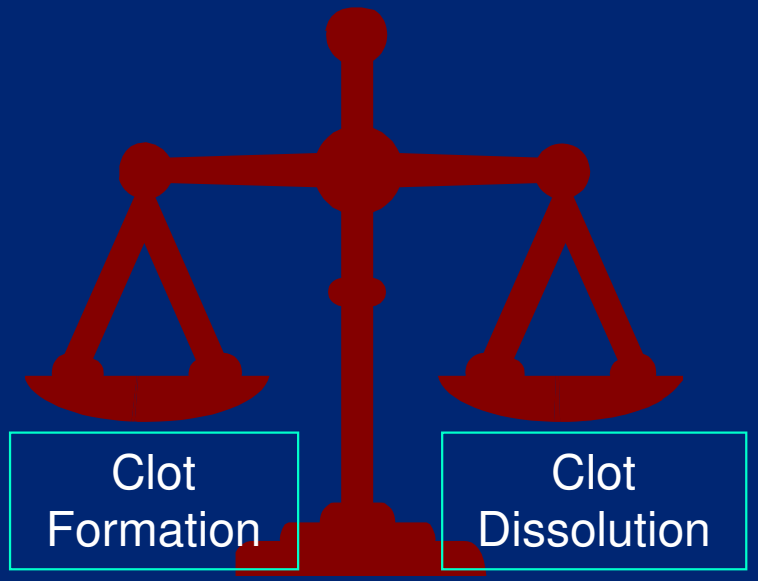
Introduction to Antithrombotic Monitoring

Topics

- What is thrombosis, and why is it significant?
- Coagulation Cascade
  - Pathways of coagulation, anticoagulation, and fibrinolysis
- Thrombophilia screening
  - Hereditary & Acquired Risk Factors
  - Laboratory Evaluation of Thrombotic Risk
- Monitoring anticoagulation and anti platelet therapy
  - Heparin Monitoring (UFH & LMWH)
  - Oral Anticoagulant Monitoring (Warfarin, Dabigatran,Rivaroxaban)
  - Atiplatelet drugs monitoring

Hemostasis

- **Hemostasis:** The balance between clotting and bleeding



- Components of Hemostasis:
  - Vasculature
  - Coagulation proteins
  - Platelets

Venous Thromboembolism – Incidence & Significance

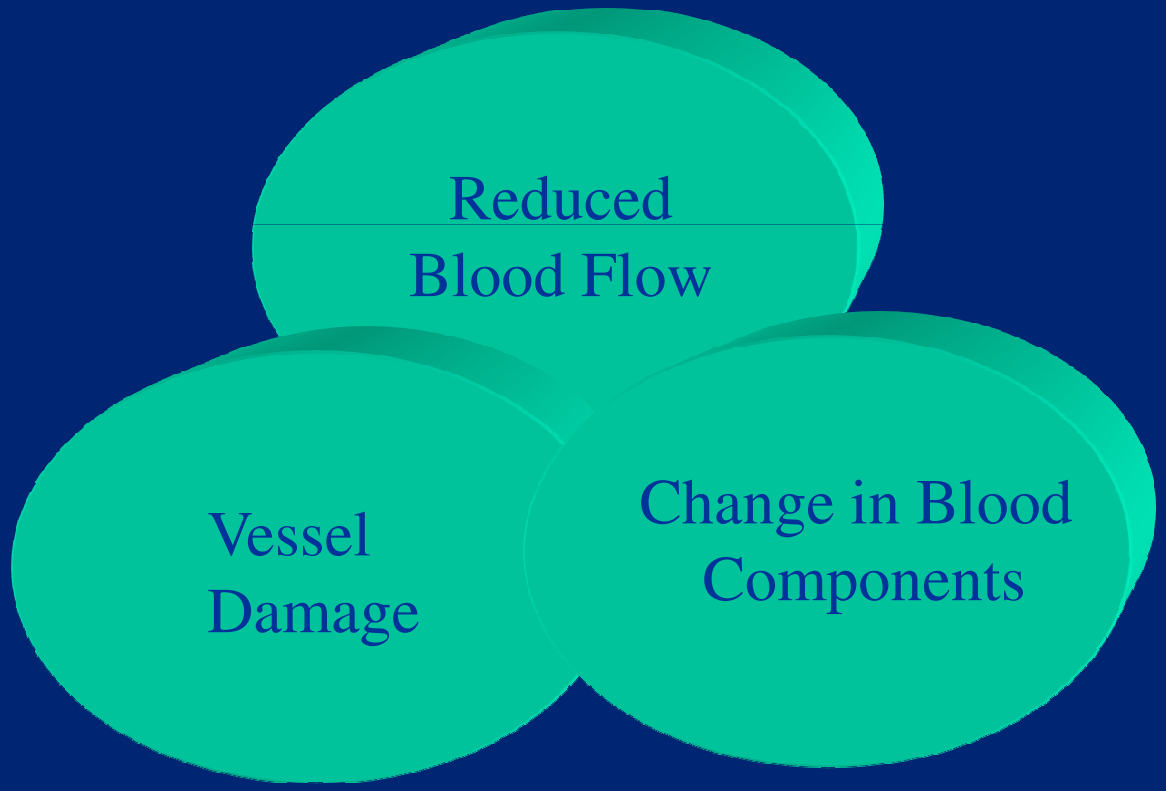
- Complications from DVT kill up to 500,000 people per year in the E.U.
  - More than AIDS and breast cancer combined!
- Third most common vascular disease
- Pulmonary Embolism (PE) leading preventable cause of death
- Incidence of DVT about 135 per 100,000 annually
- Incidence of PE about 69 per 100,000 annually

Complications of DVT

- Permanent vascular damage
- Post-phlebitic syndrome
- Pulmonary embolism (PE)
- Pulmonary hypertension

VENOUS THROMBOEMBOLISM

Virchow's triad for venous thromboembolism:



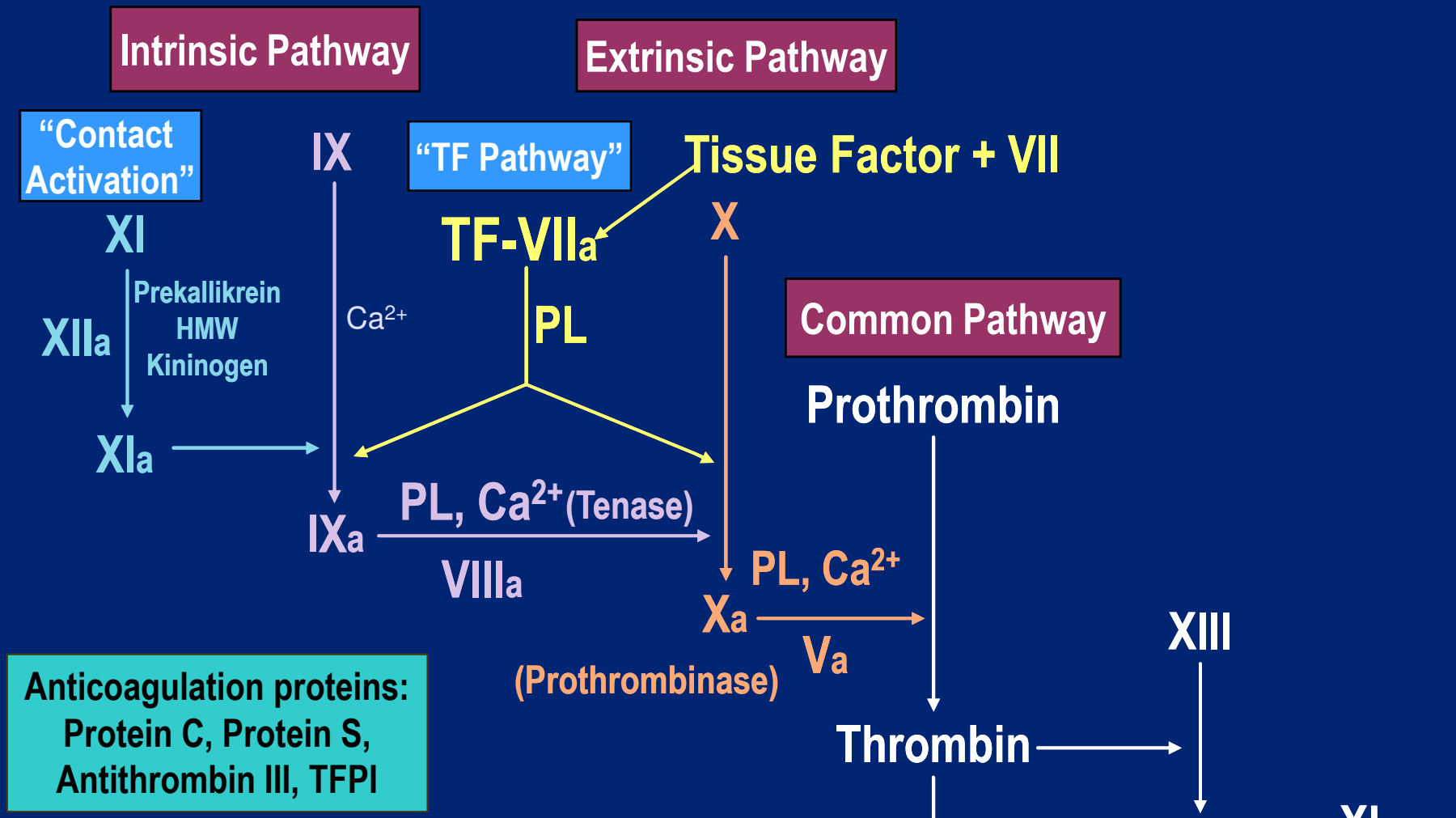
Venous Thromboembolism

- Complex, multi-causal disease
  - Physiological factors
    - Age, hormonal influence (i.e. pregnancy)
  - Acquired risk factors
    - Cancer, surgery, O.C., obesity, trauma, immobility, antiphospholipid syndrome (SLE)
  - Hereditary (genetic) risk factors
    - Deficiencies in anticoagulation proteins (AT,PC,PS)
    - Elevated coagulation proteins (FVIII)
    - Gene mutations – FII, F V Leiden

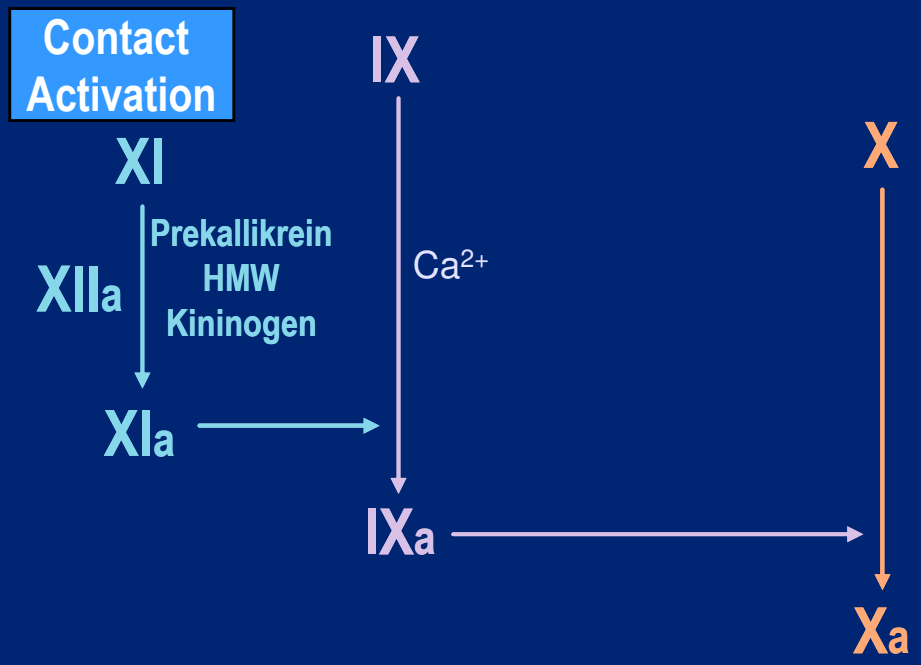
Coagulation Cascade

- Vascular damage initiates the coagulation cascade.
- Results in the generation of thrombin at the site of injury.
- Thrombin catalyzes the conversion of fibrinogen to an insoluble fibrin (clot) matrix.

