Hypercoagualable states (thrombophilia)

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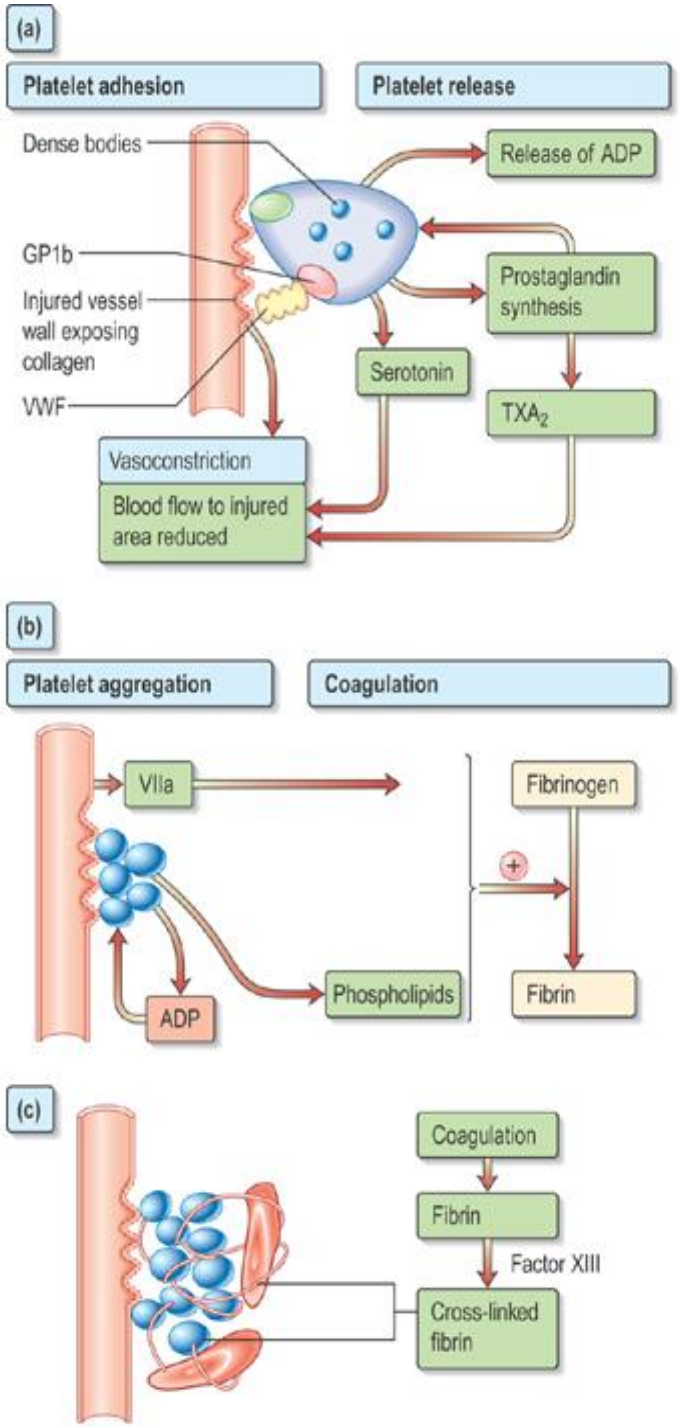
Sourced include: 427 team, slides, notes from the lecture, step up and Kumar.

You need to understand the coagulation process and platelet aggregation very well to understand minor details in this lecture; we tried making it as easy as possible.

The first 3 pages are general information you need to understand to comprehend this lecture and the bleeding disorders lecture. The lecture starts at page 5.

Normal coagulation and platelet aggregation



(A) Vessel wall injury occurs → platelets come into contact with and adhere to collagen and von Willebrand factor that is bound below the endothelium. This is mediated through glycoprotein Ib (GPIb).

This binding activates platelet prostaglandin synthesis which stimulates release of ADP from the dense bodies.

Vasoconstriction of the vessel occurs as a reflex and by release of serotonin and thromboxane A2 (TXA2) from platelets.

(b) Release of ADP from platelets induces platelet aggregation and formation of the platelet plug. The coagulation pathway is stimulated leading to formation of fibrin.

