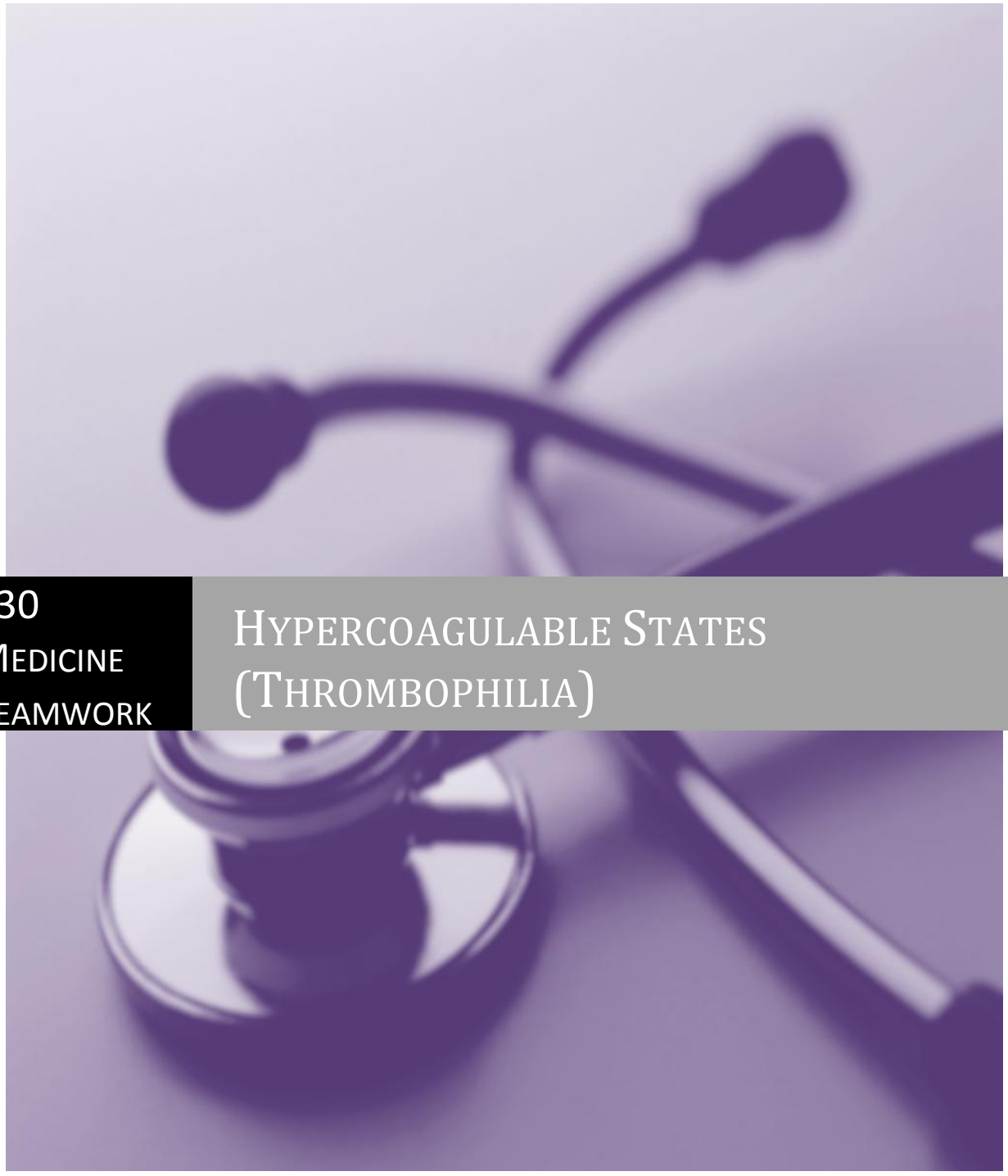
"He who studies medicine without books sails an uncharted sea, but he who studies medicine without patients does not go to sea at all." – William Osler



