

VYŠETŘOVACÍ METODY U HEMOKOAGULACE A FIBRINOLÝZY

Ústav patologické fyziologie
1. LF UK
2010 – 2015 – 2023 - ...



I. Fyziologie



Hemostáza

= je mechanismus umožňující udržení oběhu po poruše cévní stěny.

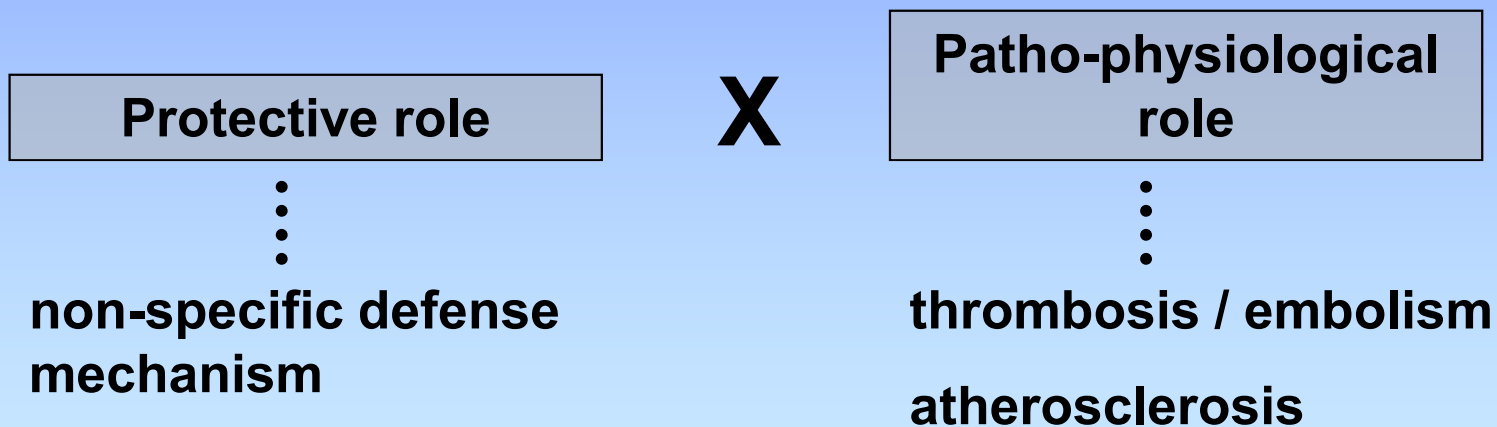
Sestává z těchto **fází**:

1. **Klidová fáze** – Udržování krve v **tekutém stavu** v cirkulaci
2. **Aktivační fáze** - **Zástava krvácení** v místě poranění pomocí hemostatické zátky
3. **Fáze restituce- rekanalizace** - Zajišťuje **odstranění hemostatické zátky** na konci reparačních procesů

Hemostasis

Hemostasis is an integral part of

- stress reaction
- inflammatory response



Hemostasis

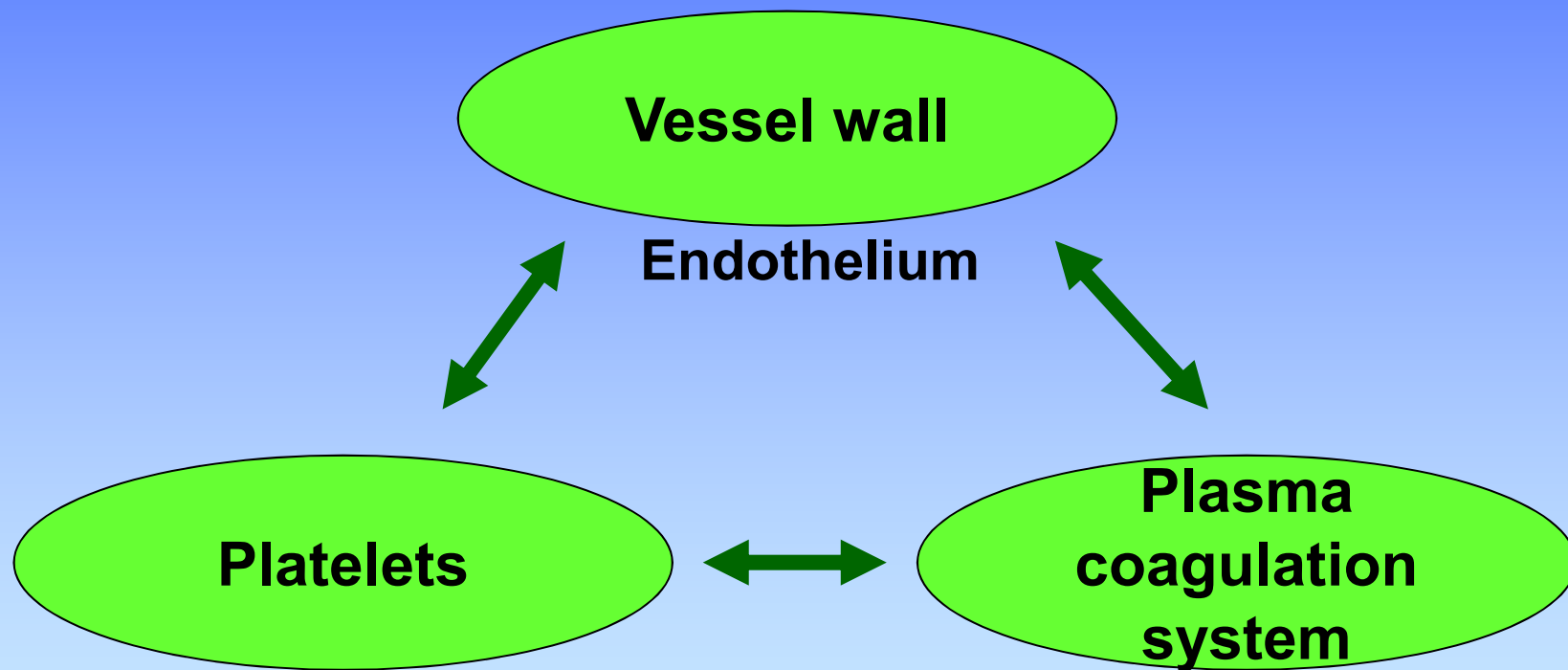
Hemostasis as a physiological process must be:

1. Rapid
2. Localized
3. Reversible

Inappropriate hemostasis:

- Thrombosis / embolism
- DIC (disseminated intra-vascular coagulation)
- bleeding / blood loss

Hemostasis



Obečné mechanismy, popis regulace

Negativní (záporná) zpětná vazba

y ...regulovaná veličina, v/v

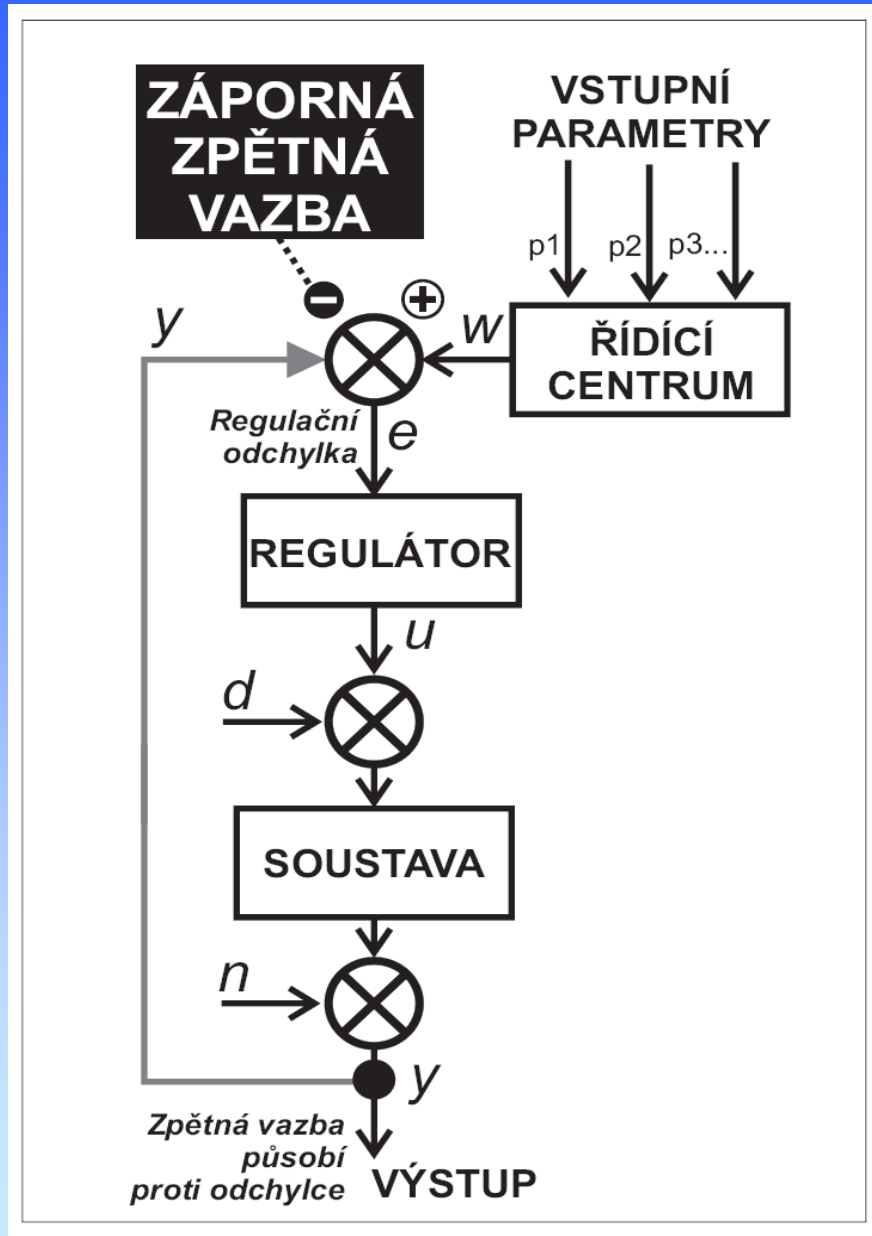
w ...požadovaná hodnota

e ...regulační odchylka/ signál

u ...akční veličina

d, n ...poruchové veličiny

U **záporné** zpětné vazby regulační odchylka e použitá k regulaci vznikne **odečtením** akční veličiny $(-y)$ od požadované hodnoty $(+w)$,
 $e = w - y$.



Pozitivní (kladná) zpětná vazba

y ...regulovaná veličina, v/v

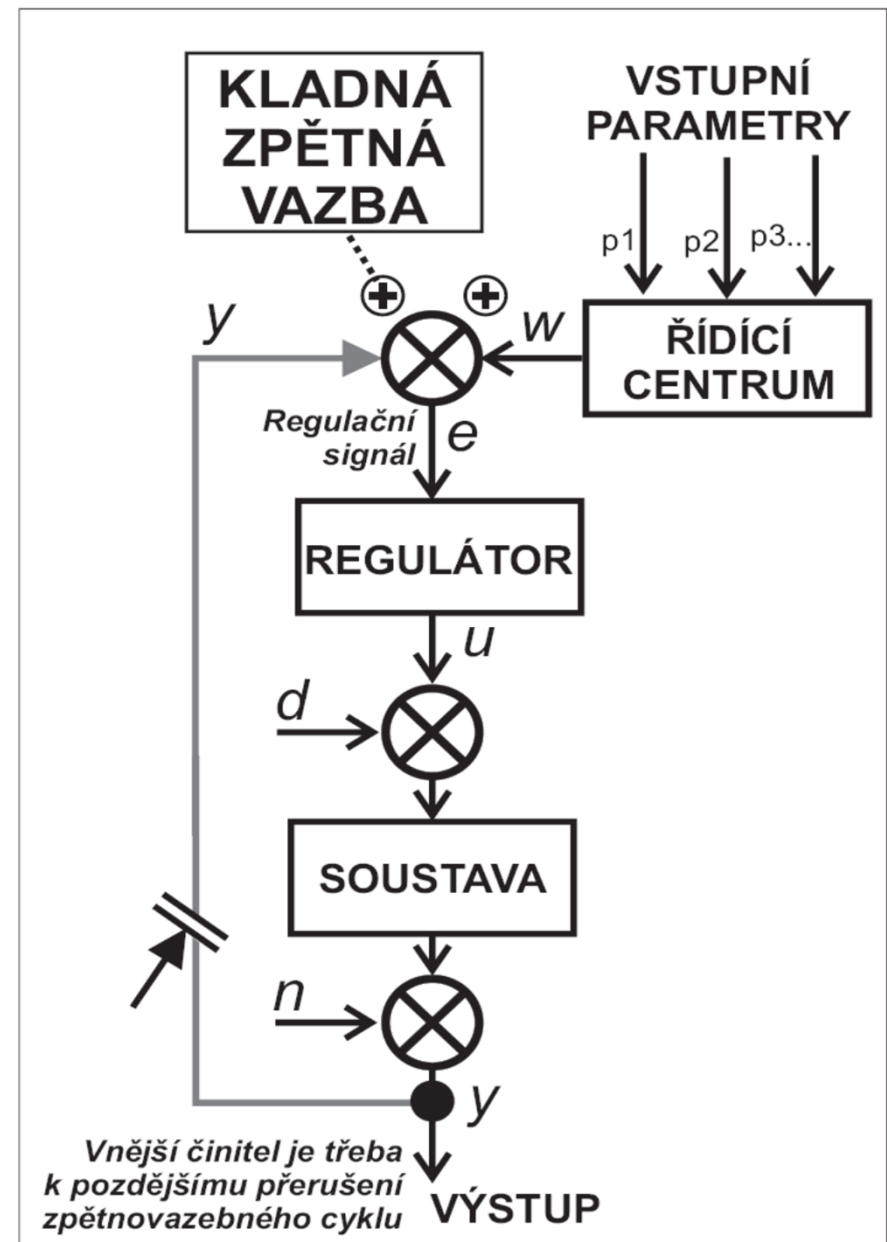
w ...požadovaná hodnota

e ...regulační odchylka/ signál

u ...akční veličina

d, n ...poruchové veličiny

U **kladné** zpětné vazby regulační signál e vznikne **přičtením** akční veličiny $(+y)$ k požadované hodnotě $(+w)$, $e = w + y$.



