Examination of Blood Clotting

Pathophysiological Aspects

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Outline

- **Hemostatic And Coagulation Abnormalities**

- **Thrombosis**
  - Basic mechanisms
  - DVT/PE
  - Laboratory approach to hypercoagulation
- **Hemorrhage**
  - Primary hemostasis (platelets and vessel wall defects)
  - Secondary hemostasis (Plasma coagulation factors)
  - Case Report

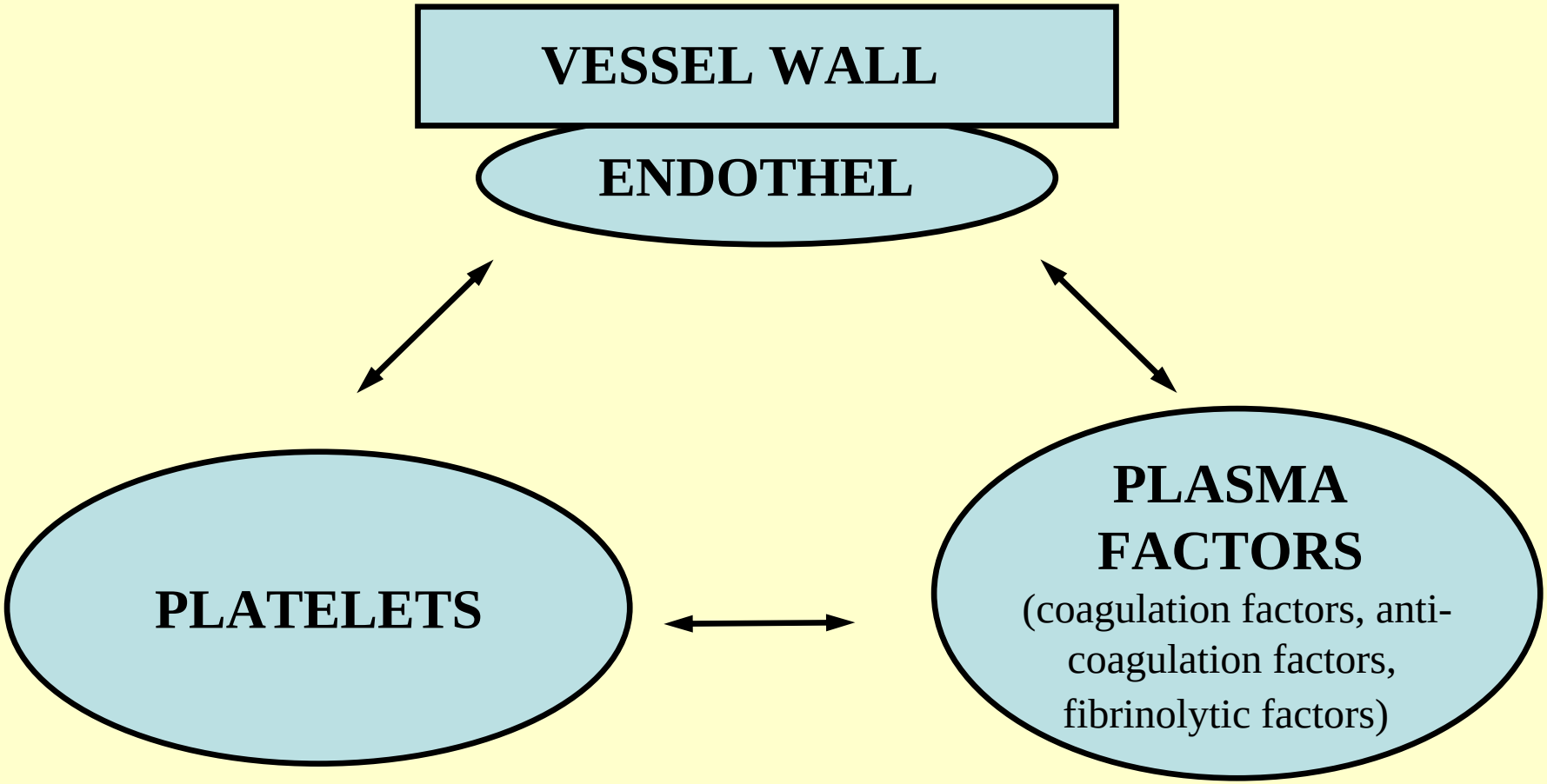
Hemostatic And Coagulation Abnormalities

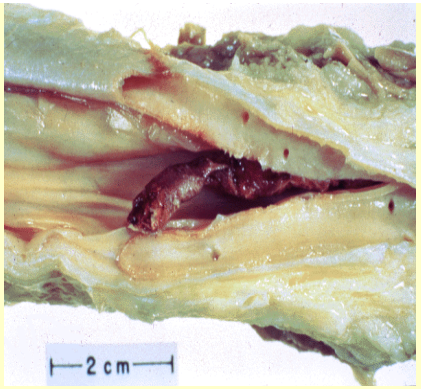
Hemostatic abnormalities can result in procoagulation or/and anti-coagulation conditions

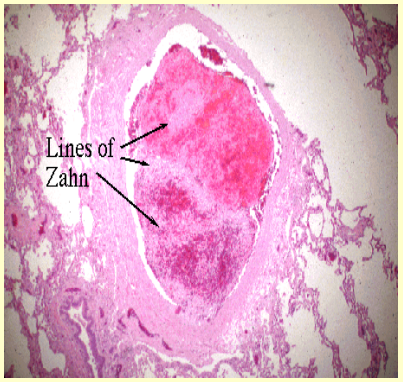
- **Thrombophilia - Thrombosis / embolia**
- **Hemorrhage**

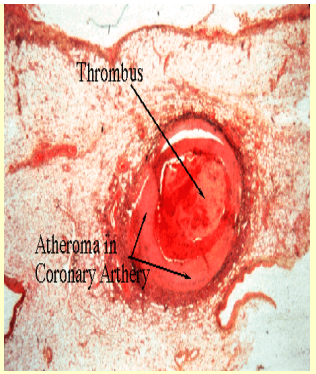
Hemostasis is a integral part of inflammatory response

HEMOSTASIS = the arrest of bleeding from an injured vessel



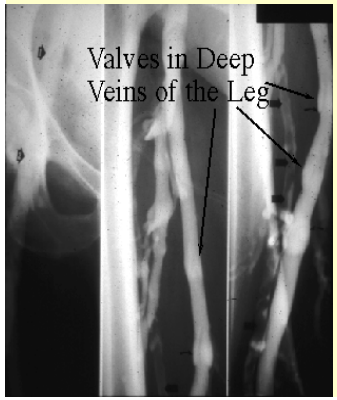






Thrombosis and Embolia

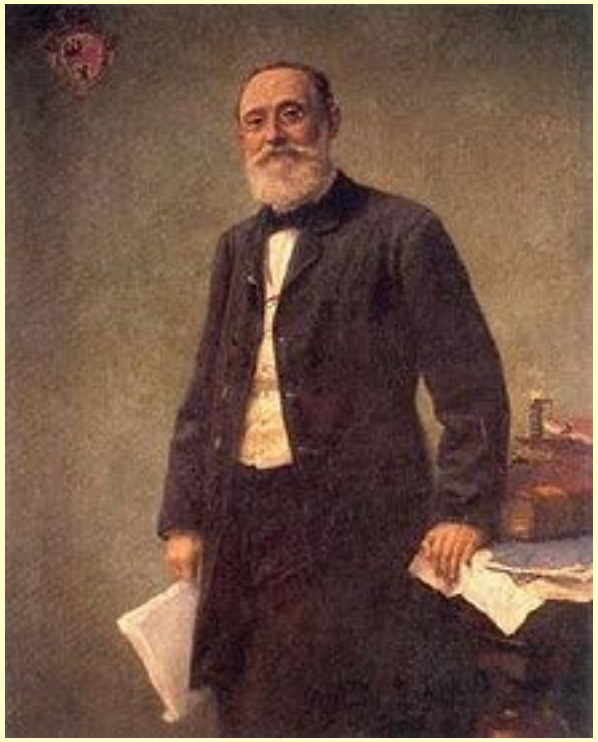
Thrombophilia (hypercoagulability) is the predisposition to develop thrombosis due to an abnormality in the system of hemostasis



Pathogenesis of Thrombosis
Virchow's triad

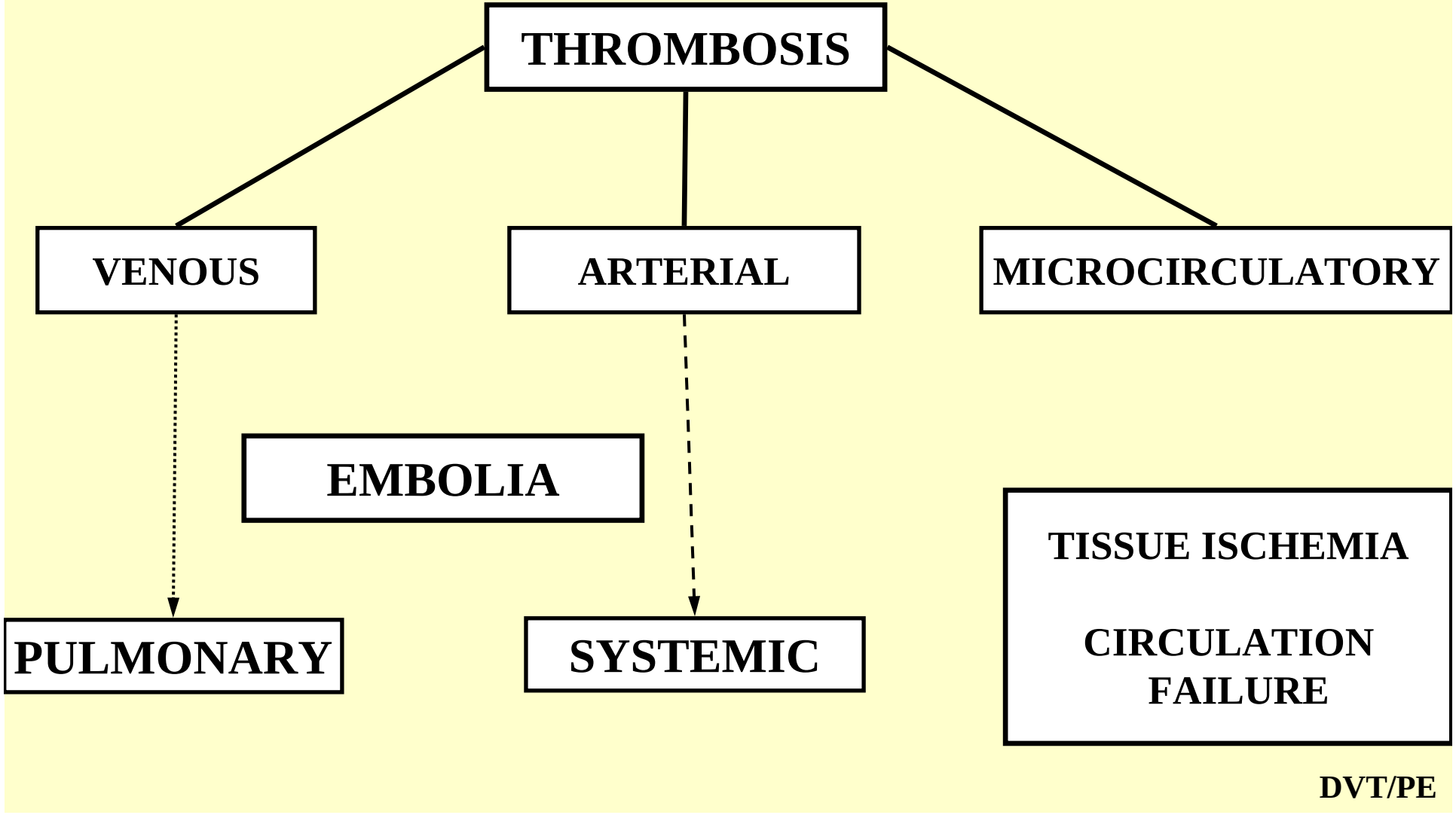
Thrombi are formed as a result of the one or more abnormalities in:

- blood vessels
- blood flow
- blood coagulability

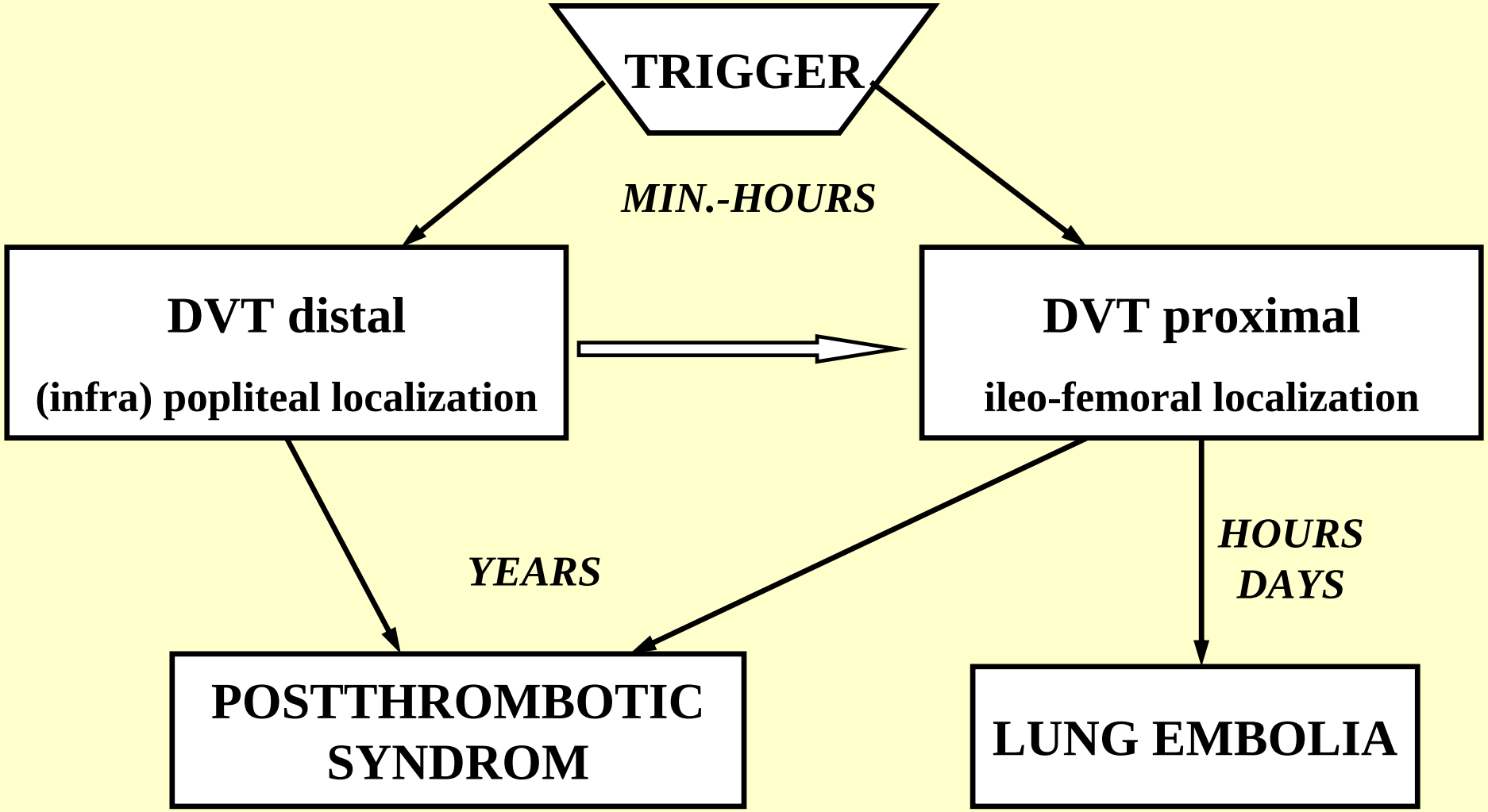


Rudolf Virchow (1821-1902)

Thrombosis And Embolia



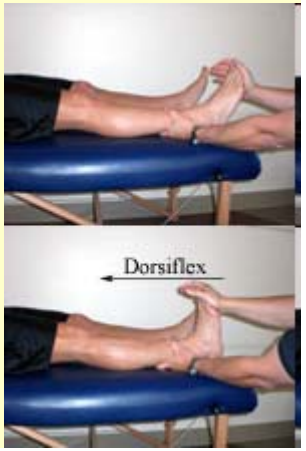
VENOUS THROMBOSIS



Deep Venous Thrombosis (DVT)

CLINICAL MANIFESTATION OF DVT

- Asymptomatic:
  - cause of > 50% of PE
- Symptomatic:
  - swelling
  - pain or palpation pain
  - changes in color and temperature of skin
  - Homan's sign
    - Increased resistance or calf pain during dorsiflection of foot



Postthrombotic syndrome
(CLINICAL MANIFESTATION OF DVT)

- sign of chronic DVT
- Usually develops 2-15 years after the episode DVT
  - varicose surface veins
  - erythema, dermatitis
  - skin ulcerations (lower extremities)

The incidence of PTS after symptomatic iliofemoral DVT
- 25-50% at 2 years
- 70-90% after 7-10 years

Laboratory markers of thrombosis

Fibrin(ogen) degradation products concentration

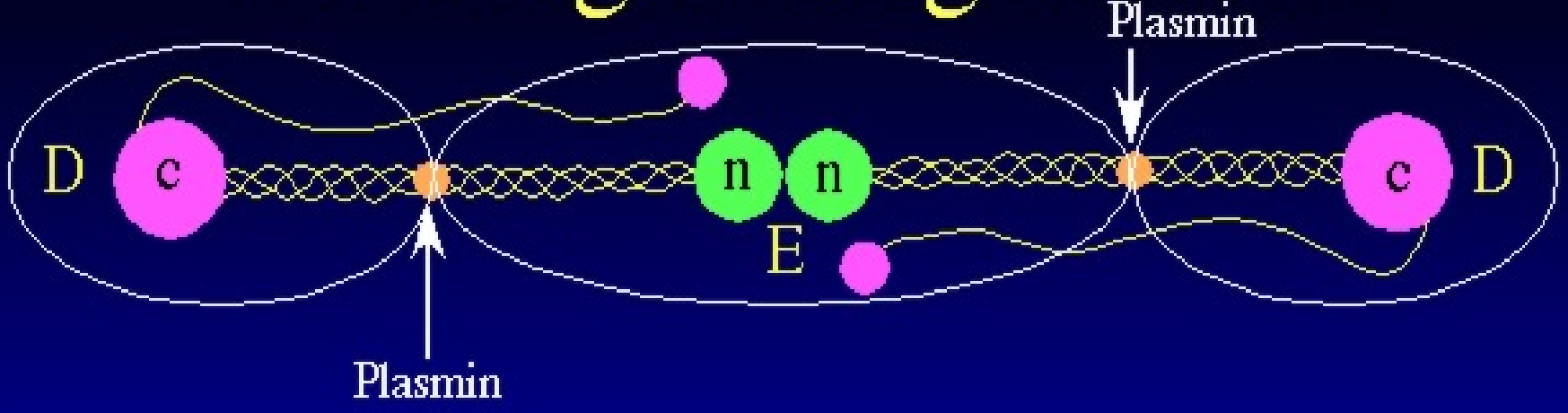
(FDP) <1000 μg/L)

- haemocoagulation failure
- ELISA or semi quantitative agglutination methods

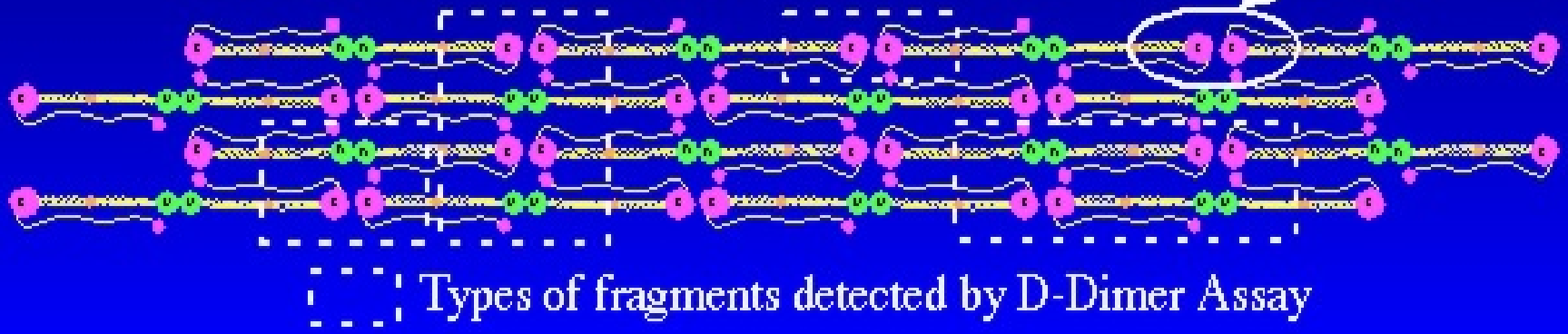
D-dimer: < 500 μg/L

- cross-linked FDPs specific for stabile fibrin
- high sensitivity but low specificity for DVT/PE