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MEDICINE
TEAMWORK

HYPERCOAGULABLE STATES (THROMBOPHILIA)

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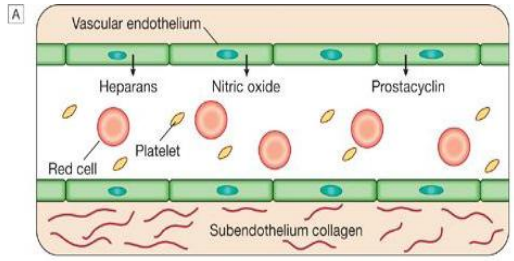
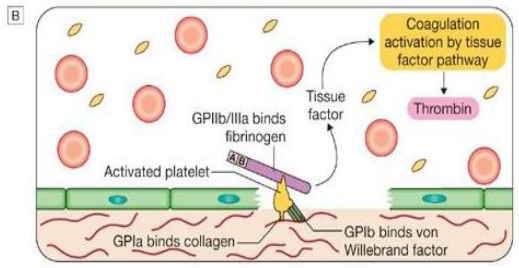
Hypercoagulable States (Thrombophilia)

The stages of normal haemostasis (for your knowledge):

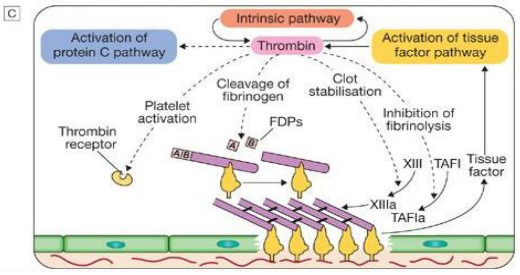
A- The vascular endothelium produces substances (including NO, prostacyclin and heparans) to prevent adhesion of platelets and white cells to vessel wall. Platelets and coagulation factors are in non activated state.



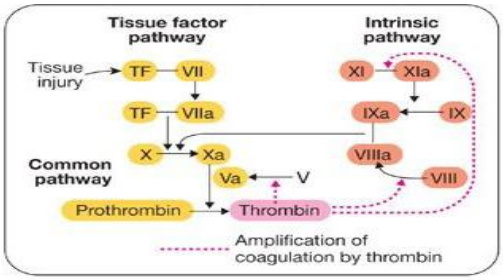
B- Tissue injury → coagulation activated → tissue factor released → binds to factor VII (extrinsic pathway activated)→ activate factor X → prothrombin to thrombin.



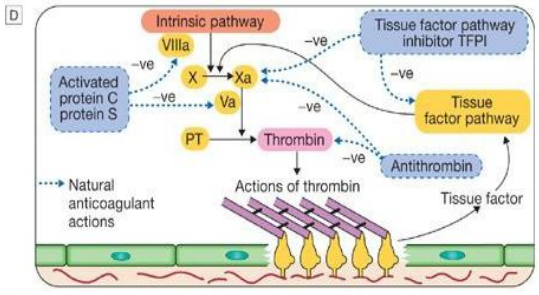
C- Thrombin amplifies its own production → intrinsic pathway become activated (VIII, IX, XI) also factor X → large amount of thrombin generated → thrombin enhances clot formation by cleaving fibrinogen into fibrin.



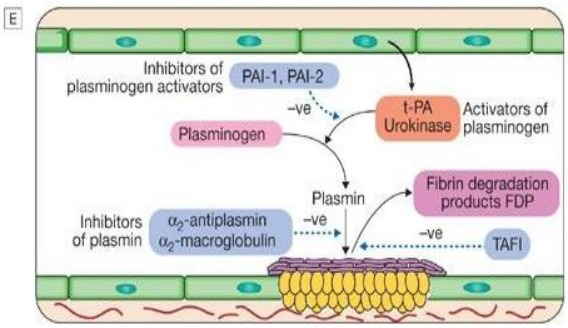
Thrombin also regulates clot formation by:
1- activation of protein C pathway which reduces further coagulation.
2- Activation of thrombin activatable fibrinolysis inhibitor (TAFI) which inhibits fibrinolysis.



