Haematology

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Haemostasis & Thrombosis

- haemostasis & thrombosis inter-related & dependant on:
  - endothelium
  - platelets
  - coagulation cascade

- haemostasis =
  - physiologic process
  - maintain blood in a fluid, clot free state in norm vessels
  - can produce rapid localized plug at site of inj if required

- thrombosis =
  - pathological
  - inappropriate activation of haemostatic mechanisms in
    - uninjured vessels
    - thrombosis in minor injury

Normal Haemostasis

- following injury:
  - arteriolar vasoconstriction:
    - reflex neurogenic mechanism
    - augmented by local secretion of endothelin
    - effect only transient
    - stops exsanguination in massive injury
    - slows flow to allow platelet & coag cascade to initiate
  - platelet adhesion & activation:
    - subendothelial ECM exposed which highly thrombogenic
    - platelets adhere
    - platelets activate = change shape, release secretory granules
    - platelet aggregation ⇒ plug
    - procoagulant activity
      - primary haemostasis
  - activation of coagulation cascade
    - driven by tissue factor:
      - membrane bound procoagulant lipoprotein
• synthesized by endothelium & exposed after injury
  • culmination of cascade = activation of thrombin
  • thrombin:
    • fibrinogen to insoluble fibrin ⇒ fibrin deposition
    • further platelet aggregation & granule release
  ⇐ secondary haemostasis
  o activation of counter-regulatory mechanisms eg t-PA which restrict clot to specific site

Platelets
• Platelets activated once contact with ECM beneath injured ECs
• Activation:
  o Adhesion [no ATP required]
  o Shape change [active process]
  o Secretion (release reaction) [active]
  o Aggregation

Adhesion
• Mediated through vWF
• Bridges gap between platelet receptors (mostly glycoprotein Ib) & exposed collagen
  ⇐ cofactors serum V & IX
• Other adhesion reactions but vWF only one strong enough to overcome shear force of blood flow
• Deficiency vWF = vW disease
• Deficiency GpIb receptor = Bernard-Soulier Syndrome

Platelet Granule Activation/Secretion
• Both granules release shortly after adhesion
• Alpha granules contain:
  o P-selectin = adhesion molecule on their membranes
  o Contain fibrinogen, fibronectin, factor V, VIII, PDGF, transforming growth factor B
  o vWF
• Dense bodies contain:
  o ADP & ATP
  o Ionized Ca
  o Histamine
  o Serotonin
  o Adrenaline
• Dense body release imp:
  o Ca required in coagulation cascade
  o ADP =
    • Potent ↑ platelet aggregation
    • ↑ ed release of ADP from other platelets
• platelet activation ⇒ surface expression phospholipid complexes:
  o nucleation & binding site for Ca & clotting factors in intrinsic coag cascade

Platelet Aggregation
• stim of aggregation =
  o ADP
  o Thromboxane A2 – from platelets
  ⇐ together ⇒ autcatalytic reaction ⇒ aggregating platelets ⇒ primary plug
• Primary plug = reversible
• Thrombin from coag cascade binds to PAR (platelet surface receptors)
  ⇐ further potentiates aggregation while also creating fibrin ⇒ cementing plug in place
At same time platelet contraction ⇒ viscous metamorphosis
↓ irreversible definitive secondary plug

∴ thrombin essential for thrombi

noncleaved fibrinogen also imp cofactor in aggregation:
  o ADP activation ⇒ change in conformation of platelet GpIIb-IIIa receptors to allow fibrinogen to bind
  o Fibrinogen binding ⇒ connection of multiple platelets ⇒ large aggregates
    ↓ GpIIb-IIIa deficiencies ≈ Glanzmann thrombasthenia bleeding disorder & therapeutic target

Erythrocytes & leukocytes also aggregate in haemostatic plugs:
  o Leukocytes adhere via P selectin ⇒ contribute to inflam response

Summary Platelet Effects

Adhere to ECM at site of endothelial injury ⇒ activated

On activation:
  o Secrete granules eg ADP
  o Synthesise TxA2

Platelets expose phospholipid complexes which imp in intrinsic coag pathway

Injured or activated ECs expose tissue factor ⇒ extrinsic coag pathway

ADP ⇒ formation of primary plug

Primary plug converted to secondary plug by
  o ADP
  o Thrombin
  o TxA2

Fibrin deposition stabilises & anchors the aggregated platelets

PGI2 & TxA2

PGI2 =
  o Endothelium derived
  o VD
  o Inhibit platelet aggregation

TxA2=
  o Platelet derived
  o VC
  o Activates aggregation

Aspirin blocks COX pathway ⇒ ↓TxA2 synthesis ⇒ ↓aggregation
Endothelium
• endothelium modulate opposing factors of haemostasis

Antithrombotic Properties
• essential to localise coagulation to where is a problem ie where original platelet plug was formed
• occurs by:
  o cascade of reactions limited to where platelets adhered
  o series of inhibitors which restrict coag to site of injury:
    ▪ circulating factors eg antithrombin & heparin molecules
    ▪ endothelium derived factors eg TFPI
    ▪ thrombomodulin system
      ⬤ all described below
• antiplatelet effects:
  o non activated platelets do not adhere to endothelium
  o Endothelial cells secrete:
    • PGI₂ (endothelial prostacyclin) & NO:
      ▪ Inhibit activated platelets from adhering to surrounding uninjured endothelium
      ▪ Potent VDs
      ▪ Inhibit aggregation
      ▪ Synthesised by endothelial cells
      ▪ Synthesis ↑ed by factors from coagulation cascade ie thrombin & cytokines
    • Adenosine diphosphatase:
      ▪ Degrades ADP ∴ inhibits platelet aggregation
• Anticoagulant effects:
  o Effects mediated by:
Heparin like molecules:
- Membrane associated
- Interact with antithrombin III ⇒
  - inactivate thrombin & other factors (serine proteases) eg factor 9,10,11,12
  - why heparin useful to minimise thrombosis

Thrombomodulin:
- Specific endothelial thrombin receptor binds to thrombin
- Converts it from procoagulant to anticoagulant which can activate protein C
- Activated protein C ⇒ cleavage of factor Va & VIIIa ⇒ inhibit clotting
  - factor V mutation ⇒ resistance to activated protein C ⇒ ↑thrombosis
- Inactivates inhibitor of t-PA activator (ie ↑tPA action)
- Protein C & S = Vit K dependant proteins
- Thrombomodulin mops up circulating thrombin preventing unwanted clots

Tissue factor pathway inhibitor:
- Secreted by ECs (and others)
- Cell surface protein that complexes & inhibits
  - activated tissue factor
    - factor VIIa
    - factor Xa

Fibrinolytic effects:
- endothelial cells synthesise tissue-type plasminogen activator (tPA)
  - ⇒ ↑fibrinolytic activity ⇒ clear fibrin deposits from endothelial surfaces

Prothrombotic Properties
- platelet effects:
  - endothelial presence of vWF
    - not specifically synthesised post inj, but is always there
    - vWF = cofactor for platelet binding to collagen & other surfaces
- procoagulant effects:
  - tissue factor induced by:
    - bacterial endotoxin
    - cytokines eg TNF, IL1
  - tissue factor ⇒ activates extrinsic clotting cascade
  - endothelium binds IXa, Xa ⇒ ↑clotting cascade
- Antifibrinolytic Effects:
  - ECs secrete PAIs (inhibitors of plasminogen activator) ⇒ ↓fibrinolysis
Coagulation

- 2 theories of secondary haemostasis:
  - classic coagulation cascade
  - cell based theory of coagulation

Classic Coagulation Cascade

- Old concept of extrinsic & intrinsic pathway now valid only in vitro
- In vivo theory:
  - Initiation ⇒ amplification ⇒ propagation ⇒ stabilisation
- = conversion of inactive proenzymes ⇒ activated
- culminates generation insoluble fibrin

Focus on common pathway of serine proteases:

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Cell Based Theory of Coagulation

Initiation
• clotting initiated by events similar to extrinsic pathway
• cells (not in blood vessels walls) possess tissue factor:
  o not found in vasc endothelium cells or free circulation
  o = glycoprotein which transmembrane
• initiation when these cells exposed to c irculation coagulation proteins ie vasc endothelium disrupted
• Factors 7, 9, 10 generate priming amount of thrombin
• Thrombin:
  o Responsible for initiation of coag process proper
  o Activation platelets
  o ↑assembly of coag factors on platelet surface

