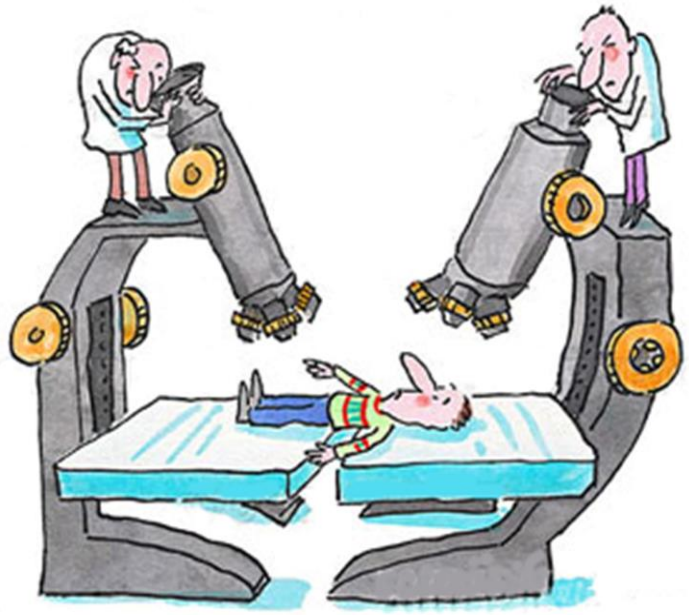
PP of hematopoietic system I – etiopathogenesis of disorders of primary and secondary hemostasis

Content

- Physiological primary plug formation and blood clotting as a higly regulated process
- Virchow‘s triad
- Bleeding disorders (bleeding diathesis) due to
  - disorders of primary hemostasis (thrombocytopenia and thrombastenia)
  - disorders of secondary hemostasis (hypocoagulation states)
- Hypercoagulation (thrombotic) disorders
  - thrombosis and embolization in typical locations



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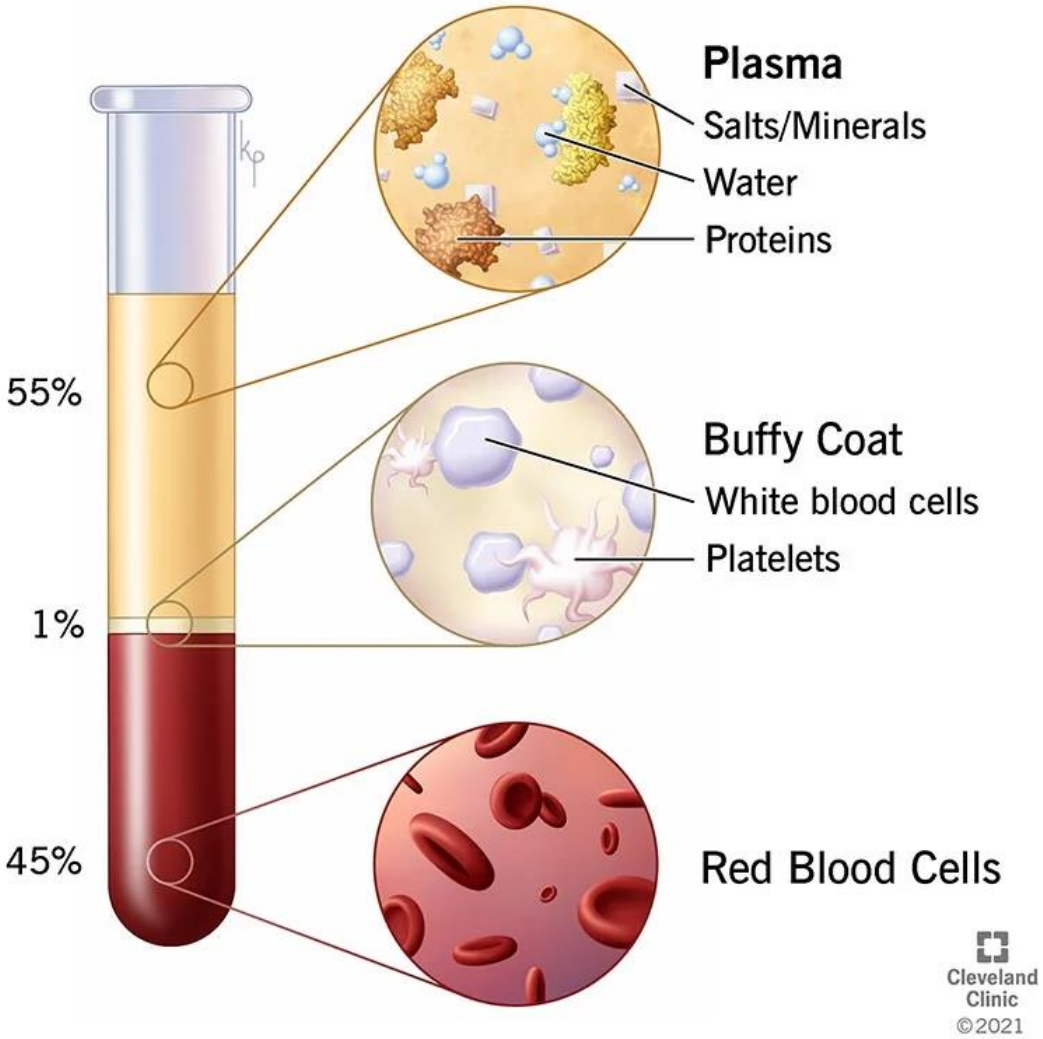
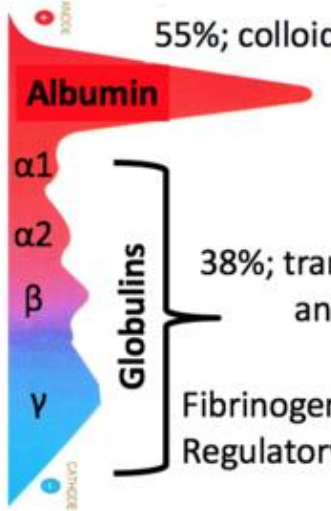
Blood – haematocrit, plasma × serum, proteins, sedimentation



Major Types:

- **Albumin** *(60%)*
  Major component of osmotic pressure of plasma
- **Globulins** *(35%)*
  Antibodies (immunoglobulin) and transport proteins
- **Fibrinogens** *(4%)*
  Functions in blood clotting
- **Other** *(<1%)*
  Various roles (α-1-antitrypsin, coagulation factors, etc.)



55%; colloid osmotic pressure or **oncotic pressure;** lipid, steroid, and hormone transport antioxidant protein (binds metals and reacts with ROS)

38%; transport ions, hormones, and immune function (γ-globulins)

Fibrinogen (7%); clotting

Regulatory Proteins (< 1%); protease inhibitors, enzymes, hormones

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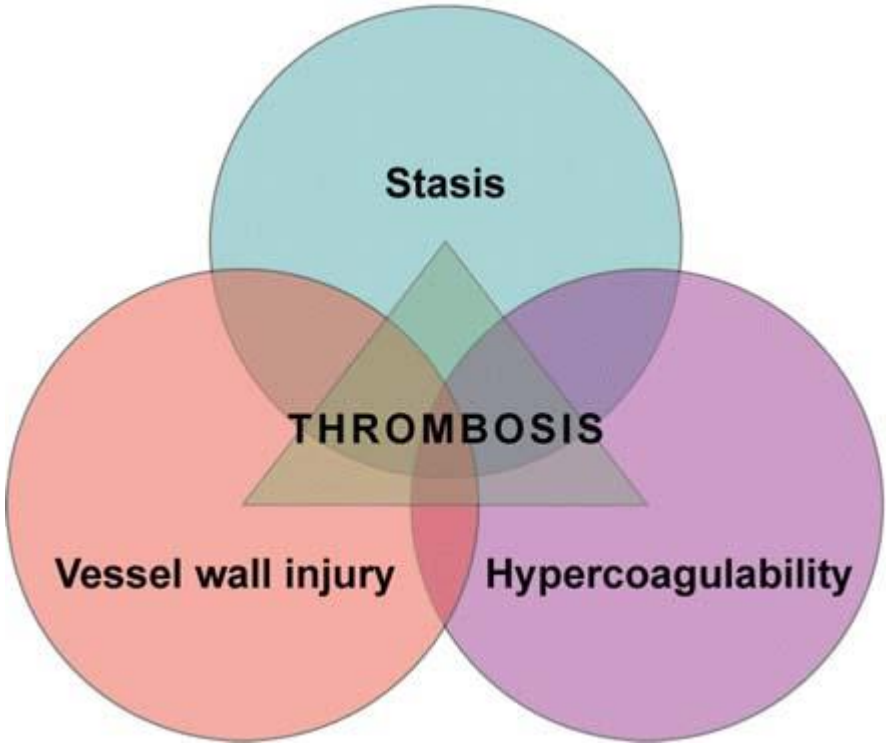
Factors keeping the blood fluidity



- physiologic blood clotting (= **haemostasis**) prevents the blood loss
  - primary
  - secondary
- **Virchow's triad**
  - vessel wall (intact endothelium)
  - blood (clotting factors in balance with anti-clotting and fibrinolytic mechanisms)
  - blood flow (preventing ptrolonged contact with verssel wall)
- alteration of any of the factors (or combination) leads to
  - pathological blood clotting (= **thrombosis**)
  - increased risk
  - spontaneous
    - examples: deep vein thrombsis (DVT), atherothrombosis
- event. subsequent occlusion by thrombus fragment = **embolization**

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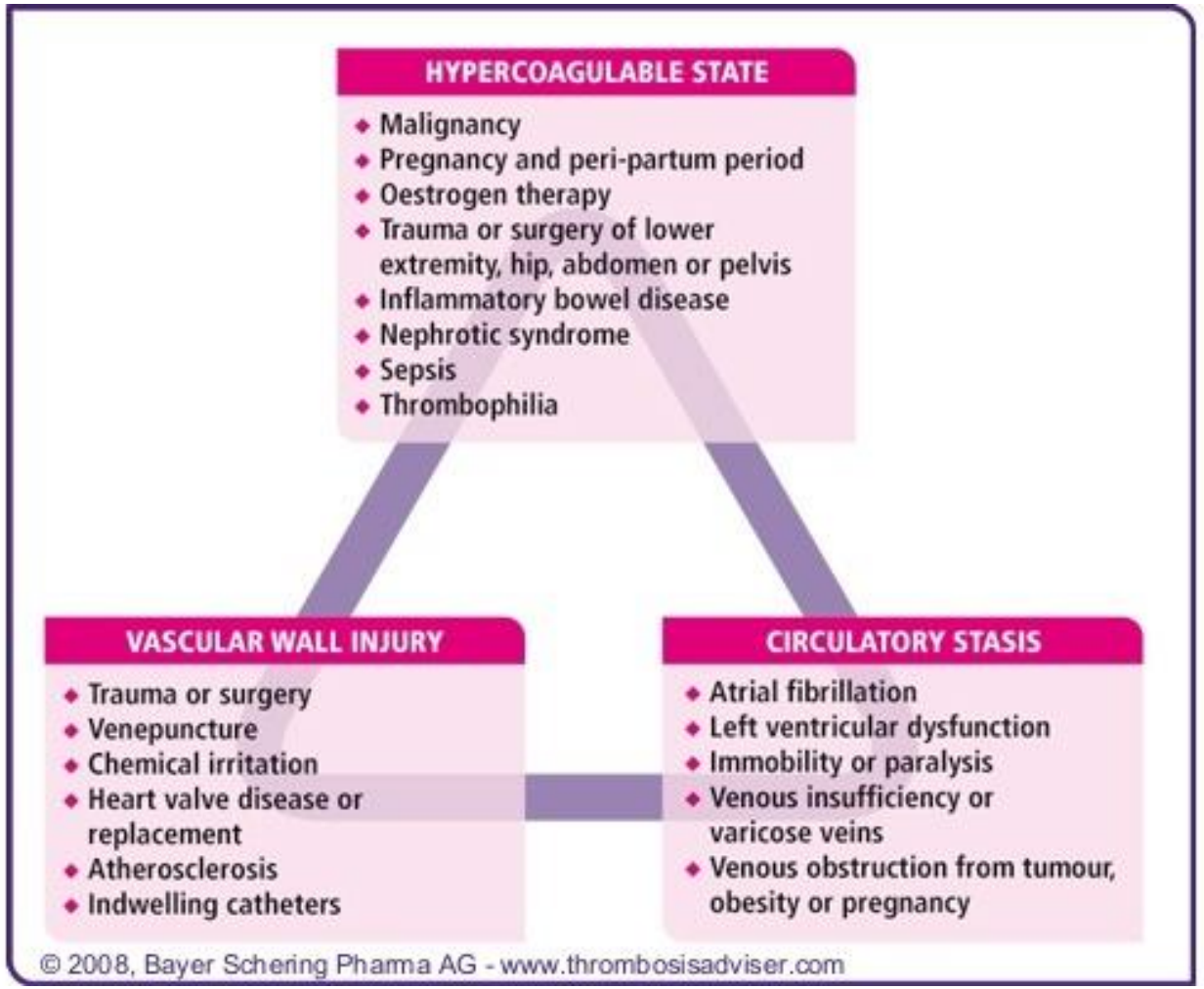
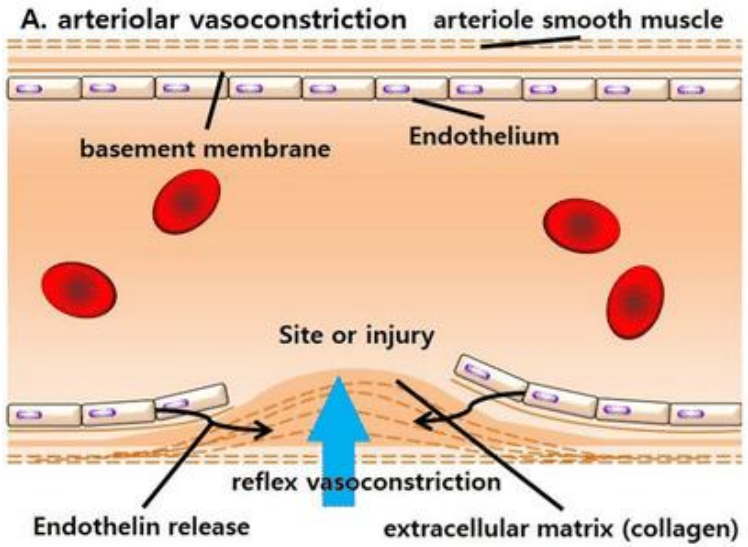
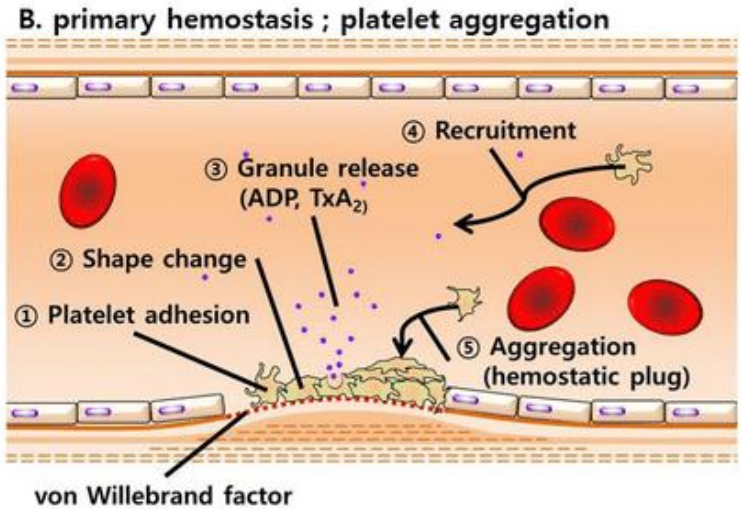
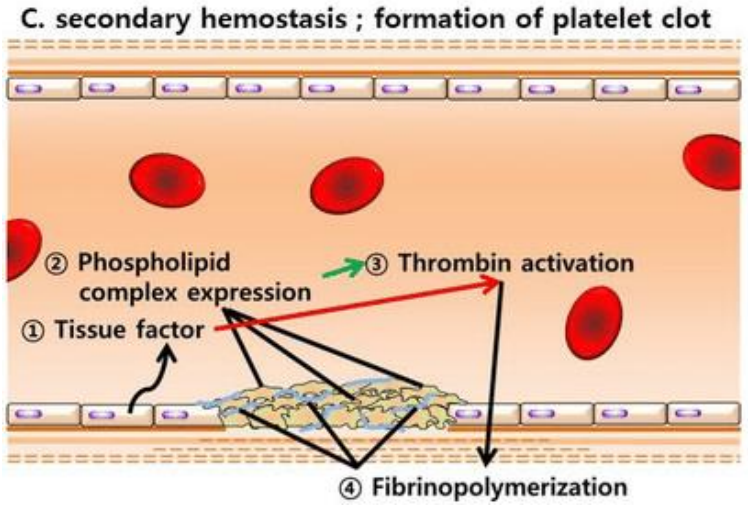
Common causes of thrombosis

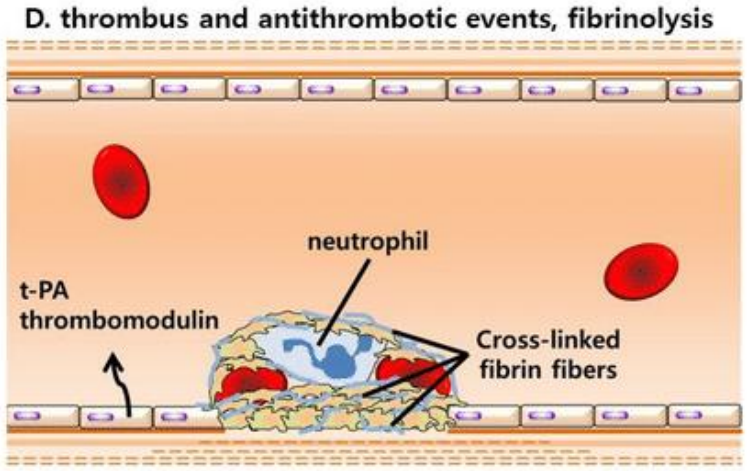


Hemostasis – phases









- (1) Arteriolar **vasoconstriction** occurs immediately by the **reflex mechanism** of the nervous system right after vascular injury, which can be enhanced by **endothelin**, a potent vasoconstrictor released from the endothelial cells constituting the vessel wall.
- (2) **Platelets** bind to the von **Willebrand factor** and **adhere** to the extracellular matrix at the site of injury, after that, they change their appearance (**activation**) and promote further recruitment and **aggregation** of platelets by releasing granules such as **ADP** and **TxA2**.
- (3) **Tissue factor** released from vascular endothelial cells expresses the platelet phospholipid complex. Through the **coagulation cascade**, they eventually activate thrombin and ultimately make the fibrin polymer to form a clot/coagulum/thrombus.
- (4) During this period, the platelet plug contains trapped neutrophils and RBCs in the blood vessels, showing permanent plugs and preventing further bleeding. In the absence of vascular injury or complete thrombus formation, the endothelial cells secrete **t-PA and thrombomodulin**, which inhibit platelet adhesion and aggregation, to exert **antithrombotic effects and fybrinolysis** that lead limitation of hemostasis.