D- Once haemostasis has been secured → antithrombin synthesized by the liver destroys activated factors such as (Xa, XIa and thrombin IIa) → tissue factor pathway inhibitor TFPI inactivates VIIa and Xa .
Thrombin binds to membrane bound thrombomodulin → activation of protein C → binds to its cofactor Protein S → cleavage of Va and VIIIa.



E- Tissue plasminogen activator (t-PA) or urokinase in the clot activate plasminogen → convert it to plasmin → hydrolyses the fibrin clot → producing fibrin degradation products including D-dimer.
Plasmin is inhibited by α2-antiplasmin and α2-macroglobulin and fibrinlolysis is further inhibited by thrombin activated (TAFI)



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

Clotting Factors:

- The coagulation system consists of a cascade of soluble inactive zymogen proteins designated by Roman numerals.
- When proteolytically cleaved and activated, each is capable of activating one or more components of the cascade.
- Activated factors are designated by the suffix 'a'.
- Some of these reactions require phospholipid and calcium.
- Coagulation occurs by two pathways; it is initiated by the extrinsic (or tissue factor) pathway and amplified by the intrinsic pathway.

Coagulation cascade:

1-Extrinsic system (factor VII)
-Factor VII is activated (factor VIIa) by tissue thromboplastin.
-Factor VIIa activates factor X in the final common pathway.
2-Intrinsic system (factors XII, XI, IX, VIII):
3-Final common pathway: factors X, V, prothrombin (II), and fibrinogen (I)

How to keep Hemostatic balance?

A- Antithrombotic function of (Endothelium)

The most important organ in preventing thrombosis.

1- Production of prostacyclin (PGI2) → vasodilatation and antiplatelet action.
2- Removal of thrombin by Thrombomodulin. [TM is a protein which exists in the wall of endothelial cells and helps in generation and activation of thrombin →formation of thrombin-thrombomodulin complex leads to protein C activation]
3-Production of tissue plasminogen activator (t-PA) → fibrinolysis.
4- Production of nitric oxide → neutralization of excess homocysteine (causing damage to endothelial cells) and vasodilatation.
5- Production of proteoglycans (heparin-like)

Natural anticoagulants:

- Protein C
- Protein S
- Antithrombin (Previously known as ATIII)

B- Antithrombotic function of (circulation):

1- **Removal of thrombin** by liver and endothelium.
2-Inactivation and removal of **activated clotting factors**.
3-Enhancement of natural anticoagulants action.
4-Facilitaation of **fibrinolysis.**

C-Antithrombotic action of (natural anticoagulants):

1-Anti-thrombin (AT) inhibits thrombin → inhibition activated factor VIII, IX, X, XI.
2-Protein C complexes with activated factor V by forming **FV.PC** complex that neutralizes activated factor VIII.
3-Protein C also neutralizes plasminogen-activator inhibitor (PAI) → enhanced fibrinolysis.
4-Protein S enhances the action of protein C.
5-Tissue factor pathway inhibitor (TFPI) → neutralizes tissue factor → ↓ extrinsic pathway activation.

D-Fibrinolysis: (works locally on the clot itself)

1- Tissue plasminogen activator (t-PA) & uorokinase (UK) activate plasminogen to plasmin → Fibrinolysis.
2- Protein C neutralizes PAI→ enhanced fibrinolysis.