Amplification
• currently not enough thrombin generated to adequately trigger enough cleavage of fibrinogen to fibrin
• amplification involves feedback mechanisms:
  o factor 7 +ve feedback loop
  o cofactor 5 & 8 +ve feedback look to cleave more thrombin from prothrombin
  o activation of F11 & F9

Propagation
• On surface of activated platelets:
  o Ca used as co factor to ↑production of factor 10
  o Factor 5 forms prothrombinase ⇒ rapid thrombin creation
• Ultimately thrombin ⇒ cleaves fibrinogen to fibrin

Stabilisation
• Need to stabilise clot
• Fibrin creation ⇒ max thrombin generation
• Thrombin then activates:
  o factor 13 ⇒ cross link soluble fibrin to stabilise matrix
  o thrombin-activateable fibrinolysis inhibitor (TAFI) which maintains clot stability

Factors
• factors (2,7,9,10,11,12) circulate in plasma as inactive precursors
• activated factors = proteases
• a reaction results from assembly of a complex held together by Ca ions on a phospholipid complex (generally on activated platelet surface)
• composition of reaction:
  o enzyme = activated coagulation factor
  o substrate = proenzyme form of coag factor
  o cofactor = reaction accelerator
• ∴clotting remains localised to site assembly possible eg activated platelet or endothelium
Thrombin
• thrombin - effects
  o effects in final stage of coag cascade
  o wide variety of effects on local vessels & inflam via:
    ▪ binding to PARs (protease activated receptors)
      • belong to 7 transmembrane G protein coupled receptor family
      • thrombin clips extracellular end of receptor ⇒ tethered peptide ⇒ binds rest of receptor ⇒ conformational change of receptor ⇒ activate assoc G protein
        ⇐ : thrombin autocatalyses receptor which explains small amount of thrombin ⇒ big effect

Factor 8
• = large protein made of 2 components:
  o larger = F8R:AG component:
    ▪ platelet adhesion to exposed subendothelial connective tissue
    ▪ platelet aggregation
    ▪ vWF binding (F8:WF)
  o smaller F8:C
    ▪ non covalently bound to larger component
• activated by thrombin
• F8a stabilises fibrin polymer by introducing Glu-Lys isopeptide bonds between adjacent fibrin monomers

Fibrinogen
• = f2
• 3 pairs of polypeptide chains: alpha, beta, delta
• cross linked by S-S bonds
• thrombin releases fibrinopeptide A + B from α & β chains ⇒ fibrin monomer
• fibrin monomer = cross linked alpha, beta delta chains
• fibrin polymer = after spontaneous hydrogen bonds between molecules of monomer

Calcium
• essential cofactor in:
  o factor 8
  o factor 5
  o factor 13 – soluble fibrin ⇒ insoluble fibrin
• in-vivo serum Ca never get low enough to prevent coagulation as will arrest prior to this
• citrate toxicity ie hypocalcaemia: citrate in transfused blood rapidly converted to HCO₃ in liver
• might need to give CaCl if prolonged QT or ST segment changes

Von Willebrand Factor
• = large multimeric plasma protein
• actue phase protein ⇒ ↑ed stress & surgery
• produced by:
  o endothelial cells ⇒ stored in Weibel-Palade bodies
  o megakaryocytes ⇒ stored in platelets α granules
• functions:
  o adhesive protein:
    ▪ main function
    ▪ platelet adhesion to subendothelium:
      • vWF from Weibel Palade bodies bind to exposed collagen & act as middle man to allow platelet attachment
      • vWF exposes sites which can bind glycoprotein 1B of platelet
      • ∴ coating of platelets over damaged area
    ▪ or to another platelet:
      • ↑VWF binding to ↑complex GP 2b:3a in platelet membrane ⇒ plt:plt adhesion
  o protect factor 8:
    ▪ circulates in plasma bound to F8 (F8R:AG)
    ▪ prevents it from degradation by eg activated protein C

Anticoagulants
• vitamin K dependant clotting factors = 2, 7,9,10, protein C & S
Fibrinolysis

This system works on top of factors already present to inhibit thrombosis
- amplification system for limitation of clot size & dissolution of stable fibrin
- fibrinolysis = breakdown of fibrin by proteolytic enzymes
- plasminogen activated to ⇒ plasmin = key serine protease involved
- Fibrinolytic cascade:
  - Plasmin generated from:
    - factor XII dependant pathway OR
    - bacterial product of streptokinase OR
    - plasminogen activators – 2 types:
      - u-PA (urokinase-like PA)
        - present in plasma & tissues
        - activates plasmin in fluid phase
        - uses amplification loop
      - t-PA (tissue-type PA)
        - most impt
        - synthesised by endothelial cells
        - most active when attached to fibrin
        - affinity for fibrin means targeted to site recent clot
  - Plasmin actions:
    - cleave fibrin & interferes with its polymerization ⇒ fibrin degradation products (also act as weak anticoagulants)
    - trigger complement cascade
    - plasmin then released into circulation again
    - [DIC = excess of free plasmin ⇒ large amount of D Dimer ⇒ activate factor 5 & 8]
  - Functional plasmin activity restricted to site of thrombosis by:
    - t-PA activates plasminogen most effectively when bound to fibrin meshwork via lysine binding sites
    - free plasmin rapid neutralized by serum a₂-anti-plasmin
• endothelium further modulates anticoag by
  o releasing PAIs (plasminogen activator inhibitors)
    \( \uparrow \) block fibrinolysis by inhibiting t-PA binding to fibrin
  o PAI release:
    \( \uparrow \) ed by:
      • Thrombin
      • Cytokines – why severe inflam \( \Rightarrow \) intravascular thrombosis
    \( \downarrow \) ed by:
      • protein C
• variations in fibrinolysis responses:
  o more active in arterial circulation & deep veins, upper limbs
  o pregnancy:
    \( \uparrow \) fibrinogen & plasminogen levels
    \( \downarrow \) t-PA, \( \alpha_2 \)-plasma inhibitor
    \( \downarrow \) overall fibrinolysis is reduced
  o neurohormonal stress (corticosteroids, catecholamines, ADH) \( \Rightarrow \) \( \uparrow \) transient \( \uparrow \) fibrinolysis
  o venous occlusion \( \Rightarrow \) \( \uparrow \) fibrinolysis – explaining MOA of calf squeezers preventing DVT

**Thrombosis**
• inappropriate activation of clotting in uninjured vasculature or thrombotic occlusion following only minor inj
• Virchow’s triad:
  o Endothelial inj
  o Stasis or turbulent flow
  o Blood hypercoagulability

**Endothelial Injury**
• Clotting caused by:
  o Exposed subendothelial ECM & tissue factor
  o Adherence of platelets
  o Imbalance of clotting factors
    \( \downarrow \) PGI2, t-PA
    \( \uparrow \) PAI, \( \uparrow \) platelet adhesion molecules
• can cause thrombosis just be self
• injury following:
  o haemodynamic stress eg HTN, turbulent flow over scarred valves
  o bacterial endotoxins
  o homocystinuria
  o HCL
  o Radiation
  o Smoke

Flow Problems
• Norm flow = laminar:
  o Cells flow in centre of lumen
  o Outside clear plasma zone
• turbulence ⇒ eddy currents with pockets of stasis
• stasis ⇒
  o platelets into contact with endothelium
  o prevent dilution of activated clotting factors
  o ↓inflow clotting inhibitors
  o ↑endothelial cell activation
• stasis predominates:
  o venous circ
  o cardiac chambers eg mitral valve stenosis & AF ⇒ dilated L atrium
  o arterial aneurysms
• turbulence:
  o arteries
  o direct ⇒ endothelial inj & dysfunction
• hyperviscocity syndromes or deformed rbcs ⇒ small vessel stasis ⇒ ↑risk thrombosis
  ◗ eg polycythaemia or sickle cell anaemia
**Blood**

- Blood =
  - 8% body weight
  - 5.6L in 70kg man
  - 55% of this volume = plasma

**Haemopoiesis**

- Pluripotential haemopoietic stem cells (PHSC) ⇒
  - Rbcs
  - Leucocytes
  - Platelets

