By Adam Hollingworth

Haematology

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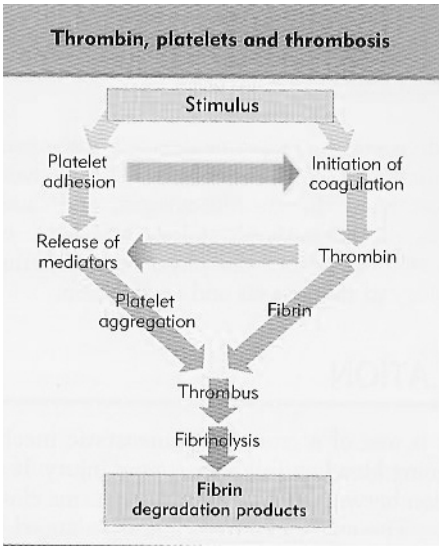
Haemostasis & Thrombosis



- 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
      ↳potent endothelium derived VC
    - 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**

- 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:
  - Adhesion [no ATP required]
  - Shape change [active process]
  - Secretion (release reaction) [active]
  - 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:
  - P –selectin = adhesion molecule on their membranes
  - Contain fibrinogen, fibronectin, factor V, VIII, PDGF, transforming growth factor B
  - vWF
- Dense bodies contain:
  - ADP & ATP
  - Ionized Ca
  - Histamine
  - Serotonin
  - Adrenaline
- Dense body release impt:
  - Ca required in coagulation cascade
  - ADP =
    - ▪Potent ↑platelet aggregation
    - ▪↑ed release of ADP from other platelets
- platelet activation ⇒ surface expression phospholipid complexs:
  - nucleation & binding site for Ca & clotting factors in intrinsic coag cascade

Platelet Aggregation

- stim of aggregation =
  - ADP
  - Thromboxane A2 – from platelets

  ↳together ⇒ autocatalytic 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 impt cofactor in aggregation:
  - ADP activation ⇒ change in conformation of platelet GpIIb-IIIa receptors to allow fibrinogen to bind
  - Fibrinogen binding ⇒ connection of multiple platelets ⇒ large aggregates
    ↳GpIIb-IIIa deficiences ≈ Glanzmann thrombasthenia bleeding disorder & therapeutic target
- Erythrocytes & leukocytes also aggregate in haemostatic plugs:
  - Leukocytes adhere via P selectin ⇒ contribute to inflam response

Summary Platelet Effects

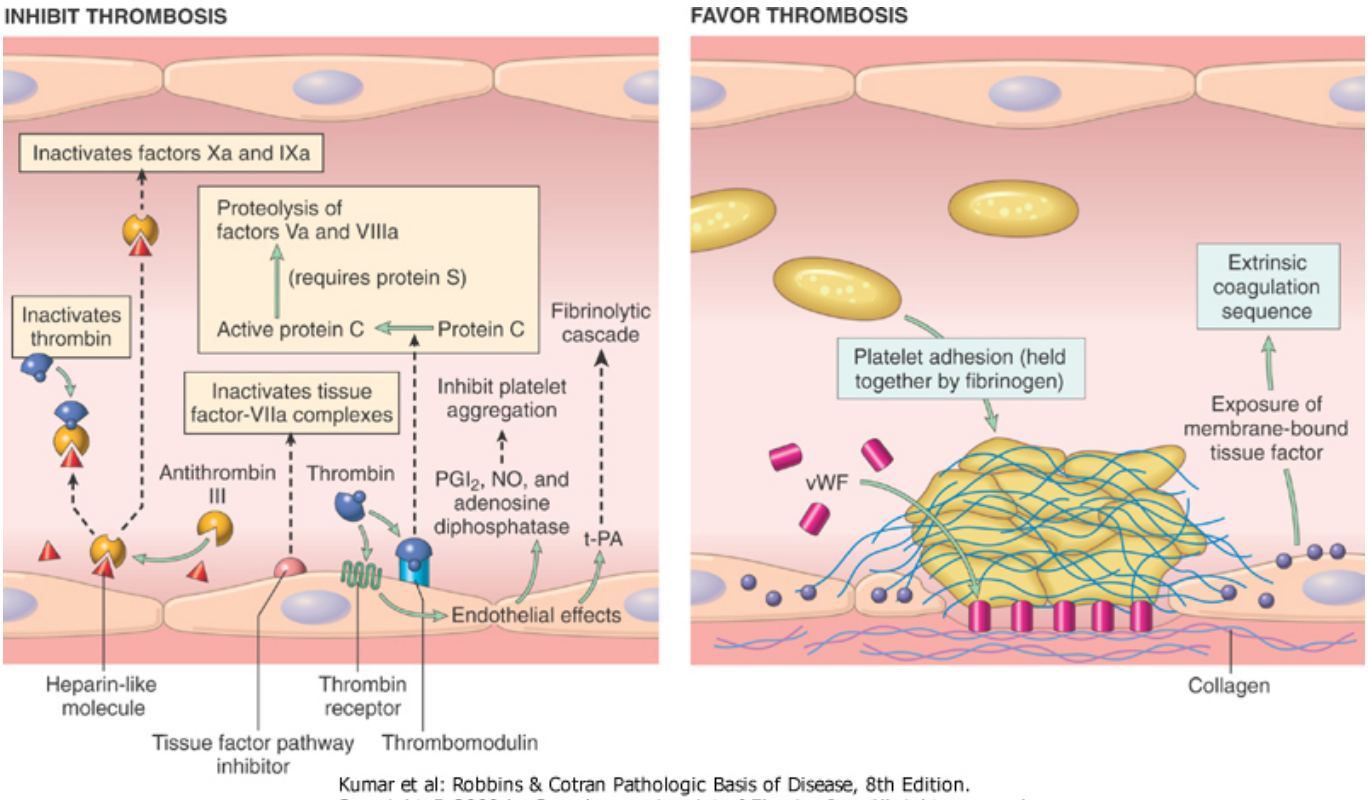
- Adhere to ECM at site of endothelial injury ⇒ activated
- On activation:
  - Secrete granules eg ADP
  - Synthesise TxA2
- Platelets expose phospholipid complexes which imp in intrinsic coag pathway
- Injured or activated ECs expose tissue factor ⇒ extrinsic coag pathway
- ADP ⇒ formation of primary plug
- Primary plug converted to secondary plug by
  - ADP
  - Thrombin
  - TxA_2
- Fibrin deposition stabilises & anchors the aggregated platelets