(We will talk about coagulation later)

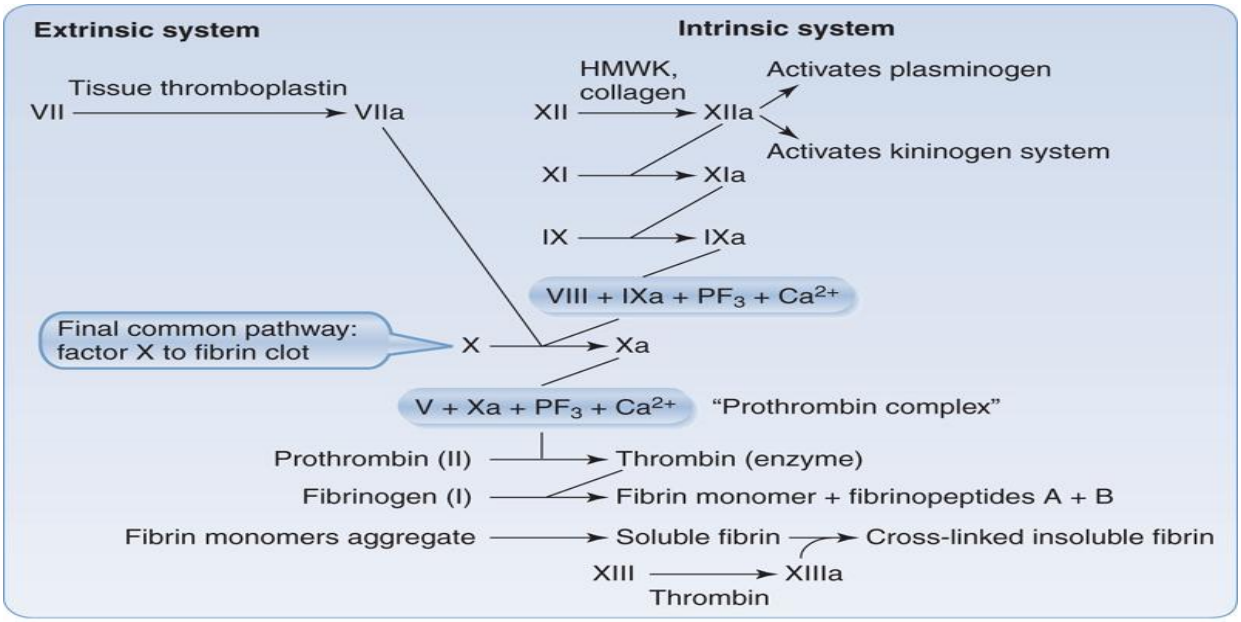
(c) Fibrin strands are cross-linked by factor XIII and stabilize the haemostatic plug by binding platelets and red cells.

Coagulation

The ultimate purpose of the coagulation cascade is to form fibrin from fibrinogen to form a clot.

Coagulation cascade

- Extrinsic system (factor VII)
  - Factor VII is activated (factor VIIa) by tissue thromboplastin.
  - Factor VIIa activates factor X in the final common pathway.
- Intrinsic system (factors XII, XI, IX, VIII)
- Final common pathway : factors X, V, prothrombin (II), and fibrinogen (I)
  - Prothrombin complex
    - (1) Four-component system consisting of factor Xa, factor V, PF3(platelet factor 3), and calcium
    - (2) Calcium binds factor Xa, a vitamin K-dependent coagulation factor.
    - (3) Complex cleaves prothrombin into thrombin (enzyme).
  - Functions of thrombin
    - (1) Acts on fibrinogen to produce fibrin monomers plus fibrin peptides A and B
    - (2) Activates fibrin stabilizing factor XIII
      - (a) Factor XIIIa converts soluble fibrin monomers to insoluble fibrin.
      - (b) Enhances protein-protein cross-linking to strengthen the fibrin clot
      - Factor XIII: cross-links insoluble fibrin monomers



(1)Coagulation is initiated by tissue damage. This exposes tissue factor (TF) which binds to factor VII. The TF-factor VII complex directly converts factor X to active factor Xa

(2) Activated factor X activates the prothrombin complex which turns it into thrombin