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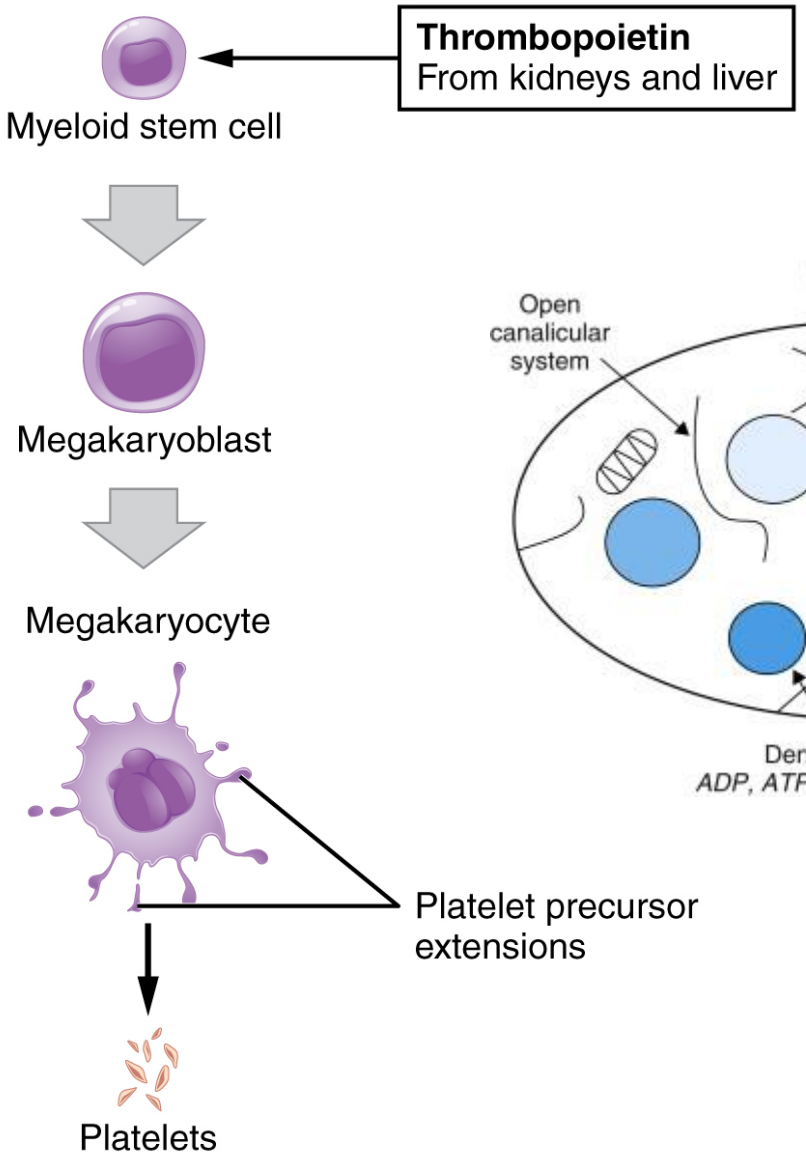
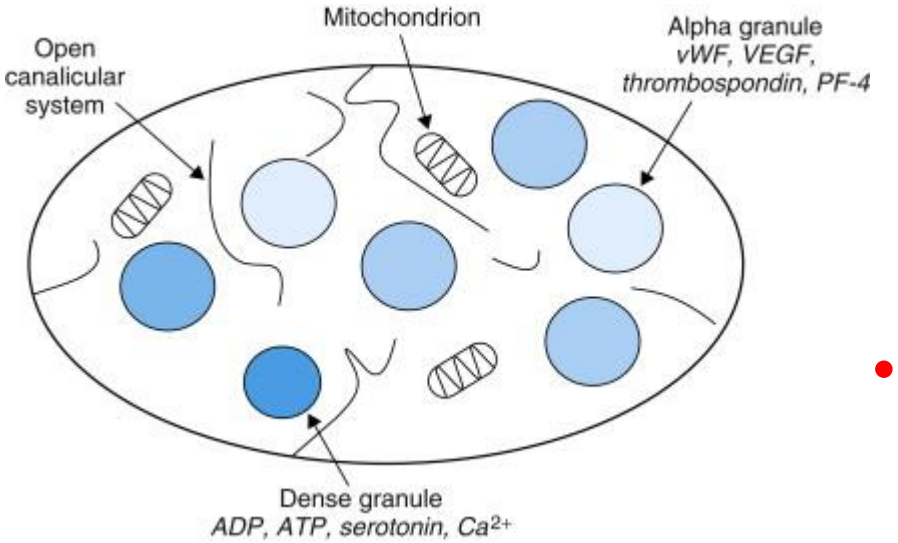
Platelet formation & structure





- **alpha granules** - contain proteins necessary for platelet adhesion
  - vWF, fibrinogen and vitronectin contribute to thrombus formation and stabilization
  - angiogenic factors - VEGF, EGF and PDGF
  - angiogenesis inhibitors - angiostatin, thrombospondin and endostatin
  - regulators of inflammation or cytokines - platelet factor 4 (PF4), CCL5 (RANTES), and interleukin-8 (IL-8)
- **dense granules** - platelet activation and recruitment and thrombus stabilization at a site of injury
  - ATP, ADP, serotonin, and calcium
- GPIIb-IIIa is expressed exclusively by megakaryocytes and platelets and is essential for both initiating and propagating thrombus formation

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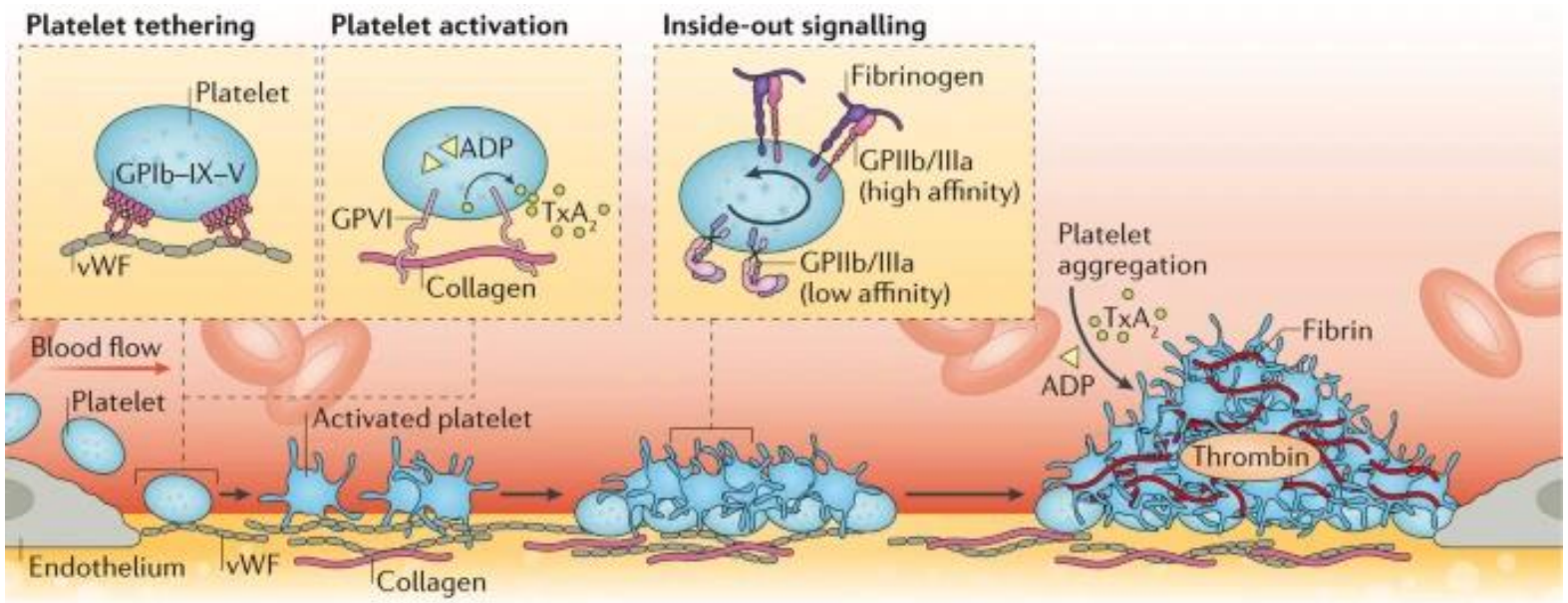
Primary hemostasis



Nature Reviews | Cardiology

Mechanisms of platelet adhesion and aggregation: Erosion or rupture of an atherosclerotic plaque exposes the thrombogenic subendothelial matrix proteins von Willebrand factor (vWF) and collagen. vWF binds to exposed collagen and uncoils, exposing multiple binding sites for platelet glycoprotein (GP)Ib–IX–V. Under arterial shear stress, the capture of platelets is mediated by interactions between GPIb and vWF (platelet tethering), which allows GPVI to bind to collagen. The binding of collagen to GPVI results in platelet activation and the release of the soluble agonists ADP and thromboxane A2 (TxA2), leading to GPIIb/IIIa activation via inside-out signalling. Activated GPIIb/IIIa changes conformation from the resting low-affinity state to a state of high affinity for its major ligand, fibrinogen. GPIIb/IIIa bound to fibrinogen acts as a bridge for platelets to aggregate. The nascent platelet thrombus is reinforced by the release of soluble agonists, such as ADP and TxA2. Thrombin generation is initiated by the release of tissue factor from the plaque and occurs on the surface of highly activated (procoagulant) platelets, resulting in a fibrin network that stabilizes the platelet thrombus.

- endothelium normally inhibits haemostasis by secretion of inhibitors of platelet aggregation and coagulation
  - nitric oxide
  - prostacyclin
  - thrombomodulin
  - heparansulfate
  - tPA
- endothelial damage stimulates platelets to **adhere** to vWf expressed on exposed subendothelium by their receptors (GPIb-IX)
- following platelet **activation** leads to release of mediators from granules
  - thromboxane, PAF, ADP, serotonin → activation of more thrombocytes (**aggregation**), vasoconstriction
  - expression of integrins (GPIIb/IIIa) → binding of fibrin and formation of definitive clot
- thrombocytes participate in the secondary haemostasis
  - by releasing tissue fator and by formation of thrombin on their surface (FIIa)

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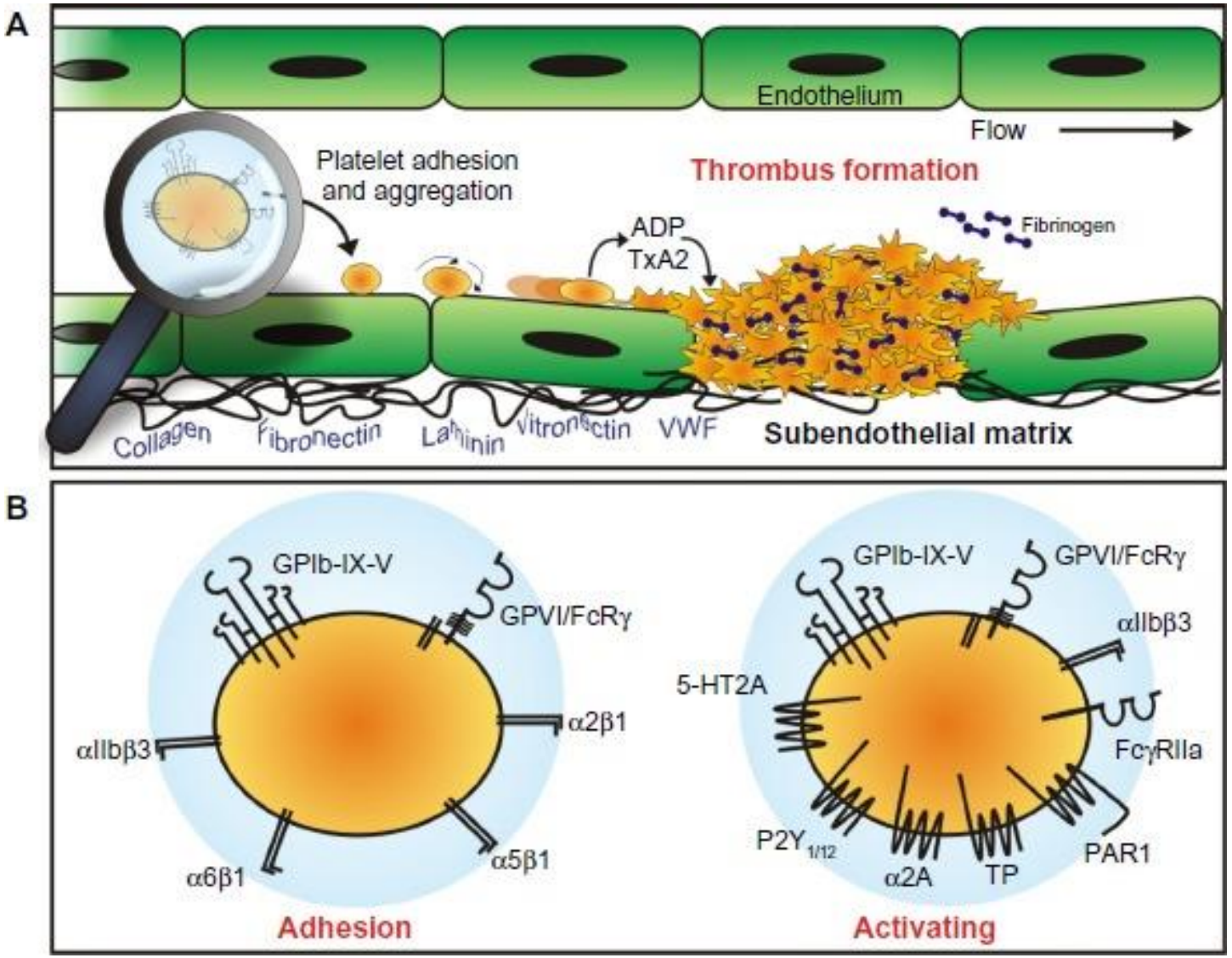
Primary platelet plug



- Platelet adhesion and aggregation:
  - (A) Platelets normally circulate through the vasculature in a nonadhesive state. Upon the detection of an exposed subendothelial matrix, platelets are induced to come into close contact with the vessel wall and roll, then arrest, at the site of vessel injury. The process of adhesion is orchestrated by the platelet adhesion receptors GPVI and GPIb-IX-V. The release of soluble agonists, such as ADP and thromboxane A2 (TxA2) amplify platelet activation. Platelet adhesion and activation, results in the formation of a platelet plug (thrombus).
  - (B) Platelet engagement with the blood vessel wall is predominantly mediated by GPVI and GPIb-IX-V; however, the platelet surface possesses receptors that can engage matrix proteins. Additional involvement of these other adhesion proteins, including integrins α2β1, α5β1, and α6β1, which bind collagen, fibronectin, and laminin, respectively, and αIIbβ3 that binds VWF and fibrinogen, among others, help to stabilize the initial attachment and facilitate platelet recruitment and thrombus growth. Platelet activation occurs following agonist binding to GPIb-IX-V and GPVI, integrin αIIbβ3, FcγRIIa, and the G protein-coupled receptors for serotonin (5-HT2A), ADP (P2Y1/12), epinephrine (α2A adrenergic receptor), TxA2 (TP), and thrombin (PAR1). Abbreviations: ADP, adenosine diphosphate; VWF, von Willebrand factor; GP, glycoprotein.

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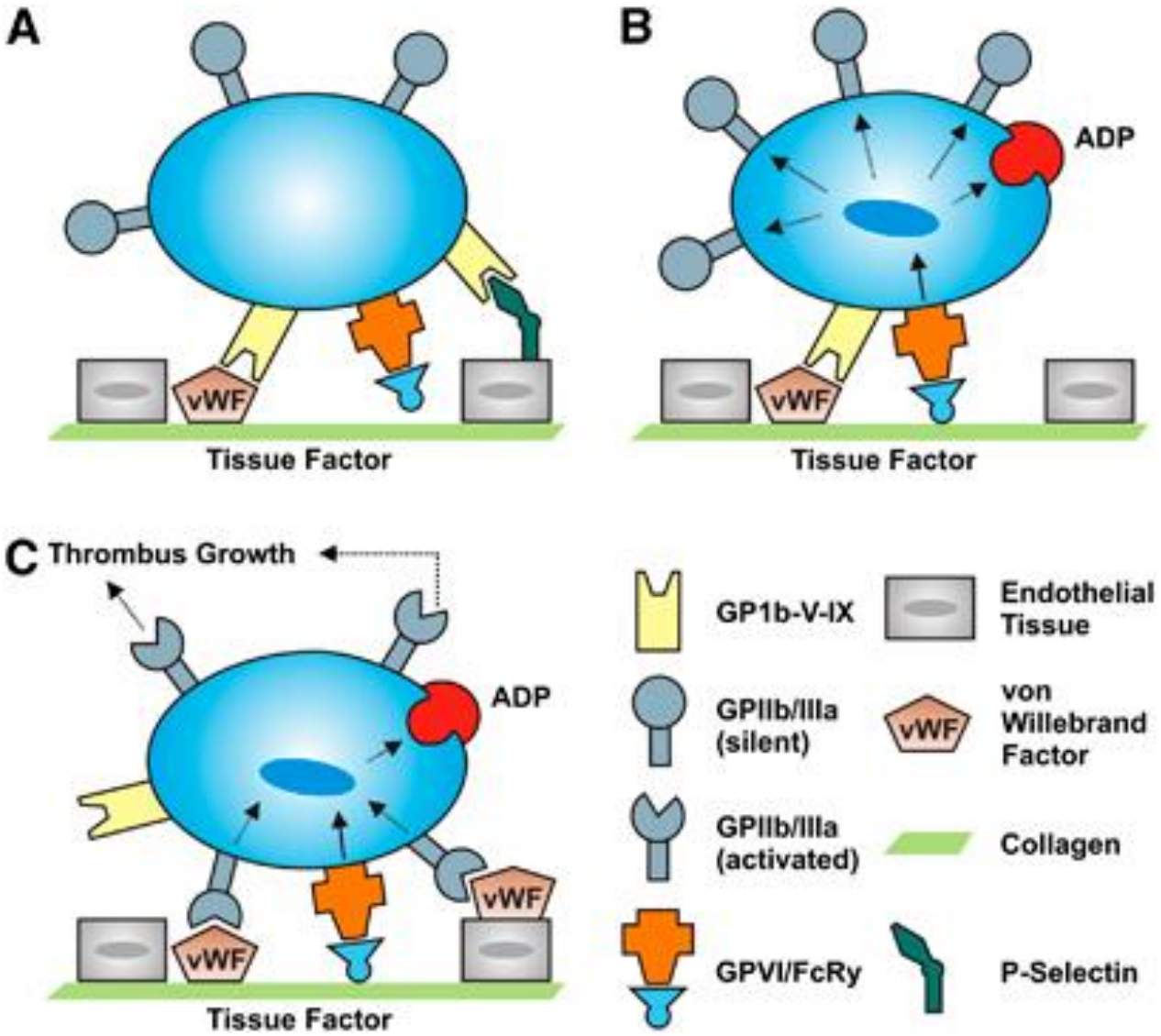
Primary platelet plug



- Distinct steps of platelet adhesion, activation, and aggregation at the activated endothelium. (A) The initial adhesion of platelets (tethering) is mediated by the binding of the glycoprotein (GP)Ib-V–IX receptor complex to the A1 domain of the von Willebrand factor (VWF) on endothelial cells. Additionally, binding to P-Selectin can enhance platelet recruitment to the intact vessel wall. (B) In a second step, interactions between GPVI and collagen stabilize the thrombus. Moreover, it comes to a cellular activation with secretion of platelet agonists (e.g., adenosine diphosphate, ADP) and transformation of the GPIIb/IIIa receptors to a state with high affinity. (C) The common final pathway of the platelet activation via the GPIIb/GPIIIa (integrin–fibrinogen) pathway culminates in an irreversible platelet aggregation and subsequent thrombus growth.

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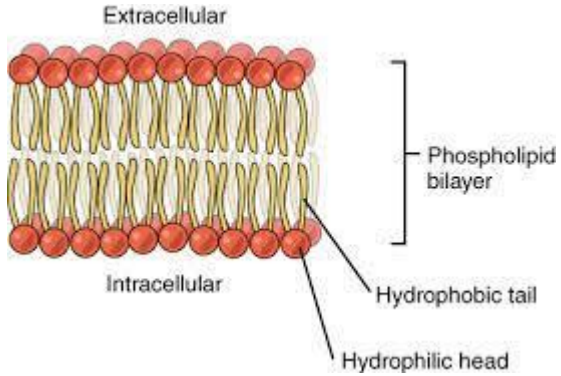


membrane phospholipids

phospholipase A2

steroids

ARACHIDONIC ACID

Cyclooxygenase Pathway

aspirin, NSAIDs

COX-1 / COX-2

selective COX-2 inhibitors

Prostaglandin G2 (PGG2)

Prostaglandin H2 (PGH2)