PGI_2 & TxA_2

- PGI2 =
  - Endothelium derived
  - VD
  - Inhibit platelet aggregation
- TxA2=
  - Platelet derived
  - VC
  - 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:
  - cascade of reactions limited to where platelets adhered
  - 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:
  - non activated platelets do not adhere to endothelium
  - Endothelial cells secrete:
    - PGI_2 (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 ∴ ⇒ inhibit platelet aggregation
- Anticoagulant effects:
  - 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 synthesize 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 plasminongen activator) ⇒↓fibrinolysis

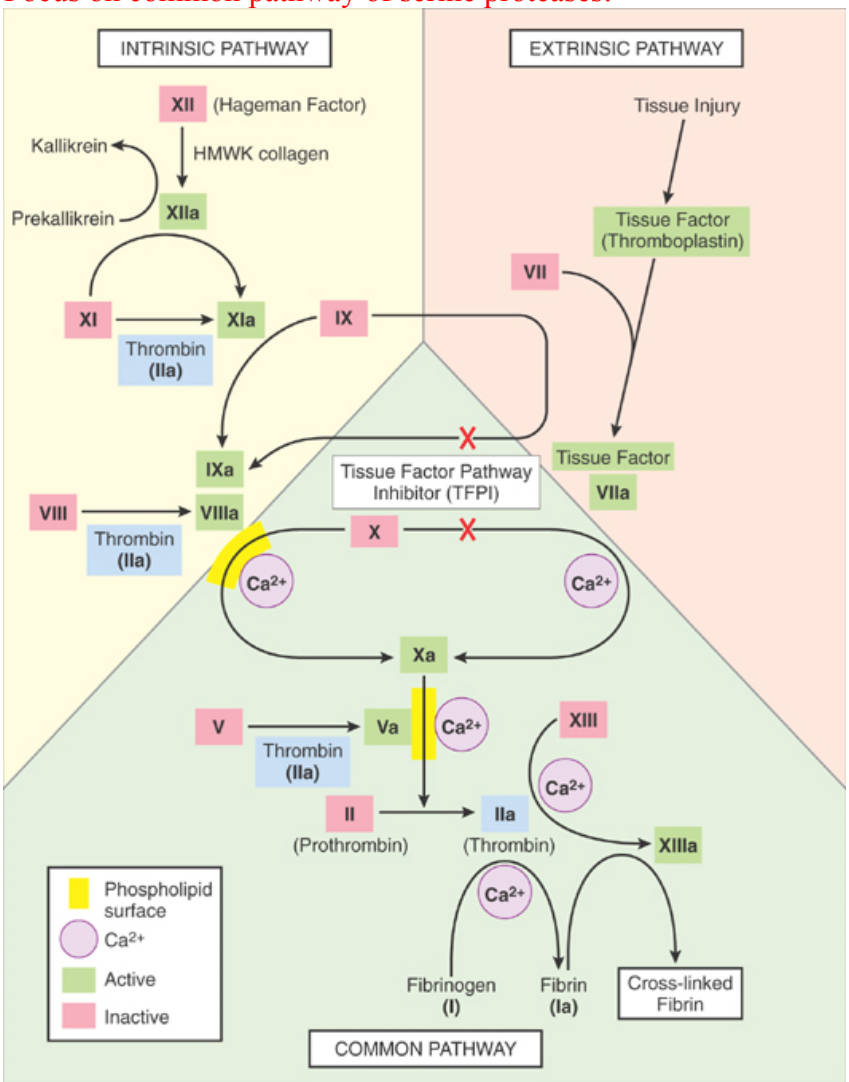
Coagulation

- 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 ⇒ propogation ⇒ stabilisation
- = conversion of inactive proenzymes ⇒ activated
- culminates generation insoluble fibrin

Focus on common pathway of serine proteases:



Cell Based Theory of Coagulation

Initiation

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

Amplification

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

Propogation

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

Stabilisation

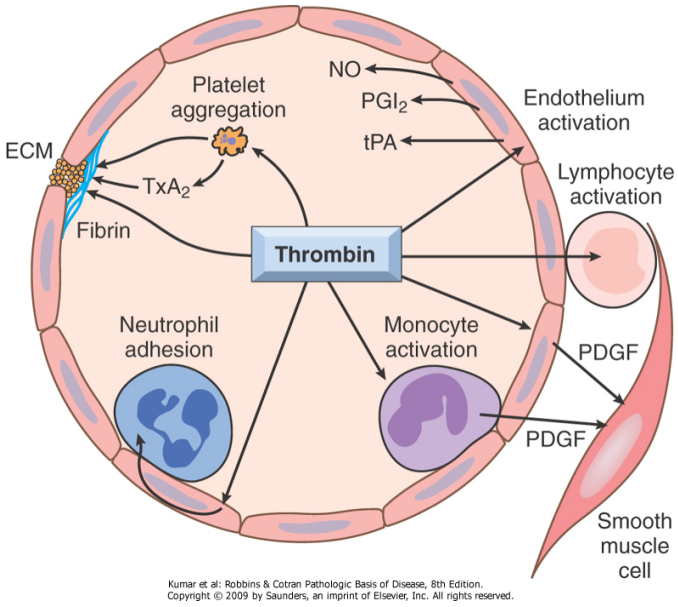
- Need to stabilise clot
- Fibrin creation ⇒ max thrombin generation
- Thrombin then activates:
  - factor 13 ⇒ cross link soluble fibrin to stabilise matrix
  - thrombin-activateable fibrinolysis inhibitor (TAFI) which maintains clot stability

