ Guillain-Barré syndrome ▪ Poliomyelitis ▪ Multiple sclerosis ▪ Severe hypokalemia

Features

Signs and symptoms of respiratory acidosis

Central nervous system	Respiratory system	Cardiovascular system
Cerebral vasodilation	Breathlessness	Flushing, bounding pulse
Increased intracranial pressure	Cyanosis	Cor pulmonale
Headache, confusion, agitation	Pulmonary hypertension	Systemic hypotension
Hallucinations, transient psychosis		Arrhythmias
Myoclonic jerks, flapping tremor, extensor plantars, depressed reflexes		Initially good cardiac output, then decreases
Papilloedema, constricted pupils		
Seizures, coma		

Mechanism

- Alveolar hypoventilation → CO₂ retention

ABG picture

- pH : low
- PCO₂: elevated
- HCO₃: Expected compensatory response: ↑

Treatment

- Consider noninvasive or invasive mechanical ventilation.

Respiratory alkalosis

Mechanism

- ↑ Respiratory rate and/or tidal volume → alveolar hyperventilation → CO₂ washout

Causes

- Anxiety leading to hyperventilation (Hyperventilation will result in carbon dioxide being 'blown off', causing an alkalosis.) → high PH , low PCO₂ , normal PO₂.
 - ⇒ not associated with hypoxia.
- pulmonary embolism
- Acute severe asthma
 - ⇒ associated with hypoxia and normal or rising CO₂
- Drugs (salicylates, progesterone)
 - ⇒ salicylate overdose leads to a mixed respiratory alkalosis and metabolic acidosis.
 - **Early stimulation of the respiratory centre** leads to a **respiratory alkalosis** whilst later the **direct acid effects of salicylates** (combined with acute renal failure) may lead to an **acidosis**.
- CNS disorders: stroke, subarachnoid haemorrhage, encephalitis
- High altitude
- **Pregnancy**
- Pain
- Excessive mechanical ventilation.
- Hepatic failure

ABG picture

- pH : elevated
- PCO₂: low
- HCO₃: Expected compensatory response: ↓

Differential diagnosis of respiratory alkalosis with type 1 respiratory failure (low pO₂ and low pCO₂):

- Chronic venous thromboembolism (most likely).
- Pulmonary fibrosis (but basal crackles may be expected).

Calcium metabolism see endocrinology

Hypercalcaemia see endocrinology

Hypocalcaemia see endocrinology

Vitamin D see endocrinology

Hyperkalaemia

Definition

- Serum potassium level > 5 mEq/L

Regulation

- Plasma potassium levels are regulated by a number of factors including:
 - ⇒ Aldosterone
 - ⇒ acid-base balance
 - ⇒ insulin levels.
- Metabolic acidosis is associated with hyperkalaemia as hydrogen and potassium ions compete with each other for exchange with sodium ions across cell membranes and in the distal tubule.

Causes

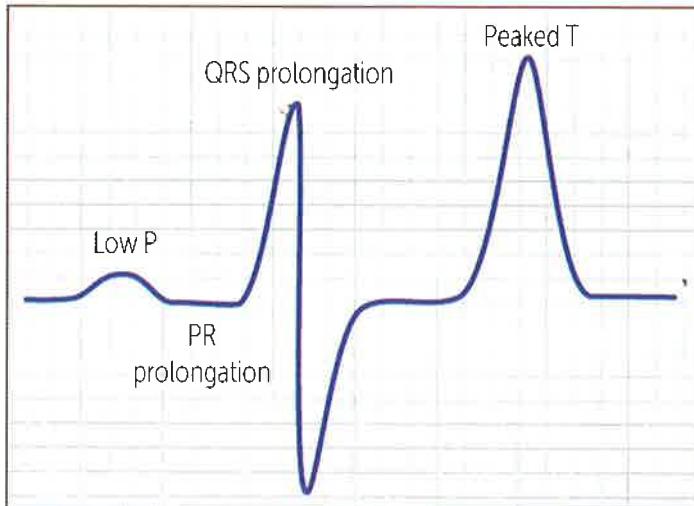
- Potassium excess:** due to altered K⁺ metabolism or intake
 - ⇒ **Reduced excretion:** acute and chronic kidney disease
 - ⇒ **Endocrine causes:** hypocortisolism, hypoaldosteronism
 - ⇒ **Drugs:** potassium-sparing diuretics, ACE inhibitors, angiotensin receptor blockers, NSAIDs, and trimethoprim-sulfamethoxazole
 - ⇒ **Type IV renal tubular acidosis**
 - ⇒ **Increased intake**
 - High potassium diet, e.g., bananas, oranges, kiwi fruit, avocado, spinach, tomatoes
 - K⁺ containing IV fluids
- Extracellular shift**
 - ⇒ **Acidosis** → ↑ extracellular H⁺ → inhibition of the Na⁺/H⁺ antiporter → ↓ intracellular Na⁺ → ↓ sodium gradient inhibits the Na⁺/K⁺-ATPase → ↑ extracellular K⁺ concentration
 - Hyperkalemia → ↑ extracellular K⁺ concentration → ↑ potassium gradient stimulates the Na⁺/K⁺-ATPase → ↑ extracellular Na⁺ → ↑ sodium gradient stimulates the Na⁺/H⁺ antiporter → ↑ extracellular H⁺ → acidosis
 - Exceptions: In renal tubular acidosis and acetazolamide toxicity, findings include hypokalemia and metabolic acidosis.
 - ⇒ **Hyperosmolality**
 - ⇒ **Insulin deficiency** (manifests with hyperglycemia)
 - ⇒ **Drugs**
 - **Beta blockers**
 - Succinylcholine: (esp. when given with preexisting burns and/or muscle trauma),
 - Digoxin: inhibits the Na⁺/K⁺-ATPase → ↑ extracellular K⁺ concentration
- Extracellular release**
 - ⇒ **Pathological cell lysis**
 - Rhabdomyolysis
 - Tumor lysis syndrome
 - Hemolysis
 - ⇒ **High blood cell turnover:** e.g., thrombocytosis, erythrocytosis, leukocytosis
 - ⇒ **Pseudohyperkalaemia:** resulting from iatrogenic red blood cell lysis
 - Blood drawn from the side of IV infusion or a central line without previous flushing
 - Prolonged use of a tourniquet

- Fist clenching during blood withdrawal
- Delayed sample analysis

When K⁺ shifts out of the cell, it's a BAD LOSS! – Beta blockers, Acidosis, Digoxin, Lysis, hyperOsmolality, high Sugar, Succinylcholine

Features

- May be asymptomatic
- Nausea, vomiting, diarrhea
- **Cardiac:** Arrhythmias (e.g., atrioventricular block, ventricular fibrillation)
- **Neuromuscular:** Muscle weakness, paralysis, paresthesia, ↓ Deep tendon reflexes
⇒ Weakness and fatigue are the most common complaints
- **ECG changes**
 - ⇒ **Early changes** (typically seen at a serum potassium level of 5.5-6.5 mEq/L)
 - tall, peaked T waves
 - shortened QT interval
 - ST-segment depression.
 - ⇒ At a serum potassium level of 6.5-8.0 mEq/L, in addition to peaked T waves:
 - Decreased or disappearing P wave
 - Prolonged PR interval
 - Widening of the QRS
 - Amplified R wave



Treatments

Immediate treatment principles include:

1. Providing calcium salts to reduce the risk of arrhythmia ('protect the heart');
2. Administering intravenous glucose and insulin ('shift potassium into cells');
3. Reducing intake and increasing output of potassium ('remove potassium from the body').

- Precipitating factors should be addressed (e.g. acute renal failure) and aggravating drugs stopped (e.g. ACE inhibitors).
- **Mild chronic hyperkalaemia (eg: 5.6 mmol/l) is well tolerated and not a cause for concern.** If serum potassium rise to >6.0 mmol/l, standard practice would be to stop the ACEi and - if K >6.0 mmol/l were to persist - to advise a low potassium diet.
- **Stabilisation of the cardiac membrane**
 - ⇒ intravenous 10 ml 10% calcium gluconate (or calcium chloride)
 - ⇒ The effects of intravenous calcium occur within 1 to 3 minutes but last for only 30 to 60 minutes.
- **Short-term shift in potassium from extracellular to intracellular fluid compartment**
 - ⇒ Combined insulin/dextrose infusion:
 - ⇒ The most effective agent .
 - ⇒ In hyperglycaemic patients (serum glucose >15 mmol/L) insulin may be given without additional intravenous glucose.
 - ⇒ The dose: **10 units of soluble insulin**
 - ⇒ Nebulised salbutamol
 - Less effective than iv insulin and glucose (not recommended as monotherapy)
 - Patients prescribed beta blockers may be 'resistant' to the hypokalaemic effects of salbutamol.
- **Removal of potassium from the body**
 - ⇒ Calcium resonium (orally or enema)
 - ⇒ Loop diuretics
 - ⇒ Dialysis

May 2020 exam: H/O muscle weakness and lethargy. K+ = 6.3 mmol/l. What is the most appropriate initial treatment to lower the serum potassium level?

→ Insulin/dextrose infusion

Pseudohyperkalaemia

High cell counts and high potassium: consider pseudohyperkalaemia

Causes

- Haemolysis during venepuncture
- Delay in the processing of the blood specimen
- Abnormally high numbers of platelets, leukocytes, or erythrocytes (such as myeloproliferative disorders or **essential thrombocythosis**)
- Familial causes

Management

- **Re-check a fresh sample at the hospital**
- Measuring an arterial blood gas will give a quick and accurate measure of true serum potassium.

Hypokalaemia and acid-base balance

Hypokalaemia - U waves on ECG

Definition

- Serum potassium (K+) level < 3.5 mEq/L

Causes

Hypokalaemia with alkalosis

- Vomiting
- Diuretics
- Cushing's syndrome
- Conn's syndrome (primary hyperaldosteronism)

Hypokalaemia with acidosis

- Diarrhoea
- **Renal tubular acidosis**
- Acetazolamide
- Partially treated diabetic ketoacidosis

Drug induced hypokalaemia

- Intracellular shifts of potassium with normal total body potassium, for example:
- ⇒ theophylline
- ⇒ β -agonists
- ⇒ caffeine
- ⇒ insulin

Other causes

- Loss of potassium stores, for example: chronic diuretic use
- **Magnesium deficiency** may also cause hypokalaemia. In such cases, **normalizing the potassium level may be difficult until the magnesium deficiency has been corrected**

In hyperthermia, as body temperature increases, what is the earliest biochemical abnormality?

⇒ **Hypokalaemia**

- As body temperature increases, such as occurs in hyperthermia due to heatstroke, the earliest abnormality is hypokalaemia.
- This is thought to be due to increased K⁺ uptake by muscles as catecholamines stimulate the NA-K-ATPase transporter.
- As the body temperature rises further, hyperkalaemia can develop with rhabdomyolysis and lactic acidosis.
- The acid-base picture is of metabolic acidosis with compensatory respiratory alkalosis.

K⁺ acts like H⁺: Hypokalemia leads to alkalosis and vice versa

Hypomagnesemia can lead to refractory hypokalemia

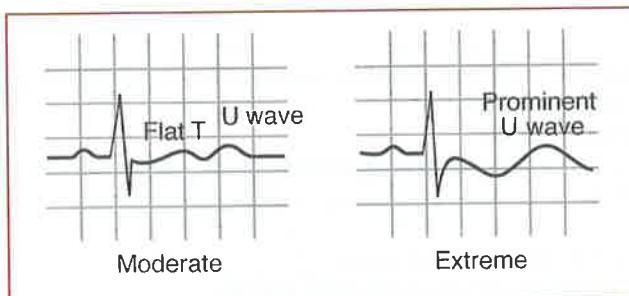
Features

- Cardiovascular : cardiac arrhythmias
- Neuromuscular:
 - Muscle cramps and spasms
 - Muscle weakness

- Decreased deep tendon reflexes
- Gastrointestinal: Constipation

ECG findings in hypokalemia

- Mild to moderate hypokalemia
 - ⇒ T-wave flattening or inversion
 - ⇒ ST depression
 - ⇒ Prolonged PR interval
- Moderate to severe hypokalemia
 - ⇒ QT prolongation
 - ⇒ Presence of U waves



Treatment

- If $K > 2.5$ with no symptoms or ECG changes → oral potassium
- If $K < 2.5$ with symptoms or ECG changes → IV potassium
- In life-threatening cases → 1L IV 0.9% NaCl with 40 mmol/l KCl infused over four hours**
 - ⇒ Cardiac monitoring.
 - ⇒ Potassium should be given in NaCl.
 - ⇒ Concentration should not exceed 40 mmol/l
 - ⇒ No more than 10-20 mmol/hour should be given.

In patients with hypokalemia, avoid solutions containing dextrose, which can increase insulin secretion and worsen hypokalemia.

Daily maintenance requirements (NICE guidelines):

- Water → 1500-2500 ml/day (25-30 ml/kg/day)
- Potassium, Sodium and Chloride → **1 mmol/kg/day**
 - ⇒ **Sodium → 70 mmol**
 - ⇒ **potassium → (40-80 mmol/day)** In the absence of kidney disease or hyperkalaemia (around 1 mmol/kg per day)

Estimation of total body potassium loss:

- a drop in 1 mmol/L K^+ of serum potassium is approximately equivalent to a 200 mmol K^+ total body loss.**

Hypernatraemia

Hypernatraemia associated with **hypovolaemia** occurs due to a free water deficit. Common causes include reduced water intake (e.g. elderly), GI losses (e.g. vomiting and diarrhoea), skin losses (e.g. burns), and renal losses (e.g. osmotic diuresis)

Hypernatraemia associated with **hypervolaemia** can occur due to hypertonic saline, hypertonic sodium bicarbonate, excess salt in diet, or hyperaldosteronism

Causes

- Insufficient water
- free water loss:
 - ⇒ renal (**diabetes insipidus**, diuretics, osmotic diuresis as with hyperglycaemia),
 - ⇒ GI (diarrhoea, vomiting),
 - ⇒ skin (sweating, burns)
- Salt overload e.g. acute salt poisoning (hypertonic saline, hypertonic sodium bicarbonate), hyperaldosteronism

Treatment

- Treatment is aimed at the underlying cause.
- Hypernatraemia should be corrected with great caution.
- Although brain tissue can lose sodium and potassium rapidly, lowering of other osmolytes (and importantly water) occurs at a slower rate, predisposing to cerebral oedema, resulting in seizures, coma and death.
- acute hypernatraemia can be corrected quickly but if chronic (>24 hours) then it should be corrected at <0.5 mmol/L/hr.
- Fluid resuscitation should involve oral water, 0.45% saline or 5% dextrose IV.

Hyponatraemia (serum sodium less than 135 mEq/L)

Mechanisms of causes

1. Water excess
2. Sodium depletion.
3. Pseudohyponatraemia:
 - ⇒ hyperlipidaemia (increase in serum volume)
 - ⇒ hyperproteinemia (e.g. myeloma)
 - ⇒ taking blood from a drip arm.

Cause of hyponatraemia

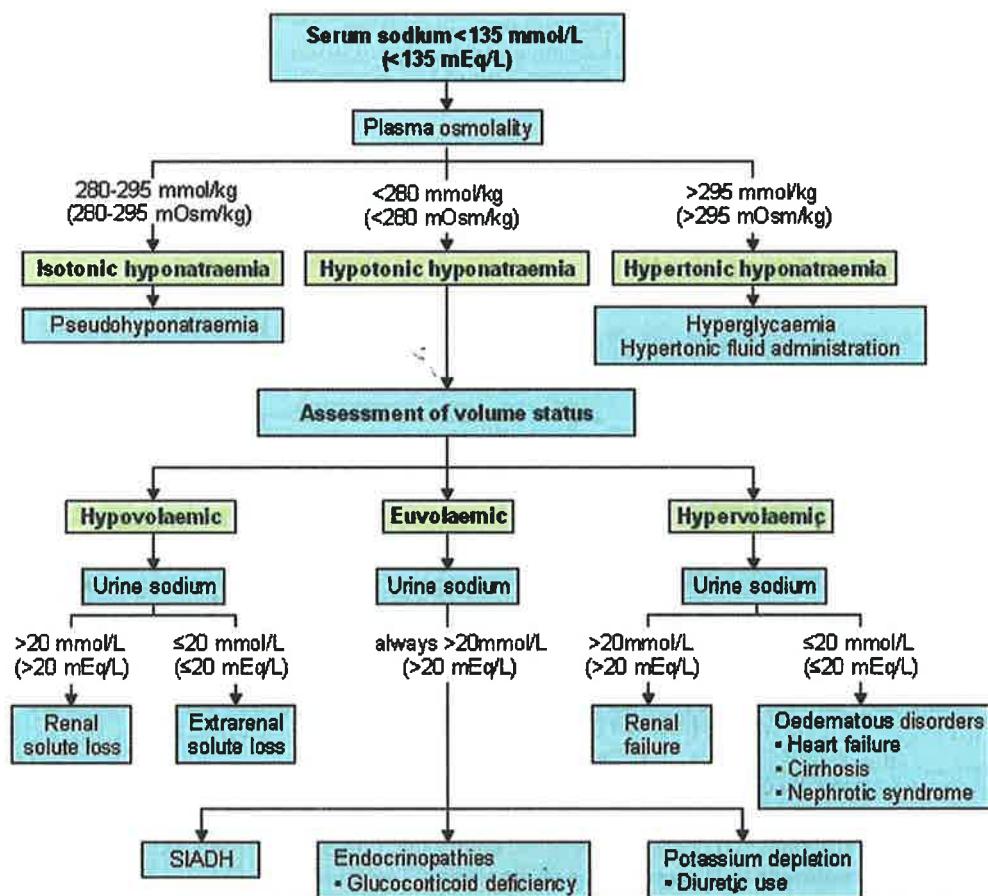
Urinary sodium > 20 mmol/l	Urinary sodium < 20 mmol/l
Sodium depletion, renal loss (patient often hypovolaemic)	Patient often euvolaemic
<ul style="list-style-type: none"> • diuretics • Addison's • diuretic stage of renal failure 	<ul style="list-style-type: none"> • SIADH (urine osmolality > 500 mmol/kg) • hypothyroidism
	Sodium depletion, extra-renal loss (hypovolaemic)
	<ul style="list-style-type: none"> • diarrhoea, vomiting, sweating • burns, adenoma of rectum
	Water excess (patient often hypervolaemic and oedematous)
	<ul style="list-style-type: none"> • secondary hyperaldosteronism: heart failure, cirrhosis • reduced GFR: renal failure • IV dextrose, psychogenic polydipsia

Features

- Fatigue
- Muscle weakness
- Gait disturbance
- Falls
- Disorientation
- Cerebral oedema
- Seizures

Investigations

- Urinary sodium and osmolarity levels aid making a diagnosis.
 - ⇒ urinary sodium
 - Reduced urinary sodium excretion [less than 30 mmol/l] may indicate total body sodium depletion even if plasma sodium levels are normal.
 - may be misleading in the presence of renal impairment or diuretic therapy.



Management

- It is important with hyponatraemia to ascertain volume status as this will determine management.
- The management of each is as follows:
 - ⇒ **Hypovolaemic hyponatraemia**
 - Diagnosis may supported by an elevated urea suggesting dehydration.
 - rehydration with sodium chloride 0.9% or a balanced crystalloid (Hartmann's)
 - avoid rapid correction of sodium in order to reduce the risk of osmotic complications such as central pontine myelinolysis
 - ❖ The rate of correction of hyponatremia should not exceed **eight** mEq/L per day.
 - ⇒ **Euvolaemic hyponatraemia**
 - check urine and serum osmolality. Does the patient meet the criteria for SIADH?
 - treat the underlying cause where possible in SIADH
 - fluid restriction (500-750mls/day)
 - monitor fluid balance and perform daily weights
 - consider demeclocycline or tolvaptan (under specialist supervision). Both inhibit the action of antidiuretic hormone.
 - ⇒ **Hypervolaemic hyponatraemia**
 - fluid and salt restriction
 - consider diuretics
 - treat the underlying cause (e.g. cardiac failure)

Hyponatraemia: correction

Acute hyponatraemia is that which occurs within a duration of 48 hours.

Acute hyponatraemia

- predisposing factors to acute hyponatraemia:
 - ⇒ Over consumption of fluids,
 - ⇒ prolonged race duration and inadequate training
- Pathophysiology
 - ⇒ When hyponatraemia develops over a short duration the ability of the brain to adapt is exceeded and cerebral oedema can result which may lead to confusion, seizures and coma. As a result patients may die from brain herniation.
- Treatment
 - ⇒ The correct treatment to give is hypertonic saline.
 - ⇒ Decompressive craniotomy would help alleviate raised intracranial pressure due to cerebral oedema however is not an appropriate first line treatment.
 - ⇒ A small, quick increase in the serum sodium is required in order to decrease intracranial pressure. **Hypertonic saline (3%) boluses are the most appropriate treatment to improve neurological status in such patients.**

Hyponatremia in patients with advanced cirrhosis

- Mechanism
 - ⇒ **systemic vasodilation**, (The most important factor) which leads to activation of endogenous vasoconstrictors including antidiuretic hormone (ADH); ADH promotes the water retention that is responsible for the fall in serum sodium.
- Tolvaptan (Vasopressin receptor antagonists) should not be used in patients with cirrhosis, because of its known potential for hepatotoxicity.

Central pontine demyelinolysis

Central pontine myelinolysis (CPM):

- Due to rapid correction of hyponatraemia
- the classical presentation is spastic quadripareisis, pseudobulbar palsy, and emotional lability (pseudobulbar affect) (locked in syndrome.)

- **Definition:** damage to the myelin sheath of the white matter in the CNS caused by a sudden rise in serum osmolality (rapid correction of chronic hyponatremia)
- Affects the central region of the pons
- **Pathophysiology:** rapid sodium correction → Sudden rises in plasma osmolarity → fluid shift from the cerebral intracellular space to the extracellular space (loss of water from the intracellular compartment) → cerebral shrinking and demyelination → end result is central pontine myelinolysis (CPM).
- **Features**
 - ⇒ Symptoms first develop several days after the correction of hyponatremia.
 - ⇒ Central pontine myelinolysis
 - Altered level of consciousness, including coma
 - Locked-in syndrome
 - Impaired cranial nerve function: dysarthria, dysphagia, and diplopia
 - Worsening quadripareisis
- **Diagnosis:** MRI brain
- **Treatment:** supportive care
- **Prevention:** Avoid hypernatremia
 - ⇒ Many authorities recommend that increases in serum sodium of **<12 mmol/24 hours** are likely to be safe for the majority of patients.
 - ⇒ Certain patients with hypokalaemia, liver disease, poor nutritional state or burns are at higher risk of demyelination and should have a rate of sodium correction of **<8 mmol/24 hours**.

"Saline depletion, for example due to excessive diuretic exposure, is best managed with a balanced electrolyte solution such as Hartmann's."

Osmolar gap

- **Osmolar gap is the difference between the calculated osmolarity and the measured osmolarity.**
- The normal value is 10-15 but may be increased in the presence of unmeasured 'abnormal' osmotically active ions in the plasma.
- An elevated osmolar gap provides indirect evidence for the presence of an abnormal solute that may be present in significant amounts.
- Ethanol, ethylene glycol (anti-freeze), acetone and methanol are solutes that will cause elevation of the osmolar gap in this way.
- **Calculated osmolarity = 2 (Na + K) + Glucose + Urea (all in mmol/L).**
- Normal serum osmolarity is 285-295 mOsm/L.
- **Osmolality is measured in the laboratory using an osmometer.**

Hypomagnesaemia

Definition

- Low magnesium below 0.7 mmol/L.

Overview

- Normal plasma magnesium (0.7-0.9 mmol)
- **The thick ascending limb (TAL) of the loop of Henle is the major site of reabsorption (60-70%)** (unlike most ions, those reabsorbed in the proximal convoluted tubule)
- **In the TAL, magnesium is passively reabsorbed.** In the distal convoluted tubule, magnesium is reabsorbed via an active, transcellular **TRPM6** channel .

Uses for magnesium include:

- polymorphic ventricular tachycardia (torsade de pointes),
- acute asthma
- prevention/treatment of seizures in pre-eclampsia.
- **Magnesium salts can be given as laxatives**

Causes of low magnesium

- **Inadequate intake:**
 - ⇒ Malnutrition, and
 - ⇒ Alcohol dependence. Hypomagnesemia is the most common electrolyte abnormality observed in alcoholic patients
 - ⇒ **Total parenteral nutrition**
- **Malabsorption:**
 - ⇒ Inflammatory bowel disease
 - ⇒ Long term PPI therapy
 - ⇒ Gluten enteropathy
 - ⇒ Intestinal bypass, and
 - ⇒ Radiation enteritis.
- **Renal tubular disease:**
 - ⇒ Hyperaldosteronism
 - ⇒ Hyperparathyroidism
 - ⇒ Obstructive uropathy
 - ⇒ Potassium depletion, and
 - ⇒ Drugs (including diuretics, amphotericin, cisplatin, cyclosporine, amikacin, gentamicin, laxatives, and tacrolimus).
- **Intracellular shift:**
 - ⇒ Post myocardial infarction
 - ⇒ Post parathyroidectomy
 - ⇒ Recovery from diabetic ketoacidosis (K^+ and PO_4^{2-} also enter cells)
 - ⇒ Refeeding syndrome (PO_4^{2-} also enters cells),
 - ⇒ Acute pancreatitis.
- **Drugs:**
 - ⇒ **cisplatin**
 - ⇒ **diuretics**
 - ⇒ **cyclosporine**
 - ⇒ **cardiac glycosides**
 - ⇒ **Colorectal cancer treatment with cetuximab/panitumumab (EGF receptor inhibitors)** $\rightarrow \downarrow TRPM6 \rightarrow$ hypomagnesemia.
 - ⇒ **Omeprazole** \rightarrow hypomagnesemia \rightarrow hypoparathyroidism \rightarrow hypocalcaemia.

- Diarrhoea
- Metabolic acidosis
 - ⇒ Chronic metabolic acidosis → ↓ renal TRPM6 expression in the DCT → ↓ Mg reabsorption → ↓ serum Mg.
- **Hypercalcaemia**
 - ⇒ Hypercalcemia → activation of **calcium-sensing receptor (CaSR)** → ↓ Mg reabsorption
- Hypokalaemia, hypocalcaemia
- **Genetic diseases**

Features

- **General**
 - ⇒ **lack of appetite.**
 - ⇒ Lethargy
 - ⇒ fatigue
- **neuromuscular hyper-excitability**
 - ⇒ **muscle weakness including fasciculations**
 - ⇒ **changes in personality**
 - ⇒ **paraesthesia**
 - ⇒ **tetany**
 - ⇒ **seizures**
- **cardiac**
- **arrhythmias**
- **ECG features similar to those of hypokalaemia**
- **The ECG change most typically associated with hypomagnesaemia is QT prolongation.**
- **exacerbates digoxin toxicity**
- **decreased PTH secretion → hypocalcaemia**
- **Hypokalemia (in 40-60%)**

Associations with hypomagnesemia

- Hypoparathyroidism
 - ⇒ ↓ Mg → ↓ magnesium-dependent adenyl cyclase generation of cyclic adenosine monophosphate (cAMP) → ↓ PTH → hypoparathyroidism
- DM (↓ Mg → ↓ insulin sensitivity and secretion)
- Cardiac: CAD, Hypertension (Mg plays a role in BP regulation), cardiac arrhythmia (prolongation of the QT interval , Torsade de pointes)

Investigation

- blood magnesium levels can guide but do not accurately reflect total body magnesium status. Attempts to find a marker of cellular magnesium status include measuring erythrocyte or monocyte Mg but these are not generally available.
- Urine Mg excretion is a useful guide. When there is inadequate intake or malabsorption, the kidneys would normally conserve Mg, giving urine Mg concentrations <7 mmol/24 hours. The reference range is around 2-7 mmol/24 hours.

Treatment

- <0.4 mmol/l
 - ⇒ intravenous replacement is commonly given. An example regime would be 40 mmol of magnesium sulphate over 24 hours
- >0.4 mmol/l
 - ⇒ oral magnesium salts (10-20 mmol orally per day)
 - ⇒ diarrhoea can occur with oral magnesium salts

Hypermagnesaemia

Overview

- Hypermagnesaemia is much less common than hypomagnesaemia and is often iatrogenic in cause.

Causes of hypermagnesaemia

- **Iatrogenic:**
 - ⇒ Treatment with magnesium sulphate to prevent/treat seizures in patients with eclampsia or pre-eclampsia
 - ⇒ Treatment with Mg containing antacids
 - ⇒ Use of citrate-glucuronic acid solutions to dissolve renal calculi either through bladder irrigation or via a nephrostomy tube
 - ⇒ Over-zealous IV treatment of hypomagnesaemia
 - ⇒ Chronic use of Mg-containing enemas.
- **Other causes:**
 - ⇒ **Acute or chronic renal failure**
 - release of Mg from tissues,
 - Mg in dialysate,
 - Mg in phosphate binding drugs
 - ⇒ Familial hypocalciuric hypercalcemia.

Lithium can cause **hypermagnesaemia**

Features

- Mild hypermagnesemia often asymptomatic
- Nausea, Lethargy
- Reduced deep tendon reflexes
- Blurry vision
- Cardiac: Vasodilatation, Hypotension, Bradycardia
- ECG changes: ↑ PR interval, ↑ QRS duration, ↑ QT interval
- Blurry vision
- Hypocalcemia
- Severe hypermagnesemia
 - ⇒ Muscle paralysis (flaccid quadriplegia)
 - ⇒ Bradycardia, Cardiac arrest
 - ⇒ Respiratory failure

Treatment

- If mild/moderate and iatrogenic, often it is enough to identify and stop the cause.
- In an emergency, dialysis or administration of IV calcium glucuronate (10 ml of 10%) will reduce the effects of hypermagnesaemia.

Hypophosphataemia

Definition

- serum phosphate level of less than 2.5 mg/dL (0.8 mmol/L).

Causes

Causes	Consequences
<ul style="list-style-type: none"> alcohol excess acute liver failure diabetic ketoacidosis refeeding syndrome primary hyperparathyroidism osteomalacia Hyperventilation 	<ul style="list-style-type: none"> red blood cell haemolysis white blood cell and platelet dysfunction muscle weakness and rhabdomyolysis central nervous system dysfunction

Mechanisms

- The three major mechanisms of hypophosphataemia are:

- Redistribution of extracellular phosphate into cells
 - hyperventilation** → respiratory alkalosis → activating phosphofructokinase → moves phosphate into cells → stimulates intracellular glycolysis.
 - Glycolysis leads to phosphate consumption as phosphorylated glucose precursors are produced.
 - Any cause of hyperventilation (eg, sepsis, **anxiety**, **pain**, heatstroke, alcohol withdrawal, diabetic ketoacidosis [**DKA**], hepatic encephalopathy, salicylate toxicity, neuroleptic malignant syndrome [**NMS**]) can precipitate hypophosphatemia.
- Decreased intestinal absorption,
 - chronic diarrhea,
 - malabsorption syndromes,
 - severe vomiting,
 - nasogastric (NG) tube suctioning.
- Depletion due to increased urinary loss.
 - the most common cause of hypophosphatemia
 - primary and secondary hyperparathyroidism.
 - Osmotic diuresis, such as seen in hyperosmolar hyperglycemic syndrome (HHS)
 - Fanconi syndrome (proximal tubule dysfunction)
 - X linked hypophosphataemic rickets
 - Oncogenic hypophosphataemic osteomalacia

MRCPUK- part-1-Sep 2017: what is the mechanism of Hypophosphataemia during treatment of DKA?

→ Shift from extracellular to intracellular space

MRCPUK-part-1-Sep 2017: what is the mechanism of Hypophosphataemia in alcoholic patients after hospital admission ?