Coagulation Cascade



Intrinsic Pathway



Intrinsic Pathway

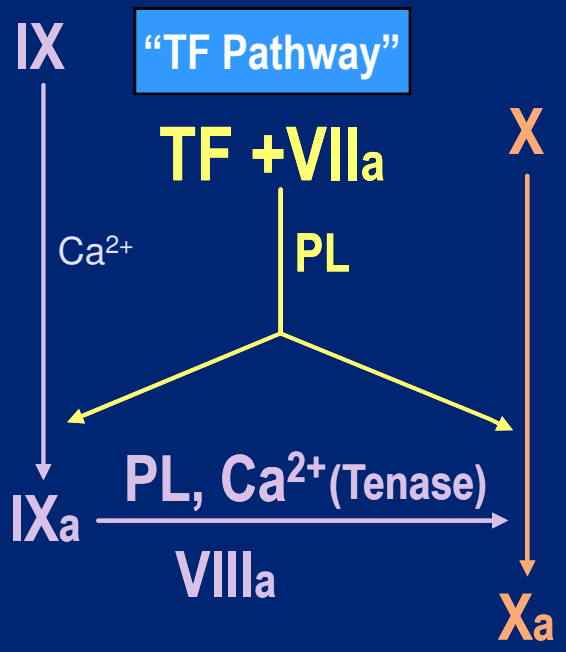
"Contact Activation": Initiated by the activation of FXII involving contact factors on negatively-charged phospholipid surfaces (glass or kaolin in vitro)

- Factors XII, XI, IX, VIII, prekallikrein, HMW kininogen
- Measured with aPTT clotting assay

Intrinsic Pathway - APTT

- The Activated Partial Thromboplastin Time (APTT): The clotting time in seconds of a mixture of citrated plasma, Ca^{2+}, contact activator, and phospholipid – norm 35 sec
- Tests for deficiencies of pro-coagulant factors in the INTRINSIC and COMMON pathways –FXII,XI,IX,VIII
- Heparin, dabigatran (Pradaxa), Factor Inhibitors, Lupus Anticoagulant can prolong the APTT

Extrinsic Pathway



Extrinsic Pathway
"Tissue Factor Pathway"

Initiated when blood is exposed to TF released from damaged endothelium

- Measured with PT clotting assay (INR 0,8-1,2)

Extrinsic Pathway - PT

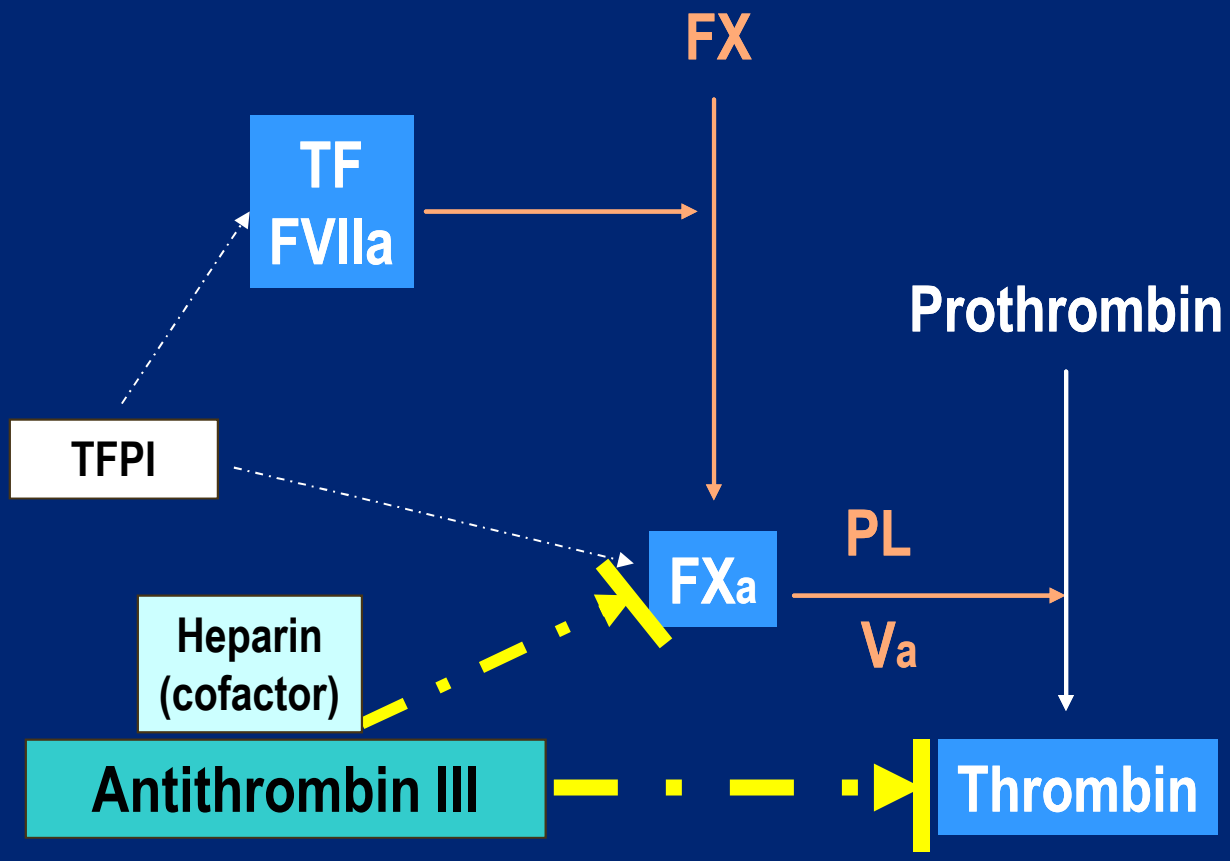
- Prothrombin Time (PT): clotting time in seconds or in INR .

- Tests for deficiencies of pro-coagulant factors of the EXTRINSIC and COMMON pathways- FVII,FX,FII, warfarin (INR!), rivaroxaban(Xarelto), apixaban(Eliquis)

Anticoagulation Pathways - Antithrombin

- Antithrombin is the major inhibitor of thrombin, accounting for approximately 80% of thrombin inhibitory activity in plasma
- Antithrombin primarily inhibits Thrombin and FXa

Anticoagulation Pathways - Antithrombin



Activated Protein C (APC) + cofactors

APC has two known cofactors: Protein S and Factor V.

Protein S:

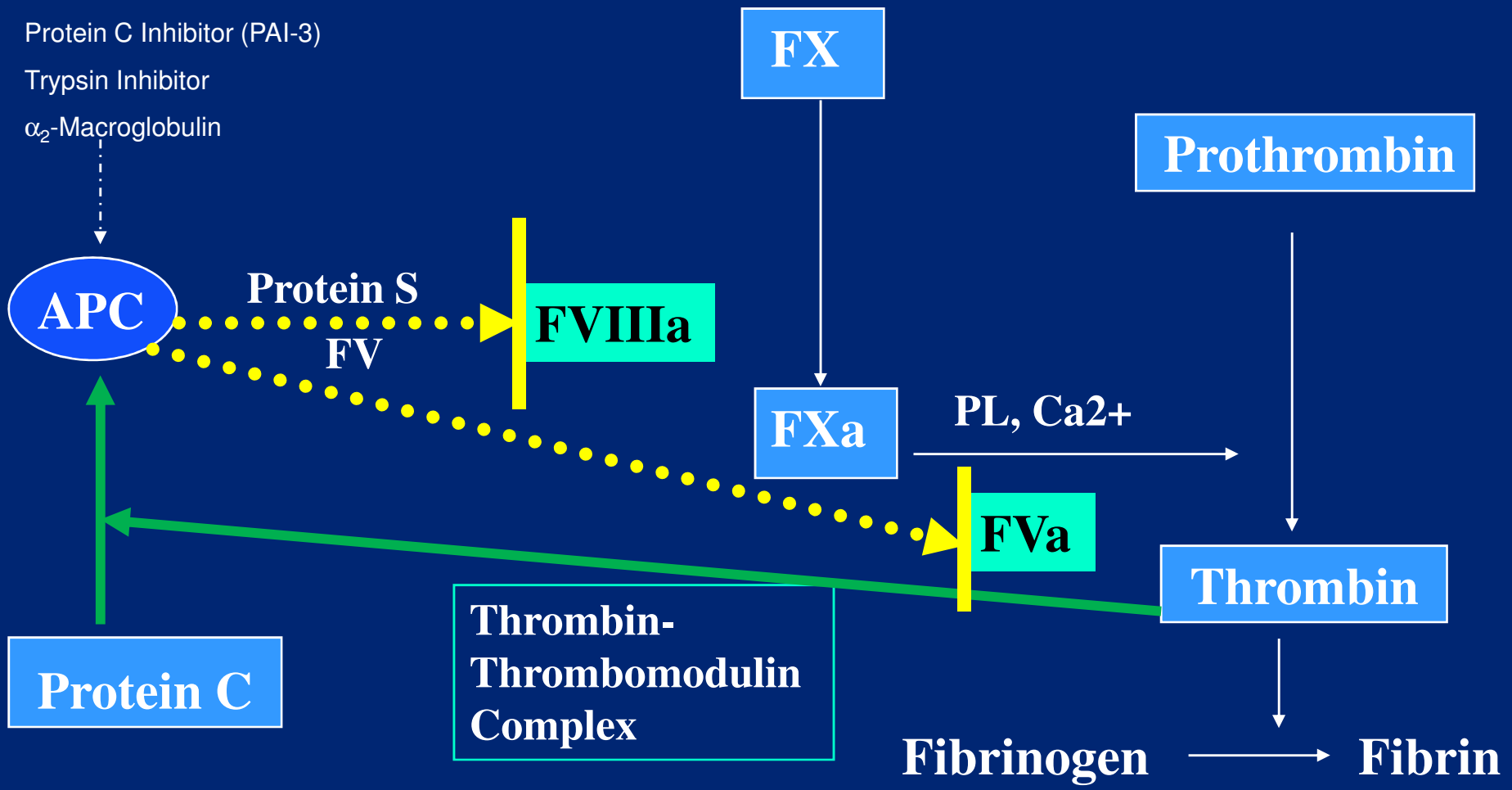
Protein S enhances binding of APC to the phospholipid of platelets and endothelial cells.

Only free protein S has a APC cofactor function. 60% of protein S is bound to C4bBP.

Factor V

Factor V together with Protein S makes APC degrade FVIIIa and FVa more effectively.