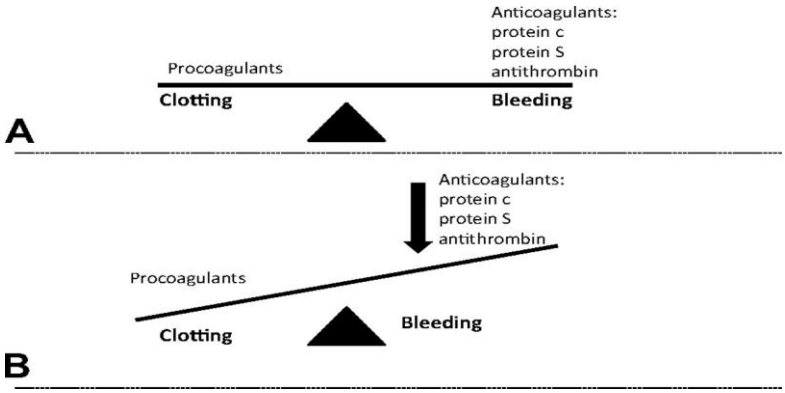
Vitamin K dependant factors: (imp)
1-Factors II, VII, XI, X, protein C and protein S.
2-Synthesized in the liver as non-functional precursor proteins.
In vitamin K deficiency these factors are affected, because they need it to work.

Thrombotic disorders (Thrombophilia):

- Patients with hyperoagulable states are more likely to develop clots, venous and arterial thrombosis, than healthy individuals.
- There is often a history of recurrent thromboembolism, thrombosis at a young age, and a family history of thrombosis.
- Venous thrombosis and pulmonary embolism are associated with significant morbidity and mortality.
- Thrombophilias are inherited hypercoagulable disorders.

Pathophysiology:

- Thrombosis can be due to increased procoagulant factors, impaired fibrinolysis, venous stasis, and endothelial cell injury (e,g pregnancy).
- The risk of thrombosis is increased in patients on hormonal replacement therapy. However, whether this risk is due to increased procoagulants or the presence of an underlying thrombophilia is not clear.
- Several underlying hereditary risk factors exist for thrombosis.
- A pathway that neutralizes activated factor V may be impaired by deficiencies in protein C & protein S.
- Factor V Leiden is the most common basis for activated protein C resistance (APCR) since the mutant factor V is resistant to inactivation by protein C and protein S.
- The neutralization of activated factor Xa and thrombin are impaired in antithrombin III (ATIII) deficiency.
- A mutant prothrombin is associated with an increase in venous thrombosis.



Clinical manifestations:

There are no specific clinical symptoms or signs directly attributable to thrombophilic disorders. Rather, the clinical expressions of an underlying thrombophilia are predominantly venous thrombosis and pulmonary embolism.

Causes and risk factors for hypercoagulability and thrombosis can be hereditary or acquired:

Hereditary Thrombophilia:

- These disorders should be suspected in patients who have a history of recurrent venous thromboembolism, venous thrombosis in a person younger than 40 years, a familial history of venous thromboembolism, and thrombosis in unusual sites (eg, mesenteric vein, renal vein, hepatic and cerebral thrombosis).
- Thrombophilic disorders are usually associated with venous thrombosis. However, protein S, protein C, ATIII deficiencies, and lupus anticoagulants have been associated with arterial thrombosis.

Normally

1- The thrombin thrombomodulin complex activates protein C and this produces APC (activated protein C).
2- APC cleaves the product of the factor 5 gene and forms a complex that inhibits factor 8.

Factor V Leiden:

- Discovered in Leiden collage, 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!!
- 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 resistance.
- Protein C levels become high due to compensatory effect. [So, both high and low levels of PC lead to thrombosis].

- There is dual action of factor V Leiden and that causes increased coagulation:
  - ↓ inactivation of factor VIIIa →↑ thrombin generation, (↓ anticoagulation)
  - ↓ PAI inactivation → ↓ fibrinolysis.

Protein C and protein S deficiency: (congenital/acquired)

- 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 (of protein C) will lead to fibrin synthesis.
- ↓ PAI neutralization → impaired fibrinolysis
- 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:** (common)
    - Protein S deficiency may be induced by OCPs, pregnancy, or nephrotic syndrome. >> severely impaired protein C action.
    - Protein C levels are low in vitamin K deficiency, DIC , liver disease, warfarin use etc
    - Treatment: give the patient protein C or S.