Endothelium

Antithrombotic Properties

Anti-platelet activities:

- Endothelium covers highly thrombogenic basal membrane
- Uninjured endothelium does not bind platelets
- PGI₂ (prostaglandin) and NO (nitric oxide) from endothelium inhibit platelet binding
- ADPase counters the platelet aggregating effects of ADP

Endothelium

Antithrombotic Properties

Anticoagulant activities:

- Heparin-like molecules ... activate anti-thrombin III (inactivates active proteases)
- Thrombomodulin ... changes specificity of thrombin (activates protein C , which inactivates factors Va and VIIIa)
- tPA (tissue plasminogen activator) ... activates fibrinolysis via plasminogen to plasmin

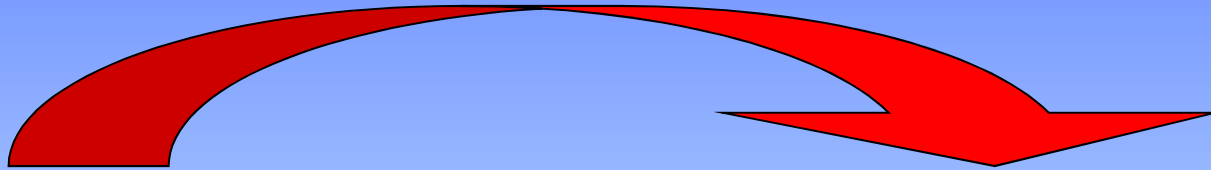
Endothelium

Prothrombotic Properties

- Synthesis of von Willebrand factor
- Release of tissue factor
- Production of PAI (plasminogen activator inhibitors)
- Membrane phospholipids bind and facilitate activation of clotting factors via Ca^{2+} bridges

Endothelium

Vessel injury

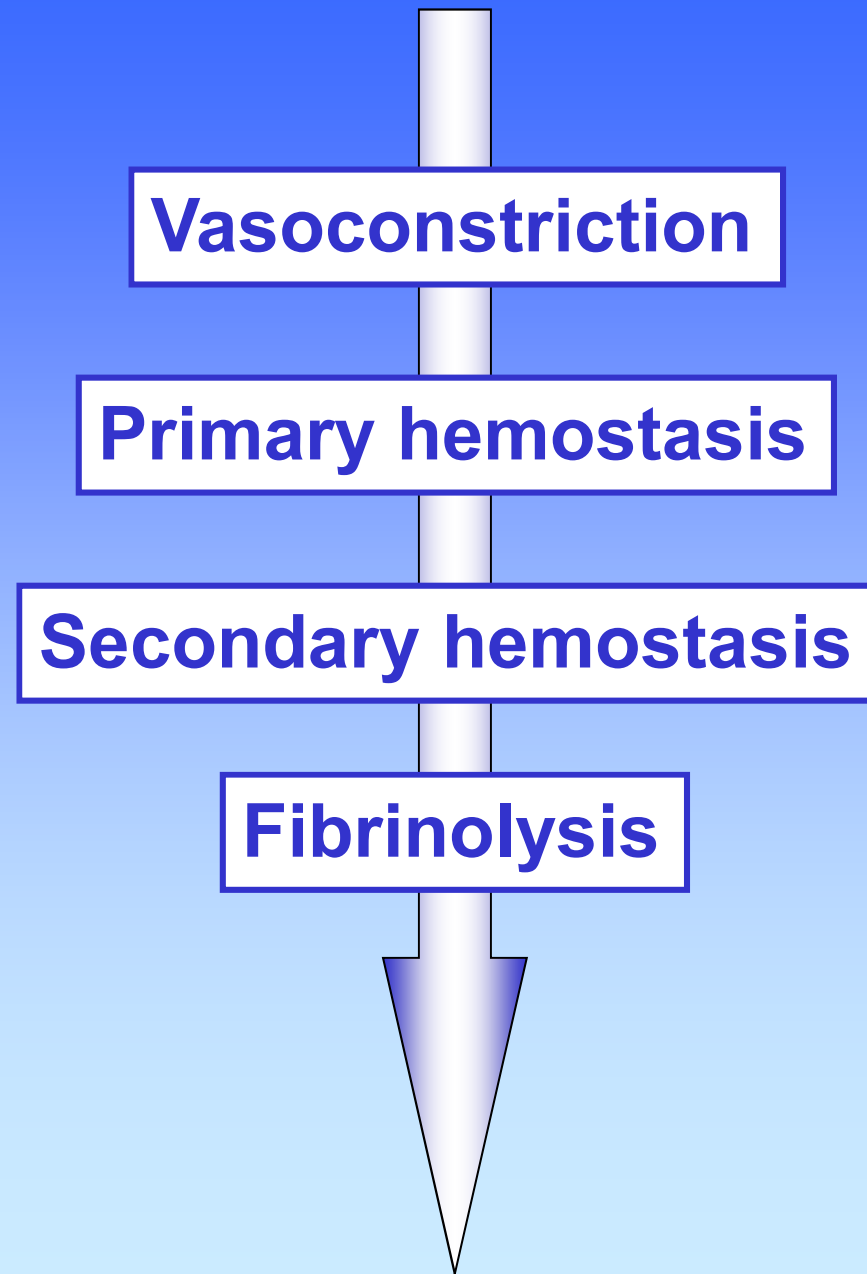


Antithrombogenic

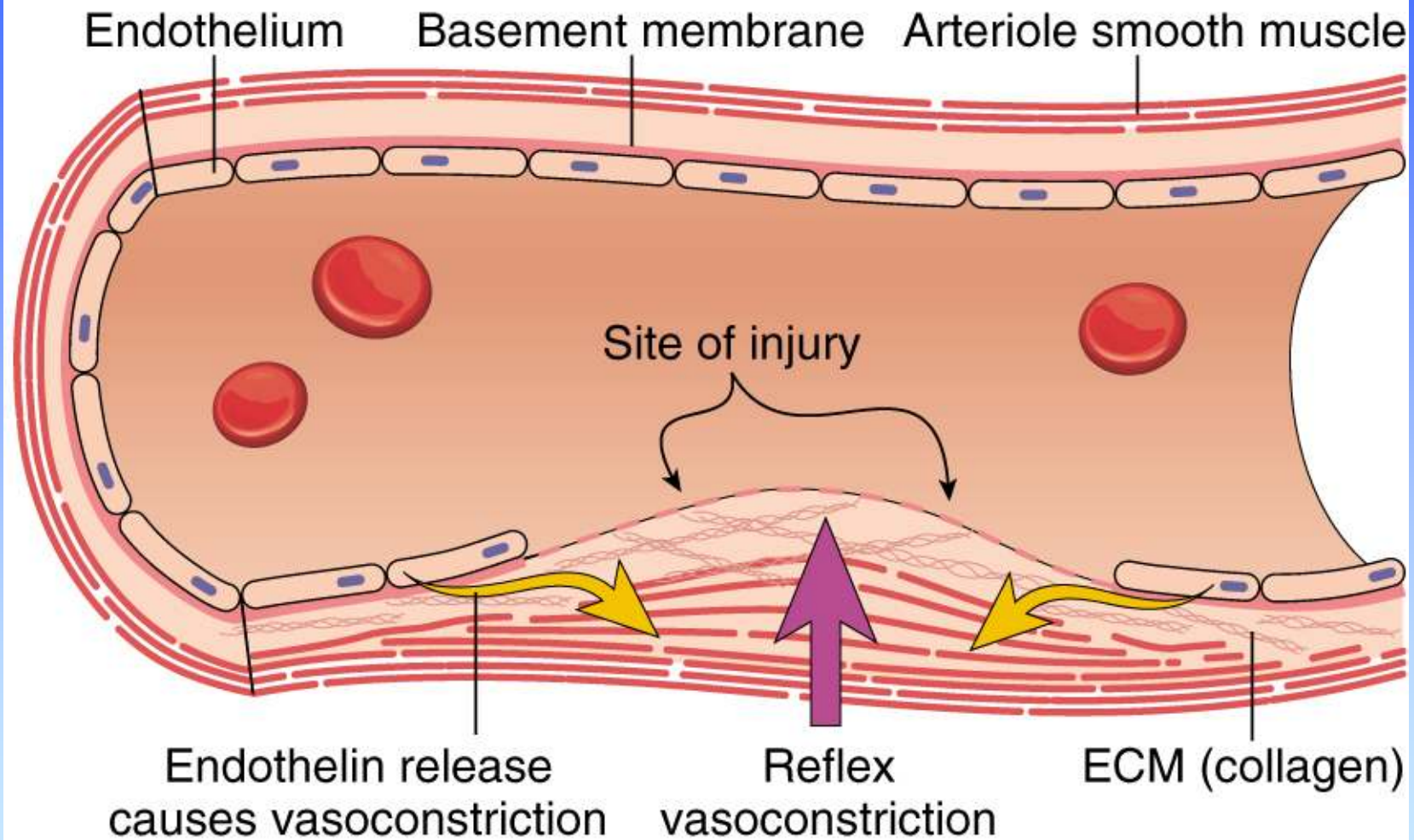
(Favors fluid blood)

Thrombogenic

(Favors clotting)

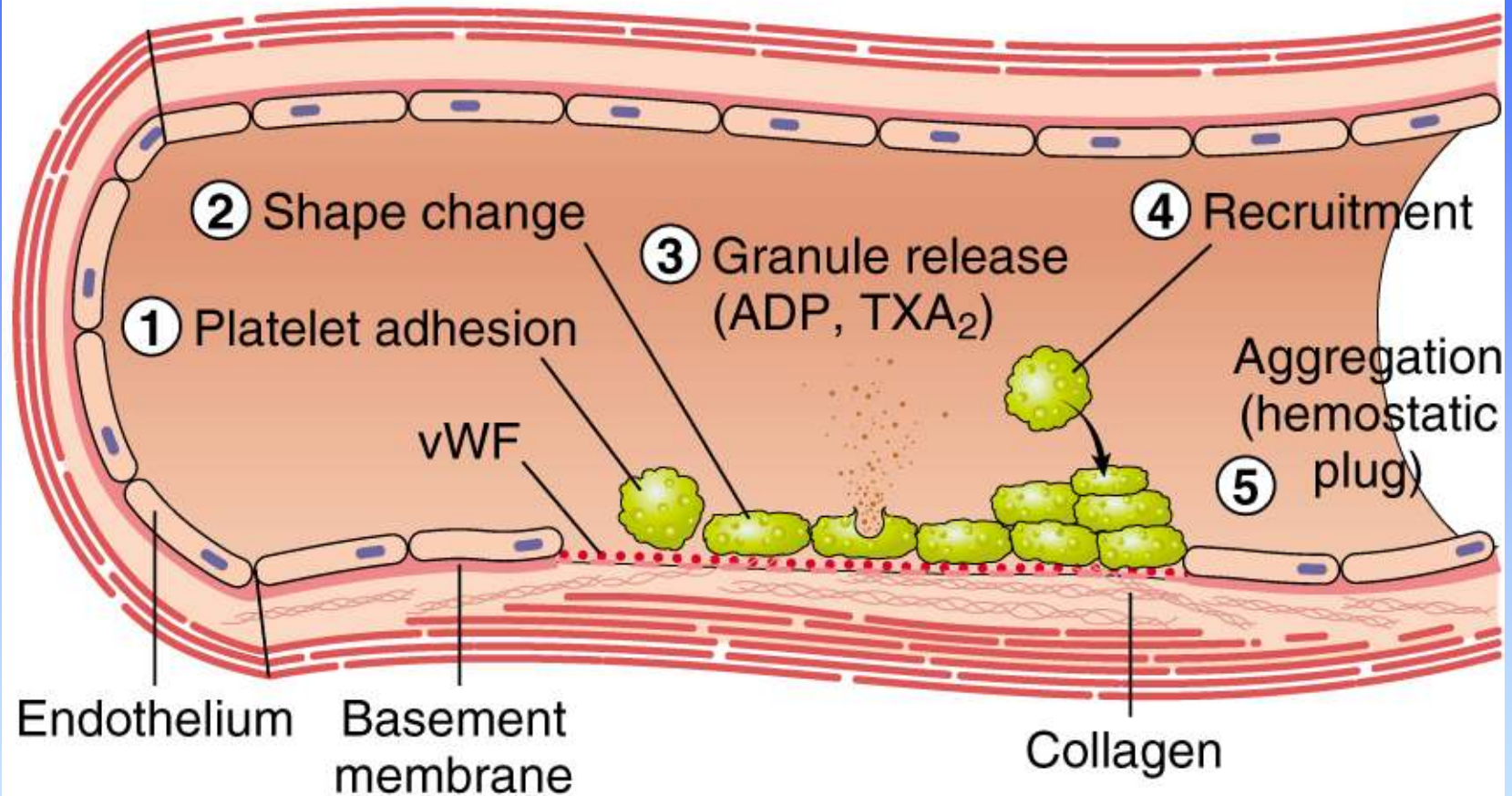


A. VASOCONSTRICTION

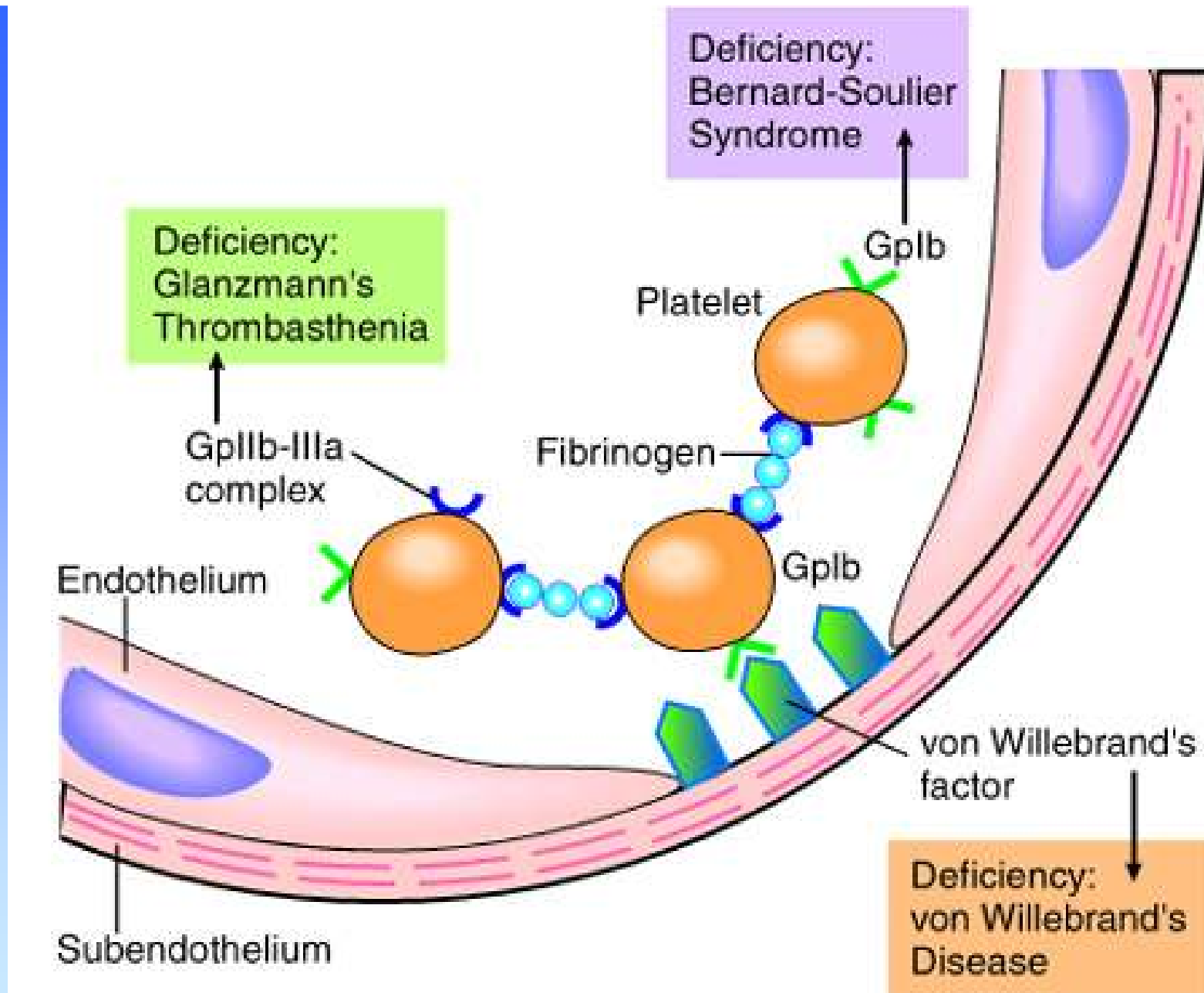


ECM (=ExtraCellular Matrix)