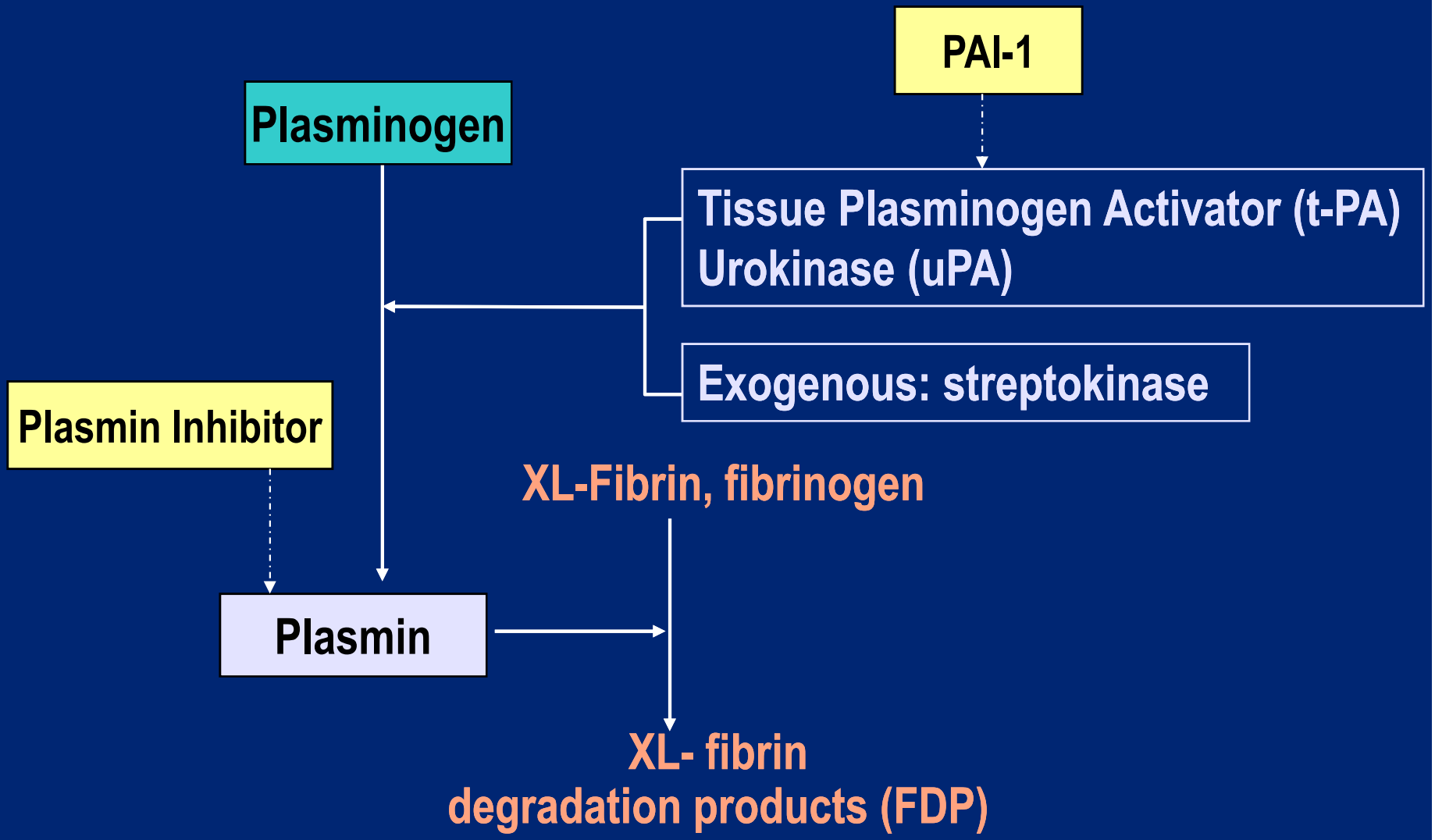
Anticoagulation Pathways – Protein C



Fibrinolytic Pathway

Fibrinolysis is initiated when fibrin is formed and eventually dissolves the clot.

Fibrinolytic Pathway



Laboratory Tests to Diagnose thrombophilia and VTE

- Due to the high prevalence of DVT and PE, and in order to prevent the morbidity and mortality associated with such diseases, a reliable and rapid diagnosis of thrombophilia state is required.

Laboratory Tests to Diagnose VTE

- The current standard methods for the diagnosis of

  DVT : doppler ultrasound

  PE : pulmonary angiography

- Both DVT and PE : D-dimer

D-Dimer

- D-Dimer, a measure of fibrin degradation products.
- Utility is in its Negative Predictive Value =

  none DVT or PE
- Elevated levels of D-Dimer:
  - Found in pathological conditions such as deep vein thrombosis (DVT) ++++, more than 500 ng/l
  - pulmonary embolism (PE) +++++, more than 1000 ng/l

Hereditary & Acquired Risk Factors

- There are several well-established risk factors and corresponding assays to test for them
  - Most of these risk factors can be hereditary or acquired

Hereditary & Acquired Risk Factors

- **Inherited Risk Factors**
- APC resistance-Factor V Leiden
- AT deficiency
- Protein C deficiency
- Protein S deficiency
- Prothrombin Mutation
- Dysfibrinogenemia (rare)

Inherited or Acquired Risk Factors:

- Hyperhomocystenemia
- Elevated levels of FVIII, IX,XI

- **Acquired Risk Factors**
- Age
- Malignancy
- Immobilization
- Trauma, Post-op
- Pregnancy
- Estrogen use
- Antiphospholipid Antiboides
- Long distance flights
- Hematologic Diseases

Laboratory Evaluation of Thrombotic Risk

Risk Factor	Laboratory Assay
Antithrombin Deficiency	AT activity
Protein C Deficiency	Protein C Deficiency PC activity (clotting or chromogenic)
Protein S Deficiency	Protein S Free Antigen (ELISA, LIA)
APC Resistance / Factor V Leiden Mutation	APC Resistance (aPTT); FV Leiden genetic test if abnormal
Prothrombin Mutation G20210A	Genetic Test
Hypherhomocysteinemia (elevated homocysteine)	EIA, Mass Spec, HPLC
Elevated Factor VIII Activity	Factor VIII activity (clotting or chromogenic)
Lupus Anticoagulant	Russel viper diluted Clotting Assay
Anticardiolipin Antibody, IgG / IgM	aCL IgG/IgM Antigen ELISA (cardiolipins)

Antithrombin, Protein C, Protein S Deficiencies

- Loss-of-Function Abnormalities
- Deficiencies of AT, PC, and PS are most commonly seen in the heterozygous state
- Levels are about 30 – 60% of normal
- Risk potential of each of these deficiencies is 10 – 25 fold
- Individuals with AT, PC, or PS deficiencies typically have a thrombotic event at a fairly young age

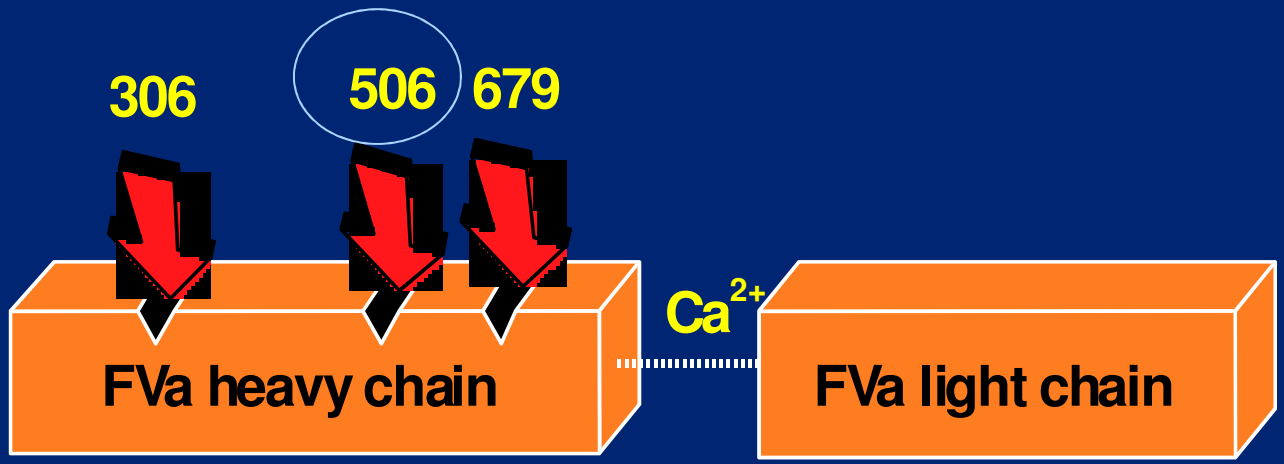
APC Resistance

- Common in the general population (8%)
- Most common cause of hereditary thrombophilia - F V Leiden

- APC Resistance alone is not a significant risk factor(HR 2-3x). Having FVL combined with other risk factors, however, greatly increases risk of thrombosis- i.g. FVL heterozyg. + O.C. = HR 30 x

INACTIVATION OF NORMAL FVa

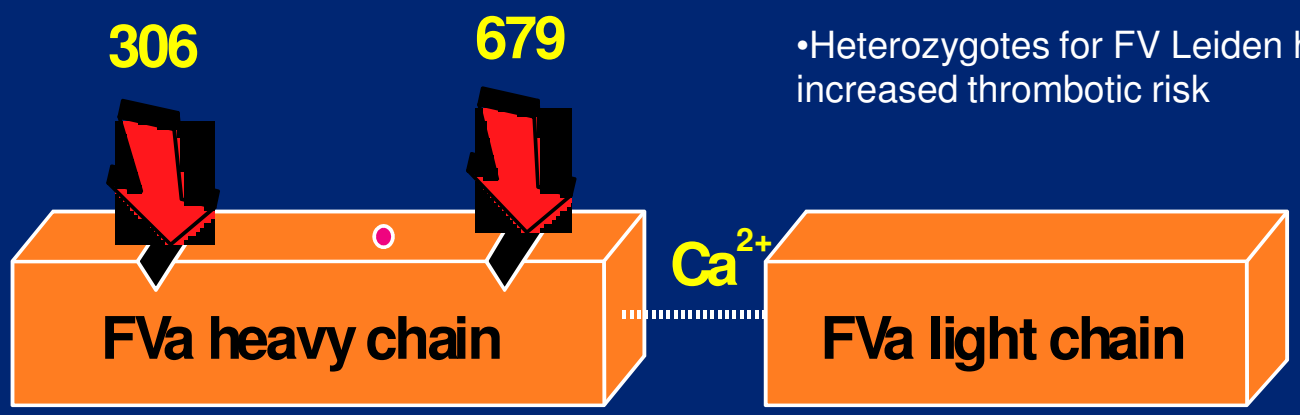
Activated PC



INACTIVATION OF MUTANT FVa:Q^{506}

FV Leiden Mutation

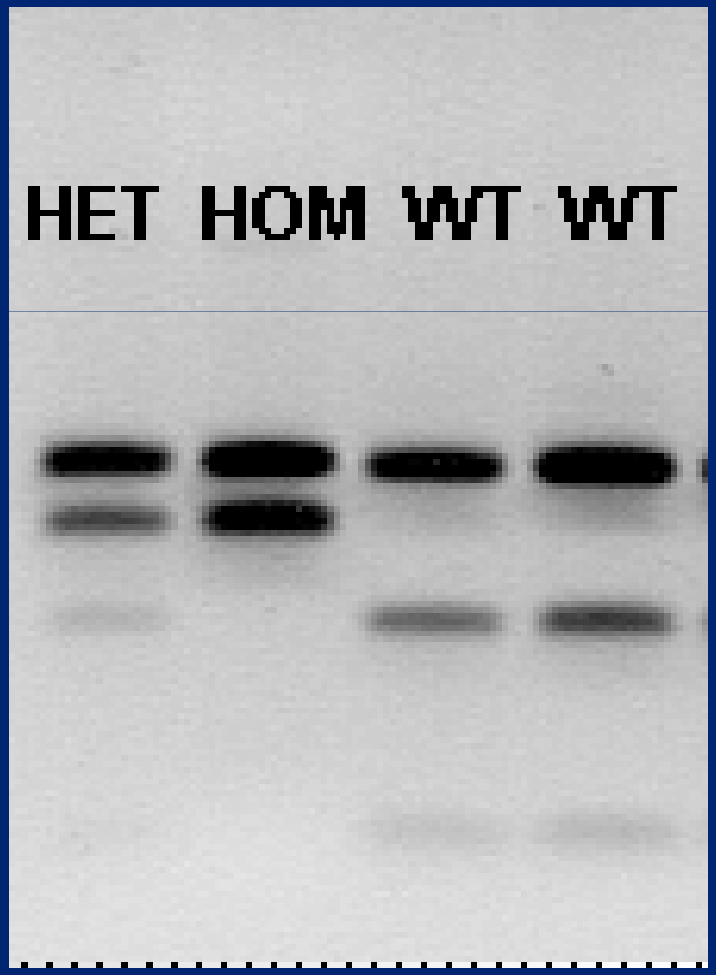
- Accounts for approx. 90% of APC Resistance
- Prevalent in about 2 – 13% of general population
- Accounts for about 20 – 60% of VTE cases
- Heterozygotes for FV Leiden have 2 – 5 fold increased thrombotic risk