Antithrombin deficiency: (congenital/acquired)

- Is a potent inhibitor of thrombin and other serine proteases of the coagulation cascade, so deficiency leads to impaired inhibition of activated factor VIII, IX, X, & XI and 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 with AT deficiency, and in 33% in the postpartum period.

Prothrombin gene mutation:

- Normal prothrombin (Factor II) circulates as Vitamin K-dependent cofactor with ½ life of 3-5 days.
- Elevated plasma levels of Factor II
- Heterozygote has a 30% higher plasma prothrombin level compared to normal.
- Prothrombin 20210A mutation is the 2nd most common prothrombotic mutation (→ ↓ thrombin inactivation).

Mutation of Factor II → antithrombin resistance (inability of Antithrombin to bind to thrombin → inefficient thrombin neutralization (AT dysfunction and accumulation) → both high and low anti thrombin levels lead to thrombosis.

Hyper-homocystenemia:

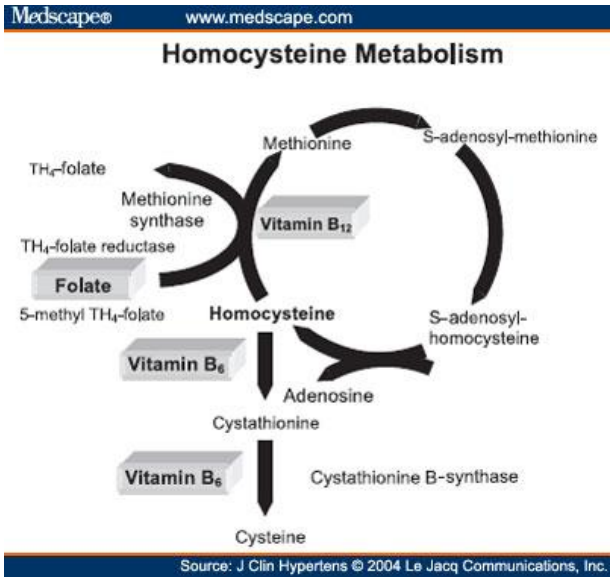
- Acquired is more common than inherited.
- Rare autosomal recessive disorder: Characterized by developmental delay, osteoporosis, ocular abnormalities, VTE, and severe premature CAD.
- Excess homocycten overcomes the NO →damage the endothelium → direct platelet aggregation and clotting factor activation.
- Also causes lipid auto oxidation → release of free radicals.
- Mutation leads to Folate inactivation →B12 inactivation →homocysten accumulation.
- 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 due (↓ vit.B12) and TB treatment deplete (vit B6).
- Implicated in both arterial and venous thrombosis.
- Treatment is by administering folic acid.

Causes of Hyperhomocystenemia:

- ↓ vit.B12, ↓ Folate, ↓ vit. B6 , ↓ Thymine, MTHFR (methyline tetra hydro folate reductase)Mutation ,INH (TB medication).

So why is homocystine thrombogenic?
Theories include:

- Direct toxicity to endothelial cells.
- Inhibits Protein C activation.
- Promotes endothelial tissue factor expression.
- Suppresses endothelial cell surface heparin sulfate.



Acquired causes for thrombotic disorders:

- Advanced age.
- Immobilization.
- Inflammation.
- Pregnancy.
- Oral contraceptive use.
- Obesity.
- Diabetes.
- Hormonal replacement therapy.
- Cancer (especially adenocarcinoma).
- Lupus anticoagulant.
- Sickle cell anemia and other hemolytic anemias

Anti phospholipid syndrome is the most important acquired cause of thrombotic disorders:

- General considerations:
  It is an autoimmune Prothrombotic Disorder that inhibits the antithrombotic function of endothelial phospholipids' including PGI2 production & release, protein C activation.
  - ✓ Damages phospholipids in cell membranes of endothelium, causing platelet activation.
  - ✓ Gives falsely 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.
  - ✓ Very High Risk for recurrent thromboembolic disease both venous and arterial. → ↑ platelet activation → arterial vasoconstriction
  - ✓ It should be considered in patients who have:
    1. Thrombosis
    2. Recurrent miscarriages
       - ➢ Antiphospholipid syndrome is when you have 1,2 and positive for antiphospholipid antibodies
  - ✓ Ischemic strokes occur in about 20% of patients and deep vein thrombosis in about 40%.
  - ✓ 27% of women who have had two or more spontaneous miscarriages have APS

To finalize the diagnosis you should have: (APS)

One or more of clinical criteria of the following:

1. ≥ 1clinical episode of vascular thrombosis.