B. PRIMARY HEMOSTASIS

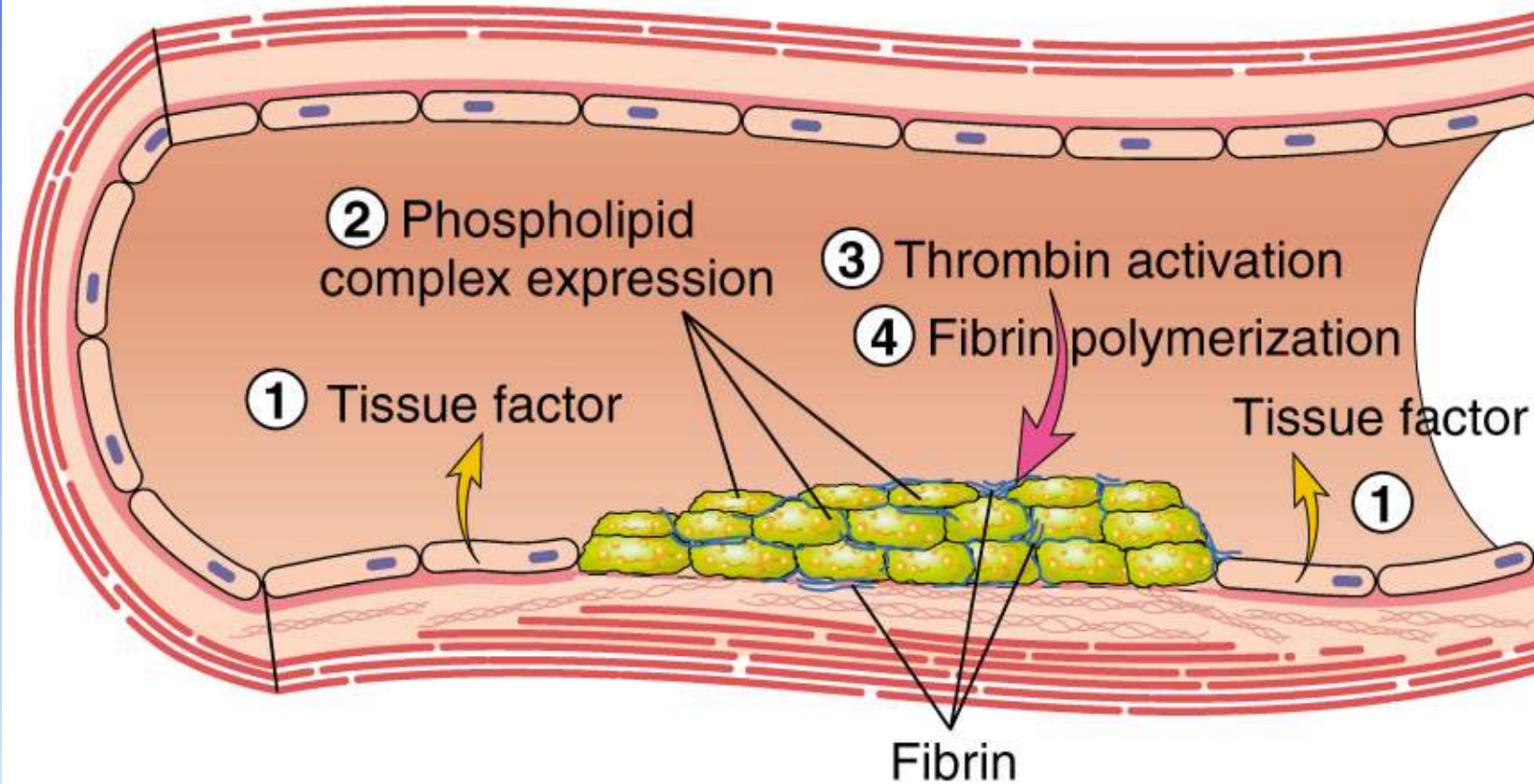


TXA₂ (thromboxane A₂, lipid)



Gp – G-protein coupled receptors

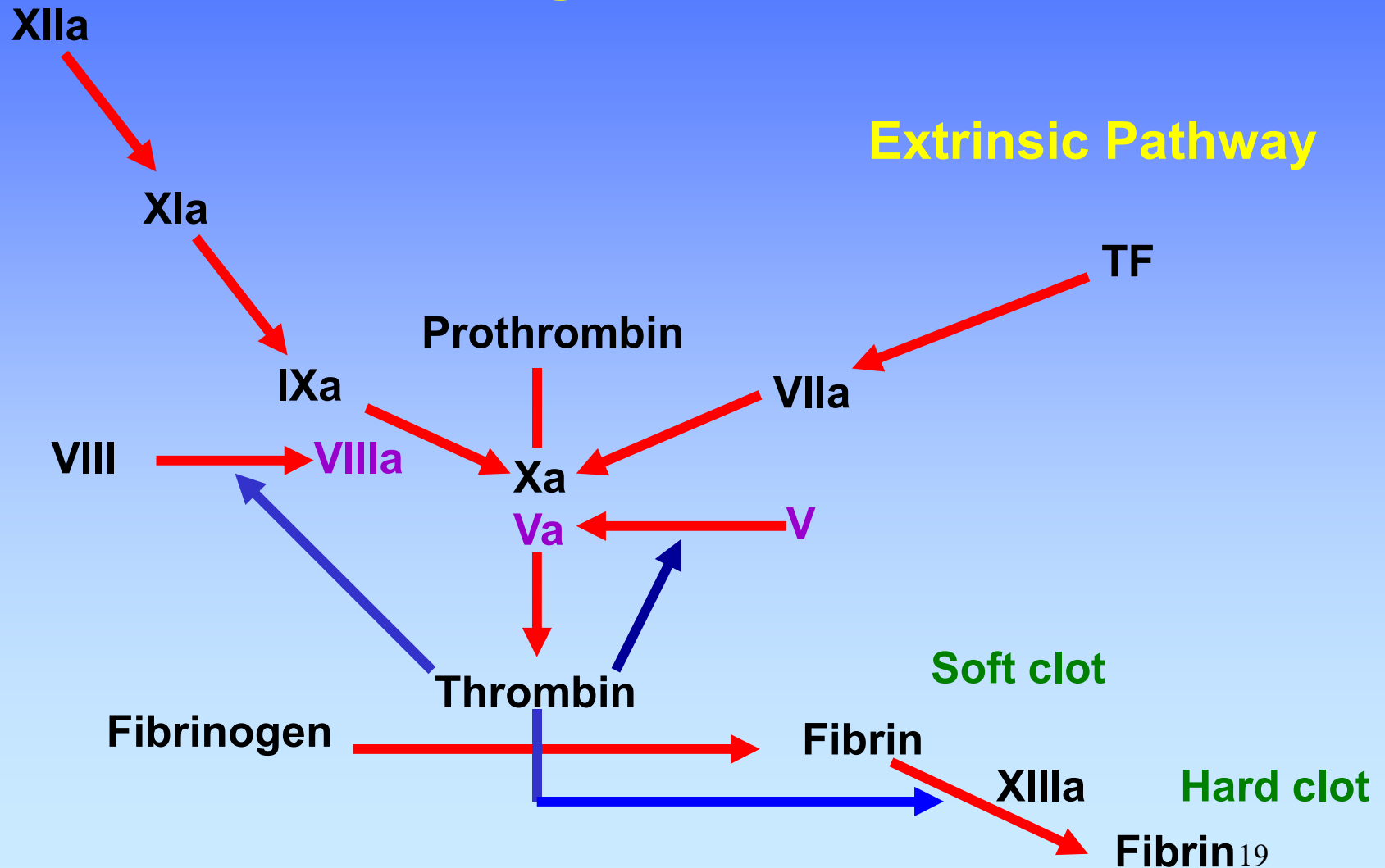
C. SECONDARY HEMOSTASIS

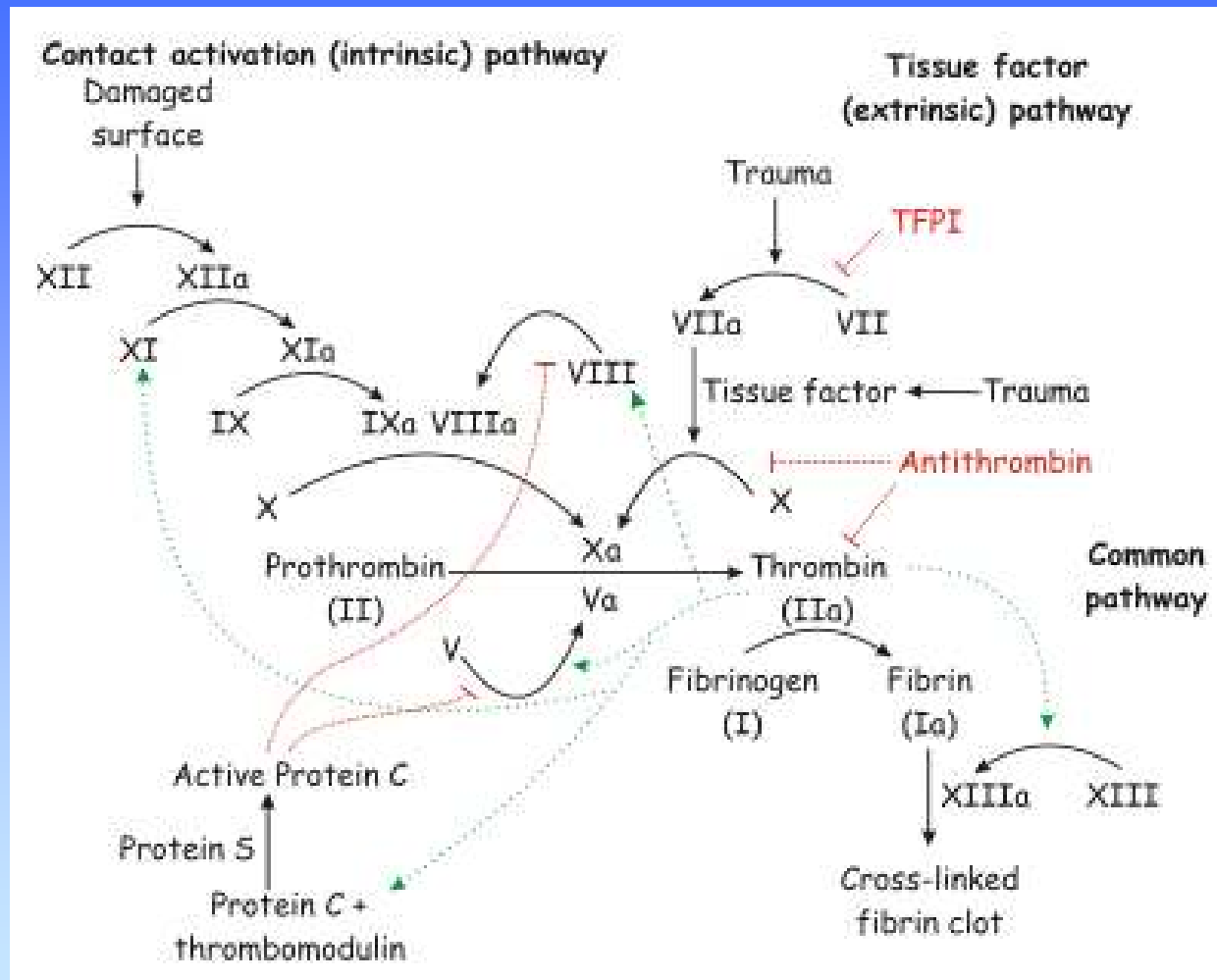


Intrinsic pathway

Coagulation

Extrinsic Pathway





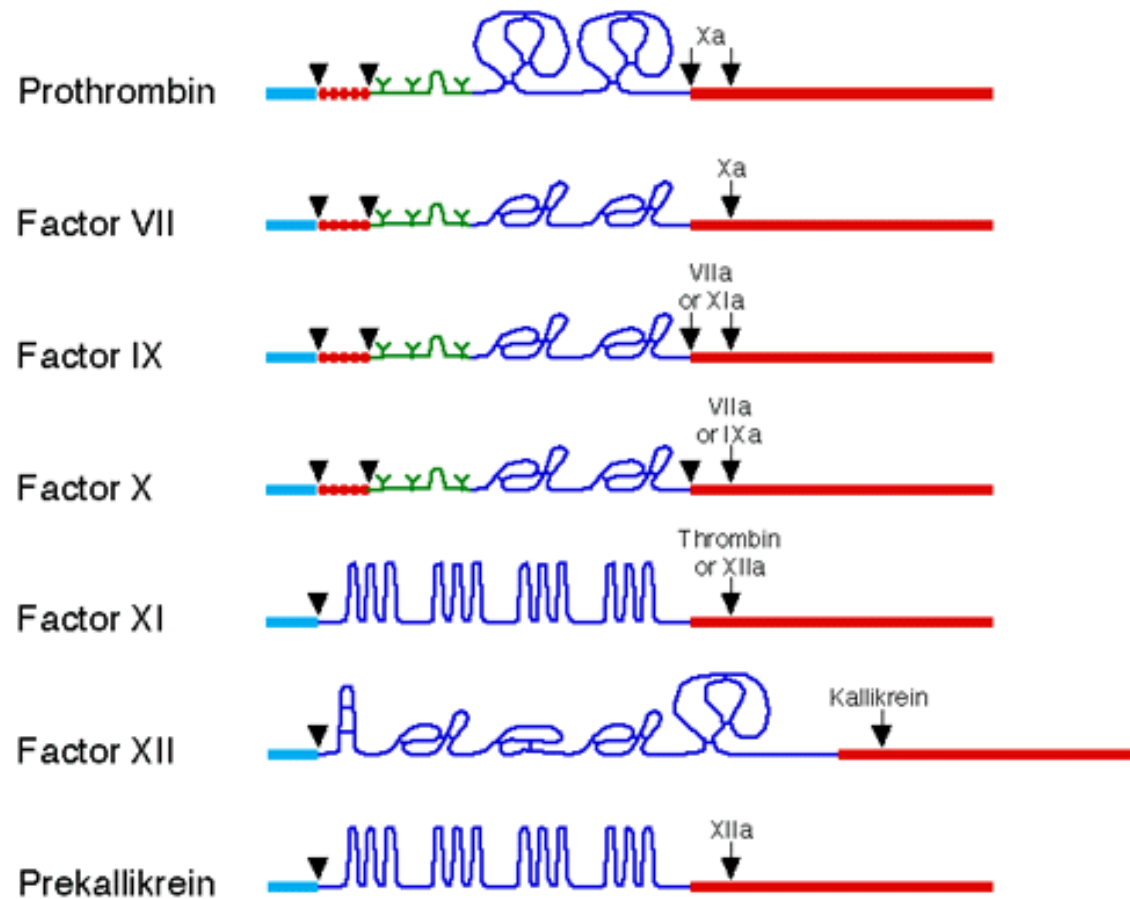
Coagulation

- **Enzymatic cascade (amplification)**
- **Several serine proteases**
- **Produced by liver (most)**
- **Require vitamin K (several, 2, 7, 9, 10, C, S)**
- **Requires Ca^{2+} (the same, 2, 7, 9, 10, C, S)**
- **3 protein cofactors (not enzymes)**
- **Reversible (via production of plasmin)**