(3) Thrombin then changes fibrinogen into fibrin and forms the clot

Vitamin K dependant factors: (IMP)

- Factors II, VII, IX, X, protein C, and protein S
- Synthesized in the liver as non-functional precursor proteins

In vitamin K deficiency these factors are affected, because they need it to work

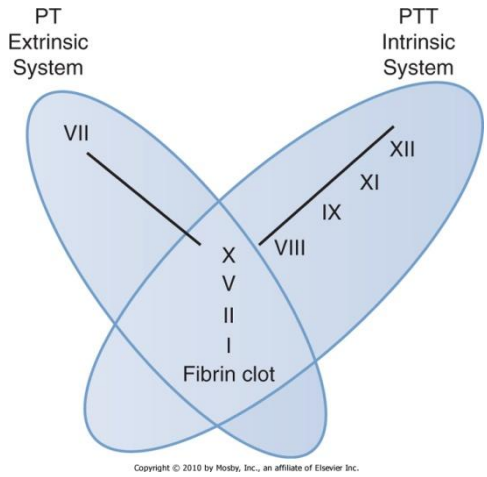
Laboratory tests:

Platelet tests:

a. Platelet count: Normal count is 150,000 to 400,000 cells/mm3
b. Bleeding time:
    i. Evaluates platelet function up to the formation of the temporary platelet plug
    ii. Normal reference interval is 2 to 7 minutes
c. Tests for vWf
    i. Ristocetin cofactor assay
        1. Evaluates vWF function
        2. Abnormal assay
            a. Classic von Willebrand disease (deficiency of vWF)
            b. Bernard-Soulier disease (absent GpIb receptor)
    ii. vWF antigen assay
        1. Measures the quantity of vWF regardless of function
        2. Decreased in classic von Willebrand disease

Coagulation tests:

a. PT – prothrombin time
    iii. Evaluates the extrinsic system down to formation of the fibrin clot: Factors evaluated include VII, X, V, II, and I
    iv. Normal reference interval for PT is 11 to 15 seconds
    v. INR: standardizes PT for warfarin therapy (normal is 2-3)
    vi. Uses of PT
        1. Follow patients who are taking warfarin for anticoagulation
        2. Evaluate liver synthetic function: Increased PT indicates severe liver dysfunction.
        3. Detect factor VII deficiency



b. Partial thromboplastin time (PTT)
    vii. Evaluates the intrinsic system down to formation of a fibrin clot: Factors evaluated include XII, XI, IX, VIII, X, V, II, and I.
    viii. Normal reference interval for PTT is 25 to 40 seconds.
    ix. Uses of PTT
        1. Follow heparin therapy
            a. Heparin enhances ATIII activity.
            b. PTT is not required to follow low-molecular-weight heparin therapy.
        2. Detect factor deficiencies in the intrinsic system
c. Thrombin time (TT)
    x. Normal is 12-14 seconds
    xi. Prolonged in fibrinogen deficiency

Fibrinolytic system tests:

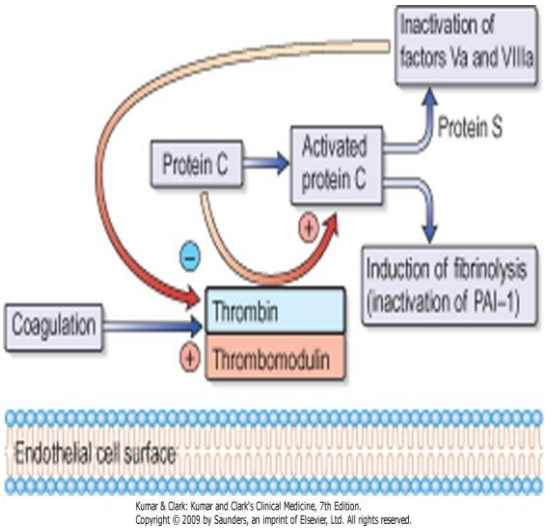
a. Fibrin(ogen) degradation products (FDPs):
    xii. Detects fragments associated with plasmin degradation of fibrinogen or insoluble fibrin in fibrin clots
b. D-dimer:
    xiii. Detects cross-linked insoluble fibrin monomers in a fibrin clot