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:
  - enzyme = activated coagulation factor
  - substrate = proenzyme form of coag factor
  - cofactor = reaction accelerator
- ∴clotting remains localised to site assembly possible eg activated platelet or endothelium

Thrombin

- thrombin - effects
  - effects in final stage of coag cascade
  - wide variety of effects on local vessels & inflam via:
    - binding to PARs (protease activated receptors)
      - belong to 7 transmembrane G protein coupled recptor 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:
  - larger = F8R:AG component:
    - platelet adhesion to exposed subendothelial connective tissue
    - platelet aggregation
    - vWF binding (F8:WF)
  - 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 & ß chains ⇒ fibrin monomer

↳ by proteolysis

- fibrin monomer = cross linked alpha, beta delta chains
- fibrin polymer = after spontaneous hydrogen bonds between molecules of monomer

Calcium

- essential cofactor in:
  - factor 8
  - factor 5
  - 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

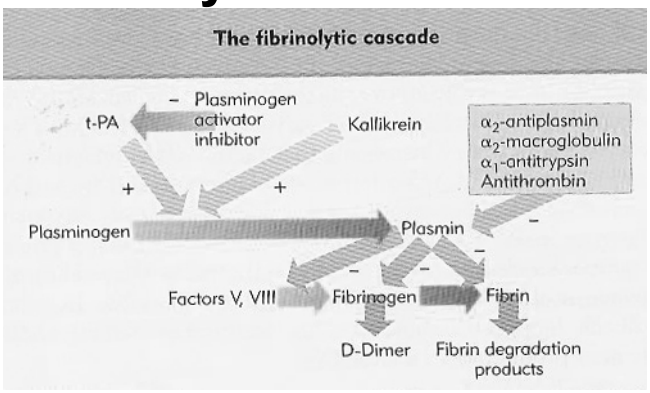
Von Willebrand Factor

- = large multimeric plasma protein
- actue phase protein ⇒ ↑ed stress & surgery
- produced by:
  - endothelial cells ⇒ stored in Weibel-Palade bodies
  - megakaryocytes ⇒ stored in platelets α granules
- functions:
  - 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
  - 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

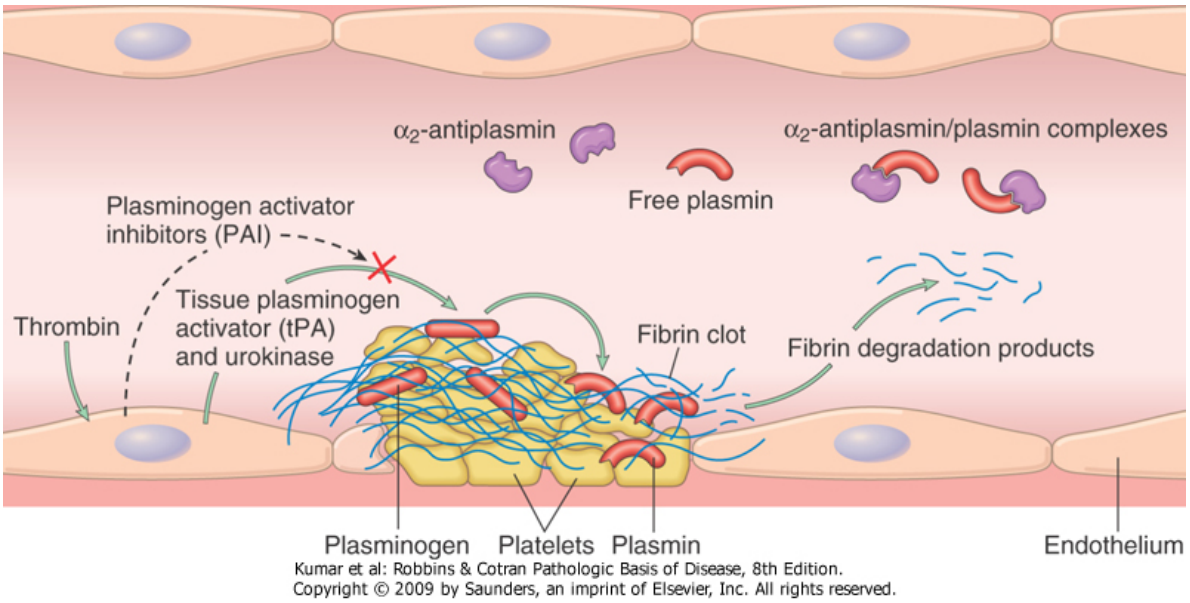


- This system works on top of factors already present to inhibit thrombosis
- =amplification system for limitation of clot size & dissolution of stable fibrin
- fibrinolysis = breakdown of fibrin by proteolytic enzymes
- plasminogen activated to ⇒ plasmin = key serine protease involved
- Fibrinolytic 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) ↳D Dimers
        - 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 a_2-anti-plasmin



- endothelium further modulates anticoag by
  - releasing PAIs (plasminogen activator inhibitors)
    ↳block fibrinolysis by inhibiting t-PA binding to fibrin
  - PAI release:
    - ↑ed by
      - Thrombin
      - Cytokines – why severe inflam ⇒ intravascular thrombosis
    - ↓ed by:
      - protein C
- variations in fibrinolysis responses:
  - more active in arterial circulation & deep veins, upper limbs
  - pregnancy:
    - ↑fibrinogen & plasminogen levels
    - ↓t-PA, α2-plasma inhibitor