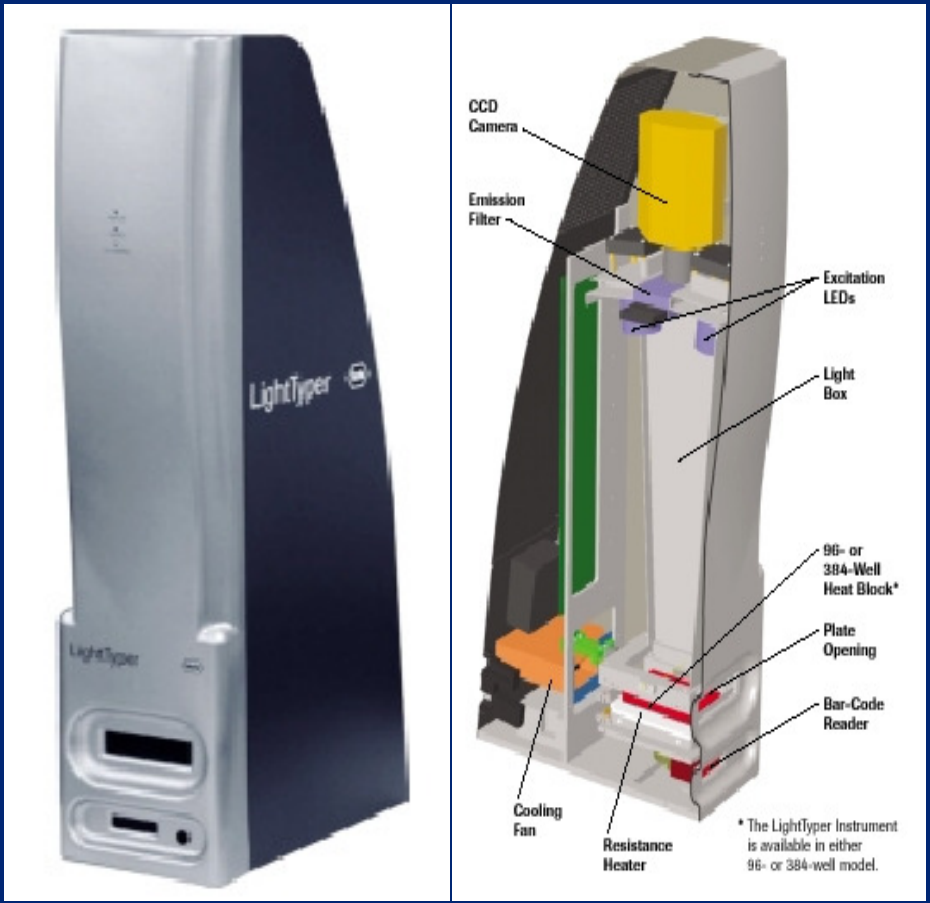
- **Mutant**

Mutation results in a 10-fold lower inactivation rate of FVa

Detection by PCR : FV Leiden (Arg506Gln)



Now : real time – PCR
Light typer (Roche)



- reaction volumes of 10 -100 µl.
- Software automates genotype assignments.
- Data captured directly from its CCD camera.
- Melting curve analysis enables the rapid, straight-forward, and reliable genotyping of wild-type sequence, heterozygotes, and homozygous SNPs.
- Duration of PCR 40 minutes

APC RESISTANCE test : INTEPRETATION OF RESULTS

- APC- ratio = $\frac{\text{Clot time APC/CaCl}_2}{\text{Clot time CaCl}_2}$

- **APC Resistance is when the APC ratio is below or equal to 2,0.**

Prothrombin G20210A mutation

- Prevalence in normal population approximately 3%
- G $\rightarrow$ A translation at nucleotide 20210 in prothrombin gene
  - Leads to an increase in Factor II (prothrombin) levels more than 130%
- Increased risk of venous thrombosis
- Detection by PCR

Hyperhomocysteinemia

- Homocysteine : an amino acid metabolite – toxic for endothel
- Conversion of homocysteine to either metabolite dependent on a number of enzymes (MTHFR) with cofactors: folic acid, vitamins B12 and B6
  - Elevated homocysteine (> 15 µmol/l) is a risk factor for AIM, stroke and recurrent VTE

Elevated Factor VIII

- **FVIII activity > 1.5 IU/mL results in 5-6-fold higher risk for DVT, especially recurrent DVT, than FVIII activity ≤ 1.0 IU/mL**
- **Confirmation of risk not associated with acute phase response**
- **Elevated FVIII persistent over time**
- **Familial trait observed – no explanation so far**

Lupus Anticoagulant / Anti-Phospholipid Syndrome (APS)

- Auto-antibodies against phospholipids or phospholipid-binding proteins
- Also known as Lupus Anticoagulant
- Major risk for recurrent miscarriage
- Major risk factor for venous thrombosis
  - Presence of antiphospholipid antibodies increases risk 9-fold
  - Thrombotic event in about 30% of patients with aPS

Anti-Phospholipid Syndrome-Laboratory Assays

Diagnosis of a definite syndrome: meet at least one clinical and one laboratory criteria.

- Clinical criteria:
  - Occurrence of thrombotic event – venous or arterial
  - Recurrent miscarriage, fetal death, or premature birth

- Laboratory criteria:
  - Lupus anticoagulant
    - Prolonged APTT, DRVVT assays
  - Anti-cardiolipin Antibodies IgG or IgM
    - Perform testing on two or more occasions, 6 weeks apart
    - Can also perform panel including anti-β_2-GPI & anti-Prothrombin

Treatment of DVT

- Once DVT is diagnosed, Unfractionated Heparin or Low Molecular Weight Heparin is administered, followed by an oral anticoagulant drug such as warfarin
- Or – Rivaroxaban (Xarelto) – direct inhibitor of FXa

Heparin Monitoring – Unfractionated Heparin (UFH)

- UFH is typically monitored with an APTT test
- Ideally, the therapeutic range of APTTs for each reagent (~1.5 – 2.5) should correspond to anti-Xa activity of 0.3 – 0.7 U/ml

LMWH Monitoring

- Low Molecular Weight Heparin (LMWH) has been shown as effective in preventing recurrent VTE and cause less bleeding
- Generally LMWH does not need monitoring
- When monitoring is required, a chromogenic anti-Xa assay is required :
- Therapy : 0,6 -1,2 IU/ml anti FXa
- Prophylaxis: 0,2-0,4 IU/ml anti F Xa

Long Term Therapy

- Warfarin is the most commonly used oral anticoagulant (OAC)
- Warfarin is a vitamin K antagonist - Impairs the generation of active vitamin K, decreasing the amounts of vitamin K dependent coagulation factors (FII, FVII, FIX, FX)
- Treatment time :
- DVT and PE : 3 months, 6 months, 12 months, lifetime
- Atrial fibrillation : lifetime ?

Monitoring Warfarin Therapy is a Balancing Act!

Warfarin Monitoring

Why monitor? Need to balance proper anticoagulation without bleeding risk.

Monitored with PT, expressed as INR:

$$INR = [Patient\ PT / Mean\ Normal\ PT]^{ISI}$$

- Where ISI = international sensitivity index, assigned by each thromboplastin manufacturer

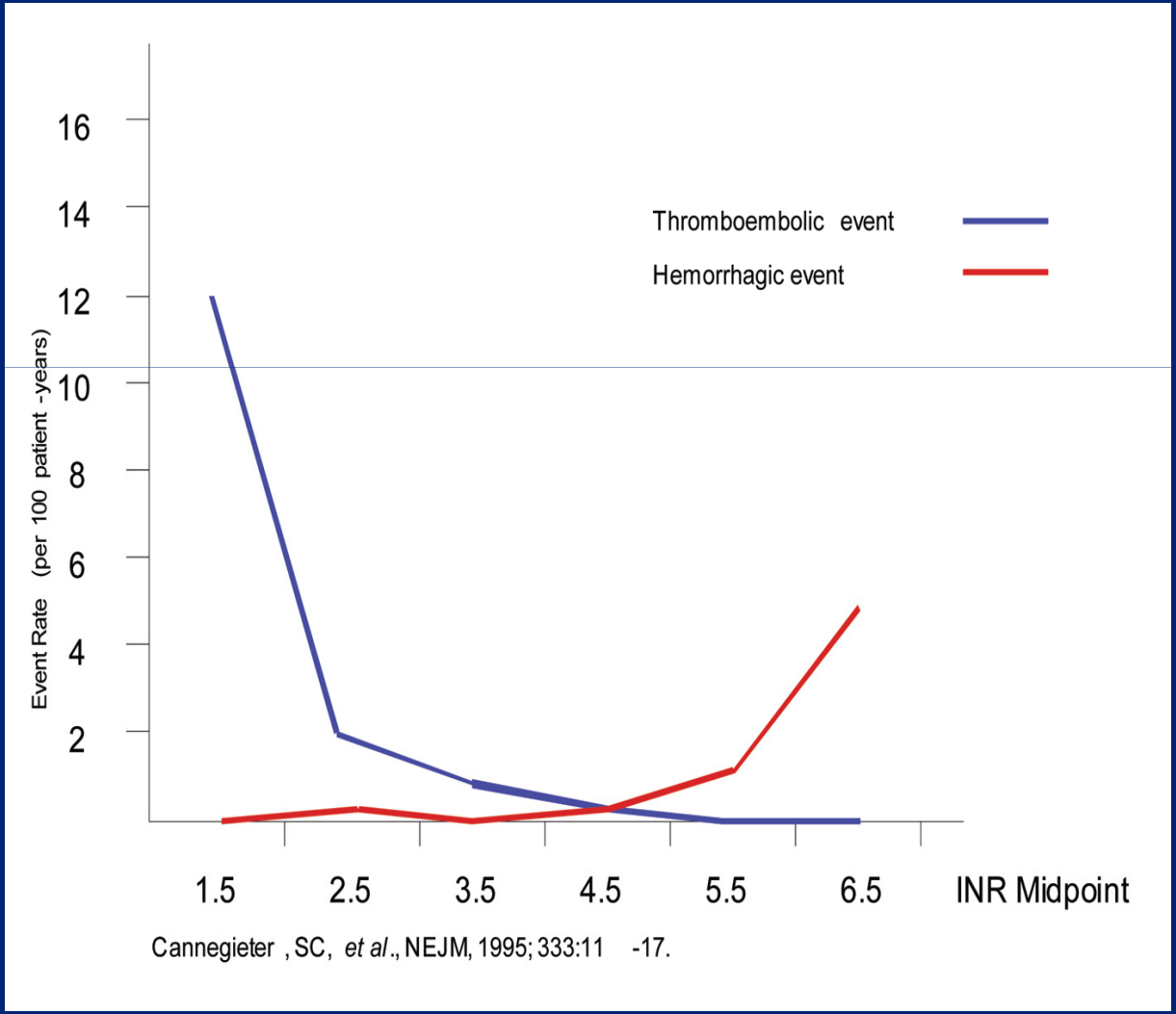
Warfarin is given orally and titrated to achieve an INR of typically 2.0 – 3.0