Introduction

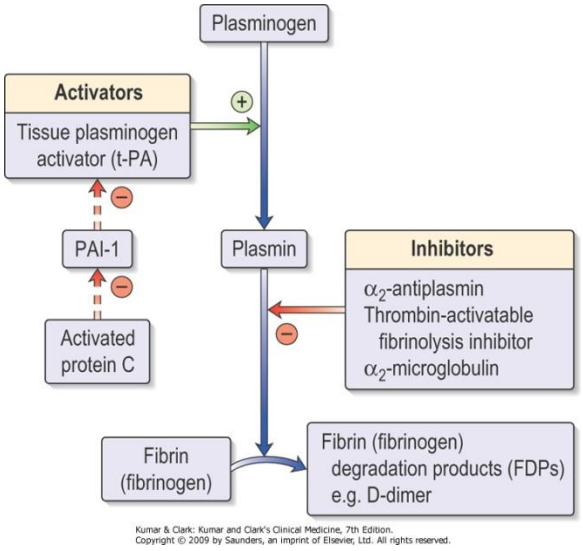
Normally blood homeostasis is keeps blood in its liquid form and only clots at the place of insult due to three factors:

Blood vessel wall (endothelium)

- **Has thrombomodulin on its surface:** the job of thrombomodulin is to **remove thrombin from the blood and stop its action**
  - What are the normal factors in the body that remove thrombin from the body? The liver and thrombomodulin.
  - When thrombin + thrombomodulin join together they form a complex this complex in turn activates protein C
  - Activated protein C does 2 things:
    - Forms a complex with factor 5 and this inhibits factor 8
    - Inhibits PAI-1 this promotes fibrinolysis



- **Secretes prostacyclin: (works opposite to thromboxane A2)**
  - Vasodilatation
  - Inhibits platelet aggregation
- **Production of tissue plasminogen activator (t-PA):**
  - What is the action of plasminogen? Plasminogen is the inactivated form of plasmin.
  - Plasmin is a protein that changes fibrin clots into degradation products also called FDPs
  - So t-PA turns plasminogen into plasmin and that breaks the fibrin clot.
  - Once the clot is broken anti-plasmin waits by the clot and catches the excess plasmin and stops it from working
  - What else activates plasminogen? Drugs: urokinase, streptokinase
  - What inhibits plasminogen?
    - Anti-plasmin stops plasmin after clot lysis
    - PAI (plasminogen activator inhibitors)
      - PAI-1: any inflammatory condition
      - PAI-2: placenta
      - PAI-3: in antiphospholipid syndrome



- **Secretes nitric oxide (NO):**
  - Neutralizes homocystine
  - Vasodilatation
- **Secretes heparin like proteoglycans (heparans) that activate anti-thrombin**
- **Contains phospholipids that help binding and activation of clotting factors via Ca++ bridges**
- **Tissue factor pathway inhibitors (TFPI),** tissue factors activate factor 7 and are secreted after cell damage

Platelets (mentioned earlier)

Coagulation factors and natural anticoagulants

- Coagulation factors were explained before
- Natural anticoagulants include:
  - **Antithrombin**
    - potent inhibitor of coagulation
    - action is greatly potentiated by heparin

- **protein C**
  - Activated by the thrombin- thrombomodulin complex
  - Activated protein C inactivates factor V and factor VIII, reducing further thrombin generation
- **Protein S**: cofactor for protein C, which acts by enhancing binding of activated protein C to the phospholipids surface.
- **Thrombomodulin**: binds to thrombin and activates protein C
- What are some of the non-physiological inhibitors of coagulation?
  - Vitamin K antagonist (works inside the body only)
  - Calcium chelators like EDTA, citrate and oxalate (work inside the test tube only)
  - Heparin (works in both the body and the tube)

General considerations:

- **Synonyms**:
  - Hypercoagulable states,
  - prethrombotic states,
  - thrombogenic states
- **So thrombophilia is defined as**:

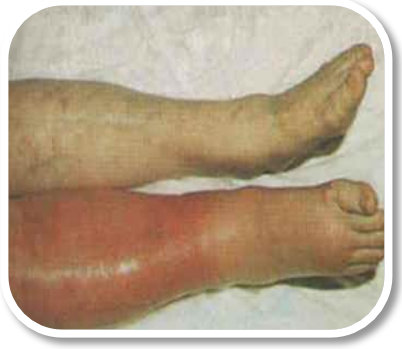
> *Alteration in the haemostatic balance between blood fluidity and clot formation due to genetic or acquired disorders which shift the balance toward excessive platelet aggregation and thrombin generation that lead to thrombosis.*

- Balance of bleeding and clotting: Imbalance in one direction can lead to: Hypocoagulable state → bleeding OR Hypercoagulable state → thrombosis

Presentation of patients:

The two most common presentations are:

1. DVT (deep venous thrombosis): Painful, swollen, warm, plethoric extremity with reduced pulse volume
2. Pulmonary embolism: Cough , shortness of breath, hemoptysis and tachycardia
3. If the clot forms in arterial vessels:
   a. Carotid artery: cerebrovascular accident, Transient ischemic attack
   b. Femoral artery: intermittent claudications
   c. Coronal artery: acute MI, angina



Causes of hypercoagulable states:

- Classified into hereditary (primary) and acquired (secondary)
- Before we start listing them you should know concept in pathology called "**Virchow's triad**" which is: to have blood thrombosis one of the following must be present:
  - Alterations in blood flow (i.e., stasis)
    - Disrupted laminar flow allows greater interaction between platelets and endothelial surface
    - Prevents dilution of locally activated clotting factors
    - Prevents inflow of clotting factor inhibitors
    - Promotes endothelial cell damage and activation

- Vascular endothelial injury or dysfunction
  - Causes exposure of sub-endothelium and release of tissue factor, thereby activating coagulation cascade
- Alterations in constituents of blood (i.e., hypercoagulability)
  - Acquired vs. inherited coagulopathies
  - Predisposing factors for thrombus formation
- In short either the blood flow is altered, endothelium is injured or not working and clotting factors are not functioning well

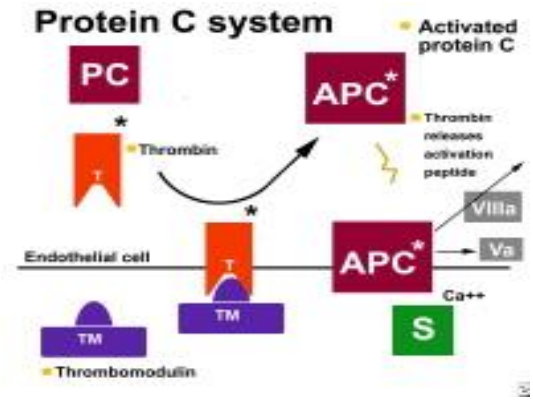
Prothrombotic states occur in cases of:

1. Vascular (Endothelial dysfunction)
   a. Endothelial cell antithrombotic properties- PGI_2, NO_2, TFPI, PAI-1, heparans, thrombomodulin
   b. Genetic predisposition and acquired defects in these functions increase the risk of arterial and venous thrombosis
2. Platelets (↑ activity and/or numbers)
3. Coagulation factors (↑)
   a. Sometimes inflammatory conditions increases factor 8
4. Natural anticoagulants (↓ and/or dysfunction)
   a. Antithrombin
   b. Protein C and S
5. Fibrinolytic system (↓)
6. Pathological conditions, (e.g.: hyperhomocysteinemia, antiphospholipid syndrome, Contraceptive pills etc.)

Inherited causes or risk factors for thrombosis:

- Inherited thrombophilias have a prevalence of 10% from total thrombophilias
- Total incidence of an inherited thrombophilia in subjects with DVT range from 24 – 37%

Factor V Leiden:



- Discovered in Leiden, the Netherlands (1993) amongst a group of subjects with unexplained VTE
- Most common form of inherited thrombophilia (~50% of cases)
- This is an inherited mutation of factor 5
- The mutation causes active protein C resistance
- **When patients with factor 5 Leiden use estrogen pills this causes** SEVERE THROMBOSIS IMP!!
- What happens in factor V Leiden(figure) :
  - Normally
    - the thrombin thrombomodulin complex activates protein C and this produces APC (activated protein C)
    - APC cleaves the product of the factor 5 gene and forms a complex that inhibits factor 8
  - Factor V Leiden: The gene is mutated so APC cannot cleave factor 5 and then factor 8 will not be inhibited and this will cause thrombosis
  - ***Protein C is the key and factor 5 is the lock, if we change the lock we cannot open the door so we have protein C is resistance***
  - ***Protein C levels become high due to compensatory effect***
- There is a dual action of factor V Leiden and that causes increased coagulation due to:
  - ↓ inactivation of factor FVIIIa

- ↑ thrombin generation, (↓ anticoagulation)
- Also ↓ PAI inactivation → ↓ fibrinolysis

Protein C and protein S deficiency:

- Protein C and S Are the 2 major cofactors responsible for regulating the amplification of the clotting cascade
- Inhibit activated cofactors Va and VIIIa, so deficiency will lead to fibrin synthesis
- Clinical expression of hypercoagulability is variable, and do not necessarily correspond with absolute concentration of Protein C
- Deficiency could be
  - Inherited autosomal dominant (heterozygous): causes ↑ risk of thrombosis
  - Inherited but homozygous: causes neonatal puprura fulminans > fatal without immediate replacement
  - Acquired:
    - Protein S deficiency may be induced by OCPs, pregnancy, or nephrotic syndrome
    - Protein C is consumed and levels are low in ***vitamin K deficiency, DIC , liver disease, warfarin use*** etc
- Treatment: give the patient protein C or S

Antithrombin deficiency:

- Is a potent inhibitor of thrombin and other serine proteases of the coagulation cascade, so deficiency leads to thrombosis
- typically occurs in a Autosomal Dominant inheritance pattern, thereby affecting both sexes equally and has multiple mutations
- ***Most thrombogenic disorder!*** IMP
- Overall incidence of AT deficiency is low
- Females with AT deficiency are at particularly high-risk for VTE during pregnancy
  - DVT occurred in 18% of pts w/ AT deficiency, and in 33% in the postpartum period

Prothrombin gene mutation:

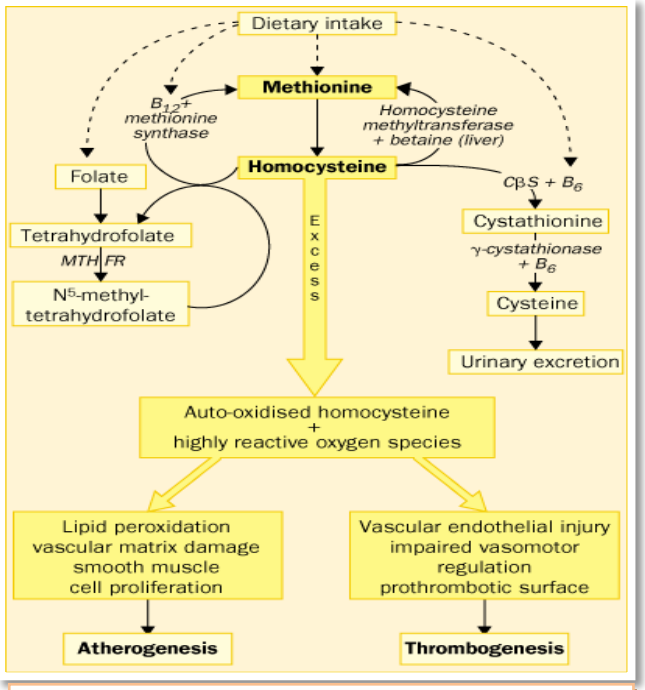
- Normal prothrombin (Factor II) circulates as Vitamin K-dependent cofactor w/ ½ life of 3-5 days
- transition (G→A) at nucleotide 20210 (G20210A)
- elevated plasma levels of Factor II
  - Heterozygote have a 30% higher plasma prothrombin level compared to normal
- Prothrombin 20210A mutation is the 2nd most common prothrombotic mutation (→ ↓ thrombin inactivation)