→ Shift from extracellular to intracellular space

- The alcoholic patient often has chronic phosphate depletion, and, after admission to the hospital, is prone to severe hypophosphatemia resulting from redistribution of extracellular phosphate into the cells.
- Two factors may contribute to this shift:
 1. I.V therapy with dextrose-containing solutions or refeeding → ↑Glucose → ↑ insulin release → ↑ phosphate uptake by the cells
 2. alcohol withdrawal → hyperventilation →acute respiratory alkalosis →intracellular alkalosis → stimulates intracellular phosphofructokinase →↑ glycolysis → movement of phosphate into cells

Hyperphosphataemia

Overview

- The healthy adult usually ingests about **8400 mg per week of phosphate through their diet**
- **Absorption** occurs mainly in the jejunum
- **Renal reabsorption:** the **majority** (70%) of filtered phosphate is reabsorbed by **type 2a sodium phosphate cotransporters** located on the apical membrane of the renal **proximal tubule**.
- The normal adult range for phosphorus is 2.5-4.5 mg/dL (0.81-1.45 mmol/L).
- **Renal excretion :** **About 5400 mg of phosphate is excreted per week through the kidneys.**

Causes

- Usually iatrogenic
- ↓calcium + ↑ phosphate levels seen in (**decreased phosphate excretion**)
 - ⇒ **renal failure**
 - ⇒ hypoparathyroidism, and pseudohypoparathyroidism
- ↑calcium + ↑phosphate seen in
 - ⇒ **vitamin D intoxication** (↓PTH + ↑ vitamin D)
 - ⇒ milk-alkali syndrome (↓PTH + ↓vitamin D)
- **Disorder that shifts intracellular phosphate to extracellular space**
 - ⇒ Tumor lysis
 - ⇒ Rhabdomyolysis
- **Increased phosphate intake** (e.g., phosphate-containing enemas)
 - ⇒ Laxative (Phospho-soda) abuse
 - ⇒ **Foods that are characteristically rich in phosphate include: dairy products, (Cheddar cheese), fibre rich foods, chocolate, and processed meats.**

Features

- Often asymptomatic
- High PO₄³⁻ levels cause the formation of an insoluble compound with calcium, which can lead to:
 - ⇒ Hypocalcemia → hypocalcemic symptoms (muscle cramps, tetany, and perioral numbness or tingling).
 - ⇒ Nephrolithiasis
 - ⇒ Calcifications in the skin

Management

- Treat the underlying cause.
- Discontinue phosphate intake (dietary or medication).
- Give phosphate binders (e.g., aluminium hydroxide, calcium carbonate).
- Consider dialysis (especially in severe cases of hyperphosphatemia in patients with renal failure).

Collagen Types

Types of collagen		
	Tissue distribution	Related conditions
Type I collagen (90% of body collagen)	Bone (produced by osteoblasts), skin, tendons, ligaments, fascia, dentin, cornea, internal organs, scar tissue (late stages of wound healing)	Osteogenesis imperfecta type I: decreased production
Type II collagen	Cartilage (including hyaline), vitreous humor of the eye, intervertebral discs (nucleus pulposus)	Achondrogenesis (type II)
Type III collagen (reticulin)	Reticular fibers in skin, blood vessels, granulation tissue, uterus, scar tissue (early stages of wound healing), fetal tissue in early embryos and throughout embryogenesis	Ehlers-Danlos syndrome (vascular type): decreased production
Type IV collagen	Basement membranes, lens	Alport syndrome: decreased production Goodpasture syndrome: autoantibodies target type IV collagen
Type V collagen	Bone, skin, fetal tissue, placenta	Ehlers-Danlos syndrome (classic type)

Vitamin B3 (Niacin) deficiency

Causes

- Malnutrition
- Heavy drinking (more common in alcoholics)
- Conditions associated with tryptophan deficiency
 - ⇒ Hartnup disease: decreased renal and intestinal tryptophan absorption
 - ⇒ Carcinoid syndrome (if metabolically active): increased tryptophan metabolism
- Vitamin B6 deficiency (e.g., **due to treatment with isoniazid**): decreased niacin synthesis from tryptophan.

- Chronic consumption of grains that have not been processed by nixtamalization (common cause in developing countries)

Features

- Atrophic glossitis**
 - the tongue is pink or red
 - appears glossy and smooth due to the atrophy of papillae.
 - can be painful.
- Pellagra (caused by severe deficiency)**
 - Characteristic dermatitis
 - Circular broad collar rash on the neck (Casal necklace); affects dermatomes C3 and C4
 - Hyperpigmented skin lesions in **sun-exposed areas** (especially on the limbs)
 - Diarrhea and vomiting
 - Neurologic symptoms (e.g. dementia, hallucinations, anxiety, insomnia, encephalopathy)

Pellagra

-  The classical features are the 3 D's - Dermatitis, Diarrhoea and Dementia.
 Caused by nicotinic acid (niacin) deficiency.

Vitamin C (ascorbic acid) (scurvy)

- Vitamin C is a water soluble vitamin.
- Dehydroascorbic acid, the oxidative product of ascorbic acid metabolism, **passively penetrates cellular membranes** and is the preferred form for erythrocytes and leukocytes.

Functions

- Antioxidant (Ascorbic acid provides electrons needed to reduce molecular oxygen. These anti-oxidant capabilities also **stabilize vitamin E and folic acid.**)
- It is a cofactor for reduction of folate to dihydro-and-tetrahydrofolate.
 - Therefore **macrocytic anaemia** in scurvy may occur due to two reasons:
 - oxidative hemolysis and
 - folate metabolism defects.**
- collagen synthesis: acts as a cofactor for enzymes that are required for the hydroxylation proline and lysine in the synthesis of collagen
 - Vitamin C deficiency (scurvy) leads to defective synthesis of collagen resulting in capillary fragility (bleeding tendency) and poor wound healing
- facilitates iron absorption**
- cofactor for norepinephrine synthesis
- cofactor for reduction of folate to dihydro-and-tetrahydrofolate.

Causes

- occurs in people with poor dietary intake, who eat little or no fruit and vegetables, commonly alcoholics and elderly people existing on a 'tea and toast' diet.
- Pregnancy, lactation and thyrotoxicosis increase ascorbic acid requirements and may precipitate scurvy.

Features vitamin C deficiency

- gingivitis, loose teeth
- poor wound healing**

- bleeding from gums, haematuria, epistaxis
- general malaise
- anaemia
 - ⇒ **macrocytic anaemia in scurvy may occur due to two reasons: oxidative hemolysis and folate metabolism defects.**
 - ⇒ normochromic, normocytic anaemia reflects bleeding into tissues

Continued deficiency leads to:

- | | |
|---|--|
| <ul style="list-style-type: none"> • Anaemia • Myalgia • Bone pain • Bruising • Petechial and perifollicular haemorrhages • Corkscrew hairs • Mood changes | <ul style="list-style-type: none"> • Fragility • scleral icterus (late, probably secondary to haemolysis), and • pale conjunctiva. • Fractures, dislocations and tenderness of bones are common in children. • Bleeding into muscles and joints may be seen |
|---|--|

Late stages can lead to:

- | | |
|--|--|
| <ul style="list-style-type: none"> • Generalised oedema • Severe jaundice • Haemolysis • Haemorrhage | <ul style="list-style-type: none"> • Neuropathy • Convulsions, and • Death. |
|--|--|

The classical skin manifestations of scurvy are:

- perifollicular hyperkeratotic papules
- perifollicular haemorrhages
- purpura, and
- ecchymoses.

Treatment

- vitamin C supplementation,
- recovery is usually complete within three months.

Vitamin B12 deficiency

Overview

- Vitamin B12 is mainly used in the body for red blood cell development and also maintenance of the nervous system.
- It is absorbed after **binding to intrinsic factor** (secreted from parietal cells in the stomach) and is actively **absorbed in the terminal ileum**.
- A small amount of vitamin B12 is passively absorbed without being bound to intrinsic factor.
 - ⇒ Approximately 1 percent of a large oral dose of vitamin B₁₂ is absorbed by this second mechanism. This pathway is important in relation to oral replacement.
- Once absorbed, vitamin B₁₂ binds to **transcobalamin II** and is transported throughout the body.
- Exhaustion of vitamin B12 stores usually occurs after 12 to 15 years of absolute vitamin B12 deficiency.

Causes

- **Malabsorption**
 - ⇒ ↓ **Intrinsic factor (IF)**
 - **Atrophic gastritis** due to
 - ☞ Autoimmune atrophic gastritis: **most common cause of vitamin B12 deficiency**

- ☞ H. pylori infection
- **Gastrectomy**
- ⇒ **Reduced uptake of IF-vitamin B12 complex in terminal ileum** due to:
 - Alcohol use disorder
 - Crohn disease, celiac disease
 - Pancreatic insufficiency
 - Surgical resection of the ileum
 - Diphyllobothrium latum (tapeworm) infection
 - Bacterial overgrowth
 - Enteritis
 - Achlorhydria
- **Malnutrition**
 - ⇒ Strict vegan diets: occurs only after years of a strict diet that excludes all animal products (unlike folate deficiency, which occurs within a few months of insufficient intake)
- **Increased demand:** e.g., during pregnancy, breastfeeding, fish tapeworm (*Diphyllobothrium latum*) infection
- **Metformin** (Chronic metformin use results in vitamin B12 deficiency in 30% of patients)

Features

- **Macrocytic anaemia**
- Sore tongue and mouth
- **Neurological symptoms:**
 - ⇒ Peripheral neuropathy
 - ⇒ Subacute combined degeneration of spinal cord
 - ⇒ The neurological symptoms can occur without anemia
- **Autonomic dysfunction:** impotence and incontinence
- **Psychiatric disorders symptoms:** including impaired memory, irritability, depression, dementia and, rarely, psychosis
- **Cardiovascular effect:**
 - ⇒ Similar to folic acid deficiency, vitamin B₁₂ deficiency produces hyperhomocysteinemia, which is an independent risk factor for atherosclerotic disease.
 - ⇒ Serum high concentrations of homocysteine and low levels of folic acid and vitamin B₁₂ are significantly correlated with the categories of coronary artery diseases

Investigations

- Serum cobalamin levels are the initial test
 - ⇒ A normal serum cobalamin level does not exclude cobalamin deficiency.
- **Diagnosis of vitamin B₁₂ deficiency is typically based on measurement of serum vitamin B₁₂ levels; however, about 50 percent of patients with subclinical disease have normal B₁₂ levels.**
- **A more sensitive method of screening for vitamin B₁₂ deficiency is measurement of serum methylmalonic acid and homocysteine levels, which are increased early in vitamin B₁₂ deficiency.**
 - ⇒ **elevated methylmalonic acid level is more specific for vitamin B₁₂ deficiency than an elevated homocysteine level.**
 - ⇒ Vitamin B₁₂ or folic acid deficiency can cause the homocysteine level to rise, so folic acid levels also should be checked in patients with isolated hyperhomocysteinemia.
 - ⇒ two enzymatic reactions are known to be dependent on vitamin B₁₂.

1. methylmalonic acid is converted to succinyl-CoA using vitamin B₁₂ as a cofactor. Vitamin B₁₂ deficiency, therefore, can lead to **increased levels of serum methylmalonic acid**.
2. homocysteine is converted to methionine by using vitamin B₁₂ and folic acid as cofactors. In this reaction, a deficiency of vitamin B₁₂ or folic acid may lead to **increased homocysteine levels**.

Management

- if no neurological involvement 1 mg of IM hydroxocobalamin 3 times each week for 2 weeks, then once every 3 months
 - ⇒ **oral vitamin B₁₂ has been shown to have an efficacy equal to that of injections** in the treatment of pernicious anemia and other B₁₂ deficiency states.
 - Although the daily requirement of vitamin B₁₂ is approximately 2 mcg, the initial oral replacement dosage consists of a single daily dose of 1,000 to 2,000 mcg. This high dose is required because of the variable absorption of oral vitamin B₁₂ in doses of 500 mcg or less.
- if a patient is also deficient in folic acid then it is important to treat the B12 deficiency first to avoid precipitating subacute combined degeneration of the cord
 - ⇒ Large amounts of **folic acid can mask the damaging effects of vitamin B12 deficiency** by correcting the megaloblastic anemia caused by vitamin B12 deficiency without correcting the neurological damage that also occurs

Sep 2017 part 1: Which structure in the body are able to synthesize vitamin B12?

↪ **gut bacteria**

- It is synthesized by gut bacteria in humans, but humans cannot absorb the B₁₂ made in their guts, as it is made in the colon which is too far from the small intestine, where absorption of B₁₂ occurs.
- Therefore diet is the only source of vit B12.

Vitamin B1 (Thiamine) deficiency

Overview

- the biologically active form of this vitamin is **thiamine pyrophosphate (TPP)**
- the most important biochemical reactions requiring the availability of thiamine includes glycolysis and tricarboxylic acid (TCA) cycle.
- There are three enzymes that require the presence of thiamine pyrophosphate as a co-factor:
 1. α-ketoglutarate **dehydrogenase**
 2. branched chain amino acid **dehydrogenase**
 3. pyruvate **dehydrogenase**

Causes

- Heavy alcohol drinking
- Malnutrition, starvation
- Malabsorption
- Malignancy

Pathophysiology

- Thiamine deficiency → impaired glucose breakdown → ATP depletion → tissue damage that primarily affects highly aerobic tissues (e.g., brain, heart)
- High-dose glucose infusions lead to increased ATP depletion, which can trigger Wernicke encephalopathy.
 - ⇒ In malnourished individuals and chronic alcohol users/heavy drinkers, thiamine should be administered before glucose infusions.

Features

- Beriberi:** inadequate thiamine uptake due to malnutrition, heavy drinking, or increased demand (e.g., hyperthyroidism, pregnancy)
 - ⇒ **Dry beriberi**
 - Symmetrical peripheral neuropathy (sensory and motor)
 - Progressive muscle wasting
 - Paralysis
 - Confusion
 - ⇒ **Wet beriberi**
 - Oedema
 - High-output cardiac failure (**dilated cardiomyopathy**)
- Wernicke encephalopathy**
 - ⇒ The triad of: Encephalopathy, Ataxia and Oculomotor dysfunction (usually nystagmus)
- Korsakoff's psychosis**
 - ⇒ characterised by both anterograde and retrograde amnesia with **confabulation**

What happens if you do not give the thiamine first before starting an intravenous glucose infusion?

- ATP failing to be adequately generated
- The inability of pyruvate to enter the TCA cycle → accumulate of pyruvate → pyruvate converted to lactate in order to be able to maintain glycolysis → **acidosis**.
- Inability of the pentose phosphate pathway to protect the cell from reactive oxygen species** that damage cellular structures, results in either **cell death or activation of apoptosis**.

Vitamin function as a co-factors:

- ⇒ Biotin for carboxylase reactions.
- ⇒ **Thiamine for dehydrogenase reactions**
- ⇒ B9 (folate) for transferases.
- ⇒ Vit C for hydroxylases.

Vitamin E deficiency

Active form: tocopherol

Function

- Lipid-soluble antioxidant in the glutathione peroxidase pathway → removes the free radical intermediates → protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction.

Therapeutic uses → Nonalcoholic steatohepatitis

Features

- **Neurologic dysfunction**
 - ⇒ Demyelination of the posterior column and spinocerebellar tract → ↓ proprioception and vibration sensation; ataxia
 - ⇒ Neurologic symptoms are similar to vitamin B12 deficiency, except that vitamin E deficiency does not lead to hypersegmented neutrophils, megaloblastic anemia, and increased methylmalonic acid levels.
- **Hemolytic anemia**; increased fragility of erythrocytes and membrane breakdown
- **Acanthocytosis**
- **Muscle weakness**

Hypervitaminosis E

- **interfere with vitamin K metabolism** → **vitamin K deficiency** → **increased tendency to bleed.**

Vitamin K Deficiency

Sources of vitamin K

- Leafy green vegetables (vitamin K1)
- Synthesized in small amounts by intestinal bacteria

Functions

- Cofactor for γ -carboxylation of glutamate residues on vitamin-K-dependent proteins involved in:
 - ⇒ Coagulation: maturation of **factors II (prothrombin), VII, IX, and X, protein C, protein S**
 - ⇒ Bone formation: osteocalcin (bone Gla protein), matrix Gla protein

Causes

- Liver failure
- Fat malabsorption
- Prolonged broad-spectrum antibiotic therapy
- Vitamin K antagonists (e.g., warfarin)

Features

- Hemorrhage (e.g., petechiae, ecchymoses)
- Vitamin K deficiency bleeding (VKDB)
 - ⇒ ↑ PT and aPTT, normal bleeding time
 - ⇒ Postnatal prophylaxis: vitamin K injection at birth

Vitamin A deficiency

Over view of vitamin A

- **Active forms:** Retinal and Retinoic acid
- **Sources**
 - ⇒ Plant sources; yellow and leafy vegetables
 - ⇒ Animal sources: in storage form; liver

Causes

- Disorders associated with fat malabsorption: inflammatory bowel disease (e.g., Crohn disease), celiac disease, cystic fibrosis, pancreatic insufficiency

Features

- Ocular manifestations
 - ⇒ Night blindness (nyctalopia)
 - ⇒ Xerophthalmia
 - ⇒ Keratomalacia
 - ⇒ Bitot spots: gray, triangular, dry patches on the bulbar conjunctiva, covered by a layer with a foamy appearance
 - Typical sign of vitamin A deficiency
 - Caused by squamous cell metaplasia and keratinization of the conjunctiva
- Keratinizing squamous metaplasia of the bladder (pearl-like plaques on cystoscopy)
- Xerosis cutis (dry skin)
- Immunosuppression

Vitamin A toxicity

- ⌚ Causes: increased intake via supplements or drugs
- ⌚ Acute toxicity: Nausea, vomiting, Vertigo, Blurred vision
- ⌚ Chronic toxicity:
 - Alopecia, Dry skin, scaling
 - Arthralgias
 - Hepatosplenomegaly, hepatic toxicity
 - Pseudotumor cerebri

Which substances in vitamin A is most likely to be maximally involved in correcting the visual disturbance?

- ⌚ Retinaldehyde
 - Retinaldehyde is derived from the oxidation of retinol

What would you give the patient who taking long term steroids to help his wound heal faster?

- ⌚ Vitamin A
 - Vitamin A is believed to counteract the effect of steroids on slowing wound healing by stimulating TGF-beta and IGF-I, as well as collagen production. However, high levels (which can accumulate because vitamin A is fat soluble) can also be toxic and inhibit collagen synthesis, such as in the skin.

Vitamin deficiency

The table below summarises vitamin deficiency states

Vitamin	Chemical name	Deficiency state
A	Retinoids	Night-blindness (nyctalopia), dry skin.
B1	Thiamine	Beriberi <ul style="list-style-type: none"> • polyneuropathy, Wernicke-Korsakoff syndrome • heart failure (dilated cardiomyopathy)
B2	(riboflavin)	Angular stomatitis, cheilosis, corneal vascularization
B3	Niacin	Pellagra <ul style="list-style-type: none"> • dermatitis • diarrhoea • dementia
B6	Pyridoxine	Anaemia, irritability, seizures
B7	Biotin	Dermatitis, seborrhoea
B9	Folic acid	Megaloblastic anaemia, deficiency during pregnancy - neural tube defects
B12	Cyanocobalamin	Megaloblastic anaemia, peripheral neuropathy
C	Ascorbic acid	Scurvy <ul style="list-style-type: none"> • gingivitis • bleeding • poor wound healing
D	Ergocalciferol, cholecalciferol	Rickets, osteomalacia
E	Tocopherol, tocotrienol	↑ fragility of RBCs. Mild haemolytic anaemia in newborn infants, ataxia, peripheral neuropathy
K	Naphthoquinone	Haemorrhagic disease of the newborn, bleeding diathesis
Selenium	Selenium	Keshan disease (cardiomyopathy).

Zinc deficiency

Features

- **perioral dermatitis:** red, crusted lesions
- (rough and dry skin)
- acrodermatitis
- alopecia
- short stature (dwarfism)
- hypogonadism
- hepatosplenomegaly
- geophagia (ingesting clay/soil)
- cognitive impairment

Treatment

- Zn supplementation has been shown to improve neuropsychological function in Chinese children.
- Zn deficiency is associated with adverse pregnancy outcomes.

Pyruvate kinase

- Pyruvate kinase is the rate-limiting step in glycolysis and gluconeogenesis
- It catalyses the transfer of a phosphate group from phosphoenolpyruvate to ADP, yielding a molecule of pyruvate and a molecule of ATP
- Deficient pyruvate kinase activity may result in the development of hereditary haemolytic anaemias

Which biochemical processes is likely to contribute most to energy creation in long distance running?

→ Fatty acid oxidation

Third edition

Notes & Notes

For MRCP part 1 & 11

By

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Basic sciences **Immunology**

Updated

2022

Chapter 15 Immunology

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Leukotrienes / Acute phase proteins	569	Complement deficiencies	588
ANCA / Antibodies and immunological markers	572	Heredity angioedema	589
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Interferon	575		
Tumour necrosis factor (TNF)			

Human leukocyte antigen (HLA)

Overview

- The human leukocyte antigen (HLA) is a gene complex that encodes the major histocompatibility complex (MHC) proteins.
- MHC proteins play a vital role in initiating immune responses as they present antigen fragments to T cells and bind T-cell receptors.
- Found on chromosome 6
- 2 classes:
 - ⇒ Class I → HLA A, B, C
 - expressed on all cells, except erythrocytes and trophoblasts
 - interact with CD8+
 - ⇒ class II → HLA DP, DQ, DR
 - expressed on B cells, dendritic cells, and monocytes
 - most important in transplant → (DR)

MHC I-associated loci (HLA-A/-B/-C) only have 1 letter after the hyphen, while MHC II-associated loci (HLA- DR/- DP/- DQ) have 2 letters.

MRCP- part-1-2018: Which HLA subtypes is usually implicated with respect to matching for avoiding hyperacute rejection?

⇒ **HLA-C**

- Anti-HLA-C IgG antibodies are usually implicated in hyperacute rejection; specifically,
- HLA-CW5 subtype antibodies have been implicated most in hyperacute rejection of renal transplant.

HLA associations

- The most important HLA associations are listed below:

HLA type	Associated diseases
HLA-A3	⦿ Hemochromatosis
HLA-B5	⦿ Behcet's disease HLA B51 is a split of B5
HLA-B47	⦿ 21-hydroxylase deficiency
HLA-CW6	⦿ Psoriasis
HLA-DR3 + DR4 combined	⦿ Diabetes mellitus type 1 (but more with HLA-DR4)
HLA-DR7	⦿ steroid-responsive nephrotic syndrome
HLA-DR2	⦿ Narcolepsy ⦿ Goodpasture's ⦿ hay fever, ⦿ systemic lupus erythematosus, ⦿ multiple sclerosis.
HLA-DR4	⦿ Felty's syndrome (90%) → most common ⦿ Rheumatoid arthritis (70%) ⦿ Diabetes mellitus type 1 (> DR3) ⦿ Drug-induced SLE ⦿ IgA nephropathy ⦿ HOCM
HLA-B27	⦿ Ankylosing spondylitis ⦿ Post-gonococcal arthritis ⦿ Reiter's syndrome (reactive arthritis) ⦿ Acute anterior uveitis
HLA-DR3	⦿ Autoimmune hepatitis ⦿ Primary biliary cirrhosis ⦿ Coeliac disease (95% associated with HLA-DQ2) ⦿ Diabetes mellitus type 1 ⦿ Primary Sjögren syndrome ⦿ Dermatitis herpetiformis

Cluster of Differentiation (CD Markers)

Function and usage of CDs:

- The CD system is commonly used as cell markers in **immuno-phenotyping**, allowing cells to be defined based on what molecules are present on their surface.
- often acting as **receptors** or ligands (the molecule that activates a receptor)
- cell signaling:** Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and DM
- Cell adhesion:** essential for the pathogenesis of infectious organisms, eg:
⇒ HIV has an **adhesion molecule termed gp120** that binds to **its ligand CD4**, which is expressed on lymphocyte.

The table below lists the major clusters of differentiation (CD) molecules

Cluster of differentiation	Function
CD1	MHC molecule that presents lipid molecules
CD2	Found on thymocytes, T cells, and some natural killer cells that acts as a ligand for CD58 and CD59 and is involved in signal transduction and cell adhesion
CD3	The signalling component of the T cell receptor (TCR) complex
CD4	Found on helper T cells. Co-receptor for MHC class II Used by HIV to enter T cells
CD5	Found in the majority of mantle cell lymphomas
CD8	Found on cytotoxic T cells. Co-receptor for MHC class I Found on a subset of myeloid dendritic cells
CD14	Cell surface marker for macrophages
CD15	Expressed on Reed-Sternberg cells (along with CD30)
CD21	Epstein-Barr virus uses the CD21 receptor to invade B cells.
CD28	Interacts with B7 on antigen presenting cell as costimulation signal
CD95	Acts as the FAS receptor, involved in apoptosis

Clusters of differentiation

- **CD4**
 - ⇒ Found on helper T cells.
 - ⇒ Co-receptor for MHC class II
 - ⇒ Used by HIV to enter T cells
 - GP120 → fuses to CD4 → allow GP41 to penetrate the cell membrane
- **CD8**
 - ⇒ Found on cytotoxic T cells.
 - ⇒ **Co-receptor for MHC class I**
 - ⇒ Found on a subset of myeloid dendritic cells
- **CD14** → Cell surface marker for macrophages
- **CD18** → the absence of it causes Leukocyte adhesion deficiency

GP41 play a role in the initial step for HIV entry into cells

Gp120 fuses to the CD4 receptor, this then allows GP41 to penetrate the cell membrane

Complement pathways

- Activation may occur via three pathways:

1. Classical pathway:

- Activated by IgM or IgG complexes binding to the pathogen
- C1q, C1r, and C1s activation → C1 complex → split of C4 into C4a and C4b and C2 into C2a and C2b → formation of C3 convertase (C4b2b) from C4b and C2b
- C2 is involved in activation via the classical pathway**

2. Alternative pathway:

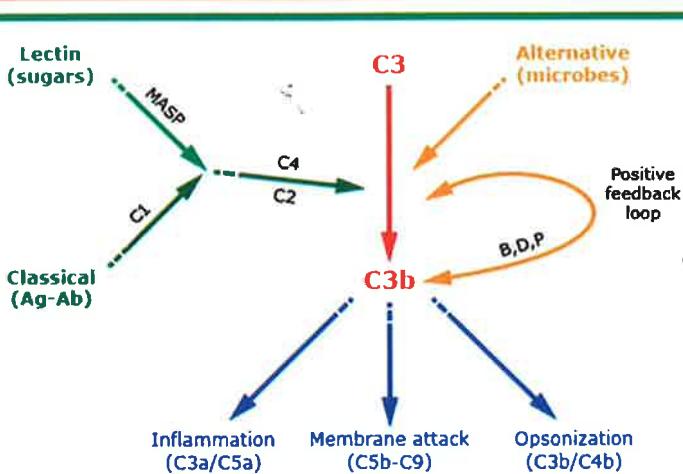
- Activated directly by pathogen surface molecules rather than by antigen-antibody complexes
- C3 is split into C3a and C3b → binding of factor B → formation of C3 convertase (C3bBb).
- Generates early innate response that does not require antibody for activation.

3. Lectin pathway:

- Activated by mannose or other sugars on pathogen surface
- Mannose-binding lectin (MBL) binds to mannose → formation of the C1-like complex, which cleaves C4 into C4a and C4b → C4b binding C2 and splitting of C2 into C2a and C2b → formation of C3 convertase (C4b2b).

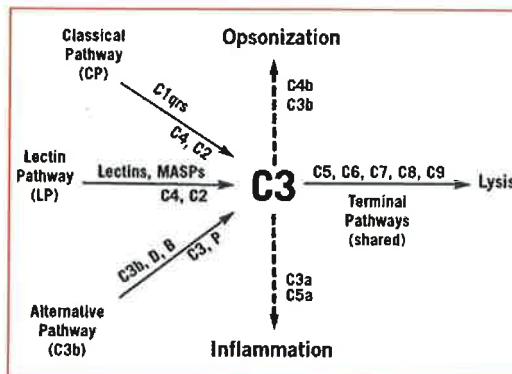
- All complement pathways have one final common pathway at C3.

IgG and IgM activate the classic pathway



The three pathways of complement activation are shown. Each leads to generation of activated C3b. The classical pathway is triggered by antibody interacting with antigen, and the lectin pathway by a lectin binding to a sugar. The alternative pathway turns over continuously. Activation of the complement system leads to inflammation (release of anaphylatoxins C3a and C5a), membrane perturbation and lysis (via the membrane attack complex, C5b-9), and opsonization (deposition of C3b and C4b).

MASP: mannose-binding lectin (MBL)-associated serine protease.



Hypersensitivity

The Gell and Coombs classification traditionally divides reactions into 4 types:

Type	Mechanism	Examples
Type I - Anaphylactic	Antigen reacts with IgE bound to mast cells (IgE-mediated)	<ul style="list-style-type: none"> Anaphylaxis Atopy (e.g. asthma, eczema and hayfever) Diagnosed by plasma tryptase (protease released from mast cell).
Type II - Cell bound	IgG or IgM binds to antigen on cell surface (antibody-mediated)	<ul style="list-style-type: none"> Autoimmune haemolytic anaemia ITP Goodpasture's syndrome Pernicious anaemia Acute haemolytic transfusion reactions Rheumatic fever Pemphigus vulgaris / bullous pemphigoid
Type III - Immune complex	Free antigen and antibody (IgG, IgA) combine (Immune complex deposition)	<ul style="list-style-type: none"> Serum sickness Systemic lupus erythematosus Post-streptococcal glomerulonephritis Extrinsic allergic alveolitis (especially acute phase)
Type IV - Delayed hypersensitivity	T-cell mediated (cell-mediated)	<ul style="list-style-type: none"> Tuberculosis / tuberculin skin reaction Graft versus host disease Allergic contact dermatitis Scabies Extrinsic allergic alveolitis (especially chronic phase) Multiple sclerosis Guillain-Barre syndrome

In recent times a further category has been added:

Type	Mechanism	Examples
Type V	Antibodies that recognise and bind to the cell surface receptors. This either stimulating them or blocking ligand binding	<ul style="list-style-type: none"> • GrAVes' disease • Myasthenia gravis

What is the hallmark signs of mast cell degranulation?

- Classical wheal and flare

Anaphylaxis

Anaphylaxis = type I hypersensitivity reaction

Anaphylaxis - serum tryptase levels rise following an acute episode

Definition

- a severe type 1 hypersensitivity reaction that can cause life-threatening and multisystem effects due to IgE-mediated mast cell activation

Pathophysiology

- Immunoglobulin E is the most common immunoglobulin involved in the pathogenesis of anaphylaxis.
- Anaphylaxis (type I hypersensitivity reaction) or anaphylactoid reactions → degranulation of mast cells → massive histamine release → systemic vasodilation → increased capillary leakage → anaphylactic shock
- Mediators involved in the development of anaphylaxis include: Tryptase, histamine, leukotrienes, prostaglandins, IL4, IL13, Heparin and platelet aggregating factor, which are generated by mast cell degranulation.
- Triggers for anaphylactic reactions: heat, cold, sexual activity, exercise.

Causes

1. Anaphylaxis (IgE mediated):

- ⇒ Food (e.g. Nuts) - the most common cause in children
- ⇒ Drugs
 - The most common IgE-mediated triggers are drugs, typically penicillin or other beta-lactam antibiotics.
 - Neuromuscular blocking agents (eg vecuronium) are responsible for 60-70% of allergic reactions related to anaesthesia.

⇒ Latex

- ⇒ Venom (e.g. Wasp sting)

2. Anaphylactoid (non-IgE mediated).

- ⇒ The reactions that produce the same clinical picture as anaphylaxis but are not IgE mediated.
- ⇒ plasma proteins or compounds, which act directly on the mast cell membrane, such as
 - Vancomycin

- Quinolone antibiotics
- Aspirin or other non-steroidal anti-inflammatory drugs
- Opiates
- Colloid plasma expanders
- Radiographic contrast media

Anaphylaxis following a blood transfusion can be due to immunoglobulin A deficiency.

Anaphylaxis VS Anaphylactoid

Is it anaphylactic OR anaphylactoid reaction?

	Anaphylactic (IgE-mediated anaphylactic reactions)	Anaphylactoid (Non IgE-mediated anaphylactic reactions)
Is sensitization required?	Yes	No
Can reaction occur in first exposure?	No	Yes
How much exposure is needed to elicit reaction?	very little (dose independent)	usually more than for anaphylaxis
Is reaction predicted by skin allergy test?	Yes	No

Which feature is the most important predictor of anaphylaxis in asthmatic patient with peanut allergy?

⇒ **Poorly controlled asthma**

- Poorly controlled asthma is an important risk factor for fatal anaphylaxis in this situation.
- Patients such as this should have their asthma well controlled and have ready access to, and knowledge of how to use, self-injectable adrenaline.

Features (Usually takes 15-30 minutes from the time of exposure to the antigen)

- **Skin or mucous membranes:** Flushing, erythema, pruritus, Swelling of the eyelids, angioedema
- **Respiratory:** hoarseness, Chest tightness, Dyspnea (due to bronchospasm or laryngeal edema), tachypnea, Stridor, wheezing, Hypoxia, cyanosis
- **Gastrointestinal:** Nausea, vomiting (especially in food allergies), Abdominal pain, diarrhea
- **Cardiovascular:** Hypotension, Tachycardia

Investigations

- **Serum mast-cell tryptase:** if elevated, supports the diagnosis of anaphylaxis
 - ⇒ has a half-life of 2 h, peaking at 1 h after anaphylaxis onset and return to baseline by 6 hours.
 - ⇒ Both sensitivity and specificity to confirm diagnosis is 95%
 - ⇒ **Normal tryptase results do not exclude anaphylaxis**
- Complement C4 levels: can be low in hereditary angioedema
- Total serum IgE level is non-specific and unhelpful.