INR

$$INR = \left(\frac{PT_{test}}{PT_{normal}}\right)^{ISI}$$

- ISI: International Sensitivity Index
- Compares local reagent with international reference preparation

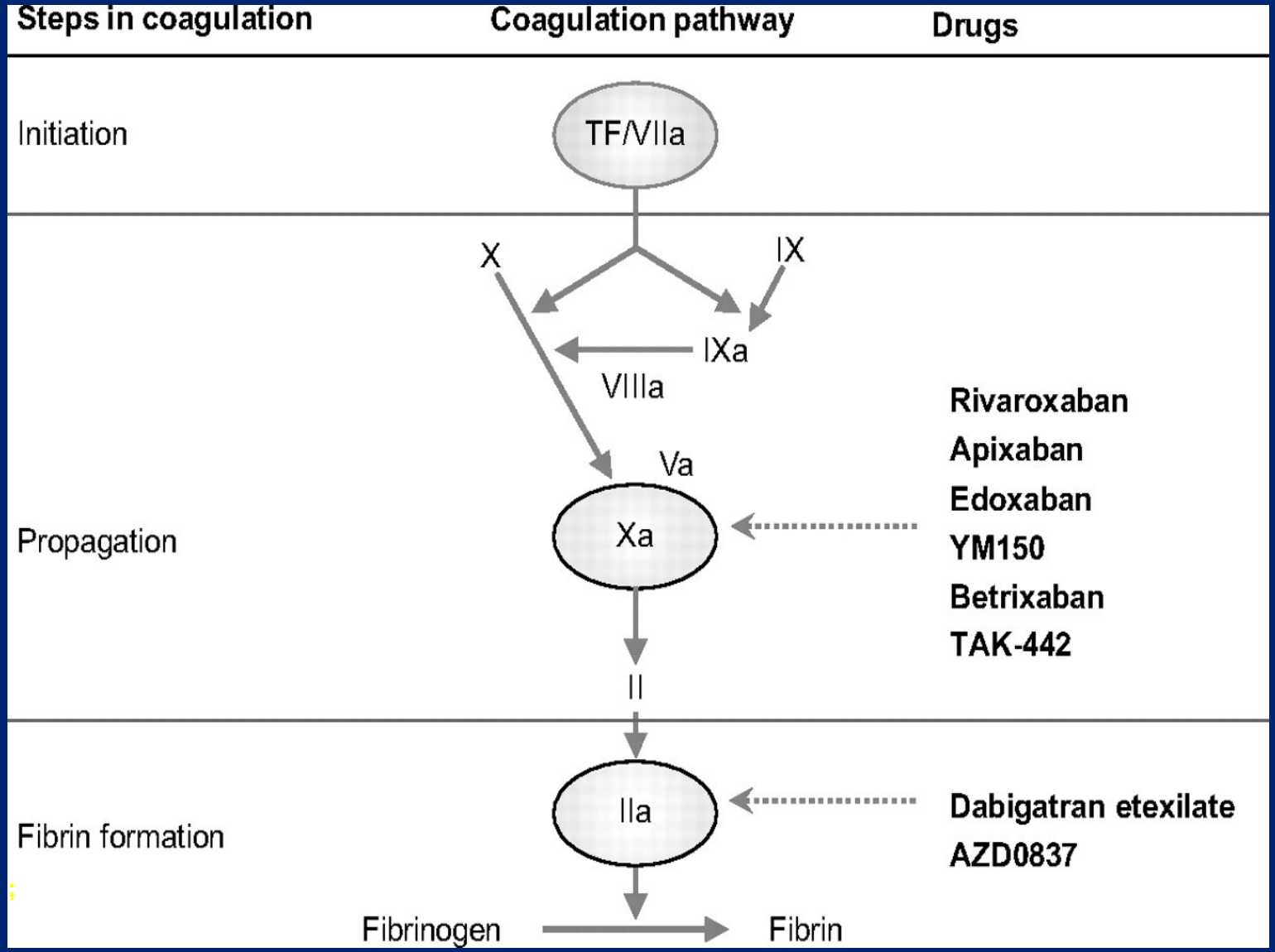
Warfarin and bleeding



New oral anticoagulants

N.O.A.C.

Illustration showing the sites of action of new anticoagulants in the coagulation cascade.



Use of NOAC (2012)

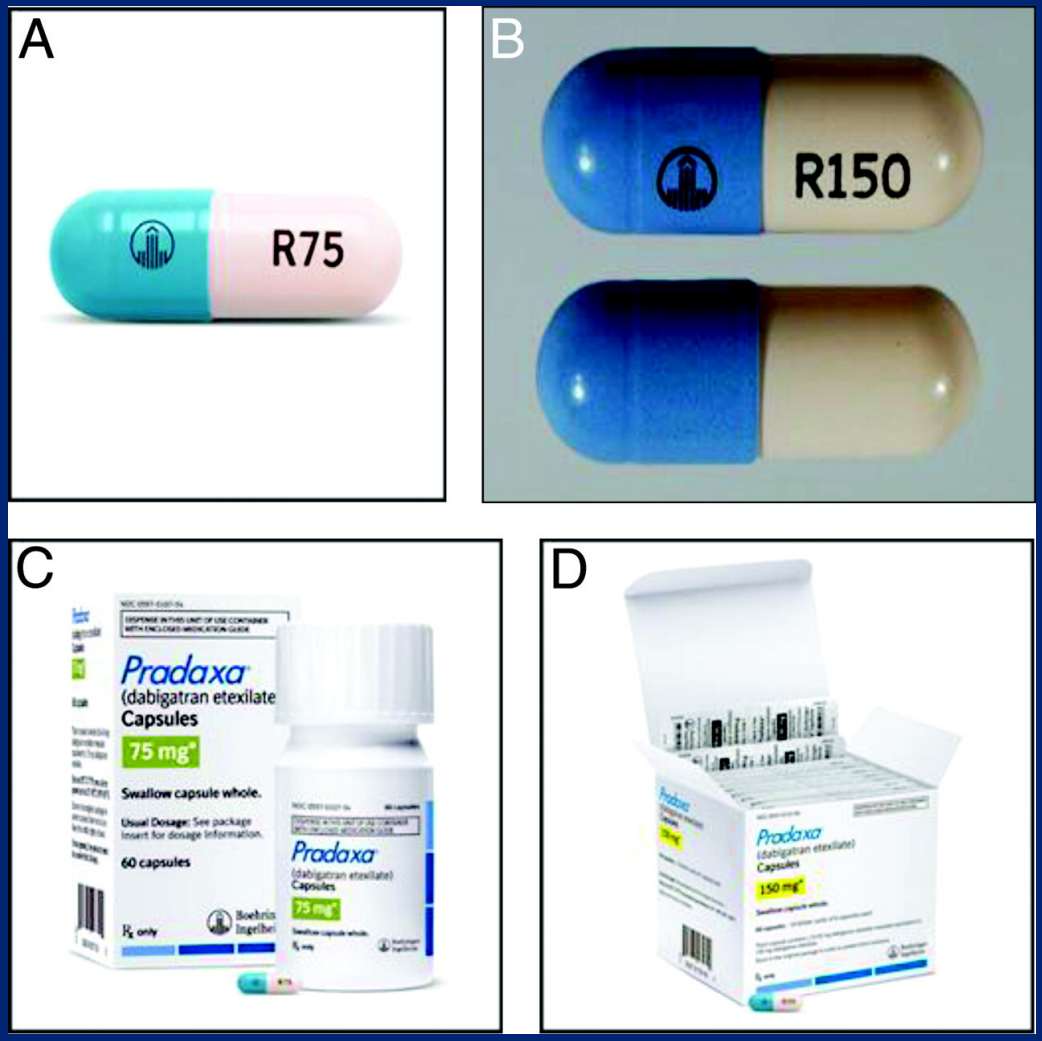
- Thromboprophylaxis in Patients Undergoing Major Orthopedic Surgery
  (all : 14 - 35 days)
- DVT and PE : rivaroxaban –
  2x15 mg / 3 weeks, after 20mg
- Atrial fibrillation (prevention of stroke):
  dabigatran, 2 x 150 mg,
  2 x 110 mg
  rivaroxaban 20 mg

How do we determine stroke risk ?

- CHADS2 (Gage, et al.: JAMA 2001)
  - **Congestive heart failure - 1pt**
  - **Hypertension - 1pt**
  - **Age > 75 - 1 pt**
  - **Diabetes - 1pt**
  - **Stroke or TIA - 2 pts**

  - **0 points – low risk** (1.2-3.0 strokes per 100 patient years)
  - **1-2 points – moderate risk** (2.8-4.0 strokes per 100 patient years)
  - **≥ 3 points – high risk** (5.9-18.2 strokes per 100 patient years)

A, Dabigatran 75,110 and 150 mg capsule.



Dabigatran and bleeding

- No reversal agent or antidote currently
- Supportive care and control of bleeding
- Eliminate by natural excretion through kidney unless renal impairment
- Plasma half life: 12 – 17 hrs

Laboratory monitoring of dabigatran

- APTT – 2x
- Specific test using a snake venom called Ecarin (Not widely available)
- Thrombin time – more 150 sec
- Thrombin dilute test