Tissue specific synthesases

PGD2 PGE2 PGF2α

PGI2 Prostacyclin — endothelia

TXA2 Thromboxane A2 — platelets

5- Lipooxygenase Pathway

zileton

Hydroperoxyeicosatetraenoic acid

5HPETE

5HETE → Leukotriene B4

Leukotriene A4 → Leukotriene C4 → Leukotriene D4 → Leukotriene E4

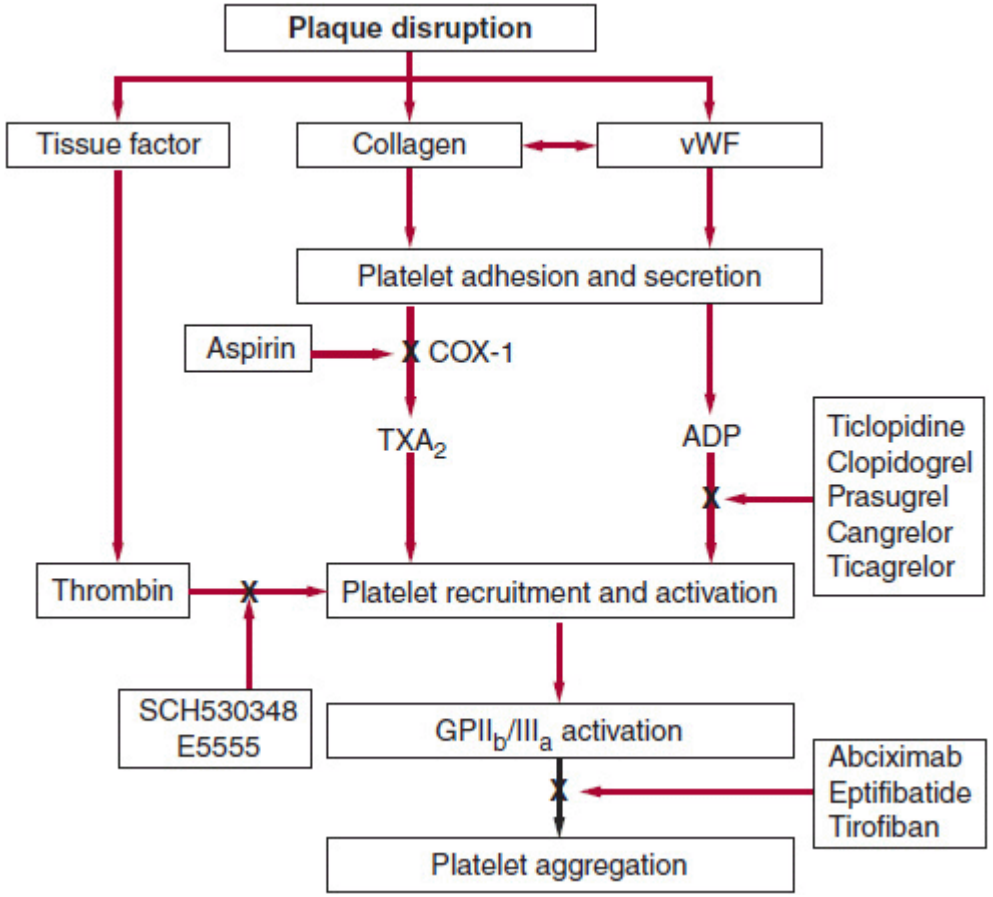
12-Lipoxygenase

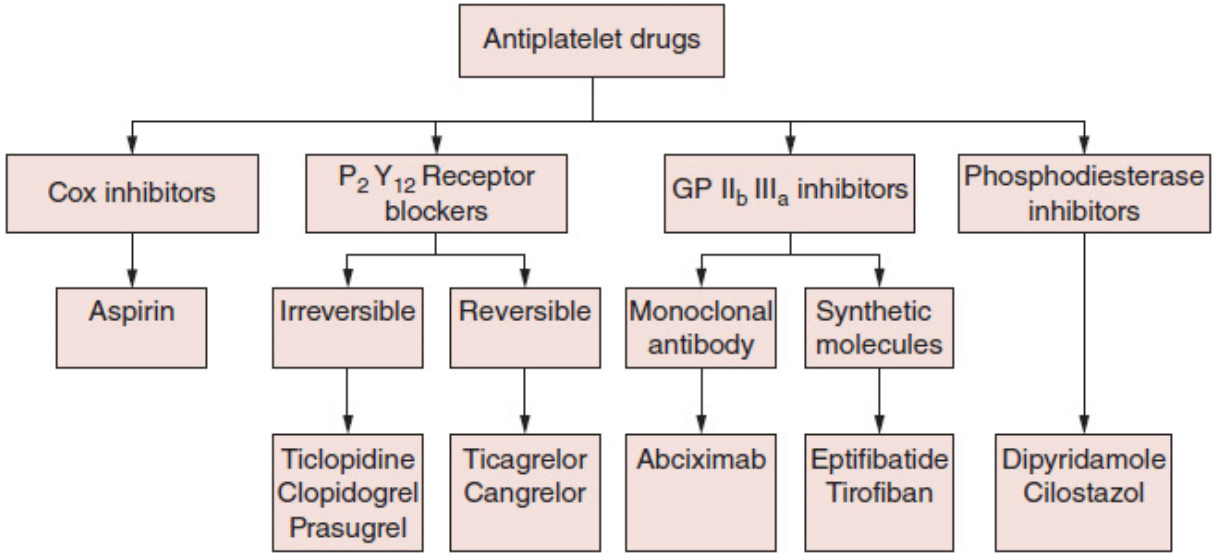
bronchial SMCs

montelukast

Lipoxin A4 Lipoxin B4

Antiplatelet therapy





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There is only 1 (2) way how to coagulate the blood, but there are many ways how to bleed

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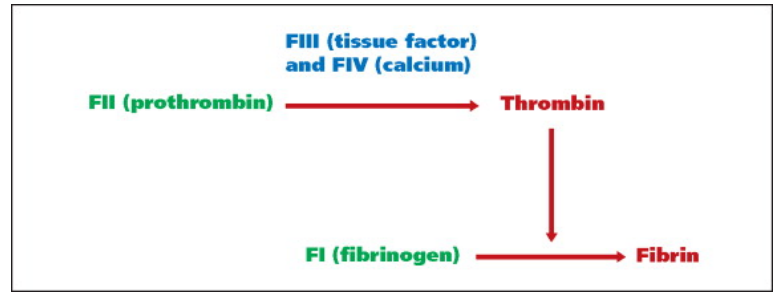
Clotting factors

No.	Name	Role
I	Fibrinogen	Clot formation
II	Prothrombin	Activation of factors I, V, VII, VIII, XI, XIII, protein C and platelets
III	Tissue factor	Cofactor VIIa
IV	Calcium	Role in binding of phospholipid coagulation factors
V	Proaccelerin	Cofactor of X – prothrombinase complex
VI		Activated form of V
VII	Proconvertin	Enables factors IX and X
VIII	Antihemophilic factor A	Cofactor of IX complex
IX	Antihemophilic factor B or Christmas factor	Enables factor X, forms the complex tensor with factor VIII
X	Stuart–Prower factor	Forms the prothrombinase complex together with factor V, which will activate factor II
XI	Antecedent of plasma thromboplastin	Activates factor IX
XII	Hageman factor	Enables factors XI, VII and prekallikrein
XIII	Fibrin stabilizing factor	Creating cross-links between fibrin monomers
XIV	Prekallikrein – Fletcher factor	Precursor of kallikrein
XV	HMWK – Fitzgerland factor	Cofactor
XVI	von Willebrand factor	Role in platelet adhesion; it is linked to factor VIII
XVII	Antithrombin III	Inhibits IIa, Xa and other proteases
XVIII	Heparin cofactor II	Inhibits IIa
XIX	Protein C	Inactivates factors Va and VIIIa
XX	Protein S	Cofactor for activated C protein

HMWK: High-molecular-weight kininogen.

- named according to their chronological discovery
  - initially Morawitz 1905
  - the cascade/waterfall model in 1964
    - model based on in vitro data!!



- all factors with the exception of TF (FIII) are present in the plasma
  - in an inactive form (zymogens)
  - only FVIIa normally circulates in small amounts in the blood
- majority of clotting factors (except TF and FVIII) are synthesized in liver
  - FII, VII, IX a X are vit. K dependent
- substrate
  - fibrinogen (FI)
- serine proteases
  - activated FII, VII, IX, X, XI, XII and prekalikrein
  - plasminogen, t-PA a u-PA
- cofactors (in tetrameric complexes)
  - HMWK, FVIII and FV

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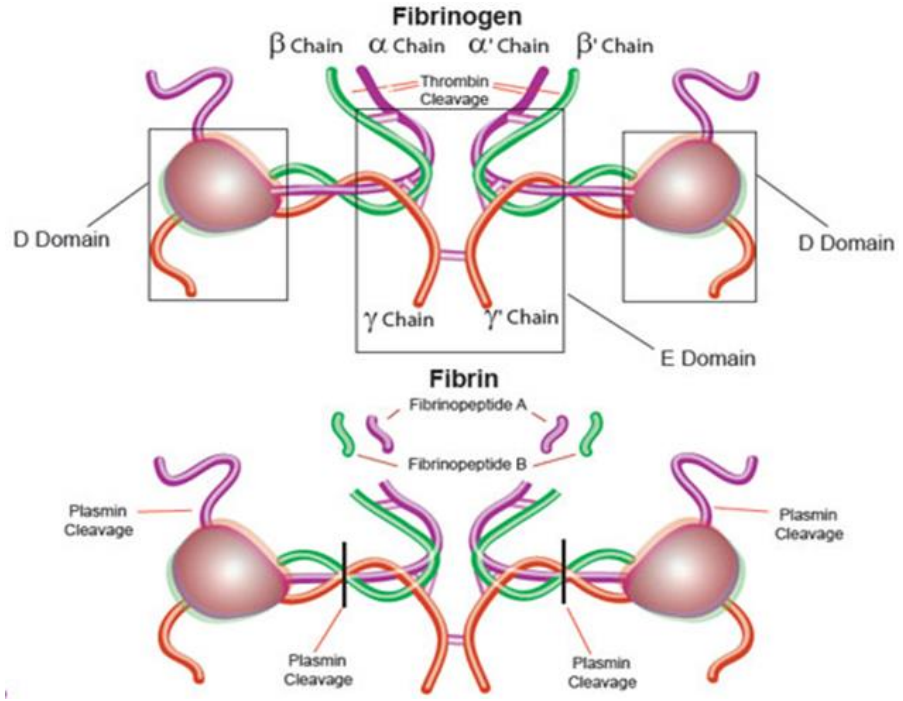
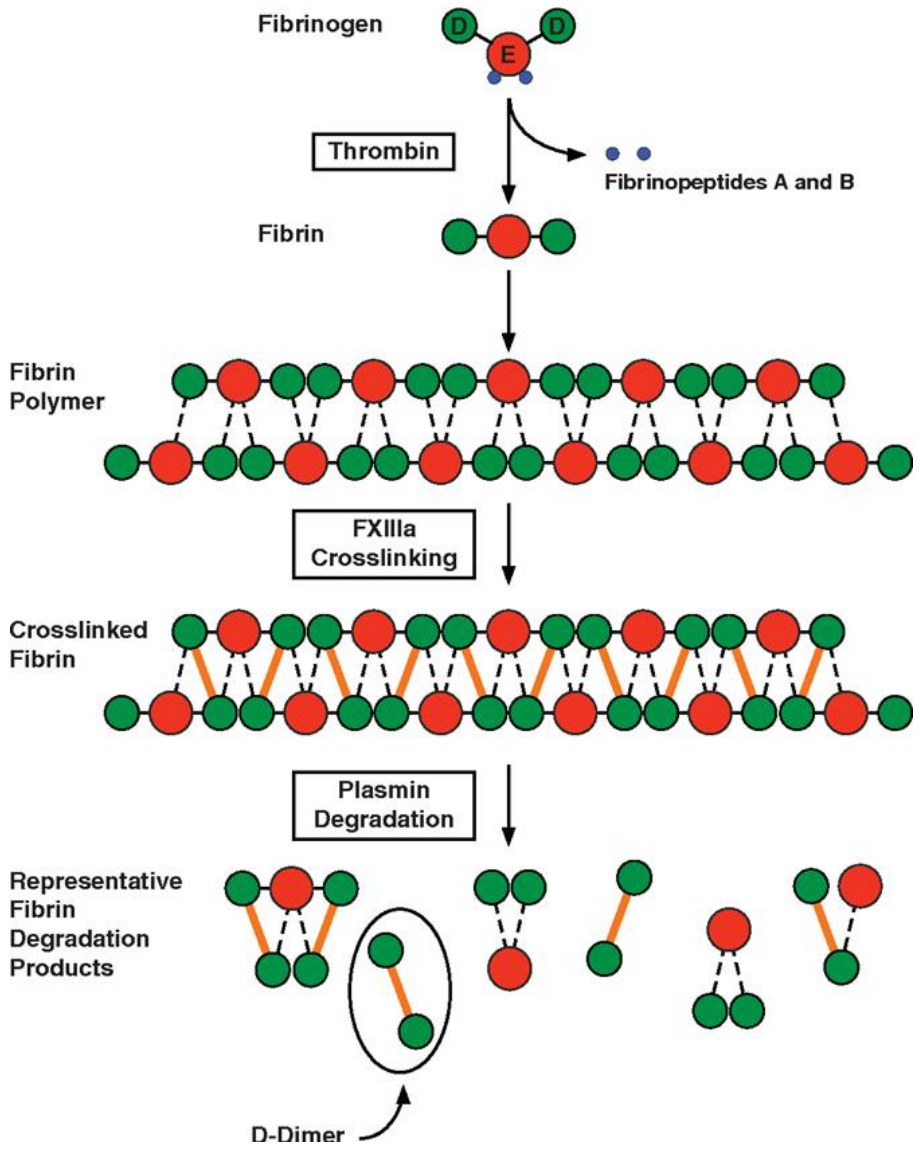
Fibrinogen - fibrin

- 3 pairs of polypeptides $([A\text{-}\alpha][B\text{-}\beta][\gamma])_2$ – 340kDa
  - D – E – D domains
- thrombin (serine protease) cleaves fibrinopeptides A and B and generates fibrin monomers $(\alpha\text{-}\beta\text{-}\gamma)_2$
- monomers spontaneously aggregate and form fibrin mesh
- fXIII (transglutaminase) further catalyses formation of cross-links between fibrin polymers
  - without fXIII (for example in hereditary deficiency) there is a unstable fragile coagulum prone to bleeding





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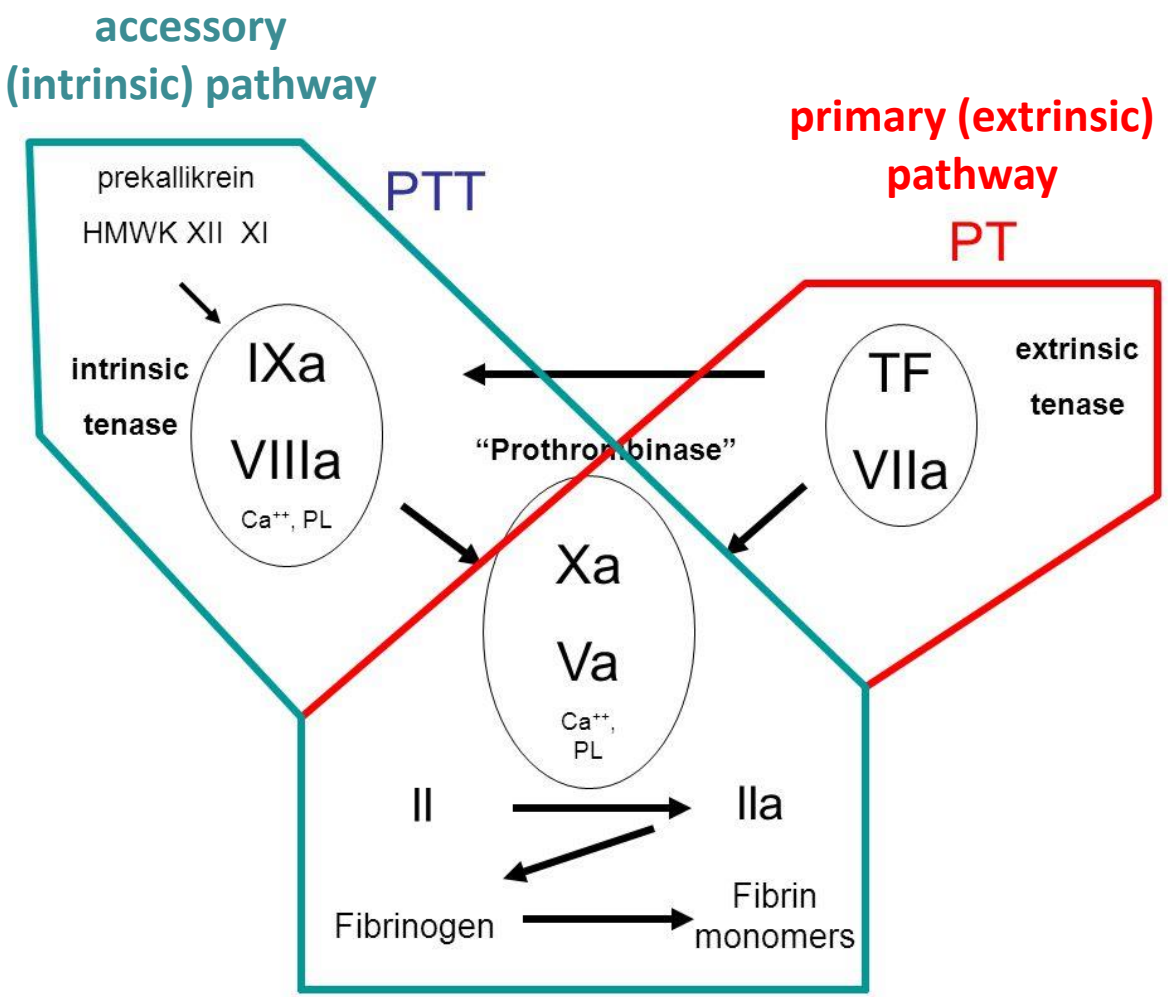
Hemostasis simply – old waterfall model



- The coagulation cascade/waterfall model has formed the basis for our understanding of coagulation for almost the past one-half century
  - it provides a logical explanation of the clotting reactions in vitro
  - in vitro laboratory studies that often used platelet-poor plasma
- prothrombin test [PT] and its international normalized ratio (INR) scalefor extrinsic and activated partial thromboplastin time [aPTT] test for intrinsic pathways were developed
  - they measure the time it takes to form an in vitro fibrin clot after a blood sample has been recalcified in the presence of appropriate reagents
- patients with specific deficiencies in the intrinsic arm of the coagulation pathway - for example, of FXII, prekallikrein (PK) and high-molecular-weight kininogen (HMWK) - have a prolonged aPTT without exhibiting increased bleeding tendencies
- patients with deficiencies in other factors associated with the intrinsic pathway—factor VIII (FVIII) and factor IX (FIX)—have a serious bleeding tendency even when the extrinsic pathway is functional
- conversely, patients with a deficiency in factor VII (FVII), an extrinsic pathway factor, also have a bleeding diathesis, even when the intrinsic pathway is not affected
- patients with deficiencies of factor X (FX) and factor V (FV), which are common pathway factors, may have impaired hemostasis, and patients deficient in factor XI (FXI) may have a bleeding diathesis that is much less predictable
- Therefore, both pathways are interdependent in vivo!!!
  - **CELL-BASED MODEL OF COAGULATION**
  - the TF/FVIIa complex is the sole initiator of coagulation in vivo

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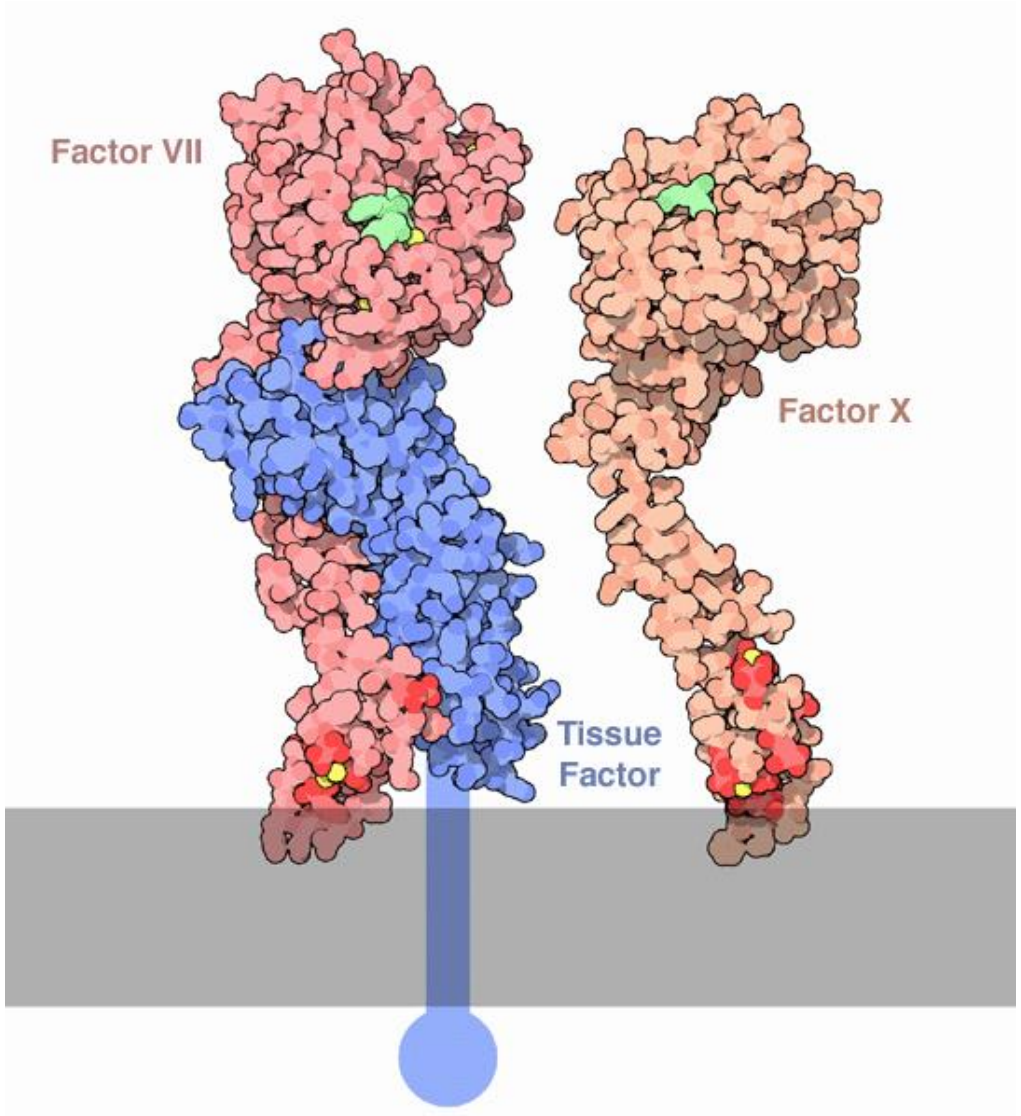
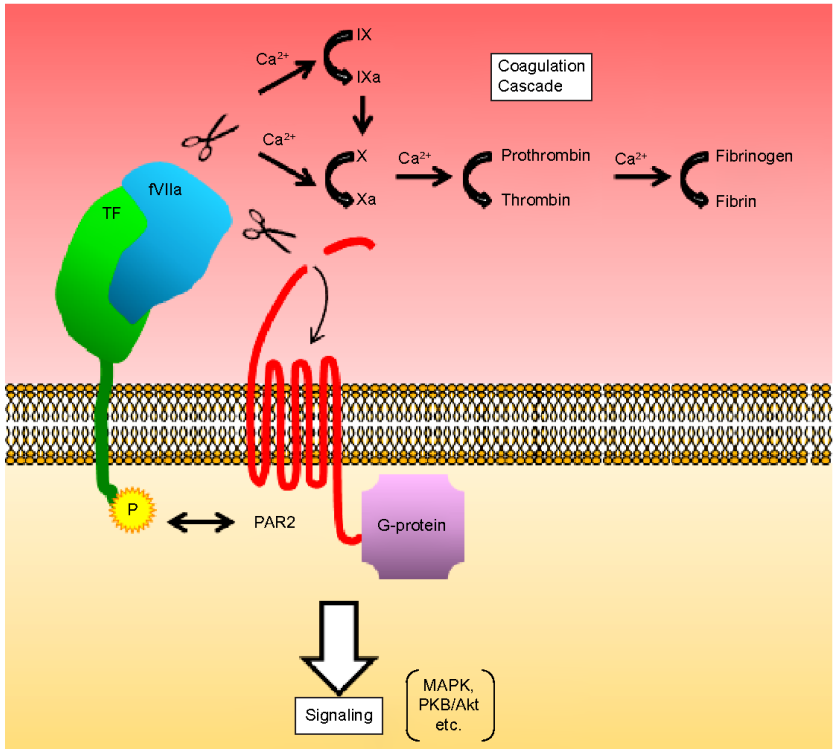
Tissue factor – coagulation and more