Fibrinogen Degradation



Plasmin degrades fibrin into fragments of many sizes, most of which are detected by the D-Dimer assay



Imaging Methods to Confirm DVT

- Real time ultrasound with Doppler
  - 24-66% sensitivity
- Magnetic resonance imaging (MRI)
- Contrast venography
  - 5-10% complication (phlebitis)
- Impedance pletysmography
- Accumulation of 125I-Fibrinogen ???
  - low specificity (false positive)

Pulmonary embolism (PE)

CLINICAL MANIFESTATION OF DVT / PE

- complication of DVT
- Symptoms and signs depend on the extend of embolism:
  - dyspnea, tachypnea, tachycardia
  - pleuritic chest pain
  - increased jugular vein filling
  - hemoptysis (expectoration of blood)
  - cardiovascular failure and sudden death

Diagnosis Of PE

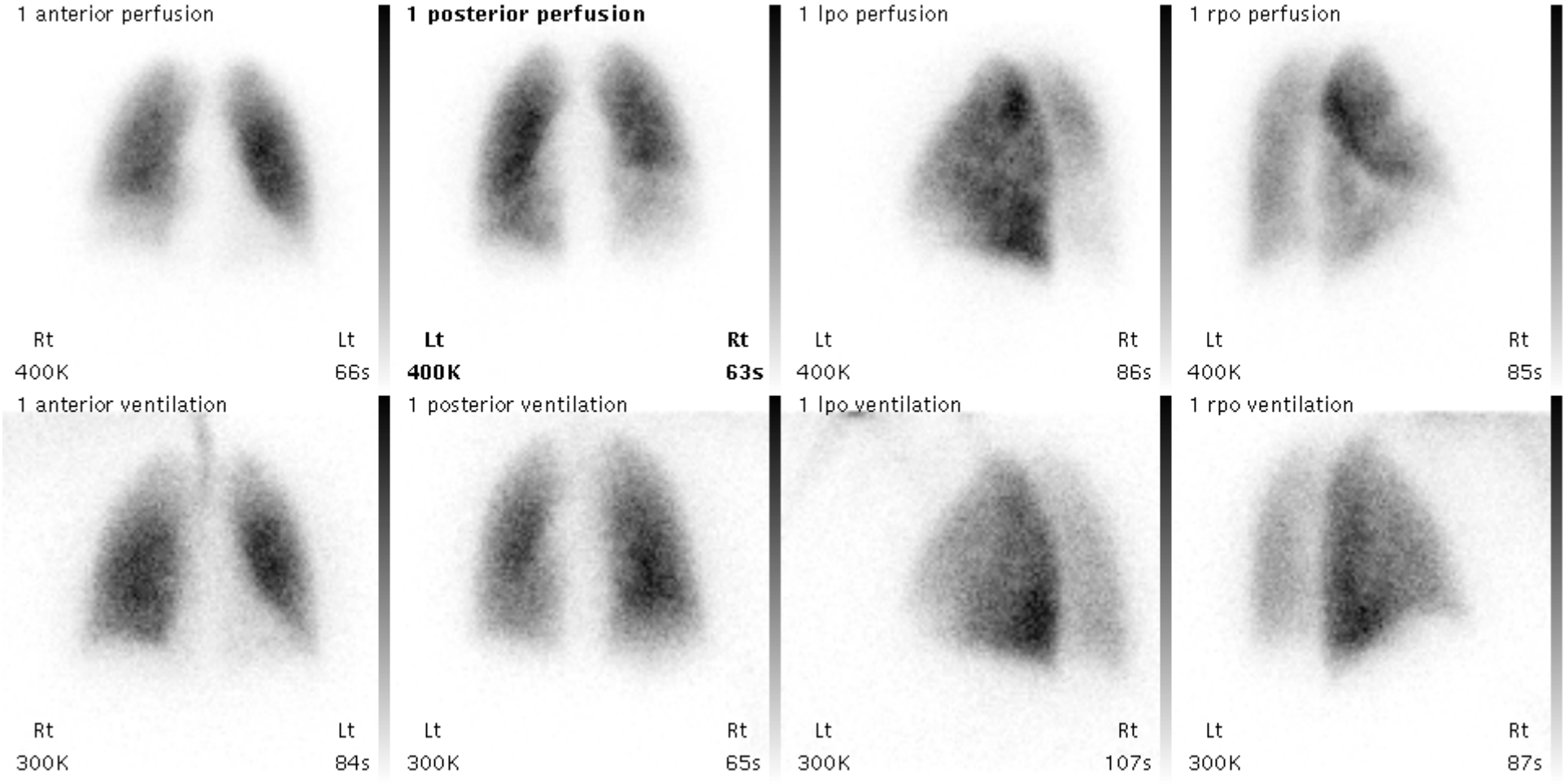
- Ventilation / Perfusion lung scan
  - recommended in all clinically stabilized pts with suspicion
- CT pulmonary angiography (CTPA)
  - referred also as CAT scan

???

- Right ventricular heart catetrization
- Echocardiography
- Blood gas analysis (ABG) ?
- ECG ?
- Chest X-ray ?

Abnormal ventilation-perfusion

(LR lobe)



Laboratory Approach to Hypercoagulation states

- **Antithrombin activity (AT):**
  - normal 100-80 % of control plasma activity
  - deficiency of AT increase the risk of thrombophilia and DIC
- APCR (Activated Protein C Resistence)
  - Factor V Leiden
    - Polymerase Chain Reaction (PCR) and Restriction fragment length polymorphism (RFLP)
- Assays of specific anticoagulant proteins