2. Pregnancy morbidity:

- ✓ ≥ 1 unexplained fetal death ≥ 10 weeks Estimated Gestational Age (EGA).
- ✓ ≥ 1 premature birth (≤ 34th week of gestation) due to eclampsia, severe pre-eclampsia, or placental insufficiency
- ✓ ≥ 3 unexplained consecutive spontaneous abortions <10 weeks EGA.

3. ≥1 positive lab test on two different occasions 6 weeks apart

- ✓ 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-β2-glycoprotein I test, which detects antibodies that bind β2-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).

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.
- Physical findings may include a palpable cord over the calf, ipsilateral edema, warmth, and/or superficial venous dilatation.
- Imaging: Duplex, spiral CT (Test of choice for PE diagnosis), Magnetic resonance venography, etc
- Echocardiography, ventilation-perfusion (V/Q) scanning, and pulmonary angiography.
- D-dimer →Useful in low pre-test probability to exclude diagnosis of VTE
  - Sensitivity and negative predictive value are high (~99%)
- Laboratory tests: PT, PTT, protein C, S, AT (almost always low with acute thromboembolic episodes), homocysteine, anticardiolipin, factor V Leiden, FII mutation, (JAK2 mutation).

TABLE 1. 5 Ps HAD CAUSED CLOTS: Differential diagnosis of hypercoagulable states

Pregnancy and postpartum
Prothrombin 20210 mutation
Protein S and protein C deficiencies
Polycythemia vera and thrombocytosis
Paroxysmal nocturnal hemoglobinuria
Smoking
Heparin-induced thrombocytopenia
Hyperhomocysteinemia
Antithrombin III deficiency
Dysfibrinogenemia
Congestive heart failure
Antiphospholipid syndrome
Uremia
Surgery
Estrogen use
Diabetes
Cancer
Leiden factor V mutation
Obesity and elevated cholesterol levels
Trauma, travel (immobility)
Thyroid disease
Thalassemia and sickle cell disease
Sepsis in disseminated infections

Treatment:

1. Anticoagulation:

a. Heparin:

- MOA: potentiates the action of antithrombin to inhibit clotting factors II and X.
- Monitored by PTT because it prolongs it.
- Half life 1 hour.
- ADRs: bleeding, heparin-induced thrombocytopenia(HIT), osteoposrosis, alopecia.
- 5-Contraindications:previous HIT, active bleeding, haemophilia, TCP, HTN, recent surgery on eyes or brain.
- 6-Antidote for overdose: protamine sulphate

b.Low-molecular weight heparin LMWH:

- MOA: inhibit factor X directly.
- Administered subcutaneous.
- They cannot be monitored by PT or PTT (they affect neither)
- Less monitoring needed, more predictable blood levels, less osteoporosis.
- Half life is variable 4-24 hours depending on product
- LMWH should be avoided in CKD; contraindicated in Stage V CKD.

c. Warfarin (oral anticoagulant)

- MOA: Vitamin K antagonist – leads to decrease in vitamin K-dependant clotting factors (II, VII, IX, X) and protein C and S.
- Monitored by PT because it is prolongs it.
- Also monitored by INR (2-3 is therapeutic).
- iv. Takes 4 or 5 days to have full effect so start heparin first.
- ADR's: TERATOGENIC (avoid in pregnancy), bleeding, skin necrosis, bleeding
- Antidote for overdose: stop warfarin and give vitamin K.
- Direct thrombin inhibitors (ximelagatran)
- Factor Xa inhibitors (fonduparinux).