- TF–fViia complex formation on the surface of endothelial cells results in the activation of the extrinsic coagulation cascade and/or Pars. The serine protease activity of the TF–fViia binary complex associated with the plasma membrane initiates activation of downstream coagulation cascades associated with coagulation factors (IX, X, prothrombin, and fibrinogen). Otherwise, this protein complex cleaves the N-terminal end of PARs. PARs are then activated via intramolecular binding between the newly created n-terminus and an extracellular loop region of the receptors. activation of G-protein coupled receptors subsequently activates downstream signalling cascades. Phosphorylation of the C-terminal end of TF could also lead to association with Par2 in a coagulation-independent manner to augment the signalling cascade

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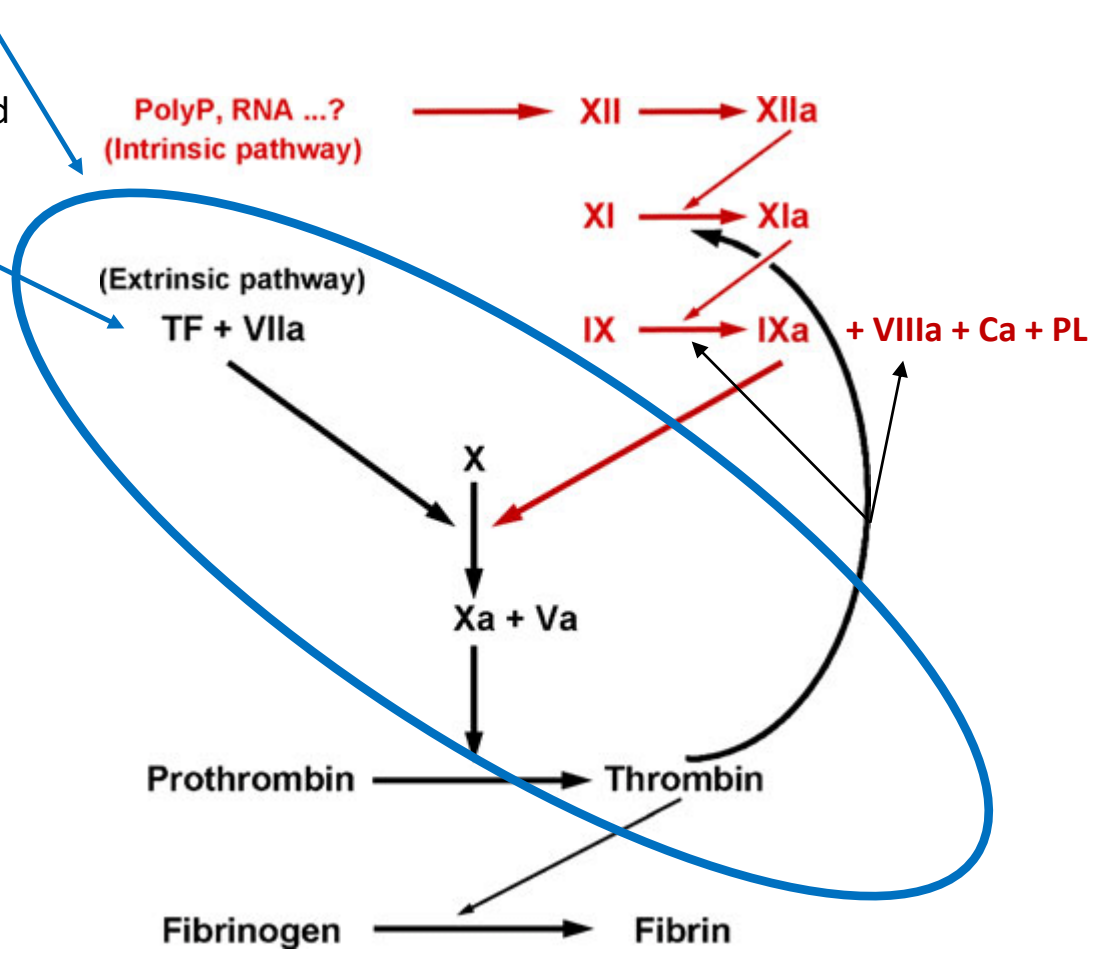
Hemostasis happens on blood cell surfaces – cell based model

- **primary** (formerly extrinsic) is fast, but less effective = **INITIATION**
  - TF is a cell receptor for FVIIa
  - TF + FVIIa + Ca + PL = **extrinsic tenase** complex
  - amount of activated thrombin is small and insufficient to convert required amount of fibrinogen, but effective to activate FXI (and FIX and cofactors FVIII and FV) and thrombocytes
  - moreover, TF is quickly inactivated by TF inhibitor (TFPI)
- following the TF blockade by TFPI the **accessory** (formerly intrinsic) pathway, that is longer but more effective = **AMPLIFICATION** and **PROPAGATION**
  - activation of FXI, FIX, FVIII and FV by thrombin
  - IXa + VIIIa + Ca + PL = **intrinsic tenase** that effectively activates FX
  - FXa in a complex with FVa, Ca and PL (= **prothrombinase**) effectively converts prothrombin to FIIa and the cleaving the fibrinogen
- negatively charged phospholipids (**PL**) necessary for the formations of complexes are provided by blood cells, namely **platelets and monocytes**
- role of HMWK, factors XII and XI (in a contact with negatively charged surfaces e.g. on.
  - exposed subendothelial collagen in vessels
  - lipoproteins (chylomicrons, VLDL)
  - bacterial wall
- is less clear in vivo (compared to the old „waterfall“ theory)
  - deficiencies of HMWK, FXII and to some extent FXI do not lead to bleeding



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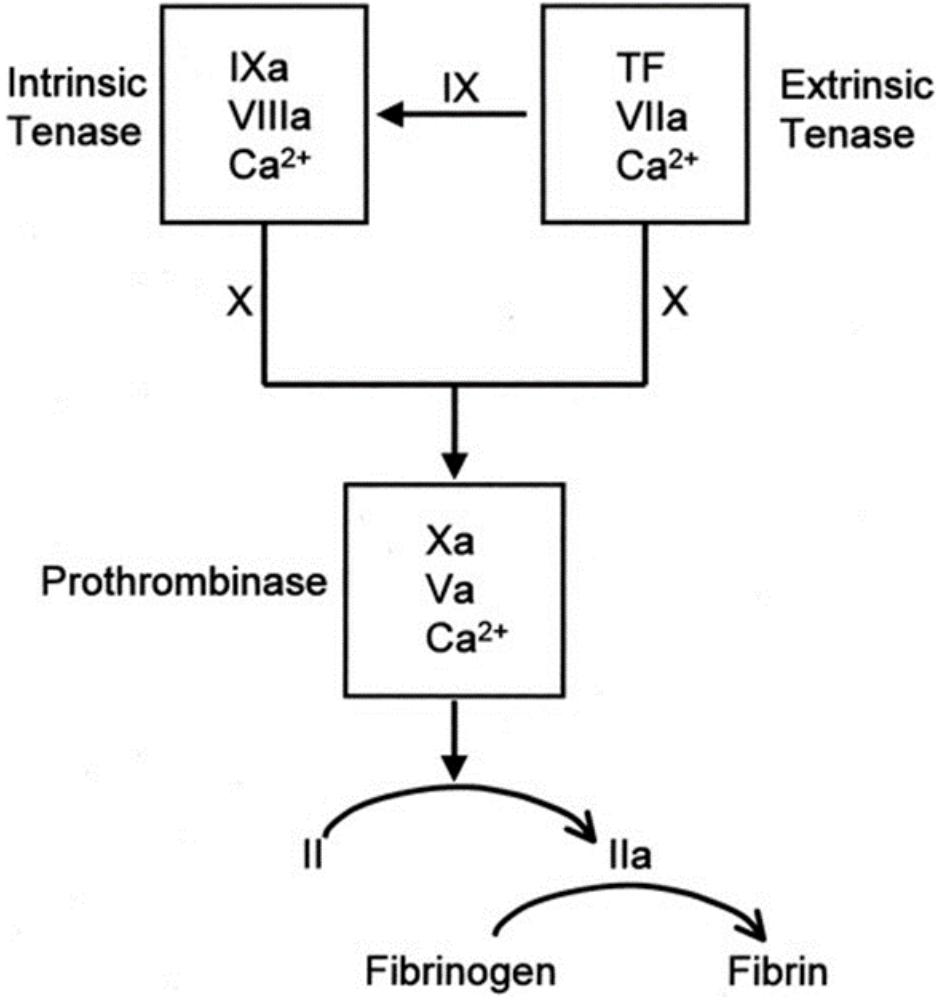
Coagulation pathways



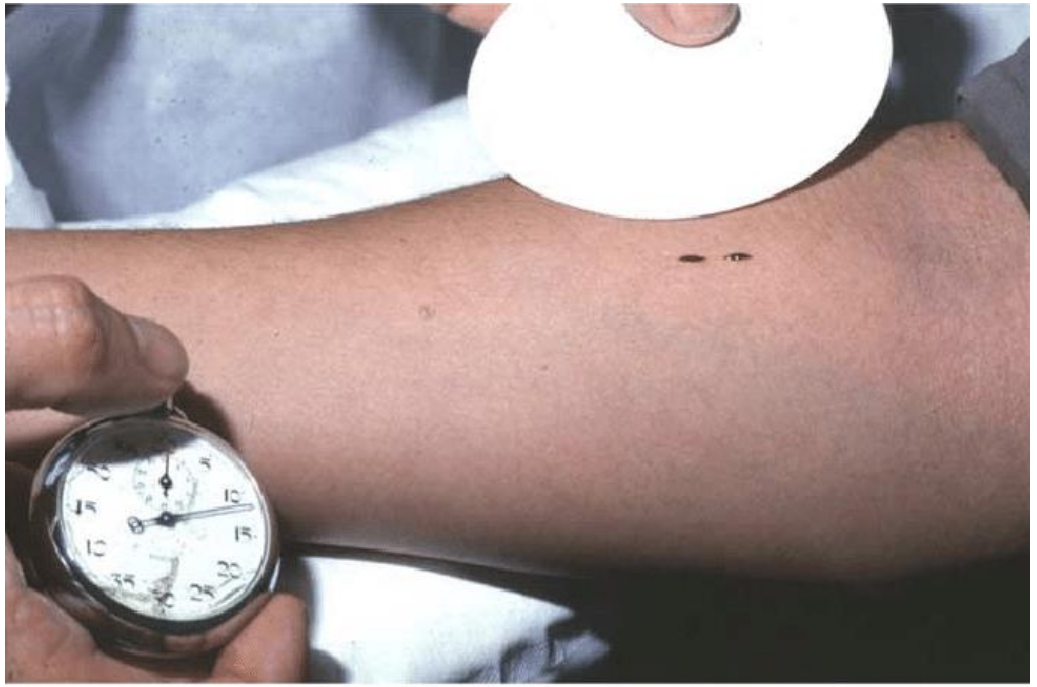
- Coagulation is initiated by extrinsic tenase, which forms when factor VIIa binds to tissue factor. Extrinsic tenase activates factors IX and X. In the presence of calcium, factor IXa binds to negatively charged phospholipid surfaces where it interacts with factor VIIIa to form intrinsic tenase, a complex that efficiently activates factor X. Factor Xa binds to factor Va on negatively charged phospholipid surfaces to form prothrombinase, the complex that activates prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin. Activated platelets or monocytes provide negatively charged phospholipid surfaces on which these clotting reactions occur.

Shannon M. Bates. Circulation. Coagulation Assays, Volume: 112, Issue: 4, Pages: e53-e60, DOI: (10.1161/CIRCULATIONAHA.104.478222)

Coagulation tests

	First-line (screening) testing	Second-line (specific) testing
Hemorrhagic disorders		
Primary hemostasis	Platelet count PFA-100	Platelet aggregation Platelet nucleotides Platelet factor 3 (PF3) von Willebrand factor (antigen and functional)
Secondary hemostasis	Activated partial thromboplastin time (APTT) Prothrombin time (PT) Fibrinogen (functional)	Intrinsic pathway factors Factor VII Fibrinogen (immunological) Factor XIII Thrombin time and/or reptilase time α_2-Antiplasmin Plasminogen activator inhibitor-1
Global (alternative) tests	Thrombin generation assays Thrombelastography/thromboelastometry Clot waveform analysis Atomic force microscopy (AFM)	

bleeding time (in vivo) – primary hemostasis (PLT function)



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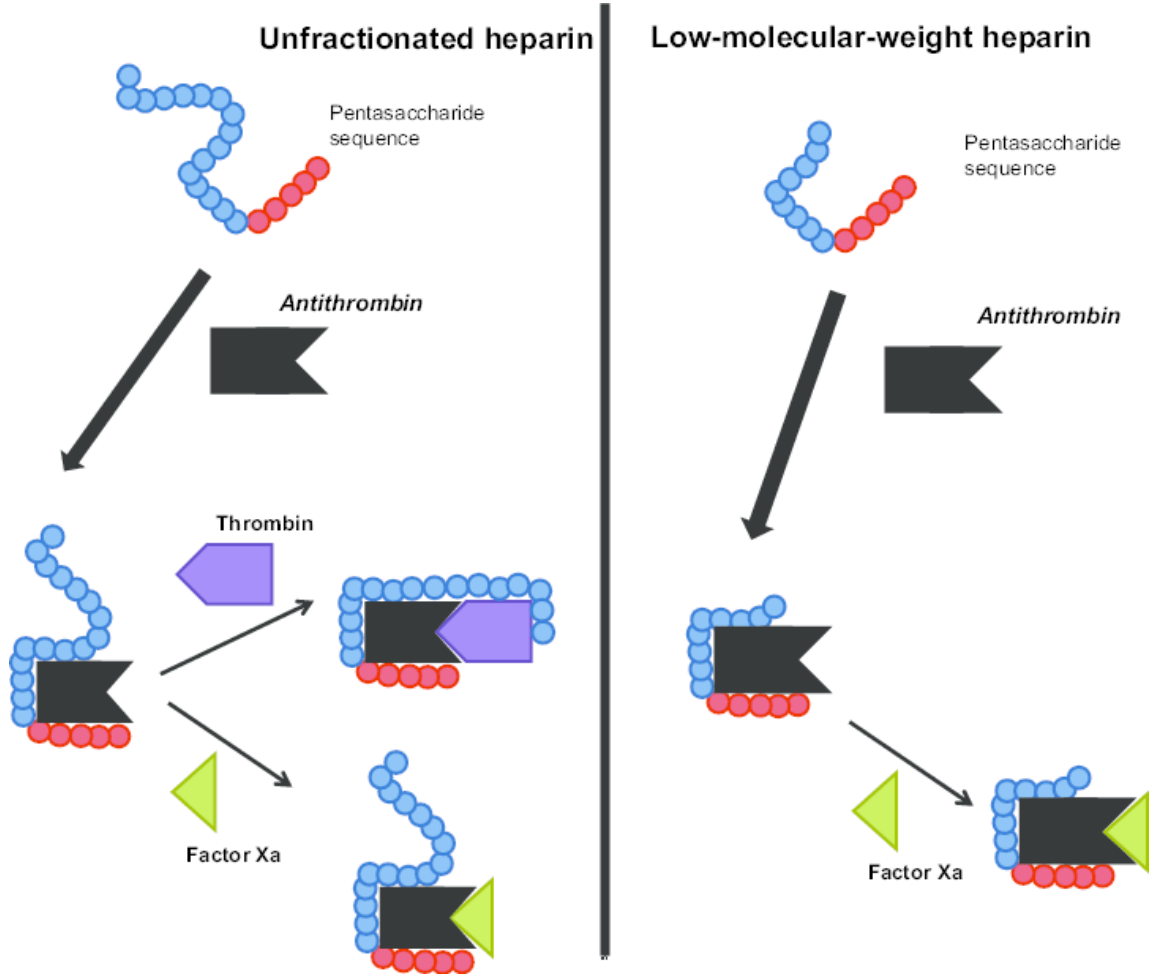
Loss of blood is a life threatening event, but the longer absence of perfusion as well

- Regulation of blood clotting
  - (1) velocity of blood flow
  - (2) intact endothelium
    - non-sticky
    - vasodilation (NO, PGI_2)
    - heparan sulfate (neutralizes serine proteases)
    - thrombomodulin (neutralizes thrombin)
    - t-PA (fibrinolysis)
  - (2) concentration of inhibitory factors
    - (a) control on the levels of thrombin
      - heparin
      - antithrombin III (and heparan sulfate)
        - inhibits thrombin and also IX, X, XI and XII
      - a2-macroglobulin
      - heparin cofactor II
      - a1-antitrypsin
    - (b) control on the level of factor Xa (inactivation of FVIII a V)
      - protein C + thrombomodulin
      - protein S
  - (3) activity of fibrinolysis



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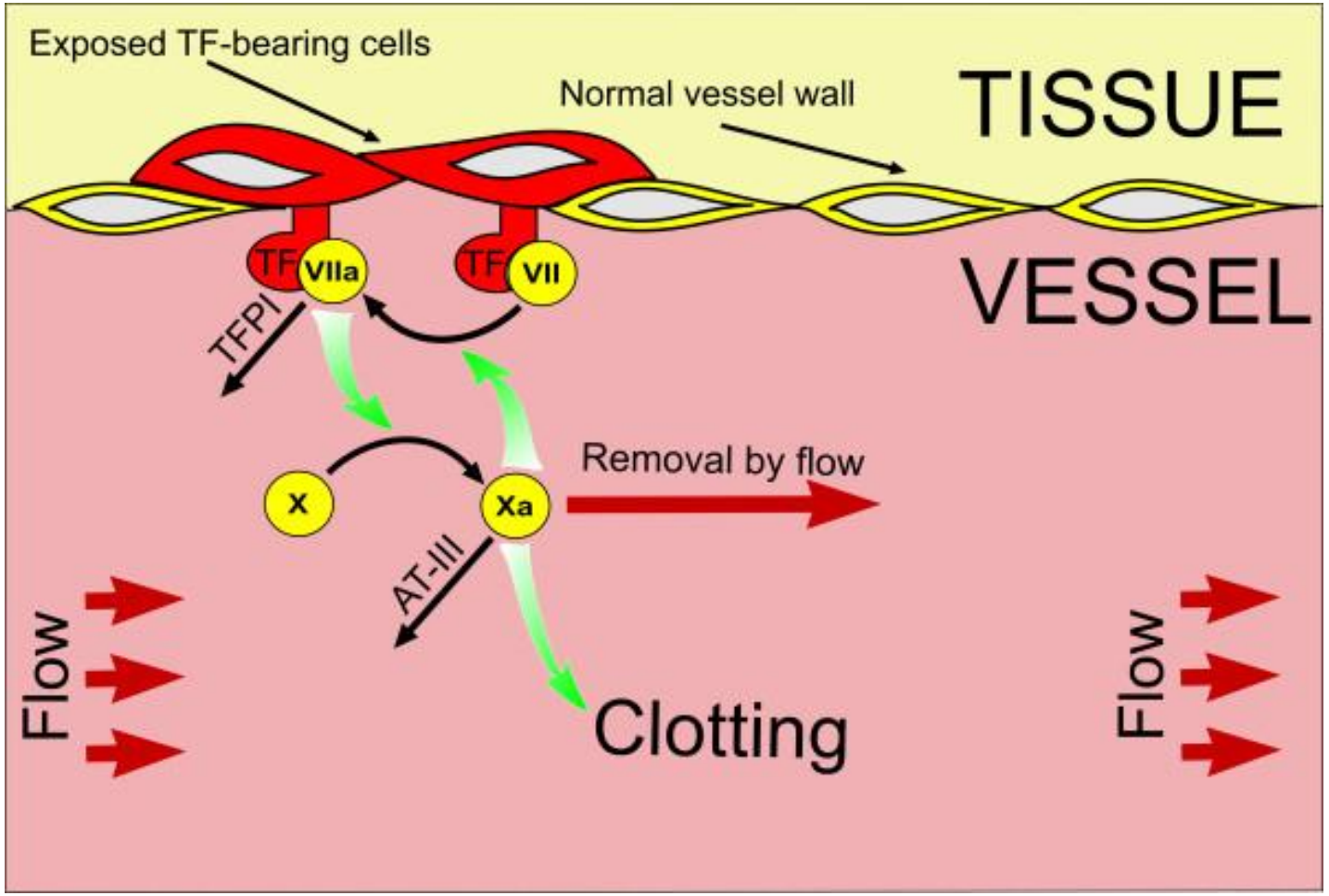
Why blood stasis may lead to thrombus (Virchow)



- Regulation of fibrin clotting by flow via the factor VII activation by factor Xa. The diagram illustrates the mechanisms controlling initiation of coagulation in the presence of flow. Factor VIIa initially present in plasma binds to TF and activates factor X; it is also inhibits by TFPI. Factor Xa can activate TF-bound factor VII in a positive feedback manner. In the absence of flow, both inhibition by TFPI and activation by factor Xa are not significant, because high concentrations of TF present on fibroblast rapidly bind factor VIIa; they do not need additional factor VII activation and are not particularly sensitive to factor VIIa-TF complex inhibition. When blood flow is present, factor Xa is rapidly removed, and the rate of factor X production becomes insufficient to create fibrin clot (factor VIIa-TF complex being inhibited by TFPI). This is when factor Xa-dependent feedback becomes important: factor Xa activates factor VII and increases its own production, counteracting the effects of flow. At higher shear rates, this feedback is insufficient, and factor Xa production is strongly inhibited. Thus, inhibition of factor VIIa-TF complex by TFPI and factor VII activation by factor Xa combine to create a threshold-like response of the system in flow: rapid clotting at low shear rates and almost no thrombus formation at higher shear rates.