- Order of organs being haemopoietically active:
  - Primitive erythroblasts 1st cells to develop in yolk sac – 2-4 weeks
  - Liver (& spleen) become – 6w – 7 months
  - BM – start at 6-7 months ⇒ 5yrs old:
    - Rbc made almost exclusively here
    - BM progressively replaced with fat in long bones until 18-20yrs
    - >20yrs confined to BM in central skeleton & prox humerus/femur

**White Blood Cells**

- granulocytes most numerous of Wbcs
  - differentiate into neuts, eosinophils, basophils – horseshoe nuclei
- lymphocytes – large round nuclei
- monocytes – kidney shaped nuclei
Platelets
- megakaryocytes \(\Rightarrow\) platelets
- no nuclei
- 60-75% circulate; rest stay in spleen
\[\Rightarrow::\text{spleenectomy} \Rightarrow \uparrow\text{serum platelet count}\]
- half life 4d

RBCs
- lose nuclei before entering circ
- av survival 120d
- each adult man = 900g haemoglobin

Production
- proerythroblast \(\Rightarrow\) series smaller normoblasts – over 5 days
- erythroblast progressively:
  - contain more Hb
  - nuclear chromatin condenses
- eventually pyknotic nucleus removed from erythroblast \(\Rightarrow\) reticulocyte
- reticulocyte =
  - 1st rbc to enter circulation
  - last 1-2 days
  - contains some RNA
  - can synthesis Hb
  - mature into rbc when RNA lost
- production regulated by EPO:
  - half life 6-9hrs
  - 90% made in kidney, 10% in liver
  - \(\uparrow\)rate of differentiation of stem cell \(\Rightarrow\) \(\uparrow\)production
- final maturation of rbc requires vit B12 + folate :
  - needed for DNA synthesis
  - deficiency = large fragile rbc with short half life
- mature rbc survive ~120 in circulation
- removed by phagocytosis in RES – chiefly spleen & BM

Structure
- biconcave disc 7.5um wide, 2um thick
- large surface area:volume to promote gas diffusion
- v deformable & can squeeze through microvessels
- rbc cell membrane = lipid bilayer containing:
  - structural proteins
  - contractile
  - enzymes
  - surface antigens
  - CHO only preset on external surface
- 4 major proteins form lattice on inner side of rbc membrane – impt in keeping biconcave shape

Hb Production
- Hb =
  - Iron containing porphyrin (metalloprotein)
Haematology

By Adam Hollingworth

- Mw ~65 kD
- Made of 4 polypeptide globin subunits & 4 haems:
  - Each subunit contains a heme conjugated to polypeptide (=globins)
    - \( \therefore = 4 \) (2 pairs) polypeptide chains in each haemoglobin

- Haem:
  - \( = \) iron-porphyrin compound. Norm in Fe++ (ferrous state)
  - Synthesis in mitochondria with series of reactions:
    - Condensation of glycine + succinyl CoA
    - \( \Rightarrow \) protoporphyrine combines + Fe++ = haem

- Globin chains = Formed in ribosomes
  - \( \therefore \) Hb = tetramer of 4 globin chains, each with own haem in a hydrophobic pocket

- Binding:
  - \( \text{O}_2 \Rightarrow \text{O}_2 \)
  - \( \text{Globin} \Rightarrow \text{CO}_2 \) & H

- In normal adult blood
  - 97.5% = Haemoglobin A – \((\alpha_2\beta_2)\):
    - 1 pair \( \alpha \) chain
    - 1 pair \( \beta \) chain – note \( \beta \) production starts after birth (see HbF)
  - 2.5% = Haemoglobin A2 \((\alpha_2\delta_2)\) (alpha, delta)
    - Also see small amounts haemoglobin A derivatives eg HbA\(_{1c}\)
    - Glucose added to terminal valine in each \( \beta \) chain

- Fetal = Hb F \((\alpha_2\gamma_2)\) (alpha, gamma)
  - Norm replaced by Hb A soon after birth
    - Switching related to O2 availability
  - Binds less 2,3DPG \( \therefore \) ↑affinity for O2 which allows O2 to move mum ⇒ fetus in placenta
    - DPG prefers B chains to Gamma chains \( \Rightarrow \) L shift OHDC

- Chromosome location for globin genes:
  - Chromosome 16 = \( \alpha \)
  - Chromosome 11 = \( \beta, \gamma, \delta \) chains

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[Diagram: Development of human hemoglobin chains]
Functions of Hb
- O2 carrier:
  - O2 loading exhibits positive cooperativity:
    - $\alpha_1\beta_1$ & $\alpha_2\beta_2$ contacts stabilise Hb molecule as O2 reacts with it
    - reaction of O2 with each subunit occurs sequentially with each facilitating the next
    - $\therefore$ ↑ing affinity as O2 loads $\Rightarrow$ sigmoid OHDC
    - myoglobin only has 1 subunit thus OMDC curve = rectangular
  - O2 unloading – vice versa:
    - $\beta$ chains pulled apart
    - 2,3-DPG enters molecule $\Rightarrow$ ↓affinity of Hb for O2
- buffering functions – see renal acid base section

RBC Metabolism
- rbc lacks mitochondria
- can generate ATP via anaerobic glycolytic pathway (Embden-Meyerhof):
  - generates:
    - 2ATP for each glucose $\Rightarrow$ lactate:
      - ATP used Na/K/ATPase to keep shape, volume, flexibility
      - NADH – needed by methaemoglobin reductase to reduce metHb $\Rightarrow$ Hb

Synthesis & Destruction of Hb
- Hb content all in red cells
  - man 16g/dl
  - woman 14g/dl
- man has 900g Hb
- destroyed:
  - 0.3g /hr
  - $\sim$50ml/day
  - 0.8% destroyed/day
  - $\sim$3 million rbc/second
- glycolysis ↓s with ↑age of rbc $\Rightarrow$ ↓ATP $\Rightarrow$ ↓cellular integrity
- old rbc's destroyed by macrphages (mainly in spleen):
  - globin portion split off $\Rightarrow$ amino acids $\Rightarrow$ re-enter aa pool
• heme $\xrightarrow{\text{heme oxidase}}$ biliverdin + CO
• biliverdin $\Rightarrow$ bilirubin $\Rightarrow$ bound to albumin $\Rightarrow$ liver
• in liver bilirubin conjugated with gluronic acid $\Rightarrow$ excreted in bile
• in GIT bili converted to stercobilin $\Rightarrow$ some reabsorbed $\Rightarrow$ excreted in urine as urobilinogen
• iron from heme reused for Hb synthesis
  • white light on skin: bilirubin $\Rightarrow$ lumirubin $\uparrow$ has shorter half life
  • without enough iron $\Rightarrow$ $\downarrow$Hb production $\Rightarrow$ iron deficiency anaemia

Iron Metabolism
• Hb contains 65-70% total body iron
• Myoglobin contains 5%
• transferrin transports iron in plasma:
  o binds 2 atoms of ferric iron (Fe$^{3+}$) / molecule
  o gets iron from RES ie destroyed rbc's or GIT
  o norm 30% saturated with iron
• dietary iron found in form of:
  o haem-protein
  o ferric protein complexes
  o ferric hydroxide
• ~10-15mg iron/days food
• 10% of this absorbed:
  $\uparrow$ in preg or iron deficiency states
• absorbed mainly in duodenum:
  o $\uparrow$absorption = gastric acid, reducing agents (keeps iron in ferrous state)
  o $\downarrow$absorption = alkali, chealting agents eg phosphates
• soluble iron enters mucosal cells in ferrous state $\Rightarrow$ portal circulation bound to transferrin
• iron storage sites:
  o liver
  o spleen
  o BM
• Stored as:
  o 65% ferritin – water soluble
  o 35% haemosiderin – insoluble
• iron losses:
  o 0.5-1g iron lost/day in faeces from desquamated GIT epithelial cells
  o urine, hair, sweat (small)
  o menstruation
  o foetus in pregnancy

Haemoglobin Reactions
• Hb + O2 $\Rightarrow$ oxyhaemoglobin
  $\downarrow$attaches to the Fe$^{2+}$ in the heme
• $\uparrow$affinity of Hb for O2:
  o $\downarrow$temp
  o $\downarrow$2,3-DPG
• $\downarrow$affinity:
  o $\uparrow$2,3-DPG
• ↑ temp
  • ↑ H+
  by shifting the position of the 4 peptide chains (quaternary structure)
  • methaemoglobin =
    • drugs & oxidising agents effect blood: Fe²⁺ ⇒ Fe³⁺
    • leads to dusky cyanosis
    • NADH system converts methaemoglobin ⇒ Hb
  • Carboxyhaemoglobin =
    • CO and Hb
    • CO has much higher affinity for Hb than O₂ thus displaces O₂
Blood Types