Management

- Airway assessment and management: Rapid sequence intubation (RSI) for airway compromise
- **Adrenaline**
 - ⇒ the most important drug in anaphylaxis and should be given as soon as possible.
 - ⇒ The dose for **adult and child > 12 years** : 500 micrograms (0.5ml **1 in 1,000**)
 - ⇒ The best site for IM injection is the anterolateral aspect of the middle third of the thigh.
 - ⇒ Adrenaline can be repeated every 5 minutes if necessary.
- Hydrocortisone 200 mg
- Chlorphenamine 10 mg
- IV fluids
 - ⇒ *Evidence from a large randomised controlled trial (RCT) suggests there is no difference between normal saline and Hartmann's solution [also known as Ringer's lactate] for resuscitation of critically ill patients.*
- Observation: It is recommended to observe patients after resolution of an anaphylactic episode for 24 hours **for possible second-phase reactivation**.

Late-phase reaction

In IgE mediated reactions such as asthma or anaphylaxis what therapy inhibits the important **late-phase reaction**? **steroids**

- The late phase reaction is due to attraction of T cell, release of leukotrienes and prostaglandins often characterised by asthma
- prevented by the administration of steroids (**Hydrocortisone**).
- Approximately 30% of deaths related to anaphylaxis occur as a consequence of this late-phase reaction

Epinephrine injections for anaphylaxis should always be given **intramuscularly** in a **concentration of 1:1,000** (as opposed to the 1:10,000 solution used in cardiac arrest). Injecting the 1:1,000 solution into a vein can lead to cardiac arrhythmia/arrest.

Antihistamines and steroids should be administered in anaphylaxis only after the initial resuscitation measures (IM epinephrine, fluids and/or vasopressors) have been given.

A lack of response to epinephrine, antihistamines, and steroids should raise suspicion of differential diagnoses such as bradykinin-mediated angioedema, which requires its own specific treatment

Exercised induced anaphylaxis

Definition

- a rare disorder in which anaphylaxis occurs after physical activity.

Features

- usually **occur around 10 minutes after exercise** and follow a sequence of pruritus, widespread urticaria and then subsequently respiratory distress and vascular collapse.

Pathophysiology

- may be related to **endorphin release during exercise** → excessive histamine release from mast cells in susceptible individuals.

Associations

- Co-factors such as foods, alcohol, temperature, drugs (eg, aspirin and other nonsteroidal anti-inflammatory drugs), humidity, seasonal changes, and hormonal changes are important in the precipitation of attacks.
- most associated with wheat ingestion.
- The foods most commonly implicated in food-dependent exercise-induced anaphylaxis are wheat, shellfish, tomatoes, peanuts, and corn.
- The patients can usually eat the causative food without problems so long as they do not exercise afterwards.

Treatment

- managed in the same manner as anaphylaxis.
- usually resolves on stopping exercise
- Reducing physical activity to a lower level may diminish the frequency of attacks.
- Patients should be instructed on the proper use of emergency injectable epinephrine** and have one available at all times.
- Patients should wear a medical alert bracelet with instructions on the use of epinephrine.

Anaphylactic reactions associated with anaesthesia

Risk factors

- Neuromuscular blocking drugs and latex appear to cause anaphylaxis more commonly in **female** patients
- Individuals with a **history of atopy, asthma or allergy to some foods** appear to be at **increased risk of latex allergy** **but not anaphylaxis to neuromuscular blocking drugs or antibiotics**
- Patients with **asthma** or taking **b-blocking drugs** may suffer a more severe reaction.

Causes

- Neuromuscular blocking agents (NMBAs)**
 - ⇒ **Most common cause**
 - ⇒ **60% of cases of anaesthesia-related anaphylaxis are due to neuromuscular blocking agents.**
 - ⇒ 80% of NMA reactions occur without prior exposure
 - ⇒ Quaternary ammonium ions (QAI) are proposed to be the allergenic epitopes in NMBAs.
 - ⇒ Common environmental chemicals such as toothpastes, washing detergents, shampoos, and **cough medicines** share these allergenic epitopes with the NMBAs, predisposed individual to become sensitised to QAI and thus be at **risk of developing anaphylaxis to NMBAs during anaesthesia**.
 - ⇒ **succinylcholine** is the NMA **most likely** to be associated with **allergic anaphylaxis** (carries the highest risk)
- Latex**
 - ⇒ Latex hypersensitivity is the second most common cause of anaesthesia related anaphylaxis in many studies (up to 20% of cases). **But now decreased due to decline in the use of latex gloves.**
- Antibiotics**
 - ⇒ Approximately 15% of anaesthesia-related anaphylactic episodes are due to **antibiotics**.
 - Skin testing is only approximately 60% predictive of clinical hypersensitivity. Penicillins and

- cephalosporins which share the b-lactam ring are responsible for approximately 70% of antibiotic-induced anaphylaxis.
- There is a higher rate of antibiotic allergy in smokers
- **Anaesthetic induction agents**
 - ⇒ Anaphylaxis to **propofol** is **very uncommon**
 - ⇒ Anaphylaxis to thiopental has become extremely uncommon, probably reflecting the decline in its use.
- **Antiseptics and disinfectants**
 - ⇒ Reactions to **chlorhexidine** have come into greater prominence in recent years.
 - ⇒ Anaphylaxis has occurred when chlorhexidine was used as an antiseptic for urological and gynaecological procedures as well as insertion of central venous and epidural catheters.
 - ⇒ Allowing chlorhexidine to dry before beginning a procedure may reduce the risk of reaction.
 - ⇒ Anaphylaxis to other antiseptics is rare.

Diagnosis

- **Timings**
 - ⇒ Type I reactions typically occur within **seconds to minutes** after i.v. **exposure**.
 - ⇒ An insidious or delayed onset may occur (e.g. with latex, antibiotics, and colloids and a tourniquet may delay onset until after surgery).
- **History of atopy and asthma has a clear link with latex allergy.**

Allergy tests

Skin prick test	<ul style="list-style-type: none"> • Most commonly used test as an easy to perform and inexpensive. • the first line for detection of allergen-specific IgE • Drops of diluted allergen are placed on the skin after which the skin is pierced using a needle. • A large number of allergens can be tested in one session. • Normally includes a histamine (positive) and sterile water (negative) control. • A wheal will typically develop if a patient has an allergy. • Can be interpreted after 15 minutes • Useful for food allergies and also pollen. It is a reliable way of excluding IgE-mediated food allergies, although the positive predictive value is around 50% or less (the sensitivity of a negative skin prick test to foods is high) • It can induce anaphylaxis, and must therefore be done in an environment where resuscitation facilities are available.
Radioallergosorbent test (RAST)	<ul style="list-style-type: none"> • Determines the amount of IgE that reacts specifically with suspected or known allergens, for example IgE to egg protein. • Results are given in grades from 0 (negative) to 6 (strongly positive) • Useful for food allergies, inhaled allergens (e.g. Pollen) and wasp/bee venom • Blood tests may be used when skin prick tests are not suitable, for example if there is extensive eczema or if the patient is taking antihistamines
Skin patch testing	<ul style="list-style-type: none"> • Useful for contact dermatitis. • Around 30-40 allergens are placed on the back. • Irritants may also be tested for. • The patches are removed 48 hours later with the results being read by a dermatologist after a further 48 hours

If a history of anaphylaxis is given it would not be appropriate to perform a skin prick test, thus Radioallergosorbent test (RAST) is the most appropriate first-line test to investigate the cause of the reaction

Reasons for a false negative RAST test

- Immediately following anaphylaxis / allergic reaction (transient drop in IgE)
- Waning of allergen-specific IgE with time following a reaction.
- Unstable allergens in the RAST substrates (especially food allergens)

Only IgE-mediated allergic reactions can be tested by skin prick testing

The wheal size resulting from the skin prick test is an excellent predictor of a positive food challenge to peanuts

Latex allergy

Definition

- A type I or type IV hypersensitivity to latex-based products (e.g., exam gloves, condoms)

Epidemiology

- 8–12% of health care workers are affected
- NHS trusts in the UK have moved away from the routine use of latex gloves precisely because of the risk of allergy. As a result, **latex allergy in hospital is now very rare in the UK.**
- Latex allergy is more common in children with myelomeningocele spina bifida.

Pathophysiology

- Sensitivity to latex may cause several problems:
 - ⇒ type I hypersensitivity (anaphylaxis)
 - **it is very unlikely that a latex allergy would explain an anaphylaxis during anaesthetic induction** (latex allergies typically used to commence when a surgeon began handling internal organs).
 - ⇒ type IV hypersensitivity (allergic contact dermatitis)
 - **Type 4 hypersensitivity is usually due to accelerators or chemicals used in the manufacturing process, whereas type 1 hypersensitivity is due to the latex proteins themselves**
 - ⇒ irritant contact dermatitis

Latex-fruit syndrome

- It is recognised that many people who are allergic to latex are also allergic to fruits, particularly **banana**, pineapple, avocado, chestnut, kiwi fruit, mango, passion fruit and strawberry. **However, bananas are the most commonly associated with latex/rubber allergy**

Latex allergy can be associated with certain foods such as bananas, avocado, kiwi and melon.

MRCPUK part-1-May 2016 exam: A nurse who is known to have an allergy to latex develops a widespread urticarial rash and facial oedema shortly after eating lunch. Which food is she most likely to have consumed? **Banana**

Serum Sickness

Definition

- Serum sickness is a classic example of a **type III hypersensitivity reaction**, which usually develops as a complication of antitoxin or antivenom administration.

Aetiology

- Antivenom or antitoxin containing animal proteins
- Medications most frequently antibiotics (e.g., penicillin, amoxicillin, cefaclor, trimethoprim-sulfamethoxazole)
- Infections: Hepatitis B virus

Pathophysiology

- exposure to an antigen (e.g., antivenom, drug) → formation of antibodies → deposition of antibody-antigen complexes in tissue → activation of the complement cascade → tissue damage and systemic inflammation

Features

- Symptoms appear 1–2 weeks following initial exposure (because antibodies take several days to form), and usually resolve within a few weeks after discontinuation of the offending agent.
- Fever
- Rash (urticarial or purpuric)
- Arthralgias
- Lymphadenopathy

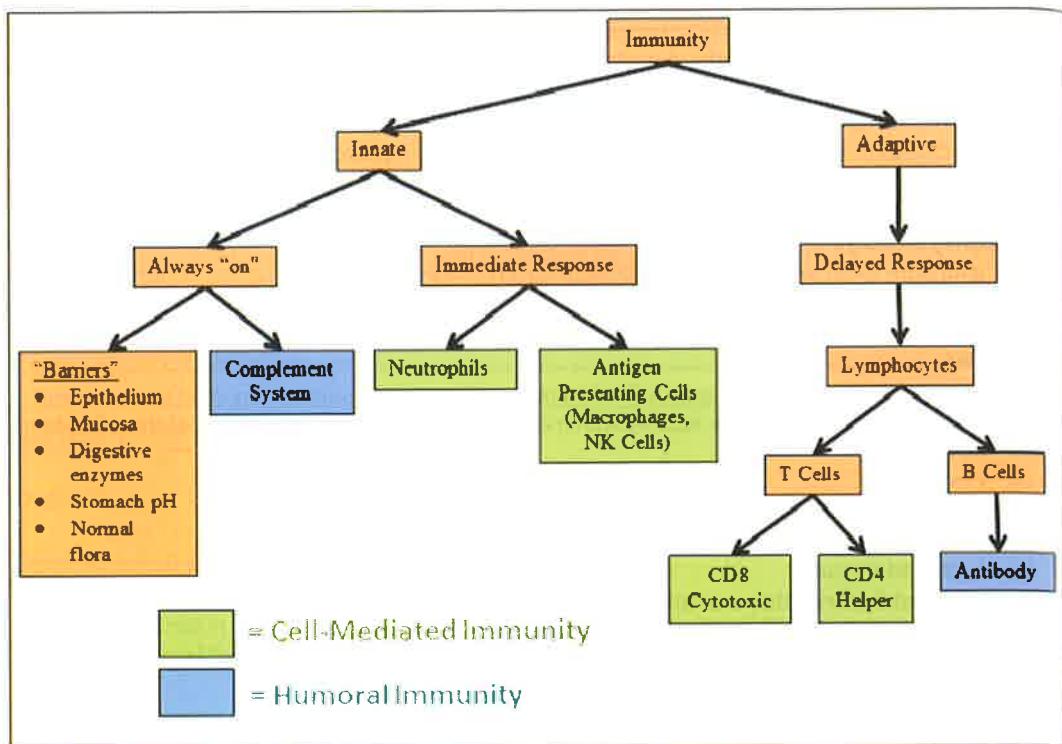
Subtypes and variants: serum sickness-like reaction

- much more common than actual serum sickness
- Aetiology: similar to that of serum sickness
- Infections (e.g., hepatitis B, rabies)
- Medications that can act as haptens (e.g., allopurinol, cephalosporins, penicillin).

Diagnostics: Urinalysis may show mild **proteinuria**.

Treatment: Stop the offending agent.

Immune system response



Innate VS Adaptive immune response

Innate (non-specific system)	Adaptive (acquired system)
Components <ol style="list-style-type: none"> Anatomical and physiological barriers Inflammatory response with leakage of antibacterial serum proteins (acute-phase proteins) and phagocytic cells Phagocytosis by neutrophils and macrophages Complement system 	Components <ol style="list-style-type: none"> Cell-mediated response effected by T cells Humeral immune response effected by B cells
Properties <ol style="list-style-type: none"> Rapid: responds within minutes to infection No antigenic specificity, i.e. the same molecules and cells respond to range of pathogens No memory, i.e. the response does not change after repeated exposure Preformed or rapidly formed components 	Properties <ol style="list-style-type: none"> Slow: response over days to weeks Antigenic specificity i.e. each cell is a programmed genetically to respond to a single antigen Immunological memory, i.e. on repeated the response is faster, stronger and qualitatively different Diversity: ability to recognize and respond to a vast number of different antigens Self/non-self-recognition: i.e. lack of response (tolerance) to self-antigens but response to foreign antigens

Overview of blood cell types involved in the innate immune response

Cell type	Functions and properties
Neutrophil	<ul style="list-style-type: none"> ⇒ Primary phagocytic cell in acute inflammation ⇒ Granules contain myeloperoxidase and lysozyme ⇒ Most common type of white blood cell ⇒ Multi-lobed nucleus
Basophil	<ul style="list-style-type: none"> ⇒ Releases histamine during allergic response ⇒ Granules contain histamine and heparin ⇒ Expresses IgE receptors on the cell surface ⇒ Bi-lobed nucleus
Mast cell	<ul style="list-style-type: none"> ⇒ Present in tissues and are similar in function to basophils but derived from different cell lines ⇒ Granules contain histamine and heparin ⇒ Expresses IgE receptors on the cell surface
Eosinophil	<ul style="list-style-type: none"> ⇒ Defends against protozoan and helminthic infections ⇒ Bi-lobed nucleus
Monocyte	<ul style="list-style-type: none"> ⇒ Differentiates into macrophages ⇒ Kidney shaped nucleus
Macrophage	<ul style="list-style-type: none"> ⇒ Involved in phagocytosis of cellular debris and pathogens ⇒ Acts as an antigen presenting cell ⇒ Major source of IL-1
Natural killer cell	<ul style="list-style-type: none"> ⇒ Induce apoptosis in virally infected and tumour cells
Dendritic cell	<ul style="list-style-type: none"> ⇒ Acts as an antigen presenting cell, but have no cytotoxic potential.

Macrophages

Overview

- Macrophages are a type of antigen-presenting cell, defined as a lymphocyte that is able to phagocytose debris, toxins, cells or pathogens.
- **Origin:** Monocytes migrate to tissue and differentiate into macrophages.
- **Activated by** γ -interferon.
- Has a long life in tissues, which differentiates it from a circulating blood monocyte
- **Important cellular component of granulomas** (eg, TB, sarcoidosis), where they may fuse to form giant cells.

Tissue-specific subtypes

- Osteoclasts (bone)
- Kupffer cells (liver)
- Microglia (brain and spinal cord)
- Histiocytes (connective tissue)

A patient undergoes liver biopsy, which shows ↑ phagocytes with kidney-shaped nuclei. What are these cells called?

- ⇒ Kupffer cells (the names of macrophages can differ in each tissue)

What signaling molecule activates macrophages?

- ⇒ γ -interferon

Important macrophage forms in various Diseases

- Lipid laden macrophage (Foam cells) = **Hyperlipidemia & Atherosclerotic plaques.**
- Hemosiderin laden macrophage(Heart failure cells) = **CHF.**
- Macrophages containing debris from ingested Lymphocytes (Tingible body macrophage) = **Benign reactive lymphadenitis.**
- Macrophages containing PAS +ve, gram +ve rod shaped bacilli within Lamina propria in small intestine = **Whipple Disease.**
- Iron trapped in Macrophages in Bone marrow = Anemia of chronic disease.
- Macrophages containing Carbon pigment along pleural lymphatics = **Anthracosis.**
- Tissue paper like macrophage = **Gaucher disease.**

The lipid A component of bacterial lipopolysaccharide (LPS) binds to CD14 on macrophages, which can trigger septic shock

Pathogenesis of atherosclerosis

1. Chronic stress on the endothelium
2. Endothelial dysfunction, which leads to
 - ⌚ Invasion of inflammatory cells (mainly monocytes and lymphocytes) through the disrupted endothelial barrier
 - ⌚ Adhesion of platelets to the damaged vessel wall → platelets release inflammatory mediators (e.g., cytokines) and platelet-derived growth factor (PDGF)
 - ⌚ PDGF stimulates migration and proliferation of smooth muscle cells (SMC) in the tunica intima and mediates differentiation of fibroblasts into myofibroblasts
3. Inflammation of the vessel wall
4. Macrophages and SMCs ingest cholesterol from oxidized LDL and transform into foam cells.
5. Foam cells accumulate to form fatty streaks (early atherosclerotic lesions).
6. Lipid-laden macrophages and SMCs produce extracellular matrix (e.g., collagen) → development of a fibrous plaque (atheroma)
7. Inflammatory cells in the atheroma (e.g., macrophages) secrete matrix metalloproteinases → weakening of the fibrous cap of the plaque due to the breakdown of extracellular matrix → minor stress ruptures the fibrous cap
8. Calcification of the intima (the amount and pattern of calcification affect the risk of complications)
9. Plaque rupture → exposure of thrombogenic material (e.g., collagen) → thrombus formation with vascular occlusion or spreading of thrombogenic material

Foam cells

- **Foam cells** are a feature of atherosclerotic plaques and **are essentially lipid-laden macrophages.**
- They may also be seen as a reaction to:
 - ⇒ silicone leakage around breast implants, and
 - ⇒ inhaled organic antigens.

MRCPUK exam- Jan-2018: You are examining tissue biopsied from around a leaking silicone breast implant. It is rich in foam cells. What is the cell lineage of foam cells?

- **Macrophage**

Fibroblasts

- The most common cell type in connective tissue
- Origin:** derived from mesenchymal stem cells
- Found in the interstitial spaces of organs.
- Histological features:** **spindle-shaped cells** arranged in a branching pattern
- Function:**
 - synthesis and organization of the extracellular matrix (ECM) and collagen
 - plays a critical role in wound healing
 - play a critical role in an immune response to a tissue injury.
 - They are **early players in initiating inflammation** in the presence of invading microorganisms. Tissue damage stimulates fibrocytes and induces the mitosis of fibroblasts.
 - Responsible for forming the cap over an atherosclerotic plaque.**
- Pathologic fibrosis** is characterized by uncontrolled fibroblast activation that results in exaggerated and persistent ECM accumulation and remodeling.

Immunoglobulins

IgD is involved in the activation of B-cells

The table below summarises the characteristics of the 5 types of immunoglobulin found in the body:

Type	Frequency	Shape	Notes
IgG	75%	Monomer	<ul style="list-style-type: none"> comprises the majority of circulating antibody in serum the major antibody produced in the secondary immune response. Enhances phagocytosis of bacteria and viruses half-life: 7-23 days Fixes classical complement can bind to NK cells for antibody-dependent cytotoxicity (ADCC). the only antibody that can cross the placenta and enter the fetal circulation Most abundant isotype in blood serum Gamma is the type of heavy chain found in IgG.
IgA	15%	Monomer/dimer	<ul style="list-style-type: none"> Found in secretions such as saliva, tears and mucous made primarily in the mucosal-associated lymphoid tissues (MALT). Provides localized protection on mucous membranes The Fc portion of secretory IgA binds to components of mucous and contributes to the ability of mucous to trap microbes. Most commonly produced immunoglobulin in the body (but blood serum concentrations lower than IgG) half-life ≈ 5 days Transported across the interior of the cell via transcytosis can activate the <u>alternative</u> complement pathway. (IgA ≈ Alpha) Low levels of IgA are associated with an increased incidence of Coeliac Disease. Alpha is the type of heavy chain found in IgA.

Type	Frequency	Shape	Notes
IgM	10%	Pentamer	<ul style="list-style-type: none"> First immunoglobulins to be secreted in response to an infection (primary response) Fixes classical complement pathway (most efficient) Anti-A, B blood antibodies (note how they cannot pass to the fetal circulation, which could of course result in haemolysis) Monomeric forms of IgM are found on the surface of B-lymphocytes as B-cell receptors or slg. half-life: about 5 days Mu is the type of heavy chain found in IgM.
IgD	1%	Monomer	<ul style="list-style-type: none"> Involved in activation of B cells (as a surface receptor on B cells) may play a role in eliminating B-lymphocytes generating self-reactive autoantibodies. Delta is the type of heavy chain found in IgD. Hyper-IgD is associated with periodic fever (attacks of fever every 4-8 weeks, with each attack lasting 3-7 days)
IgE	0.1%	Monomer	<ul style="list-style-type: none"> produced by plasma cells Mediates type 1 hypersensitivity reactions Binds to Fc receptors found on the surface of mast cells and basophils Provides immunity to parasites such as helminths Least abundant isotype in blood serum half-life of 2 days IgE may protect external mucosal surfaces by promoting inflammation, enabling IgG, complement proteins, and leucocytes to enter the tissues. Cross linking of cell-bound IgE by antigen triggers the release of vasodilators for an inflammatory response. The Fc portion of IgE made against parasitic worms and arthropods can bind to eosinophils enabling opsonization. This is a major defense against parasitic worms and arthropods. Epsilon is the type of heavy chain found in IgE. Raised IgE levels are a normal finding in 2.5%

Each day an average adult produces approximately 3gm of antibodies, about two-thirds of this IgA

Acute organ rejection is due to anti-IgG antibodies to the human leukocyte antigen (HLA) incompatible tissues with primary activation of T cells.

Blood transfusion

- Rhesus** antibodies are **IgG**, whereas **ABO** antibodies are **IgM**

Commonly recognized immunoglobulin changes in liver disease

(usually accompanied by a decrease in albumin) are:

- IgG** ↑ in: chronic active hepatitis, cryptogenic cirrhosis
- IgM** ↑ in: 1° biliary cirrhosis, alcoholic cirrhosis
- IgA** ↑ in: alcoholic cirrhosis.

Immunoglobulins (antibodies) have two functional parts: the Fc region and the Fab region

- **Fc region**
 - ⇒ Contains the **constant** region
 - ⇒ Formed by **heavy** (H) chains
 - ⇒ Recognizes and binds **complement** (IgG, IgM)
- **Fab region**
 - ⇒ Contains the **variable** region
 - ⇒ Formed by **light** (L) chains and **heavy** (H) chains
 - ⇒ Recognizes and binds to **antigens**

Immunoglobulins and complement fixation

- **IgA** can fix complement via the **alternative** pathway
- **IgG** and **IgM** can fix complement via the **classical** pathway through the Fc portion of the immunoglobulin

Protein analysis: Gamma globulins

- **Hypergammaglobulinaemia**

 ⇒ **Causes of polyclonal hypergammaglobulinaemia**

- Artefactual, e.g. prolonged venous stasis before venepuncture
- Haemoconcentration secondary to dehydration
- Chronic infection, e.g. TB, infective endocarditis, leishmaniasis
- Autoimmune disease, e.g. SLE, rheumatoid arthritis
- Ulcerative colitis and Crohn's disease
- Sarcoidosis
- Hepatic disease.

 ⇒ **Causes of monoclonal hypergammaglobulinaemia**

- Multiple myeloma, Waldenstrom's macroglobulinaemia and heavy chain disease
- Leukaemia, lymphoma or carcinoma
- Bence Jones proteinuria
- 'Benign' paraproteinaemia
- Amyloidosis.

- **Agammaglobulinemia** (e.g., Bruton agammaglobulinemia)

- **Hypogammaglobulinemia** (low IgG)

 ⇒ Nephrotic syndrome

 ⇒ Drug-induced reactions

 ⇒ Acquired humoral and congenital immunodeficiencies

Immunoglobulins: therapeutics

Basics

- formed from large pool of donors (e.g. 5,000)
- IgG molecules with a subclass distribution similar to that of normal blood
- half-life of 3 weeks

Uses

- primary and secondary immunodeficiency
- idiopathic thrombocytopenic purpura

- myasthenia gravis
- Guillain-Barre syndrome
- **Kawasaki disease**
- toxic epidermal necrolysis
- pneumonitis induced by CMV following transplantation
- low serum IgG levels following haematopoietic stem cell transplant for malignancy
- dermatomyositis
- chronic inflammatory demyelinating polyradiculopathy

Leukotrienes

Overview

- mediators of inflammation and allergic reactions
- secreted by leukocytes
- formed from arachidonic acid by action of lipoxygenase
- it is thought that the NSAID induced bronchospasm in asthmatics is secondary to the express production of leukotrienes due to the inhibition of prostaglandin synthetase

Function

- **cause bronchoconstriction,**
- **mucous production** (an important consideration in the pathophysiology of bronchial asthma)
- increase vascular permeability, attract leukocytes
- leukotriene D4 has been identified as the SRS-A (slow reacting substance of anaphylaxis) which causes bronchial wall and intestinal smooth muscle contraction

Acute phase proteins

Acute phase proteins

- CRP
- procalcitonin
- ferritin
- fibrinogen
- alpha-1 antitrypsin
- caeruloplasmin
- serum amyloid A, serum amyloid P
- haptoglobin
- complement

Negative acute phase proteins

- During the acute phase response, the liver **decreases the production of other proteins** (sometimes referred to as negative acute phase proteins). Examples include:
 - ⇒ albumin
 - ⇒ transthyretin (formerly known as prealbumin)
 - ⇒ transferrin
 - ⇒ retinol binding protein
 - ⇒ cortisol binding protein

ANCA

cANCA = Wegener's; pANCA = Churg-Strauss + others

- There are two main types of anti-neutrophil cytoplasmic antibodies (ANCA):
 1. cytoplasmic (cANCA) and
 2. perinuclear (pANCA)
- For the exam, remember:
 - ⇒ cANCA - Wegener's granulomatosis
 - ⇒ pANCA - Churg-Strauss syndrome + others (see below)

cANCA

- most common **target serine proteinase 3 (PR3)**
- some correlation between cANCA levels and disease activity
- Wegener's granulomatosis, positive in > 90%
 - ⇒ In Wegener's, the level of PR3 antibody and ANCA titre are **related to disease activity** and the antibodies typically disappear when the disease is in remission.
- microscopic polyangiitis, positive in 40%

pANCA

- most common **target is myeloperoxidase (MPO)**
- cannot use level of pANCA to monitor disease activity
- associated with immune crescentic glomerulonephritis (positive in c. 80% of patients)
- microscopic polyangiitis, positive in 50-75%
- Churg-Strauss syndrome, positive in 60%
- primary sclerosing cholangitis, positive in 60-80%
- Wegener's granulomatosis, positive in 25%
- **Other causes of positive ANCA (usually pANCA)**
 - ⇒ inflammatory bowel disease (UC > Crohn's)
 - ⇒ connective tissue disorders: RA, SLE, Sjogren's
 - ⇒ autoimmune hepatitis

MRCP-part-1-Jan-2018 exam: Which one of the following statements is true regarding cytoplasmic anti-neutrophil cytoplasmic antibodies (cANCA)?

- Associated with Wegener's granulomatosis

Rheumatoid factor (see rheumatology)

Antibodies and immunological markers

Marker	Associated condition
Antinuclear antibodies (ANA)	<ul style="list-style-type: none"> • Younger women often have low (ANAs) • increase with age • ANA positivity with antiphospholipid antibody syndrome (APL) suggests secondary APL, ie in association with a connective tissue disease. • The common tests used for detecting and screening ANAs are indirect immunofluorescence and enzyme-linked

Marker	Associated condition
	<p>immunosorbent assay (ELISA).</p> <ul style="list-style-type: none"> Although positive titres of 1:160 or higher are strongly associated with autoimmune disorders, they are also found in 5% of healthy individuals Positive titres of less than 1:160 are present in up to 20% of the healthy population, especially the elderly.
Anti-Ro (SS-A) and anti-La (SS-B)	<p>Anti-Ro</p> <p>Sjögren's syndrome (50–70%)</p> <p>SLE with cutaneous involvement (30%)</p> <p>anti-Ro can cross the placenta and cause neonatal lupus in babies.</p>
Anti-Smith (Anti-Sm)	<p>very specific marker for SLE (99%)</p> <p>sensitivity (20%)</p> <p>not associated with disease activity.</p>
Anti-nuclear ribonucleoprotein (anti-nRNP) also known as anti-U1-RNP	<p>highly associated with mixed connective tissue disease.</p> <p>SLE (30 – 40%)</p>
Anti-double stranded DNA (anti-dsDNA)	<p>very specific marker for SLE, (nearly 100%).</p> <p>sensitivity (85%).</p> <p>Correlate with disease activity in SLE.</p> <p>also linked with lupus nephritis.</p>
Anti-histone	<p>drug induced lupus (75–95%)</p> <p>idiopathic SLE (75%)</p> <p>Unlike anti-dsDNA, these antibodies do not fix complement.</p>
anti-glycoprotein-210 (anti-gp210) and anti-nucleoporin 62 (anti-p62)	primary biliary cirrhosis (PBC) (25–30%).
Anti-centromere	<p>limited cutaneous systemic sclerosis, also known as CREST syndrome,</p> <p>primary biliary cirrhosis</p>
Thyroid autoantibodies (microsomal and thyroglobulin)	<p>Hashimoto's thyroiditis (70-90% microsomal: 75-95% thyroglobulin)</p> <p>Pernicious anaemia (55% microsomal)</p>
Anti-Scl-70	<p>diffuse cutaneous scleroderma (40%),</p> <p>limited cutaneous involvement (10%).</p> <p>SLE (5%)</p> <p>The antigenic target of anti-Scl-70 antibodies is topoisomerase I</p>
Antireticulin	<p>Coeliac disease (37%)</p> <p>Crohn's disease (24%)</p>
Gastric parietal cell antibody	<p>Pernicious anaemia (>90%)</p> <p>Atrophic gastritis (60%)</p> <p>Autoimmune thyroid disease (33%)</p>

Marker	Associated condition
Anti-mitochondrial antibody	Primary biliary cirrhosis (60-94%)
Anti-smooth muscle antibody	Chronic active hepatitis (40-90%) Primary biliary cirrhosis (30-70%) Idiopathic cirrhosis (25-30%) Viral infections (80%)
Anti-sp100	primary biliary cirrhosis (PBC) (20–30%). very specific marker of the disease.
Anti-PM-Scl	polymyositis/systemic sclerosis (PM/SSc) overlap syndrome (50%).
Anti-Hu	small-cell lung cancer, neuroblastoma and prostatic cancer
Intrinsic factor antibodies	pernicious anaemia, and hence (subacute combined degeneration of the spinal cord) secondary to vitamin B12 deficiency
Anti-Ri	neuroblastoma (children) and fallopian or breast cancer (adults), resulting in paraneoplastic opsoclonus myoclonus ataxia (POMA).
Anti-Yo	gynaecological tumours and breast cancer,
Anti-Tr	Hodgkin's disease, resulting in cerebellar degeneration.
Anti-Ta (Ma2)	testicular tumours, and can lead to limbic or brain stem encephalomyelitis.
Anti-endomysial / gliadin / transglutaminase	coeliac disease, and related vitamin B-1 deficiency may lead to Wernicke's encephalopathy and Korsakoff's psychosis
Tissue transglutaminase antibody ('tTGA') & Endomysial antibody ('EMA')	The most accurate blood tests for coeliac disease
double-stranded DNA (ds-DNA) Anti-dsDNA	highly specific for SLE.
Antibodies that bind single-stranded denatured DNA (ss-DNA)	present in 90% of patients with SLE, but also in drug-induced lupus and other connective tissue disorders.
Anti-Jo	Polymyositis
Rheumatoid factor	Rheumatoid arthritis, Sjogren's (90%), SLE (30%) <i>5% of normal population</i>

The only two auto-antibodies which have a role in monitoring disease activity (there is correlation between levels and disease activity)

1. **Anti-ds DNA** antibodies in systemic lupus erythematosus (**SLE**)
2. Circulating anti-neutrophil cytoplasmic antibody (**cANCA**) in **Wegener's granulomatosis**.