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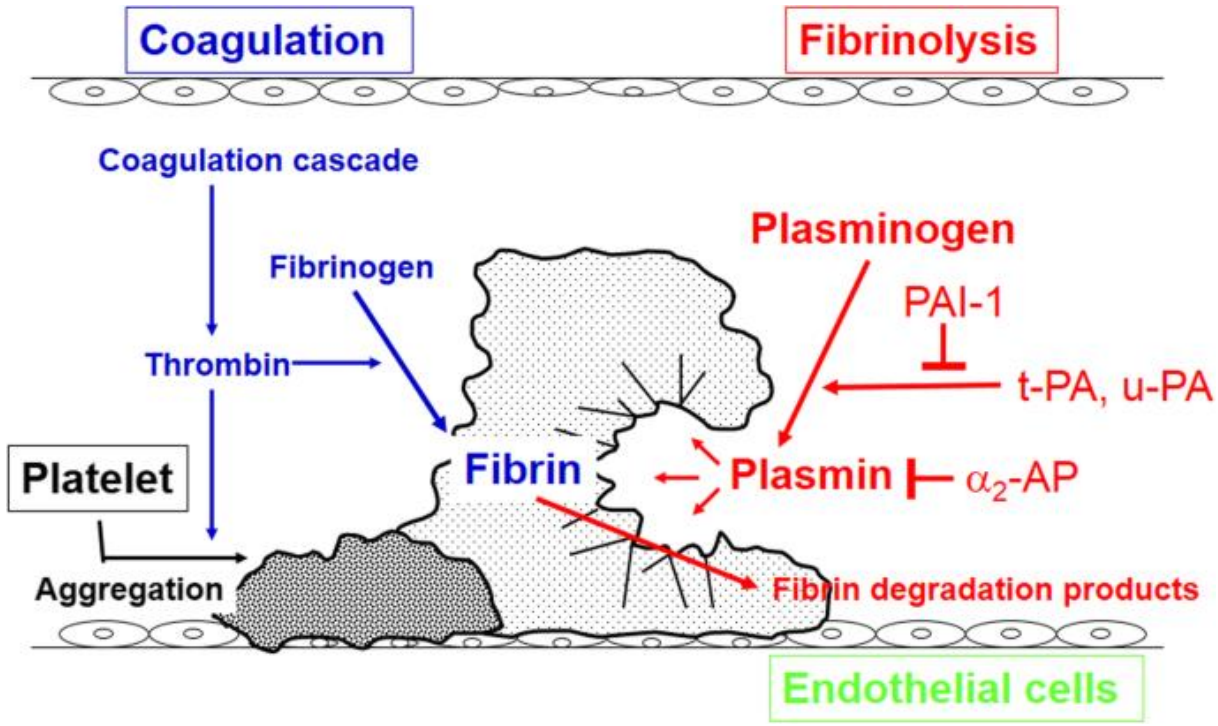
Fibrinolytic system

- plasmin (serine protease) circulates as a inactive proenzyme (plasminogen)
  - free plasmin vividly inhibited by α_2-antiplasmine
- activation of plasminogen by tissue plasminogen activator (t-PA) (from endothelium) and urokinase u-PA (from epithelia) to plasmin
- degradation of fibrin to fibrin-degradation products
  - one of which are a D-dimers used in diagnostics of thrombosis
- activity of tPA inhibited by PAI-1
  - PAI-1 is an acute phase reactant and factor increased in obesity



Hemostasis disorders

- (A) hypocoagulation states (bleeding diathesis)
  - defects of primary haemostasis
    - vessel wall diseases
    - thrombocytopenia
    - thrombocytopatia/thrombasthenia
      - incl. von Willebrand disease
  - defects of secondary haemostasis (coagulopathies)
    - hereditary
      - haemophilia A and B
      - von Willebrand disease
    - acquired
      - malnutrition, chronic liver disease etc.
- (B) hypercoagulable states (thrombophilia)
  - hereditary
    - activated protein C resistance (APCR)
  - acquired
- (C) combined
  - syndrome of disseminated intravascular coagulation (DIC)

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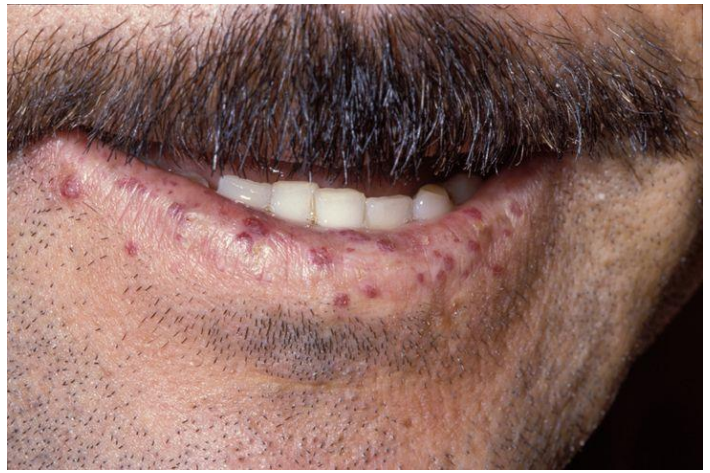
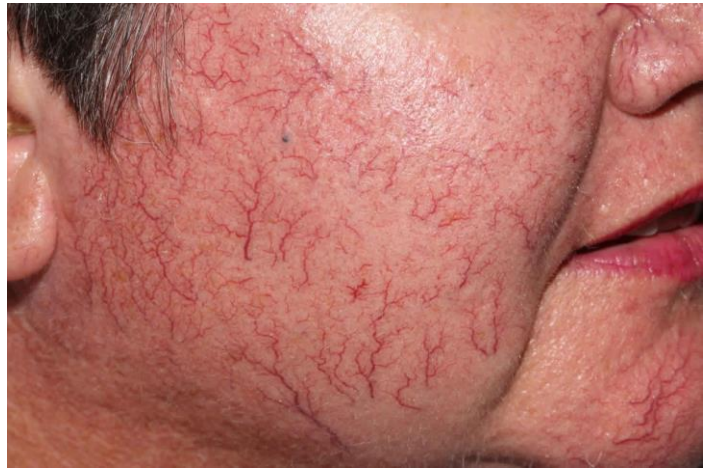
HYPO-COAGULATION DISORDERS (BLEEDING DIATHESIS)

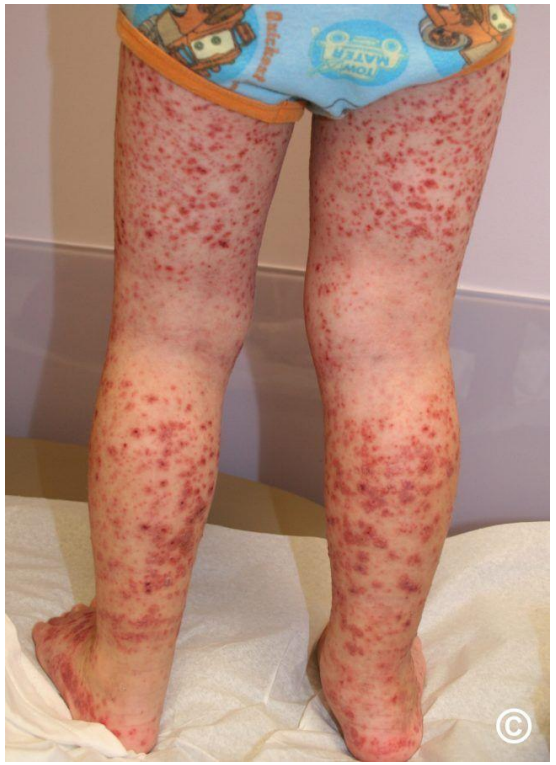
MED

Skin and mucosal bleeding in defective 1-y hemostasis

- (1) vasculopathies
  - inborn hereditary
    - telengiectasia hereditaria (m. Rendu-Osler)
      - thinning of vessel wall → telengiectasias (skin, mucosas, lungs, urogenital tract)
    - Ehlers-Danlos and Marfan syndrome
      - defective structure of connective tissue (collagen or elastin)
  - acquired
    - senile purpura
    - bacterial toxins (measles, scarlet fever)
    - vit. C deficiency (scorbutic)
    - immune complexes (Henoch-Schönlein purpura)
- (2) thrombocytopenia
- (3) thrombocytopathia/thrombasthenia
- (4) von Willebrand disease







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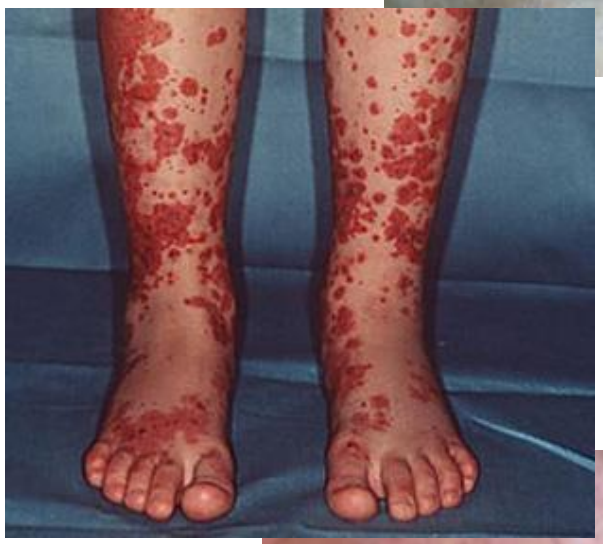
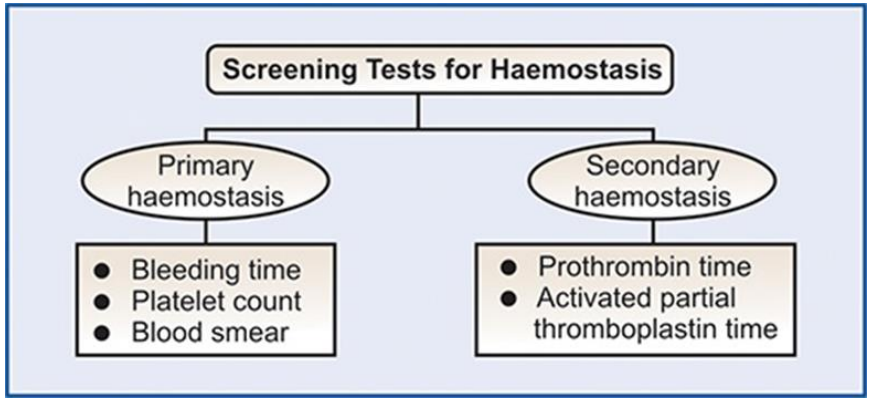
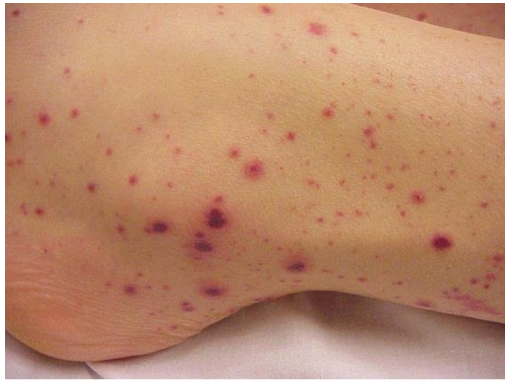
Defects of primary hemostasis

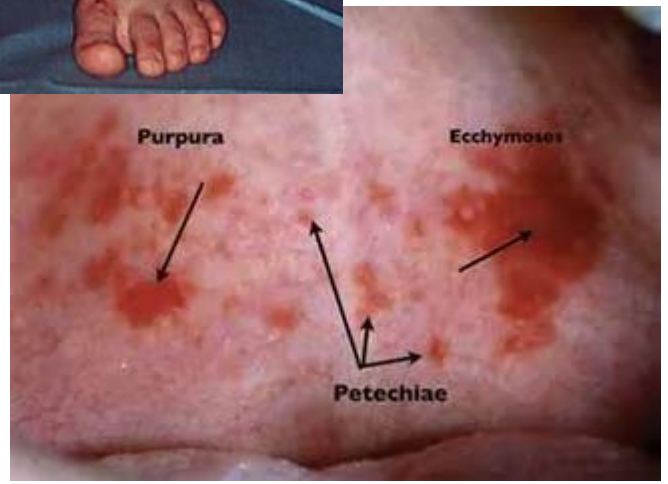
- manifestation:
  - skin: petechiae (1-2mm), purpura (>2mm), ecchymosis (>1cm) – easy bruising (worse in alcoholic malnutrition
  - mucosal membranes: epistaxis, bleeding from gums or GIT, haematuria, menorrhagia, haemoptysis
  - intracranial bleeding only in very severe disorders (e.g. intraspinal hematoma after lumbar punction)



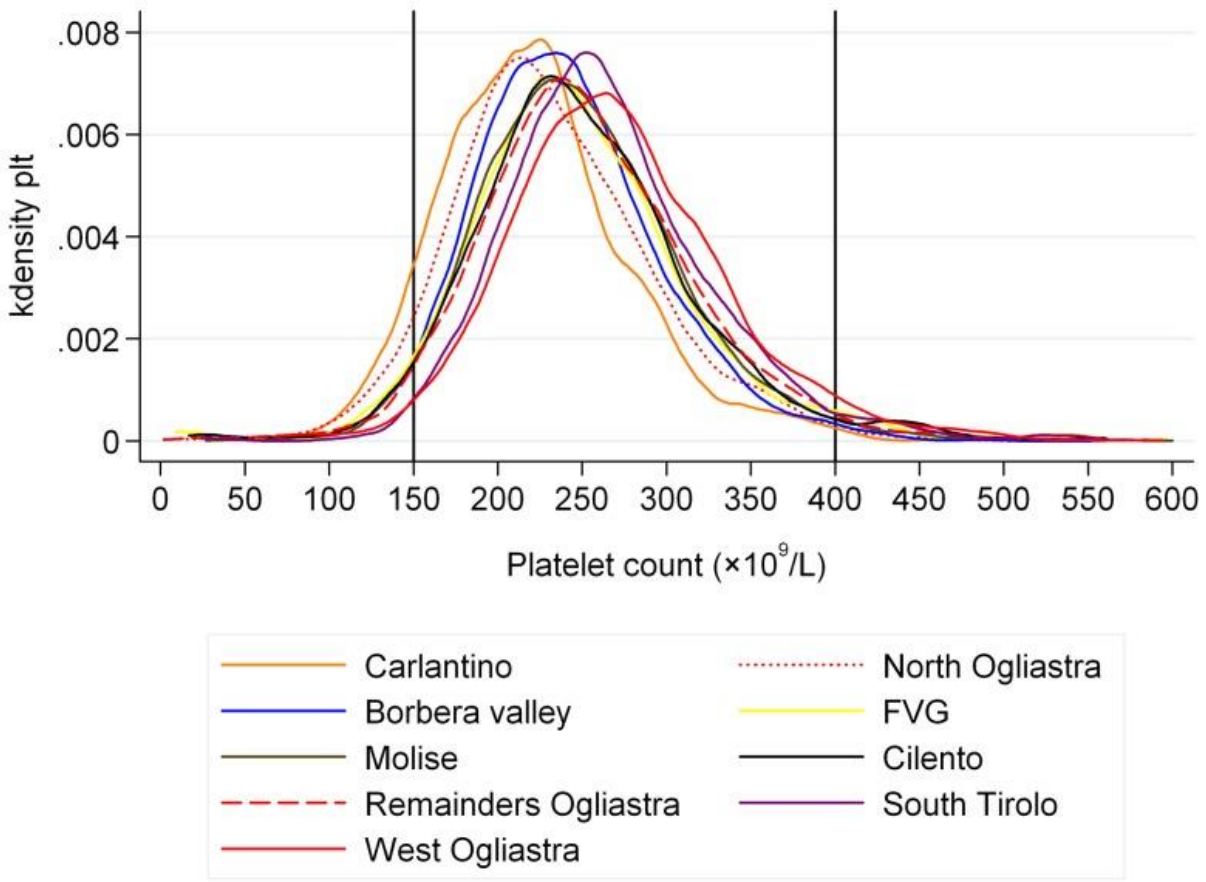


Primary hemostatic disorder	Secondary hemostatic disorder
Petechiae	Hematomas (single or multiple)
Ecchymoses	Subcutaneous bleeding
Epistaxis	Hemoperitoneum
Gingival bleeding	Hemothorax, including hemomediastinum and pulmonary parenchyma
Menorrhagia	
Hyphema	Hemarthrosis
Hematuria	Bleeding into muscles
Melena	Central nervous system hemorrhage

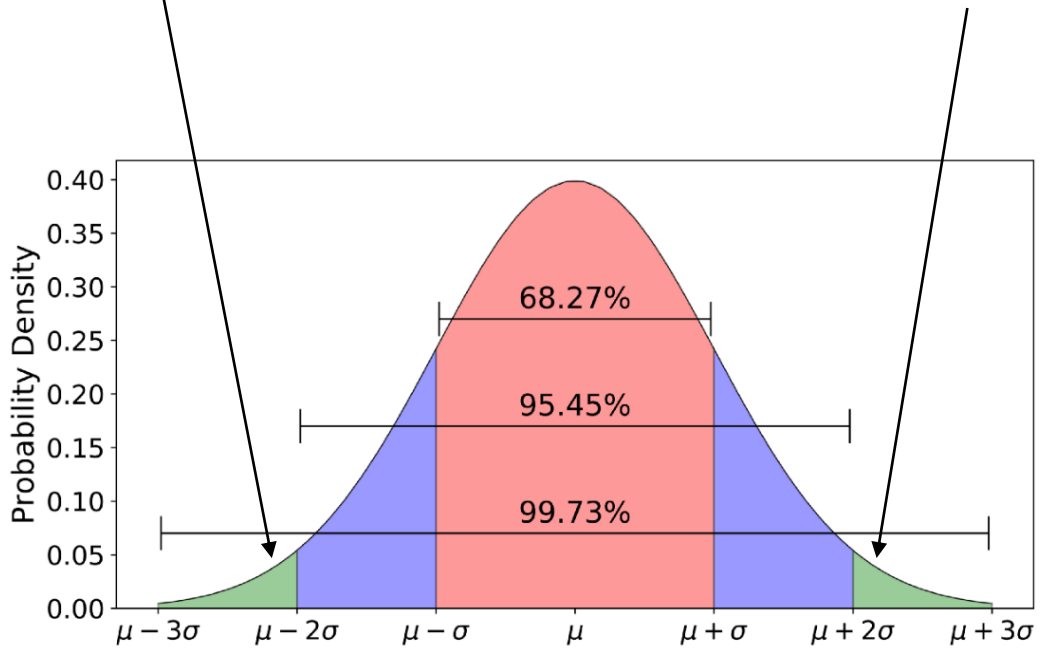




Thrombocytopenia - platelet count

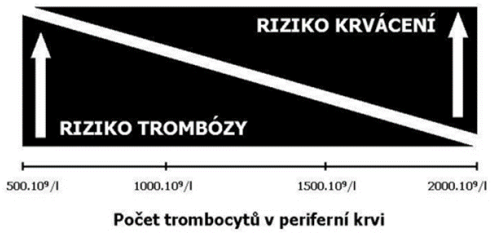


- reference range 150,000 – 400,000/μL
- clinically significant values (± 2SD)
  - bleeding <50,000/μL + symptoms
  - thrombocythemia >750,000/μL