Coagulation

Factor	Name	Molecular Weight	Plasma concentration (µg/ml)	Required for hemostasis (% of normal)	Vit K dependency	Natural source
I	Fibrinogen	330,000	3000	30	No	Liver
II	Prothrombin	72,000	100	40	Yes	Liver
III	Tissue factor		--		No	Tissue
IV	Calcium ion		--	--	No	Plasma
V	Proaccelerin	300,000	10	10-15	No	Liver
VII	Proconvertin	50,000	0,5	5-10	Yes	Liver
VIII	Antihemophilic	300,000	0,1	10-40	No	RES
IX	Thromboplastin	56,000	5	10-40	Yes	Liver
X	F. Stuart	56,000	10	10-15	Yes	Liver
XI	Prethromboplastin	160,000	5	20-30	No	Liver
XII	F. Hageman	76,000	30	0	No	Liver
XIII	Fibrin stabilizing	320,000	30	1-5	No	Liver
vWF	Von Willebrand	140,000			No	Endothelium
Prot C					Yes	Liver
PKLK	Prekallikrein	82,000	40	0		
HMWK	HMW Kallikrein	108,000	100	0		

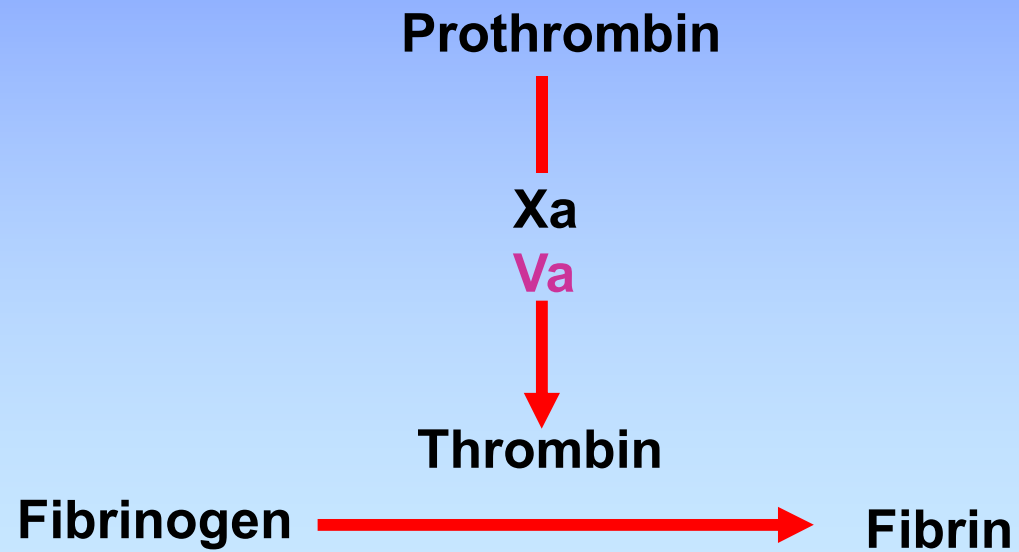
Coagulation



Coagulation

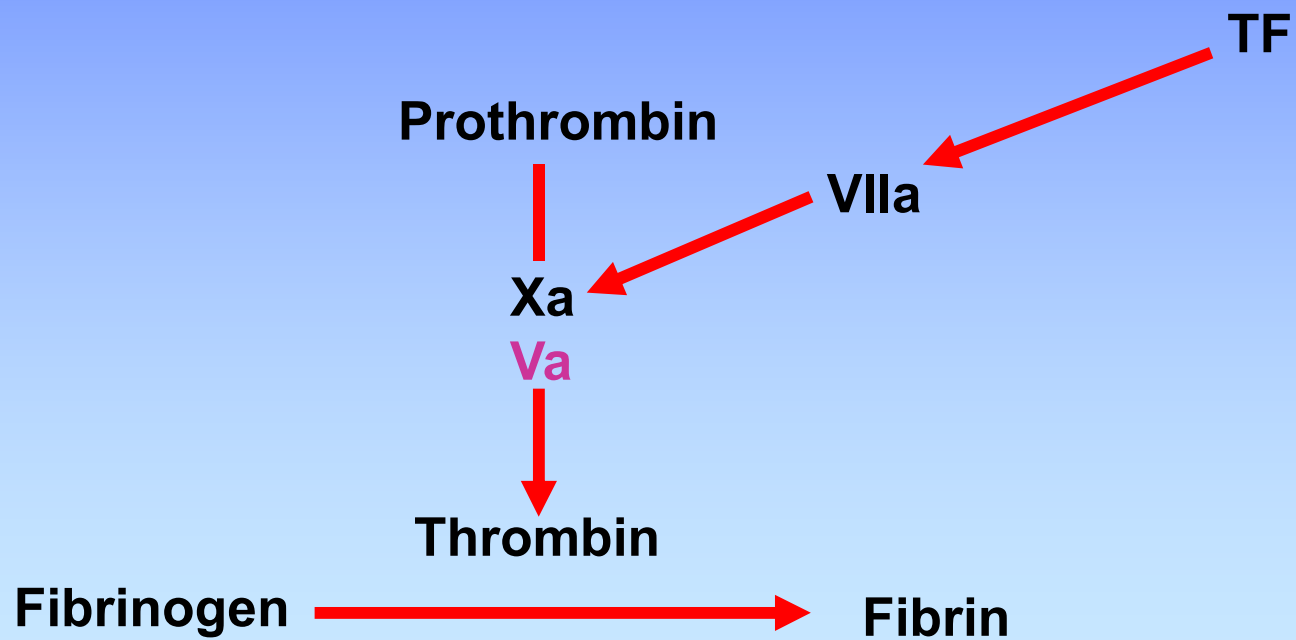
Fibrinogen $\xrightarrow{\text{Thrombin}}$ **Fibrin**