Hyperhomocystenemia

- ***Acquired is more common than inherited***
- Rare autosomal recessive disorder (MTHFR):
  - Characterized by developmental delay, osteoporosis, ocular abnormalities, VTE, and severe premature CAD.
  - Treatment is by administering folic acid
- Less marked elevations of homocysteine are more common, occurring in 5-7% of the population, and are associated with a number of clinical factors like Vitamin deficiencies (i.e., folate, Vit B6, and/or Vit B12) and vegetarians (↓ vit.B12) and TB treatment
- **Causes:**
  - ↓ vit.B12, ↓ Folate ,↓ vit.B6

- ↓ Thymine
- MTHFR (methyline tetra hydro folate reductase)Mutation
- INH (TB medication)

- Homocysteine has primary atherogenic and prothrombotic properties.
- Implicated in both arterial and venous thrombosis. So why is homocystine thrombogenic? Theories include:
  - Direct toxicity to endothelial cells
  - Inhibits Protein C activation
  - Promotes endothelial tissue factor expression
  - Surpresses endothelial cell surface heparin sulfate



Any block in the pathway will ↑ homocystine

Acquired causes or risk factors for thrombosis:

1. Pregnancy
   a. Bleeding or thrombosis
   b. PAI-2: is secreted from the placenta
   c. Protein S is very low in pregnancy (and OCP)
   d. Clotting factors are high and platelet are more active
2. Oral contraceptive or hormone replacement therapy
3. Heparin use (and subsequent HITT: heparin induced thrombocytopenia)
4. Myeloproliferative disorders like: Polycythemia Vera or essential thrombocythemia
5. Hyper viscosity syndromes: Multiple myeloma or Waldenstrom's macroglobulinemia
6. Atherosclerosis: causes Endothelial injury and dysfunction
   a. LDL cholesterol – oxidized LDL--- foam cells
   b. Diabetes mellitus – glycated LDL cholesterol
   c. Smoking – free radical production
   d. Hypertension – smooth muscle proliferation
   e. Genetic alterations – MTHFR mutations

7. Antiphospholipid antibody syndrome. VERY IMPORTANT!

a. General considerations:
   i. Autoimmune Acquired Prothrombotic Disorder
   ii. Damages phospholipids in cell membranes of endothelium, causing platelet activation
   iii. ***Gives prolonged PTT in the lab results although the patient has thrombosis:*** Tube clotting needs calcium and phospholipids, and because the antibodies are found in the sample this will make the clotting take more time because it attacks the phospholipids and then you will have prolonged PTT
   iv. Very High Risk for recurrent thromboembolic disease both venous and arterial
   v. It should be considered in patients who have:
      1. Thrombosis
      2. Recurrent miscarriages
      3. Antiphospholipid syndrome is when you have 1,2 and positive for antiphospholipid antibodies
   vi. Ischemic strokes occur in about 20% of patients and deep vein thrombosis in about 40%
   vii. Twenty-seven per cent of women who have had two or more spontaneous miscarriages have APS
b. Diagnosis: To finalize the diagnosis you should have:

i. One or more of clinical criteria of the following:
    1. ≥ 1clinical episode of **Vascular thrombosis**
    2. **Pregnancy morbidity:**
        a. ≥ 1 unexplained fetal death ≥ 10 weeks Estimated Gestational Age (EGA).
        b. ≥ 1 premature birth (≤ 34th week of gestation) due to eclampsia, severe pre-eclampsia, or placental insufficiency
        c. ≥ 3 unexplained consecutive spontaneous abortions <10 weeks EGA.

ii. ≥1 positive lab test on two different occasions 6 weeks apart
    1. The *anticardiolipin test*, which detects antibodies (IgG or IgM) that bind the negatively charged phospholipid, cardiolipin
    2. The *lupus anticoagulant test* which detects changes in the ability of blood to clot in a test tube. The *anti-β_2-glycoprotein I* test, which detects antibodies that bind β_2-glycoprotein I, a molecule that interacts closely with phospholipids.
    3. In short: Antibody is to cardiolipin in APA (ELISA assay); antibody is to *beta* 2 glycoprotein 1 and platelet phospholipids in patients with lupus anticoagulants (aPTT and/or PT).