Thrombocytosis (= thrombocythemia) = ↑ PLT count

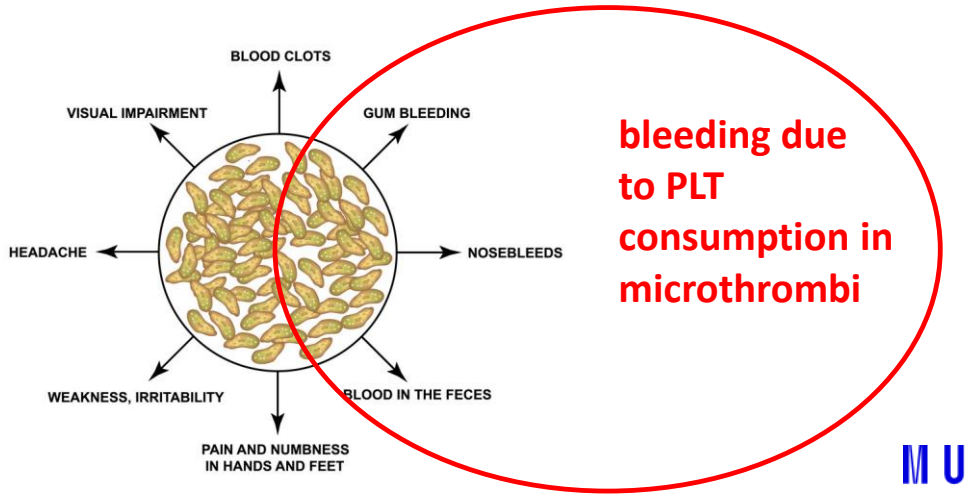
- clinically insignificant unless PLT >750,000/μL
- primary (essential) – rare
  - one of the forms of myeloproliferative disorders (disease of the elderly)
    - essential thrombocythemia or polycythaemia vera (clonal proliferation)
- secondary (reactive) – common
    - 100% in children
    - 80% in adults
  - generally due to
    - ↑ TPO (thrombopoetin)
      - (↑ IL-6 → TPO)
    - ↑ EPO (mimics the effect of TPO)
      - iron deficiency, bleeding, haemolytic anaemia, malignancy (anemia)
    - ↑ IL-6 (inflammation)
      - infection, malignancy



Clonal	Reactive	Spurious
Essential thrombocythemia	Infection	Microspherocytes
Polycythemia vera	Inflammation	Neoplastic cell fragments
Primary myelofibrosis	Iron deficiency	Schistocytes
Chronic myeloid leukemia	Hyposplenism	Bacteria
	After surgery	
	Hemolysis	
	Malignancy	
	Effect of drugs	

Source: adapted from Bleeker and Hogen[6]

SYMPTOMS OF THROMBOCYTOSIS



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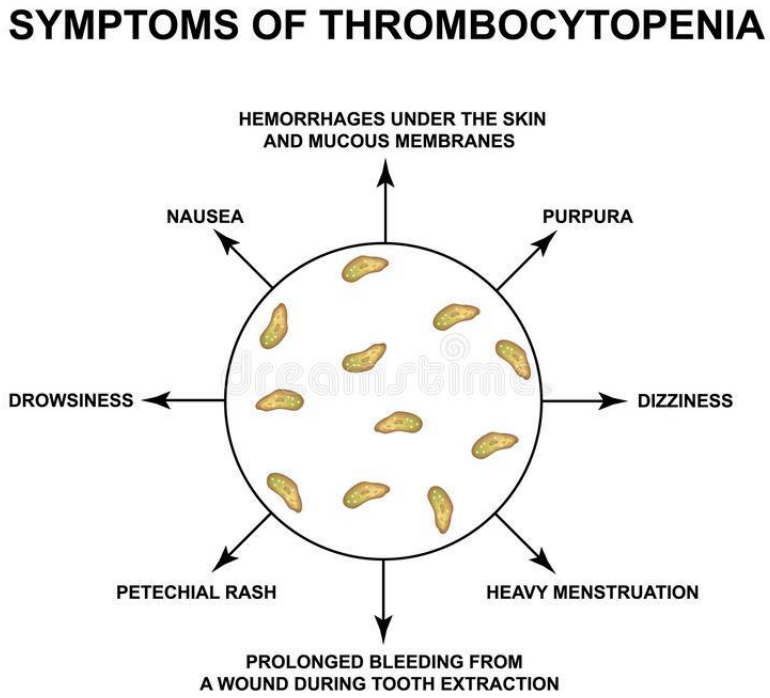
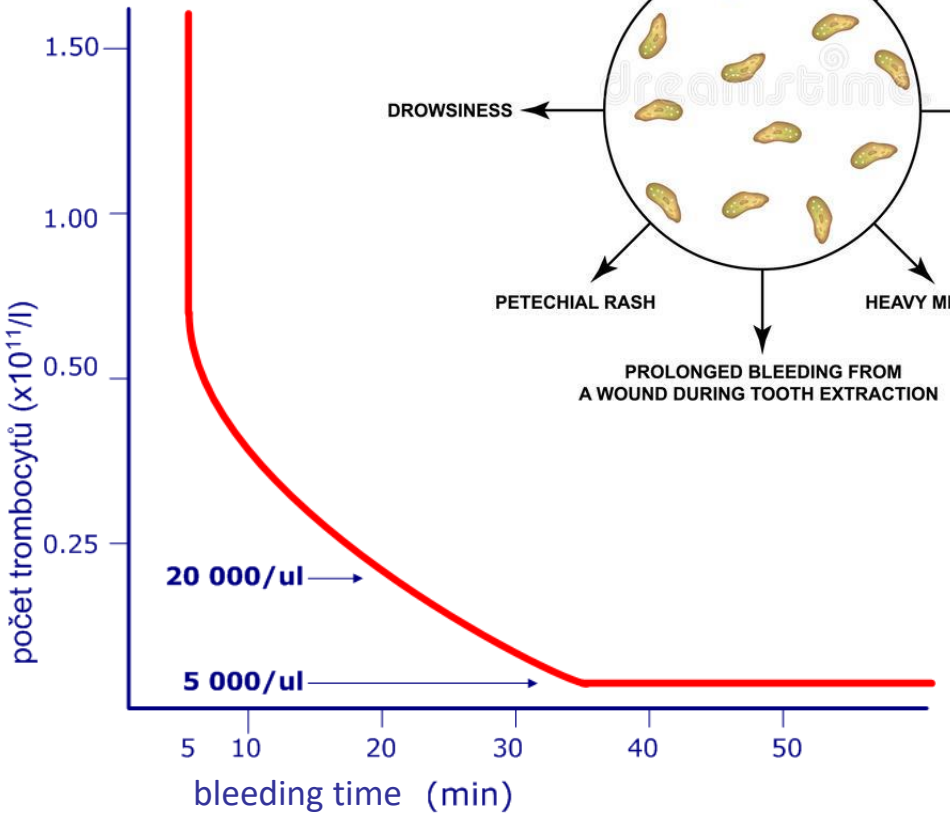
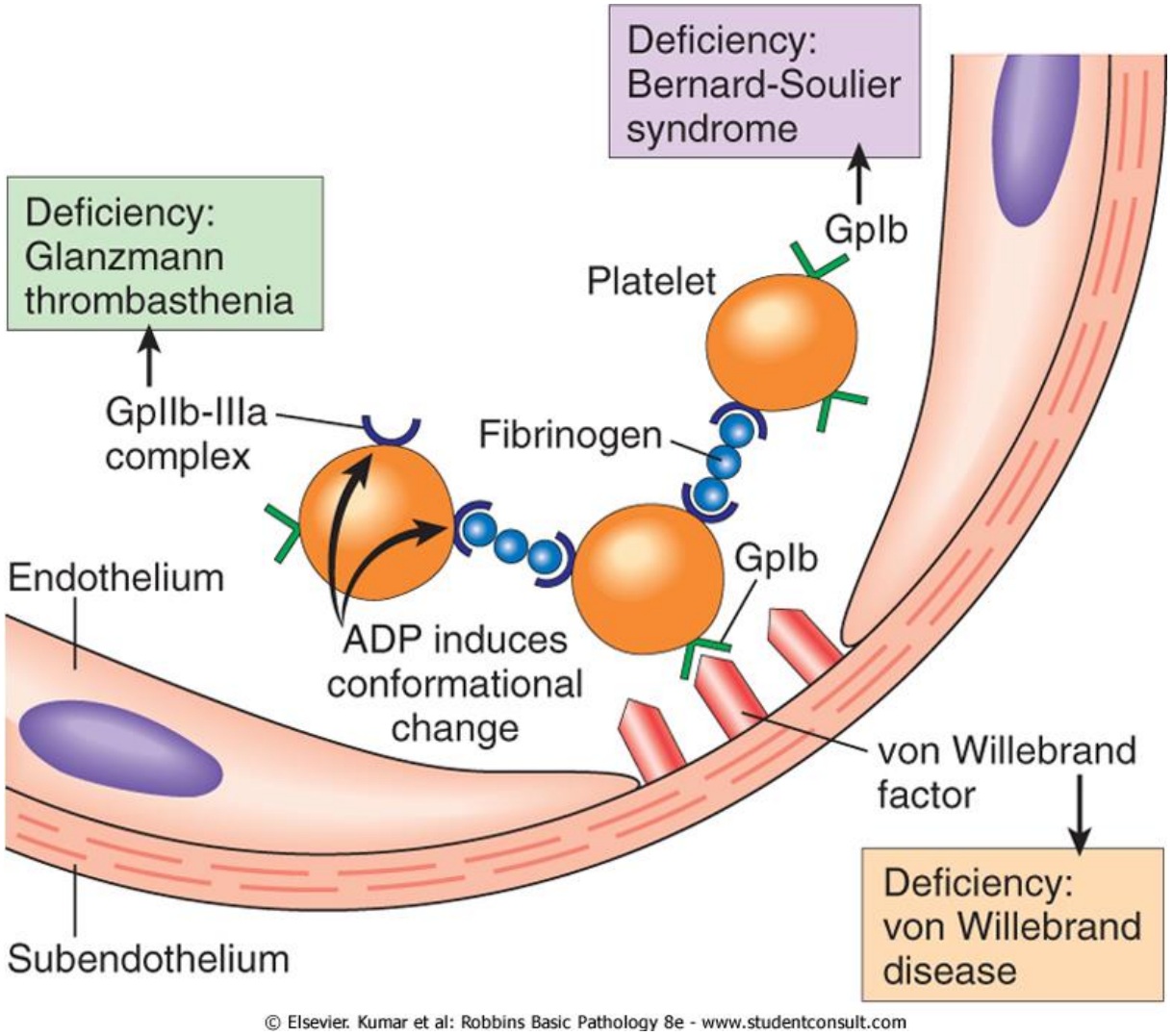
Thrombocytopenia

- PLT count normally 150 – 400 000/µl (1.5–4×1011/l)
- in circulation 8-10 days
- aetiology - primary or secondary
  - low production
    - aplastic anaemia (pancytopenia)
    - myelodysplastic syndrome
    - myelofibrosis
    - B12 a/or pholate deficiency
    - liver disease (↓ TPO)
  - destruction/consumption/splenic sequestration
    - autoimune - idiopathic thrombocytopenic purpura (ITP)
      - AIHA + ITP = Evans syndrome
    - drug-induced (valproate, methotrexate, …)
    - SLE (systemic lupus erythemoatodes)
    - APS (anti-phospholipid syndrome)
    - hypersplenism
  - high consumption
    - DIC
    - thrombotic thrombocytopenic purpura (TTP)
    - HELLP (pregnancy) = hypertension, elevated liver enzymes and low platelets
    - hemolytic-uremic syndrome (HUS)
  - dilutional
    - mostly benign, e.g. pregnancy (volume expansion)
- bleeding in trombocytopenia
  - superficial – skin and mucosla mostly
    - <50 000/µl – mild risk of bleeding
    - <20 000/µl – significant risk of bleeding
    - <5 000/µl – extremely high risk of bleeding

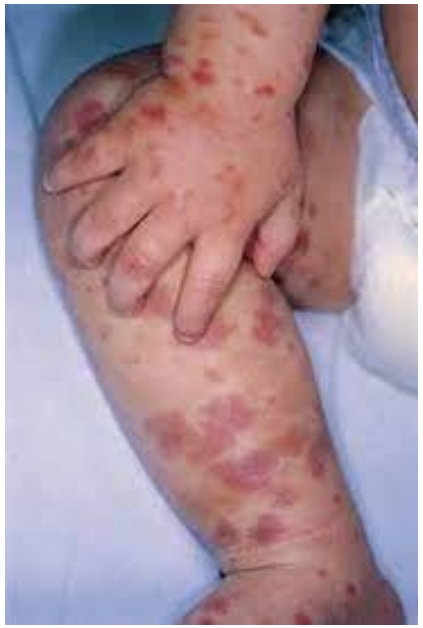




Thrombocytopathia = impaired PLT function



- abnormal adhesion or aggregation
  - Bernard-Soulier syndrome
    - receptor GPIb-IX defect
  - Glanzmann thrombastenia
    - receptor GPIIb-IIIa defect
  - von Willebrand disease
    - vWf deficiency
- abnormal degranulation
  - Heřmansky-Pudlák syndrome
  - Chédiak-Higashi syndrome



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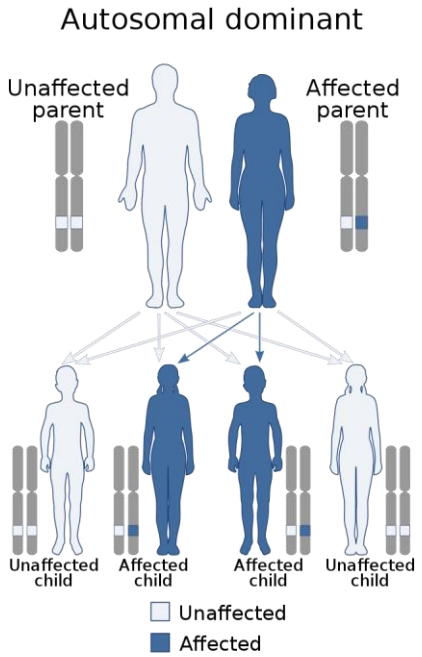
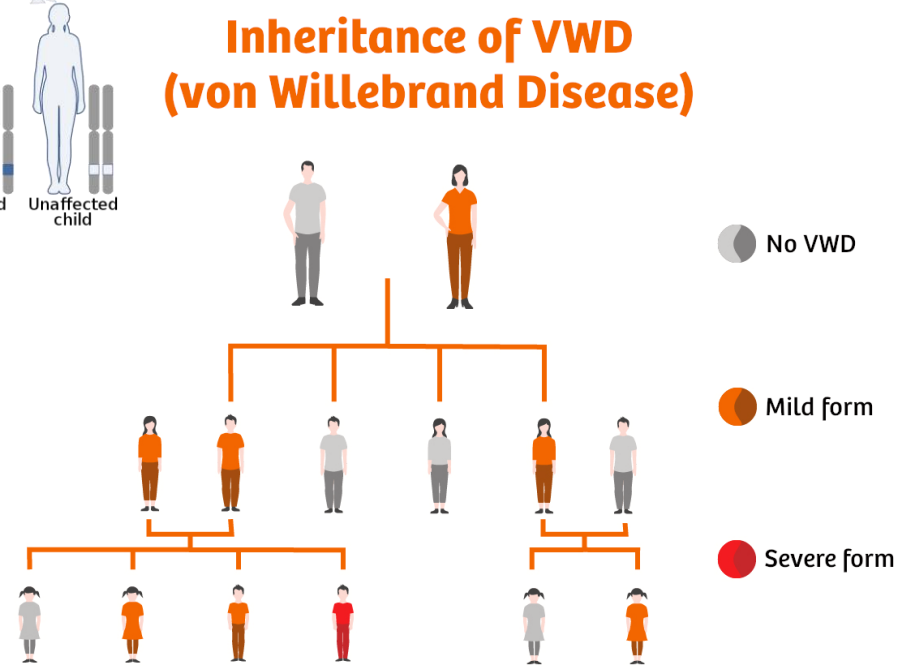
von Willebrand disease

- Von Willebrand Disease (VWD) is a genetic disorder caused by missing or defective von Willebrand factor (VWF), which help form a platelet plug during the clotting process
  - deposited in blood vessel walls and after exposure binds to platelets (alpha granules) - abnormal adhesion of platelets (primary hemostasis)
  - vWf also binds FVIII in plasma (otherwise unstable and degraded quickly) → defective secondary hemostasis
- The most common inborn disease of coagulation
- The gene for von Willebrand factor is on one of the autosomes, chromosome 12
  - since it is not on the sex chromosome (unlike hemophilia), it occurs equally in men and women
    - if only one parent has a dominant inheritance type of VWD, with each birth there is a 50% chance of having a child (boy or girl) who inherits the VWD mutation
    - a 50% chance of having a child (boy or girl) who does not inherit the VWD mutation
- several types of vW disease
  - type 1 (~75%) – low concentration of vWf
    - symptoms are usually mild
  - type 2 (~20%) – normal concentration of non-functional vWf
    - four subtypes: type 2A, type 2B, type 2M and type 2N, depending on the presence and behavior of multimers
      - binding to platelets (type 2A)
      - binding to collagen of subendothelial layer (type 2B)
      - transport of fVIII (type 2N)
    - symptoms are mild to moderate
  - type 3 – absolute deficit vWf (homozygotes)
    - symptoms are typically severe, and include spontaneous bleeding episodes, often into their joints and muscles





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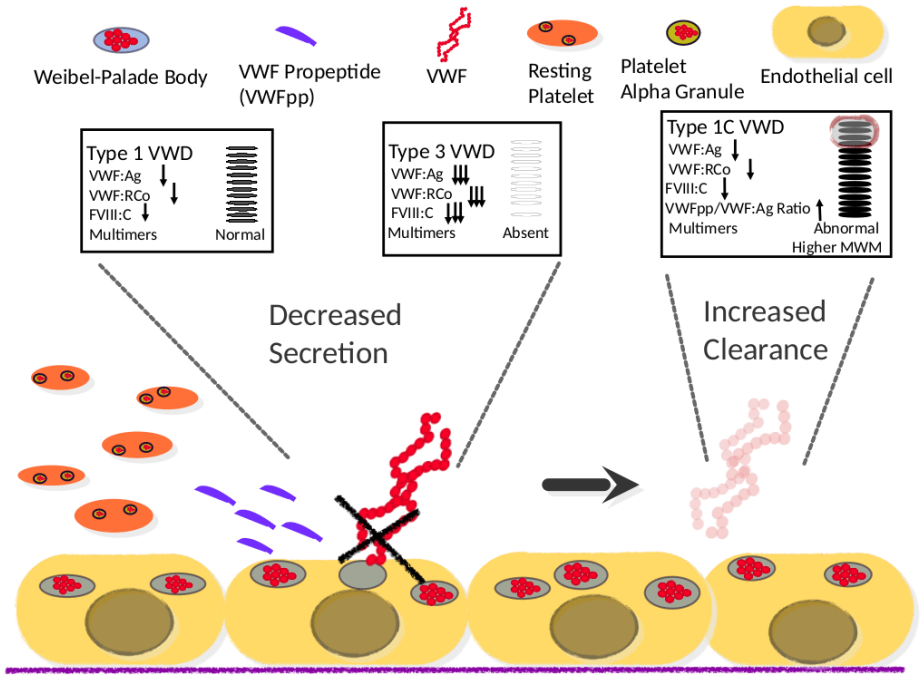
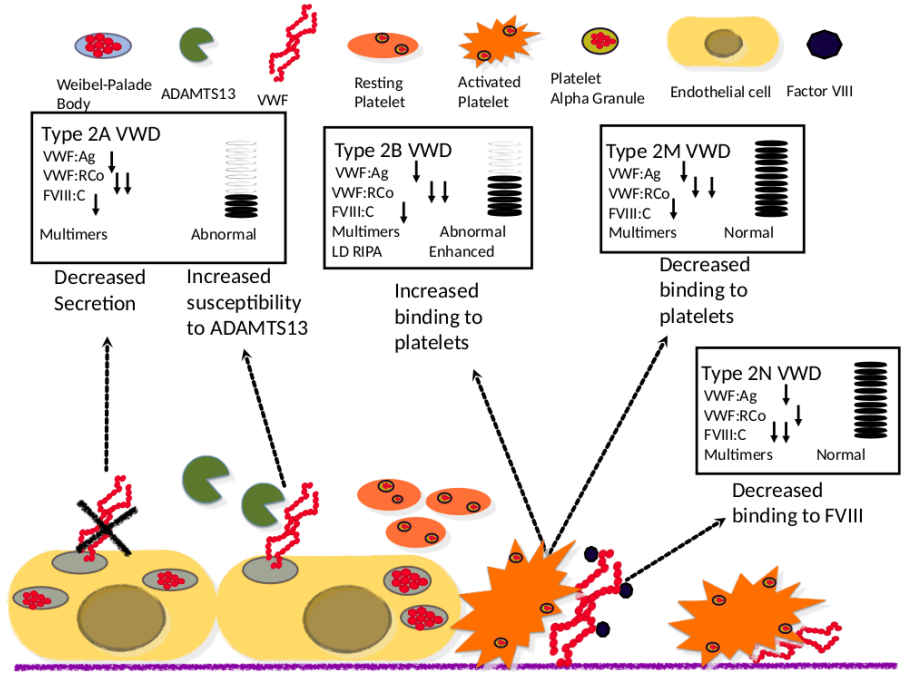
Many subtypes of vWD



Quantitative defects of von Willebrand factor, as seen in von Willebrand disease types 1 & 3. In the classic presentation, type 1 VWD sees a decrease in VWF:Ag, VWF:RCo, and FVIII:C, and multimer levels are normal. Type 3 VWD presents with the same decreases, but to a much greater degree, and multimers are absent. Types 1 & 3 both show decreased secretion. Type 1C presents similar decreases to type 1, but shows an increase in the ratio of VWFpp to VWF:Ag and an abnormally high quantity of multimers, as well as increased clearance.