Interleukins

Definition

- Interleukin are a group of signaling proteins **expressed by leukocytes** that regulate immune response as well as cellular proliferation and differentiation.

Production

- The majority of interleukins are synthesized by helper CD4 T lymphocytes, as well as through monocytes, macrophages, and endothelial cells.**

Function

- The function of the immune system depends in a large part on interleukins,
- They promote the development and differentiation of T and B lymphocytes, and hematopoietic cells.

Both cytokine overexpression and underexpression can be pathogenic:

- Production of IL-1, IL-6 and TNF due to endotoxin stimulation of macrophages following Gram-negative infection → **Septic shock**
- Chagas' disease** (Trypanosoma cruzi infection) → **reduced expression of IL-2 receptor** → marked immune suppression.

Overview of interleukins

Cytokine	Main sources	Functions
IL-1	Macrophages	Acute inflammation Induces fever
IL-2	Th1 cells	Stimulates growth and differentiation of T cell response
IL-3	Activated T helper cells	Stimulates differentiation and proliferation of myeloid progenitor cells
IL-4	Th2 cells	Stimulates proliferation and differentiation of B cells (Stimulates switching to IgE and IgG.)
IL-5	Th2 cells	Stimulates proliferation and differentiation of B cells (Stimulates switching to IgA.) Stimulates production of eosinophils
IL-6	Macrophages, Th2 cells	Stimulates differentiation of B cells Induces fever stimulates production of acute phase proteins.
IL-8	Macrophages	Neutrophil chemotaxis
IL-10	Th2 cells	Inhibits Th1 cytokine production Also known as human cytokine synthesis inhibitory factor and is an ' anti-inflammatory ' cytokine
IL-12	Dendritic cells, macrophages, B cells	Activates NK cells. stimulates differentiation of naive T cells into Th1 cells

Other cytokines

Cytokine	Main sources	Functions
Tumour necrosis factor-α	Macrophages	Induces fever Neutrophil chemotaxis
Interferon-γ	Th1 cells	Activates macrophages

Mnemonic Hot T-Bone stEAK

- ⦿ IL-1: fever (**Hot**)
- ⦿ IL-2: stimulates **T lymphocytes**
- ⦿ IL-3: stimulates **Bone marrow**
- ⦿ IL-4: stimulates **IgE**
- ⦿ IL-5: stimulates **IgA**

Interleukin 1 (IL-1)

- **Produced by macrophages and monocytes**
- **Action**
 - ⇒ Endogenous pyrogen (**one of the mediators of shock in sepsis**): promotes
 - Fever (Along with **IL-6** and **TNF**, it acts on the hypothalamus causing **pyrexia**)
 - Vasodilation → edema
 - Adhesion and diapedesis of inflammatory cells via cytokines, e.g. WBCs
 - ⇒ Co-stimulator of T cell and B cell proliferation. (Stimulation of acute phase response)
 - ⇒ Hematopoietic growth factor
 - Stimulates proliferation of granulocytes in the bone marrow and lymphocytes in the spleen
 - Inhibits hematopoiesis
 - ⇒ Induces expression of adhesion molecules in the endothelium
 - ⇒ Promotes differentiation of Th17 cells involved in autoimmunity
 - ⇒ Also known as osteoclast-activating factor: Dysregulation of IL-1 in cartilage leads to damage and osteoarthritis.
 - ⇒ **Play a role in the formation of the atherosclerotic plaque**
 - The uptake of oxidized low-density lipoproteins (LDL) by vascular endothelial cells results in → IL-1 expression → stimulates the production of platelet-derived growth factor.

Interleukin-2 (IL-2)

- **Produced by Th1 cells (mainly CD4+ cells)**
- **Functions**
 - ⇒ Stimulates proliferation and differentiation of T cells (helper, cytotoxic, regulatory T cells, and natural killer cells)
 - ⇒ Activates macrophages
 - ⇒ IL-2 is part of the body's natural response to microbial infection, and in discriminating between foreign ("non-self") and "self".
 - ⇒ there is some evidence that IL-2 may be involved in itchy psoriasis
- **Therapeutic use**
 - ⇒ **High-dose interleukin-2 can produce a high rate of response and durable remissions in patients with metastatic renal cancer.**
 - ⇒ IL-2 analog (aldesleukin): metastatic melanoma and renal cell carcinoma
 - ⇒ IL-2 antagonists (e.g., basiliximab): prevention of renal transplant rejection

Interferon

Interferon- γ is responsible for activating macrophages

To remember the use of interferon- γ , think

"Interferon gamma for granulomatous diseases!"

Overview

- Interferons (IFN) are cytokines released by the body in response to viral infections and neoplasia.
- Are a part of the innate immune system
- Have antiviral, antimicrobial, and antiproliferative properties
- Used in the treatment of chronic infections (hepatitis B and C, chronic granulomatous diseases), immune-mediated diseases (multiple sclerosis), and even tumors (leukemia, Kaposi sarcoma)
- They are classified according to cellular origin and the type of receptor they bind to.
- **IFN-alpha and IFN-beta bind to type 1 receptors** whilst IFN-gamma binds only to type 2 receptors.

Types

- **IFN-alpha**
 - ⇒ **Produced by** leucocytes
 - ⇒ **Function:** Antiviral action (Inhibits viral protein synthesis by activating ribonuclease L)
 - ⇒ **Therapeutic use:** Hepatitis B & C, Kaposi's sarcoma, metastatic renal cell cancer, hairy cell leukaemia
- **IFN-beta**
 - ⇒ **Produced by** fibroblasts
 - ⇒ **Function:** Antiviral action
 - ⇒ **Therapeutic use:** Multiple sclerosis → Reduces the frequency of exacerbations in patients with relapsing-remitting MS
- **IFN-gamma (γ)**
 - ⇒ The only member of the type II class of interferons
 - ⇒ **Produced by** Th1 cell
 - ⇒ **Function**
 - **Activates macrophages** to increase phagocytosis
 - Activates the expression of Class II major histocompatibility complex (MHC) molecules
 - Weaker antiviral action
 - ⇒ **Therapeutic use**
 - Chronic granulomatous diseases (e.g., leprosy, leishmaniasis, toxoplasmosis)

Side effects of interferon

- **Flu-like symptoms** (fever, chills)
- Depression
- Myopathy
- Neutropenia
- Interferon-induced autoimmunity

What is the MOA of Toxic Shock Syndrome Toxin (TSST-1) from *Staphylococcus aureus*?

- Bringing of MHC II and T-cell receptors in proximity to outside of the antigen binding site, thereby causing overwhelming release of **IFN-gamma** and **IL-2**

The relation between IL-12 and IFN-gamma:

How do IFN-gamma levels change in IL-12 Receptor Deficiency?

⇒ Decrease

- IL-12 → Th1 cell activation → release IFN-gamma → activates macrophages.
- No IL-12 action = no IFN-gamma release from Th1 cells

Tumour necrosis factor (TNF)

Overview

- Tumour necrosis factor (**TNF**) is a pro-inflammatory cytokine with multiple roles in the immune system
- TNF is secreted mainly by macrophages
- Act mainly in a paracrine fashion

Function

- Activates macrophages and neutrophils, acts as co-stimulator for T cell activation
- Increased acute phase proteins
- Similar properties to IL-1, induced pyrexia
- TNF is important in the pathogenesis of rheumatoid arthritis.**
 - ⇒ TNF blockers (e.g. infliximab, etanercept) are now licensed for treatment of severe rheumatoid
- A key cytokine in the pathogenesis of multi-organ failure, a Key mediator of bodies response to Gram negative septicaemia. **High concentrations of TNF induce shock-like symptoms**
- Exerts an interferon-like effect against viruses
- Enhanced HLA class I expression
- Anti-tumour effect (e.g. phospholipase activation)
- TNF-alpha binds to both the p55 and p75 receptor. These receptors can induce apoptosis. It also cause activation of NFkB
- Endothelial effects include increase expression of selectins and increased production of platelet activating factor, IL-1 and prostaglandins
- Promotes the proliferation of fibroblasts and their production of protease and collagenase.
- the prolonged exposure to **low concentrations of TNF can result in cachexia**, a wasting syndrome. This can be found, for example, in cancer patients.
- Raised levels lead to increased insulin resistance**

TNF blockers

- Used to treat IBD, rheumatoid arthritis, ankylosing spondylitis and psoriasis.
- Examples
 - ⇒ **Infliximab**: monoclonal antibody, IV administration
 - ⇒ **Etanercept**: fusion protein that mimics the inhibitory effects of naturally occurring soluble TNF receptors, subcutaneous administration
 - ⇒ **Adalimumab**: monoclonal antibody, subcutaneous administration
- Adverse effects of TNF blockers**
 - ⇒ reactivation of latent tuberculosis
 - ⇒ demyelination

- **Contraindications of usage of TNF- alpha antagonist**
 - ⇒ Active infection
 - ⇒ Active TB
 - ⇒ MS (Multiple sclerosis)
 - ⇒ Heart failure (NYHA grade 3-4).
 - ⇒ Pregnancy and Breast feeding

Nitric oxide (NO)

Nitric oxide (NO)

- ⌚ Has a half-life of only a few seconds.
- ⌚ It is not stored by the body but is synthesized as a result of activation.
- ⌚ Nitrate drugs stimulate the formation and release of NO.
- ⌚ Relaxation of smooth muscle cells in vessel walls leads to the dilation of coronary arteries, pulmonary arteries, and peripheral veins.
- ⌚ Peripheral vasodilation leads to a decrease in cardiac preload.

Overview

- It is formed from L-arginine and oxygen by nitric oxide synthetase (NOS).
- An inducible form of NOS has been shown to be present in macrophages.
- Nitric oxide has a very short half-life (seconds), being inactivated by oxygen free radicals
- Nitric oxide generates cyclic guanosine monophosphate (cGMP) as the second messenger
- Can freely diffuse across cell membranes, so NO can act as an intracellular and extracellular signalling molecule

Effects

- Acts on guanylate cyclase leading to raised intracellular cGMP levels and therefore decreasing Ca²⁺ levels
- Causes smooth muscle relaxation and subsequent dilation of blood vessels
- Inhibits platelet aggregation

Clinical relevance

- Underproduction of NO is implicated in hypertrophic pyloric stenosis
- Lack of NO is thought to promote atherosclerosis
- In sepsis increased levels of NO contribute to septic shock
- Organic nitrates (metabolism produces NO) is widely used to treat cardiovascular disease (e.g. angina, heart failure)
- Sildenafil is thought to potentiate the action of NO on penile smooth muscle and is used in the treatment of erectile dysfunctions
- N₂O, also known as 'laughing gas', is often used in obstetrics and trauma for pain relief

Endothelin-1 (ET-1)

- A 21-amino-acid **polypeptide**
- Endothelin-1 is a potent **vasoconstrictor** that is encoded by the EDN1 gene and **produced by vascular endothelial cells.**
- It is a highly potent vasoconstrictor and plays a part in the modulation of vascular tone
- It may have a role in diseases such as Raynaud's phenomenon
- Its levels increase when the endothelium is stressed, for example in trauma or oxidative stress
- **Clinical significance**
 - ⇒ Long term ET-1 exposure has been associated with hypertrophic cardiomyopathy.
 - ⇒ Endothelin-1 receptor antagonists (Bosentan) are used in the treatment of pulmonary hypertension. Inhibition of these receptors prevents pulmonary vasculature constriction and thus decreases pulmonary vascular resistance.

Kinins

Overview

- Kinins are mostly produced at inflamed or injured tissue of the body
- Kinins are potent vasoactive basic peptides involved in the inflammatory response
- **Their activation leads to release of chemotactic cytokines**

Functions

- increase vascular permeability
- cause vasodilation, pain, and the contraction of smooth muscle
- stimulate arachidonic acid metabolism

Erythrocyte sedimentation rate (ESR)

Overview

- The ESR is a non-specific marker of inflammation and depends on both the size, shape and number of red blood cells and the concentration of plasma proteins such as fibrinogen, alpha₂-globulins and gamma globulins

Causes of a high ESR

- temporal arteritis
- myeloma
- other connective tissue disorders e.g. systemic lupus erythematosus
- other malignancies
- infection
- other factors which raise ESR: increasing age, female sex, anaemia

Causes of a low ESR

- **polycythaemia**
- afibrinogenaemia/ hypofibrinogenaemia

Leukocyte alkaline phosphatase

Raised in	Low in
<ul style="list-style-type: none"> • Myelofibrosis • Leukemoid reactions • Polycythemia rubra vera • Infections • Steroids, Cushing's syndrome • Pregnancy, oral contraceptive pill 	<ul style="list-style-type: none"> • Chronic myeloid leukemia • Pernicious anemia • Paroxysmal nocturnal hemoglobinuria • Infectious mononucleosis

Thymus

T cells = Thymus

B cells = Bone marrow

The Thymus arises from the Third pharyngeal pouch

Embryology: Thymus epithelium arises from the 3rd pharyngeal pouch (endoderm).

Function: Maturation and differentiation of T lymphocytes

Location: The thymus is a gland composed of two identical lobes, located in the superior anterior superior mediastinum, in front of the heart and behind the sternum.

Clinical significance

- Thymic hypoplasia or aplasia: DiGeorge syndrome, SCID
- Thymoma: tumor of thymic epithelial cells: Seen in myasthenia gravis, pure red cell aplasia, immunodeficiency with thymoma

Thymic cortex and medulla

- The cortex is the area of the thymus that is dense and full of immature T cells.
- The medulla is the area of the Thymus that is **pale** and full of **mature T cells**

B cells (B lymphocytes)

Origin: Originate and mature in the bone marrow

Function

- Major component of the adaptive immune system: The humoral immune response of the adaptive immune system mainly consists of B cells and antibodies.
- After activation, B cells differentiate into plasma cells that produce and secrete antibodies

Surface proteins

- B cells express numerous proteins on their surface:
 - ⇒ CD19, CD20, CD21 (used by EBV), and CD40
 - ⇒ MHC II
 - ⇒ IgG
 - ⇒ B7

Plasma cells

- Plasma cells are fully differentiated cells from B-cells and hence lack these features (i.e. they lack surface-bound IgG and MHC class II and cannot undergo somatic hypermutation or isotype switching).
- plasma cells do not have surface-bound IgG (unlike B-cells).
- plasma cells cannot undergo somatic hypermutation (unlike B-cells).
- plasma cells cannot undergo isotype switching (unlike B-cells).

B lymphocytes VS T lymphocytes

	B lymphocytes	T lymphocytes
Site of production	bone marrow. germinal centre of lymph nodes and spleen.	produced in the bone marrow but mature in the thymus Paracortical region of lymph nodes and spleen.
Functions	Humoral immunity <ul style="list-style-type: none"> ⇒ antibody production (immunoglobulins) ⇒ control of pyogenic bacteria prevention of blood-borne infections. ⇒ neutralization of toxins. 	Cell-mediated immunity; <ul style="list-style-type: none"> ⇒ protection against intracellular organisms, protozoa and fungi; ⇒ graft rejection; ⇒ control of neoplasms.
% of total lymphocytes:	<ul style="list-style-type: none"> ⇒ 12% ⇒ mainly fixed. 	<ul style="list-style-type: none"> ⇒ 70-80% (the majority of circulating lymphocytes in plasma). ⇒ mainly circulating; ⇒ long-lived memory cells.

T cells (T lymphocytes)

Origin

- Originate from lymphoid progenitor cells in the bone marrow and mature in the thymus.

Distribution

- **T lymphocytes compose the majority of circulating lymphocytes in plasma.**
- **Lymph nodes:**
 - The **paracortical areas** contain T cells and accessory cells.
 - B cells are found within the cortex in follicles, which have central areas known as **germinal centres**.
 - The **medulla** contains large blood vessels and sinuses, and medullary cords that contain plasma cells secreting antibody.

Function

- A major component of the adaptive immune response
- Essential for cell-mediated immunity
 - ⇒ T lymphocytes are involved in cell-mediated acquired immune responses, whereas B lymphocytes are involved in humoral immunity and produce immunoglobulins.

Mechanism of action

- T cells recognise antigen only when presented by (self) MHC molecules on an antigen presenting cell (**Co-operation with other cell types is required for T cell recognition of antigen**)
- **Patients with HIV have a deficiency of T-cells (CD4 T-cell lymphocytes)**

T cell subtypes

- T cells are largely divided into **cytotoxic T cells (CD8+)**, **T helper cells (CD4+)**, and regulatory T cells.

What is the predominant site in the lymph node that contains T cells?

- ⇒ **Paracortex**

CD8 proteins on the surface of cytotoxic T cells interact with MHC I receptors, while CD4 proteins on the surface of T-helper cells interact with MHC II receptors.

Rule of 8:

- ⇒ MHC I x CD 8 = 8.
- ⇒ MHC II x CD 4 = 8.

T-Helper cells (CD4+)

- Activated via antigen presentation by MHC class II receptors
- There are two major subsets of T-Helper cells:
 - ⇒ **Th1**
 - involved in the cell mediated response and **delayed (type IV) hypersensitivity**
 - Immune response to **intracellular** pathogens (viruses, intracellular bacteria)
 - secrete **IFN-gamma, IL-2, IL-3**
 - ⇒ **Th2**
 - involved in mediating humoral (antibody) immunity e.g. stimulating production of IgE in asthma
 - Immune response to **extracellular** pathogens (bacteria, parasites)
 - secrete IL-4, IL-5, IL-6, IL-10, IL-13
- An increase in the Th1:Th2 ratio is associated with a reduction in the risk of allergic/hypersensitivity reactions.

MRCP-part-1-Jan- 2018 exam: What is most commonly secreted agent by T-helper cells subset 2 (Th2 cells) ?

- ⇒ **Interleukin 4**

Primary immunodeficiency

Disorders may be classified according to which component of the immune system they affect.

Neutrophil disorders

Disorder	Underlying defect	Notes
Chronic granulomatous disease	Lack of NADPH oxidase reduces ability of phagocytes to produce reactive oxygen species	Caused by a failure of intracellular killing (no respiratory burst). Causes recurrent pneumonias and abscesses, particularly due to catalase-positive bacteria (e.g. <i>Staphylococcus aureus</i> and fungi (e.g. <i>Aspergillus</i>) Negative nitroblue-tetrazolium test Screening is by the nitroblue tetrazolium (NBT) test Abnormal dihydrorhodamine flow cytometry test
Chediak-Higashi syndrome	Microtubule polymerization defect which leads to a decrease in phagocytosis	Affected children have 'partial albinism' and peripheral neuropathy. Recurrent bacterial infections are seen Giant granules in neutrophils and platelets
Leukocyte adhesion deficiency	Defect of LFA-1 integrin (CD18) protein on neutrophils	Recurrent bacterial infections. Delay in umbilical cord sloughing may be seen Absence of neutrophils/pus at sites of infection

B-cell disorders

Disorder	Underlying defect	Notes
Common variable immunodeficiency CVID	Many varying causes	Hypogammaglobulinemia is seen. May predispose to autoimmune disorders and lymphoma
Bruton's (x-linked) congenital agammaglobulinaemia	Defect in Bruton's tyrosine kinase (BTK) gene that leads to a severe block in B cell development	X-linked recessive. Recurrent bacterial infections are seen Absence of B-cells with reduced immunoglobulins of all classes
Selective immunoglobulin A deficiency	Maturation defect in B cells	<ul style="list-style-type: none"> • Most common primary antibody deficiency. • Recurrent sinus and respiratory infections • Associated with coeliac disease and may cause false negative coeliac antibody screen

T-cell disorders

Disorder	Underlying defect	Notes
DiGeorge syndrome	22q11.2 deletion, failure to develop 3rd and 4th pharyngeal pouches	Common features include congenital heart disease (e.g. tetralogy of Fallot), learning difficulties, hypocalcaemia, recurrent viral/fungal diseases, cleft palate

Combined B- and T-cell disorders

Combined B- and T-cell disorders: SCID WAS ataxic (SCID, Wiskott-Aldrich syndrome, ataxic telangiectasia)

Disorder	Underlying defect	Notes
Severe combined immunodeficiency	Most common (X-linked) due to defect in the common gamma chain, a protein used in the receptors for IL-2 and other interleukins. Other causes include adenosine deaminase deficiency	Recurrent infections due to viruses, bacteria and fungi. Reduced T-cell receptor excision circles Stem cell transplantation may be successful
Ataxia telangiectasia	Defect in DNA repair enzymes	<ul style="list-style-type: none"> Autosomal recessive. Features include: <ol style="list-style-type: none"> cerebellar ataxia, telangiectasia (spider angiomas), recurrent chest infections and 10% risk of developing malignancy, lymphoma or leukaemia
Wiskott-Aldrich syndrome	Defect in WAS gene	X-linked recessive. Features include recurrent bacterial infections, eczema, thrombocytopenia. Low IgM levels Increased risk of autoimmune disorders and malignancy

Selective IgA deficiency

The history of mucosal infections (sinus and gastrointestinal) and the family history of immune cytopenia and coeliac disease are suggestive of selective IgA deficiency.

Definition

- Most common primary immunodeficiency that is characterized by a near or total absence of serum and secretory IgA

Features

- Often asymptomatic
- Recurrent infections
 - ⇒ May manifest with sinusitis or respiratory infections (S. pneumoniae, H. influenzae)
 - ⇒ Chronic diarrhea, partially due to elevated susceptibility to parasitic infection (e.g. by Giardia lamblia)
- Associated with autoimmune diseases (e.g., **gluten-sensitive enteropathy**, inflammatory bowel disease, immune thrombocytopenia) and atopy
 - ⇒ **10-fold increased risk of coeliac disease**
 - ⇒ Pernicious anaemia and hence gastric carcinoma
 - ⇒ ↑ **Adverse reactions to blood products**
 - Patients with selective IgA deficiency should be tested for the presence of anti-IgA antibodies prior to transfusion with blood products.
- Anaphylactic reaction to products containing IgA (e.g., intravenous immunoglobulin)
- Associated with IgG2 deficiency
 - ⇒ **They are more likely than the general population to have an IgG2 deficiency, leading to recurrent bacterial infections**
 - ⇒ **The possibility of IgG2 deficiency should always be investigated in IgA-deficient individuals with a history of recurrent bacterial infections, but Staphylococcus aureus is the exception**

The Six A's of selective IgA deficiency: Asymptomatic, Airway infections, Anaphylaxis to IgA-containing products, Autoimmune diseases, Atopy

Diagnosis

- low serum IgA level, with normal IgG and IgM levels
- False-positive pregnancy tests

Treatment

- No specific treatment
- Prophylactic antibiotics
- Intravenous infusion of IgA is not recommended because of the risk of anaphylactic reactions (caused by the production of anti-IgA antibodies).

To prevent transfusion reactions, IgA-deficient patients must be given washed blood products without IgA or obtain blood from an IgA-deficient donor.

IgG subclass deficiency

Overview

- A decrease of one of IgG subclass (IgG1, IgG2, IgG3 or IgG4) in a patient whose **total IgG concentration is normal**.

IgG1 deficiency

- Almost always presents as hypogammaglobulinemia, since IgG1 normally makes up about 70 percent of total IgG. Therefore, only those patients with IgG1 deficiency with normal total IgG should be diagnosed with selective IgG1 deficiency.

IgG2 deficiency

- More common in children
- Infections with *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis* are characteristic, since IgG2 comprises most of the antibody response against

polysaccharide capsular antigens → multiple presentations with otitis media and respiratory tract infections.

- If these patients are vaccinated with Pneumovax, they are still unable to mount a response to *S. pneumoniae* antigens

IgG3 deficiency

- More common in adults.
- Infections with *Moraxella catarrhalis* and *S. pyogenes* are typical → frequent chronic sinusitis

IgG4 deficiency

- May or may not be associated with symptomatic sinopulmonary infections.

Isolated IgD deficiency

- IgD are surface receptors of B lymphocytes
- No specific signs or symptoms
- **increased viral respiratory tract infections,**
⇒ IgE deficiency leads to both **viral and parasitic infections**
- IgA, IgG and IgM levels are entirely normal
- **Isolated IgD deficiency has been identified amongst people of Basque origin, hence the link to northern Spain**
- Not require any specific treatment

Common variable immunodeficiency (CVID)

Definition

- primary immunodeficiency with low serum levels of all immunoglobulins despite phenotypically normal B cells

Epidemiology

- **The most common clinically significant primary immunodeficiency** is CVID.
⇒ IgA deficiency is more common, but most are asymptomatic.
- Sex: ♀ = ♂
- Onset: present later than other B cell defects (usually 20–35 years of age)

Pathophysiology

- Most cases are sporadic with no known family history (**No clear pattern of inheritance**)
- B cells are phenotypically normal but are unable to differentiate into Ig-producing cells, (**B-cell dysfunction**) resulting in low immunoglobulins of all classes.

Features

- Recurrent pyogenic respiratory infections, e.g., sinopulmonary infections (in rare cases, enteroviral meningitis)
- Associated with a high risk of **lymphoma**, gastric cancer, **bronchiectasis**, and autoimmune disorders (e.g., rheumatoid arthritis, autoimmune hemolytic anemia, immune thrombocytopenia, vitiligo)

Investigations

- Quantitative immunoglobulin levels: **low levels of IgG, IgA, and IgM**
- Decreased number of plasma cells
- Flow cytometry shows subsets of **normal B and T cells**
- Poor response to immunizations

Treatment

- **Intravenous immunoglobulin (IVIG)** replacement therapy (**first line**), **the best option to prevent recurrent chest infections.**
- Prophylactic antibiotics

⌚ **CVID** → B-cell Cannot differentiate into plasma cells → low immunoglobulins but **normal or decreased B cells.**

⌚ **Bruton's** → Pre-B lymphocytes are increased because there's a maturation defect.

MRCP-part-1-May-2018

H/O **recurrent Giardia lamblia diarrhea and multiple upper respiratory infections** since birth. serum analysis reveals **normal levels of mature B lymphocytes**. What other finding on serum analysis predisposes the patient to recurrent diarrheal infections?

⌚ **Deficiency In IgA**

- The patient has **common variable immunodeficiency disorder (CVID)**
- IgA prevent the binding of pathogens to the epithelial cells; thus, preventing protozoa like Giardia lamblia from causing inflammation. Its absence, therefore, leads to the increased likelihood of repeat infection of the GI mucosa

Bruton's agammaglobulinemia (X-linked agammaglobulinemia)

Live vaccines (e.g., MMR) are contraindicated in patients with Bruton agammaglobulinemia.

Pathophysiology

- **X-linked recessive** disease caused by a mutations in the gene coding for **Bruton tyrosine kinase (BTK)** leads to **complete deficiency of B lymphocytes**
- The most common genetic event is a **missense mutation** (substitution in one amino acid in a protein).

Epidemiology: occurs mainly in boys

Features

- Symptoms develop between 3 and 6 months of age when maternal IgG levels in fetal serum start to decrease.
- Hypoplasia of lymphoid tissue (e.g., tonsils, lymph nodes)
- Recurrent, severe, pyogenic infections (e.g., pneumonia, otitis media), especially with encapsulated bacteria (S. pneumoniae, N. meningitidis, and H. influenzae)
- Hepatitis virus and enterovirus (e.g., Coxsackie virus) infections

Diagnosis

- Flow cytometry
 - ⇒ Absent or low levels of B cells (marked by CD19, CD20, and CD21)
 - ⇒ Normal or high T cells
- Low immunoglobulins of all classes
- Absent lymphoid tissue, i.e., no germinal centers and primary follicles

Treatment

- IV immunoglobulins
- Prophylactic antibiotics

Severe combined immunodeficiency disease (SCID)

SCID is due to either a deficiency in IL-2R gamma chain (most common, X-linked) or deficiency in adenosine deaminase (autosomal recessive)

Overview

- Numerous genetic mutations → Combined B- and T-cell disorder → immunodeficiency
- X-linked recessive mutations → defective IL-2R gamma chain receptor linked to JAK3 (most common SCID mutation)
- Autosomal recessive → Adenosine deaminase deficiency (it aid in breakdown of deoxyadenosine, which is a breakdown product of DNA) → Accumulation of toxic metabolites (deoxyadenosine and dATP) (Deoxyadenosine is toxic to lymphocytes, thus accumulation of this leads to apoptosis of lymphocytes)

Features (usually manifests in the first year of life)

- Recurrent infections
- Diarrhea
- Dermatitis
- Failure to thrive
- Lymph nodes and tonsils may be absent

Diagnosis

- Flow cytometry: absent T cells , abnormal function of B-cells
- CXR: absent thymic shadow
- Lymph node biopsy: absent germinal centers
- ↓ Lymphocyte count (< 3000/ μ L)

Treatment

- Bone marrow transplantation (the best initial curative treatment)

Prognosis

- Without intervention, SCID usually results in severe infection and death in children by age 2 years.

DiGeorge syndrome

DiGeorge syndrome - a T-cell disorder

Definition

- A syndrome characterized by defective development of the third and fourth pharyngeal pouches leading to hypoplastic thymus and parathyroids

Pathophysiology

- Autosomal dominant; microdeletion at **chromosome 22** → Abnormal development of the third and fourth pharyngeal pouches → thymic aplasia and defective parathyroid → **T-cell deficiency and dysfunction** → primary immunodeficiency
 - ⇒ The thymus arises from the 3rd pharyngeal pouch,
 - ⇒ the parathyroid glands receive contribution from both 3rd and 4th pouches.
- It is an example of a microdeletion syndrome.

Features

- Thymus aplasia/hypoplasia → Recurrent infections (viral/fungal/PCP pneumonia) due to T-cell deficiency
- Parathyroid gland hypoplasia → hypocalcaemic tetany

- **Cardiac anomalies:** (e.g., tetralogy of Fallot, VSD, ASD)
- **Facial abnormalities:**
 - ⇒ Cleft palate
 - ⇒ Micrognathia (small lower jaw) and/or retrognathia
 - ⇒ Dysplastic ears
 - ⇒ High and broad nasal bridge

Investigations

- **Chest X-ray shows absence of the thymic shadow.**
- **Low levels of serum calcium (Ca^{2+}) and parathormone (PTH)**
- \downarrow Absolute T-lymphocyte count
- Delayed hypersensitivity skin testing
- Fluorescence in situ hybridization (FISH) → Detection of 22q11.2 deletion

MRCP-part-1- May 2019 exam: In a patient having DiGeorge syndrome, which infection is he most at risk from, secondary to his immune system dysfunction? *Cryptococcus neoformans* (T-cell dysfunction → $\uparrow\uparrow$ risk from recurrent viral and fungal infections)

Wiskott-Aldrich syndrome (WAS)

Wiskott-Aldrich syndrome: Classic tetrad of:

1. Purpura (bleeding diathesis)
2. Eczema (high risk of atopic disorders)
3. Recurrent bacterial, viral, and fungal infections (e.g., chest, otitis media)
4. \uparrow Risk of autoimmune diseases and hematological malignancies

Definition

- Wiskott-Aldrich syndrome (WAS) is defined as an X-linked hereditary disorder associated with adaptive and innate immunodeficiency, micro-thrombocytopenia, eczema, and an increased risk of autoimmune disorders and malignancy.

Pathophysiology

- "Loss-of-function" mutation in **WASP gene (X-linked recessive inheritance)** → combined **B- and T-cell** dysfunction and thrombocytopenia

Epidemiology: occurs primarily in males

Features

- **Classic tetrad**
 1. Purpura (bleeding diathesis)
 2. Eczema (high risk of atopic disorders)
 3. Recurrent bacterial, viral, and fungal infections (e.g., chest, otitis media)
 4. **Increased risk of autoimmune diseases and hematological malignancies** (e.g., lymphoma, leukemia)

Investigations

- Thrombocytopenia with small platelets
- Low IgM and IgG levels
- \uparrow IgE and IgA
- Genetic analysis (confirmatory test): mutated WASP gene

Prognosis

- The disease has **variable penetrance**, which means that life expectancy can range from 6 - 30 years.

Complement deficiencies

- ⇒ C3 deficiency is associated with recurrent bacterial infections,
- ⇒ C5 deficiency is more characteristically associated with disseminated meningococcal infection

- ⇒ Deficiencies of the **classical** complement pathway such as **C1 and C4** deficiencies are strongly associated with the development of **systemic lupus erythematosus**;
- ⇒ deficiencies of the **alternative** pathway, such as **C3 and C5–9**, are associated with increased risk of **recurrent pyogenic infections**.