Coagulation



Coagulation

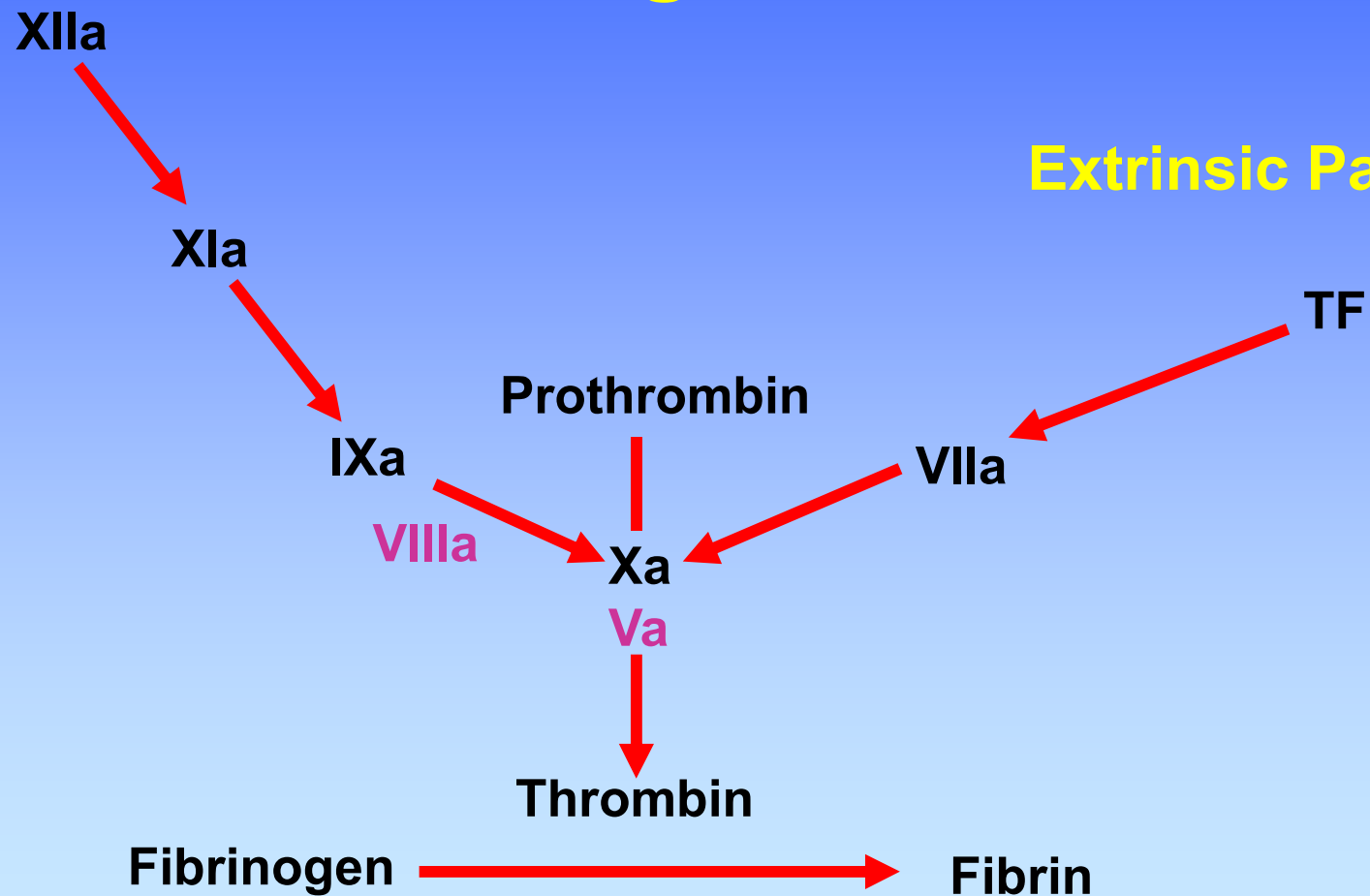
Extrinsic Pathway



Intrinsic pathway

Coagulation

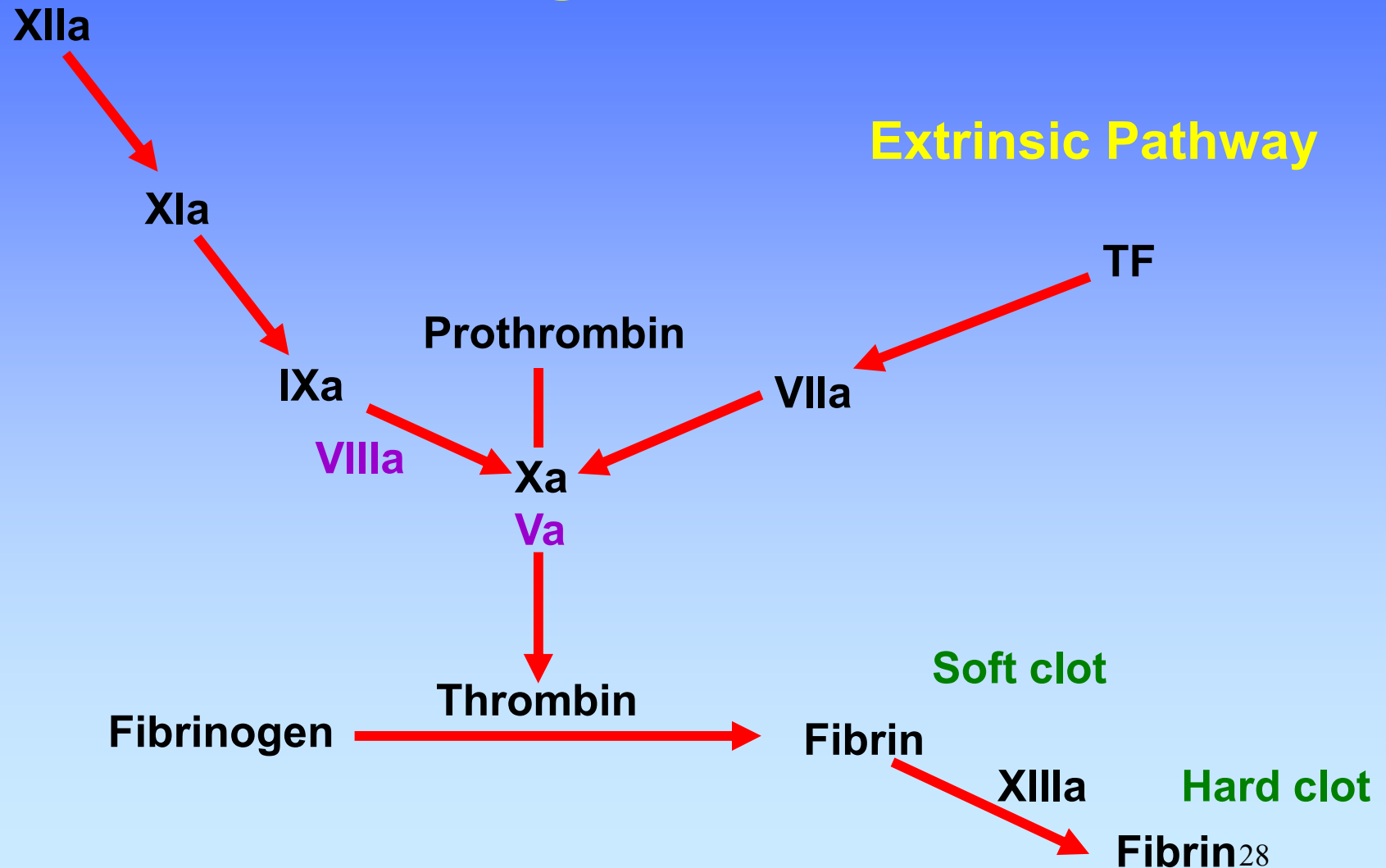
Extrinsic Pathway



Intrinsic pathway

Coagulation

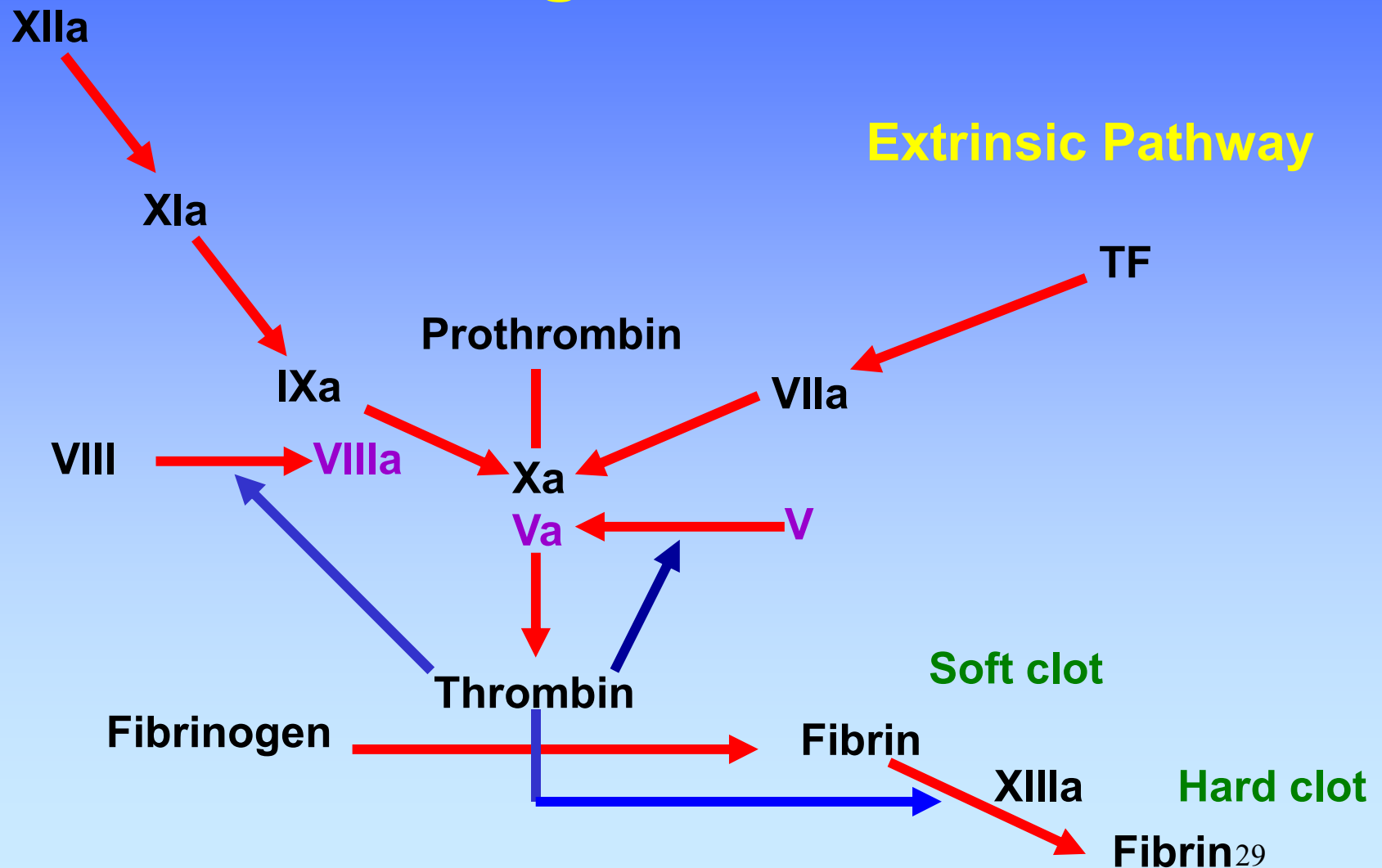
Extrinsic Pathway



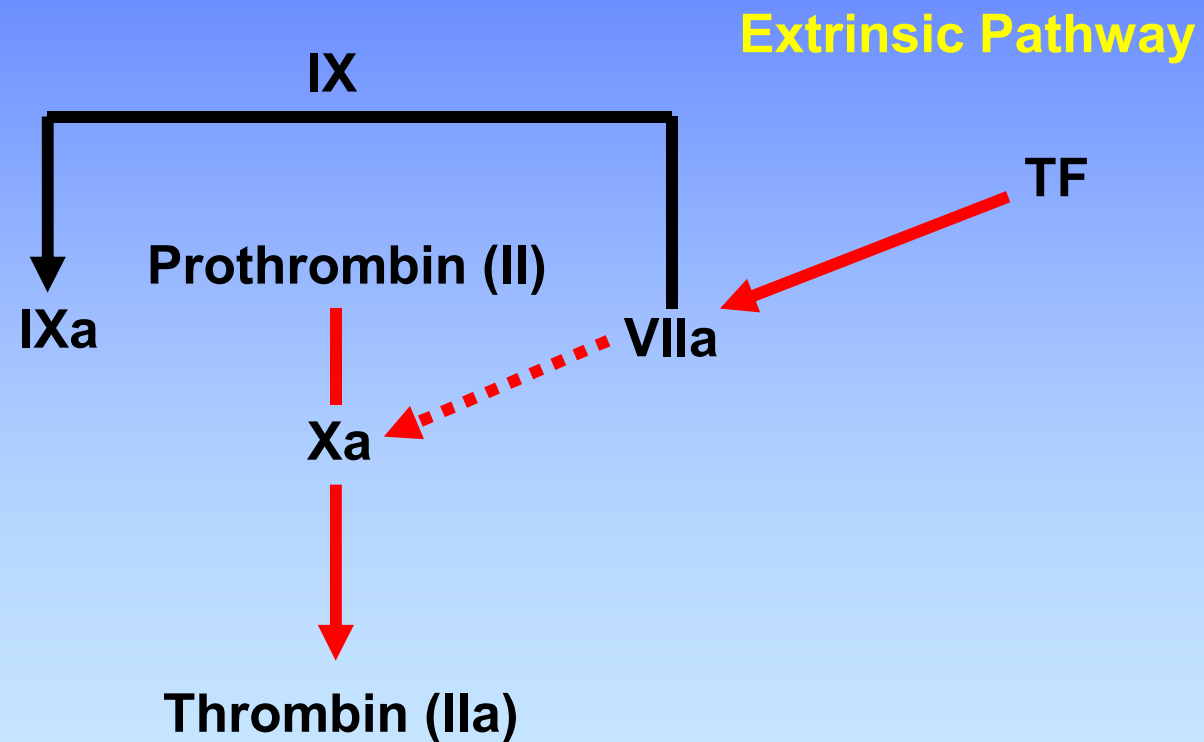
Intrinsic pathway

Coagulation

Extrinsic Pathway

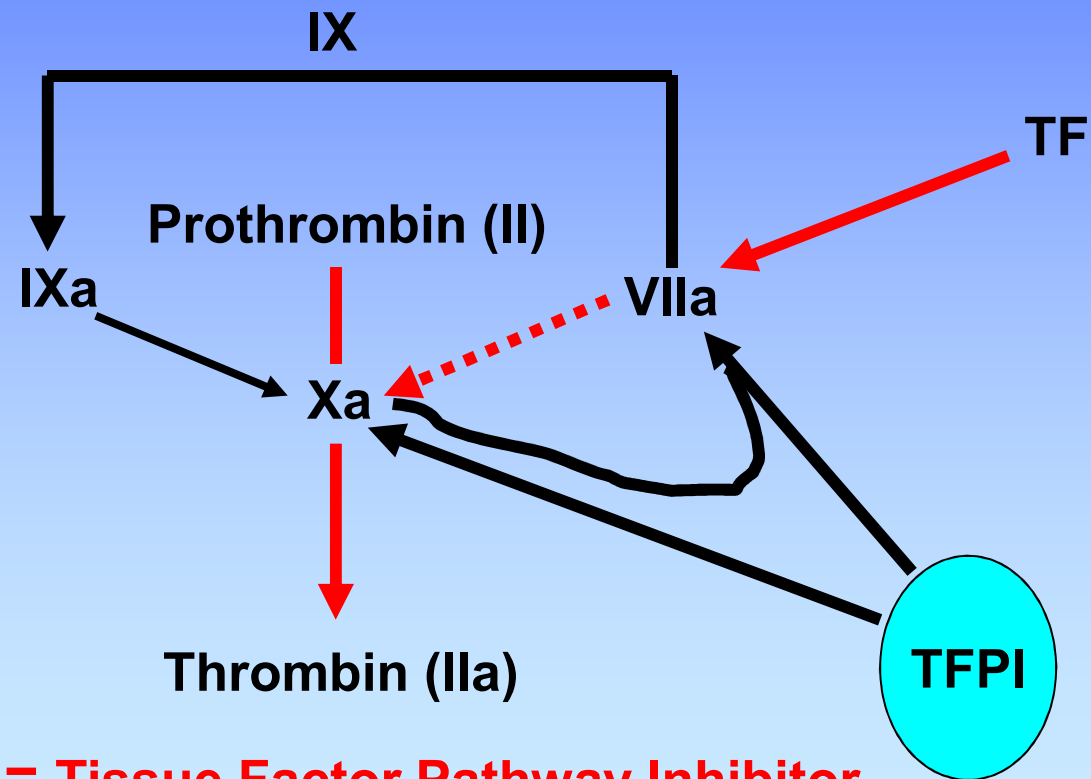


Revised tissue factor pathway



New: Production of IXa
Interaction of intrinsic and extrinsic pathways

Revised tissue factor pathway



New: TFPI = Tissue Factor Pathway Inhibitor
... inhibition of Xa and VIIa

Revised tissue factor pathway

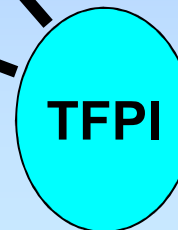
TFPI is protease inhibitor

34 and 41 kD forms in plasma (C-term truncation)

Activities:

- direct inhibition of Xa
- inhibition VIIa-TF complex in a [Xa]-dependent manner
- binding to LDL, HDL and Lp (a)

~10% present in platelets (endothelium also)



New: TFPI = Tissue Factor Pathway Inhibitor
... inhibition of Xa and VIIa

Revised tissue factor pathway

Net results:

Production of IXa

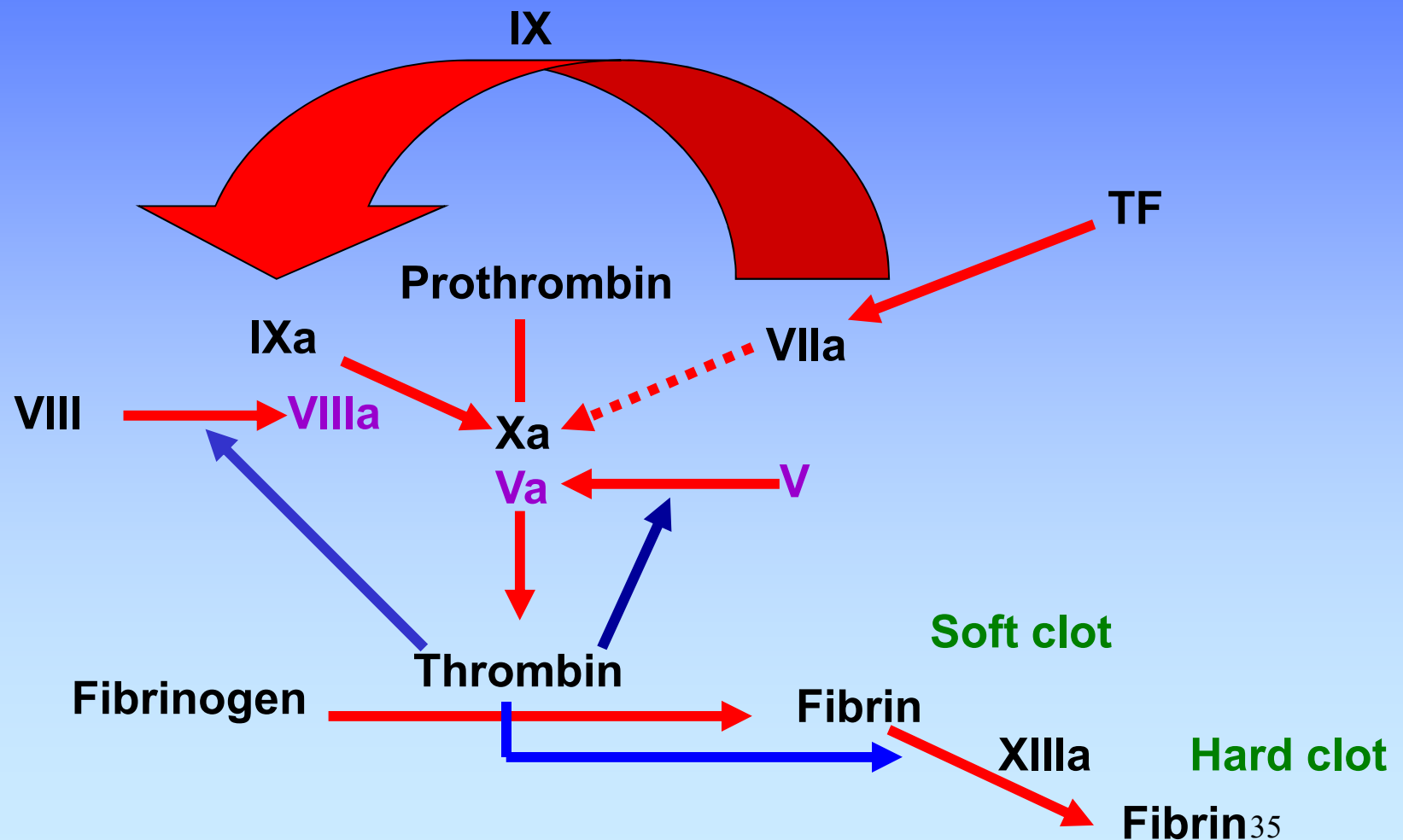
Production of small amounts of
thrombin (IIa)

No or only little fibrin formed!