2. Thrombolysis: Tissue plasminogen activators (t-PA, u-PA, urokinase, alteplase)

3. Thrombectomy (arterial).

Summary Points

- Endothelium is the most important organ in preventing thrombosis
- Fibrinolysis works on destroying the clot itself
- Thrombosis can be due to increased procoagulant factors, impaired fibrinolysis, venous stasis, and endothelial cell injury (e,g pregnancy).
- The risk of thrombosis is increased in patients on hormonal replacement therapy. However, whether this risk is due to increased procoagulants or the presence of an underlying thrombophilia is not clear.
- Patients present with thrombosis in different locations, mostly Pulmonary Embolisms and Venous Thrombosis
- Antithrombin deficiency is the most thrombogenic disorder
- Natural anticoagulants → Protein C, Protein S, Antithrombin (Previously known as ATIII)
- Protein S deficiency may be induced by OCPs, pregnancy, or nephrotic syndrome. >> Severely impaired protein C action.
- Protein C levels are low in vitamin K deficiency, DIC , liver disease, warfarin use etc
- When patients with factor 5 Leiden use estrogen pills this causes SEVERE THROMBOSIS
- Antiphospholid syndrome can be suspected in patients with:
    - Multiple miscarriages
    - Thrombosis
- Vitamin K dependant factors: (imp)
    - 1-Factors II, VII, XI, X, protein C and protein S.
    - 2-Synthesized in the liver as non-functional precursor proteins.
- Acquired causes for thrombotic disorders include Advanced age, Immobilization, Inflammation, Pregnancy, Oral contraceptive use, Obesity, Diabetes, Hormonal replacement therapy, Cancer (especially adenocarcinoma), Lupus anticoagulant, Sickle cell anemia and other hemolytic anemias
- Diagnosis depends on the state of the patients and the type of thrombosis they have.
- Treatment includes Heparin, LMWH, Warfarin, Trombolysis and Thrombectomy (arterial)
- Heparin is monitored by PTT, Warfarin is monitored by INR

From the lecture:

- 1- Vascular damage → a) release of tissue factor → activation of extrinsic pathway→ thrombosis b) platelet aggregation and activation c) interruption of endothelial antithrombotic functions
- 2- Impaired circulation → a) impaired removal of thrombin b) impaired action of natural anticoagulants, c) impaired fibrinolysis
- 3- Protein C deficiency (congenital/acquired)→ a) un opposed action of activated factor V & VIII, b) ↓ PAI neutralization → impaired fibrinolysis
- 4- Protein S deficiency (congenital/acquired) → severely impaired protein C action
- 5- Antithrombin deficiency (congenital/acquired) → inefficient thrombin neutralization and impaired inhibition of activated factor VIII, IX, X, & XI
- 6- Factor V Leiden → inability of activated protein C to complex with factor V → Protein C resistance (accumulation / dysfunction) → thrombosis (particularly with contraceptive pills)
- 7- Prothrombin (Factor II) mutation → inability of Antithrombin to complex with thrombin → inefficient thrombin neutralization (AT dysfunction and accumulation)
- 8- Excess homocysteine → Hyperhomocysteinemia → a) impaired nitric oxide action → vasoconstriction, b) endothelial injury, c)platelet activation, d) activation of clotting factors, e) lipid autoxidation & release free radicals
- 9- Anti-phospholipid syndrome (APS): primary or secondary autoimmune disorder that inhibit phospholipid function on platelets and on endothelial cells → a) impaired endothelial antithrombotic function including PGI2 production & release, protein C activation, b) ↑ platelet activation → arterial vasoconstriction, c) clotting factor activation, d) ↑ PAI → inefficient fibrinolysis
- APS is associated with recurrent thrombosis, recurrent spontaneous abortion and intrauterine growth retardation and fetal loss, thrombocytopenia and hemolytic anemia.
- Patient may present with thrombosis and falsely prolonged activated partial thromboplastin time (↑ PTT)

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

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.

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