Overview

- Complement is a series of proteins that circulate in plasma and are involved in the inflammatory and immune reaction of the body.
- Complement proteins are involved in chemotaxis, cell lysis and opsonisation.
- Most of complement deficiencies are inherited in **autosomal recessive** fashion; the exception being properdin deficiency, which is usually described as having an X-linked inheritance pattern.

C1 inhibitor (C1-INH) protein deficiency

- causes hereditary angioedema
- C1-INH is a multifunctional serine protease inhibitor
- probable mechanism is uncontrolled release of bradykinin resulting in oedema of tissues

C1q, C1rs, C2, C4 deficiency (classical pathway components)

- predisposes to immune complex disease
- e.g. SLE, Henoch-Schonlein Purpura, vasculitides
- mechanism
 - ⇒ complement activity is associated with clearance of circulating immune complexes
 - ⇒ If immune complexes are not cleared, they undergo → tissue deposition where an inflammatory process is triggered, leading to SLE

C3 deficiency

- causes recurrent bacterial infections
- Deficiencies of **C3** is more commonly associated with **haemolytic uraemic syndrome**

C5 deficiency

- predisposes to Leiner disease
- recurrent diarrhoea, wasting and seborrhoeic dermatitis

C5-9 deficiency

- encodes the membrane attack complex (MAC)
- particularly prone to ***Neisseria meningitidis*** infection
- **Absent classical and alternate pathway activity**

Membrane attack complex (MAC)

- Formed by C5b, C6, C7, C8, and multiple copies of C9 complement proteins on pathogen cell membranes
- Function → lyses pathogens
- Inhibited by CD59
 - ⇒ This exists on body cells to protect them from MAC.

- ⇒ paroxysmal nocturnal haemoglobinuria, results in red cells that lack CD59. These red cells can, therefore, be lysed by MAC.

Decay-accelerating factor (DAF) deficiency is associated with → Paroxysmal nocturnal haemoglobinuria (PNH).

Diagnosis

- CH50 assay screening test

MRCPI-part-1-jan-2017: Post splenectomy what type of immunodeficiency is occurs?

⇒ **Humoral**

- Post splenectomy there is increased susceptibility to H. Influenzae, N. Meningitidis and Strep pneumonia which are encapsulated organisms **due to the loss of splenic macrophages which are part of the humoral response.**

MRCPUK-pat-1-May 2019 exam: A 23-year-old man is admitted with sepsis. Blood cultures are reported as *Neisseria gonorrhoeae*. Which complement protein is the patient most likely to deficient in?

- C5-9

Hereditary angioedema

Hereditary angioedema - C1-INH deficiency

Hereditary angioedema - C4 is the best screening test inbetween attacks

Overview

- Hereditary angioedema is an **autosomal dominant** condition associated with **low plasma levels of the C1 inhibitor (C1-INH) protein.**
- C1-INH is a multifunctional serine protease inhibitor

Pathophysiology

- Deficiency of C1 esterase inhibitor leads to persistent activation of the classical complement pathway and C4 levels are frequently low secondary to activation and consumption.
⇒ ↓C1 inhibitor allow C1 to act on C4 and C2
- Mechanism of attacks : uncontrolled release of **bradykinin** resulting in oedema of tissues.

Investigation

- C1-INH level is low during an attack
- Low C2 and C4 levels are seen, even between attacks.
- **Serum C4 is the most reliable and widely used screening tool**
- Angioedema does not readily cause a rise in mast cell tryptase.

Features

- Painless, **non-pruritic** swelling of subcutaneous/submucosal tissues
 - ⇒ urticaria is not usually a feature
 - ⇒ attacks may be preceded by painful macular rash
- May affect upper airways, skin, genital or abdominal organs (can occasionally present as abdominal pain and vomiting due to visceral oedema)
- Triggers include stress, infection and menstruation

Management

- Acute:** **IV C1-inhibitor concentrate** (1000-1500 units given intravenously over 20-30 min),
 - ⇒ fresh frozen plasma (FFP) if this is not available
- Prophylaxis:**
 - ⇒ Anabolic steroid, synthetic androgen: Danazol may help
 - ⇒ Aminocaproic acid

Complication

- If treatment fails to normalise the C4 levels and they remain persistently low, these patients are at an **increased risk of developing SLE**.

Other Causes of angioedema

- Bradykinin-mediated angioedema**
 - ⇒ Hereditary angioedema (inherited C1 inhibitor deficiency)
 - ⇒ Acquired angioedema (acquired C1 inhibitor deficiency)
 - Often associated with lymphoproliferative diseases and B-cell malignancies
 - ⇒ ACE inhibitor-induced (rarely ARB-induced): impaired bradykinin breakdown
 - Can occur within days to 2 years after starting ACE inhibitor
- Histamine-mediated angioedema (mast cell-mediated angioedema)**
 - ⇒ Usually coexist with urticaria
 - ⇒ Salicylate- and/or aspirin-associated angioedema
 - ⇒ **Moxonidine is a centrally acting antihypertensive and is associated with angioedema**
- Idiopathic angioedema:** Possible triggers: cold, heat, stress, and exercise

Granulomatous inflammation

Definition

- A pattern of chronic inflammation. Can be induced by persistent T-cell response to certain infections (eg, TB), immune-mediated diseases, and foreign bodies.
- A granuloma is a collection of macrophages: giant cells as a nidus of chronic inflammation

Mechanism

- Macrophages** →↑cytokine secretion (eg, TNF) → formation of epithelioid macrophages and giant cells

Types of granuloma and causes

- Caseating granulomas**
 - ⇒ Granulomas with central necrosis
 - ⇒ Found in **infections** e.g., tuberculosis, fungal infections, **tertiary syphilis**, *Bartonella henselae* (cat scratch disease)
- Noncaseating granulomatous inflammation**
 - ⇒ Granulomas without central necrosis
 - ⇒ Found in **immune-mediated diseases** (e.g., sarcoidosis, Crohn disease), sarcoidosis, vasculitis, and foreign body exposure

TNF-α is important for maintaining the granuloma. It is essential to test patients for latent TB before initiating anti-TNF therapy because the drug causes breakdown of the granuloma and can result in disseminated TB.

Third edition

Notes & Notes

For MRCP part 1 & 11

By

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Basic sciences

Genetics

Updated

2022

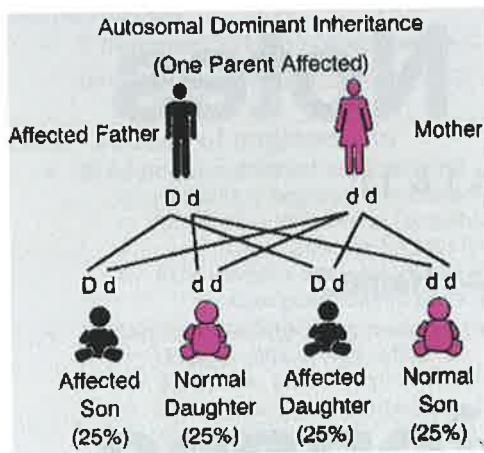
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Autosomal dominant conditions

Autosomal recessive conditions are often thought to be 'metabolic' as opposed to autosomal dominant conditions being 'structural', notable exceptions:

- some 'metabolic' conditions such as Hunter's and G6PD are X-linked recessive whilst others such as hyperlipidaemia type II and hypokalaemic periodic paralysis are autosomal dominant
- some 'structural' conditions such as ataxia telangiectasia and Friedreich's ataxia are autosomal recessive



The following conditions are **autosomal dominant**:

- **Achondroplasia**
- Acute intermittent porphyria
- Adult polycystic disease
- Antithrombin III deficiency
- Ehlers-Danlos syndrome
- **Familial adenomatous polyposis**
- Hereditary haemorrhagic telangiectasia
- Hereditary spherocytosis
- Hereditary non-polyposis colorectal carcinoma
- Huntington's disease
- Hyperlipidaemia type II
- Hypokalaemic periodic paralysis
- Malignant hyperthermia
- Marfan's syndromes
- Myotonic dystrophy
- Neurofibromatosis
- **Noonan syndrome**
- Osteogenesis imperfecta
- Peutz-Jeghers syndrome
- Retinoblastoma
- Romano-Ward syndrome
- **Tuberous sclerosis**
- Von Hippel-Lindau syndrome
- Von Willebrand's disease*

As an autosomal dominant condition, **two affected parents** can expect:

- **1 in 4 chance of an unaffected child**
- 1 in 2 chance of an affected heterozygous child
- 1 in 4 chance of an affected homozygous child.

Which disease demonstrates autosomal co-dominant inheritance?

→ **Alpha-1-antitrypsin deficiency**

*type 3 von Willebrand's disease (most severe form) is inherited as an autosomal recessive trait. Around 80% of patients have type 1 disease

Achondroplasia

Aetiology

- **Mutation in fibroblast growth factor receptor 3 gene (FGFR3) → reduced endochondral ossification**
 - ⇒ activation of fibroblast growth factor receptor 3 on chromosome 4, resulting in inhibited chondrocyte proliferation.
- **autosomal dominant**
- The homozygous form is usually fatal.

Epidemiology

- Most common type of skeletal dysplasia and disproportionate short stature (1:40,000 children worldwide affected)

Risk factor

- The incidence **increases with paternal age**.

Pathophysiology

- Epiphyseal growth cartilage fails,
- there is **normal bone formation and repair**.
 - ⇒ Therefore, **NO increased risk of fracture**.

Features

becomes obvious within the first year with disparity between a large skull, normal trunk length and short limbs.

- short stature
- short limbs (rhizomelia) with shortened fingers (brachydactyly)
 - ⇒ The fingertips may only come down to the iliac crest, and the shortness of the limbs is often most marked proximally.
 - ⇒ short stature due to shortening of the limbs, but spinal length is maintained.
 - ⇒ The limbs appear broad with deep creases.
- large head (Macrocephaly) with frontal bossing
- midface hypoplasia with a flattened nasal bridge
- 'trident' hands
- lumbar lordosis
- Normal intelligence

Complications

- Small foramen magnum → compression of the cervical medulla
- Spinal canal stenosis and radiculopathy (of the lower back)
 - ⇒ low back and leg pain,
 - ⇒ paresthesias, dysesthesia,
 - ⇒ incontinence
- Secondary scoliosis
- **Recurrent otitis media**
- Cardiopulmonary complications (due to a small chest wall)

Diagnostics

- **X-ray**
 - ⇒ **It may be diagnosed radiographically at birth**,
 - ⇒ Lateral skull
 - midface hypoplasia,
 - frontal prominence
 - ⇒ pelvis

- narrow in anteroposterior diameter with deep sacroiliac notches and short iliac wings.
- ⇒ Spine
 - progressive narrowing of the interpedicular distance from top to bottom (reverse of normal).
 - ❖ abnormally narrow interpedicular distance → spinal canal stenosis; scoliosis
- ⇒ Extremities
 - bones are short and broad;
 - short fingers
 - metaphyseal irregularity,
 - flaring in the long bones,
 - late-appearing irregular epiphyses.

Management

- medical
 - ⇒ Early administration of **growth hormone** (1–6 years)
- Surgical corrections:
 - ⇒ spinal stenosis, secondary scoliosis, genu varum, foramen magnum decompression

Osteogenesis imperfecta ("brittle bone disease")

Pathophysiology

- **Autosomal dominant mutation** in **COL1A1** or **COL1A2** genes → ↓ synthesis of normal type I collagen → impaired bone matrix formation (osteogenesis)

Features

- Growth retardation
- Skeletal deformities, brittle bones, and recurrent fractures from minimal trauma
- Blue sclerae due to visible choroidal pigment.
- Progressive hearing loss secondary to otosclerosis
- Brittle, opalescent teeth (dental imperfections) due to a lack of dentin formation.

Types

- type I: The most common, and milder form.
- Type II: most severe form; lethal perinatally or within the first year

Diagnostics

- DNA test
- Ultrasonography before birth and radiographic skeletal survey afterwards (fractures, callus, deformities)
- **Bone or skin biopsy** → type 1 collagen mutation

Therapy

- No cure available
- Bisphosphonates; decrease the risk of fractures
- Surgery for functional improvement

Individuals with osteogenesis imperfecta can't **BITE**: Bones (recurrent fractures), I ("eye" = blue sclerae), Teeth (dental abnormalities), Ears (hearing loss).

MRCPUK-part-1-May-2009 exam: A pregnant woman is known to have polycystic kidney disease. What is the chance her child will also have the disease?

→ 50% (Polycystic kidney disease is usually inherited in an autosomal dominant fashion and hence 50% of her children will be affected, regardless of gender)

Down's syndrome (trisomy 21)

Epidemiology and genetics

- the most common autosomal abnormality

Risk of Down's syndrome with increasing maternal age

Age (years)	Risk
20	1 in 1,500
30	1 in 800
35	1 in 270
40	1 in 100
45	1 in 50 or greater

One way of remembering this is by starting at 1/1,000 at 30 years and then dividing the denominator by 3 (i.e. 3 times more common) for every extra 5 years of age

Cytogenetics

Mode	% of cases	Risk of recurrence
Non-disjunction	94%	1 in 100 if under mother < 35 years
Robertsonian translocation (usually onto 14)	5%	10-15% if mother is translocation carrier 2.5% if father is translocation carrier
Mosaicism	1%	

- The chance of a further child with Down's syndrome is approximately 1 in 100 if the mother is less than 35 years old. If the trisomy 21 is a result of a translocation the risk is much higher.
- Down syndrome have one of the two karyotypes:
 - 47,XX,+21 (trisomy 21): more common
 - 46,XY,der(14;21): characterized by the presence of two normal chromosomes 21, one normal chromosome 14 and a product of Robertsonian translocation between chromosomes 14 and 21 (der(14;21); der stands for derivative).

The general risk of trisomy 21 increases with maternal age. This does not, however, apply to translocation trisomies

Features

- face: upslanting palpebral fissures, epicanthic folds, Brushfield spots in iris, protruding tongue, small ears, round/flat face
- flat occiput
- single palmar crease, pronounced 'sandal gap' between big and first toe
- hypotonia
- congenital heart defects (40-50%, see below)
- duodenal atresia **can be diagnosed by U/S at gestation → double bubble sign**
- Hirschsprung's disease

Associations

- ↑ risk for developing acute myeloid leukemia (AML)** (approximately 1-2% of children with Down syndrome develop AML, the great majority < 5 y) rather than acute lymphoblastic leukemia (ALL), which is a more common form of leukemia in children.
- Other haematological disorders associated with Down's syndrome include:
 - ⇒ Fanconi's anaemia,
 - ⇒ Patients with learning disabilities may be prone to lead poisoning due to pica.

Cardiac complications

- 50% of children with Down's syndrome have a cardiac defect.
- multiple cardiac problems may be present
- endocardial cushion defect (c. 40%, also known as atrioventricular septal canal defects)**
- ventricular septal defect (c. 30%)
- secundum atrial septal defect (c. 10%)
- tetralogy of Fallot (c. 5%)
- isolated patent ductus arteriosus (c. 5%)

Later complications

- subfertility:
 - ⇒ Males are almost always infertile due to impaired spermatogenesis.
 - ⇒ **Females are usually subfertile**, and have an increased incidence of problems with pregnancy and labour
- learning difficulties
- short stature
- repeated respiratory infections (+hearing impairment from glue ear)
- acute lymphoblastic leukaemia
- hypothyroidism
- Alzheimer's
- atlantoaxial instability

To remember the most important features associated with Down syndrome, think of the 5 A's: Advanced maternal age, duodenal Atresia, Atrioventricular septal defect, AML/ALL, early onset of Alzheimer disease.

Diagnosis

Screening tests (Prenatal)

- Combined test** (first trimester) (11–13 weeks)
 - ⇒ Maternal serum
 - ↑ Beta human chorionic gonadotropin (β -hCG)
 - ↓ Pregnancy-associated plasma protein A (PAPP-A)

- ⇒ Ultrasound
 - **Nuchal translucency; increases** due to the large amount of fluid collecting behind the neck
 - Short neck, thickened nuchal fold
 - Absent or hypoplastic nasal bone
 - Shortened middle phalanges of the fifth digits with clinodactyly
 - Shortened long bones (humerus, femur)
- **Quadruple test** (second trimester) (15–18 weeks)
 - ⇒ ↓ Free estriol
 - ⇒ ↓ Alpha-fetoprotein (AFP)
 - ⇒ ↑ Inhibin A
 - ⇒ ↑ β-hCG

Diagnostic tests (confirmatory test)

- Prenatal → Fetal karyotyping
 - ⇒ Chorionic villus sampling (9–14 weeks)
 - ⇒ Amniocentesis (15–22 weeks)
 - ⇒ Percutaneous umbilical cord sampling (18–22 weeks)
- Postnatal → Chromosome analysis

In the quadruple test, hCG and Inhibin A are both High up (\uparrow) and Estriol and α-fetoprotein are both deficient (\downarrow).

Noonan's syndrome

Overview

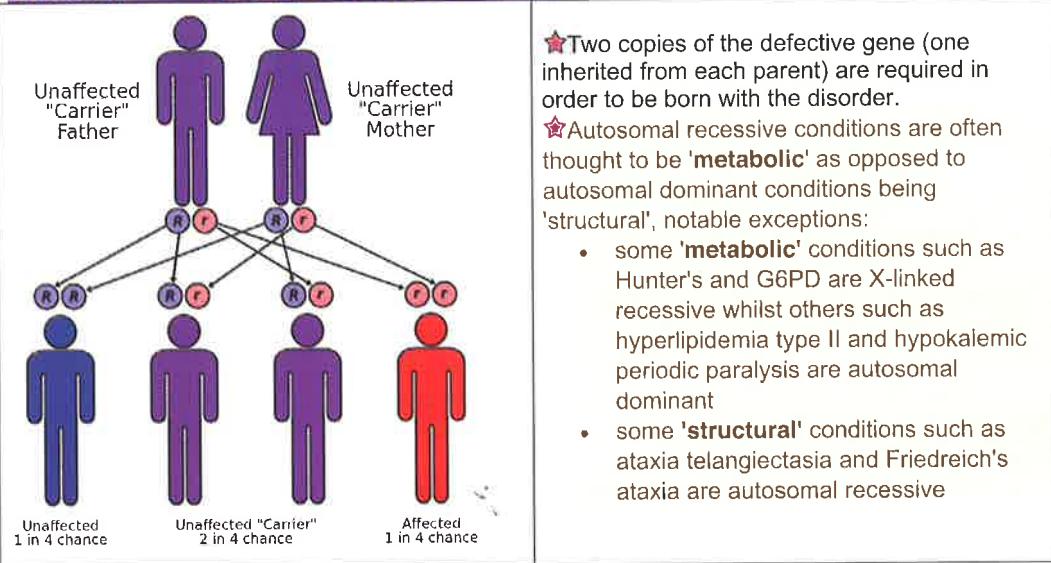
- Relatively common, autosomal-dominant inherited disorder.
- Caused by activating mutations in multiple genes in the Ras/mitogen-activated protein kinase (RAS-MAPK pathway).
- The most commonly implicated gene is PTPN11, on chromosome 12
- Often thought of as the 'male Turner's',
- In contrast to Turner's syndrome, **the karyotype is normal**
- The majority of patients lead normal lives

Feature

- features similar to Turner's syndrome:
 - ⇒ short stature,
 - ⇒ webbed neck,
 - ⇒ chest (pectus) deformity
 - widely-spaced nipples,
 - pectus carinatum and excavatum,
- characteristic features:
 - ⇒ cardiac: (occurs in 50% to 80%)
 - **typically, pulmonary valve stenosis**
 - **atrial septal defect (ASD)**
 - occasionally hypertrophic cardiomyopathy
 - ⇒ easy bruising or bleeding (due to coagulation factor deficiency or platelet dysfunction),
 - coagulation problems: factor XI deficiencies
 - ⇒ facial features,
 - triangular-shaped face
 - hypertelorism (increased distance between the eyes)

- downslanting eyes
 - vivid blue or blue-green irides
 - low-set, posteriorly rotated ears
 - ptosis
- ⇒ Boys frequently present with cryptorchidism and manifest delayed puberty.
- ⇒ learning disabilities,
- Mild cognitive impairment is found in up to 33%
 - Intellectual development may be delayed, but by adulthood intelligence is normal in 2/3 of patients.

Autosomal recessive conditions



★ Two copies of the defective gene (one inherited from each parent) are required in order to be born with the disorder.

★ Autosomal recessive conditions are often thought to be '**metabolic**' as opposed to autosomal dominant conditions being '**structural**', notable exceptions:

- some '**metabolic**' conditions such as Hunter's and G6PD are X-linked recessive whilst others such as hyperlipidemia type II and hypokalaemic periodic paralysis are autosomal dominant
- some '**structural**' conditions such as ataxia telangiectasia and Friedreich's ataxia are autosomal recessive

Autosomal recessive conditions are '**metabolic**' - exceptions: inherited ataxias
Autosomal dominant conditions are '**structural**' - exceptions: hyperlipidaemia type II, hypokalaemic periodic paralysis

The following conditions are **autosomal recessive**:

- | | |
|---|---|
| <ul style="list-style-type: none"> • Albinism • Ataxia telangiectasia • Congenital adrenal hyperplasia • Cystic fibrosis • Cystinuria • Familial Mediterranean Fever • Fanconi anaemia • Friedreich's ataxia • Glycogen storage disease | <ul style="list-style-type: none"> • Haemochromatosis • Homocystinuria • Lipid storage disease: Tay-Sach's, Gaucher, Niemann-Pick • Mucopolysaccharidoses: Hurler's • PKU • Sickle cell anaemia • Thalassaemias • Wilson's disease. |
|---|---|

MRCPUK-part-1-May 2012 exam: A man diagnosed as having hereditary haemochromatosis. His wife is not a carrier. What is the chance his child will develop haemochromatosis?

→ 0% (Haemochromatosis is an autosomal recessive condition. If one of the parents has haemochromatosis (i.e. is homozygous) and the other is not a carrier/affected, then all the children will inherit one copy of the gene from the affected parent and hence will be carriers)

Ehlers–Danlos syndrome (EDS)

The classic presentation of EDS involves hyperextensible skin, joint hypermobility, and a tendency to bleed easily.

- Ehlers–Danlos syndrome is a disorder of faulty collagen synthesis most commonly affecting collagen type III and V.
- Inheritance patterns and type of collagen affected vary (can be autosomal dominant or recessive)
- Collagen deficiencies in Ehlers–Danlos syndrome are often caused by problems with cross-linking.
- **Hypermobile Ehlers–Danlos syndrome** (EDS) is the **most common** of 13 subtypes.
 - ⇒ Most hypermobile people are not aware of the fact and assume that everyone is as flexible as they are.
 - ⇒ Most cases of hypermobile EDS, are inherited in an autosomal dominant manner.
 - ⇒ associated with hypermobile joints, but **skin features are much less prominent**
 - ⇒ Systemic features may include increased propensity to asthma, mild valve regurgitation and gastrointestinal (GI) symptoms, including constipation and hiatus hernia.
- The **most severe** form of Ehlers–Danlos syndrome is the **vascular type**.
 - ⇒ deficiencies in type **III** collagen:
 - Type III collagen also known as reticulin, and is found primarily in granulation tissue, artery walls, skin, intestines and the uterus.
 - ⇒ involves vascular and organ rupture due to type III collagen deficiency.
- The **classical type** of Ehlers–Danlos syndrome has deficiencies in **type V collagen**.
 - ⇒ in which joint and **skin manifestations predominate**
 - associated with much **more severe dermatological features**, including hyperelastic skin that splits easily and marked propensity to bruising.
- **Kyphoscoliotic EDS is usually inherited in autosomal recessive fashion.**

Features

Cardiovascular	<ul style="list-style-type: none"> • Features of heart valve defects (particularly mitral valve prolapse) • Features of aneurysms/dissections of the iliac, splenic, renal arteries, or the aorta • Berry/saccular aneurysms of the cerebral arteries → features of subarachnoid hemorrhage
Musculoskeletal	<ul style="list-style-type: none"> • Joint hypermobility with tendency to dislocate • Skeletal abnormalities (e.g., scoliosis) • features of chronic pain syndrome and marfanoid habitus
Skin	<ul style="list-style-type: none"> • Tendency to bruise easily • Skin hyperextensibility • Frequent skin lacerations and poor skin healing (e.g., following surgery)
Other	<ul style="list-style-type: none"> • Hernias • Features of organ rupture (e.g., shock, local pain), especially in vascular EDS



Elbow region of a female patient of Ehlers-Danlos syndrome: The skin of the elbow is hyperelastic (*cutis hyperelastica*), but rapidly returns to its initial position when released.



Diagnosis

- Definitive diagnosis for all subtypes of EDS, except hypermobile EDS, can be made by molecular genetic testing.
- The genetic basis of hypermobile EDS remains unknown and the diagnosis is made by clinical criteria only.
- A baseline echocardiogram with views of the aortic arch and aorta and regular reevaluations should be obtained to evaluate for mitral valve prolapse and any signs of aortic enlargement.

Prognosis

- Life expectancy is typically normal with the exception of vascular EDS, which has a reduced life expectancy of ~ 50 years.

Pseudoxanthoma elasticum (PXE)

- inherited condition (**usually autosomal recessive***) connective tissue disorder involves the elastic fibres of the eye, skin and cardiovascular system.
 - *there are reports of autosomal dominant inheritance in a minority of cases
- caused by mutations in the ABCC6 gene → lack of functional ABCC6 protein leads to ectopic mineralization that is most apparent in the elastic tissues of the skin, eyes and blood vessels.

Features

- Eye**
 - retinal angiod streaks
 - due to dystrophic calcification of Bruch's membrane
 - Visual loss can occur by infarction of the visual pathways and is likely to explain the chronic changes of optic disc atrophy
- Skin**
 - 'plucked chicken skin' appearance - small yellow papules on the neck, antecubital fossa and axillae
 - The first clinical sign
- Cardiac**
 - mitral valve prolapse,
 - increased risk of ischaemic heart disease
 - Due to loss of elastic tissue, patients have an increased incidence of mitral regurgitation, aortic regurgitation and aortic dissection.

- **Gastrointestinal haemorrhage**

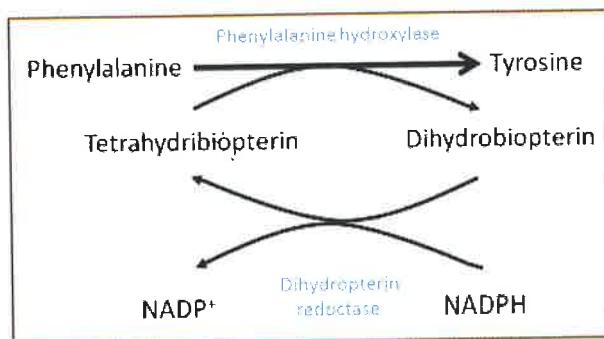
- **CNS**

- ⇒ Cerebral ischaemia in PXE is caused by small vessel occlusive disease.
- ⇒ Intracranial aneurysms
- ⇒ Subarachnoid and intracerebral haemorrhages
- ⇒ Progressive intellectual deterioration
- ⇒ Mental disturbances, and
- ⇒ Seizures.

Phenylketonuria (PKU)

Overview

- Autosomal recessive condition
- Caused by a disorder of phenylalanine (an essential amino acid) metabolism.
 - ⇒ usually **due to defect in phenylalanine hydroxylase**, an enzyme which converts phenylalanine to tyrosine .
 - ⇒ In a small number of cases the underlying defect is a deficiency of the tetrahydrobiopterin-deficient cofactor, e.g. secondary to defective dihydrobiopterin reductase.
- The gene for phenylalanine hydroxylase is located on chromosome 12.
- The incidence of PKU is around 1 in 10,000 live births.
- High levels of phenylalanine lead to problems such as learning difficulties and seizures.



- The sequence of phenylalanine metabolism is the following: phenylalanine → tyrosine → L-Dopa → dopamine → norepinephrine → epinephrine.
 - ⇒ **the neurological symptoms are most likely caused by a reduction in which neurotransmitters?**
 - Norepinephrine

Features

- usually presents by 6 months e.g. with developmental delay, seizures, typically infantile spasms
- child classically has fair hair and **blue eyes**
- learning difficulties. **Even with dietary treatment some degree of cognitive impairment is seen**
- Microcephaly, prominent maxilla, growth retardation and wide-spaced teeth are found in untreated children.

- Eczema
- partial albinism due to decreased tyrosine production.
- '**musty' odour**' to urine and sweat secondary to phenylacetate, a phenylketone

Diagnosis

- Diagnosis of classic PKU requires raised Phe levels, **increased urinary Phe metabolites** and normal cofactor (tetrahydrobiopterin) concentrations.
 - ⇒ plasma levels of tyrosine are difficult to measure, and have diurnal variation. Whilst the levels are often low in patients with PKU, the levels can be normal depending on what time of the day the sample is taken and whether or not the patients are being treated.
- Guthrie test: the 'heel-prick' test done at 5-9 days of life - also looks for other biochemical disorders such as hypothyroidism
- hyperphenylalaninaemia
- phenylpyruvic acid in urine

Management

- **Low phenylalanine and high tyrosine diet**

Prognosis

- Excellent with normal life expectancy diagnosed early and blood phenylalanine (phe) levels remain within the therapeutic range.

Alkaptonuria

The **black** discolouration of sclera and urine becoming **black** on standing should alert you to the likelihood of **Alkaptonuria**.

Pathophysiology

- Autosomal recessive disorder of phenylalanine and tyrosine metabolism
- **Caused by a deficiency of homogentisic acid oxidase** responsible for the degradation of homogentisic acid produced from phenylalanine and tyrosine.
- Accumulation of homogentisic acid causes pigmentation of the urine, sclera and connective tissues.
- Alkaptonuria is generally a benign and often asymptomatic condition.

Features

- Pigmented sclera
- **Urine turns black if left exposed to the air**
- Deposition in the joints causes cartilage pigmentation (ochronosis) and degeneration.
 - ⇒ **Patients develop arthritis at 40 years of age.**
 - ⇒ intervertebral disc calcification may result in back pain
 - ⇒ The knees and spine are commonly affected.
 - ⇒ The sacroiliac joint may be spared.
- Renal stones
- Homogentisic acid is a reducing agent, therefore it gives a false **positive Glucostix test** but normal Clinitest.

Treatment

- High-dose vitamin C
- Dietary restriction of phenylalanine and tyrosine

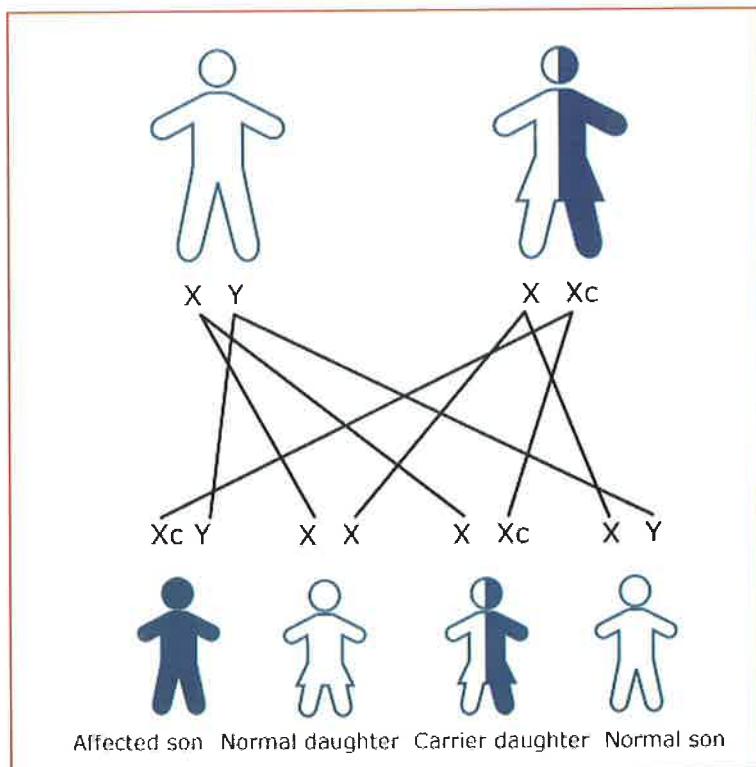
X-linked recessive

X-linked recessive conditions - there is no male-to-male transmission. Affected males can only have unaffected sons and carrier daughters.