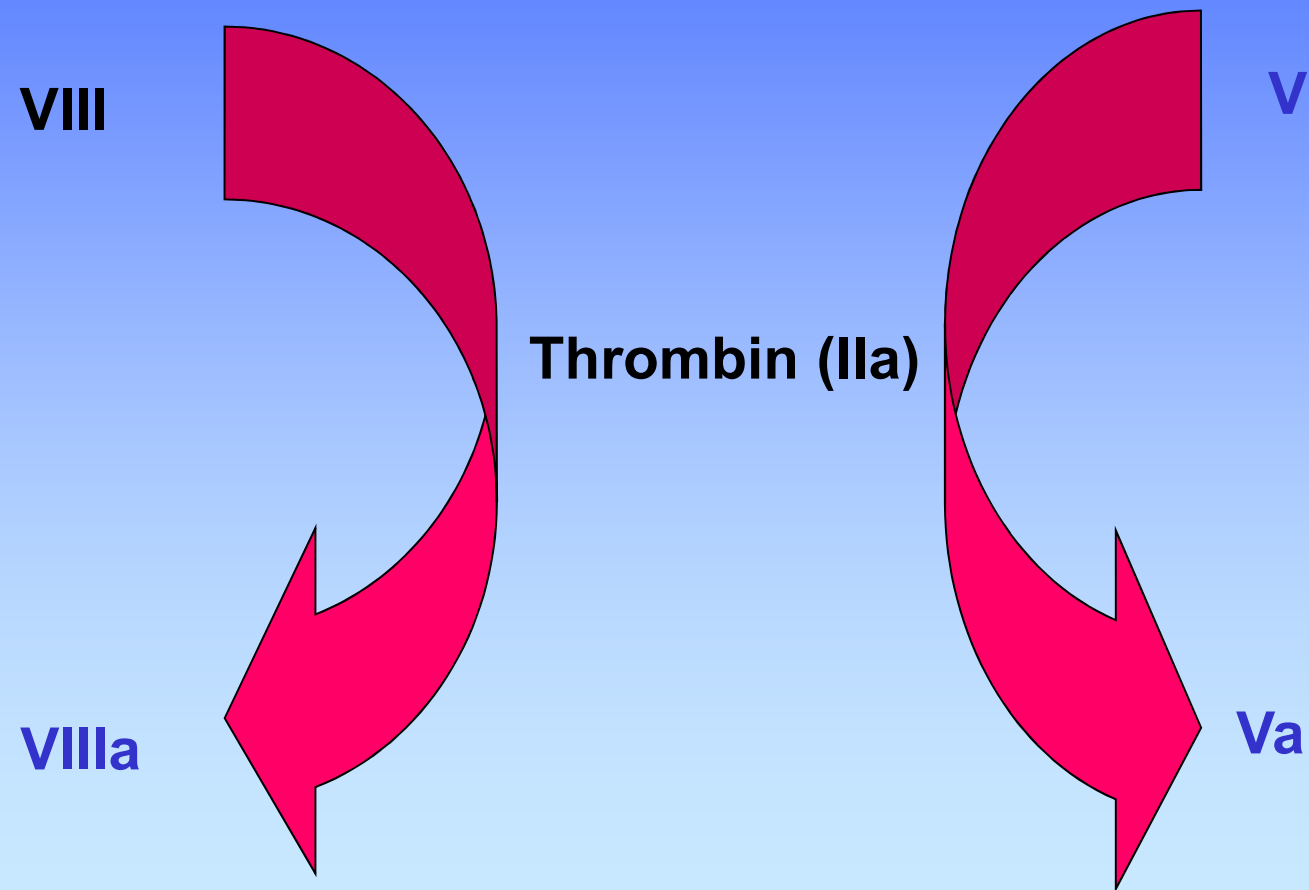
Revised tissue factor pathway

- 
- VIIa forms via binding of VII to TF
 - VIIa activates some $X \rightarrow Xa$
 - Xa converts a small amount of II to IIa; **this thrombin is used to produce small amts of VIIIa and Va**
 - As the concentration of TF-VIIa-Xa-IIa increases, **TFPI inactivates this complex** stopping further production of thrombin.
 - **IXa, with VIIIa** (produced as above), produces Xa; this Xa with Va **produces new thrombin**; this thrombin produces more VIIIa and Va and then we get lots of thrombin and fibrin.

Revised tissue factor pathway



Revised tissue factor pathway



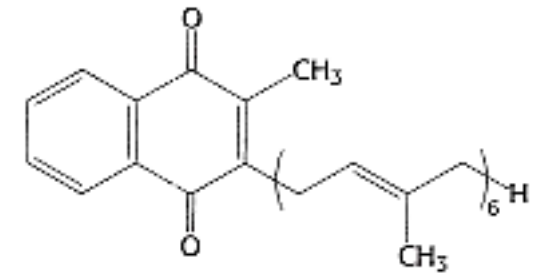
Role of vitamin K

Factors II, VII, IX, X, proteins C and S require a post-translational modification (PTM) before their activation

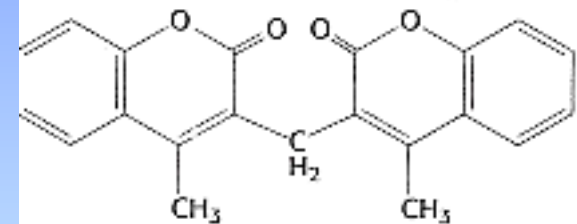
This PTM requires vitamin K

This PTM involves the addition of a COO^- to certain Glu residues in the clotting factors

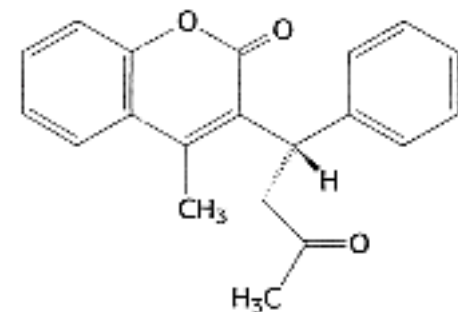
resulting in the formation of several gamma-carboxy glutamates



Vitamin K

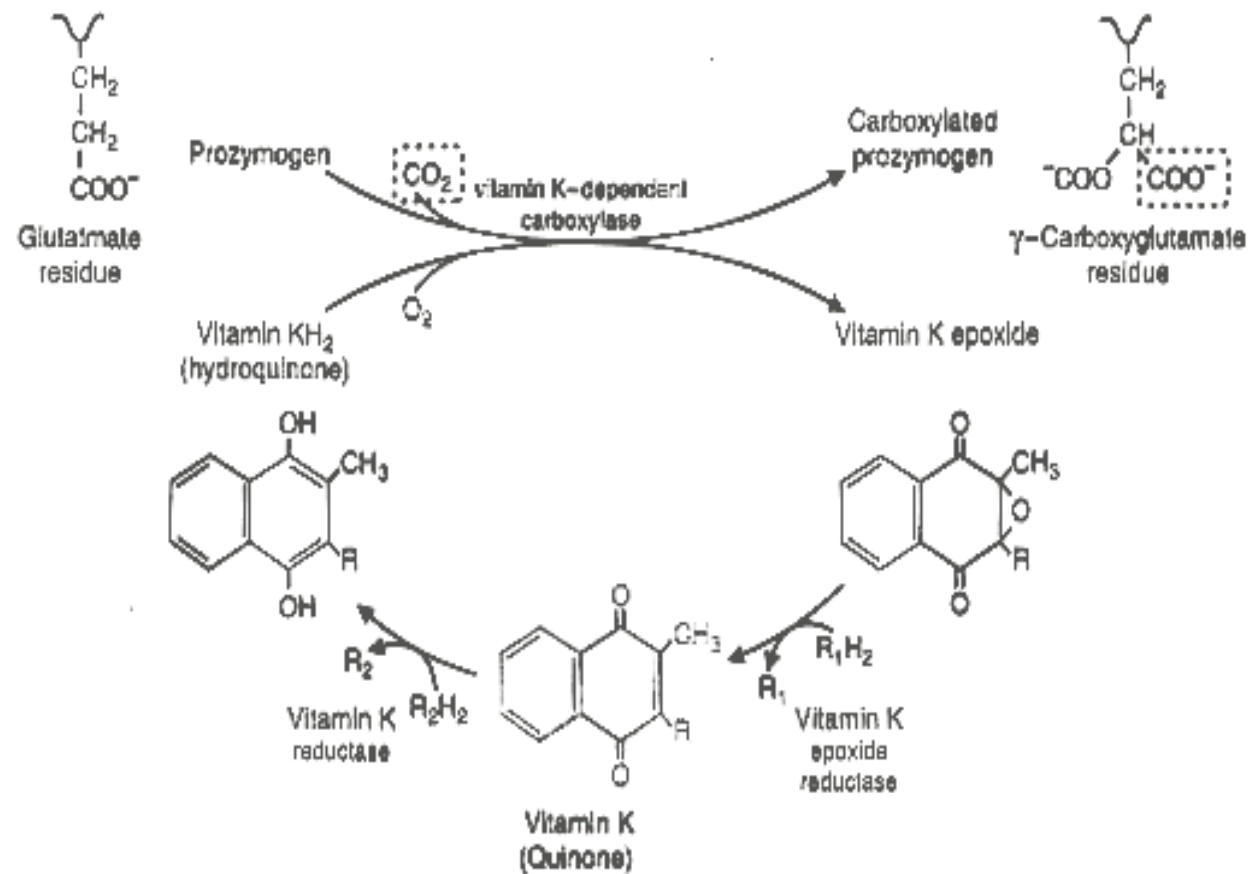


Dicoumarol



Warfarin

Role of vitamin K

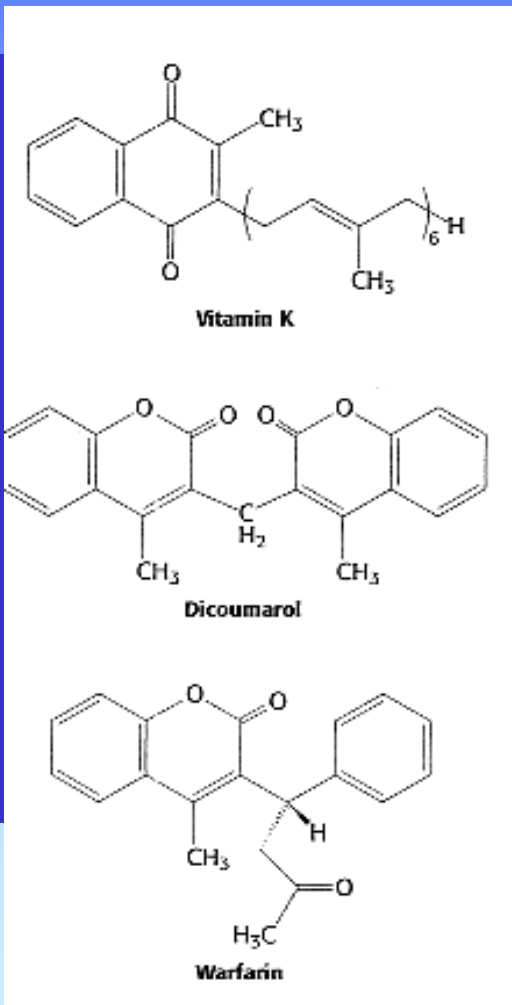


Physiologic inhibitors of coagulation

- **Antithrombin III**
 - SERPIN
- **Activated Protein C + protein S**
 - Inactivates Va and VIIIa (via proteolysis)
 - mutation: Factor V Leiden (APC resistance)
- **Thrombomodulin**
 - Binds to thrombin
 - Decreases ability to produce fibrin
 - Increases ability to activate Protein C

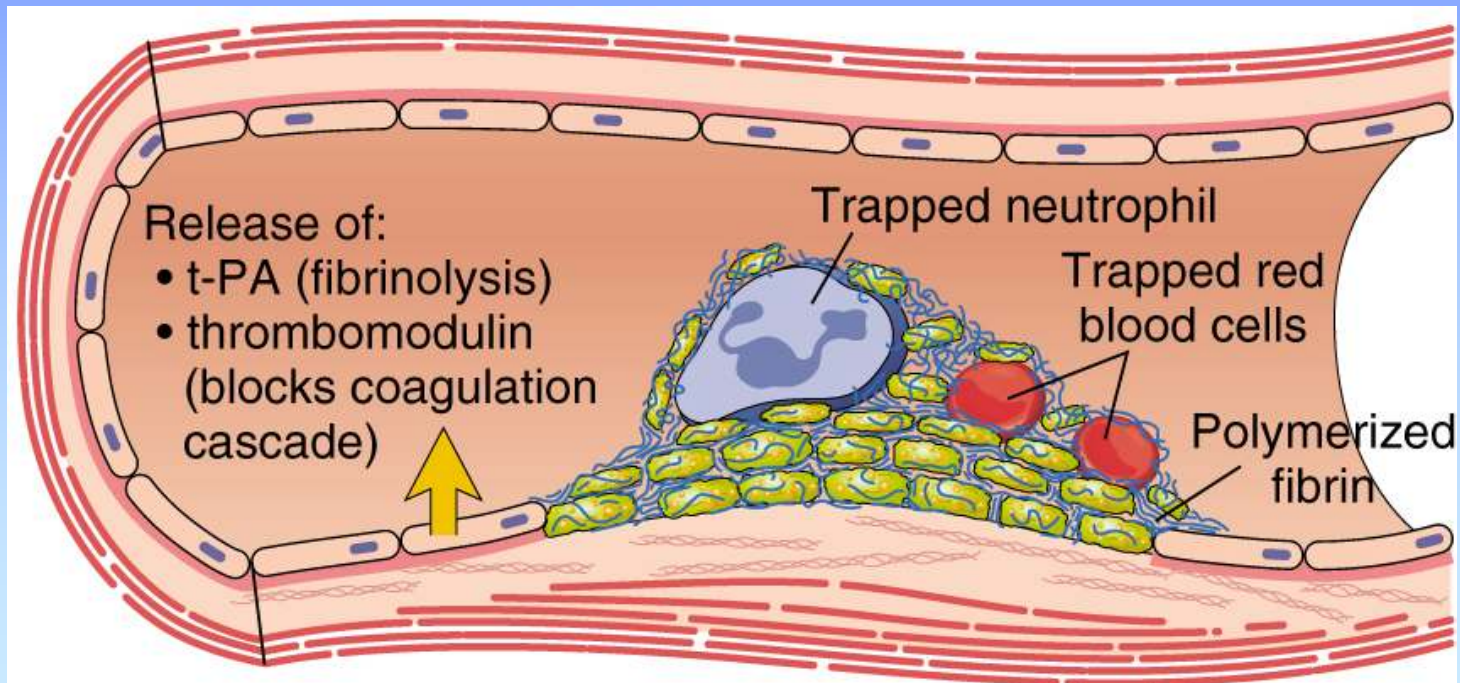
Non-physiologic inhibitors of coagulation

- **Vitamin K antagonists**
(in vivo only)
- **Ca chelators**
(in vitro only)
 - EDTA
 - Citrate
 - Oxalate
- **Heparin**
(in vivo and in vitro)