Qualitative defects of von Willebrand factor, as seen in von Willebrand disease type 2. Like types 1 & 3, all forms of type 2 VWD present with a decrease in VWF:Ag, VWF:RCo, and FVIII:C. In type 2A, there is decreased secretion of VWF and an increased susceptibility to ADAMTS13 and abnormal multimers. Type 2B presents with increased binding to platelets, abnormal multimer count, and enhanced LD RIPA. Type 2M shows decreased binding to platelets and multimer levels are normal, while type 2N presents with decreased binding to FVIII and normal multimers as well.

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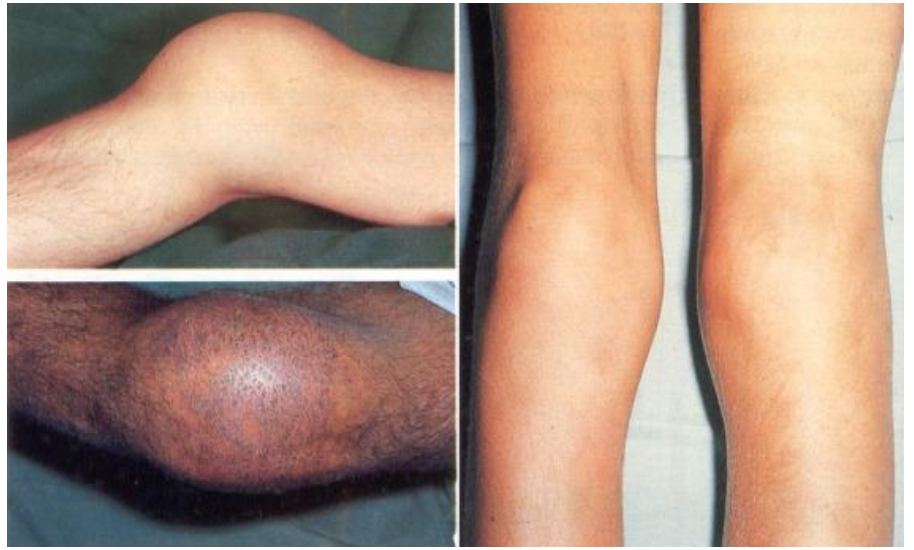
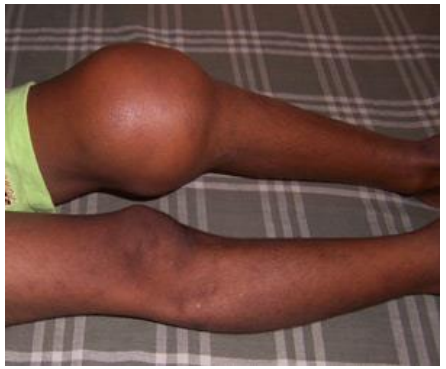
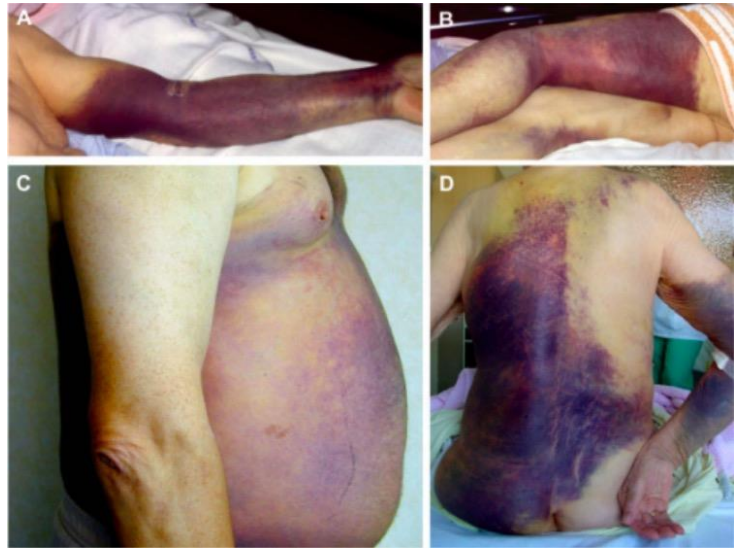
Defects of secondary haemostasis

- typically bleeding to the tissues (hematomas), e.g. joints, muscles, brain, retroperitoneal space
- no petechias or purpura
- **(A) inborn disorders**
  - **haemophilia A** (Xq-chromosome linked) – defect of fVIII
    - fVIII serves as a co-factor of activation of fX to fXa in the reaction catalysed by fIXa (intrinsic tenase)
    - decrease of concentration up to 25% of normal values does not cause coagulation disorder, decline to 25-1% mild form, <1% severe form
    - >150 point mutations in fVIII gene – large phenotypic variability!!!
      - eventually inhibitor of FVIII is responsible
    - prevalence in males 1:5,000 to 1:10,000
  - **haemophilia B** (Xq-chromosome linked) – defect of fIX
    - prevalence 10x lower than haemophilia A
    - >300 mutations in fIX gene (85% point mutations, 3% short deletions and 12% large deletions)
  - defects of other factors
    - rare, mostly autosomal recessive, clinically manifested only when severe deficit
      - afibrinogenemia (defect fI)
      - haemophilia C (defect of fXI) – Askenazy Jews
- **(B) acquired disorders**
  - liver insufficiency
  - vitamin K deficit (lipid malabsorption)
  - DIC







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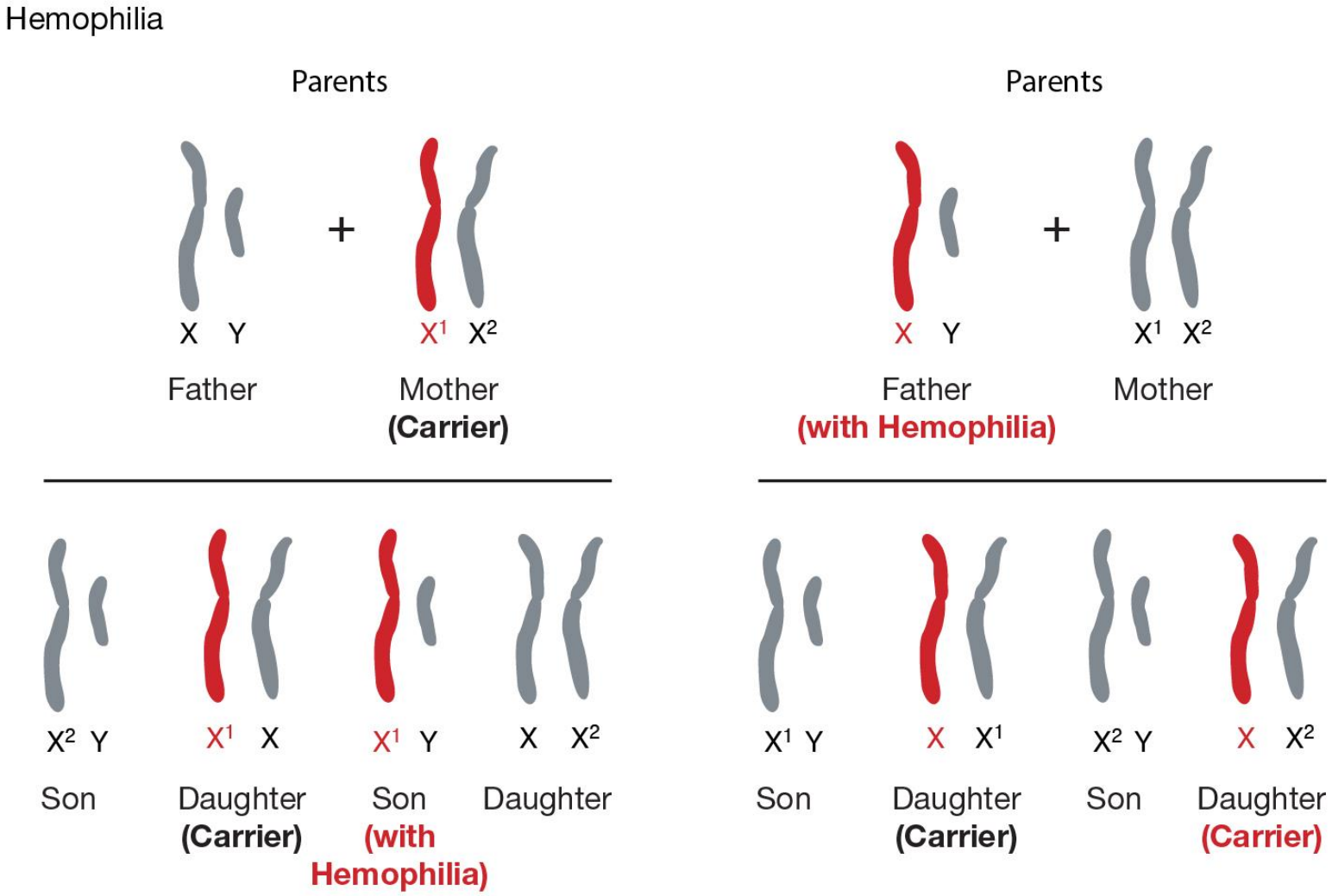
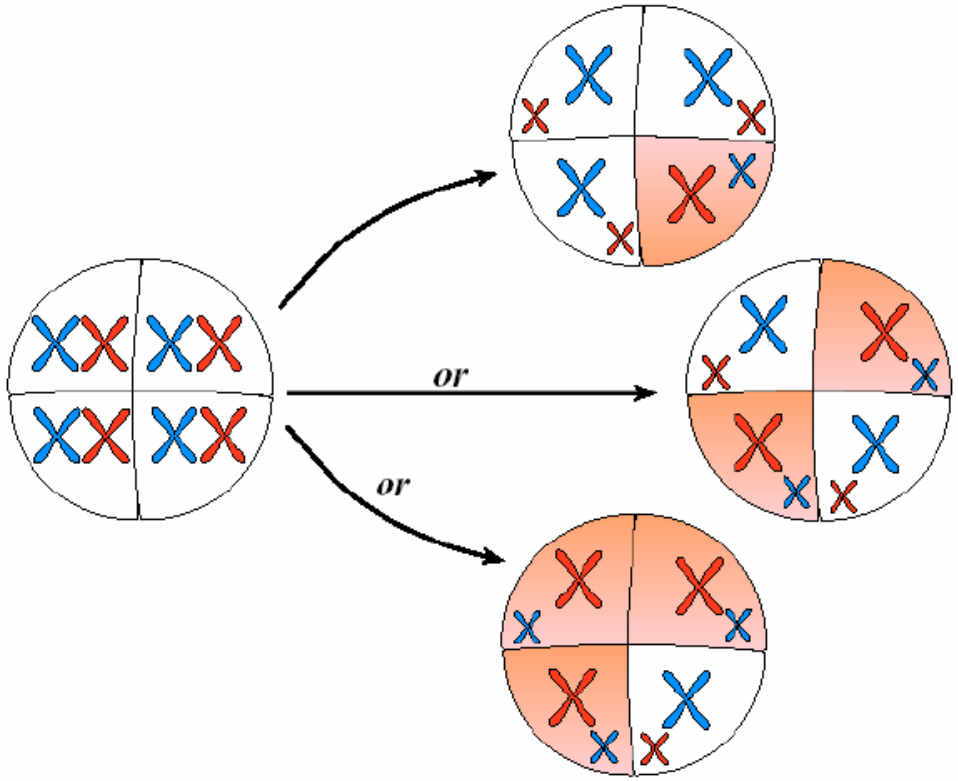
Hemophilia A and B genetics



Hemophilia in females – parents or chromosomal lyonization



- An early female embryo chooses, at random, to inactivate paternal X chromosomes (blue, normal) in some cells and maternal ones (red, with a hemophilia A mutation) in others. On average (middle right) half the cells retain the ability to make factor VIII normally and half do not. Occasionally, most cells retain the ability to make factor VIII (top right) or lose that ability (bottom right).

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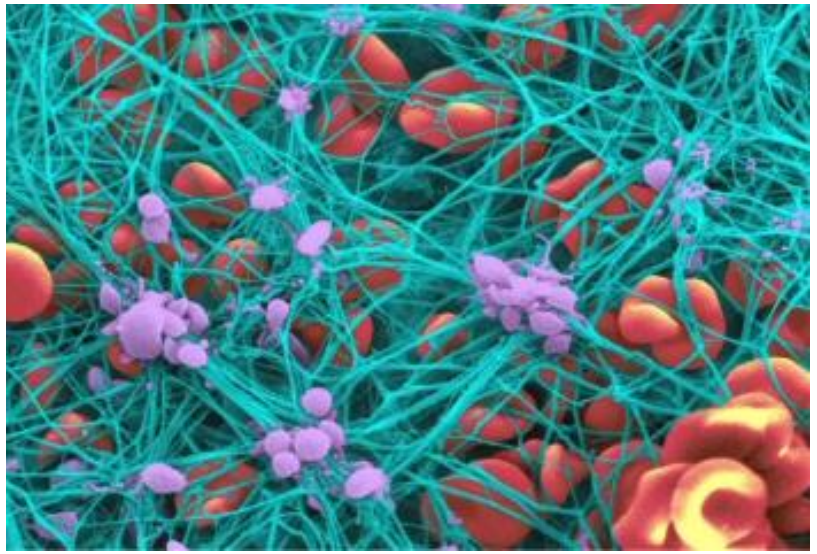
DIC (consumption coagulopathy)

- initially hypecoagulation (thrombotic state), later consumption of coagulation factors (bleeding)
- coagulation becomes non-limited in space and is not a primary reaction to injury
- pathogenesis
  - circulation doesn’t normally contain TF!!!
    - nor endothelial neither blood corpuscles do not produce it on their surface
  - some pathologic situations lead to activation of factor VII by TF (and subsequently of extrinsic pathway) - pathological sources of TF:
    - tissue cells – e.g. fetal cells during birth, extensive traumas, spreading of cancer cells etc.
    - pathological blood elements expressing TF – e.g. myelo- and lymphoproliferative diseases
    - pathologically activated endothelium and monocytes stimulated by endotoxin during sepsis to produce TF
    - TF from the cytoplasm of erythrocytes during haemolysis
- consequences
  - 1. phase - microthrombi in micro-circulation
    - ischemia or even gangrene
  - 2. phase - hypo- to afibrinogenemia, thrombocytopenia
    - bleeding to organs
  - pathologic fibrinolysis



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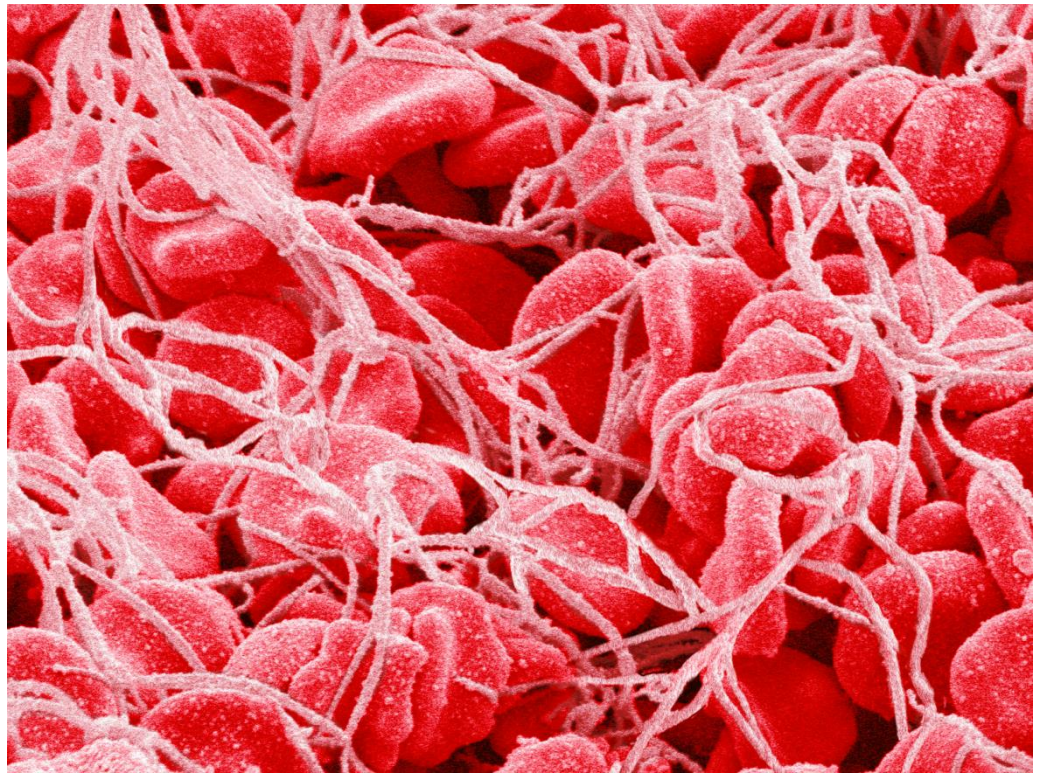

HYPERCOAGULATION STATES (THROMBOPHILIA)

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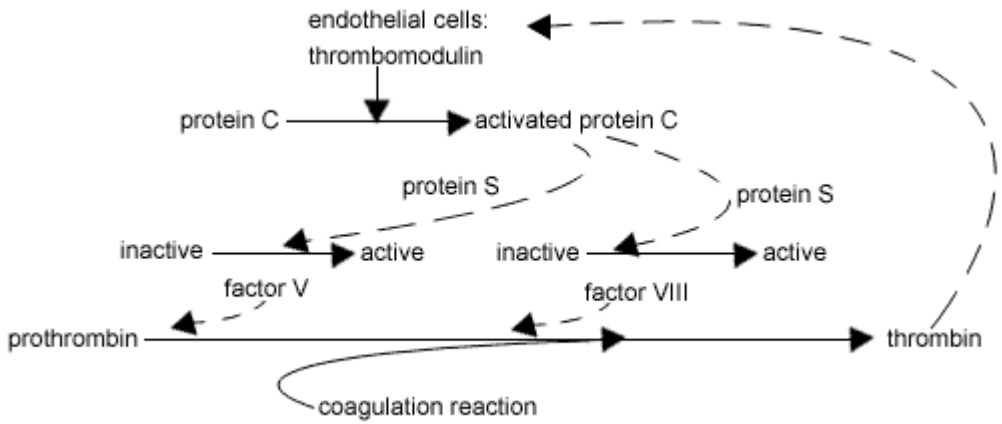
Hypercoagulation states

- increased risk or spontaneous and often recurrent venous thromboses and thrombembolisms (lung typically), event. pregnancy complications, infertility
- (A) inherited thrombophilias
  - (1) defects of coagulation inhibitors
    - AT III deficiency (AR)
    - protein C or S deficiency (AD)
    - activated protein C resistance (APCR)
      - activated protein C (with protein S as a cofactor) degrades fVa and fVIIIa and limits coagulation
      - mutated fV does not respond to activated protein C - most common inborn genet. abnormality ("Leiden" mutation of fV)
    - mutation of prothrombin gene (promotor → quantitative effect)
    - hyperhomocysteinemia (mutation in MTHFR gene)
    - antiphospholipid syndrome
      - anti-cardiolipin antibodies, lupus anticoagulans etc.
    - hypehomocysteinemia
  - (2) defects of fibrinolysis
    - ↑LP(a)
    - ↑ PAI-1 (promotor → quantitative effect)