X-linked conditions: Duchenne/Becker, haemophilia, G6PD

- The abnormal gene is carried on the X chromosome, and in the carrier female, the normal allele on her other X chromosome protects her from the disease. Since the male does not have this protection, he manifests the disease.
- only males are affected. An exception to this seen in examinations are **patients with Turner's syndrome, who are affected due to only having one X chromosome.**
- Females only occasionally show mild sign of disease
- X-linked recessive disorders are transmitted by heterozygote females (carriers) and male-to-male transmission is not seen.
- **Affected males can only have unaffected sons and carrier daughters.**
- heterozygous female carrier →
 - 50% of male children are affected
 - 50% of female children are carrier
- The possibility of an affected father having children with a heterozygous female carrier is generally speaking extremely rare. However, in certain Afro-Caribbean communities G6PD deficiency is relatively common and homozygous females with clinical manifestations of the enzyme defect are seen.
- **Many of the inherited eye disorders such as retinitis pigmentosa and ocular albinism are inherited in an x-linked recessive pattern.**
- **The following conditions are inherited in a X-linked recessive fashion:**

⇒ Androgen insensitivity syndrome ⇒ Becker muscular dystrophy ⇒ Colour blindness ⇒ Duchenne muscular dystrophy ⇒ Fabry's disease ⇒ G6PD deficiency ⇒ Haemophilia A,B	⇒ Hunter's disease ⇒ Lesch-Nyhan syndrome ⇒ Nephrogenic diabetes insipidus ⇒ Ocular albinism ⇒ Retinitis pigmentosa ⇒ Wiskott-Aldrich syndrome ⇒ Fragile X syndrome
--	---
- **The following diseases have varying patterns of inheritance, with the majority being in an X-linked recessive fashion:**
 - ⇒ Chronic granulomatous disease (in > 70%)



What is the most common genetic disorder?

⇒ **Sex-linked disorder**

- The most common genetic disorder is actually a relatively minor one, red-green colour blindness, which is seen in 2–4% of men.
- Other examples of more significant sex-linked disorders include haemophilia A and B.

X-linked dominant disorders

- ➡ No carrier (the carrier of a defective gene associated with a disorder, will have the disorder)
- ➡ affected woman
 - Half of the daughters and sons are affected
 - male will have worse symptoms than female (because women carry two X)
- ➡ affected father → all his daughters are affected but none of his sons.

- The gene responsible for a genetic disorder is located on the X chromosome, and only one copy of the allele is sufficient to cause the disorder when inherited from a parent who has the disorder.
- X linked dominant disorders are rare (for example, **vitamin D-resistant rickets**).
- They affect both sexes but females **more than males**.

- ⇒ Males can only get an X chromosome from their mother whilst females get an X chromosome from both parents. As a result, females tend to show higher prevalence of X-linked dominant disorders because they have more of a chance to inherit a faulty X chromosome.
- Homozygous mother → All children are affected.
- An affected mother with the trait → half the sons and half the daughters inherit the disorder
- when the mother alone is the carrier ; she herself will have the disorder,
 - ⇒ 50% Of her daughters and sons will have the disorder,
 - ⇒ 50% will be unaffected.
- **Affected females will transmit the condition to 50% of their children, whether male or female.**
- When the father alone is the carrier of a defective gene associated with a disorder, he too will have the disorder.
 - ⇒ 100% Of his daughters will have the disorder, since all of his daughters will receive one copy of his single X chromosome.
 - ⇒ none of his sons will have the disorder; sons do not receive an X chromosome from their father.
 - ⇒ **affected father → all his daughters are affected but none of his sons.**

Vitamin D-resistant rickets

Overview

- Vitamin D-resistant rickets is a **X-linked dominant** condition
 - ⇒ affected female will transmit the disease to 50% of her sons and 50% of her daughters.
 - ⇒ affected male will transmit the condition to all of his daughters but none of his sons.
- usually presents in infancy with failure to thrive.
- **caused by impaired phosphate reabsorption in the renal tubules**

Features

- failure to thrive
- normal serum calcium, **low phosphate**, elevated alkaline phosphatase
- x-ray changes: cupped metaphyses with widening of the epiphyses

Diagnosis

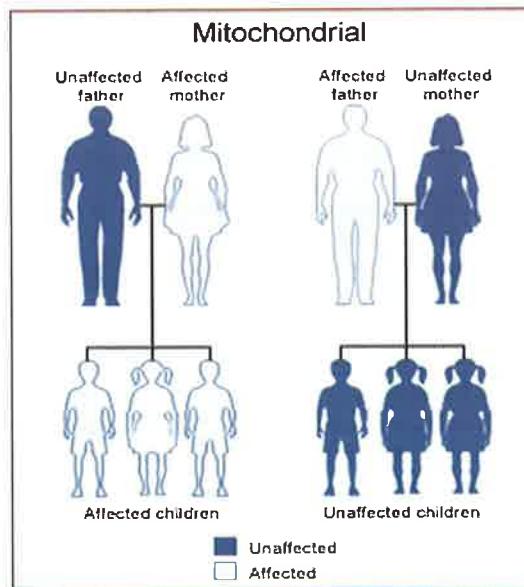
- made by demonstrating **increased urinary phosphate**

Management

- high-dose vitamin D supplements
- oral phosphate supplements

Mitochondrial diseases

Mitochondrial diseases follow a maternal inheritance pattern



- Whilst most DNA is found in the cell nucleus, a small amount of double-stranded DNA is present in the mitochondria. It encodes protein components of the respiratory chain and some special types of RNA

Characteristics: Mitochondrial inheritance has the following characteristics:

- inheritance is only via the maternal line as the sperm contributes no cytoplasm to the zygote
- all children of affected males will not inherit the disease
- all children of affected females will inherit it
- generally, encode rare neurological diseases
- poor genotype: phenotype correlation** - within a tissue or cell there can be different mitochondrial populations - this is known as heteroplasmy)

Histology

- muscle biopsy classically shows '**red, ragged fibres**' due to increased number of mitochondria

Examples

- Leber's optic atrophy**
 - ⇒ Cyanocobalamin (a form of B12) should be avoided as it may lead to blindness in Leber's disease patients.
- MELAS syndrome:** mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes
- MERRF syndrome: myoclonus epilepsy with ragged-red fibres**
 - ⇒ generalised myoclonus (60%),

- ⇒ epilepsy,
- ⇒ optic atrophy (20%),
- ⇒ short stature (10%),
- ⇒ ataxia,
- ⇒ cognitive decline
- ⇒ encephalopathy (EEG findings of generalised slow waves)
- ⇒ sensorineural hearing loss
- ⇒ impaired glucose tolerance.
- sensorineural hearing loss

Myoclonic epilepsy with ragged red fibres (MERRF)

A young patient presenting with cognitive impairment developing after a period of normal development, seizures, myoclonic jerks, Wolff-Parkinson White syndrome and worsening vision (consistent with optic atrophy).

Diagnosis → **(MERRF)**, which is a mitochondrial DNA disorder **diagnosed by → ragged red fibres on muscle biopsy.**

Kearns-Sayre syndrome

Kearns-Sayre syndrome:

- **Mitochondrial inheritance**
- **Onset < 20-years-old**
- **Triad of:**
 - ⇒ **External ophthalmoplegia**
 - ⇒ **Retinitis pigmentosa and**
 - ⇒ **Heart block.**

Overview

- **mitochondrial** DNA mutation.
- onset in patients < 20 years old

Features

- **external ophthalmoplegia → Ptosis**
- **retinitis pigmentosa**
- **heart conduction defect**
- sensorineural hearing loss is almost universal in those who survive into the fourth decade of life; this may not be fully corrected with hearing aids.
- Other associated features:
 - ⇒ cerebellar ataxia,
 - ⇒ raised cerebrospinal fluid (CSF) proteins,
 - ⇒ proximal myopathy.
 - ⇒ short stature
 - ⇒ **multiple endocrinopathies** including diabetes mellitus, hypoparathyroidism, and Addison disease.

Diagnosis

- Muscle biopsy may reveal ragged red fibers.
- Muscle histochemistry reveals deficiency of cytochrome c oxidase (mitochondrial respiratory chain enzyme).

Prognosis

- Patients rarely live beyond their 40s and there are no therapeutics currently available.

Kallman's syndrome

Kallman's – LH & FSH low – normal

Klinefelter's – LH & FSH – raised

Overview

- Kallman's syndrome is a recognised cause of delayed puberty secondary to hypogonadotrophic hypogonadism.
- It is usually inherited as an **X-linked recessive** trait.
- Caused by failure of GnRH-secreting neurons to migrate to the hypothalamus → gonadotrophin releasing hormone (GnRH) deficiency
- May arise due to abnormalities of the KAL-1 or KAL-2 gene (encoding anosmin-1 and FGF-1).
- There is isolated gonadotrophic deficiency (may be evidenced by a normal prolactin).
- The clue given in many questions is lack of smell (anosmia) in a boy with delayed puberty

Incidence

- 1 in 10,000 males
- More common in men: male to female ratio of 4:1.

Features

- **Hypogonadotrophic hypogonadism**
 - ⇒ Sex hormone levels are low
 - ⇒ LH, FSH levels are inappropriately low/normal
 - ⇒ Lack of development of secondary sexual characteristics
 - ⇒ Primary amenorrhoea.
- **Infertility**
 - ⇒ In male individuals: cryptorchidism and low sperm count
 - ⇒ In female individuals: primary amenorrhea
- **Cryptorchidism (Cryptorchidism is more suggestive of Kallman's than Klinefelter's syndrome)**
 - ⇒ Cryptorchidism is the absence of one or both testes from the scrotum (undescended testis).
- **Anosmia** present in 75% (**Lack sense of smell**) due to failure of the olfactory bulb to develop, leading to loss of gonadotropin releasing hormones.
- Patients are typically of normal or above average height
- **No mental retardation**
- **Delayed puberty:** (e.g., absent thelarche in female individuals, decreased growth spurt)

- Associated disorders
 - ⇒ Renal agenesis
 - ⇒ Cleft lip/cleft palate
 - ⇒ Visual defects : colour blindness
 - ⇒ Deafness

Diagnosis

- Diagnostic test → Fluorescent in situ hybridisation (FISH) is currently the best means of a genetic diagnosis
- Absent olfactory bulbs are present on 75% of MRI scans in these patients.
 - ⇒ The appearance on cerebral MRI → Absent olfactory bulbs

Treatment

- For a male who begin a relationship with a woman
 - ⇒ Pulsed (NOT Continuous) GnRH treatment is needed to restore LH and FSH release.
 - It needs to be continued for as long as fertility is required.
 - As natural GnRH release is pulsatile, continuous therapy fails to lead to LH and FSH release.
 - Once his family is complete, switching to testosterone therapy may be more convenient for him.
 - Although Testosterone supplementation will restores secondary sexual characteristics, it doesn't restore fertility and is therefore not appropriate here.
 - FSH can be used to induce fertility, but it is less effective than pulsed GnRH therapy.
- If fertility is not required, there is no need to stimulate spermatogenesis with (GnRH) or gonadotropins; only testosterone replacement is required.
- LH can be used in conjunction with FSH to induce fertility in women with Kallmann syndrome.
- For a woman who wants to start a family:
 - ⇒ HCG to drive production of gonadal steroid hormones, FSH to drive ovulation, harvesting of eggs, and IVF. This process is most effective in achieving successful pregnancy.

Klinefelter's syndrome

Klinefelter's? - do a karyotype

Overview

- Klinefelter's syndrome is associated with male phenotype and **47, XXY karyotype**
- the commonest form of which is XXY, is the result of chromosomal non-disjunction; as such, it does not follow a mendelian pattern of inheritance.

- it is the most common chromosomal disorder associated with male hypogonadism and infertility.
- Incidence: between **1 in 500** and 1 in 1000.
- The rate of chromosomal non-dysjunction increases with increasing maternal age and increasing paternal age, each parent contributing 50% of the risk. Around 60% of Klinefelter cases do not survive the fetal period.
- **has no specific genetic pattern of inheritance**
 ↳ **chances of inheriting the disorder → < 1%**

Features

- often taller than average
- lack of secondary sexual characteristics
- small, firm testes
- infertile, azoospermia
- gynaecomastia
 ↳ **increased incidence of breast cancer** (20 times higher than a normal male).
- elevated gonadotrophin levels ($\uparrow\uparrow LH/FSH$) due to testicular failure
 - ↳ Leydig cell dysfunction $\rightarrow \downarrow$ testosterone $\rightarrow \uparrow LH \rightarrow \uparrow$ estrogen.
 - ↳ dysgenesis of seminiferous tubules $\rightarrow \downarrow$ inhibin B $\rightarrow \uparrow FSH$.
- Low testosterone levels
- **Low HDL cholesterol**, elevated triglyceride ,normal or increased (LDL)
- increased cardiovascular risk due to lipid abnormality.
- decrease libido
- decrease bone mineral density \rightarrow increased risk of osteoporotic fractures.

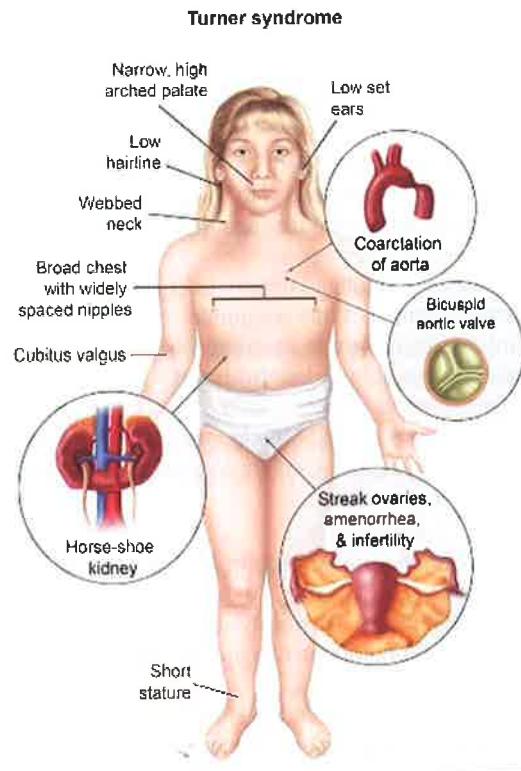
Investigation

- **Diagnosis is by chromosomal analysis**
- **the most appropriate investigation in suspected cases → FSH, LH**
 - ↳ Both FSH and LH are raised in Klinefelter syndrome, and elevation would be a strong pointer to confirming the underlying diagnosis.
 - ↳ more useful than Testosterone (wouldn't indicate whether the defect was at the level of the pituitary or the testes)

Treatment → Testosterone

- **Testosterone is known to improve bone mineralization and is the treatment of choice**

Turner's syndrome



Turner's syndrome - most common cardiac defect is bicuspid aortic valve

Overview

- affects around 1 in 2,500 females.
- caused by either the presence of only one sex chromosome (X) or a deletion of the short arm of one of the X chromosomes.
- denoted as 45,XO or 45,X**

Features

- short stature
- shield chest, widely spaced nipples
- webbed neck
- cardiac defects:
 - ⇒ **bicuspid aortic valve (15%)**,
 - ⇒ coarctation of the aorta (5-10%)
 - hypertension and systolic murmur

- **primary amenorrhoea**
- **associated absent uterus and streak ovaries**
- cystic hygroma (often diagnosed prenatally)
- high-arched palate
- short fourth metacarpal
- multiple pigmented naevi
- **keloid scars**
- lymphoedema in neonates (especially feet)
- **Horseshoe kidney** is strongly associated with Turner's syndrome
 - ⇒ often initially presents with stone disease, pelviureteric junction (PUJ) obstruction, trauma, infections and tumors.
 - ⇒ In a pediatric patient with multiple urinary tract infections or renal stones, imaging must be performed to rule out this congenital anomaly.
 - ⇒ **Which anatomical structures is responsible for horseshoe kidney anomaly during normal embryological development?**
 - ➡ Inferior mesenteric artery
 - ➡ occurs when the isthmus of the kidney becomes trapped behind the inferior mesenteric artery as the kidneys ascend during embryonic life.

Associated conditions

- autoimmune diseases:
 - ⇒ autoimmune thyroiditis (**hypothyroidism (much more common in Turner's)**)
 - ⇒ and Crohn's disease
- **Hypertension is quite common in Turner syndrome (10%) and is typically idiopathic - essential.** In a **small proportion** causes can include:
 - ⇒ coarctation of the aorta
 - ⇒ and renal dysfunction due to horsehoe kidney.
- metabolic abnormalities (dyslipidaemia and glucose intolerance)
- recurrent otitis media.
- Diabetes mellitus
 - ⇒ Although the incidence of diabetes mellitus is increased in patients with Turner syndrome, it is thought to be driven by insulin resistance and is very responsive to weight loss.

Diagnosis

- karyotype → identification of 45X0 .

Prognosis

- What condition is responsible for most of the excess mortality associated with Turner syndrome?
 - ⇒ Thoracic aortic aneurysm rupture

Marfan's syndrome

Marfan's syndrome is caused by a mutation in a protein called fibrillin-1

Overview

- **autosomal dominant** connective tissue disorder.
- caused by a defect in the fibrillin-1 gene on chromosome 15
 - ⇒ Mutation of **FBN1** that encodes **Fibrillin-1**.

- affects around 1 in 3,000 people.
- may occur as a spontaneous mutation, (1/3rd of cases), and this occurs more commonly to offspring of older males.

Features

- Skeletal
 - ⇒ tall stature with arm span to height ratio > 1.05
 - ⇒ high-arched palate
 - ⇒ arachnodactyly
 - ⇒ pectus excavatum
 - ⇒ pes planus
 - ⇒ scoliosis of > 20 degrees
 - ⇒ crowded teeth.
 - ⇒ Dural ectasia:
 - ballooning of the dural sac at the lumbosacral level
 - Dural ectasia affects around 60% of patients with Marfan's syndrome.
 - **It may cause lower back pain associated with neurological problems such as bladder and bowel dysfunction.**
 - ⇒ **ligamentous/joint laxity resulting in multiple joint dislocations**, hypermobile joints
- Heart:
 - ⇒ **Dilation of the aortic sinuses (seen in 90%)** which may lead to aortic aneurysm, aortic dissection, aortic regurgitation, mitral valve prolapse (75%).
- Lungs: repeated pneumothoraxes
- Eyes:
 - ⇒ **Upwards** lens dislocation (superotemporal ectopia lentis) seen in 50% of patients
 - ⇒ **Retinal detachment**
 - ⇒ Blue sclera, myopia, early glaucoma, and early cataracts.

Diagnosis

- Unfortunately, **DNA testing for fibrillin gene mutations, whilst helpful, cannot exclude a diagnosis of Marfan because a number of mutations exist (at least 130).**
- Hence **diagnosis is made on the major and minor features associated with the syndrome.**

Prognosis & treatment :

- The life expectancy of patients used to be around 40-50 years.
- With the advent of regular echocardiography monitoring and beta-blocker/ACE-inhibitor therapy this has improved significantly over recent years.
 - ⇒ Treatment with **β -blockers** reduces the rate of aortic dilatation and the risk of rupture
- Aortic dissection and other cardiovascular problems remain the leading cause of death however.
- Pregnancy is associated with increased risk of aortic rupture.

A mutation of which gene is most closely associated with Marfan's syndrome?

→ **FBN-1 mutation**

- FBN-1 gene mutation → Defect in fibrillin → Marfan's

Homocystinuria

Marfanoid skeletal abnormalities (tall and thin, elongated limbs, arachnodactyly) + mental retardation → Homocystinuria

Overview

- **Autosomal recessive** disease
- Caused by **deficiency of cystathione beta synthase** results in an accumulation of homocysteine
 - ⇒ cystathione beta synthase is responsible for converting homocysteine to cystathione. Cystathione is later converted to cysteine,
 - so, patients who have this enzyme deficiency **need to supplement their diets with exogenous cysteine.**
 - Levels of homocysteine and methionine accumulate

Types

- **Homocystinuria type 1** → a defect in **cystathione synthetase** is responsible.
- Homocystinuria type 2 → defects in **methylene tetrahydrofolate reductase**
 - ⇒ However, individuals with this condition rarely survive the neonatal period or, if they survive longer than this, they often have more severe mental retardation.

Features

- fine, fair hair
- musculoskeletal: may be similar to Marfan's - arachnodactyly etc
- neurological: learning difficulties, mild to moderate mental handicap ,seizures
- ocular: downwards (inferonasal) dislocation of lens
 - ⇒ The sudden visual deterioration could either be due to a thrombotic episode or to the lens dislocation associated with this condition.
- **increased risk of arterial and venous thromboembolism** (atherosclerosis, thrombosis, MI)
 - ⇒ the most common cause of death.
- malar flush,
- **livedo reticularis**

Diagnosis

- made by the **cyanide-nitroprusside test**, which is also positive in cystinuria
 - ⇒ addition of sodium nitroprusside to urine → urine changes color to an intense red
- Guthrie test is used for screening the neonates for the presence of homocystinuria.

Treatment

- Dietary modification aim to: reduce intake of methionine and increase intake of cysteine.
- vitamin B6 (pyridoxine) supplements
 - ⇒ 50% of patients respond to large doses of pyridoxine (vitamin B₆)
- Folate and vitamin B12 supplements
 - ⇒ facilitate the conversion of homocysteine to methionine.
 - ⇒ homocysteine levels (homocysteinemia) are more commonly tested in diagnosis of Vitamin B12 Deficiency.

Homocystinuria VS Marfan's

	homocystinuria	Marfan's syndrome
inheritance	autosomal recessive	autosomal dominant
lens dislocation	downward lens dislocation	upward lens dislocation
aortic incompetence	heart rarely affected	aortic incompetence may occur
intellectual development	mental retardation (nearly 50%)	normal
livedo reticularis	seen due to the venous thrombosis in the small vessels of the skin	NO
other principle features	osteoporosis , recurrent thromboembolism; characteristic laboratory features : plasma methionine and homocystine levels are elevated, homocystine is excreted in the urine, plasma cystine levels are reduced, positive urine cyanide-nitroprusside test; response to treatment with pyridoxine	flat feet, herniae, scoliosis; there is a 50% reduction in life expectancy

Fragile X syndrome

Overview

- Fragile X syndrome is a disorder affecting the methylation and expression of the fragile X mental retardation 1 gene.
- genetic inheritance → X-linked dominant with variable penetration
- Patients affected by fragile X syndrome usually have over 200 CGG trinucleotide repeats.

Features

- moderate to severe mental retardation
- prognathism
- face: (**long** face, **prominent** forehead, **large** jaw (prognathism) and **large** ears
- macro-orchidism
 - ⇒ **In post pubertal males, abnormally large testes** are a distinctive feature.
- speech delays
- double-jointedness
- autistic symptoms,
- occasional self-mutilation.
- Otitis media, strabismus, and dental problems may be present
- hyperextensible joints
- hypotonia,
- heart problems, including mitral valve prolapse.

Management

- Treatment focused on preventing common medical problems such as gastroesophageal reflux, sinusitis, and otitis media, +
- speech, occupational, and physical therapy.

Trinucleotide repeat disorders

Anticipation in trinucleotide repeat disorders = earlier onset in successive generations

Definition

- Trinucleotide repeat disorders are genetic conditions caused by an abnormal number of repeats (expansions) of a repetitive sequence of three nucleotides.
- These expansions are unstable and may enlarge which may lead to **an earlier age of onset in successive generations - a phenomenon known as anticipation**. In most cases, an increase in the severity of symptoms is also noted
- **Friedreich's ataxia is unusual in not demonstrating anticipation**

Examples (note dominance of neurological disorders):

- Fragile X (CGG)
- **Huntington's (CAG)**
- myotonic dystrophy (CTG)
 - ⇒ CTG repeats in the DMPK gene
- Friedreich's ataxia* (GAA)
 - ⇒ (*Friedreich's ataxia is unusual in not demonstrating anticipation)
- spinocerebellar ataxia
- spinobulbar muscular atrophy
- **Kennedy disease**, also known as 'X-linked bulbospinal neuronopathy'
- dentatorubral pallidoluysian atrophy

Trinucleotide repeat disorders mnemonic:

Try (trinucleotide) hunting for my fried eggs (X).

Genetic anticipation

Anticipation: successive generations present with symptoms at an earlier age

- **Definition:** The 'classic' definition of anticipation is **earlier onset** in successive generations. However, in most cases, an increase in the severity of symptoms is also noted. If both options (earlier onset and sever symptoms) are presented, then the earlier onset should be chosen
- **Example:** A man aged 33 presents with features of Huntington's disease (depression, weight loss and choreiform movements). He informs you that his father had similar symptoms aged 50, his grandfather aged 75 and both deteriorated in terms of mobility and mental state, and eventually died.
- **Occur in:**
 - ⇒ Huntington's disease
 - ⇒ Myotonic dystrophy
 - ⇒ Fragile X syndrome

Polygenic diseases

- **Definition:**
 - ⇒ genetic disorder that is caused by the **combined action of more than one gene**.
 - ⇒ Because such disorders depend on the presence of several genes, they are not inherited as simply as are single-gene diseases.
- **Examples:**
 - ⇒ hypertension,
 - ⇒ coronary heart disease,
 - ⇒ diabetes
 - ⇒ **Amyotrophic lateral sclerosis (ALS)**

Lysosomal storage diseases

Definition

- Lysosomal storage diseases are a group of inherited metabolic disorders caused by a deficiency of specific enzymes. This causes an accumulation of abnormal substances that are usually degraded within lysosomes, resulting in cell damage and death.

Risk factors

- Ashkenazi ethnicity
- Male sex
 - ⇒ Fabry's disease is X-linked, but heterozygous females typically (>75%) do have symptoms, although less severe, more variable in expression, and at a later age of onset.

Key features

- Hyperacusis → Characteristic of Tay-Sachs disease.
- Optic atrophy or retinitis pigmentosa are seen in juvenile form of Tay-Sachs disease.
- hx of renal failure → Found in adult Fabry's disease.
- Hepatosplenomegaly → common in Gaucher's disease
- onset in adulthood (Fabry's, Gaucher's type 1, Pompe's)

Diagnosis

- **Enzyme assay (1st investigations to order)**

Gaucher's disease

Gaucher

- **Glucocerebrosidase deficiency**
- **Glucocerebroside accumulation**

Features involve multiple systems: Blood (pancytopenia, anaemia, recurrent infections,) bones, hepatosplenomegaly, lung (cough) → think of Gaucher's disease

Pathophysiology

- Autosomal recessive mutation in the glucocerebrosidase (GBA) gene located on chromosome 1 → Deficiency of β -glucocerebrosidase → accumulation of glucocerebroside (sphingolipid found in cell membranes that can accumulate in the lysosome of macrophages) in the brain, liver, spleen, and bone marrow (i.e., Gaucher cells).

Epidemiology

- Gaucher's disease is the most common lysosomal storage diseases.
- About one in 100 people in the United States are carriers of the most common type of Gaucher disease (**type I**).
- The carrier rate among Ashkenazi Jews is 8.9% while the birth incidence is one in 450.

Consequences

- Parkinson's disease is more common in Gaucher's disease patients** (the most commonly known genetic risk factor for Parkinson's)
- Cancer risk may be increased, particularly myeloma.

Types

- GD type I (Chronic non-neuropathic; adult Gaucher disease)**
 - Most common form
 - Associated with a normal lifespan
- GD type II (Acute neuropathic; infantile Gaucher disease)**
 - typically begins within 6 months of birth
 - Symptoms include progressive brain damage, spasticity and seizures.
 - carries the worst prognosis, affected children **usually die by age two**.
- GD type III (Subacute neuropathic; juvenile Gaucher disease)**
 - can begin at any time in childhood or even in adulthood
 - characterized by slowly progressive, but milder **neurologic symptoms** compared to type II.
 - Patients often live into their early teen years and adulthood

Features

- Hepatosplenomegaly (**massive splenomegaly**)
- Bone pathology (bone crises, osteoporosis, aseptic necrosis) **the chief complaint is of bone pain in an adult**.
- Blood abnormalities: anemia, thrombocytopenia
- diffuse infiltrative pulmonary disease
- Growth delays
- Yellowish-brown skin and scleral pigmentation (**Characteristic yellow or yellow-brown papules (pingueculae) develop at the sclerocorneal junctions**).

Diagnosis

- Enzyme analysis (Enzyme studies of blood leucocytes)** → Reduced glucocerebrosidase activity in leukocytes or fibroblasts
- Accumulation of glucocerebroside in leukocytes or fibroblasts
- Gaucher cell: lipid-rich macrophages with an enlarged cytoplasm with inclusions that resemble crumpled tissue paper on microscopy

Treatment

- Recombinant glucocerebrosidase.



The slide shows yellow papules (pingueculae) in the cornea; these are characteristic of Gaucher disease.

Common exam questions

- Features of anaemia, recurrent pneumonia, bone pain and hepatosplenomegaly.
Which of the following is the most likely diagnosis?
⇒ Gaucher's disease
- Features of anaemia, recurrent pneumonia, bone pain and hepatosplenomegaly.
.Which of the following is the most likely enzyme deficiency found in this patient?
⇒ Glucocerebrosidase

Gaucher disease causes massive splenomegaly

Fabry's disease

Pathophysiology (a lysosomal storage disorder)

- X-linked recessive mutation → α -Galactosidase A deficiency → accumulation of trihexoside ceramide (a glycolipid found in multiple body tissues) in the endothelium of vessels, in the epithelium of many organs, and in smooth muscle cells → disorder affecting many organ systems.

Epidemiology

- Typical onset is during childhood but may also appear in 60–80-year-old adults
- Mainly affects boys

Features

- Early features
 - ⇒ Peripheral neuropathy: Periodically occurring dysesthesia in the hands and feet caused by small fiber neuropathy, which manifests as burning pain (Fabry crises)
 - ⇒ Anhidrosis or hypohidrosis (decreased sweating)
 - ⇒ Angiokeratomas (warty skin lesions with telangiectasia and hyperkeratinized covering)
 - ⇒ Corneal clouding
 - ⇒ Cataract

- **Late features**
 - ⇒ Restrictive cardiomyopathy
 - ⇒ Cerebrovascular lesions (TIA and stroke)
 - ⇒ Fabry nephropathy, causing progressive renal failure (the first manifestation of renal insufficiency in Fabry disease is proteinuria.)

The disorder has three distinct clinical entities:

1. Classical presentation in the male homozygote with early presentation in childhood - angiokeratomas, heart failure, cataracts and renal disease
2. **Male homozygotes with atypical presentation in adulthood with proteinuria, acroparesthesia, angiokeratomas and cardiomegaly**
3. Female heterozygotes can present again in adulthood with similar mild symptoms.
 - An X linked recessively inherited condition can exist in female carriers who may exhibit mild to moderate symptoms. This is due to variable expression according to random X inactivation of the affected gene in embryogenesis

most common symptoms → peripheral neuropathy, angiokeratomas, and hypohidrosis.

Diagnosis

- **Absent or deficient levels of alpha-galactosidase A** in leucocytes, plasma or cultured fibroblasts.
- Gene analysis of alpha-galactosidase A (GLA) gene (**the gold standard for the diagnosis**)
- **Slit-lamp examination** of the cornea → microscopic lipid deposits
- Microscopy of the spun urine sediment may demonstrate 'Maltese cross' lipid globules

In Fabry disease, tissue accumulation of which is most likely to occur?

- ⇒ **Trihexosyl ceramide**

Treatment

- Enzyme replacement therapy with α-galactosidase A

Mucopolysaccharidoses (MPS) (Hurler's & Hunter's syndromes)

Pathophysiology

- Mutations in lysosomal enzymes → impaired breakdown of glycosaminoglycans → Accumulation of glycosaminoglycans, i.e., heparan sulfate (HS) and dermatan sulfate (DS)

Features

- Occur in both conditions (typically milder in Hunter syndrome):
 - ⇒ Developmental delay
 - ⇒ Facial dysmorphisms: frontal bossing, elongated skull, flattened nasal bridge, broad nasal tip, thickened gingiva, anteverted nostrils, constant nasal discharge, spaced and protruded eyes.
 - ⇒ Airway obstruction
 - ⇒ Hepatosplenomegaly

Diagnosis

- Increased urinary levels of dermatan sulfate (DS) and heparan sulfate (HS)
- Enzyme assay to confirm specific enzyme deficiency (definitive test)

Treatment

- Enzyme replacement therapy
- Bone marrow transplantation

	Hurler syndrome (mucopolysaccharidosis type I)	Hunter syndrome (mucopolysaccharidosis type II)
Inheritance	Autosomal recessive	X-linked recessive
Pathophysiology	Deficiency of α -L-iduronidase (enzyme responsible for the hydrolysis of glycosaminoglycans)	Deficiency of iduronate-2-sulfatase
Features	<ul style="list-style-type: none"> ⌚ Corneal clouding ⌚ Inguinal hernia 	<ul style="list-style-type: none"> ⌚ Aggressive behavior, Hyperactivity ⌚ No corneal clouding ⌚ Carpal tunnel syndrome

Which feature suggests a diagnosis of Hurler's syndrome rather than Hunter's syndrome?
→ Cloudy cornea.

Hunter syndrome presents as Hurler syndrome, but patients with Hunter syndrome have normal vision and aggressive behavior.