RBC Antigens
- 400 rbc antigens known
- inherited simple Mendelian fashion
- major antigens=
  - ABO
  - Rh
- Other antigens less impt:
  - Weak antigens & antibodies only develop after multiple exposures or cold temperatures (cold agglutinins (aka antibodies)
- people produced antibodies to antigens they don’t have ie they express self tolerance of their own antigens. Failure of this system = haemolysis
- role of antigens is unknown

RBC Antibodies
- naturally occurring when lack corresponding antigen
- most impt = ABO
- ABO antibodies develop >3months age
- Natural antibody creation gp A & B antigens enter body via bacteria & food ⇒ antibody creation
  - usually IgM, reactive at 37deg C but optimal reactivity at 4deg
- Immune antibody creation occurs:
  - Trans-placental passage of antigens – only IgG can get across. Most impt = Rh antibody (antiD)
  - Transfusion
  - IgG = react optimally at 37deg

ABO System
- Antigen – on rbc cell
  - also found in plasma, saliva, gastric juice, tears, bile (not CSF)
  - unlike Rh which only on rbcs
- Antibodies – in blood serum
- Transfusion of packed red cells = transfusion of cells not serum
- ABO system named after antigens on rbc cell
- Varieties & frequency (Caucasian) of blood types – named after antigens
  - A = A antigen; anti B antibody (45%) ⇒ give A or O
  - B = B antigen; anti A antibody (10%) ⇒ give B or O
  - AB = A & B antigen; no antibody (4%) ⇒ give anything
  - O = have no antigens; anti A & B antibodies (43%) ⇒ give O only
  - thus O = universal donor; AB = universal recipient
- Antigens in intestinal bacteria & food very similar to agglutinins
  - thus: soon develop antibodies to antigens not already in their own blood

Transfusion Reactions
- Plasma in donor transfusion of packed red cells is extremely diluted once placed inside recipient
  - thus any antibodies don’t significantly activate onto against host rbcs antigens
but if recipients plasma has antibodies against donor rbcs ⇒ agglutinate & haemolyse ⇒ free Hb into plasma

- Transfusion reaction vary
  - minor ↑ bilirubin
  - severe jaundice
  - renal tubular damage ⇒ anuria ⇒ death

**Inheritance ABO System**

- autosomal dominant inheritance:
  - phenotype B: genotype BO or BB
- thus both parents B – can have children:
  - BB
  - BO
  - OO
  - can use this to say a child is not a fathers, but not to prove he is

**Other Antibodies**

- Exist many other rbc antibodies eg Rh, Duffy etc

**Rh System**

- Named after rhesus monkey
- C, D, E antigens only on rbcs
- D is the most antigenic and most common ~85%
- Rh antibodies =
  - Rarely occur naturally:
    - anti C & anti E
    - but no natural anti D exists
  - Usually
    - Immune created,
    - Warm
    - IgG in origin ie can cross placenta (actively)
- Problem when Rh–ve mother exposed to fetal Rh +ve blood in 1st pregnancy:
  - Needs D antibody (antiD) <72hrs to mop up/destroy Rh D+ antigens which could have crossed placenta/entered maternal circulation
  - ⇒ this prevents formation of maternal antiD IgG which would cause haemolysis of next pregnantys Rh+fetus (erythroblastosis fetalis)
  - haemolysis ⇒ death in utero, kernicterus, anaemia, jaundice, hyrdops fetails
  - bilirubin depositioned in basal ganglia
- 85% whites = Rh +ve
- 99% Asians Rh +ve

**Other Blood Groups**

- clinically less imp
  - P, Lewis, MN systems:
    - Naturally occurring antibodies only react at low temps
    - Antigens low antigenicity
  - Kell system:
    - 3rd most imp after ABO, Rh
    - k antigen:
present on rbcs, WBCs, platelets
is immunogenic but low frequency ∴ only impt if multiple transfusions

**Anaemia**

- Anaemia is deemed as a reduction in red cell mass below the normal range.
- The normal range varies with age, sex, environment and pregnancy

**Physiological consequences of acute and chronic anaemia.**

- Acute blood loss ⇒
  - rapid fluid shift from the interstitial compartment to the intravascular compartment. 
    - usually supplemented by IV fluid.
  - ⇒ rapid fall in red cell count due to dilution. Effects of this:
    - ↓ viscosity of blood
    - ↓ oxygen carrying capacity of blood:
      - Oxygen carrying capacity = ([Hb] x SaO2 x 1.34) + 0.003 x PaO2,
        - oxygen flux = Delivery is carrying capacity x cardiac output
      - ∴ fall in Hb from 150 g/l to 100 g/l results in a fall in oxygen carrying capacity from 20 ml/100 ml to 14 ml/100 ml.
      - If metabolic rate is unchanged, this requires a
        - lower mixed venous PO2 ie ↑O2 extraction and
        - increased cardiac output to maintain oxygen flux.
          - Both of these changes occur - the rise in CO facilitated by ↓ viscosity
    - ↑ production of 2,3DPG ⇒ R shift OHDC (↑O2 unloading)
    - ↑RR: some increase in PAO2.
    - ↑rbc production:
      - Within hours of acute blood loss
      - stim by the impairment of tissue oxygenation ⇒ ↑erythropoietin.
      - ↑reticulocyte count to 10-15% over a week
    - ↑ platelet and WCC occur as they are mobilized from marginal sites.

- chronic anaemia depend partly on the cause of the anaemia.

  - Reduction in oxygen carrying capacity is always present and results in the same physiological responses as acute anaemia:
    - increased ventilation,
    - ↑CO,
    - ↑2,3DPG
    - ↓ mixed venous PO2.
    - haematological changes depend on the cause of the anaemia

**Classification**

- chronicity –
  - acute
  - chronic
- MCV
- Cause:
  - Blood loss
Haemolytic anaemias
- Anaemia of ↓ed erythropoesis

**Chronicity**
- Acute:
  - Haemorrhage
  - Haemolysis
- Chronic:
  - Everything else

**MCV**
- Can be classified under MCV terms

<table>
<thead>
<tr>
<th>Red Cell Appearance Indices</th>
<th>Small cells (microcytic)</th>
<th>Normal Cells (normocytic)</th>
<th>Large Cells (macrocytic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low MCV &lt;80</td>
<td>Iron deficiency</td>
<td>Acute blood loss</td>
<td>Vit B12 def.</td>
</tr>
<tr>
<td></td>
<td>o ↓Diet</td>
<td>o Renal failure</td>
<td>o Alcohol</td>
</tr>
<tr>
<td></td>
<td>o malabsorption</td>
<td>o Marrow failure</td>
<td>o Liver disease</td>
</tr>
<tr>
<td></td>
<td>o bleeding</td>
<td>o Haemolytic anaemias</td>
<td>o Reticulocytosis</td>
</tr>
<tr>
<td></td>
<td>o growth/pregnancy</td>
<td>o Endocrine disease:</td>
<td>o Hypothyroid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o Hypothyroid</td>
<td>o Hypoadrenal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o hypopituitary</td>
<td>o Hypoendocrine</td>
</tr>
</tbody>
</table>

**Bone Marrow Diagnosis**
- Thalassaemia
- Sideroblastic disease
- Anaemia of chronic disease
- • Iron deficiency
  - o ↓Diet
  - o malabsorption
  - o bleeding
  - o growth/pregnancy
- • Acute blood loss
- • Renal failure
- • Marrow failure
- • Haemolytic anaemias
- • Endocrine disease:
  - o Hypothyroid
  - o Hypoadrenal
  - o hypopituitary

**Cause**
- Blood loss:
  - Acute
  - Chronic blood loss ⇒
    - iron reserves depleted or
    - rate of loss > rate of replenishment
- Haemolytic anaemias:
  - intrinsic abnormalities of rbcs:
    - hereditary: eg
      - disorders of membrane cytoskeleton
      - enzyme deficiencies: eg
        - hexokinase deficiency
        - G6PD deficiency
      - Disorder Hb synthesis:
        - Thalassaemia
        - Sickle cell anaemia
    - Acquired:
      - Membrane defect eg paroxysmal nocturnal haemoglobinuria
  - Extrinsic abnormalities
    - Antibody mediated:
      - Isohaemagglutinins eg transfusion reactions
    - Autoantibodies:
o Idiopathic
o Drug
o SLE