8. Prior thrombotic event
9. Recent major surgery, especially orthopedic.
10. Presence of a central venous catheter
11. Trauma
12. Immobilization
13. Malignancy (GI, lung, pancreas and ovaries)
    a. Risk for thrombosis is multifactorial.
    b. Predominantly venous thrombosis - stasis, tumor invasion of vessels, chemotherapy effects superimposed on acquired or primary defects in homeostasis.
    c. Distinct procoagulant (cysteine protease) found in many patients which can activate Factor X directly.

Diagnosis of thrombophilia:

- Usually they are diagnosed with venous thrombotic embolization
- DVT and pulmonary embolism are the two most common manifestations of the same disease: VTE
    - 90% of cases of acute PE are due to emboli emanating from the proximal veins of the lower extremities
- Clinical examination (non-specific)
    - Physical findings may include a palpable cord over the calf, ipsilateral edema, warmth, and/or superficial venous dilatation
- Contrast venography
- Non-invasive testing
    - Impedance plethysmography
    - Compression ultrasonography
        - Recommended in moderate to high pre-test probability
    - D-dimer
        - Useful in low pre-test probability to exclude diagnosis of VTE
        - Sensitivity and negative predictive value are high (~99%)
    - Magnetic resonance venography
    - Computed tomography
    - Echocardiography, ventilation-perfusion (V/Q) scanning, and pulmonary angiography

Treatment:

1. Anticoagulation:
    a. Heparin:
        i. MOA: potentiates the action of antithrombin to inhibit clotting factors II and X
        ii. Monitored by PTT because it prolongs it
        iii. Half life 1 hour
        iv. Recommended dose 75U/kg
        v. ADRs: bleeding, heparin-induced thrombocytopenia(HIT), osteoposrosis, alopecia
        vi. Contraindications: previous HIT, active bleeding, haemophilia, TCP, HTN, recent surgery on eyes or brain
        vii. Antidote for overdose: protamine sulphate
    b. Low-molecular weight heparin LMWH:
        i. MOA: inhibit factor X directly
        ii. Administered subcutaneous
        iii. They cannot be monitored by PT or PTT (they affect neither)
        iv. Less monitoring needed, more predictable blood levels, less osteoporosis.
        v. Half life is variable 4-24 hours depending on product
        vi. Dose is adjusted to body weight
        vii. LMWH should be avoided in CKD; contraindicated in Stage V CKD
    c. Warfarin (oral anticoagulant)
        i. MOA: Vitamin K antagonist – leads to decrease in vitamin K-dependant clotting factors (II, VII, IX, X) and protein C and S
        ii. Monitored by PT because it is prolongs it
        iii. Also monitored by INR (2-3 is therapeutic)
        iv. Takes 4 or 5 days to have full effect so start heparin first
        v. ADR's: TERATOGENIC (avoid in pregnancy), bleeding, skin necrosis, bleeding
        vi. Antidote for overdose: stop warfarin and give vitamin K
    d. Direct thrombin inhibitors (ximelagatran)
    e. Factor Xa inhibitors (fonduparinux)
2. Thrombolysis
    a. Tissue plasminogen activators (t-PA, u-PA, urokinase, alteplase)
3. Thrombectomy (arterial)

MCQs

1. Factor V Leiden is characterized by:
    a. Protein s deficiency
    b. Bleeding disorder
    c. Hypercoagulable status*
2. New concept of blood coagulation --> tissue factor is the main factor --> activates factor VII --> X --> Thrombin
3. Which of the following is not a recognized cause for acquired hypercoagulable states:
    a. OCP use
    b. Malignancy
    c. Antiphospholipid antibody
    d. Protein C deficiency
    e. Post surgery on knee joint
4. The following approach is recommended for excessive heparin overdose.
    a. Fresh frozen plasma.
    b. Stored plasma.
    c. Vitamin K intravenously
    d. Protamine sulfate orally
    e. Protamine sulfate intravenously after heparin discontinuation.
5. Which drug can be used for treatment of warfarin toxicity?
    a. Heparin
    b. Allopurinol
    c. Coumarin
    d. Vitamin K
    e. Vitamin E
6. Non Vit. K related Factor is : a. VII b. VIII * c. IX d. X e. Prothrombin