Hemorrhage

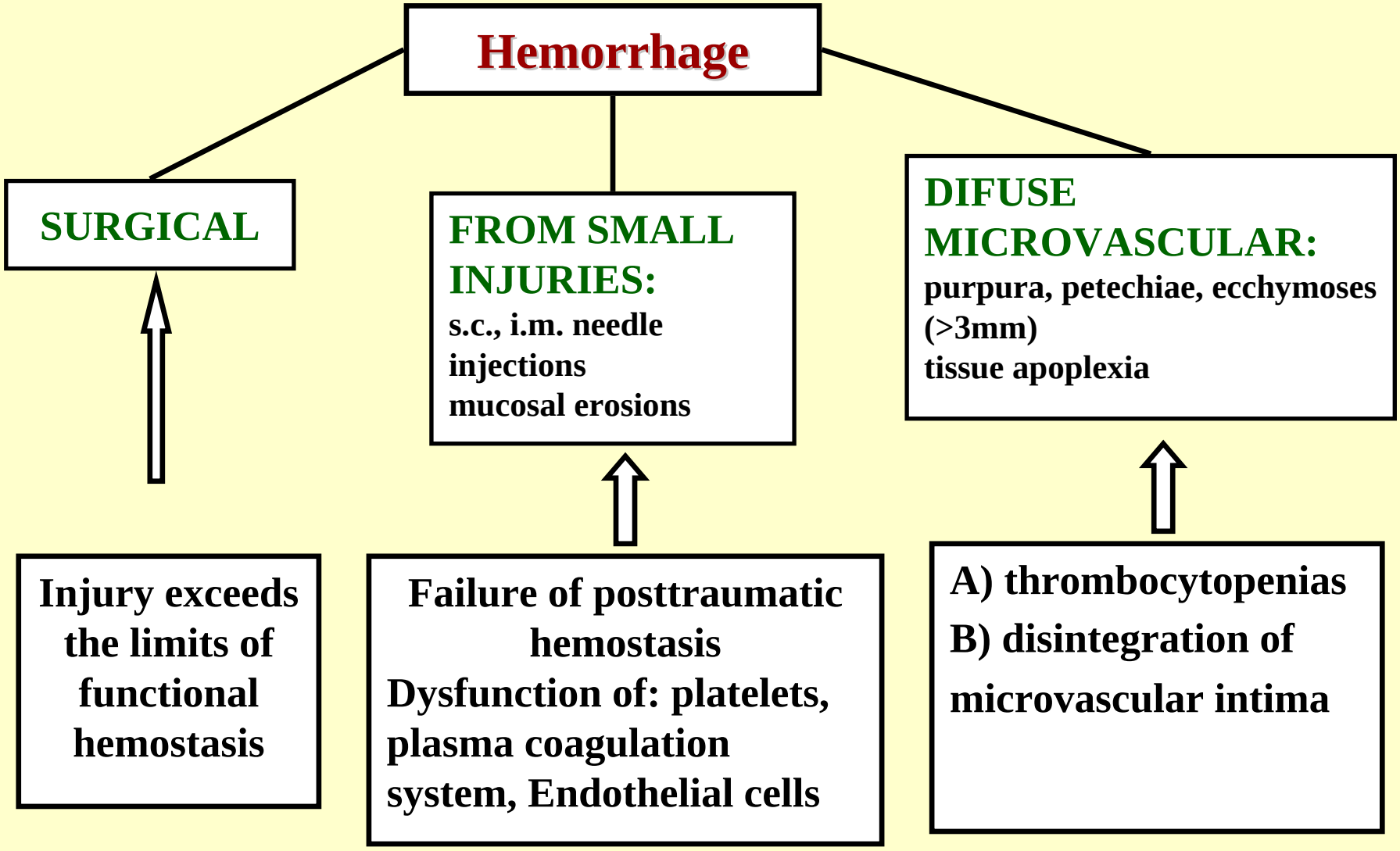
Primary hemostasis

- **Platelets and vessel wall defects**

Secondary hemostasis

- **Plasma coagulation factors**

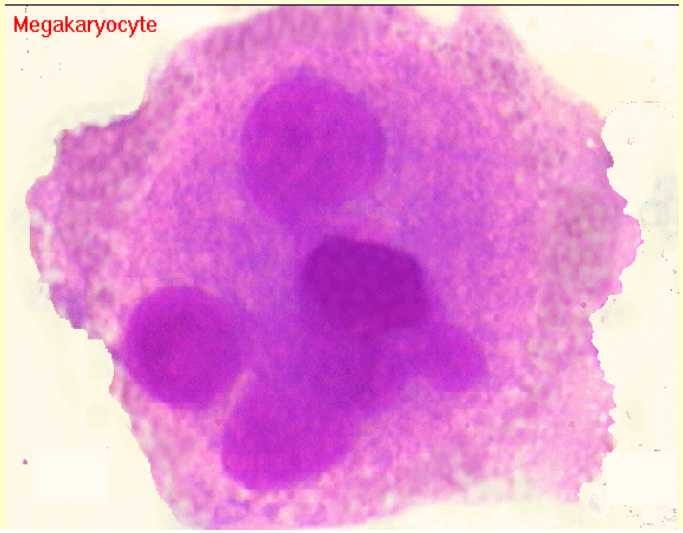
Hemorrhage

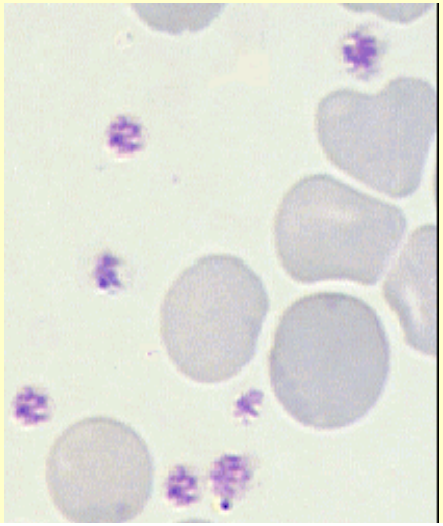

Hemorrhage
SURGICAL
FROM SMALL INJURIES:
s.c., i.m. needle injections
mucosal erosions
DIFUSE MICROVASCULAR:
purpura, petechiae, ecchymoses (>3mm)
tissue apoplexia
Injury exceeds the limits of functional hemostasis
Failure of posttraumatic hemostasis
Dysfunction of: platelets, plasma coagulation system, Endothelial cells
A) thrombocytopenias
B) disintegration of microvascular intima

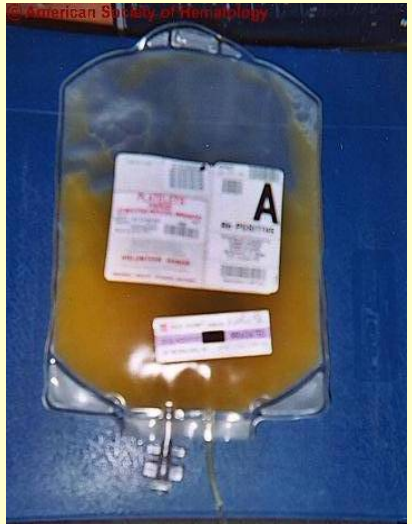
PRIMARY HEMOSTASIS

complex defense reaction which involve platelets and vessel wall

VASOCONSTRICTION AND AGGREGATION







Platelets and von Willebrand factor

Platelet count (PLT) - 150k - 400k /μL

- for surgery optimal > 100k /μL
- severe thrombocytopenia PLT < 20k /μL

Mean platelet volume (MPV) - 6 - 8 fL

- some hereditary thrombocytopaties have large PLT

Aggregometry

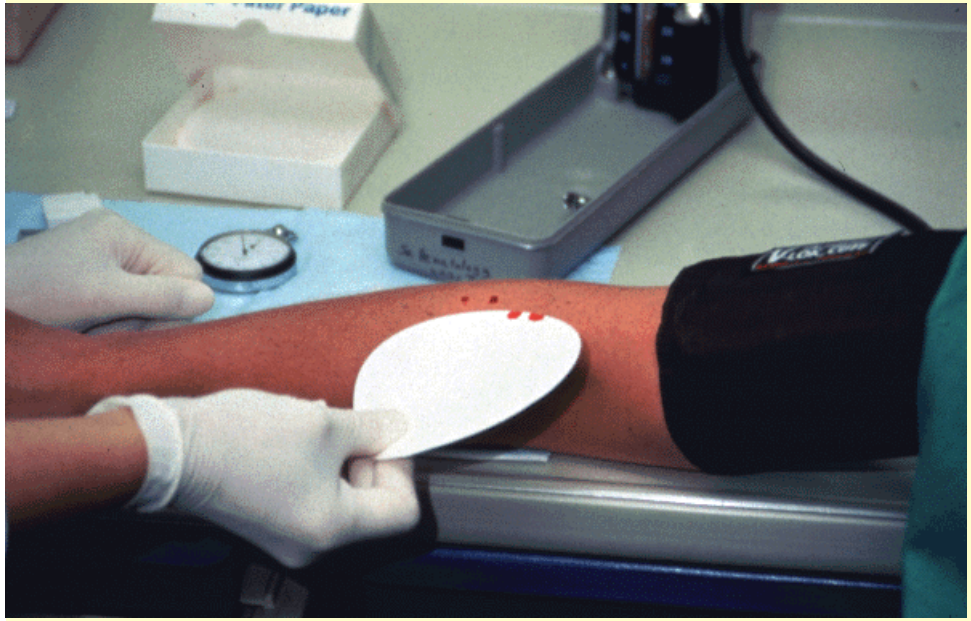
- used for classification of congenital platelet disorders
- Aggregation is induced in PLT rich plasma or in WB collected to sodium citrate by the addition of activator (ADP, thrombin, collagen)

Bleeding Time (Duke 1910, Ivy 1941)

- Functional test of primary hemostasis (platelets)
- Time required for spontaneous hemostasis after the standard injury

- Duke: Standard incision of ear auricle 2-5 min.
- Ivy: Standard incision (6x1 mm) on the volar aspect of the forearm, with the BP cuff inflated to 40 mm Hg (Ivy) 4-8 min

Bleeding Time - Ivy



- Standard incision 6x1 mm
- BP cuff inflated to 40 mm Hg
- Normal value: 4-8 min

Prolonged:

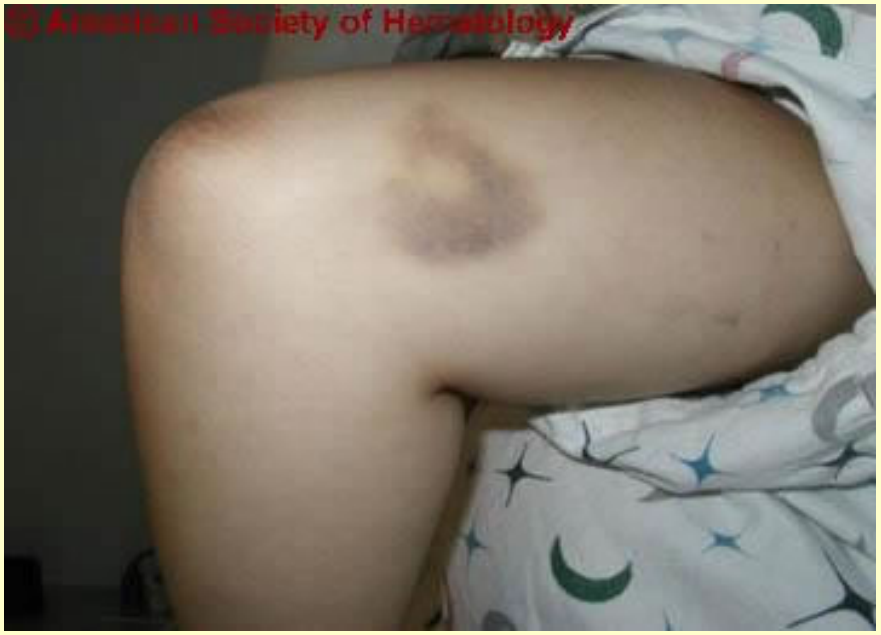
- thrombocytopenia (< 100k/mL)

-Disorders of platelet function

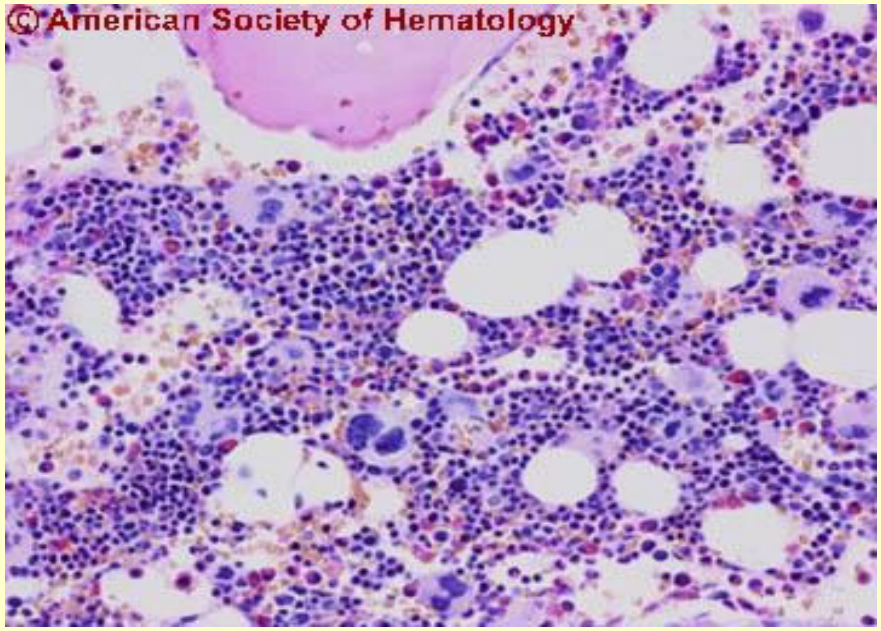
- von Willerand disease

-aspirin (>5 days)

Idiopathic Thrombocytopenic Purpura



Large ecchymotic area over the thigh following minor trauma. The platelet count at the time was 7000/uL