- Mechanical trauma to rbc:
  - Microangiopathic:
    o TTP
    o DIC
  - Cardiac traumatic haemolytic anaemia

- Infections eg malaria
- Chemical injury eg lead poisoning
- Sequestration in phagocyte system eg hypersplenism

- ↓rbc production:
  o disturbance of stem cells:
    - aplastic anaemia
    - anaemia of renal failure
    - endocrine disorders
  o disturbance of erythroblasts:
    - ↓B12/folic acid
    - iron deficiency
    - thalassaemia

- unknown or many mechanisms:
  o sideroblastic
  o anaemia chronic infections

**Hereditary Spherocytosis**
- autosomal dominant
- deficiency in spectrin & ankyrin meshwork protein on inner rbc cell membrane
- rbc's ⇒ more spheroidal, less deformable ⇒ splenic sequestration
- infections can trigger:
  o haemolytic crisis
  o aplastic crisis
- >50% develop gallstones from chronic ↑bili

**G6PD Deficiency**
- G6PD produces glutathione & NADPH as part of hexose monophosphate shunt
- Glutathione protects rbc's from oxidative injury
- Oxidant stresses ⇒ Hb denaturation in form of:
  o Heinz bodies
  o ↓deformability ⇒ splenic sequestration
- X linked disorder
- 10% American blacks – less severe. Susceptible to oxidant drugs eg anti-malarials
- Mediterranean form –
  o G6PD levels v low ∴ haemolytic episodes more severe
  o Ingestion fava beans/legumes = oxidants

**Sickle Cell Disease**
- Sickle Cell Anaemia = mutant chains:
  o Hb S (α2βs)²= mutant β chain (one glutamic acid replaced by a valine)
  o 8% American blacks heterozygous for HbS
HbS polymerises into long stiff chains at low O2 tensions (deoxygenated) ⇒
- Rbc changes from biconcave disc to crescent shape
- ↑ fragility ⇒ thrombus & aggregation of rbc
- benefit is protection against malaria
- common in Africa, Arabia, India

Determinants of severity of sickling:
- amount of HbS in rbc
- interaction with other Hb chains in rbc
- mean corpuscular Hb concentration (MCHC):
  - ↓ MCHC
- capillary transit times = proportional to amount of O2 extraction
  - sluggish ⇒ ↑ O2 extraction ⇒ ↑ deoxygenation ⇒ sickling

Heterozygotes:
- 40% HbS; rest HbA
- HbA reacts poorly with HbS ⇒ resisting aggregation
- HbF reacts even less with HbA ∴ delayed presentation of sickle cell until >6 months

Consequences:
- R shift of OHDC
- Chronic haemolysis – rbc life span shortened to ~20d
- Microvascular occulsions ⇒ hypoxia & infarction

**Thalassaemia**
- Thalassaemia = normal structure of chains but different or absent amounts
- = imbalance between α & β chains of haemoglobin:
  - α thalassaemia =
    - deficiency α synthesis
    - due to deletion α globin genes
    - ⇒ excess non-α globins:
      - free β chains unstable & damage cell membranes
      - free gamma chains = stable but bind O2 very avidly ⇒ tissue hypoxia
    - classification:
      - silent carrier = barely detectable ↓ α chains
      - trait
      - HbH disease = deletion of 3 α globin genes ⇒ unstable tetramers of β globin
      - Hydrops fetalis = all 4 α globins deleted ⇒ free gamma chains ⇒ in-utero death
  - β thalassaemia =
    - deficiency β synthesis
    - total absence or ↓ed but detectable β globin synthesis
    - caused by point mutations affecting transcription or translation
    - ⇒ excess α chains form aggregates which damage cell membrane causing:
      - ineffective erythropoiesis
      - haemolysis
    - features:
      - skeletal abnormalities – overactive marrow
      - iron overload – from over absorption & repeated transfusions
• clinically divided based on severity of anaemia (genetic defect & whether homozygous or heterozygous) into:
  • minor – symptomless carrier state
  • intermedia – rarely requires transfusions
  • major – regular transfusions req’d otherwise quick death

• thalassaemia Rx’s:
  o long term folic acid supplements
  o blood transfusions
  o splenectomy with vaccinations & long term proph. Antibiotics
  o Stem cell transplant

Paroxysmal Nocturnal haemoglobinuria
• Chronic intravascular haemolysis
• Only acquired haemolytic anaemia
• Rbc’s have ↑susceptibility to complement mediated lysis
• Due to X linked mutation

Immune Haemolytic Anaemias
• Due to anti red cell antibodies
• Classification occurs based on Coombs test – detects
  o Serum antibodies
  o Complement on rbcs
• Types:
  o Warm antibody haemolytic - IgG
    ▪ Primary = Idiopathic
    ▪ Secondary =
      • SLE
      • Lymphomas
      • Hodgkins
      • Carcinomas
  o Cold agglutinin (antibody) immune haemolytic anaemia – IgM
    ▪ Primary = idiopathic
    ▪ Secondary:
      • Infections eg infectious mononucleosis
      • lymphomas
  o Cold haemolysis haemolytic - IgG

Methaemoglobin
• =small portion of Fe irons in Hb exist in Fe+++ state (ferric)
• unable to carry O2
• causes:
  o congenital deficiency of enzyme converting ferric ions to ferrous state
  o drugs eg SNP, prilocaine
• = a functional anaemia

Sulphaemoglobin
• also unable to carry O2

(Myoglobin)
• haem containing O2 binding protein present in skeletal mm
• has a role as O2 store
• Contains a single globin chain
By Adam Hollingworth

• Dissociation curve has a rectangular hyperbola shape
• Curve lies very L of Hb ie much higher affinity for O2
  ↓ allows optimal loading/unloading of O2 at PO2 levels which occur in muscle

Marrow Failure
• = aplastic anaemia
• idiopathic in 65% cases
• there are leukaemic, cancerous or other abnormal cells in blood or bone marrow
• can be:
  o acquired - more common
  o inherited - uncommon
• occurs due to reduction in stem cell numbers ↓ all cell lines

Clinical Features
• anaemia
• bleeding – minimal trauma, blood blisters in mouth
• infection – mouth infections

Vitamin B12 Deficiency
• diminished erythropoiesis
• B12 & folate needed for production of thymidine ⇒ building block of DNA
• Anaemia 2nd to
  o ↓production
  o abnormal rbcs ⇒ premature removal by phagocytes
• Causes:
  o Pernicious anaemia – most common
  o Pancreatitis
  o Coeliac /crohns disease
  o metformin
    \{ Uncommon, and mild B12 deficiency

Complications
• unRx’ed can ⇒ marrow failure ie pancytopenia

Pernicious Anaemia
• = autoimmune attack of gastric mucosa ⇒ ↓ intrinsic factor secretion⇒vit B12 malabsoprtion

Pathogenesis
• more common in females
• assoc with AID:
  o thyroid – 33% correlation
  o addison’s
  o vitiligo
• parietal & chief cells of stomach are replaced by mucin secreting cells

Clinical Features
• Insidious gradual onset
• Polyneuropathy: - demyelination of spinal cord tracts ⇒ spastic paresis & sensory ataxia
  \{ no neuro symptoms with folate deficiency
  o Symetrical parathesiae in fingers, toes
  o Loss vibration sense, proprioception
  o Progressive weakness
By Adam Hollingworth

Investigations
- blood film
- bone marrow
- serum bilirubin – raised due to ineffective erythropoesis
- serum B12
- vit B12 absorption test (Schilling):
  - IM injection overnight of B12
  - Take radiolabelled B12 with intrinsic factor & without
  - Look for labelled B12 in urine
  - +ve for PA if ↑B12 in urine WITH intrinsic factor

Treatment
- **intramuscular B12**
  - x6 over 1st 2wks
  - then 3monthly for life
- **oral B12 supplements**

Folate Deficiency
- found in green vegetables eg spinach, broccoli or liver & kidney
Causes
- nutritional:
  - poor intake
  - alcohol excess
  - anorexia
- antifolate drugs eg methotrexate, phenytoin, trimethoprim
- excess utilization:
  - physiological - eg pregnancy, lactation
  - pathological:
    - haematological disease eg excess rbc destruction
    - malignancy
    - inflam disease
  - malabsorption