Fibrinolysis

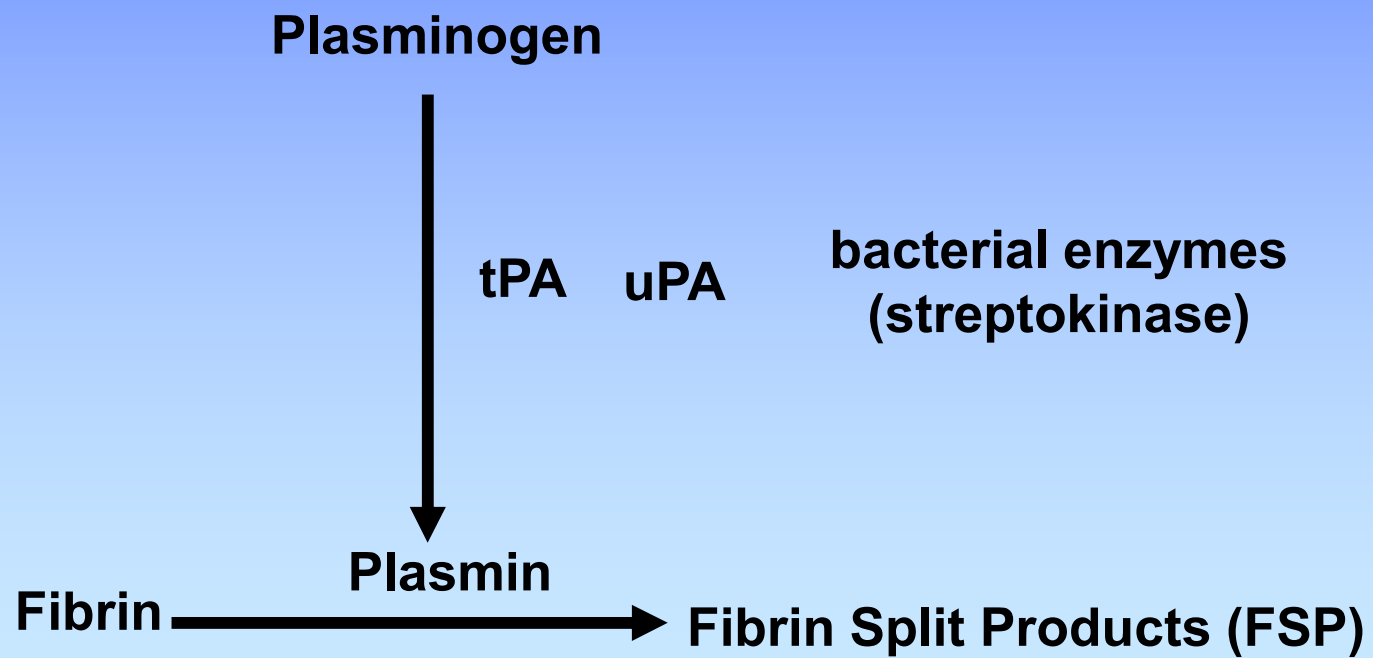
... Clot removal



Fibrinolysis

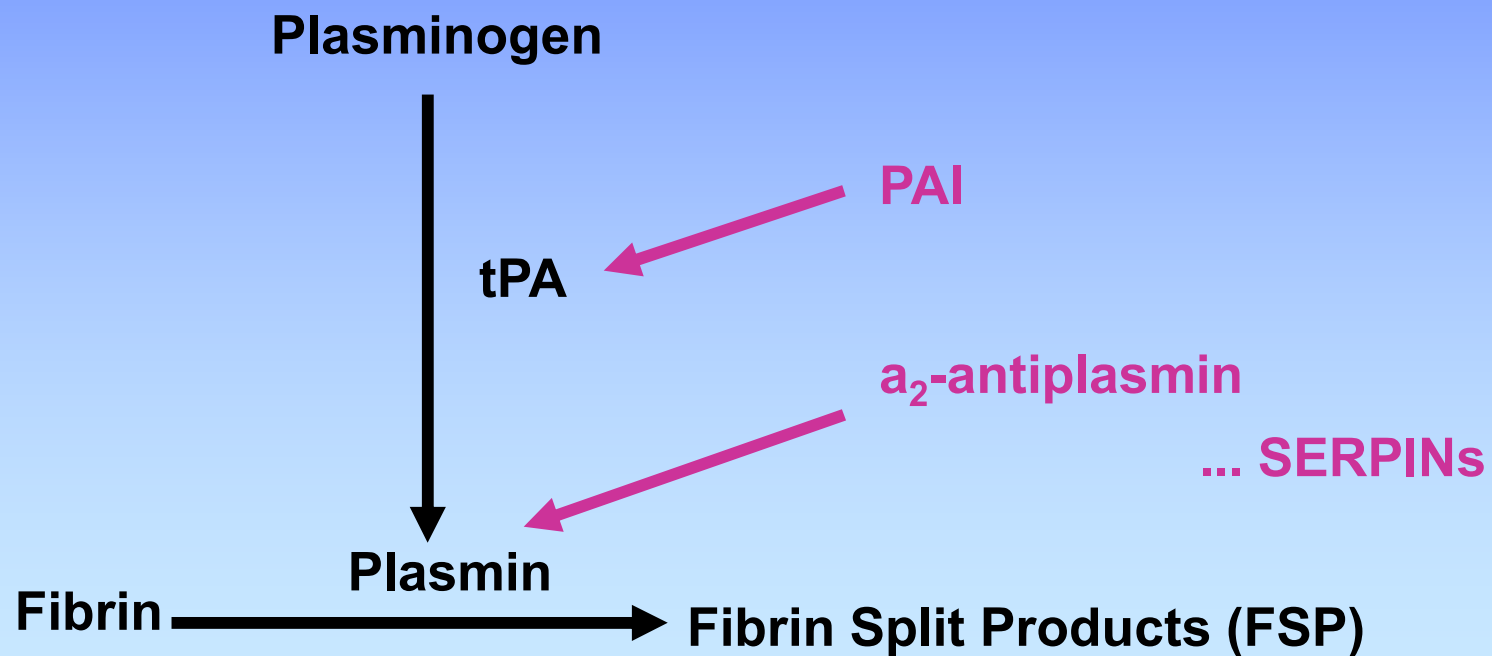
Fibrin $\xrightarrow{\text{Plasmin}}$ **Fibrin Split Products (FSP)**

Fibrinolysis

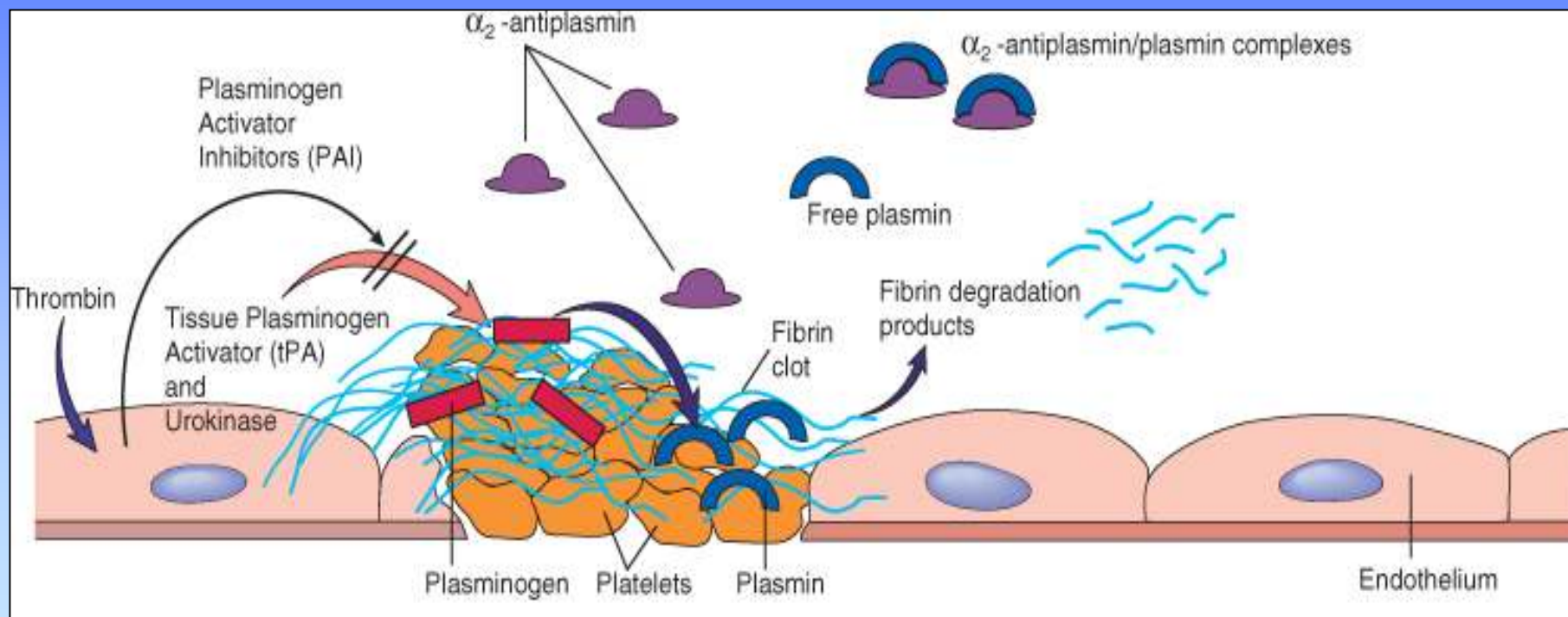


Fibrinolysis

Inhibitors of fibrinolysis



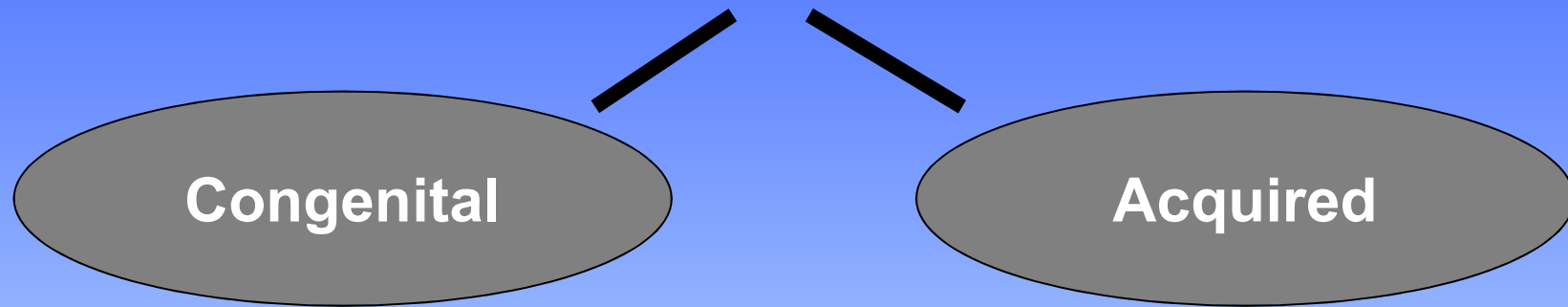
Fibrinolysis



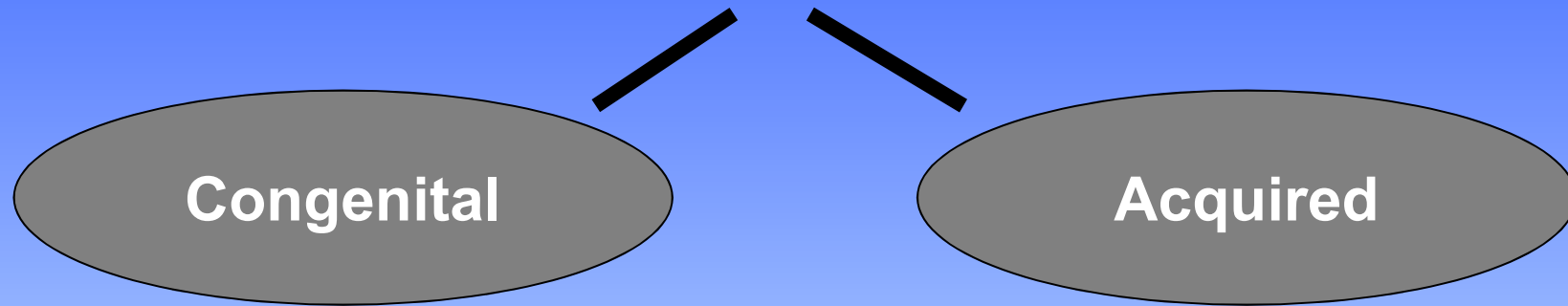
II. Pathology



Coagulopathies

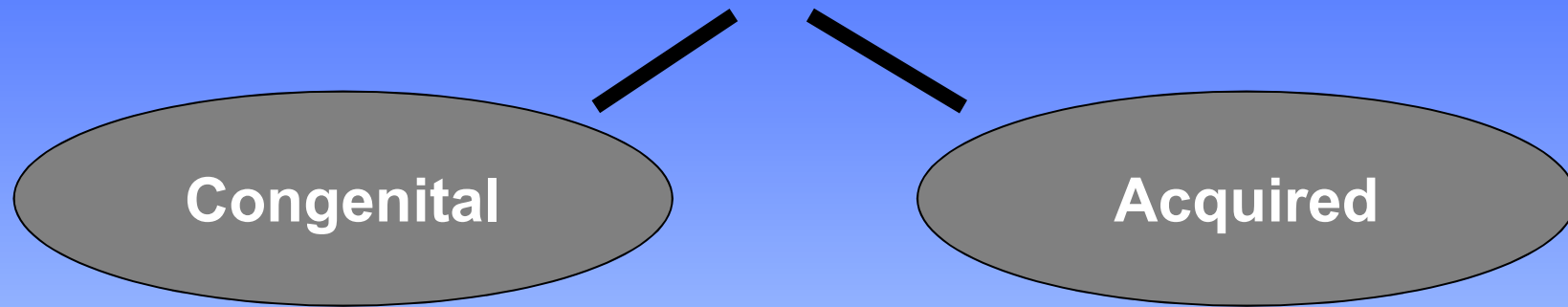


Coagulopathies



Hemophilia A ... f VIII
Hemophilia B ... f IX
Hemophilia C ... f XI
Dys- / A- fibrinogenemia
F V defic. (parahemophilia)
F XIII defic.
APC resistance

Coagulopathies



Liver proteosynthesis

Vitamin K defic.

- obstructive icterus
- intestin. resorption

Anticoagulant therapy

- Dicumarol
- Heparin

Vasculopathies

Congenital

Mb. Rendu-Osler-Weber

= hereditary hemorrhagic
teleangiectasia
AD, TGFbeta1 rec.

Ehlers-Danlos Sy.