    ↳overall fibrinolysis is reduced
  - neurohormonal stress (corticosteroids, catecholamines, ADH) ⇒ ↑transient ↑fibrinolysis
  - venous occlusion ⇒ ↑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:
  - Endothelial inj
  - Stasis or turbulent flow
  - Blood hypercoagulability

Endothelial Injury

- Clotting caused by:
  - Exposed subendothelial ECM & tissue factor
  - Adherence of platelets
  - Imbalance of clotting factors
    - ↓PGI2, t-PA
    - ↑PAI, ↑platelet adhesion molecules

- can cause thrombosis just be self
- injury following:
    - haemodynamic stress eg HTN, turbulent flow over scarred valves
    - bacterial endotoxins
    - homocystinuria
    - HCL
    - Radiation
    - Smoke

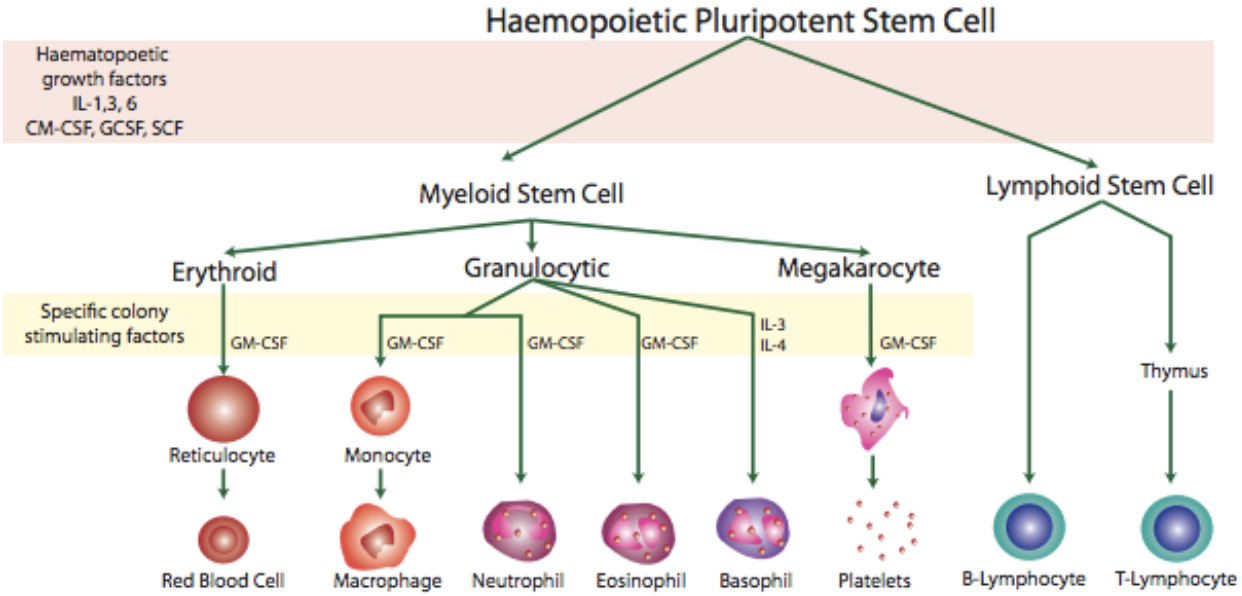
Flow Problems

- Norm flow = laminar:
    - Cells flow in centre of lumen
    - Outside clear plasma zone
- turbulence ⇒ eddy currents with pockets of stasis
- stasis ⇒
    - platelets into contact with endothelium
    - prevent dilution of activated clotting factors
    - ↓inflow clotting inhibitors
    - ↑endothelial cell activation
- stasis predominates:
    - venous circ
    - cardiac chambers eg mitral valve stenosis & AF ⇒ dilated L atrium
    - arterial aneurysms
- turbulence:
    - arteries
    - 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
  - Leuckocytes
  - 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 humerous/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 ⇒ platelets
- no nuclei
- 60-75% circulate; rest stay in spleen
  ↳∴ spleenectomy ⇒ ↑serum platelet count
- half life 4d

RBCs

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

Production

- proerythroblast ⇒ series smaller normoblasts – over 5 days
- erythroblast progressively:
  - contain more Hb
  - nuclear chromatin condenses
- eventually pyknotic nucleus removed from erythroblast ⇒ = 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
  - ↑rate of differentiation of stem cell ⇒ ↑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)

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

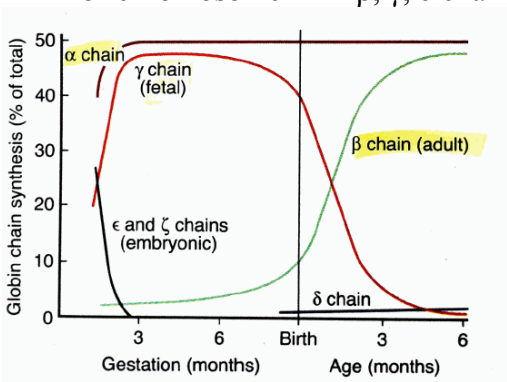
- Haem:
  - = iron-porphyrin compound. Norm in Fe++ (ferrous state)
  - synthesis in mitochondria with series of reactions:
    - condensation of glycine + succinyl CoA
    - ⇒ protoporphyrine combines + Fe++ = haem
- Globin chains = Formed in ribosomes