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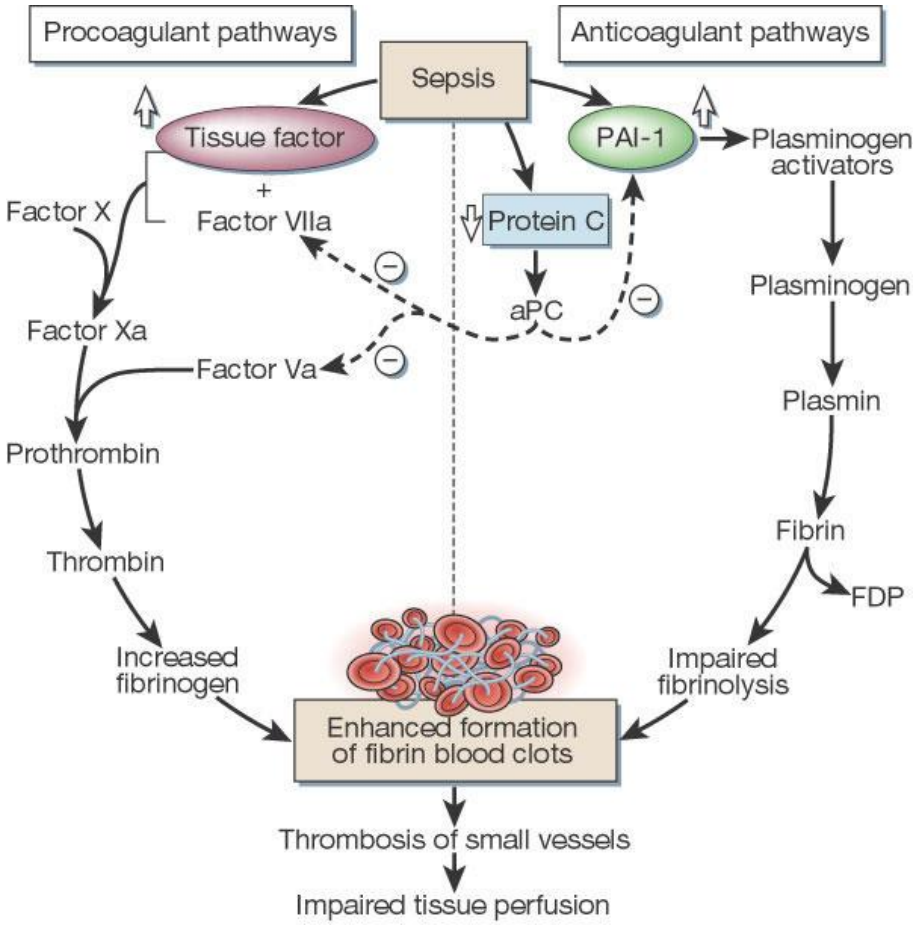
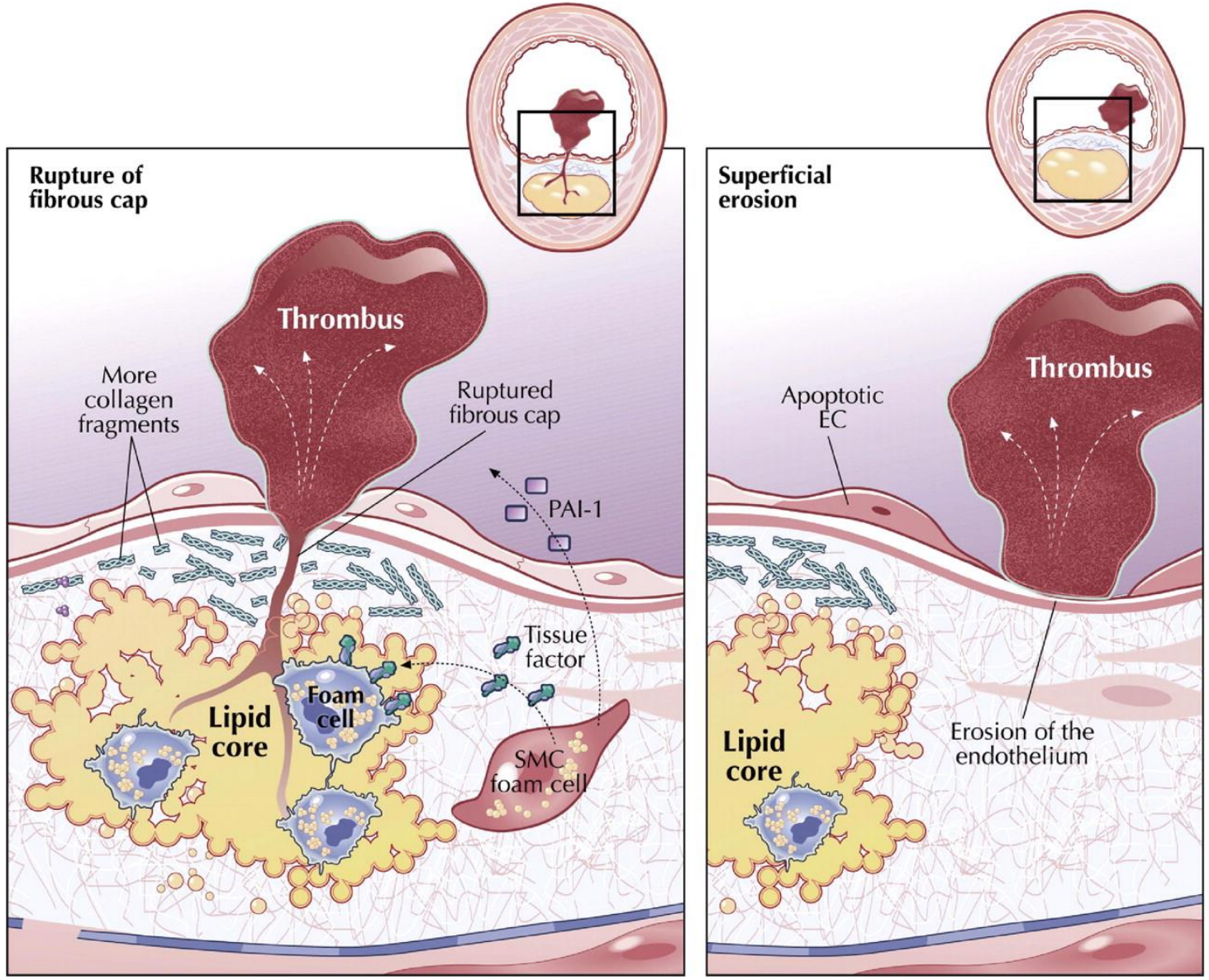
Hypercoagulation states

- (B) acquired thrombophilias
  - (1) clin. situations and complications of therapy
    - immobilisation
    - hyperoestrogenic states
      - pregnancy
      - oral contraception
      - HRT
  - (2) pathologic situations
    - atherosclerosis
    - obesity (↑ PAI-1)
    - hyperviscous syndromes
      - polycythemia vera, thrombocythemia, sec. polyglobulia, gamapatias)
    - tumours
    - heart failure
    - sepsis
    - hyperlipidemias
    - nephrotic syndrome
    - venous insufficiency
    - hypehomocysteinemia



Atherosclerosis x coagulation



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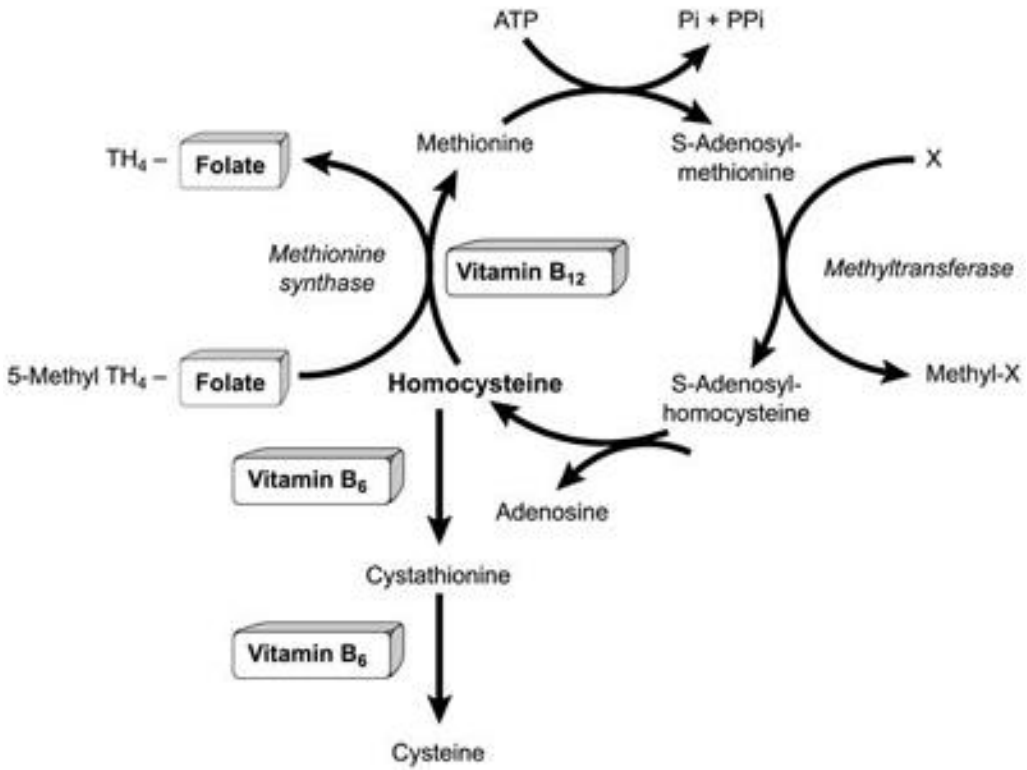
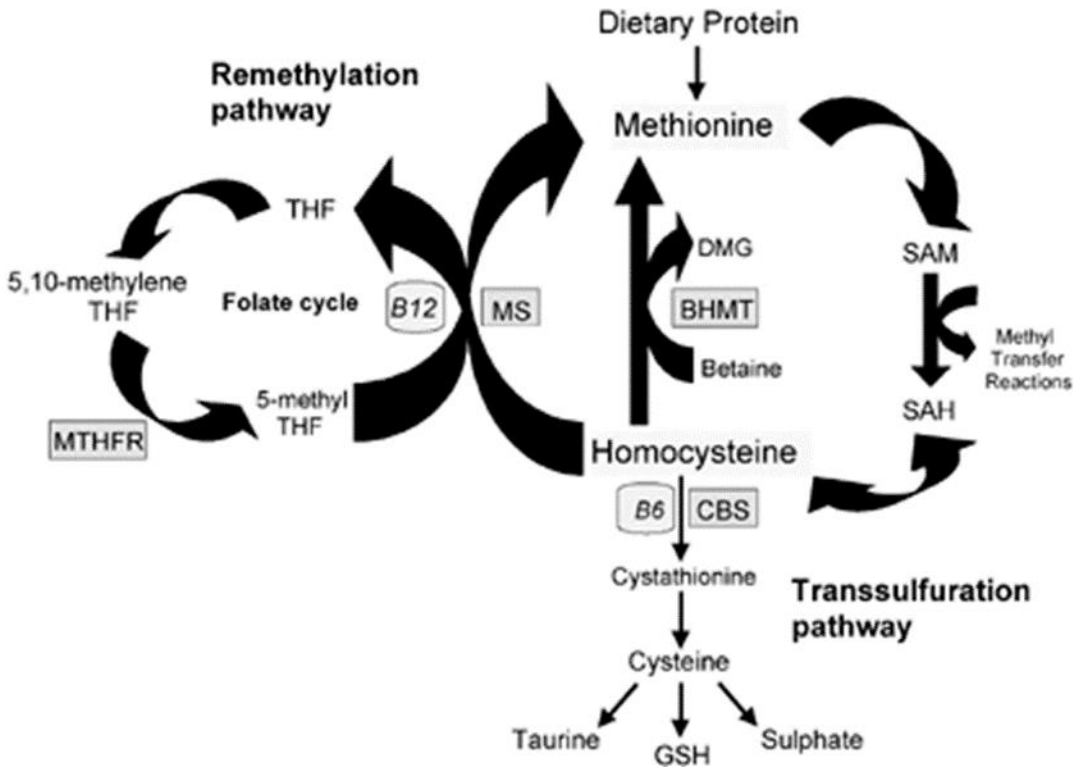
Hyperhomocysteinemia (HHcy)

- homocystein is an intermediate product in the methionine cycle
  - either further metabolised to cysteine
  - or remethylated back to methionine (in the folate cycle)
- several enzymes and their cofactors are needed (vitamins B, folic acid)
- the reasons for abnormal metabolism of homocysteine and subsequent HHcy can be genetic or nutritional or both
  - mutation in genes encoding enzymes
  - deficient intake of vitamin B6, B12 and folic acid
- HHcy = pathologic increase of plasm. concentrations of homocysteine
- Hhcy is an independent risk factor of atherosclerosis and thrombembolism, fertility disorders and some congenital defects and neurological abnormalities (cleft spine)
- homocysteine induces endothelial dysfunction and initiates apoptosis
- (A) monogenic HHcy
  - deficit of cystathionin-β-synthase (in homozygotes) leads to extreme elevation of plasma Hc
  - prematured atherosclerosis
  - rare disease
- (B) mild HHcy
  - polymorphism in gene encoding enzyme methylen tetrahydrofolate reductase (MTHFR)



Homocysteine metabolic pathways



- Dietary methionine is converted to the methyl donor S-adenosylmethionine (SAM) and is demethylated to S-adenosylhomocysteine (SAH) and homocysteine. In the transsulfuration pathway, homocysteine is converted to cystathionine by the enzyme cystathionine -synthase (CBS) and the cofactor vitamin B6 (pyridoxyl phosphate). Once formed from cystathionine, cysteine can be utilized in a number of cellular functions, including protein synthesis and glutathione (GSH) production. Homocysteine can also be remethylated through the folate cycle. This pathway requires the enzyme methionine synthase (MS) and vitamin B12 as well as the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) and folic acid, which enters the cycle as tetrahydrofolate (THF). In liver and kidney, homocysteine is also remethylated by the enzyme betaine homocysteine methyltransferase (BHMT), which transfers a methyl group to homocysteine via demethylation of betaine to dimethylglycine (DMG)

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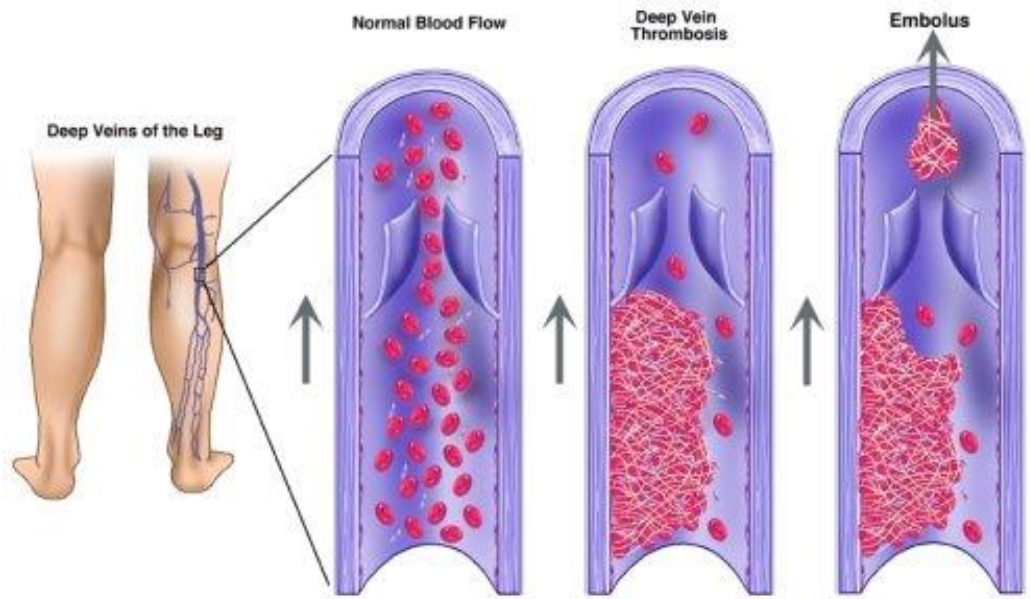
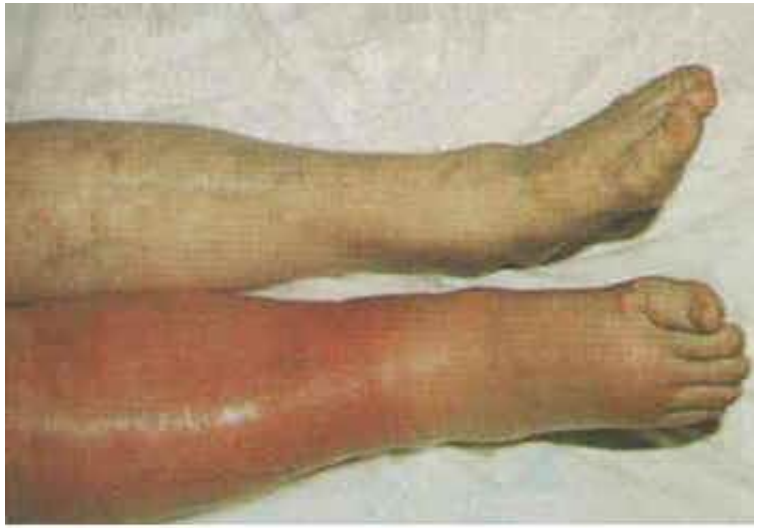
Deep vein thrombosis





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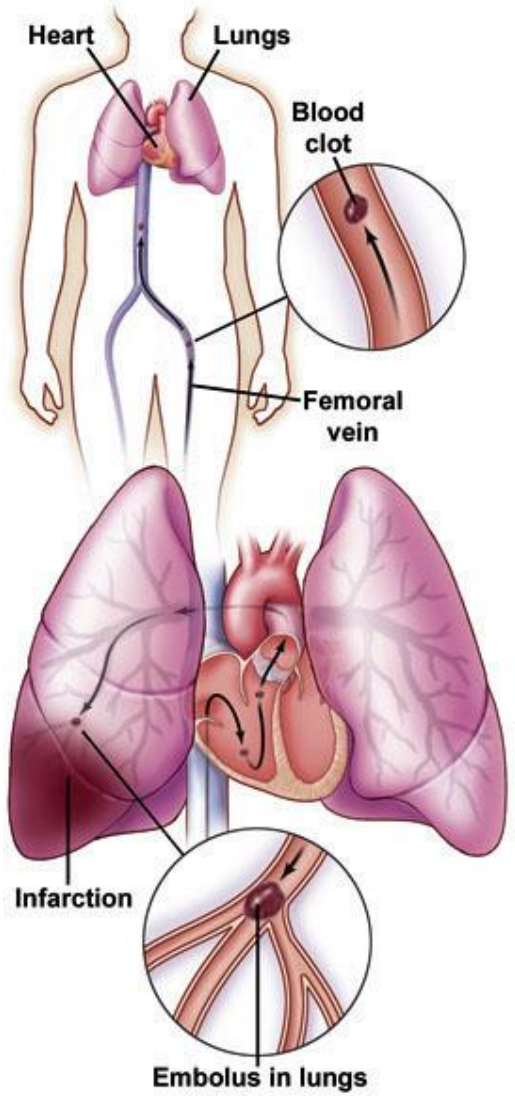
Pulmonary embolism



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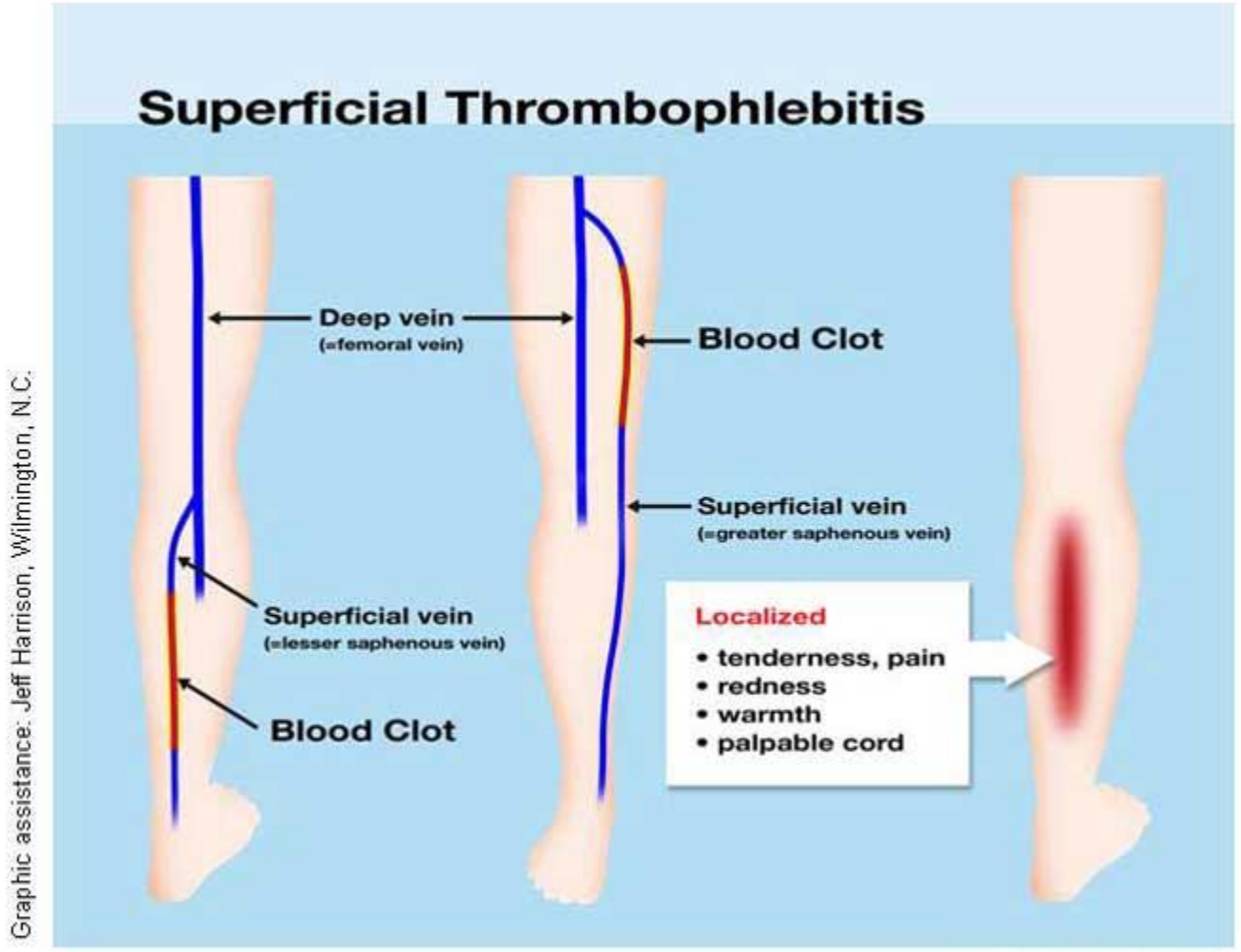
Superficial vein thrombosis



I agree O-positive is rather nice,
but my favourite by far is AB-negative...

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