<u>Bone marrow biopsy</u> showing both megakaryocytic and erythroid hyperplasia

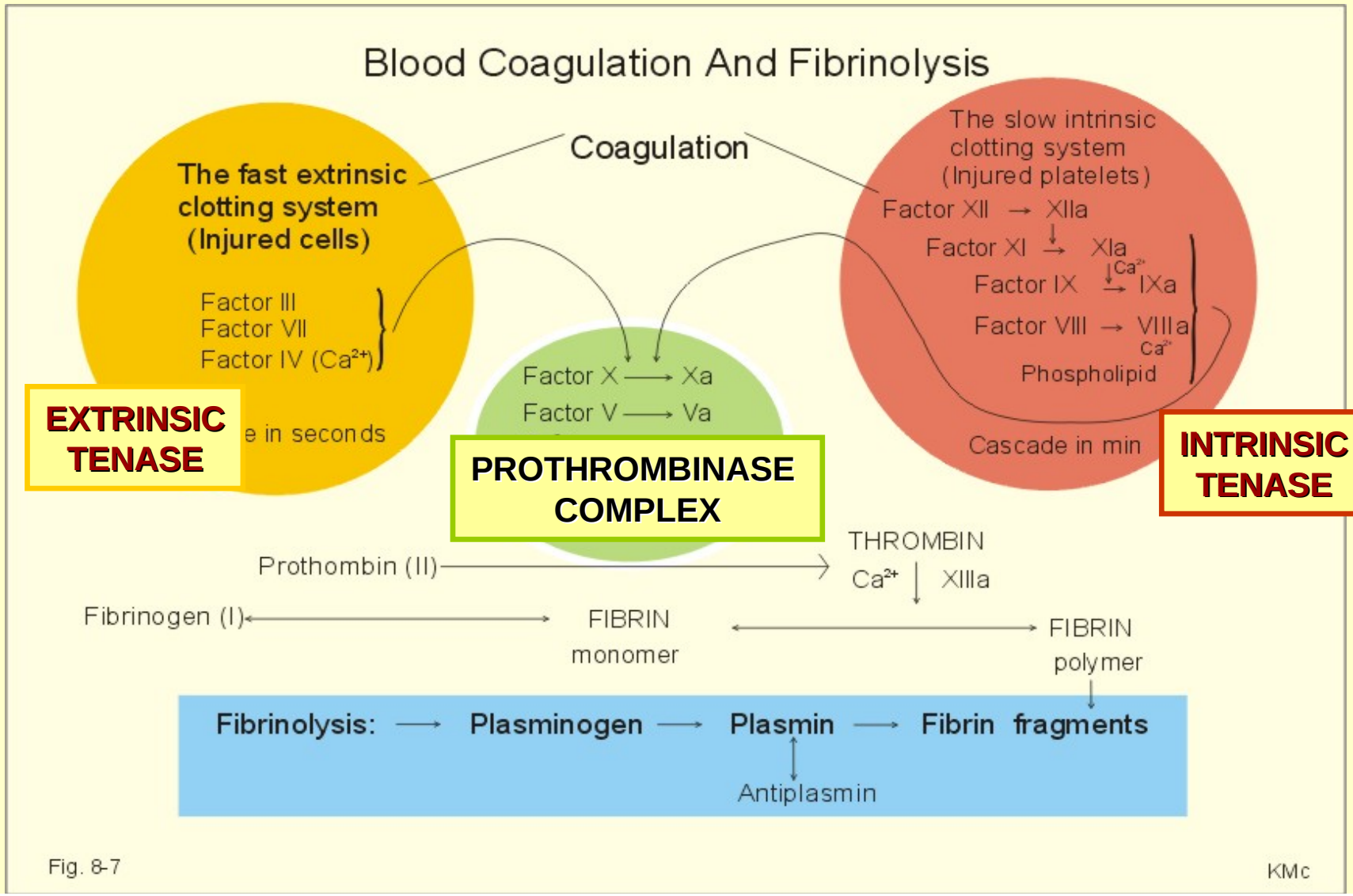
Capillary resistance test (Rumpel-Leede)

- Test of capillary vessel wall integrity
  - diagnosis of hereditary purpuras (e.g. Weber-Rendu-Osler pupura)
- Number of petechiae on the forearm area (4x4 cm) after the application of standard pressure of 10,5 kPa for 10 min using a BP cuff
- > 5 petechiae indicates increased fragility of capillaries -

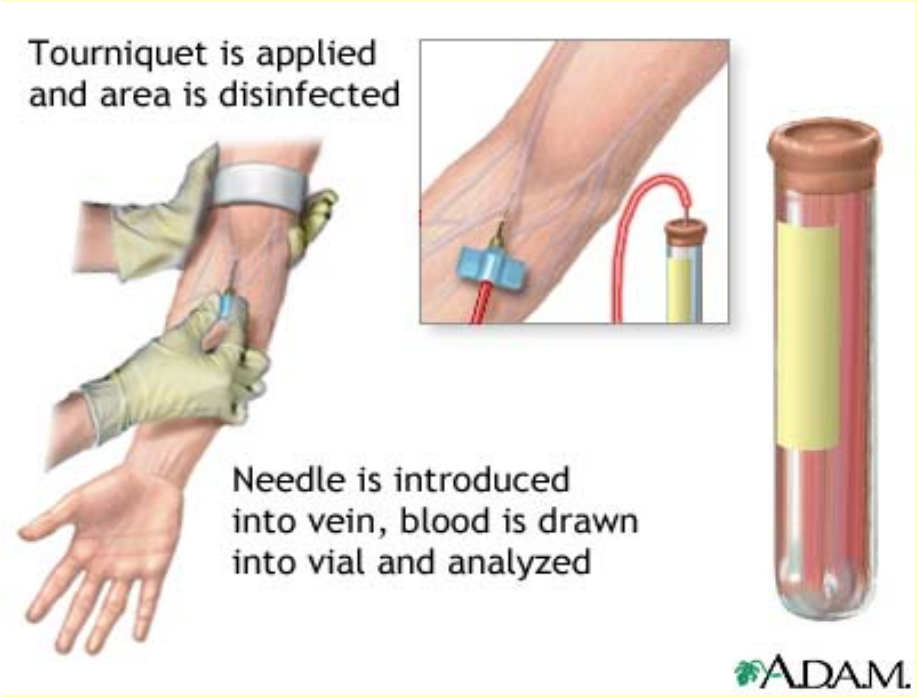
SECONDARY HEMOSTASIS

- Proteolytic cleavage of plasma coagulation factors

Plasma Coagulation Factors



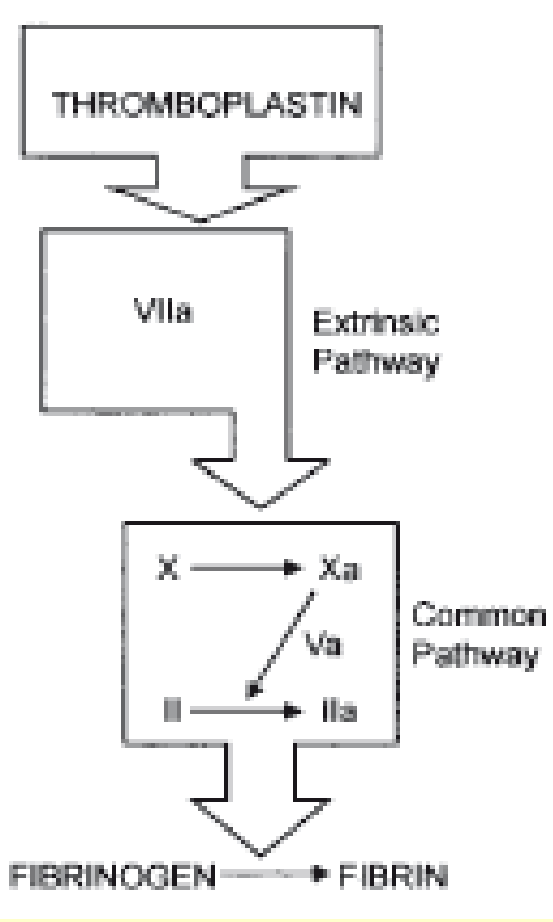
Venepuncture



Blood is collected into tubes with acid citrate dextrose (ACD) and centrifuged to obtain platelet poor plasma (PPP)

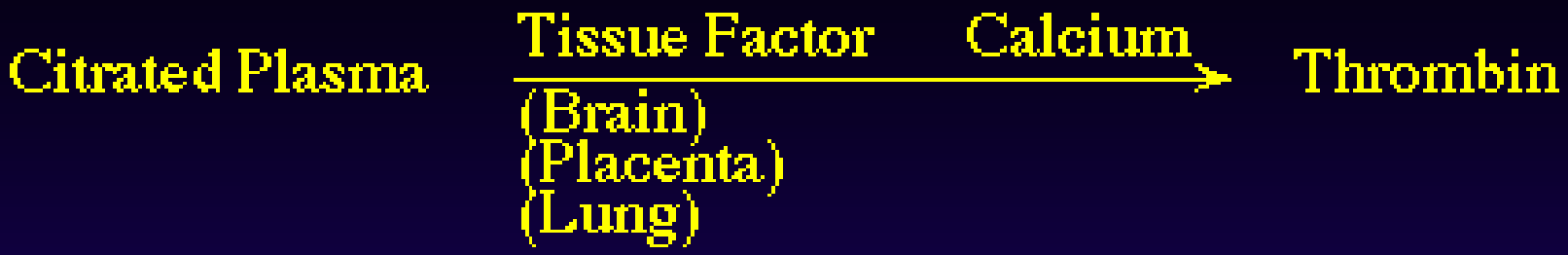
Prothrombin Time = PT (Quick's test)

- Fibrin fibers appearance (i.e. Clotting time) is measured after the addition of thromboplastin (TF and phospholipids) to recalcified ($CaCl_2$) plasma
- Use:
  - monitor therapy with coumarin (max. **INR = 4.5**)
  - test of the liver biosynthesis
  - screening for disordered coagulation



Prothrombin Time

(PT)



Factors: VII, X, V, II, Fibrinogen

International Normalized Ratio

$$INR = \left(\frac{\text{Patient Pro Time}}{\text{Mean Normal Pro Time}} \right)^{ISI}$$

ISI = A function of the relationship between working and WHO thromboplastins

Ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the ISI value for the analytical system used.