Glycogen storage disorders (GSD)

Key feature of glycogen storage disorders:

- Tay-Sachs commonly has a 'cherry red spot' macula
- Pompe disease leads to cardiomyopathies
- McArdle's disease leads to rhabdomyolysis after exercise and lactic acidemia
- Von Gierke disease leads to hypoglycemia and hepatomegaly

Pompe trashes the Pump (heart)

Glycogen

- Glycogen is the storage form of carbohydrate, found predominantly in muscle and liver.
- **Chains of glucose residues are linked by alpha-1,4 glycosidic bonds**, i.e. between the first carbon of one glucose and the fourth carbon of the next. Branches occur about every ten residues, and are formed by alpha-1,6 glycosidic linkages.
- Glycogen synthesis and degradation occur at the tips of branches, with the branching structure increasing the number of sites at which glucose residues can be added or removed.

Pompe's disease or acid maltase deficiency (glycogen storage disorder type 2): is a deficiency in alpha-glucosidase. It produces a myopathy , restrictive cardiomyopathy and hepatomegaly.

Glycogen storage disorders:

- Muscle involvement (muscle glycogenoses): Types II, III, IV, V
- Liver involvement (liver glycogenoses): Types I, III, IV
- Types III and IV (late-onset type) may present with both muscle and liver involvement
- **NO liver involvement → V**

- **Autosomal recessive**

- All types of glycogen storage diseases result in abnormal metabolism and product accumulation within cells.
- Type IV (Andersen's disease) is the only one **GSD** involved in Glycogen Synthesis. The rest are involved in Glycogen degradation.

Diagnosis

- **Periodic acid-Schiff stain is helpful in diagnosing glycogen storage disorders.**

Type I (Von Gierke's disease)

- Relative frequency: ~25%
- Deficient enzyme
 - ⇒ **Type 1a** → Glucose-6-phosphatase
 - Role of the enzyme → Hydrolysis of glucose-6-phosphate to glucose and inorganic phosphate
 - ⇒ **Type 1b** → Glucose-6-phosphate translocase
 - Role of the enzyme → Transport of glucose-6-phosphate into the endoplasmic reticulum where it is hydrolyzed by glucose-6-phosphatase
- Characteristic features
 - ⇒ Hepatomegaly
 - ⇒ Severe fasting hypoglycemia, mild ketosis
 - ⇒ Severe hyperlipidemia → doll-like facies
 - ⇒ Hyperuricemia
 - ⇒ Lactic acidosis
 - ⇒ Anemia
 - ⇒ Failure to thrive

Type II (Pompe's disease)

- Relative frequency: ~15%
- Deficient enzyme: Lysosomal acid maltase deficiency
- Role of the enzyme: Glycogenolysis within the lysosome
- Characteristic features
 - ⇒ Hypertrophic cardiomyopathy and/or conduction blocks
 - ⇒ Proximal myopathy
 - ⇒ Macroglossia
 - ⇒ Failure to thrive

Type III (Cori's disease)

- Relative frequency: ~25%
- Deficient enzyme: debranching enzyme (alpha-1,6-glucosidase).
- Role of the enzyme: Glycogenolysis
- Characteristic features
 - ⇒ Generalized muscle weakness and/or cramps
 - ⇒ Hepatomegaly
 - ⇒ Possibly cirrhosis (ascitis, splenomegaly)
 - ⇒ Mild, fasting hypoglycemia and ketosis
 - ⇒ Hyperlipidemia

Type IV (Andersen's disease)

- Relative frequency: ~3%
- Deficient enzyme: **Glycogen branching enzyme**
- Role of the enzyme: **Glycogenesis**
- Characteristic features
 - ⇒ Proximal myopathy
 - ⇒ Hepatomegaly
 - ⇒ Possibly cirrhosis (ascites, splenomegaly)

Type V (McArdle's disease)

- Relative frequency: ~2%
- Deficient enzyme: Muscle phosphorylase (myophosphorylase)
- Role of the enzyme: Glycogenolysis
- Characteristic features
 - ⇒ Generalized muscle weakness, exercise intolerance (with a second wind phenomenon),
 - ⇒ Rhabdomyolysis and myoglobinuria

McArdle's disease (Type V glycogen storage disease)

Often presents in adolescence with exercise intolerance, cramps and weakness

A history of painful muscle cramps that occur within a few minutes of initiating activity and which subside rapidly with rest, in conjunction with a raised serum CK, is highly suggestive of McArdle's disease

Pathophysiology

- Autosomal recessive mutation in myophosphorylase (PYGM) gene on chromosome 11 → **myophosphorylase deficiency** (myophosphorylase is involved in the breakdown of glycogen to glucose) → unable to release glucose from glycogen in muscle (**decreased muscle glycogenolysis**).

Features

- **Muscle pain and stiffness following exercise** (reversible)
 - ⇒ in the first few minutes of activity.
 - ⇒ Characterised by '**second wind' phenomenon**'
 - after about 8 minutes most patients achieve a 'second wind' and can then continue exercise with less difficulty.
 - **Second wind** is a phenomenon in distance running, such as marathons (an athlete who is too tired to continue suddenly finds the strength to press on at top performance with less exertion).
 - **Mechanism** → **metabolic switch**
 - ❖ When non-aerobic glycogen metabolism is insufficient to meet energy demands, physiologic mechanisms utilize alternative sources of energy such as fatty acids and proteins via aerobic respiration.
 - ❖ muscle fibers use fat as a source of energy.

Investigations

- Creatine kinase levels are elevated in more than 90%
- NO increase in venous lactic acid levels following exercise testing.
- Urine study → **Myoglobinuria** following exercise
- Muscle and/or liver biopsy → ↑glycogen → **PAS-positive granules** (initial tests)
- **DNA testing for the gene defects (Gene sequencing):** the **gold standard** for the diagnosis

Management

- No specific treatment
- Dietary therapy (e.g., uncooked corn starch, glucose preparations) with the aim of preventing hypoglycemia and/or muscle symptoms
- Foods rich in **fructose** and **galactose** should be avoided in patients with GSD type I
- Advised to ingest snacks containing **sucrose** before exercise.
- Tourniquets should not be used during operative procedures

The exertional thigh cramps, the presence of myoglobin and change in colour of urine after exercise suggests glycogen storage disease type V - McArdle's syndrome. the next most appropriate investigation → Muscle biopsy which reveals subsarcolemmal deposits of glycogen appearing at the periphery of fibres.

Linkage disequilibrium

- Linkage disequilibrium is the non-random association of alleles at different loci in a given population.
- Loci are said to be in linkage disequilibrium when the frequency of association of their different alleles is higher or lower than what would be expected if the loci were independent and associated randomly.
- Consider the scenario of two separate genetic loci A and B, where each locus carries two possible alleles. If these two loci A and B are in linkage disequilibrium → An individual with locus A is likely to have locus B
- Linkage disequilibrium almost always, occurs between alleles at genetic loci that are closely linked in the genome.

Imprinting

Definition

- imprinting is a phenomenon by which certain genes are expressed in a parent-of-origin-specific manner.
 - ⇒ the term 'imprinting' refers to → Differential expression of alleles contingent on their parental origin
 - If the allele inherited from the father is imprinted, it is thereby silenced, and only the allele from the mother is expressed.
 - If the allele from the mother is imprinted, then only the allele from the father is expressed.

Mechanism

- poorly understood but does involve DNA methylation.
- Disease may occur as a result of a defect in one allele if the other allele is imprinted and hence not expressed.

Examples

- diseases involving genomic imprinting include:
 - ⇒ Prader-Willi syndrome (paternally imprinted)
 - ⇒ Angelman syndrome (maternally imprinted)

Prader-Willi syndrome

Deletion of chromosome 15

- Prader-Willi – paternal
- Angelman syndrome - maternal

Overview

- Prader-Willi syndrome is an example of **genetic imprinting** where the phenotype depends on whether the deletion occurs on a gene inherited from the mother or father:
 - ⇒ Prader-Willi syndrome if gene deleted from father
 - ⇒ Angelman syndrome if gene deleted from mother
- Prader-Willi syndrome is associated with the absence of the active **Prader-Willi gene on the long arm of chromosome 15**, this may be due to:
 - ⇒ **Microdeletion of paternal 15q11-13 (70% of cases)**
 - ⇒ Maternal uniparental disomy of chromosome 15
- **The mode of inheritance is → Non-Mendelian**

Features

- **Hypotonia during infancy**
- Dysmorphic features
- **Short stature** (Growth hormone deficiency)
- **Hypogonadism and infertility**
 - ⇒ (risk factor for osteoporosis)
- **Cryptorchidism** (undescended testis)
- **Learning difficulties**
- **Childhood obesity due to Hyperphagia** (abnormally desire for food → overeating → obesity)
- Behavioural problems in adolescence
- Associated with elevated ghrelin
 - ⇒ Ghrelin is a hormone produced in the fundus of the stomach and in the pancreas
 - ⇒ Ghrelin levels increase before meals and decrease afterwards
 - ⇒ Receptors for ghrelin are found in the arcuate nucleus and the hypothalamus

Treatment

- Administration of **growth hormone and sex hormones (testosterone)** is the treatment of choice
- Calorie restriction

MRCPUK-part-1-September 2017 exam: Which one of the following is the most common genetic cause of Prader-Willi syndrome?

→ **Microdeletion of the paternal 15q11-13**

Chromosome 15 is implicated in Prader-Willi, Angelman, and Marfan syndromes.

Angelman syndrome

Overview

- Angelman syndrome is a genetic condition characterized by a mutation on the **maternal copy of chromosome 15**.
- occurs as a result of a phenomena known as genomic **imprinting**.
- The imprinted copy of the gene is silenced through methylation or histone modification.
- Normally, certain paternal alleles on chromosome 15 are silenced and only the maternal alleles are expressed. However, in Angelman syndrome, the maternal alleles are mutated. Hence, the patient will have disease since only the mutated maternal alleles are active.

Features

- Developmental delay
- Intellectual disability
- **Seizures, Ataxia**
- **Unprovoked laughter**
- Large mouth with tongue protrusion.
- Hypo-pigmentation with blond hair

Diagnosis

- genetic studies showing loss of function of the UBE3A gene.

Mutations

- **Missense mutation**
 - ⇒ substitution in one amino acid in a protein
 - ⇒ e.g: glutamic acid is substituted by valine in sickle-cell disease
- **Nonsense mutation**
 - ⇒ the altered DNA sequence prematurely signals the cell to stop building a protein.
 - ⇒ This type of mutation results in a shortened protein that may function improperly or not at all.
- **Insertion mutation**
 - ⇒ changes the number of DNA bases in a gene by **adding a piece of DNA**. As a result, the protein made by the gene may not function properly.
- **Frameshift mutation**
 - ⇒ insertions or deletions of nucleotides
 - ⇒ e.g: cystic fibrosis
- **Point mutation**
 - ⇒ a change in a single nucleotide
 - ⇒ e.g: C282Y mutation responsible for haemochromatosis
- **Splicing mutation**
 - ⇒ results in larger nonfunctional protein
 - ⇒ e.g: β-thalassemia
- **Large Segment Deletion**
 - ⇒ Unequal crossover at meiosis results in loss of large segment of DNA
 - ⇒ Loss of function mutation
 - ⇒ e.g., α-thalassemia (deletion of α-globin gene)
- **Termination mutation**
 - ⇒ generation of a premature stop codon
 - ⇒ e.g: Hurler syndrome

Chromosome abnormality

- Chromosome anomalies usually occur when there is an error in cell division following meiosis or mitosis.

Types

- Numerical disorders**

- ⇒ called **aneuploidy** (an abnormal number of chromosomes), occurs when an individual either is:
 - missing a chromosome from a pair (monosomy)
 - e.g: Turner syndrome, (born with only one sex chromosome, an X).
 - has more than two chromosomes of a pair (trisomy, tetrasomy, etc.).
 - e.g: Down syndrome (trisomy 21)
- ⇒ **Unbalanced autosomal translocation**
 - most likely to cause a severe phenotype**
 - As a rule, the clinical effects of a chromosome abnormality reflect the amount of imbalance of genetic material. For example: all autosomal monosomies and most autosomal trisomies are incompatible with life, the exceptions being trisomy 13 (Patau syndrome), trisomy 18 (Edward syndrome) and trisomy 21 (Down syndrome); only the last of these carries a reasonable life expectancy.
- ⇒ **Sex chromosome aneuploidy:**
 - This is associated with comparatively **less severe phenotypes**, e.g. Klinefelter syndrome (XXY) and Turner syndrome (XO).

- Structural abnormalities:** e.g:

- ⇒ Duplications:
 - A portion of the chromosome is duplicated, resulting in extra genetic material.
 - e.g: Charcot-Marie-Tooth disease type 1A, caused by duplication of the gene encoding peripheral myelin protein 22 (PMP22) on chromosome 17.

List of common Chromosomal disorders:

Chromosome	disorders
Chromosome 1	variegate porphyria
chromosome 3	von Hippel Lindau (VHL)
Chromosome 4	Polycystic Kidney Disease (PKD2) Huntington's disease Achondroplasia
Chromosome 6	hereditary haemochromatosis
Chromosome 7	Cystic Fibrosis
Chromosome 9	Fredrich's ataxia
Chromosome 11	Sickle Cell Disease Beta-Thalassemia
Chromosome 12	Phenylketonuria von Willebrand's disease
Chromosome 13	Patau Syndrome. Wilson Disease. retinoblastoma
Chromosome 15	Marfan's Syndrome Angelman Syndrome Prader-Willi Syndrome Tay-Sachs Disease.
Chromosome 16	Polycystic Kidney Disease (PKD1) alpha- Thalassemia
Chromosome 17	Celiac Disease. Charcot-Marie-Tooth Disease. Neurofibromatosis (NF1)
Chromosome 18	Edward Syndrome
Chromosome 19	Myotonic Dystrophy
Chromosome 21	Down Syndrome
Chromosome 22	DiGeorge Syndrome. Neurofibromatosis (NF2)

McCune-Albright syndrome (MAS)**McCune-Albright syndrome:**

- Triad of patchy skin pigmentation, bone abnormalities, and endocrine abnormalities.
- McCune-Albright syndrome is a form of mosaicism
- Due to a mutation in the GNAS1 gene

Notes & Notes

For MRCP part 1 & 11

By

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Basic science

Biostatistics & EBM

Updated

2022

Chapter 17 Genetics

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Significance tests

Null hypothesis (H_0)

- A null hypothesis (H_0) states that two treatments are equally effective (and is hence negatively phrased).
- A significance test uses the sample data to assess how likely the null hypothesis is to be correct.
- The null hypothesis is always that there is no difference between the variables we would like to test for a difference.
- For example: 'there is no difference in the prevalence of colorectal cancer in patients taking low-dose aspirin compared to those who are not'

The alternative hypothesis (H_1)

- is the opposite of the null hypothesis, i.e. There is a difference between the two treatments

P value

- The **p value** is the probability of obtaining a result by chance at least as extreme as the one that was actually observed, assuming that the null hypothesis is true.
- It is therefore equal to the chance of making a type I error (see below).
- **the p-value is the probability of obtaining the observed results or results which are more extreme if the null hypothesis is true**

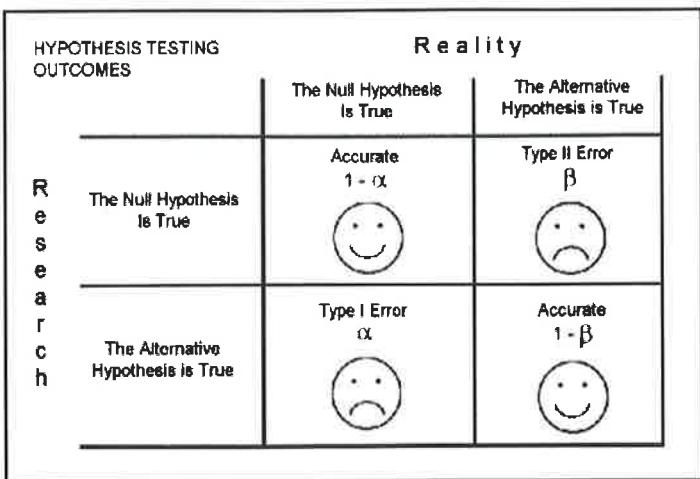
- **Example: if $p=0.03$. What does ' $p=0.03$ ' mean?**
- **It means → the probability that a difference between the two sample groups occurred by chance is 3%**

Statistical errors

- Two types of errors may occur when testing the null hypothesis
 1. type I:
 - **the null hypothesis is rejected when it is true,**
 - ☞ 'the null hypothesis is falsely rejected'.
 - ☞ i.e. Showing a difference between two groups when it doesn't exist,
 - ☞ a false positive.
 - This is determined against a preset significance level (termed alpha).
 - As the significance level is determined in advance the chance of making a type I error is not affected by sample size.
 - It is however increased if the number of end-points are increased. For example if a study has 20 end-points it is likely one of these will be reached, just by chance. i.e. the result is just a statistical fluke.
 2. type II:
 - **the null hypothesis is accepted when it is false,**
 - ☞ 'the null hypothesis is falsely accepted'.
 - ☞ i.e. **Failing to spot a difference when one really exists,**
 - ☞ a false negative.
 - The probability of making a type II error is termed beta.
 - It is **determined by both sample size** and alpha. This can happen if the sample size is too small.
 - ☞ **Increasing the sample size** reduces the standard error, meaning the estimate is more precise and the **probability of a type-2 error is reduced.**
 - This type of error **can be avoided by** making explicit power calculations before embarking on any study. This will answer the question 'if I am studying an

outcome that occurs in (say) 20% of a conventionally treated group and want to show a (say) halving in the rate of this outcome, then how many patients do I need to study?"

	Study accepts H_0	Study rejects H_0
Reality H_0		Type 1 error (alpha)
Reality H_1	Type 2 error (beta)	Power ($1 - \beta$)



Error: type I (alpha) vs. type II (beta)

➡ Type I (Alpha) Error: "There Is An Effect" where in reality there is none.

The power

- The power of a study is the probability of (correctly) rejecting the null hypothesis when it is false, i.e. the probability of detecting a statistically significant difference
 - ⇒ power = $1 - \beta$ – the probability of a type II error
 - ⇒ power can be increased by increasing the sample size
- As the power decreases, type II error (= 1-power) will increase. Therefore, the chance of type II error will increase if the same sample size is used.**
- The statistical power will decrease if the standard deviation increases.
- 'Power of the study' refer ➔ The probability of a statistically significant treatment effect if the true treatment difference is at a prespecified level**
- Power is determined by sample size, effect size, and its standard error.
- The statistical significance** of a result is the probability ('p value') that the observed relationship (eg between variables) or a difference (eg between means) in a sample occurred by pure chance and that in the population from which the sample was drawn, no such relationship or differences exist
- The sample size can be reduced if the level of significance is increased.
- The power increases with the set level of significance, if other variables remain the same.

Significance tests: types

Correlation

- parametric (normally distributed): Pearson's coefficient
- non-parametric: Spearman's coefficient

- The type of significance test used depends on whether the data is **parametric** (something which can be measured, usually normally distributed) or non-parametric
 - ⇒ **Parametric tests (the data follow normal distribution)** (quantitative variables)
 - Student's t-test - paired or unpaired*
 - Pearson's product-moment coefficient (**Pearson correlation coefficient**)
 - ⇒ used to assess **correlation** (strength of association) between two variables
 - ⇒ **Non-parametric tests**
 - Mann-Whitney U test - unpaired data
 - ⇒ used to compare **medians or rank orders** of **two groups** with non-normal distribution.
 - Wilcoxon signed-rank test:
 - ⇒ compares two sets of observations on a **single sample**
 - ⇒ The data in the study is **non-parametric, paired** and comes from the same population.
 - chi-squared test:
 - ⇒ used to compare **proportions** or **percentages** (eg: prevalence) between two categorical variables
 - ⇒ for example, comparing the **proportion** of children developing measles between a group receiving a new measles vaccine and a group not given the vaccine
 - ⇒ Should be used for 2 **independent** samples.
 - Spearman, Kendall rank:
 - ⇒ measures the **correlation** between the ranks of **two** variables which do not follow a normal distribution.
 - ⇒ **compares ranks** and not values, such as the perception of pain (**ranked on a scale of 1-10**)

In a scenario looks at whether the values are correlated, and the data is non-parametric, (e.g: pain scale), Spearman's rank correlation coefficient should be used.

- Paired data refers to data obtained from a single group of patients, e.g. Measurement before and after an intervention. Unpaired data comes from two different groups of patients, e.g. Comparing response to different interventions in two groups

Choosing the appropriate test

- Choosing of a test to examine a statistical problem depends upon the scale of measurement (nominal, ordinal, interval, ratio) and the type of question being asked
- a non-parametric test would give less power

Student's t-test

- **Paired t-test**
 - ⇒ Compares a **single measure** (variable) recorded **on a single group** of individuals **on two different occasions**.
 - ⇒ Is used to compare **means in a single sample**, **for example, before and after treatment**.
 - ⇒ → comparing **means** (**not proportions**) in the **same subjects**
 - ⇒ **paired t-test is used to compare post-treatment and pre-treatment result of a single group.**
 - ⇒ eg: the same subject measured before and after a process change, or the same subject measured at different times.
 - ⇒ As both sets of measurements were made on the same patients, the measurements are not independent
- **Unpaired t-test (Independent sample t-test)**
 - ⇒ is the most appropriate statistical test to compare means of two independent samples.
 - compare the **means of two** different populations
 - ⇒ An independent sample t-test may be used in a study of **two independent** treatment groups, and the **sample sizes are relatively large** (>30 in each group) and the variable is **Normally distributed**.
 - ⇒ eg: Blood pressure is a continuous variable which is normally distributed; as such Student's t test is the most appropriate way to test for **differences in the mean BPs between the two groups**.
 - ⇒ For example, suppose we are evaluating the effect of a medical treatment, and we enroll 100 subjects into our study, then randomly assign 50 subjects to the treatment group and 50 subjects to the control group. In this case, we have two independent samples and would use the unpaired form of the t-test.
 - ⇒ eg: 2 groups (treatment group & placebo group) In a randomised controlled trial of drug A for treatment of hypercholesterolemia

Log-rank test

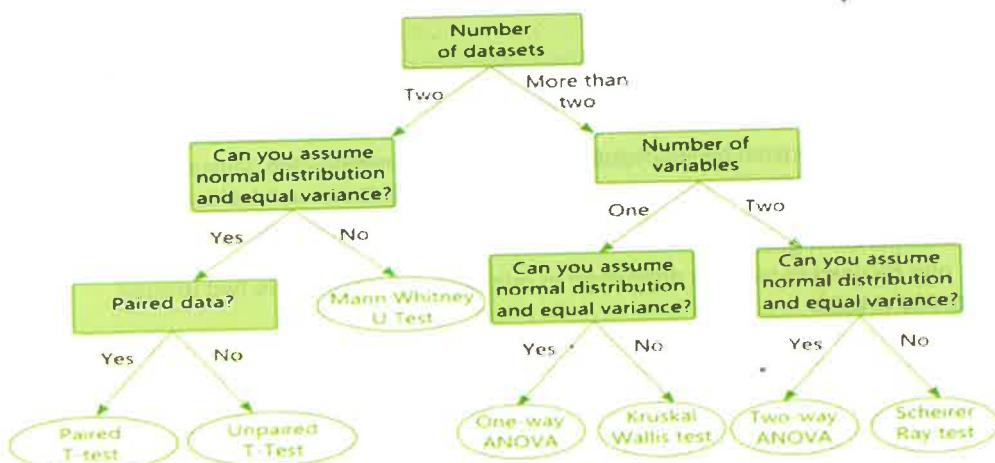
- Is the most appropriate test to compare two survival curves with censored data.
- Log-rank test should be used to compare survival data between two groups, but not compare median survival. Mean survival is not known unless all patients have died.
- If a question presented survival data and some observations are censored(ex: not came for follow up) and the outcomes are not known. We need to use survival analysis for such data and the log-rank test is the appropriate test to use to compare survivals in two independent groups.
- can be used to test the difference in relapse rate between the two groups

McNemar's test

- is applied to binary data, but is only applicable to paired data, used to compare proportions
- McNemar's test is used to compare paired samples - either case control studies where each case is matched to a control, or to studies where two treatments are given to matched subjects.
- It cannot be used where the sample size differs.
- is used to test for agreement of repeated observations.

Regression techniques

- are used to predict the value of one variable based on the other
- **Multiple regression**
 - ⇒ is used to analyse the relationship between one dependent variable and one or more independent variables
- **Logistic regression (Log regression analysis)**
 - ⇒ It is used to describe the relationship between one dependent binary variable and one or more metric independent variables.
 - ⇒ It is commonly used to assess plasma concentrations of a drug as it allows examination of the relationship between possible confounding factors such as renal function or age.
 - ⇒ This would allow us to determine whether one variable is dependent on another, ex: in case whether drug concentration was dependent on body surface area.
 - **ANOVA (analysis of variance)** is an example of logistic regression analysis.
 - is a statistical test which tests for co-variance between populations and is useful when variables such as age, sex or race may be expected to affect the treatment's effectiveness.
 - tests for a difference in mean values between a **number of groups**
 - **Is the most appropriate to compare the means of more than two groups. (used for more than two means)**
 - **One-way analysis of variance** is identical mathematically to the unpaired Student t-test when just two groups are being compared.
 - **The one-way (analysis of variance) (ANOVA) compares the means of the groups**
 - The means should be presented with confidence intervals to give the reader an idea of whether the differences between the groups were significant
- **The Cox (proportional odds) regression (Cox proportional hazards regression):**
 - ⇒ this method was devised specifically for the type of study in which many patients fail to reach the end-point (ie in statistical terms, are 'censored') and in which follow-up time varies.
 - ⇒ Cox regression is designed specifically for the analysis of time to an event occurring.



Parametric tests and analogous nonparametric procedures

Analysis Type	Example	Parametric Procedure	Nonparametric Procedure
Compare means between two distinct/independent groups	Is the mean systolic blood pressure (at baseline) for patients assigned to placebo different from the mean for patients assigned to the treatment group?	Two-sample t-test	Wilcoxon ranksum test
Compare two quantitative measurements taken from the same individual	Was there a significant change in systolic blood pressure between baseline and the six-month followup measurement in the treatment group?	Paired t-test	Wilcoxon signedrank test
Compare means between three or more distinct/independent groups	If our experiment had three groups (e.g., placebo, new drug #1, new drug #2), we might want to know whether the mean systolic blood pressure at baseline differed among the three groups.	Analysis of variance (ANOVA)	Kruskal-Wallis test
Estimate the degree of association between two quantitative variables	Is systolic blood pressure associated with the patient's age?	Pearson coefficient of correlation	Spearman's rank correlation

- Categorical variables are not continuous, e.g. drug / placebo, dead / alive. They should be described as percentages or proportions and compared with a Chi-squared test.
- Normally distributed continuous data should be described as mean and standard deviation and compared with a Student's t-test.
- Skewed continuous data should be described as median and range and compared using a test such as the Wilcoxon rank-sum test or the Mann-Whitney U-test.

MRCPUK-part-1-May-2017 exam: A study is designed to assess severity of snoring before and after using a new mandibular device. What is the most appropriate statistical test to apply to this data?

→ Wilcoxon signed-rank test

Normal distribution

- The normal distribution is also known as the Gaussian distribution or 'bell-shaped' distribution. It describes the spread of many biological and clinical measurements
- Properties of the Normal distribution
 - symmetrical i.e. Mean = mode = median
 - 68.3% of values lie within 1 SD of the mean
 - 95.4% of values lie within 2 SD of the mean
 - 99.7% of values lie within 3 SD of the mean
 - this is often reversed, so that within 1.96 SD of the mean lie 95% of the sample values
 - the range of the mean - (1.96 *SD) to the mean + (1.96 * SD) is called the 95% confidence interval, i.e. If a repeat sample of 100 observations are taken from the same group 95 of them would be expected to lie in that range

MRCPUK-part-1-January 2019 exam: A study is designed to assess the efficacy of a new antihypertensive drug. Two groups of patients are randomly assigned, one to take the established drug for 3 months whilst the other takes the new drug for 3 months. blood pressure is measured before and 3 months .After period off medication the drug swapped around and again, blood pressure is measured before and 3 months later. Which one of the following significance tests is it most appropriate to apply?

- Student's paired t-test (comparing parametric data from the same patients (they swapped medication halfway through the study))

Standard deviation

SD = square root (variance)

Remember that around two-thirds of values lie within 1 SD of the mean, one-third will therefore lie outside 1 SD, and half of these (one-sixth) will be less than 1 SD below the mean

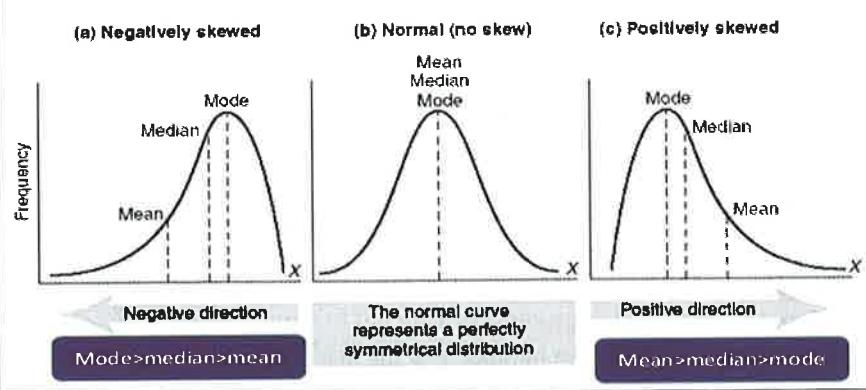
- the standard deviation (SD) is a measure of how much dispersion exists from the mean
- **It is a measure of the spread of the sample distribution**
- $SD = \text{square root (variance)}$
- **The standard deviation** is a sort of average of the deviations of each observation from the mean, whereas **the range** is simply the difference between the largest and smallest observations.
- The standard deviation is affected by outliers and would be larger than expected if outliers are present
- If the data are skewed, the standard deviation will tend to overestimate the spread in the data
- If the standard deviation is reduced, the sample size required is smaller.
- **If SD increased the power of study is reduced .**
- **The standard deviation would give the best estimate of a spread of a measurement about the mean**
- Variance is the square of standard deviation. Standard deviation is the square root of variance.

Skewed distributions

Skewed distributions

- alphabetical order: mean - median - mode
- '>' for positive, '<' for negative
- Normal (Gaussian) distributions: mean = median = mode (**bell-shaped**)
- Positively skewed distribution: mean > median > mode
- Negatively skewed distribution mean < median < mode
- To remember the above note how they are in alphabetical order, think positive going forward with '>', whilst negative going backwards '<'

Position of mean median mode



- Mean, median and mode are measures of central tendency
- Descriptive statistics provide mean, median and mode values for a distribution**

Example: The annual numbers of reported cases of leptospirosis in the USA over the 5-year period from 1985 to 1990 were: 2, 1, 3, 4, 1, . What was the mean, median and modal number of cases per year?

Answer:

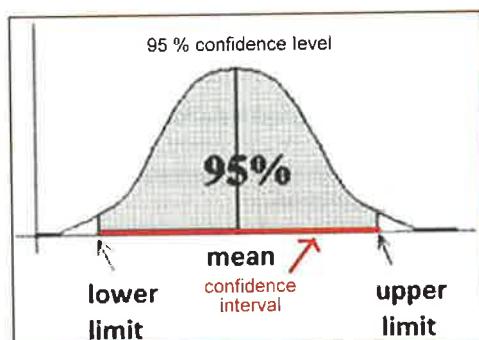
- The mean** is found by summing all the values and dividing by 5; this gives a mean = $11/5=2.2$
 - ⇒ **The mean is the average value of observations, and therefore very sensitive to extreme values in a distribution**
 - ⇒ If the mean is greater than the median, this indicates a positive skew.
- For the median and mode ⇒ rewrite the values in ascending order: ie 1,1,**2**,3,4,
- The median** is the middle value when the values are placed in order = **2**
 - ⇒ For an even number of values it is halfway between the two middle values,
 - ⇒ If you forgot to sort the values before looking for the middle value, you will have got the incorrect answer = 3
 - ⇒ The median is the observation that divides the frequency distribution by half and is equal to the 50th centile (lies exactly between each end of a range of values)
 - ⇒ It responds to the number of extreme observations but not their value, and therefore is useful as a measure of central tendency in extremely skewed distributions
 - ⇒ In a normal distribution the median equals the mean
- The mode** is the most common value; this is → 1 , which occurs twice, whereas all other values occur only once
 - ⇒ **mode is the most commonly observed value**

- The distribution sample means will be normally distributed even if the population values are not normally distributed.
- The random sampling distribution of means would always tend to be normal, irrespective of the population distribution for which the samples were drawn. Hence, even if the population distribution is skewed or in any non-normal distribution, the sample means would be normally distributed.'
- the mean of the random sampling distribution of means is equal to the mean of the original population.
- In a distribution skewed by the presence of a number of positive outliers → Mean increases, median may increase, mode remains the same

Confidence interval and standard error of the mean

Standard error of the mean = standard deviation / square root (number of patients)

- Definition of **confidence interval**
 - ⇒ a range of values for a variable of interest constructed so that this range has a specified probability of including the true value of the variable.
 - ⇒ The specified probability is called the confidence level, and the end points of the confidence interval are called the confidence limits*
 - ⇒ in simpler terms: a range of values within which the true effect of intervention is likely to lie
- A confidence interval is needed for almost all statistical estimates, including sensitivity or specificity of a diagnostic test.
- If the confidence interval includes the number 1, → the trial did not find a statistically significant difference between the variables (this does not mean there was no difference)
 - ⇒ This means the association is not statistically significant and therefore the p value should be above 0.05.