↳∴ Hb = tetramer of 4 globin chains, each with own haem in a hydrophobic pocket

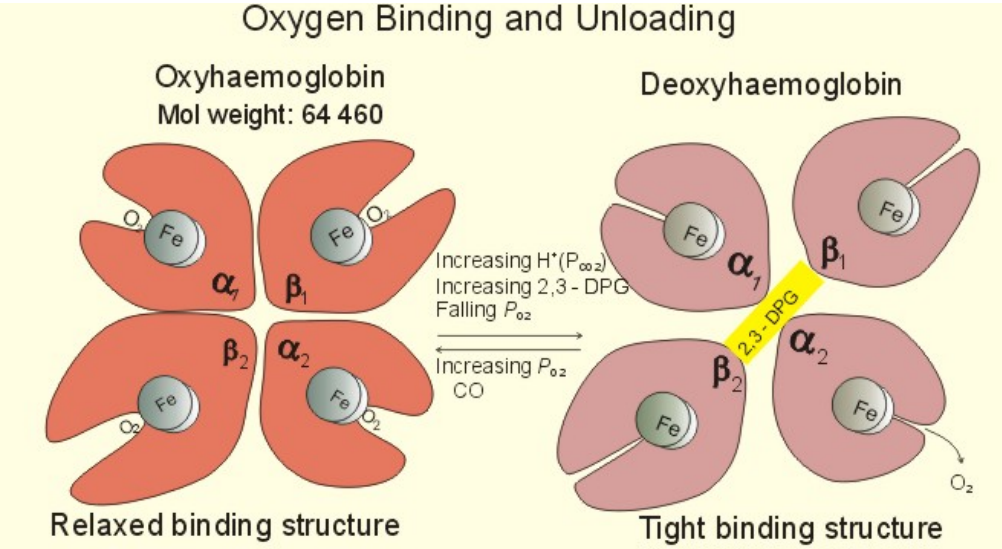
- Binding:
  - Haem ⟹ O2
  - Globin ⟹ CO2 & H
- In normal adult blood
  - 97.5% = Haemoglobin A – ($\alpha_2\beta_2$):
    - x1 pair α chain
    - x1 pair β chain – note ß production starts after birth (see HbF)
  - 2.5% = Haemoglobin A_2 ($\alpha_2\delta_2$) (alpha, delta)

↳also see small amounts haemoglobin A derivatives eg HbA_{1c}
↳glucose added to terminal valine in each β chain

- Fetal = Hb F (α2γ2) (alpha, gamma)
  - norm replaced by Hb A soon after birth
    ↳switching related to O2 availability
  - binds less 2,3DPG ∴ ↑affinity for O2 which allows o2 to move mum ⇒ fetus in placenta
    ↳ DPG prefers B chains to Gamma chains ⟹ L shift OHDC
    -
- chromosome location for globin genes:
  - chromosome 16 = α
  - chromosome 11 = β, γ, δ chains



JRE 32–8 **Development of human hemoglobin chains.**



Functions of Hb

- O2 carrier:
  - O2 loading exhibits positive cooperativity:
    - α1ß1 & α2ß2 contacts stabilise Hb molecule as O2 reacts with it
    - reaction of O2 with each subunit occurs sequentially with each facilitating the next
    - ∴ ↑ing affinity as O2 loads ⇒ sigmoid OHDC
      ↳myoglobin only has 1 subunit thus OMDC curve = rectangular
  - O2 unloading – vice versa:
    - ß chains pulled apart
    - 2,3-DPG enters molecule ⇒ ↓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 ⇒ lactate:
      - ATP used Na/K/ATPase to keep shape, volume, flexibility
    - NADH – needed by methaemoglobin reductase to reduce metHb ⇒ 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
  - ~50ml/day
  - 0.8% destroyed/day
  - ~3 million rbc/second
- glycolysis ↓s with ↑age of rbc ⇒ ↓ATP ⇒ ↓cellular integrity
- old rbcs destroyed by macrphagues (mainly in spleen):
  - globin portion split off ⇒ amino acids ⇒ re-enter aa pool

  - heme $\xrightarrow{\text{heme oxidase}}$ biliverdin + CO
  - biliverdin ⇒ bilirubin ⇒ bound to albumin ⇒ liver
  - in liver bilirubin conjugated with gluronic acid ⇒ excreted in bile
  - in GIT bili converted to stercobilin ⇒ some reabsorbed ⇒ excreted in urine as urobilinogen
  - iron from heme reused for Hb synthesis
- white light on skin: bilirubin ⇒ lumirubin
  ↳has shorter half life
- without enough iron ⇒ ↓Hb production ⇒ iron deficiency anaemia

Iron Metabolism

- Hb contains 65-70% total body iron
- Myoglobin contains 5%
- transferrin transports iron in plasma:
  - binds 2 atoms of ferric iron (Fe3+) / molecule
  - gets iron from RES ie destroyed rbcs or GIT
  - norm 30% saturated with iron
- dietary iron found in form of:
  - haem-protein
  - ferric protein complexs
  - ferric hydroxide
- ~10-15mg iron/days food
- 10% of this absorbed:
  ↳↑ed in preg or iron deficiency states
- absorbed mainly in duodenum:
  - ↑absorption = gastric acid, reducing agents (keeps iron in ferrous state)
  - ↓absorption = alkali, chealting agents eg phsophates
- soluble iron enters mucosal cells in ferrous state ⇒ portal circulation bound to transferrin
- iron storage sites:
  - liver
  - spleen
  - BM
- Stored as:
  - 65% ferritin – water soluble
  - 35% haemosiderin – insoluble
- iron losses:
  - 0.5-1g iron lost/day in faeces from desquamated GIT epithelial cells
  - urine, hair, sweat (small)
  - menstruation
  - foetus in pregnancy

Haemoglobin Reactions

- Hb + O2 ⇒ oxyhaemoglobin
  ↳attaches to the Fe2+ in the heme
- ↑affinity of Hb for O2:
  - ↓temp
  - ↓2,3-DPG
- ↓affinity:
  - ↑2,3-DPG