= defects in collagen
synthesis



Acquired

Purpura Henoch-Schönlein

Scorbut

Steroid purpura

Purpura simplex and senilis



Rizikové faktory a příklady žilních uzávěrů

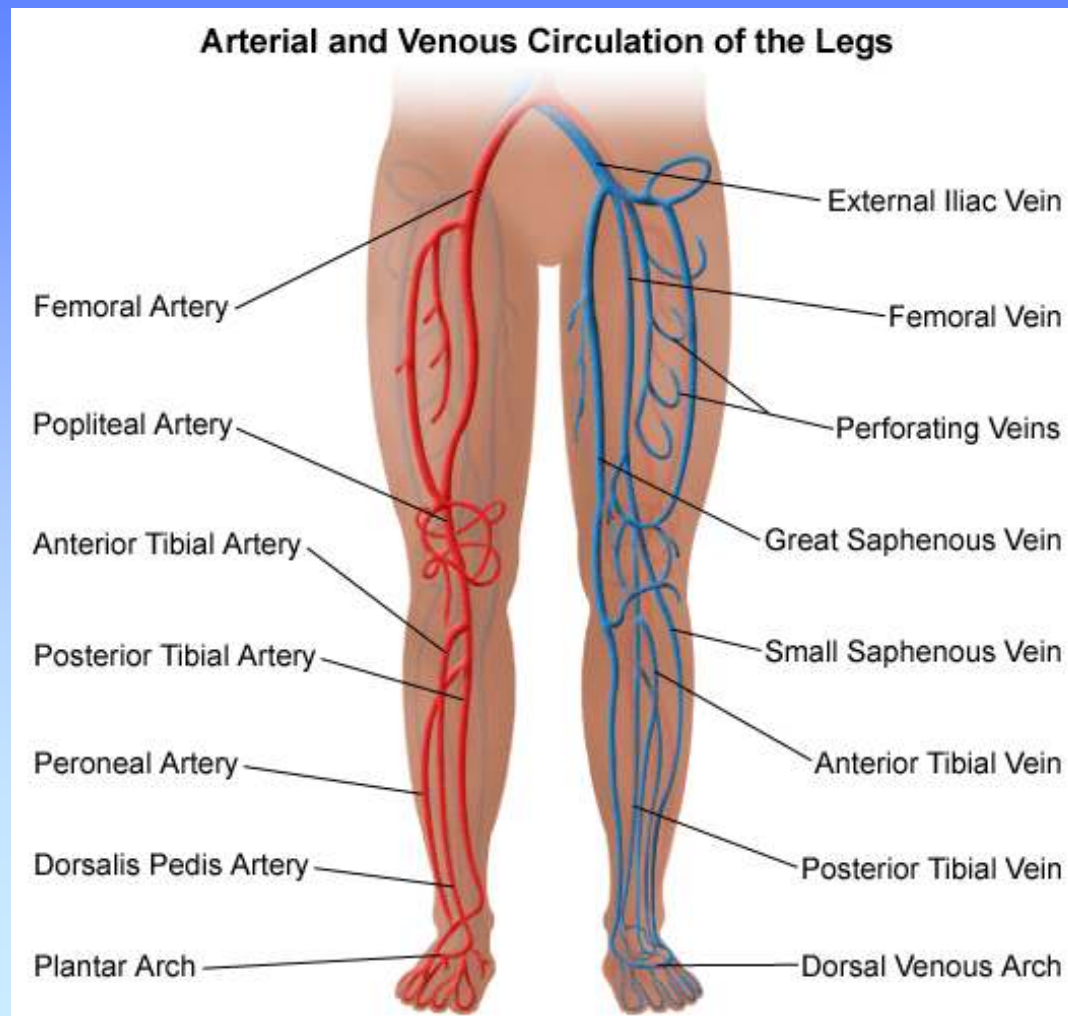
Rizikové faktory vzniku žilní trombózy:

- útlak cévy zevnějšku (např. trombóza hlubokých žil levé dolní končetiny je cca 3x častější než pravé dolní končetiny.... Proč?)
- hyperviskozita (dehydratace, polyglobulie, leukémie, hyperfibrinogénémie aj.)
- městnání krve v žilním systému (deficientní chlopně žil, útlak žil zevnějšku)
- imobilita
- obezita
- nadměrná aktivace sekundární hemostázy (např. u infekcí, zánětů, malignit, v těhotenství)
- samostatnou kapitolou jsou vrozené trombofilní stavy.

Příklady žilních uzávěrů:

- flebotrombóza DK**= trombóza **hlubokých** žil dolních končetin
- tromboflebitida DK**= trombóza **povrchových** žil dolních končetin
- plicní tromboembolismus**
- trombóza viscerálních žil** (např. trombóza vrátnicové žíly, trombóza jaterních žil: tzv. **Budd-Chiariho syndrom**)
- Trousseauův příznak** (migrující tromboflebitida u nádorových onemocnění)
- samostatnou kapitolou jsou žilní uzávěry u **chronických hemolytických anémií** a **klonálních poruch krvetvorby** (MPN, PNH)

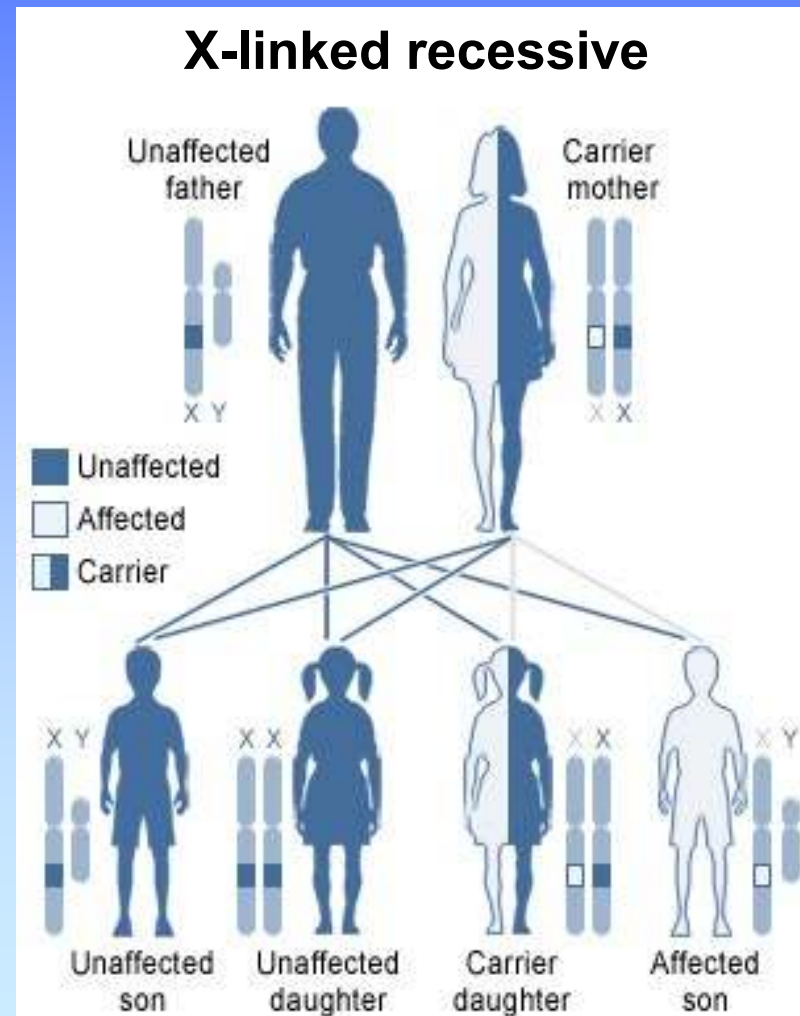
Trombóza hlubokých žil levé dolní končetiny
vzniká cca 3x častěji než trombóza pravé dolní
končetiny



Genetic examination

Hemophilia A

1 : 10 000



Clinical signs



Hemophilia

Large hemorrhage after a small injury
Arthral hemorrhage
Secondary arthropathy

Clinical signs



Thrombocytopenia



Petechiae, pigmentation

Clinical signs



Henoch-Schonlein

Clinical signs



F XIII deficiency

Late bleeding
Keloid scarring

Clinical signs



Deep venous thrombosis

Pulmonary embolism

III. Diagnostics and monitoring



Standard tests in Faculty General Hospital

Quick time, INR	0,8 - 1,2
Act.Part.Thromb.Time	27-35 s
Thrombin time	12 - 14 s
Fibrinogen	2 - 4 g/l
Antithrombin III	> 70%
Ethanol test	neg.
D-dimers (FDP)	neg.

Prothrombin Time (Quick test)

Principle: Stimulation of extrinsic (main) coag. system

Citrate plasma ... add TF (in excessive amount) + CaCl_2 ... fibrin fibre

Normal: PT = 12 - 15 s

$\text{INR} = (\text{PT}_p)^{\text{ISI}} / \text{PTN}$

ISI = international index of sensitivity of used thromboplastin (commonly > 1)

Prolongation: defic. vit. K dep. FII, VII, X, $\downarrow\downarrow$ Fbg

Usage: screening, monitoring of oral anticoagulants, liver proteosynthesis

Normal range

INR 0,8 - 1,2

Therapeutic range

INR = 2,5 - 4,5

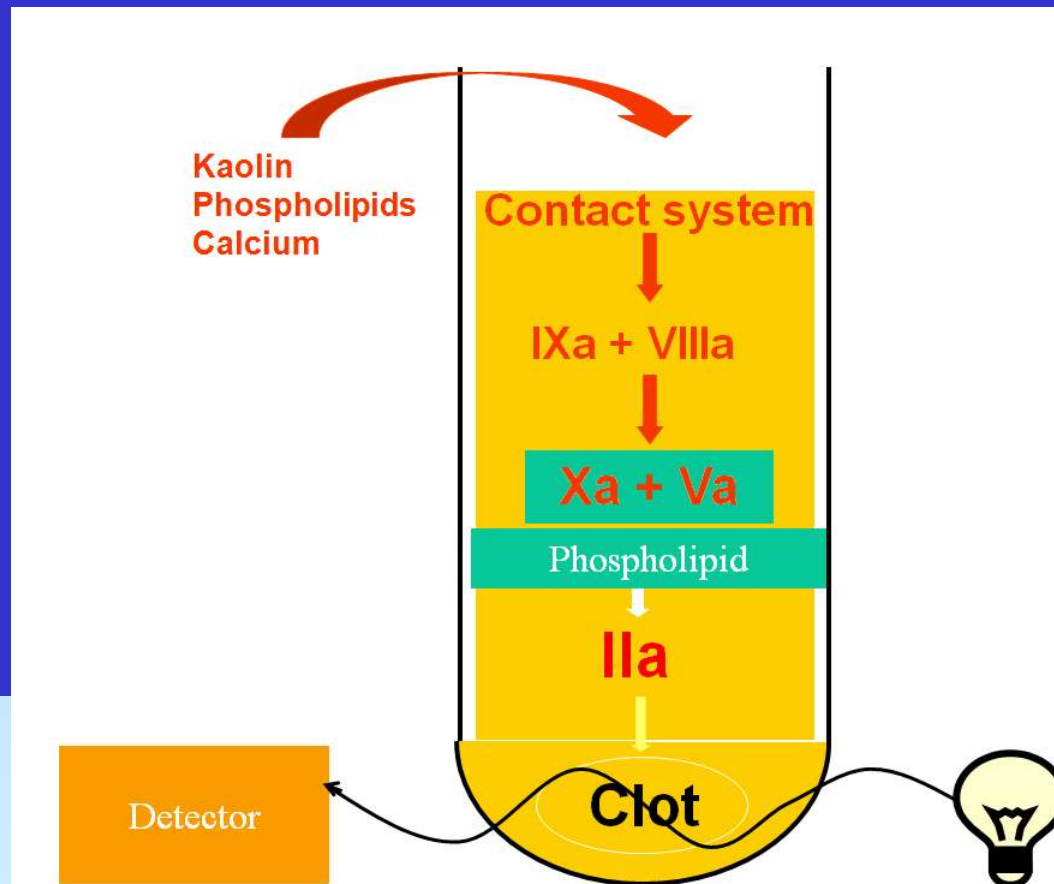
Surgery

INR < 1,6

APTT, Activated partial thromboplastin time

Principle: Stimulation of intrinsic (contact) way of coag. system

Citrate plasma ... add contact activator (e. g. kaolin) + CaCl_2 ... fibrin fibre



APTT, Activated partial thromboplastin time

Principle: Stimulation of intrinsic (contact) way of coag. system

Citrate plasma ... add contact activator (e. g. kaolin) + CaCl_2 ... fibrin fibre

Normal: APTT = 27 - 35 s

**Prolongation: defic. of VII, V, X, XII, VIII, XI, IX
(hemophilia A,B,C), $\downarrow\downarrow$ Fbg, $\uparrow\uparrow$ FDP**

Shortening: prothrombotic status

**Usage: screening, diagnostics of coagul. deficits,
monitoring of heparin therapy**

Therapeutic range 1,2 - 2,5 x

Lee-White test

Cloting time of whole blood

Whole blood without anticoagulants (CaCl_2) ...
polystyrene or glass tube, 37°C ...
spontaneous stimulation of intrinsic

Normal: 4 - 10 min.

Usage: Basic, rough orientation in acute status

Thrombin Time

Whole blood without anticoagulants (CaCl_2) ... add thrombin in standard amount, 37°C ... fibrin fibre

Normal: 12 - 14 s

Prolongation:

↓↓ Fbg (acute stage of DIC)
antithrombins
fibrinolysis

Usage: DIC

monitoring of fibrinolytic therapy

Fibrinogen, Fbg

Normal plasma levels = 2 - 4 g /l
Functional of immunological detection

High: Inflammation

DM

Smoking

Low: Low synthesis (congenital or low liver function)

Consumption (DIC)

Hypofibrinogenemia

Dysfibrinogenemia

FDP

Total degradation products of fibrin(-ogen)

ELISA or agglutination semiquantitative methods

**High: Recent coagulation activity
(thrombo/ embolism, bleeding, surgery, DIC ...)**

High sensitivity, low specificity

Paracoagulation tests (Ethanol, Protamin)

Principle: Ethanol catalyzes conversion of fibrin monomers + PDP → fibrin polymers

Low sensitivity and specificity

Usage: 1st stage of DIC

Duke test

Duke, 1910

Estimation of bleeding time

**Time of spontaneous cutoff of bleeding after
standard puncture to auricle of ear**

Limits: 2 - 5 min., or 4 - 8 min. (depends on methods)

**Prolongation - Disturbance of primary hemostasis:
Plt < 20 000 or Plt dysfunction, vW disease**



Rumpel - Leede test

Capillary resistance

Number of petechia on forearm (area 4 x 4 cm) after a standard pressure (cuff 10,5 kPa for 10 min.) or after underpressure (Brown, 1949)

Limits: > 5 petechia ... higher capillary fragility (e.g. hereditary purpura Weber-Rendu-Osler)

Presumable results

Diagnosis	Plt	Duke	APTT	Quick	TT
Thrombocytopenia	↓	↑	N	N	N
Hemophilia A	N	N	↑	N	N
Hemophilia B	N	N	↑	N	N
Hemophilia C	N	N	↑	N	N
vWd	N	↑	N / ↑	N	N

Presumable results

Diagnosis	Plt	Duke	APTT	Quick	TT
F V defic.	N	N	↑	↑	N
F II defic.	N	N	↑	N	N
F VII defic.	N	N	N	↑	N
Warfarin / vit. K def.	N	N	↑	↑	N
Heparin i. v.	N	N / ↑	↑	N / ↑	↑
Heparin s. c.	N	N	N	N	N

Presumable results

Diagnosis	Plt	Ethan	APTT	Quick	TT
DIC 1 st stage	↓	+	↑	↑	N
DIC 2 nd stage	↓ ↓	-	↑ ↑ ↑	↑ ↑ ↑	↑ ↑

Standard tests in Faculty General Hospital

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D-dimers (FDP)	neg.

Risc factors and examples of VTE (venous thrombo-embolism)

Risc factors:

- vessel oppression (e.g. phlebo-thrombosis of left lower extremity is circa 3 times more common than phlebo-thrombosis of right lower extremityWhy is that so?)
- dehydration
- hyperviscosity
- stasis syndrom (e.g. right heart insufficiency, long airplane flight)
- immobility
- obesity
- activation of secondary hemostasis, e.g. Inflammation, infection, trauma, malignancies
- inborn hypercoagulable states

Examples:

- phlebothrombosis** of deep veins of lower extremities
- thrombophlebitis** of superficial veins of lower extremities
- lung thrombembolism**
- thrombosis of large visceral veins** (e.g. thrombosis of vena portae, hepatic vein thrombosis= **Budd-Chiari syndrome**)
- Trousseau symptom** (migratory thrombophlebitis in malignancies)
- thrombotic complications in **chronic hemolytic anemias** (sickle cell anemia, thalassemias) and **clonal disorders of hematopoiesis** (MPN, PNH)

Souhrn/ Dotazy/ komentáře ?

**Děkuji
Vám za pozornost**

- Toto je v jakékoliv formě
(PDF, PPT, PPTX atd.)
neoficiální výukový materiál
- pro interní potřebu
- nešířit
- pro dotazy kontaktujte:
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