Key point

- A 95% confidence interval:
 - ⇒ Most commonly, the 95% confidence level is used
 - ⇒ What is the best interpretation of the 95% confidence interval?
 - We are 95% confident that the mean in the value is between confidence limits
 - ⇒ confidence interval at the 95% confidence level means that the confidence interval should contain the true effect of intervention 95% of the time.

- ⇒ A 95% confidence interval means that there is only a 5% chance that the true mean value for the variable lies outside the ranges quoted
- ⇒ The 95% confidence limits will be the mean plus or minus 1.96 standard errors
 - lower limit = mean - (1.96 * SEM)
 - upper limit = mean + (1.96 * SEM)
- ⇒ For example, in a study the mean height in a sample taken from a population is 183cm. You know that the standard error (SE) (the standard deviation of the mean) is 2cm. This gives a 95% confidence interval of 179-187cm (+/- 2 SE).
- ⇒ meaning that there is a 5% chance that the true population mean is not included in this range, in other words a 95% chance that the true population mean is included within this range
- ⇒ If the 95% confidence interval does not include 0 (zero), the difference is statistically significant
- ⇒ If the p value is less than 0.05, → statistically significant → the 95% confidence interval should not include 0.
- Standard error of the mean (SEM)
 - ⇒ The standard error of the mean (SEM) is a measure of the spread expected for the mean of the observations - i.e. how 'accurate' the calculated sample mean is from the true population mean
 - ⇒ **SEM = SD / square root (n)**
 - where SD = standard deviation and n = sample size
 - therefore, the SEM gets smaller as the sample size (n) increases
 - ⇒ **standard error of the mean → Gets smaller as the sample size increases**
 - ⇒ **Increasing the sample size will reduce the standard error of the mean and the width of the confidence interval.**
 - ⇒ The standard error is → Smaller than the standard deviation

Assessment of significance (is the result statistically significant?)

- If confidence interval does not include 1, this means the association is statistically significant and therefore the p value should be below 0.05.
- A narrow confidence interval emphasises the significance of the result, but it is the p-value that describes significance, not the confidence interval around it.
- If there is no significant P-value given in the question, we can conclude that the association in the question is significant if the 95% confidence interval is very narrow (its range does not include the value 0). (e.g: 95% confidence interval 2 to 8)

Confounding variable

- Is an extraneous variable in a statistical model that correlates (directly or inversely) with both the dependent variable and the independent variable.
- To give a hypothetical example of a confounding variable:
- A study shows that wearing sunglasses and putting on sun cream are linked - increases in sun cream sales are higher when sales of sunglasses increase. It could be that sun cream makes individuals wear sunglasses or that wearing sunglasses reminds people that they need to put on sun cream. However, there is a third "confounding" variable that affects

BOTH sales of sunglasses and sun cream - the weather. It could be that hot, sunny weather makes people both put on sunglasses and apply sun cream.

- **Another example:** In a case-control study on the association between cola drinking and type 2 diabetes => **BMI is likely to be a confounding variable**
- In general, **a randomised controlled trial eliminates confounding** by known and unknown factors.
- **Stratified analysis eliminates the confounding of the stratified data.**
- Multivariable logistic regression can control and minimise confounding by simultaneous adjustment for multiple factors.

Correlation and linear regression

Overview

- The terms correlation and regression are related but are not synonymous.
- Correlation is used to test for association between variables (e.g. whether salary and IQ are related).
- Once correlation between two variables has been shown regression can be used to predict values of other dependent variables from independent variables.
- **Regression is not used unless two variables have firstly been shown to correlate.**

Correlation

- The degree of correlation is summarised by the correlation coefficient (r). This indicates how closely the points lie to a line drawn through the plotted data. In parametric data this is called Pearson's correlation coefficient and can take any value between -1 to +1.
- **The value of ' r ' (coefficient of variation) ranges from -1 to +1**
- For example
 - $r = 1$ - strong positive correlation (e.g. systolic blood pressure always increases with age)
 - $r = 0$ - no correlation (e.g. there is no correlation between systolic blood pressure and age)
 - $r = -1$ - strong negative correlation (e.g. systolic blood pressure always decreases with age)
- Whilst correlation coefficients give information about how one variable may increase or decrease as another variable increases they do not give information about how much the variable will change. They also do not provide information on cause and effect.
- Correlation is summarised when using parametric variables by Pearson's correlation coefficient (represented by a small r).
- In the situation of non parametric variables, Spearman's correlation coefficient is used. Spearman's correlation coefficient is usually represented by the Greek letter ρ (rho), or by r_s .
- In the case of dichotomous variables logistic regression is used.
- Linear (or simple linear) regression is used when looking for association between two continuous variables, and multiple regression is used when looking for association between more than two continuous variables.

Linear regression

- In contrast to the correlation coefficient, linear regression may be used to predict **how much one variable changes when a second variable is changed**.
- A regression equation may be formed, $y = a + bx$, where:

- y = the variable being calculated
- a = the intercept value, when $x = 0$
- b = the slope of the line or regression coefficient. Simply put, how much y changes for a given change in x
- x = the second variable

Correlation coefficient

- The correlation coefficient measures the strength (and direction, if linear) of the relationship between two variables.
- Correlation coefficient does not follow normal distribution.
- Calculation of correlation coefficient does not need to assume normal distribution.
- If there is perfect linear relationship with positive slope between the two variables, the correlation coefficient is 1.
- If there is a perfect linear relationship with negative slope between the two variables, the correlation coefficient is -1.
- A correlation coefficient of 0 means that there is no linear relationship between the variables.
- The correlation is not necessarily linear
- Correlation coefficient describes the linear relationship between two variables. If the relationship between them is not linear, it can be misleading and should not be used.
- **The correlation coefficient does not depend on sample size.** Increasing the sample size will not change the correlation coefficient as its value does not depends on sample size.
- **The correlation coefficient can be a negative number.**
- The correlation coefficient can range from -1 to +1.
- Correlation and regression are different.
 - ⇒ **Correlation** describes how closely two variables are associated.
 - ⇒ **Regression** allows you describe one variable with respect to the other in terms of an equation.

Screening test statistics

Sensitivity = true positives / (true positives + false negatives)

Specificity = true negatives / (true negatives + false positives)

The rule of thumb is that a high sensitivity helps to **rule out** disease (SnOut) and a high specificity helps to **rule in** (Spln) disease (Mnemonic "spin and snout")

Contingency tables

- also known as 2 * 2 tables, are used to illustrate and calculate test statistics such as sensitivity.
- TP = true positive; FP = false positive; TN = true negative; FN = false negative

	Disease present	Disease absent
Test positive	TP	FP
Test negative	FN	TN

The table below lists the main statistical terms used in relation to screening tests:

Measure	Formula	Plain English
Sensitivity	TP / (TP + FN)	Proportion of patients with the condition who have a positive test result
Specificity	TN / (TN + FP)	Proportion of patients without the condition who have a negative test result
Positive predictive value	TP / (TP + FP)	The chance that the patient has the condition if the diagnostic test is positive
Negative predictive value	TN / (TN + FN)	The chance that the patient does not have the condition if the diagnostic test is negative
Likelihood ratio for a positive test result	sensitivity / (1 - specificity)	How much the odds of the disease increase when a test is positive
Likelihood ratio for a negative test result	(1 - sensitivity) / specificity	How much the odds of the disease decrease when a test is negative

Sensitivity and specificity

- Essentially a knowledge of the sensitivity/specificity is based on the disease state itself, whereas predictive values are based on the test result.
- Sensitivity and specificity will not change with sample size. They will change only with:
 - composition of the sample (especially if subjects in the sample have different risks of disease)
 - performance of the test
 - diagnostic threshold, and
 - The "gold standard" to be compared with.
- The reliability of estimates of sensitivity, specificity, positive and negative predictive value will all increase with increasing sample size, which will reduce their confidence intervals.
- Increasing the cut-off of a positive test result will decrease the number of false positives and hence increase the specificity.

Positive and negative predictive values

- Positive and negative predictive values are prevalence dependent.
 - ⇒ The positive predictive value will increase and negative predictive value will decrease if the prevalence of the disease increases.

Likelihood ratios

- Likelihood ratios are not prevalence dependent.
- If the sensitivity increases, the likelihood ratio of a positive test will increase. If the specificity decreases, the likelihood ratio of a positive test will decrease.
- The likelihood ratio of negative test will increase if the specificity of the test is decreased.
- The lower the likelihood ratio of a negative test, the less likely is the presence of disease
- The likelihood ratio of a positive test helps to rule in disease and the likelihood ratio of a negative test helps to rule out disease.

Posterior probability

- Posterior probability = posterior odds / (1 + posterior odds)
 - ⇒ Posterior odds of having disease = prior odds × likelihood ratio.
 - ⇒ Prior odds of having disease = Prevalence(P) / (1 - P)

Precision

- quantifies a tests ability to produce the same measurements with repeated tests.

MRCPUK-part-1-September 2009 exam: What is the correct formula to calculate the negative predictive value of a screening test?

→ $TN / (TN + FP)$

Incidence and prevalence

Incidence is the number of new cases per population in a given time period.

Prevalence is the total number of cases per population at a particular point in time.

- These two terms are used to describe the frequency of a condition in a population.
- The **incidence** is the number of new cases per population in a given time period.
- For example, if condition X has caused 40 new cases over the past 12 months per 1,000 of the population the annual incidence is 0.04 or 4%.
- The **prevalence** is the total number of cases per population at a particular point in time.
- For example, imagine a questionnaire is sent to 2,500 adults asking them how much they weigh. If from this sample population of 500 of the adults were obese then the prevalence of obesity would be 0.2 or 20%.

Relationship

- prevalence = incidence * duration of condition
- in chronic diseases the prevalence is much greater than the incidence
- in acute diseases the prevalence and incidence are similar. For conditions such as the common cold the incidence may be greater than the prevalence

Relative risk

Relative risk ratio (RRR) = EER / CER

- **Relative risk (RR)** is the ratio of risk in the experimental group (experimental event rate, EER) to risk in the control group (control event rate, CER).
 - ⇒ EER = rate at which events occur in the experimental group
 - ⇒ CER = rate at which events occur in the control group
- For example, if we look at a trial comparing the use of paracetamol for dysmenorrhoea compared to placebo we may get the following results

	Total number of patients	Experienced significant pain relief
Paracetamol	100	60
Placebo	80	20

- Experimental event rate, EER = $60 / 100 = 0.6$
Control event rate, CER = $20 / 80 = 0.25$
Therefore the relative risk ratio = EER / CER = $0.6 / 0.25 = 2.4$
- If the risk ratio is > 1 then the rate of an event (in this case experiencing significant pain relief) is increased compared to controls. It is therefore appropriate to calculate the relative risk increase if necessary (see below).
- If the risk ratio is < 1 then the rate of an event is decreased compared to controls. The relative risk reduction should therefore be calculated (see below).
- **The relative risk is always positive**
- **Relative risk reduction (RRR) or relative risk increase (RRI) is calculated by dividing the absolute risk change by the control event rate**
Using the above data, RRI = $(EER - CER) / CER = (0.6 - 0.25) / 0.25 = 1.4 = 140\%$
- **Relative risk reduction = $1 - \text{relative risk}$**

Remember that risk and odds are different. If 20 patients die out of every 100 who have a myocardial infarction, then the risk of dying is $20 / 100 = 0.2$ whereas the odds are $20 / 80 = 0.25$.

Numbers needed to treat and absolute risk reduction

$$\text{NNT} = 1/\text{absolute risk reduction}$$

$$\text{Absolute risk reduction} = (\text{Control event rate}) - (\text{Experimental event rate})$$

- **Numbers needed to treat (NNT)** is a measure that indicates how many patients would require an intervention to reduce the expected number of outcomes by one.
- **Example: if a study for stroke reveals that 20 patients need to be treated to prevent one event.**
- **That means, if you treat a 1000 patients then you will expect to have 50 fewer strokes**

- It is calculated by $1/(Absolute\ risk\ reduction)$
- **Experimental event rate (EER)** = (Number who had particular outcome with the intervention) / (Total number who had the intervention)
- **Control event rate (CER)** = (Number who had particular outcome with the control) / (Total number who had the control)
- **Absolute risk reduction = CER-EER or EER-CER**
- ARR = risk in control group - risk in treated group.
 - ⇒ **For example:** If a drug reduces the incidence of heart attacks from 12% to 8% then:
 - The control event rate (CER) is 12%
 - The experimental event rate (EER) is 8%
 - The relative risk reduction (RRR) is 33% ($[EER-CER/CER] \times 100$)
 - The absolute risk reduction (ARR) is 4% (CER-EER)
 - The number needed to treat (NNT) is 25 ($[1/ARR] \times 100$)

Number needed to harm

- For many studies now, papers quote the number needed to harm. This uses the same principle to establish the difference in absolute risk of an adverse event occurring between two treatment strategies, **calculating a number needed to harm by dividing 100 by the absolute risk.**

Hazard ratio

- The hazard ratio (HR) is similar to relative risk but is used when risk is not constant to time. It is typically used when analysing survival over time
- Example: A study is performed comparing two chemotherapy regimes for patients with small cell lung cancer. The end point of the study is survival time. Which one of the following types statistical measures is it most appropriate to compare survival time with? → Hazard ratio

Odds and odds ratio

Odds - remember a ratio of the number of people who incur a particular outcome to the number of people who do not incur the outcome

NOT a ratio of the number of people who incur a particular outcome to the total number of people

- **Odds are a ratio of the number of people who incur a particular outcome to the number of people who do not incur the outcome.** The odds ratio may be defined as the ratio of the odds of a particular outcome with experimental treatment and that of control.

Odds vs. probability

In contrast, probability is the fraction of times you'd expect to see an event in many trials. When expressed as a single number probability is always between 0 and 1. So, if we take the example of rolling a dice:

- the probability of rolling a six is $1/6$ or 0.166666
- the odds of rolling a six is $1/5$ or 0.2
- Odds ratios are the usual reported measure in case-control studies. It approximates to relative risk if the outcome of interest is rare.

For example, if we look at a trial comparing the use of paracetamol for dysmenorrhoea compared to placebo we may get the following results

	Total number of patients	Achieved = 50% pain relief
Paracetamol	60	40
Placebo	90	30

The odds of achieving significant pain relief with paracetamol = $40 / 20 = 2$

The odds of achieving significant pain relief with placebo = $30 / 60 = 0.5$

Therefore the odds ratio = $2 / 0.5 = 4$

Pre- and post- test odds and probability

Pre and post-test odds

- **Pre-test odds**
 - ⇒ The odds that the patient has the target disorder before the test is carried out
 - ⇒ **Pre-test odds = (pre-test probability/[1 – pre-test probability]).**
- **Post-test odds**
 - ⇒ The odds that the patient has the target disorder after the test is carried out
 - ⇒ **Post-test odds = (pre-test odds x likelihood ratio).**
 - ⇒ the likelihood ratio for a positive test result = sensitivity / (1 - specificity).

Pre and post-test probability

- **Pre-test probability**
 - ⇒ the proportion of people with the target disorder in the population at risk at a specific time (point prevalence) or time interval (period prevalence).
 - ⇒ For example, the prevalence of rheumatoid arthritis in the UK is 1%.
- **Post-test probability**
 - ⇒ The proportion of patients with that particular test result who have the target disorder
 - ⇒ **Post-test probability = (post-test odds/[1 + post-test odds]).**

Screening: Wilson and Junger criteria

1. The condition should be an important public health problem
2. There should be an acceptable treatment for patients with recognised disease
3. Facilities for diagnosis and treatment should be available
4. There should be a recognised latent or early symptomatic stage
5. The natural history of the condition, including its development from latent to declared disease should be adequately understood
6. There should be a suitable test or examination
7. The test or examination should be acceptable to the population
8. There should be agreed policy on whom to treat
9. The cost of case-finding (including diagnosis and subsequent treatment of patients) should be economically balanced in relation to the possible expenditure as a whole
10. Case-finding should be a continuous process and not a 'once and for all' project

R-values

- A **positive R-value** means that as one variable increases, so does the other
- A **negative R-value** means that as one variable decreases, the other increases ie the correlation is inverted (**A negative R-value indicates an inverse association**)
- association or lack of association is indicated by how close the value of R is to zero
- statistical significance is denoted by its **p-value**
- P-values < 0.05 are considered to be significant

Scales of measurement

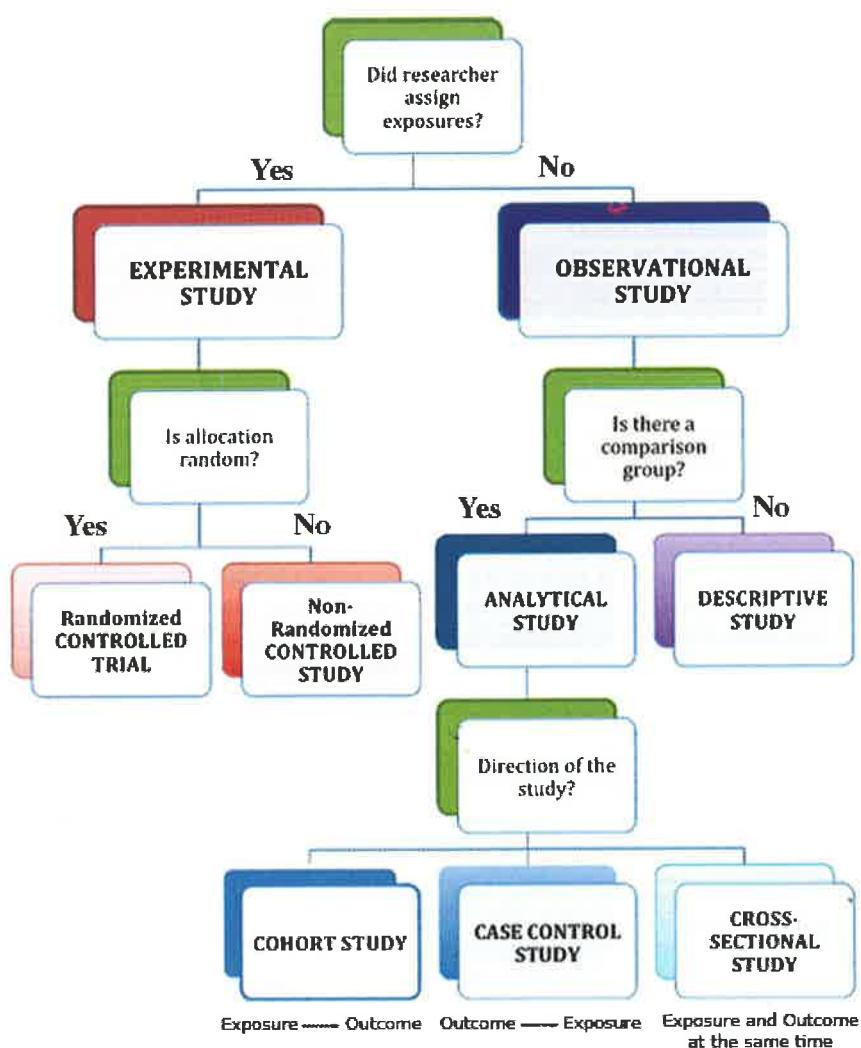
Data always come in one of the four scales of measurement:

Nominal	Data are divided into qualitative groups, such as hot/cold, with no implication of order.
Ordinal	Data are placed in an order (hot/hotter/hottest), although the absolute levels are unknown and no conclusion can be made about the size of the interval.
Interval	dividing a continuous measurement into groups (eg age groups). Data are placed in an order; and the exact value of the measurement is given, usually in measured quantities representing the difference between two measurements (81-90/91-100/101-110 °C). That is, differences between arbitrary pairs of measurements can be meaningfully compared. Ratios between numbers of the scale are not meaningful, so operations such as multiplication and division cannot be carried out directly. But ratios of differences can be expressed; for example, one difference can be twice Another If the measurement scale does not have an absolute zero (ie no numbers exist below the zero) this is called interval data.
Ratio	Here, there is a value of 0 kelvin, and it isn't possible to get below this (ie absolute zero), therefore, the ratio between the values is meaningful, eg 271-280/281-290/291-300 kelvin.

Select Study Design to Match the Research Goals

Objective	Study design
Describe of disease or spectrum	Case series or report Cross sectional study
Determine operating characteristics of a new diagnostic test	Cross sectional study
Describe prognosis	Cohort study
Determine cause-effect	Cohort study Case control study
Compare new interventions	Randomised clinical trial
summarize literature	Meta-analysis

Select Study Design



The following table highlights the main features of the main types of study:

Study type	Key features
Randomised controlled trial	Participants randomly allocated to intervention or control group (e.g. standard treatment or placebo) Practical or ethical problems may limit use
Cohort study	Observational and prospective. Two (or more) are selected according to their exposure to a particular agent (e.g. medicine, toxin) and followed up to see how many develop a disease or other outcome. The usual outcome measure is the relative risk. Examples include Framingham Heart Study
Case-control study	Observational and retrospective. Patients with a particular condition (cases) are identified and matched with controls. Data is then collected on past exposure to a possible causal agent for the condition. The usual outcome measure is the odds ratio. Inexpensive, produce quick results Useful for studying rare conditions Prone to confounding
Cross-sectional survey	Provide a 'snapshot', sometimes called prevalence studies Provide weak evidence of cause and effect

Systematic review (meta-analysis)

Funnel plots - show publication bias in meta-analyses

- a study of studies.
- statistical (quantitative) combination of results from two or more studies addressing the same research question.
- Metanalysis= systematic reviews + Quantitative measures.
- Usually used to treatment studies.
- **A 'meta-analysis' would look at combining all previous data. This is likely to be the quickest option to complete, and also produces the highest level of evidence.**
- **rapid and efficient**
- **Publication bias might be present** (positive results are published more often than the negative ones).
- Publication bias can be examined by funnel plots if a sufficient number of studies is found.
- Non-randomised or other studies may or may not be included.
- However, randomised controlled trials usually have lower risk of bias and hence give us more confidence about validity of results and are preferred primary sources for systematic review.
- Critical appraisal is an important part of systematic review and it has to be objectively performed using well-defined criteria or appraisal tools.

- Meta-analysis, that is, combining results numerically in a statistically appropriate way, though desirable, is not always feasible, depending on the availability of usable data and heterogeneity. (**Meta-analysis is not always performed**)
- The search strategy in systematic review should be comprehensive involving electronic databases and other sources and using well-defined search terms.
- **Case-control studies are not usually included in the search of literature in systematic review**
- The research question is always focused
- there are at least two authors to independently appraise the search results and primary studies.
- It is not mandatory to exclude studies with missing data.
- The effect size should not affect the weight of each study, although it will affect the final result.
- Trial quality is usually not incorporated into meta-analysis nowadays since the weightings can be subjective and arbitrary.
- **The weight of each study should depend on the sample size**
- **Funnel plots**
 - ⇒ show publication bias in meta-analyses
- **Forest plot**
 - ⇒ **The most appropriate way of graphically depicting the results of meta-analysis.**

Fixed vs random effect model for meta-analysis

The fixed effect model	The random effects model
the most commonly used model for meta-analysis. Provides the best estimate of the treatment effect	
attempts to provide one single best estimate of treatment effect.	attempts to find an average treatment effect.
assumes there is no heterogeneity between the trials.	assumes heterogeneity
assumes a single treatment effect	allows multiple treatment effects.

Randomised controlled trial (RCT)

Overview

- **The purpose of randomisation is to prevent systematic differences (bias) between treatment groups.**

Aim: to determine the possible effect of a specific intervention on a given population

Advantages

- Minimizes bias
- Can demonstrate causality

Disadvantages

- Cannot be used to evaluate **rare diseases**

- ⇒ For rare diseases and exposures, **case control studies are the best option.**
Although cohort studies are good for rare exposures, they are not good for rare diseases.
- Cannot be used when treatments have well-known adverse side effects
- Expensive and time-consuming

Uses

- the 'gold standard' for evaluating a new intervention
- May be used to **test an efficacy** of a drug

Study method

- **Randomization:** Study participants are randomly allocated to either the treatment/intervention group or the control group to ensure that both groups have approximately the same baseline characteristics.
- **Blinding:** the practice of not informing an individual or group about which study participants are part of the control group and which are part of the treatment group (used to reduce bias)
- **Classic errors in randomisation**
 - ⇒ Consecutive sampling, which may well not be representative if the study time is short.
 - ⇒ Convenience sampling: strong potential for bias, with volunteers generally healthier than others.
 - ⇒ Judgmental sample: including those that you want only. The potential for systematic error is enormous.

Methods of analysis for randomized controlled trials

- **Intention to treat analysis (ITT)**
 - ⇒ Intention to treat analysis is a method of analysis for **randomized controlled trials** in which all patients randomly assigned to one of the treatments are analysed together, regardless of whether or not they completed or received that treatment.
Include the patients who drop out in the final data set
 - ⇒ Intention to treat analysis is done to avoid the effects of crossover and drop-out, which may affect the randomization to the treatment groups.
 - ⇒ **ITT** helps to reduce bias by sticking to the original allocation of treatment and analysing the patient in that treatment group even if they do not receive the treatment
 - ⇒ **ITT** is considered to be the analysis which is least subject to bias. **Considered the most robust**
- **Per protocol analysis**
 - ⇒ A **per protocol analysis** may exclude patients who suffered an event but then did not follow the protocol accurately, for example, a patient treated with the diabetes agent who was admitted to hospital, but missed one to two doses of medication.

Observational study

- Disadvantages

⇒ From association in an observational study, we cannot infer cause and effect

Biological assays

- Biological assays are designed to measure the relative potency of different preparations.

Sequential trial

- a trial in which the data are analysed after each participant's results become available and the trial continues until a clear benefit is seen in one of the comparison groups, could also be used to assess efficacy, but there would have to be a large expected difference from placebo.
- 'Sequential' trial would be comparing one therapy to another sequentially (usually with wash out periods in between).

Crossover trial

- ⇒ The principle of a crossover design is that a patient has one drug or treatment, then a washout period, and then another drug, and the effect is compared between the two in a single individual.
- ⇒ For this reason it is a good **study design for treatment of chronic conditions (eg: comparing analgesics in arthritis)** but not appropriate for acute conditions.

- In a crossover trial, the patient (who usually has a chronic stable disease) receives one drug (or placebo) and then the other drug after a washout period
- Each patient will usually receive all drugs within the study
- In this way, confounding can be greatly reduced
- If a drug had long-lasting effects it may not be easy to see which of the trial drugs was having an effect
- A self-limiting illness is difficult to study in this way
- Because each person is acting as their own control, it is usually possible to use smaller numbers to get the same power.
- If any treatment in a cross-over trial is a disease-modifier (in the most extreme case, kills or cures the patient), then the interpretation of results in any subsequent period becomes impossible. This is because disease modification implies that one course of the drug will permanently change the future timecourse of that patient's disease in some way, making a cross-over study un-interpretable. In this case a parallel trial is the only appropriate option.

Sampling

- Sampling error arises when only a portion of the population is studied
- **Random sampling** implies that the sample has been selected from a sampling frame in such a way that every individual has the same chance of being selected
- **The standard error of the mean** is the standard deviation divided by the square root of the sample size, hence it must always be smaller than the standard deviation
- **Inference** is the process of drawing conclusions about the population using the sample information
- **a sample statistic is a point estimate of a population parameter**

Bias (Systematic error)

Definition

- An error in the study design or the way in which the study is conducted that causes systematic deviation of findings from the true value

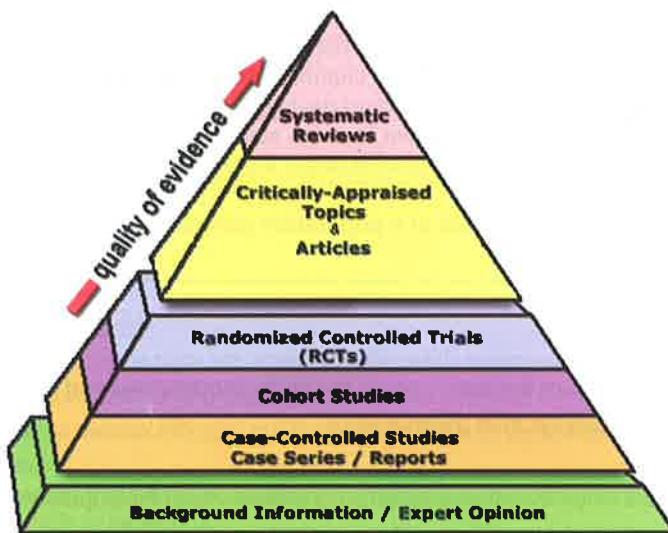
Types

- **Selection bias** occurs when the study population is different from the population to whom the results will be applied and there is therefore said to be
- **Allocation bias** occurs when patients are not randomly assigned to a particular treatment.
- **Assessment bias** occurs when the observer knows which treatment the subject is taking.
- **Observer bias** is when one observer consistently under or over reports a particular variable.
- **Recall bias** applies to case-control studies when a patient is more likely to remember a particular detail of exposure if they go on to develop the disease.

Study design: evidence and recommendations

Levels of evidence

- Ia - evidence from meta-analysis of randomised controlled trials
- Ib - evidence from at least one randomised controlled trial
- IIa - evidence from at least one well designed controlled trial which is not randomised
- IIb - evidence from at least one well designed experimental trial
- III - evidence from case, correlation and comparative studies
- IV - evidence from a panel of experts



Grading of recommendation

- Grade A - based on evidence from at least one randomised controlled trial (i.e. Ia or Ib)
- Grade B - based on evidence from non-randomised controlled trials (i.e. IIa, IIb or III)
- Grade C - based on evidence from a panel of experts (i.e. IV)

Study design: new drugs

Superiority trial → a large sample size is required to demonstrate a significant difference

When a new drug is launched there are a number of options available in terms of study design. One option is a placebo-controlled trial. Whilst this may provide robust evidence it may be considered unethical if established treatments are available and it also does not provide a comparison with standard treatments.

Compare a new drug to an existing treatment

- The statistician need to decide whether the trial is intended to show superiority, equivalence or non-inferiority:
- Superiority**
 - one problem is the large sample size needed to show a significant benefit over an existing treatment
- Equivalence**
 - an equivalence margin is defined (-delta to +delta) on a specified outcome. If the confidence interval of the difference between the two drugs lies within the equivalence margin then the drugs may be assumed to have a similar effect

- **Non-inferiority**
 - ⇒ similar to equivalence trials, but only the lower confidence interval needs to lie within the equivalence margin (i.e. -delta).
 - ⇒ Small sample sizes are needed for these trials.
 - ⇒ Once a drug has been shown to be non-inferior large studies may be performed to show superiority
- It should be remembered that drug companies may not necessarily want to show superiority over an existing product. If it can be demonstrated that their product is equivalent or even non-inferior then they may compete on price or convenience.

Phases of new drug development

phase	goal	notes
Animal trial	Safety for testing the drug in humans	
Phase I	<ul style="list-style-type: none"> Initial safety <ul style="list-style-type: none"> most frequent side effects How the drug is metabolized and excreted. 	<ul style="list-style-type: none"> conducted in healthy volunteers. The number of subjects ranges from 20 to 80.
Phase II	Effectiveness (RCTs)	The number of subjects ranges from a few dozen to about 300.
Phase III	Comparative efficacy (Effectiveness compared to commonly used treatment)	<ul style="list-style-type: none"> The number of subjects ranges from several hundred to about 3,000 The best study for phase 3 is a randomised control study.
Phase IV (post marketing)	Side effects	Enrolls a large number of patients, typically several thousands.

Graphical representation of data

Charts and diagrams

Quantitative data	Qualitative data
Histogram Scatter diagram	Bar diagram Pie diagram

The interpretation of novel findings in a published clinical research study

- The trustworthiness of a study should depend solely on its scientific validity, that is, whether it is free of bias.
- **The conclusion should be treated with skepticism even if it is extensively peer-reviewed**