  - ↑temp
  - ↑H+

  ↳by shifting the position of the 4 peptide chains(quaternary structure)
- methaemoglobin =
  - drugs & oxidising agents effect blood: $Fe^{2+} \Rightarrow Fe^{3+}$
  - leads to dusky cyanosis
  - NADH system converts methaemoglobin ⇒ Hb
- Carboxyhaemoglobin =
  - CO and Hb
  - CO has much higher affinity for Hb than O_2 thus displaces O_2

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
  ↳∴ 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 ⇒ agglutinante & 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 corssed placenta/entered maternal circulation
  - ⇒ this prevents formation of maternal antiD IgG which would cause haemolysis of next pregnancys Rh+fetus (erythroblastosis fetalis)

    ↳haemolysis ⇒ death in utero, kernicterus, anaemia, jaundice, hyrdops fetails (oedema)

    ↳bilirubin depositied in basal ganglia
- 85% whites = Rh +ve
- 99% Asians Rh +ve

Other Blood Groups

- clinically less impt
- P, Lewis, MN systems:
  - Naturally occurring antibodies only react at low temps
  - Antigens low antigenicity
- Kell system:
  - 3rd most impt 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

Red Cell Appearance	**Small cells (microcytic)**	**Normal Cells (normocytic)**	**Large Cells (macrocytic)**	
Indices	Low MCV <80	Norm MCV	High MCV >96	
Bone Marrow Diagnosis			Megaloblastic	Normoblastic

	• Iron deficiency
	  ○ ↓Diet
	  ○ malabsorption
	  ○ bleeding
	  ○ growth/pregnancy
	• Thalassaemia
	• Sideroblastic disease
	• Anaemia of chronic disease
		• Acute blood loss
		• Renal failure
		• Marrow failure
		• Haemolytic anaemias
		• Endocrine disease:
		  ○ Hypothyroid
		  ○ Hypoadrenal
		  ○ hypopituitary
			• Vit B12 def.
			• Folate deficiency
				• Alcohol
				• Liver disease
				• Reticulocytosis
				• Hypothyroid

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:

        - Idiopathic
        - Drug
        - SLE
    - Mechanical trauma to rbcs:
        - Microangiopathic:
            - TTP
            - DIC
        - Cardiac traumatic haemolytic anaemia
    - Infections eg malaria
    - Chemical injury eg lead poisoning
    - Sequestration in phagocyte system eg hypersplenism
- ↓rbc production:
    - disturbance of stem cells:
        - aplastic anaemia
        - anaemia of renal failure
        - endocrine disorders
    - disturbance of erythroblasts:
        - ↓B12/folic acid
        - iron deficiency
        - thalassaemia
- unknown or many mechanisms:
    - sideroblastic
    - anaemia chronic infections

Hereditary Spherocytosis

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

G6PD Deficiency

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

Sickle Cell Disease

- Sickle Cell Anaemia = mutant chains:
    - Hb S ($\alpha_2\beta^s_2$)= mutant β chain (one glutamic acid replaced by a valine)
    - 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 rbcs
  - 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):
    - ↳ ie chance of HbS interacting with other HbS & aggregating
    - ↑dehydration ⇒ ↑MCHC
    - thalassaemia ⇒ ↓MCHC
  - capillary transit times = proportional to amount of O2 extraction
    - sluggush ⇒ ↑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 >6months
- Consequences:
  - R shift of OHDC
  - Chronic haemolysis – rbc life span shorterned 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 ß 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 geners ⇒ unstable tetramers of ß globin
      - Hydrops fetalis = all 4 α globins deleted ⇒ free gamma chains ⇒ in-utero death
  - ß thalassaemia =
    - deficiency ß synthesis
    - total absence or ↓ed but detectable ß 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:
    - long term folic acid supplements
    - blood transfusions
    - splenectomy with vaccinations & long term proph. Antibiotics
    - 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
    - Serum antibodies
    - Complement on rbcs
- Types:
    - Warm antibody haemolytic - IgG
        - Primary = Idiopathic
        - Secondary =
            - SLE
            - Lymphomas
            - Hodgkins
            - Carcinomas
    - Cold agglutinin (antibody) immue haemolytic anaemia – IgM
        - Primary = idiopathic
        - Secondary:
            - Infections eg infectious mononucleosis
            - lymphomas
    - Cold haemolysis haemolytic - IgG

Methaemoglobin

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

Sulfhaemoglobin

- 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

- 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:
  - acquired - more common
  - 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 erythropoesis
- B12 & folate needed for production of thymidine ⇒ building block of DNA
- Anaemia 2nd to
  - ↓production
  - abnormal rbcs ⇒ premature removal by phagocytes
- Causes:
  - Pernicious anaemia – most common
  - Pancreatitis
  - Coeliac /crohns disease
  - metformin

  } Uncommon, and mild B12 deficiency

Complications

- unRx'ed can ⇒ marrow failure ie pancytopaenia

Pernicious Anaemia

- = autoimmune attack of gastric mucosa ⇒ ↓intrinsic factor secretion⇒vit B12 malabsoption

Pathogenesis

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

Clinical Features

- Insidious gradual onset
- Polyneuropathy: - demyelination of spinal cord tratcs ⇒ spastic paresis & sensory ataxia
  ↳ no neuro symptoms with folate deficiency
  - Symetrical parathesiae in fingers, toes
  - Loss vibration sense, proprioception
  - Progressive weakness

- ataxia

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

↳= IM

Treatment

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

↳should ↑Hb and ↓LDH

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
  - pathologcial:
    - 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