Clin Features
- same as B12 but do not get gastric atrophy or neurological changes
Treatment
- 5mg folic acid daily

Iron Deficiency

Causes

<table>
<thead>
<tr>
<th>Diet Intake</th>
<th>IDA</th>
<th>Anaemia of Chronic Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>rare cause in Western diet</td>
<td>Ferritin ↓</td>
<td>↑ or norm</td>
</tr>
<tr>
<td>Major sources = Cereals &amp; meats</td>
<td>Iron ↓</td>
<td>↓</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>TIBC ↑</td>
<td>↓</td>
</tr>
<tr>
<td>Small bowel resection esp duodenum &amp; jejunum</td>
<td>Blood Loss ↑ demand</td>
<td></td>
</tr>
<tr>
<td>Pregnancy/infancy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Haematology - 28
• Most commonly from GI, uterine bleeding
• Abroad: hookworm infestation of GI tract ⇒ blood loss

Clinical Features
• Signs of iron deficient anaemia:
  o Brittle nails
  o Spoon shaped nails (koilonychia)
  o Smooth Atrophic tongue
  o Angular stomatitis
  o Brittle hair
  o Syndrome dysphagia & glossitis (Plummer-Vinson syndrome)
• Symptoms from history:
  o ↓dietary intake
  o self medication with NSAIDS ⇒ GI bleeding
  o blood in faeces – from Ca lower bowel/haemorrhoids
  o duration of periods in women – Norm. = 3-5 towels/tampons per day

Investigations
• FBC & ferritin & tibc
• Blood film
• Iron Studies – electrophoresis of Hb
• Bone Marrow studies

Classification of Haemoglobinopathies
• Classification
  o Structural hemoglobinopathies
    ▪ Sickle cell anaemias
    ▪ Hb C and M
    ▪ Low and high O2 affinity Hb
  o Thalassaemias
    ▪ Alpha thalassaemia variants
    ▪ Beta thalassemia variants
  o Combined structural/thalassaemias
  o Hereditary persistance of fetal Hb (HPFH)
  o Aquired Haemoglobinopathies
    ▪ Methemoglobinemia
    ▪ Leukaemia induced disorders of Hb
Assessment of Coagulation, Platelet Function & Fibrinolysis

Bleeding Time
- Functional test of clotting
- Standardised cut made on the skin & time of bleeding measured
- Difficult to calibrate
- Good test of platelets – primary haemostasis usually reaction stopping the bleeding
  but if time is prolonged doe not indicate nature of clotting defect

Platelet Count
- Good predictive value of risk of bleeding
- Platelets need to known to have norm function
- Results:
  - <50x10^9 = assoc prolonged bleeding
  - <20x10^9 = assoc spont dangerous haemorrhages

Prothrombin Time or INR
- Assesses extrinsic & common pathways
- Method:
  - Specimen of plasma at 37deg is citrated – to bind any ionized Ca
  - Start of test = Tissue factor & Ca added
  - Time taken to coagulate = result
- Normal range 0.9-1.2
- Prolonged if:
  - Warfarin
  - Vit K deficiency
  - Liver disease
- Most commonly used to assess coumarin anticoagulants ie 7, 9, 10, prothrombin

Activated partial Thromboplastin Time (APTT)
- Ax intrinsic & common pathways
- Method:
  - Citrated plasma at 37deg combined with kaolin & cephalin
  - Excess of Ca added ⇒ time to coag measured
- Screens for adequacy of factors 9, 11, 12, PK, HMWK
- Used to adjust heparin dose
- Norm 35-45 sec
- Prolonged in:
  - Heparin
  - Haemophilia

Thrombin Time
- Assesses common pathway ie fibrinogen ⇒ fibrin
- Method:
  - Thrombin added to plasma
Time to coagulate measured

- Activated Clotting Time
  - Automated device used to assess for supratherapeutic heparinisation
  - Different brands used which have different norm values (e.g., 80-160 seconds)
  - Norm value = no heparin effect
  - Only relevant to UFH
  - Measures intrinsic pathway
  - Linear response to ↑ACT with ↑heparin
  - Works by adding factors to blood to activate clotting, e.g., kaolin or glass beads
  - False long reading seen in lupus anticoagulation

-Thromboelastogram (TEG)
  - Sample of blood placed in a cup which is then gently rotated 6x/min to simulate sluggish venous flow
  - Thin wire probe in the middle used to measure degree of coagulation
  - Clot forms around the wire
  - ↑speed of onset & strength of clot measured and displayed in different ways

- Main variables determined:
  - R time = long → ↑time to evidence of first clot ⇒ give FFP
  - K value = long → ↓speed of clot formation ⇒ give cryo
  - α angle = ↓ed angle → ↓speed of clot formation ⇒ give cryo
  - MA (max amplitude) = ↓ed size → ↓clot strength ⇒ give platelets
  - A30 (amplitude at 30min) = ↓ed size → too much fibrinolysis ⇒ give TXA
Deficiencies of Above Tests

- None will assess function:
  - Factor 13
  - Alpha2 antiplasmin deficiency
  - vWF deficiency
- ∴ always risk of excessive bleeding
- is a functional reserve in concentration of clotting factors:
  - haemophilia A = no symptoms until factor 8 level <5%
- to determine specific cause for defective clotting need to do
  - specific factor assays
  - tests for anti-factor antibodies

Fibrinolytic System

- Assessed using clot lysis time
  - is shortened in alpha2 antiplasmin deficiency
- Circulating fibrin degredation products can be assayed ⇒ some info about clot lysis
- Fibrin crosslinking can be assessed by clot solubility in 5M urea
  - ↑ed time in factor 13 deficiency
Plasma Proteins

- proteins=
  - albumin
  - globulin
  - fibrinogen
  - caeroplasmin
  - CRP
  - transferrin

- function:
  - Proteolytic (complement, coagulations, fibrinolysis)
  - Role in acid base (buffering) ~15% of total
  - Oncotic pressure ~25mmHg
  - Transport
  - Enzyme systems (α1 antitrypsin)
  - Immunological
  - Metabolic (store of amino acids/energy source)

Origin

- antibodies from lymphocytes
- other proteins mostly from liver
- albumin:
  - approx 40% intravascular
  - rest mostly in skin
  - 5-10% degraded every day; replaced hepatic synthesis 200-400mg/kg/day
    - carefully regulated
  - transported to extravascular stores by capillary vesicular transport mechanisms
  - makes up 80% of oncotic pressure
  - primary transporter of many substances:
    - bili, Ca, hormones (T3 & T4)
    - CO₂ – as carbamino compounds
    - drugs – 2 main binding sites – BZ & warf sites

- Globins:
  - α1 -
    - acid glycoprotein (αag)–
      - acute phase reactant
      - carrier for most basic drugs
      - low capacity/low conc system
  - α2 eg haptoglobin – scavenges globins from Hb
  - β eg haemopexin – scavenges free haem
  - γ – Igs – from B/plasma cells

- Others
  - coag factors
  - CRP
  - complement
  - cytokines
Hypoproteinaemia
• stores used up before hypoproteinaemia occurs
• causes:
  o prolonged starvation
  o malabsorption syndromes
  o liver disease
  o nephrosis
  o afibrinogenemia – congen poor blood clotting
Blood Products & transfusion Medicine

- transfusion involves safe & compatible blood/products from donor to recipient
- compatibility between donor rbc antigens & recipient plasma antibodies is vital to prevent haemolytic reactions

Donors

- criteria for donor:
  - voluntary, healthy, unpaid
  - <13% volume to be taken
  - 18-60 or 70 (if regular)
  - Hb >135g male, 125g female
- Self deferral eg Hx HIV/HBV/HCV, malaria, fever, foreign travel, body piercing, tattoos
- Blood testing:
  - HBV:
    - HBsAg - low infective carrier
    - antiHBc = evidence of past infection
  - HCV – anti HCV
  - HIV, anti HIV1+2, p24 antigen
  - Treponema – also serves as marker for other STDs
  - HTLV 1+2 antibodies
  - CMV – antibodies

Blood Grouping (ABO & Rh)

- Testing of ABO & Rh(D) on donor & recipient
- testee rbc suspended in saline ie no serum
- serum with known antibodies added to test solution
- watch for agglutination ∴ work out grouping
- this done with
  - IgM solutions (ABO)
  - IgG solution (rhesus)
- serum containing IgM antibodies - anti A, anti-B, anti-AB
- serum with known gp A, B, O rbcs (reverse grouping)
- anti serum containing an IgG potent enough agglutinate Rh(D) +ve cells in saline
  - weak agglutination due to D variants may be missed