  HEMOCLOT > 220 ng/l

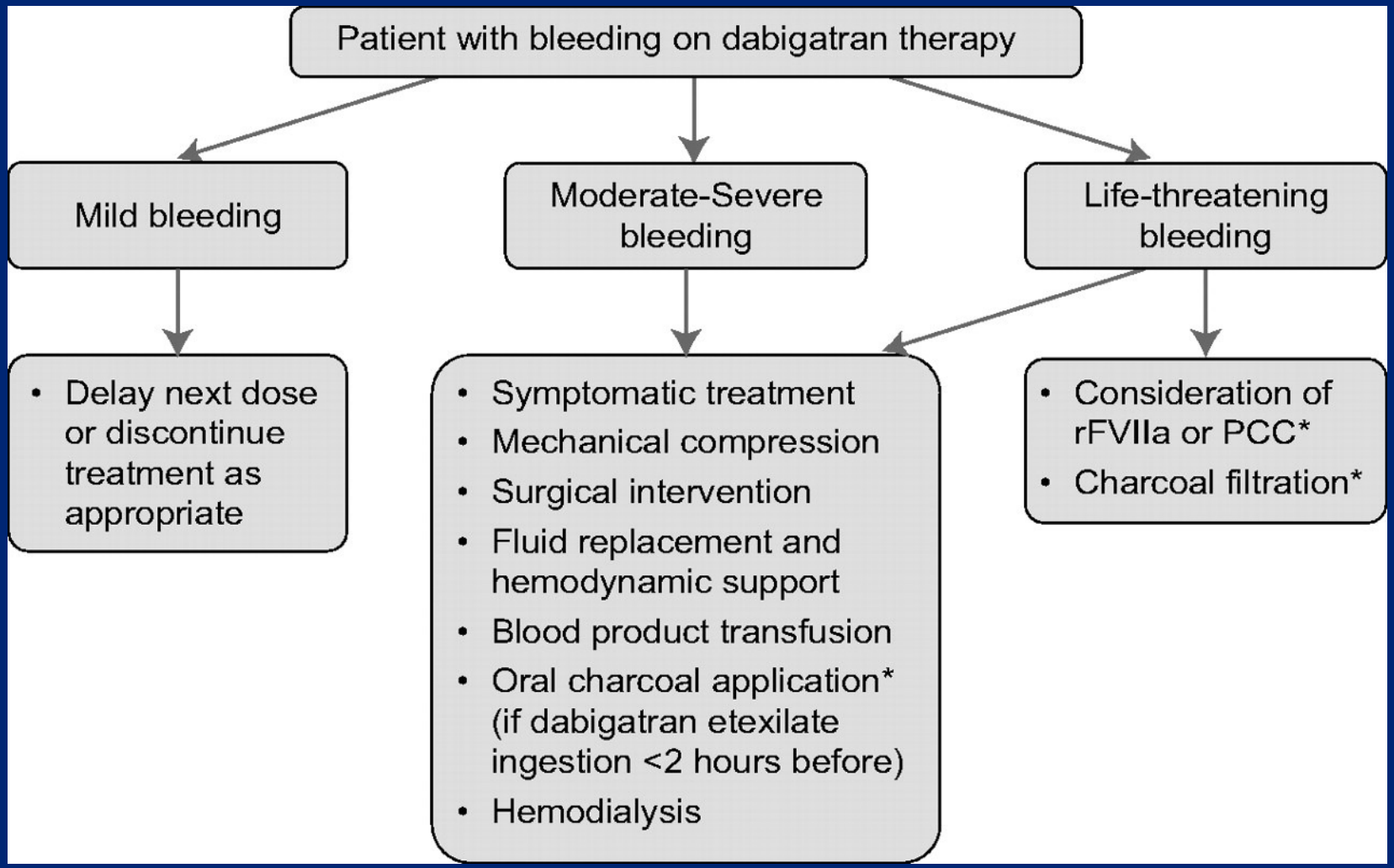
Rivaroxaban

- Once daily
- As effective or better than warfarin
- Less hemorrhagic stroke than warfarin
- Similar reduction in ischemic stroke
- Less bleeding than warfarin
- No routine lab testing
- No reversal
  - Half life 5-9 hours

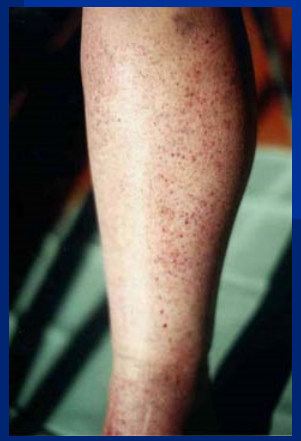
Laboratory monitoring of rivaroxaban

- Coagulation testing: aPTT no !,
- only PT (sec),
- best test - inhibition of F Xa

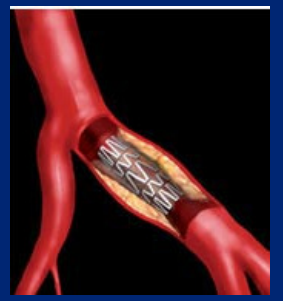
Guide to the management of bleeding in patients taking NOAC.

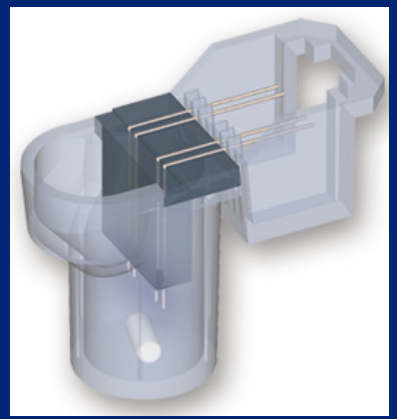


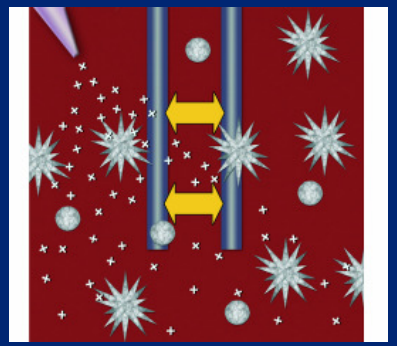
Hankey G J , Eikelboom J W Circulation 2011;123:14361450

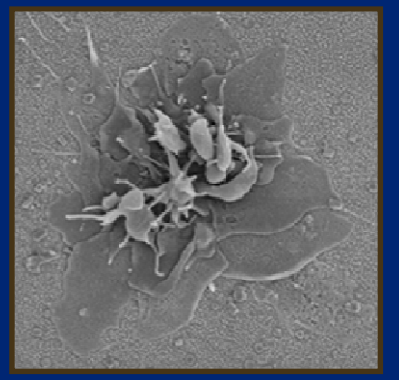


Anti platelet therapy

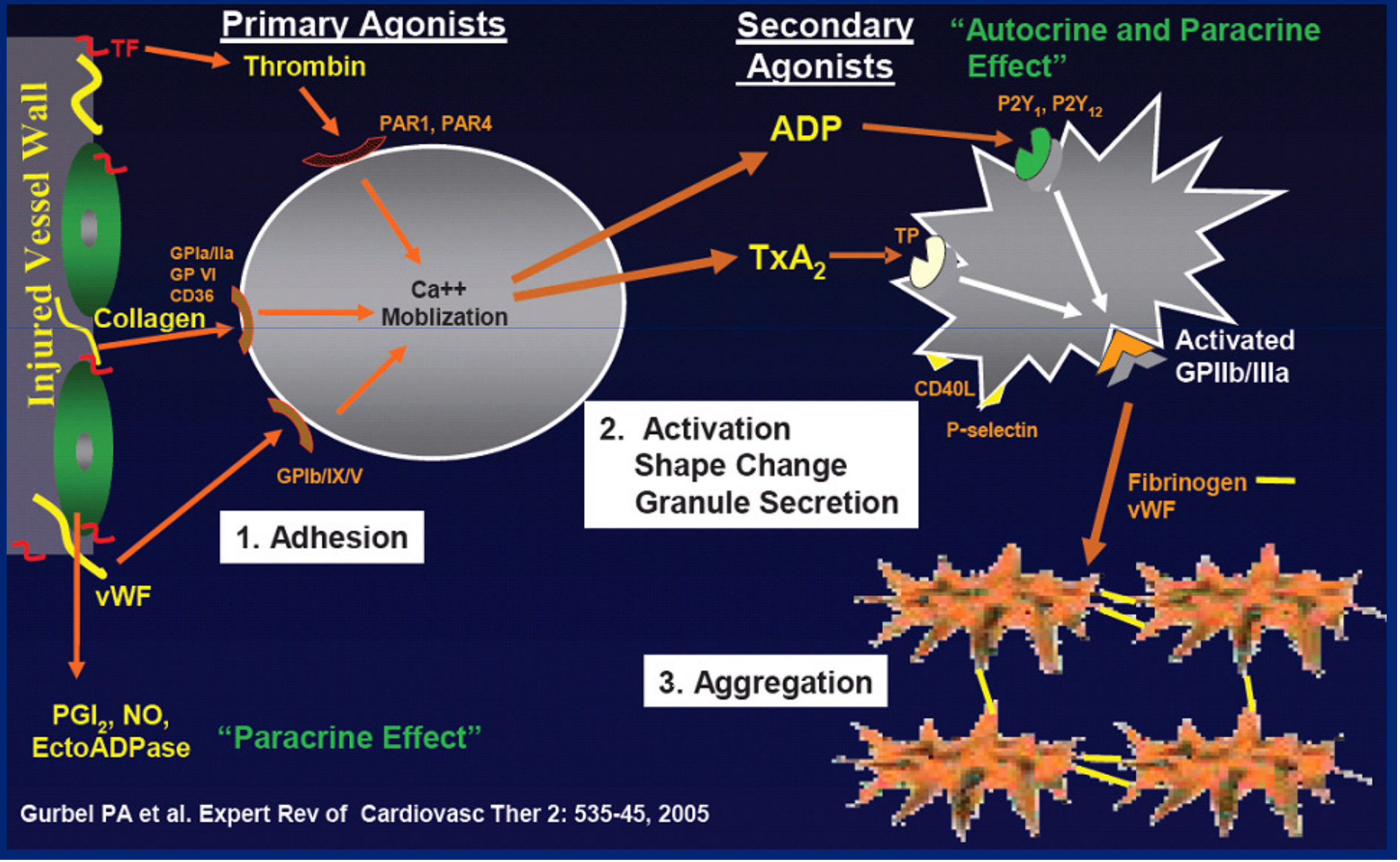








Platelet function
Mechanisms



Gurbel PA et al. Expert Rev of Cardiovasc Ther 2: 535-45, 2005

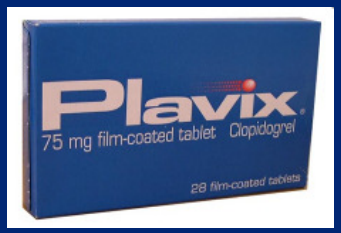
Anti-platelet drugs
Main classes

Cyclooxygenase Inhibitors

- Aspirin

Antagonists of ADP receptor

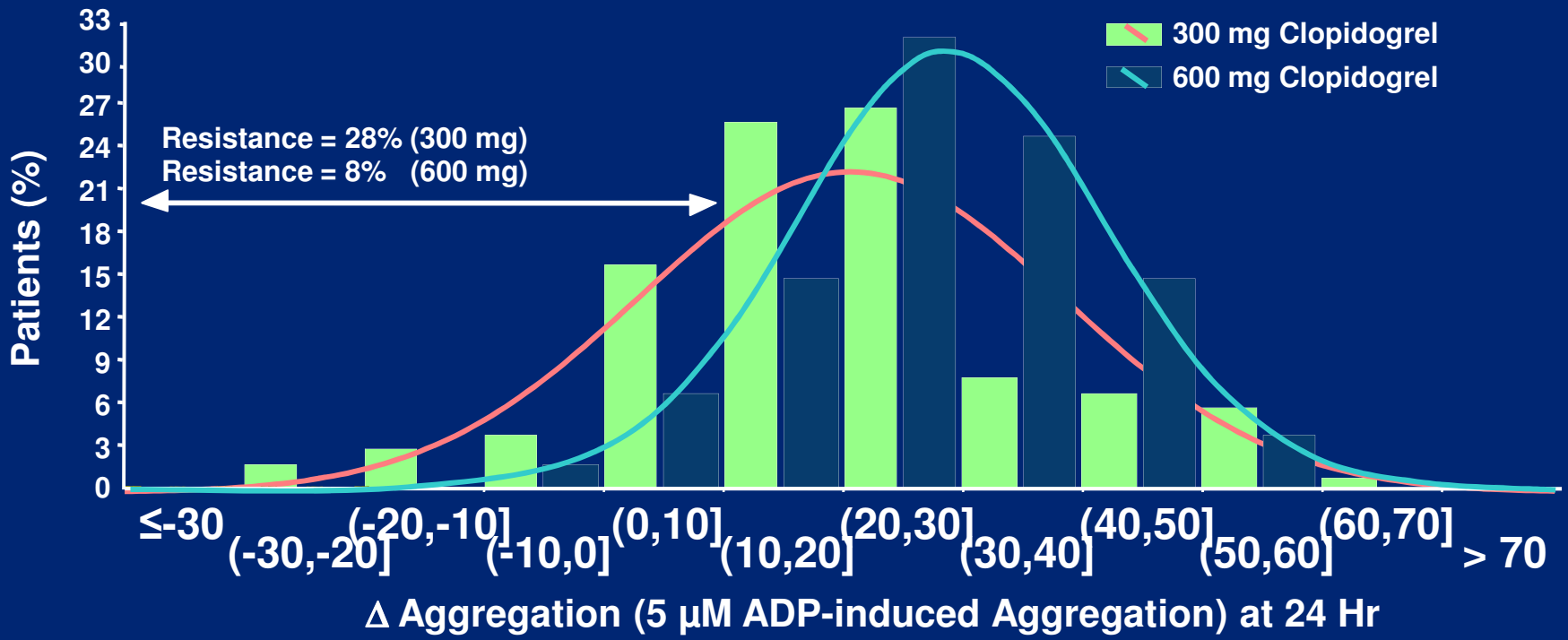
- Ticlopidin
- Clopidogrel
- Prasugrel (Efient)
- Ticagrelor