Diet Intake

- rare cause in Western diet
- Major sources = Cereals & meats

Malabsorption

- Small bowel resection esp duodenum & jejunum

Blood Loss/↑demand

- Pregnancy/infancy

	IDA	Anaemia of Chronic Disease
Ferritin	↓	↑ or norm
Iron	↓	↓
TIBC	↑	↓

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

Clinical Features

- Signs of iron deficient anaemia:
  - Brittle nails
  - Spoon shaped nails (koilonychia)
  - Smooth Atrophic tongue
  - Angular stomatitis
  - Brittle hair
  - Syndrome dysphagia & glossitis (Plummer-Vinson syndrome)
- Symptoms from history:
  - ↓dietary intake
  - self medication with NSAIDS ⇒ GI bleeding
  - blood in faeces – from Ca lower bowel/haemorrhoids
  - 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
  - Structural hemoglobinopathies
    - Sickle cell anaemias
    - Hb C and M
    - Low and high O2 afinity Hb
  - Thalassaemias
    - Alpha thalassaemia variants
    - Beta thalassemia variants
  - Combined structural/thalaessemias
  - Hereditary persistance of fetal Hb (HPFH)
  - 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
  - $<20\ x10^9$ = 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 adequancy 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

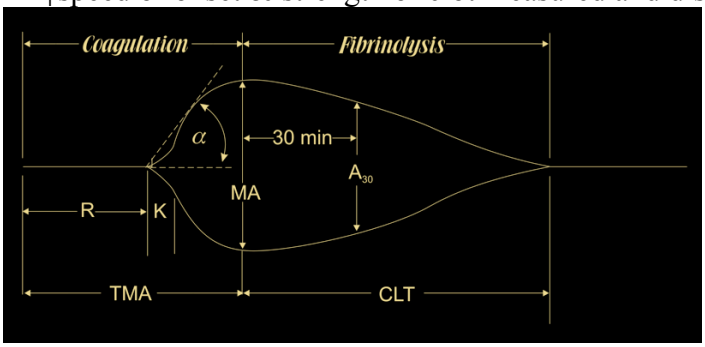
  - Time to coagulate measured
    ↳ie Ca not required
- Normal range 10-15 sec
- Prolonged in
  - Heparin Rx
  - DIC
  - Afibrinogenaemia
  - Excessive dabigitran

Activated Clotting Time

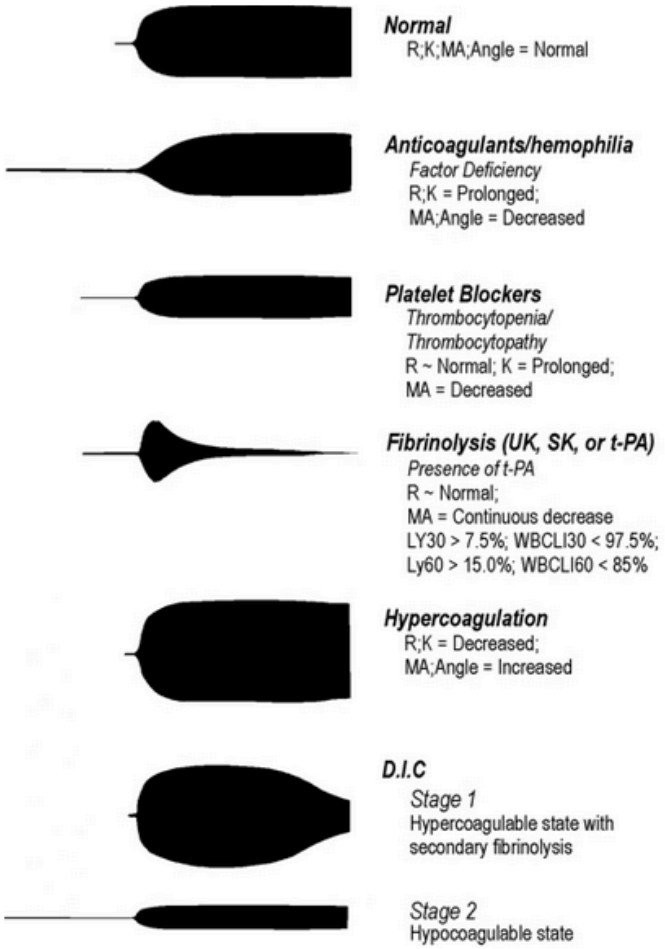
- Automated device used to assess for supratherapeutic heparinisation
- different brands used which have diff norm values ie 80-160 secs
  - ACT >400sec used for bypass
- Norm value = no heparin effect
- only relevant to UFH
- measures intrinsic pathway
- linear response to ↑ACT with ↑heparin
- works by adding factors to blood to activate clotting eg 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 x6/min to simulate sluggish venous flow
- thin wire probe in middle used to measure degree of coagulation
- clot forms around 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:
  - P roteolytic (complement, coagulations, fibrinolysis)
  - R ole in acid base (buffering) ~15% of total
  - O ncotic pressure ~25mmHg
  - T ransport
  - E nzyme systems (α1 antitrypsin)
  - I mmunological
  - M etabolic (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
  - ß 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:
    - prolonged starvation
    - malabsorption syndromes
    - liver disease
    - nephrosis
    - 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

Extent tested:	**Relative safety:**
• ABO-compatible	99.4%
• ABO + Rh compatible	99.8% (1:1000 react)
• ABO + Rh + neg antibody screen **aka group & screen**	99.94% (1:10 000)
• ABO + Rh + neg ab screen + Coombs' test ("full X-match")	99.95% (1:500 000)

- ∴, 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 while blood
- ~200-250mls plasma removed
- has HCT ≥0.75