ISI usually between 1.0 and 1.4

Prothrombin Time = PT (Quick's test):

Longer PT:

- deficiency of FV or vit. K dependent FII, VII, IX, X
- severe FBG deficiency
- hi FDP

- In some settings is not influenced by heparin (to 1 U/mL)
- Normal values: 11-14 s (INR 0.9-1.1)

Activated partial tromboplastin time = aPTT

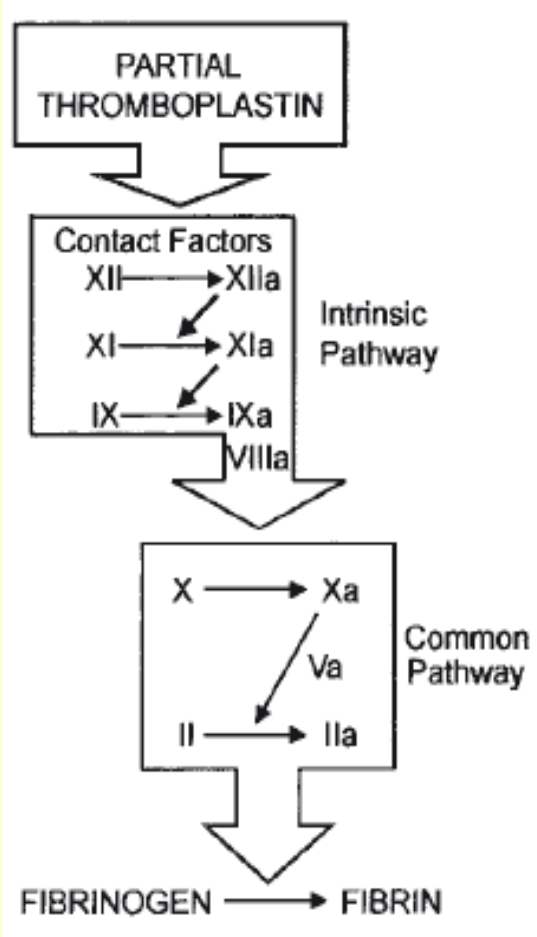
-The clotting time is measured after the addition of phospholipids into the recalcified plasma that has been preincubated with particulate material (e.g. micronized silica or kaolin) to initiate activation of contact system

-**Use:**

-monitoring of heparin therapy (1.5 - 2.5 fold prolongation)

-contact (intrinsic) factor deficiency XII, PK, HMWK, FXI (hemofilia C), FIX (B) , FVIII (A)

-Lupus anticoagulants



Activated Partial Thromboplastin Time (aPTT)

Factors: HMWK, PreKal. XII, XI, IX, VIII, X, V, II, Fibrinogen

Activated partial tromboplastin time = aPTT

Typically 28-36 s

Results are presented as a patients aPTT vs. normal aPTT

-**Prolonged test time:**

- Deficiency of FII, V, X;
- Deficiency of contact system (FXII, PK, HMWK); FXI,FIX, FVIII (hemophilia C, B, A)
- lupus anticoagulants
- severe deficiency of FBG; high levels of FDP
- disordered conversion of FBG

- **Shorter test time:** Prothrombotic situations

Evaluation of PCS function in acute medicine

Bedside examination

Lee-White test

- coagulation time of whole blood w/o anticoagulant
- - polystyrene or glass tube at 37oC.
- - Normal time 4-10 min. (depends on setting)
- - Function of PCS - fast orientation

Thrombin time of whole blood

- - coagulation time of whole blood collected to the tube with standard amount of thrombin
- - Presence of fibrinogen YES / NO
- - DIC decompensation- fast screening method

Activated coagulation time (ACT)

- blood drown w/o anticoagulant is transferred to the tube with contact activator (silica or kaolin) and mixed at 37oC until coagulum is present (normal about 150 s).
- Routine use for
  - the heparinization control of extracorporeal circulation or hemodialysis (required 180-300 s)
  - the heparinization control of extracorporeal circulation during heart surgery > 600 s

CASE REPORT: KOA 1

F, 55 years

At 26

- 10 days after 3rd delivery - DVT of lower right extremity and SVT of lower left extremity
- Therapy with heparin then OA with warfarin 6 month

At 41, 43

- 2 uncomplicated gyn. surgeries (ovarial cyst)

At 45

- Episodes of techycardia: ECHO showed mitral stenosis

At 46, 53, 54: SVT of lower extremities

Clinical diagnosis

Thrombophilia

What is the cause of thrombophilia?

Inherited causes

Acquired causes

Acquired thrombophilia

- Increased viscosity of the blood (dehydration, polycythemia)
- Increased central venous pressure and reduced leg veins flow – e.g. neoplasm, **pregnancy**, stenosis
- Oral contraceptive use
- Vitamin K deficiency
- Cancer
- Anti-phospholipid antibodies
- DIC (acute, chronic)
- Liver failure
- Massive injury
- Acute infectious disease
- Immobilization longer than 3 days
- etc

Inherited thrombophilia

Factor	General Population	People With Thrombosis
APCR: factor V Leiden mutation	3-8% of Caucasians	20-25%
Prothrombin G20210A	2-3% of Caucasians	4-8%
Antithrombin deficiency	1 in 2-5000	1-1.8%
Protein C deficiency	1 in 300	2.5-5.0%
Protein S deficiency	Unknown	2.8-5.0%
Hyperhomocysteinemia	11%	13.1-26.7%

Inherited anatomical vein variations

KOA 1/II

Test for APC resistance: APC ratio

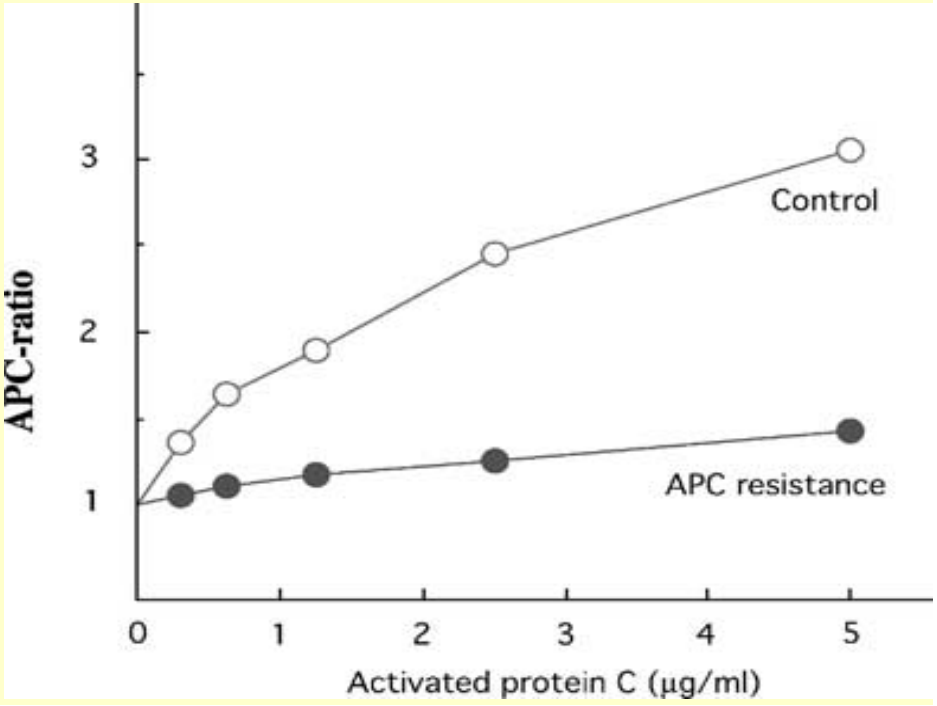
- aPTT test with and w/o addition of APC

APC ratio = (aPTT w APC / aPTT w/o APC)

Normal APC ratio: 2 - 5

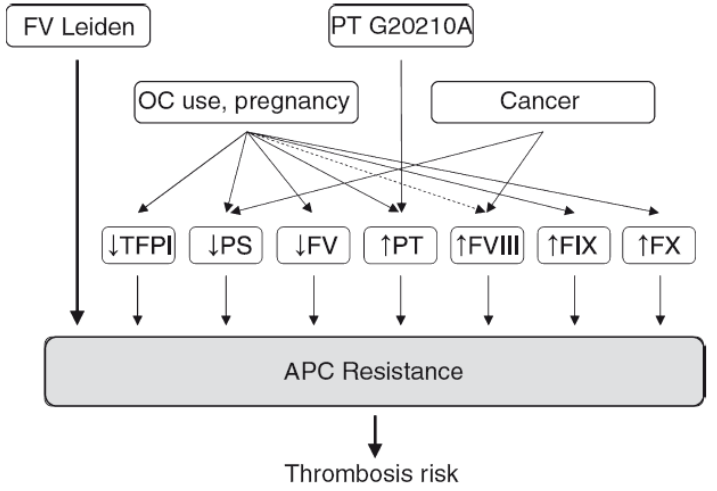
APC resistance < 2

Pacient APC ratio = 1.15



APC resistance

- **Factor V (Leiden) mutation**
- Oral contraceptive use
- Pregnancy
- Elevated prothrombin levels due to the G20210A mutation
- Cancer