Antagonists of GP IIb/IIIa

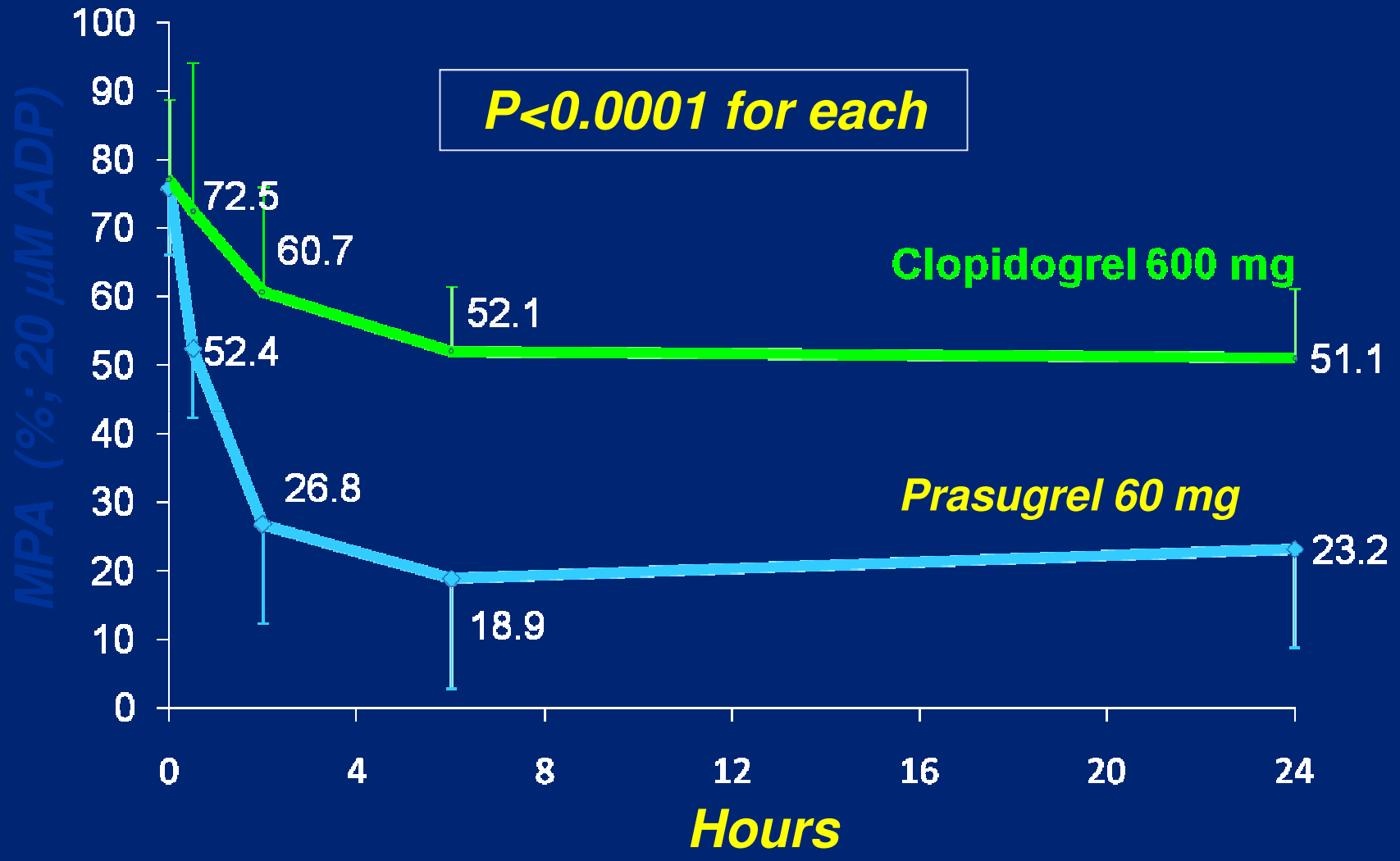
- Abciximab (Reopro)
- Tirofiban (Aggrastat)
- Eptifibatide (Integrilin)

Clopidogrel Response variability - resistance to therapy



Gurbel PA et al. *J Am Coll Cardiol.* 2005;45:1392-1396.

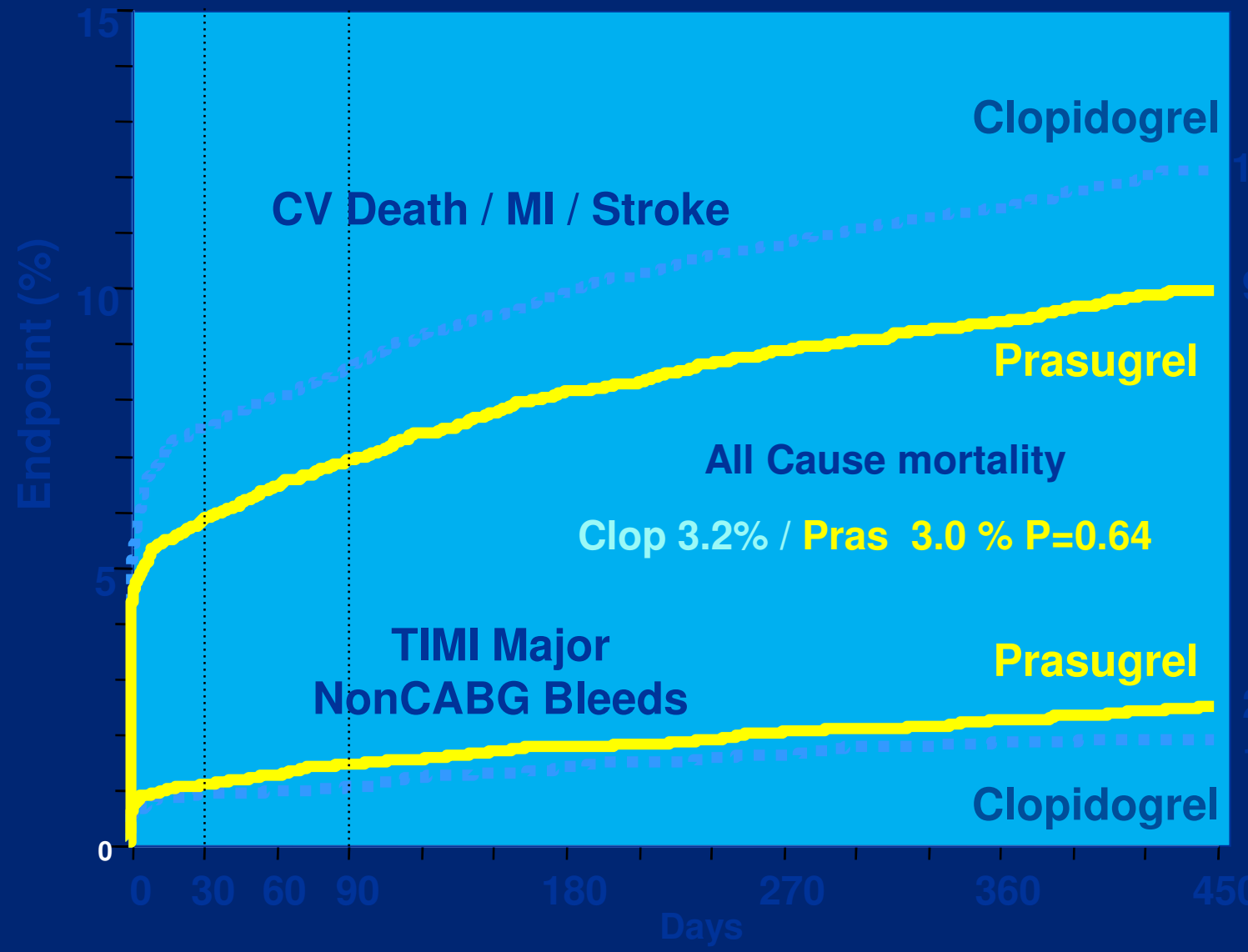
Clopidogrel vs. Prasugrel: Anti-platelet effects of the loading doses