Blood Screening

- testing of recipient +/- donor blood
- testee serum taken; rbc which are group matched BUT with known minor antibodies (Kell/Duffy) are added.
  - Agglutination proves presence of minor antibodies

Coombs Test

- done to test for unexpected IgG weak antibodies
- done as indirect test
  - testee serum added to Coombs rbc’s - this binds IgG onto rbc
  - Coombs rbc’s with antibodies bound are washed away from testee serum
Coombs reagent added to cells which contains anti-human antibodies which bind to IgG on rbc ⇒ agglutination = positive Coombs test

- control sample also done to check activity of Coombs reagent
- does not add much safety to group & screen – see next

Cross Match
- involves:
  - group testing – saline agglutination test (as above)
  - screen – as above
  - Coombs Test
- rarely done in ANZ as only adds 0.01% extra of safety on top of group and screen

Prior to Administration of Blood Products
- donor:
  - self deferral
  - disease testing
  - group & screen
- recipient:
  - group and screen

Safety of Blood Transfusion & Degree of Compatibility testing

<table>
<thead>
<tr>
<th>Extent tested</th>
<th>Relative safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO-compatible</td>
<td>99.4%</td>
</tr>
<tr>
<td>ABO + Rh compatible</td>
<td>99.8% (1:1000 react)</td>
</tr>
<tr>
<td>ABO + Rh + neg antibody screen aka group &amp; screen</td>
<td>99.94% (1:10 000)</td>
</tr>
<tr>
<td>ABO + Rh + neg ab screen + Coombs’ test (“full X-match”)</td>
<td>99.95% (1:500 000 )</td>
</tr>
</tbody>
</table>

∴ Coombs’ test adds very little xtra and is usually omitted in routine testing.

Blood Products

Whole Blood
- ~400-500ml blood taken
- 63ml anticoagulant added:
  - citrate-phosphate-dextrose (CPD)
  - CPD-adenine
  - SAG-M or ADSOL: saline, adenine, glucose + mannitol
  - dilutes plasma by ~20%
- Additives:
  - Citrate: combines with & neutralises Ca ∴ anticoagulates blood
  - Phosphate: added as buffer + source of phosphate for metabolism
  - Adenine: provides substrate for ATP synthesis ∴ prolongs shelf life to ~35ds
  - Dextrose: for rbc metabolism – glycolysis – (rbc has no mitochondria)
- Blood stored at 4-6deg C
  - low temp inhibits metabolism & inhibits bacterial growth
- Properties of whole blood depend on
  - anticoag added
duration of storage
• get in massive transfusion protocols – contains all clotting factors

**Packed Red Cells**
• obtained by centrifugation or sedimentation of 1 unit of whole blood
• ~200-250mls plasma removed
• has HCT ≥0.75

**RBC Substitutes**
• stroma free Hb=
  o special Hb characteristics:
    ▪ cross linked,
    ▪ surface conjugated
    ▪ polymerized
    ▪ encapsulated
  o ⇒ ↑half life & ↓nephrotoxicity
  o problems:
    ▪ ↑oncotic pressure
    ▪ half life 6hrs
• perflurocarbon emulsions:
  o advantages:
    ▪ long shelf life
    ▪ stored at room temp
    ▪ subjected to viral inactivation
    ▪ universal biocompatibility
    ▪ religious acceptance
  o problems:
    ▪ half life 24-28hrs
    ▪ require Fio2 100%
    ▪ can interfere with many lab tests
• applications of substitutes:
  o trauma/military
  o surgery +/- acute normovolaemic haemodilution

**Platelets**
• available as:
  o standard unit = from single donor or pooled from 4-6 units blood
  o adult dose = apheresed from single donor = 5-6std units
• special storage conditions = extend shelf life to ~5days
  o temp 20-26deg – usually 22deg
  o special packs made from polyolefin plastic = allows aeration
  o constant agitation needed
• 1 std unit contains ~6x10^10 platelets .∴ 1 std unit transfused ⇒ ↑plt count by ~10x10^9/L per m2 body s.a.
• risks:
  o plts express HLA class I antigen
  o contamination by wcc & rbc's can cause allo-immunisation – esp with repeated transfusions
    ⇐⇒ refractoriness to subsequent platelet transfusions
  • ∴ ABO & Rh compatible plts are usually used
• HLA matched plts used for plts with HLA antibodies
  o Disease transmission – sepsis quoted 1:12,000
• 1/3 of transfused plts are sequestered in spleen

ASA Recommendations on Plt transfusion
• ↑consumption ie ITP = prophylactic platelet transfusion rarely effective
• surgery =
  o <50 - give platelets if high risk surgery
  o 50-100 = determine risk eg aspirin, renal disease, type of surgery
  o >100 = Rarely needed if >100
• if low risk surgery of norm vaginal delivery can consider even if platelets <50
• consider platelet t/f if known platelet dysfunction or risks of despite platelet count:
  o CPB
  o Renal failure
  o uraemia

FFP
• Prepared from fresh blood ⇒ frozen rapidly to -30deg (must be frozen <8hrs post collection)
• Collected from single donor – via separation or apheresis
• Undergoes viral inactivation = UV light/methylene blue/pasteurization/solvent
• Lasts 1yr
• Contains:
  o Factors (labile 5&8) and
  o Stabile factors (1,2,7,9,10,11,12, AT3, protein C+S)
  o Plasma lipids
• 1 unit FFP ⇒ ↑all coag factors by 2-3%
• indications:
  o reversal of warf 5-8ml/kg
  o Antithrombin 3 deficiency – with heparin Rx
  o TTP & HUS
  o Rx of immunodeficiencies
  o Massive blood transfusions

Cryoprecipitate
• Made from freshly separated plasma by
  o freezing at -70degs
  o rapid thawing at 4degs
• stored at -30deg, shelf life 1yr
• contains rich amounts :
  o f8 = 80unuts
  o fibrinogen – 250mg
  o fibronectin
  o vWF
  o F13
• 1 unit ⇒ ↑fibrinogen by 0.5g/l
• indications:
  o vWF unresponsive to DDAVP
  o congen fibrinogen deficiencies – rare
  o DIC
Factor VIIa

- Mode of action:
  - Activated factor 7 effectively bypasses steps coagulation steps needed f8 & f9 by upregulating extrinsic pathway in conjunction with tissue factor → now thought unlikely mechanism
  - Haemostatic function by platelets activation

- Is a vit K dependant factor

- Indications:
  - Severe refractory bleeding (unlicensed & controversial)
  - Haemophilia A or B – not responding to specific factor administration 2nd to antibody creation/inhibitors
  - Congen factor 7 deficiency

- Risks:
  - Arterial thrombosis
  - 50-90mcg/kg

Changes during Blood Storage

- platelets:
  - non functional within 48hrs if stored at 4deg
  - platelets in massive transfusion more impt than coag factor deficiency (dilutional thrombocytopenia)

- WCC:
  - Granulocytes lose phagocytic + bactericidal properties within 4-6hrs post collection
  - Antigenic properties remain

- Rbc:
  - ↑spherical with time ⇒ ↑fragility ⇒ ↑ed chance haemolysis ⇒ ↑free Hb
  - if rbc’s transfused at max recommended storage time (35d) = 10-20% destroyed ≤24hrs

- ↓2,3DPG (& ↓ATP):
  - in CPD-A blood:
    - @14days =50% 2,3DPG
    - @28days =5% 2,3DPG, ATP 75%

- microaggregate formation:
  - made by platelets + leucocytes (10-40um)
  - can cause pulmon dysfunction
  - microfilters does not help

- coagulation factors:
  - f5 & f8 = labile factors:
    - f5 @14d = 50%
    - f8 @24hrs = 50%, @21d = 6%
  - f8 should be produced endogenously anyway with stresses (if not haemophiliac)
  - levels of other factors not ↓ed up to 21days
  - use of packed cells ie less plasma will ⇒ factor dilution

- biochemical:
  - ↑serum K:
    - @7days K – 12mmol/L
    - 30days = 30mmol
    - not a problem after transfusion as
      - restoration of rbc metabolism ⇒ reuptake of K into rbc
      - catecholamines ⇒ K uptake
- dilutional effect via distribution through ECF
- slow transfusion ⇒ time for above processes
  - ↑rbc intracellular sodium
  - ↓pH – 6.7 @28days
  - ↓calcium