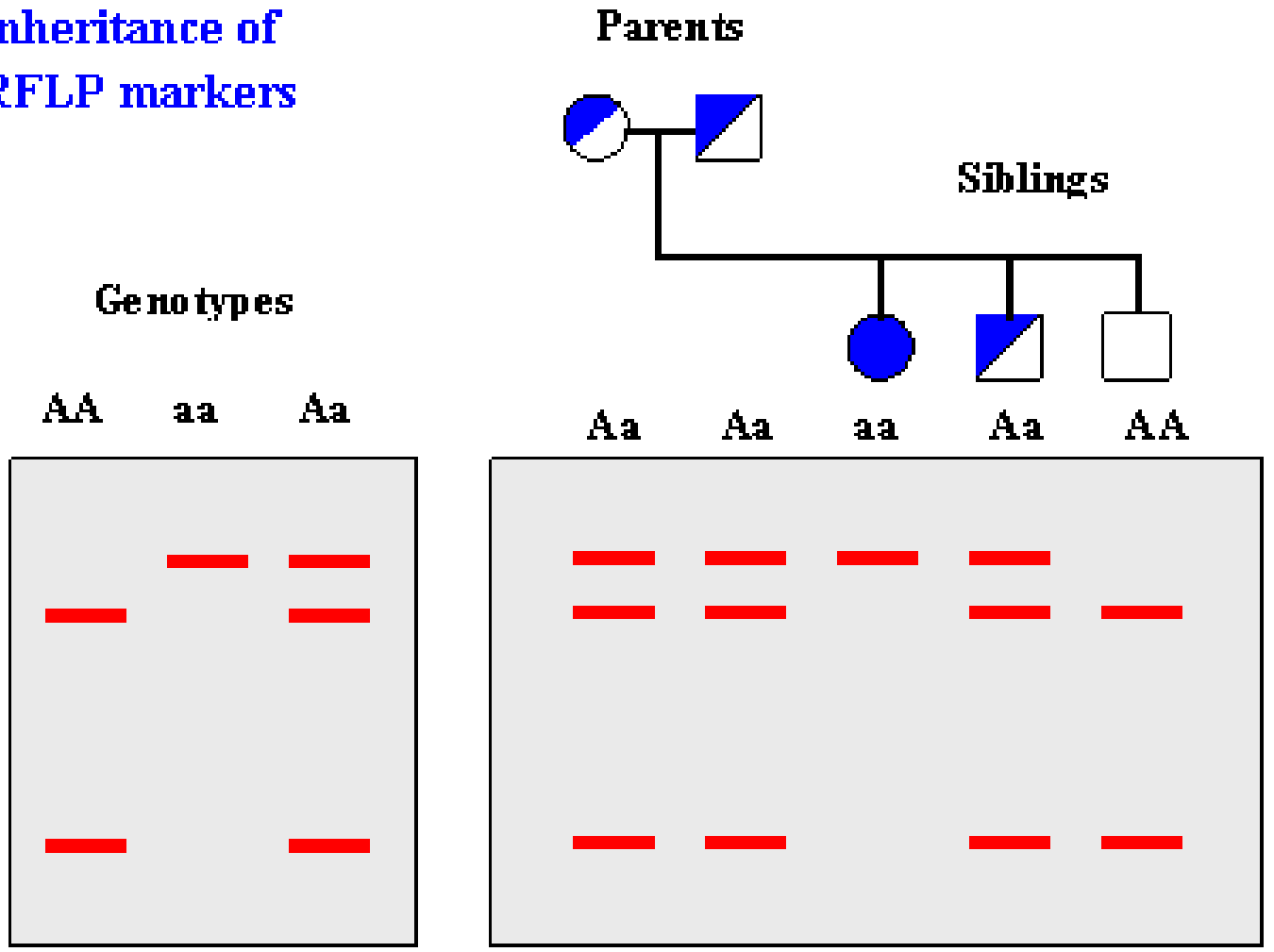


KOA 1/II

Mutation analysis of FV:

- RFLP PCR (Restriction fragment length polymorphism)
  - PCR amplification of FV DNA fragment (which include 1691) (1691G→A substitution) and subsequent cleavage of PCR product by restriction enzyme MnII
  - MUTATION CHANGES THE CLEAVAGE SITE = ENZYME DOES NOT CUT THE FRAGMENT

Inheritance of RFLP markers



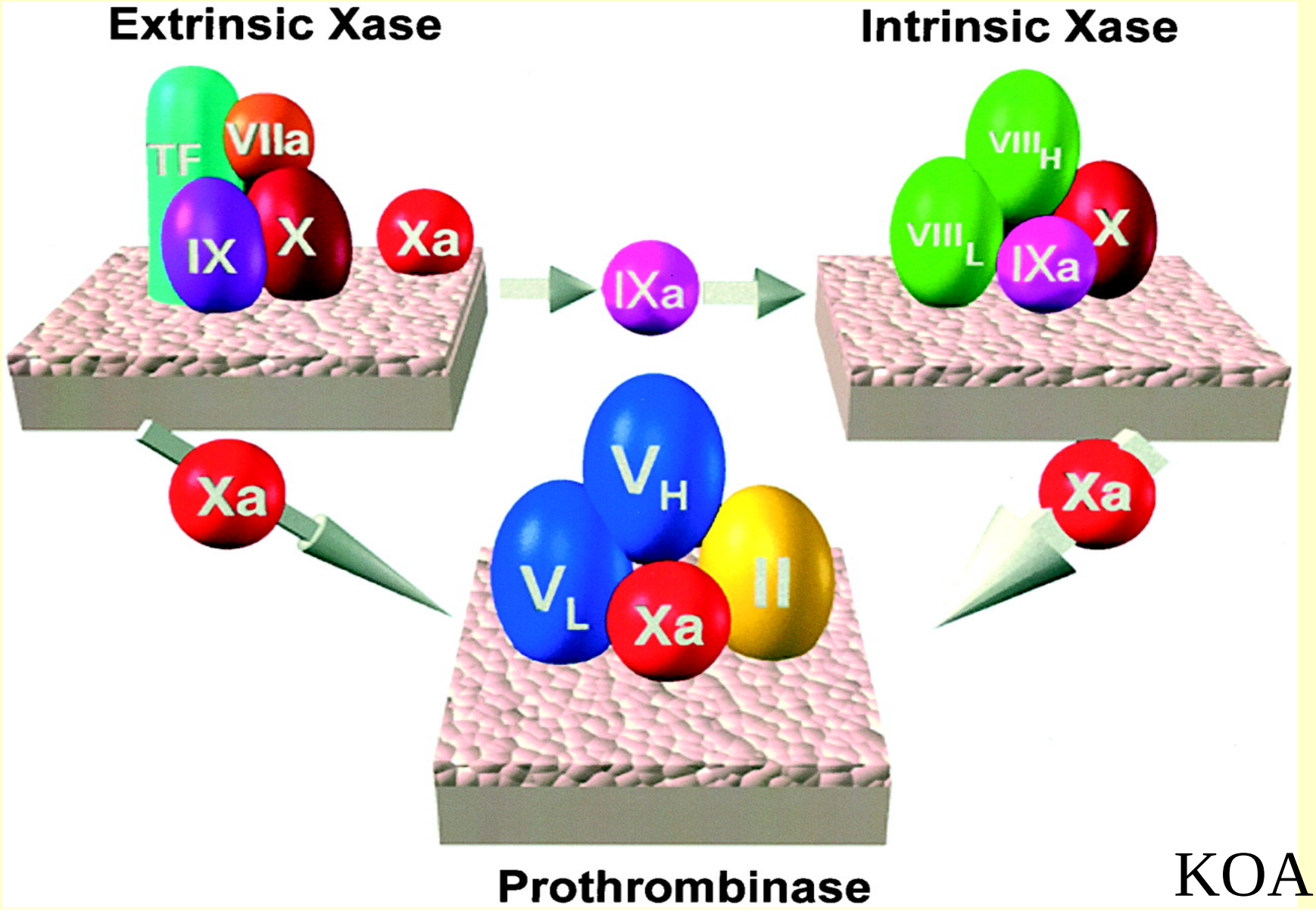
KOA 1/II

Mutation analysis of FV:

- **ARMS PCR** (amplification refractory mutation system)
  - PCR using mutation specific primers

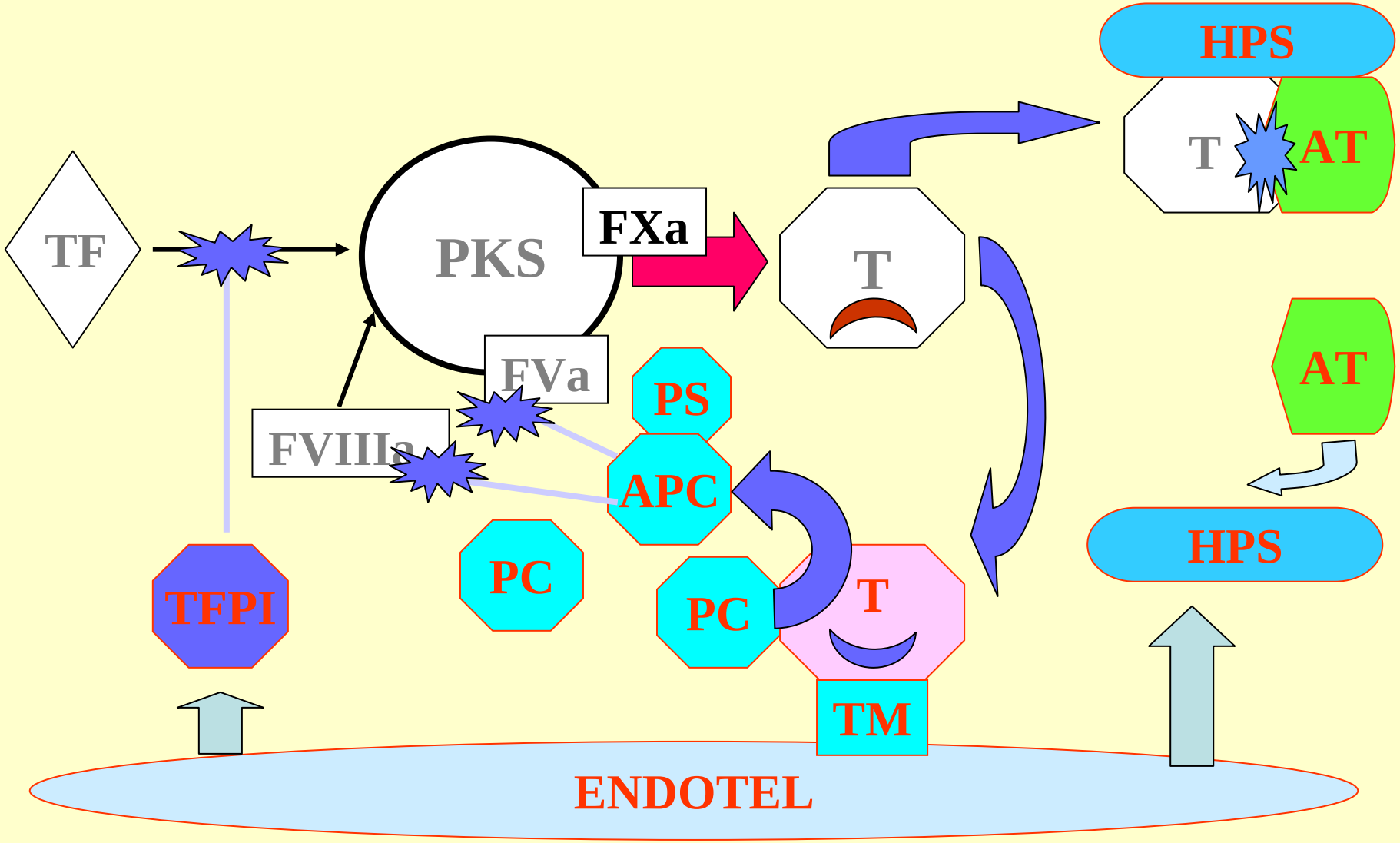
LAB RESULT homozygose mutation of FV (Leiden mutation) (Arg506Gln)

What is the role od (APC) v PS?