RBC Substitutes

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

Platelets

- available as:
  - standard unit = from single donor or pooled from 4-6 units blood
  - adult dose = apheresed from single donor = 5-6std units
- special storage conditions = extend shelf life to ~5days
  - temp 20-26deg – usually 22deg
  - special packs made from polyolefin plastic = allows aeration
  - 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:
  - plts express HLA class 1 antigen
  - contamination by wcc & rbcs 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
        - All is less of an issue with leucodepleted irradiated blood
    - 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 =
    - <50 - give platelets if high risk surgery
    - 50-100 = determine risk eg aspirin, renal disease, type of surgery
    - >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:
    - CPB
    - Renal failure
    - 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:
    - Factors (labile 5&8) and
    - Stabile factors (1,2,7,9,10,11,12, AT3, protein C+S)
    - Plasma lipids
- 1 unit FFP ⇒ ↑all coag factors by 2-3%
- indications:
    - reversal of warf 5-8ml/kg
    - Antithrombin 3 deficiency – with heparin Rx
    - TTP & HUS
    - Rx of immunodeficiencies
    - Massive blood transfusions

Cryoprecipitate

- Made from freshly separated plasma by
    - freezing at -70degs
    - rapid thawing at 4degs
- stored at -30deg, shelf life 1yr
- contains rich amounts :
    - f8 = 80unuts
    - fibrinogen – 250mg
    - fibronectin
    - vWF
    - F13
- 1unit ⇒ ↑fibrinogen by 0.5g/l
- indications:
    - vWF unresponsive to DDAVP
    - congen fibrinogen deficiencies – rare
    - 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 thrombocytopaenia)
- WCC:
  - Granulocytes lose phagocytic + bactericidal properties within 4-6hrs post collection
  - Antigenic properties remain
- Rbcs:
  - ↑spherical with time ⇒ ↑fragility ⇒ ↑ed chance haemoylsis ⇒ ↑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 rractions
- 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 fr 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
  - Only selected units tested then kept for neonates, immunosuppressed
    ↳anti-CMV antibodies
  - Risk <1%/unit
- Bacterial contamination:
  - Esp:
    - Gram +ves
    - Yersinia
    - Pseudomonas
  - Far more common than risk of viral transmission
  - Risk 1:50 - 66,000
- Other:
  - HTLV 1+2, malaria, NVCJD

2. Transfusion reactions

- Allergic:
  - ?against incompatible plasma proteins
  - mild = common
    - rash/pruritis/fever
    - slow infusion rate
  - moderate:
    - stop, antihistamine
    - use washed rbcs/platelets for subsequent transfusions
  - severe:
    - anaphylaxis
    - Due to infusion of IgA to IgA deficient pt who has anti-IgA antibodies (1:700)
    - use washed rbcs/platelets in future
  - less common with leucodepletion
- febrile reactions:
  - (non-haemolytic type)
  - usually occurs <4hrs
  - caused by
    - recipient antibodies against donor leucocytes
    - induced by cytokines in donor rbc or platelets
  - unusual fever >38, headache, N&V, rigor, CP
  - mild: slow rate, antipyrexic, tramadol for shivers
  - severe: stop. Future transfusions:
    - buffy coat rbcs
    - leucodepleted
    - HLA compatible platelets
  - (multips get more severe reactions than primips)
  - less common with leucodepletion
- Haemolytic reactions:
  - 2nd to ABO/Rh incompatibility
  - 50% caused by clinical error
  - 1:250,000 – 1million
  - 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

  - Rx:
    - stop stat. send donor & recipient sample to lab for repeat typing
    - maintain UO – IVF, furosemide, mannitol
    - optimise DO2
- delayed haemolytic reactions:
  - 1:1000 ⇒ 1:250 000
  - 2nd to antibodies against minor donor rbc antigens
  - usually 10-14days post
  - supportive Rx

3.Metabolic/Electrolyte Reactions (~storage lesion)

- ↓pH:
  - due to:
    - lactic acid production from rbcs
    - citrate
  - pH blood 6.9-7 @21days
  - but uncommon & usually only in massive transfusions
  - more common is slight met alkalosis: citrate metabolised to HCO3
- ↓2,3DPG:
  - ⇒ L shift OHDC
  - usually not impt
- ↑K:
  - blood @21days = 30mmol/L
  - usually not an issue
  - give Ca if needed
- ↓Ca:
  - citrate toxicity
  - not problem unless >1unit/5min
  - 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:
  - ↑incidence bacterial infections
  - recurrence of some cancers
  - (but good post organ transplants)
- leucodepletion may ↓immunomodulation

6. TRALI

- non cardiogenic pulmon oedema – similar to ARDS
- = SOB, hypoxia, ↓bp, fever
- causes:
  - HLA antigens cause severe acute microvascular injury
  - 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 lymphcotyes engraft in host ⇒ immune response against host cells
  - Rash, ↓ECC, ↓plts ⇒ sepsis, death
  - impt in:
    - immunocompromised
    - prem babies
- lecuodepletion 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)
    - ↑K:
        - only issue if v rapid, pt acidotic, hyperK already
        - . Give Ca, insulin/dextrose
    - ↓clotting factors/platelets
        - esp low platelets & labile factors 5&8
        -
    - hypothermia –
        - drop 0.5degC/unit of blood unless warmer
        - ↓temp leads to:
            - malignant arrhythmias
            - ↓DO2 via Bohr effect
            - aggravation of citrate toxicity
    - ↓2,3 DPG – use of CPD-adenine ↓s problem as 2,3 ↓s slower
    - acidosis
    - or alkalosis – citrate metabolised to bicarbonate
    - microaggregates : pulmon 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 = wbcs $<5x10^6$/6units
- advantages:
    - ↓febrile reactions
    - ↓sensitisation with human WBC antigens – esp impt in bone marrow pts
    - ↓plts refractoriness
        ↳= <7 rise post 2 standard units
    - ↓/prevent CMV/NVCJD transmission
    - possible:
        - ↓HTLV1+2 transmission
        - ↓immunomodulation
        - ↓TRALI
        - ↓bacterial contamination
- disadvantages:
    - loss of rbc & platelets
    - release of bradykinin – only an issue with bedside