**Complications of Transfusion**
- ~3% react to blood
- fatal reaction = rare : 1 : 50,000 transfusions

**Classification:**
- by type
- by time

**By Type**
- disease transmission
- transfusion reactions
- metabolic/electrolyte abnormalities
- microaggregates
- immunomodulation
- transfusion related acute lung injury (TRALI)
- other

1. **Disease Transmission**
- HCV:
  - Anti HCV antibodies
  - Nucleic acid amplification test (NAT):
    - Has ↓ed window period for missing diagnosis of
      - HIV (22d ⇒ 10d)
      - HCV (70d ⇒ 10d)
  - Risk 1:250,000/unit ANZ
  - Responsible for 90% transfusion hepatitis
  - Needlestick 1.8% risk of getting HCV
- HBV:
  - Tests:
    - Hbs-Ag
    - Anti-HBV antibodies
  - Risk = 1:400,000/unit
  - Responsible for ~10% transfusion hepatitis
  - Needlestick ~30% risk!!
- HIV:
  - Tests:
    - Anti-HIV 1+2 antibodies
    - P-24 ag
    - NAT
  - Risk 1:1,000,000/unit (aus) – no known transmission in NZ
  - Needlestick ~1%
- CMV:
  - Most common viral transmitted disease via blood transfusion
usually fairly innocuous for most people
o Only selected units tested then kept for neonates, immunosuppressed
  ➔ anti-CMV antibodies
o Risk <1%/unit
• Bacterial contamination:
o Esp:
  ▪ Gram +ves
  ▪ Yersinia
  ▪ Pseudomonas
o Far more common than risk of viral transmission
o Risk 1:50 - 66,000
• Other:
o HTLV 1+2, malaria, NVCJD

2. Transfusion reactions
• Allergic:
o Against incompatible plasma proteins
o Mild = common
  ▪ rash/pruritis/fever
  ▪ slow infusion rate
o Moderate:
  ▪ stop, antihistamine
  ▪ use washed rbcs/platelets for subsequent transfusions
o Severe:
  ▪ anaphylaxis
  ▪ Due to infusion of IgA to IgA deficient pt who has anti-IgA antibodies (1:700)
  ▪ use washed rbcs/platelets in future
o Less common with leucodepletion
• Febrile reactions:
o (non-haemolytic type)
o Usually occurs <4hrs
o Caused by
  ▪ recipient antibodies against donor leucocytes
  ▪ induced by cytokines in donor rbc or platelets
o Unusual fever >38, headache, N&V, rigor, CP
o Mild: slow rate, antipyrexic, tramadol for shivers
o Severe: stop. Future transfusions:
  ▪ Buffy coat rbcs
  ▪ Leucodepleted
  ▪ HLA compatible platelets
o (multips get more severe reactions than primips)
o Less common with leucodepletion
• Haemolytic reactions:
o 2nd to ABO/Rh incompatibility
o 50% caused by clinical error
o 1:250,000 – 1million
o Symptoms:
  ▪ Initial: fever/rigor. Restlessness, chest pain, ↓bp
  ➔ NB fever & rash more likely to be allergic reaction (not ABO)
  ▪ Later: Haemolysis of bloods (anaemia, ↑unconjugated bili, ↓haptoglobin), renal failure
  from stromal & lipid contents precipitating in kidney

By Adam Hollingworth
o Rx:
  ▪ stop stat. send donor & recipient sample to lab for repeat typing
  ▪ maintain UO – IVF, furosemide, mannitol
  ▪ optimise DO2

• delayed haemolytic reactions:
  o 1:1000 ⇒ 1:250 000
  o 2nd to antibodies against minor donor rbc antigens
  o usually 10-14 days post
  o supportive Rx

3. Metabolic/Electrolyte Reactions (~storage lesion)

• ↓pH:
  o due to:
    ▪ lactic acid production from rbc
    ▪ citrate
  o pH blood 6.9-7 @21 days
  o but uncommon & usually only in massive transfusions
  o more common is slight met alkalosis: citrate metabolised to HCO3

• ↓2,3DPG:
  o ⇒ L shift OHDC
  o usually not impt

• ↑K:
  o blood @21 days = 30mmol/L
  o usually not an issue
  o give Ca if needed

• ↓Ca:
  o citrate toxicity
  o not problem unless >1 unit/5 min
  o risk factors:
    ▪ liver dysfunction
    ▪ hypothermia
    ▪ hyperventilation

• ↓Mg

4. microaggregates

• clumping of plts & WBCs in storage (10-40um) ⇒ pulmonary dysfunction
• no fix

5. Immunomodulation

• caused by sensitisation to donor wbc’s
• causes:
  o ↑incidence bacterial infections
  o recurrence of some cancers
  o (but good post organ transplants)
• leucodepletion may ↓immunomodulation

6. TRALI

• non cardiogenic pulmon oedema – similar to ARDS
• = SOB, hypoxia, ↓bp, fever
• causes:
  o HLA antigens cause severe acute microvascular injury
  o High antigen titre in donor plasma reacts with recipients neutrophils
already localised in pulmon vasculature

- develops <2-4hrs ⇒ resolve 4days
- 90% recovery
- much less common 2nd to leucodepletion

7. Other
- volume overload
- DIC/ARDS
- Religious issues
- Graft vs Host:
  - Live transfused lymphocytes engraft in host ⇒ immune response against host cells
  - Rash, ↓ECC, ↓plts ⇒ sepsis, death
  - impt in:
    - immunocompromised
    - prem babies
- leucodepletion not that helpful but gamma irradiation is – must do if to high risk pt
- 90% mortality

By Time
Early (<24hr)
- include:
  - acute haemolytic reactions eg ABO or rhesus incompatibility
  - bacterial contamination:
  - febrile (non haemolytic) reactions – from HLA antibodies
  - allergic reaction:
    - Anapylaxis
  - fluid overload:
    - transfusion related lung injury (TRALI) –

Late (>24hrs)
- include:
  - delayed haemolytic –
  - infections (viruses hep B/C, HIV, bacterial sepsis, protozoa, prions)
  - iron overload
  - graft versus host disease
  - post transfusion purpura =
    - ↓platelet count 5-7days post transfusion:
      - antibodies to platelet specific antigen
      - usually women who have been pregnant
      - need IV immunoglobulin & platelet transfusion
    - potentially fatal
  - immune modulation

Massive Transfusions
- >10 units in 24 hours or transfusion of entire circulating blood volume in 24hrs
- complications:
  - citrate toxicity (=↓Ca)
    - if t/f rate >1litre/10min ie 3units
    - tremor/tetany/ST & QT prolongation
(note Ca level never low enough to contribute to bleeding)

- $\uparrow$K:
  - only issue if very rapid, pt acidotic, hyperK already
  - Give Ca, insulin/dextrose

- $\downarrow$ clotting factors/platelets
  - esp low platelets & labile factors 5&8

- hypothermia –
  - drop 0.5 degC/unit of blood unless warmer
  - $\downarrow$ temp leads to:
    - malignant arrhythmias
    - $\downarrow$DO2 via Bohr effect
    - aggravation of citrate toxicity

- $\downarrow$2,3 DPG – use of CPD-adenine $\downarrow$s problem as 2,3 $\downarrow$s slower

- acidosis
- or alkalosis – citrate metabolised to bicarbonate

- microaggregates: pulmonary damage +/- ARDS

- volume overload

**Universal Leucodepletion**

- bedside vs lab
  - lab = better as better quality control, cost effective & hygiene
- blood passed through a filter 20-40um
- leucodepleted = wbc $<5\times10^6$/6units

- advantages:
  - $\downarrow$ febrile reactions
  - $\downarrow$sensitisation with human WBC antigens – esp impt in bone marrow pts
  - $\downarrow$plts refractoriness
    - $\leftarrow<$ rise post 2 standard units
  - $\downarrow$/prevent CMV/NVCJD transmission
  - possible:
    - $\downarrow$HTLV1+2 transmission
    - $\downarrow$immunomodulation
    - $\downarrow$TRALI
    - $\downarrow$bacterial contamination

- disadvantages:
  - loss of rbc & platelets
  - release of bradykinin – only an issue with bedside