Extrinsic Xase
Intrinsic Xase
TF
VIIa
IX
X
Xa
IXa
VIII H
VIII L
IXa
X
Xa
Xa
V H
V L
Xa
II
Prothrombinase

KOA 1

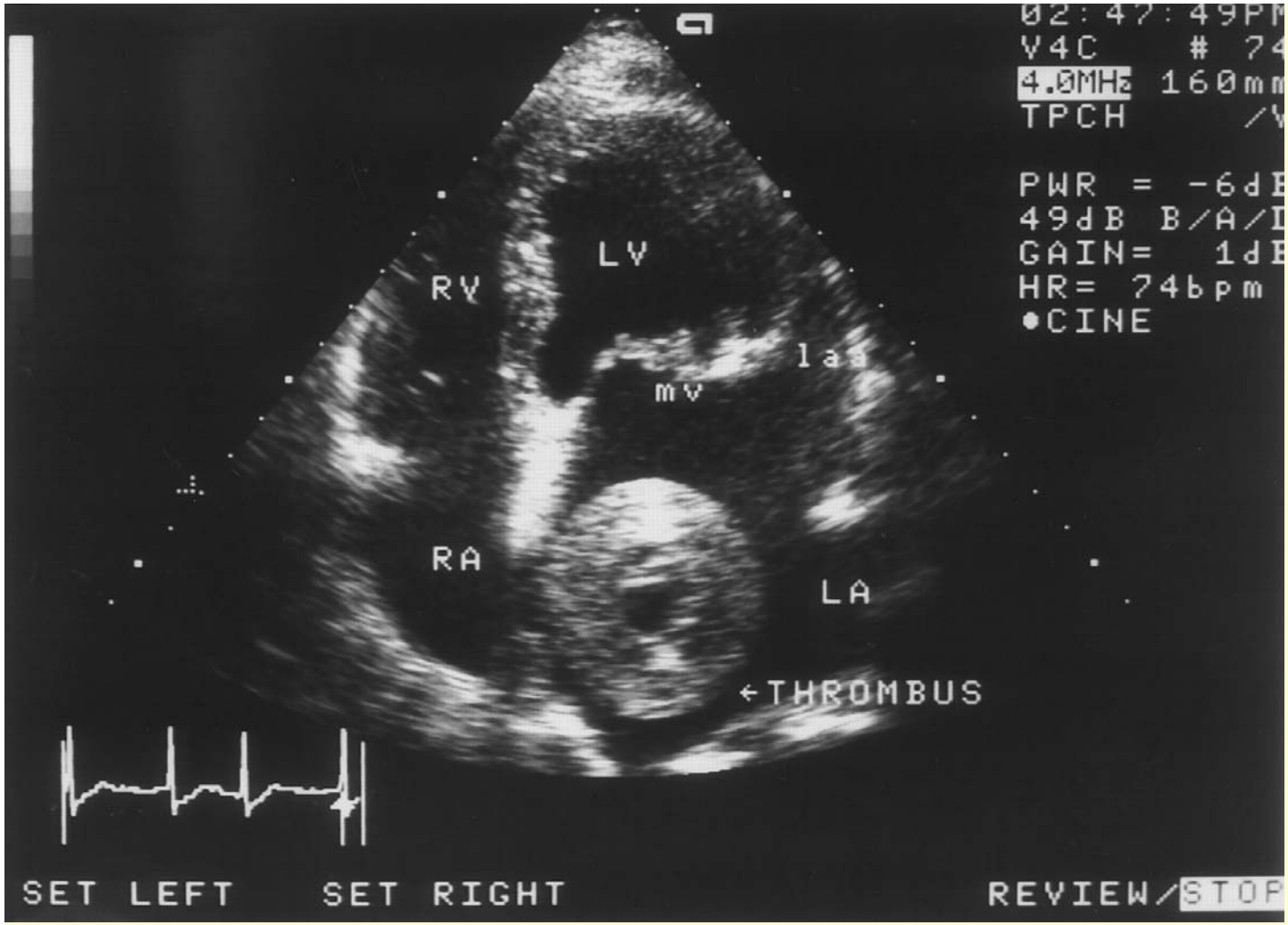
KOA 1/III Regulation of Thrombin Activity



KOA 1/IV

During hospitalization acute episodes of atrial fibrilation: ECHO revealed thrombus in the LA plus mitral stenosis

Cardiosurgery: Thrombectomy and mitral valve replacement



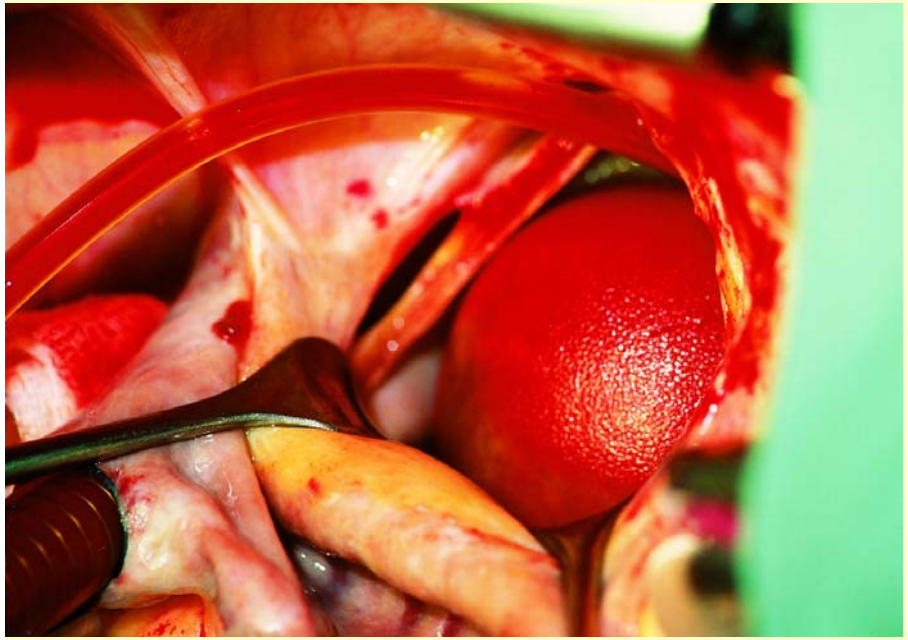
How it may look like

Wright-Smith G R et al. Circulation 1998;98:931-932

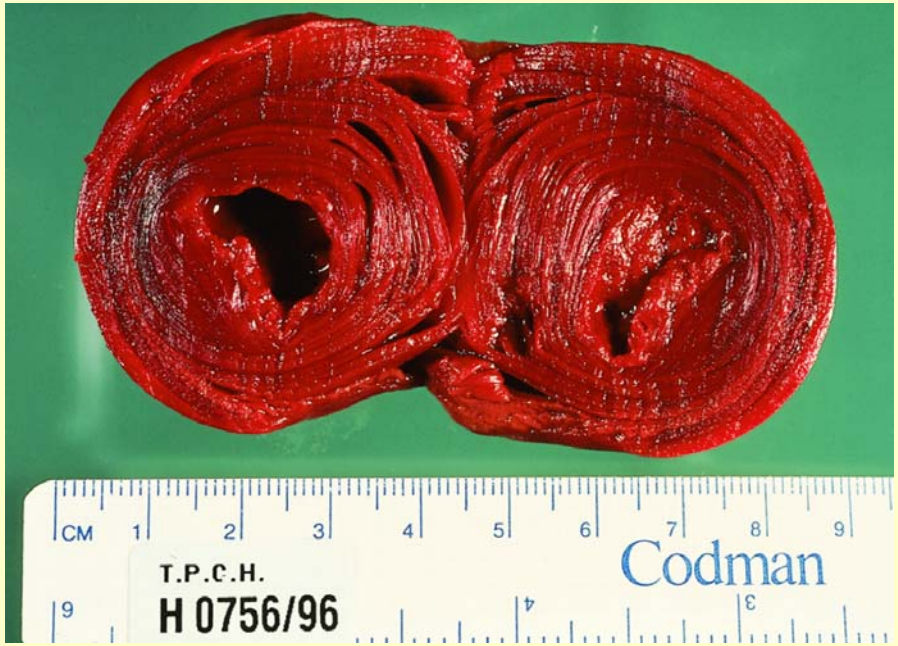
Transthoracic echocardiogram showing a large, ball-shaped mass, with an echolucent center, that was freely mobile within the left atrial cavity.

American Heart Association
Learn and Live

How it may look like



At surgery, the mass was not adherent to the atrial wall and was easily removed from the left atrium.



Cut section of the mass revealed laminated thrombus, giving an onionskin appearance with central cavitation.

American Heart Association
Learn and Live

Wright-Smith G R et al. Circulation 1998;98:931-932

Subsequent therapy

Long term therapy with anticoagulants with INR = 3.5 – 4.5

Vit.Kdef./OA

PLT	BT	aPTT	PT	TT	FBG
N	N	P	P	N	N

KOA 1

Factor V^{LEIDEN}

- Risk of thrombosis
  - heterozygous mutation (Factoru V^{LEIDEN}) 7-fold increased risk compared to wt
  - homozygous mutation (Factoru V^{LEIDEN}) 80-fold increased risk compared to wt

Approximately 1 person in 20 develops a DVT in the course of his or her lifetime (~20-25% of them would have Leiden mutation)

To Remember

- Hemostasis is part of inflammatory response
- Bleeding time
- aPTT
- PT (Quick)
  - INR
- Thrombin time (DIC fast screen)
- D-dimer
- APC resistance

Thank you

Principle of the Mixing Study