PRINCIPLE TIMI 44

TRÎTON TIMI-38

Clopidogrel vs. Prasugrel: Phase III study results-ACsy



↓ 138 events

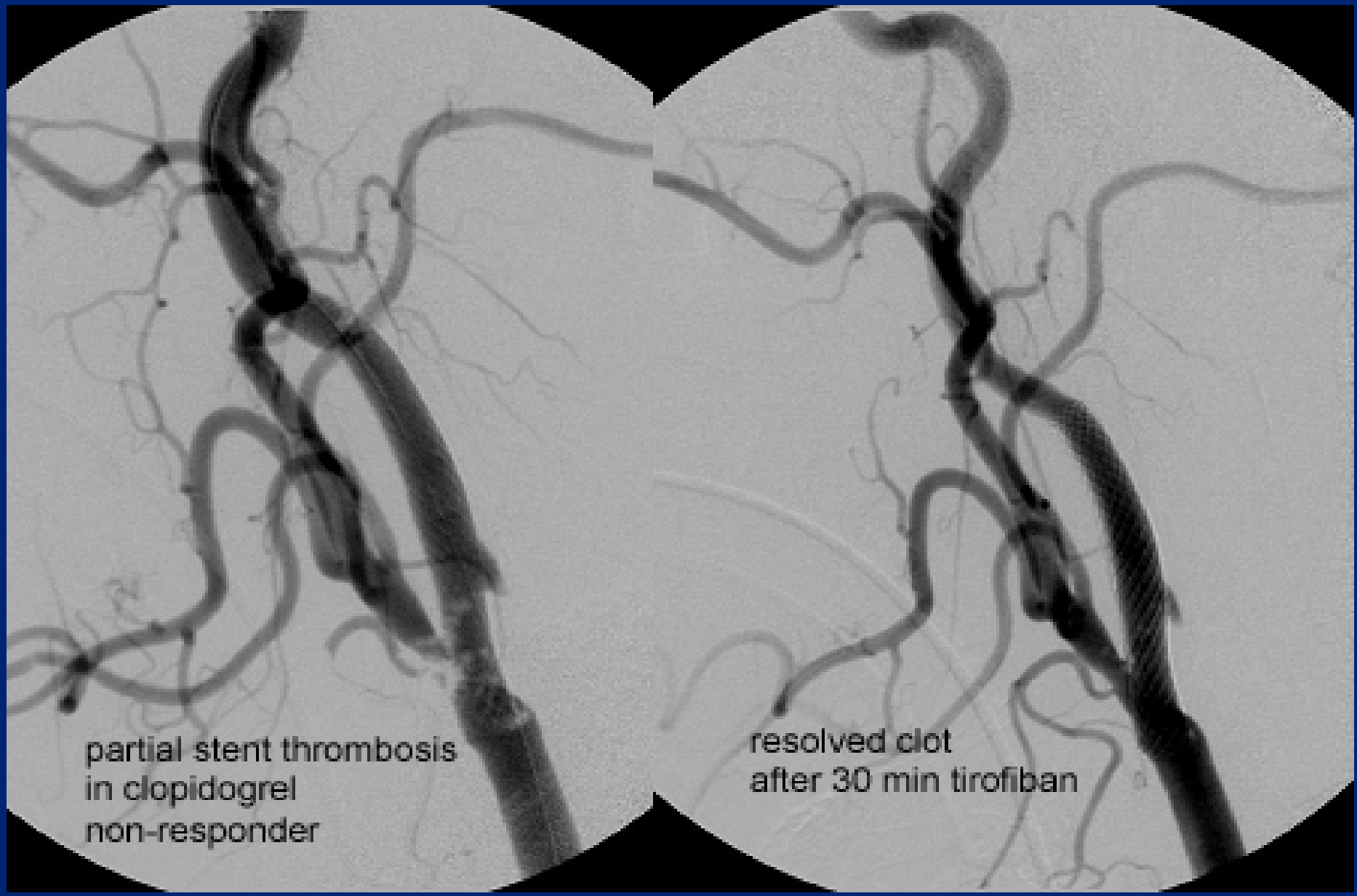
HR 0.81
(0.73-0.90)
P=0.0004

↑ 35 events

HR 1.32
(1.03-1.68)
P=0.03

ADP receptor antagonists
Comparison

Drug	Administration		Prodrug	ttPeak plt inhibition	Reversibility (half-life)
	Route	Frequency			
Clopidogrel	Oral	Once daily	yes	2-6h (after 600 mg loading dose)	No
Prasugrel	Oral	Once daily	yes	2h	No
Ticagrelor	Oral	Twice daily	No	2h	**Yes (12h)**


partial stent thrombosis
in clopidogrel
non-responder
resolved clot
after 30 min tirofiban

aggregometry

Platelet function testing

Platelet function testing

Verifynow

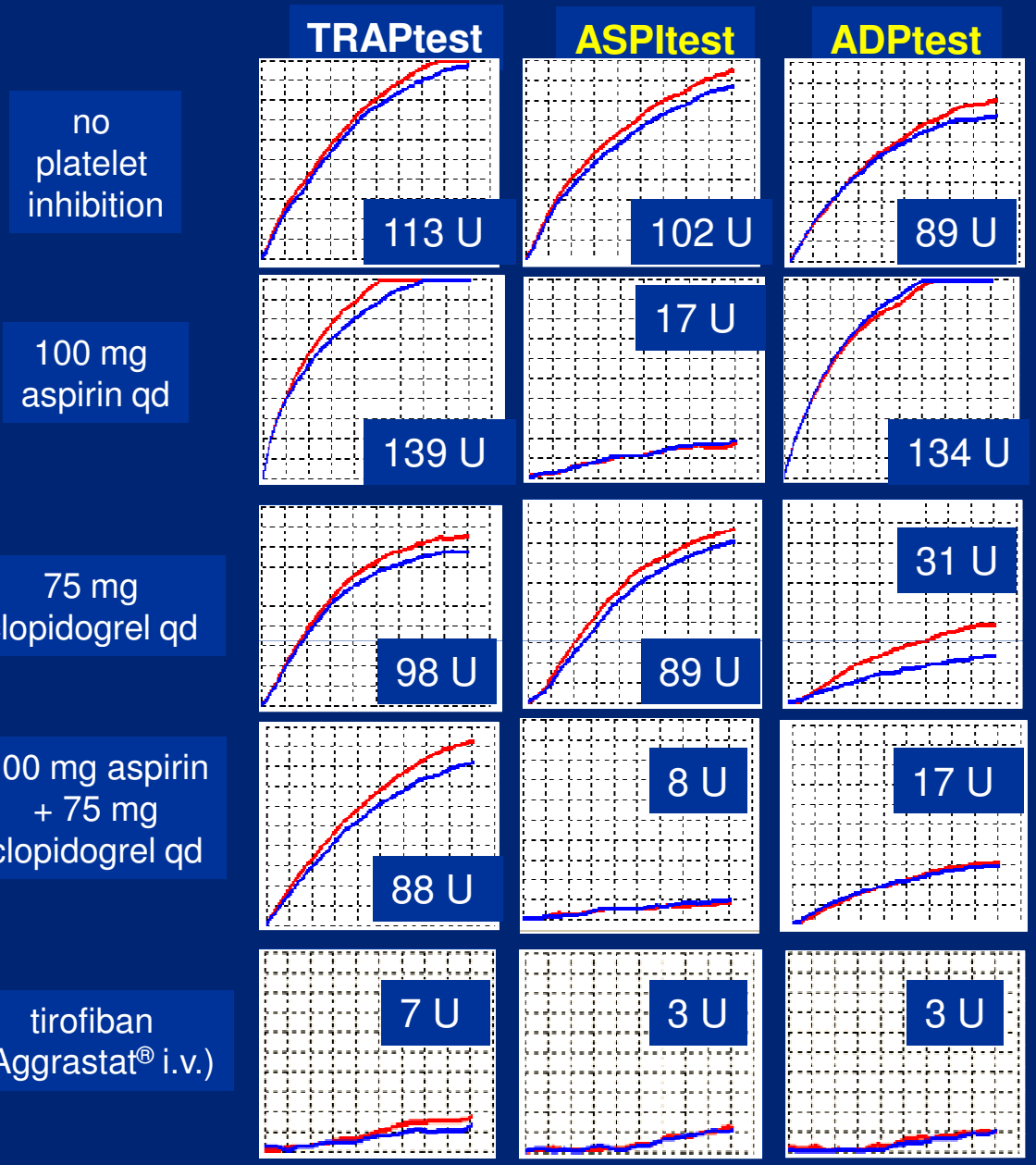
VASP

LTA

Multiplate

→ **highest relative risk for clopidogrel „resistant“ patients using the Multiplate analyzer**

Multiplate tracings
Examples



Interpretation of MEA results for patients on dual anti-platelet therapy (analysis of hirudin blood)

ADPtest

≤ 45 U

Reduced platelet aggregation compared to average non-clopidogrel treated individuals. Result is in accordance with sufficient P2Y12 blockade.

> 45 U

Aggregation is higher than expected for a clopidogrel-treated individual.

Check compliance. If patient is treated with Clopidogrel 75 mg or Clopidogrel 150 mg / d consider switching to Prasugrel 10 mg /d (as long as there is no contraindication to Prasugrel treatment).

From Clopidogrel 75 mg / d also a switch to Clopidogrel 150 mg / d can be performed.

ASPItest

≤ 50 U

Reduced platelet aggregation compared to average non-aspirin treated individuals. Result is in accordance with a regular COX blockade.

> 50 U

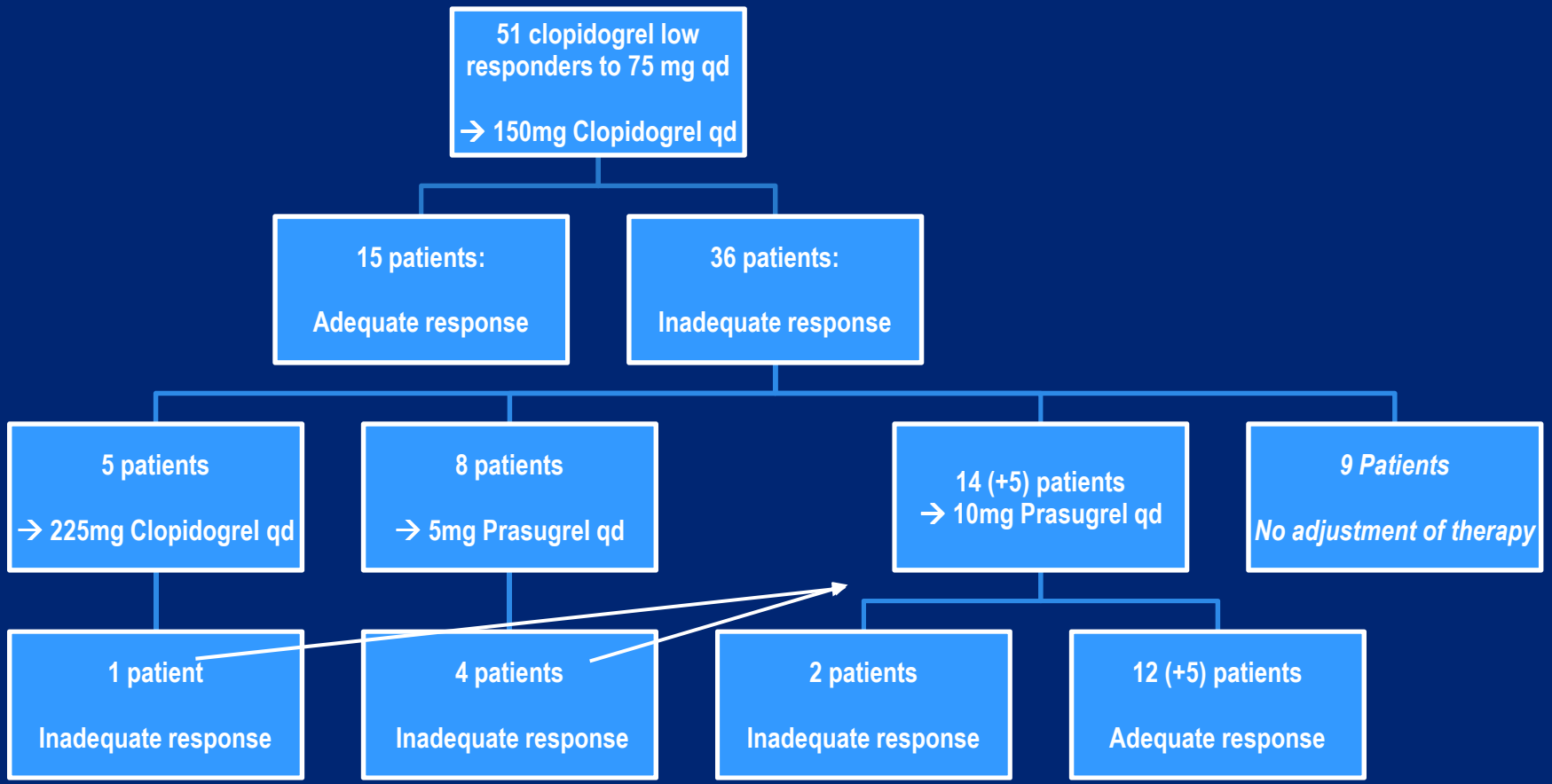
Aggregation is higher than expected for an aspirin-treated individual.

Check compliance. If the patient is treated with < 100 mg Aspirin / d or with coated aspirin, consider switching to Aspirin 100 mg / d uncoated.

So far there is no clear evidence that this finding is related to an increased risk of thromboembolic events.

NB: The antiplatelet treatment of the patient should always be acceptable regarding the clinical status of the patient in the light of current recommendations by the clinical societies, also without reference to the Multiplate result. Intensifying anti-platelet therapy can increase bleeding.

Repetitive MEA and individual adjustment



Schuhmann C, Krötz F, Cardiology, Munich University Clinic