DISORDERS OF HEMOSTASIS

INTRODUCTION

- Hemostasis is the process of blood clot formation at the site of vessel injury.
- When a blood vessel wall is disrupted, the hemostatic response must be
  - quick
  - localized
  - carefully regulated
- Abnormal bleeding or a propensity to nonphysiologic thrombosis may occur when specific elements of these processes are missing or dysfunctional.

Introduction...

- The pathways of thrombin-stimulated fibrin clot formation and plasmin-induced clot lysis are linked and carefully regulated
- When they work in coordinated harmony, a clot is laid down initially to stop bleeding, followed by eventual clot lysis and tissue remodeling.
- Abnormal bleeding can result from
  - diminished thrombin generation (factor VIII deficiency)
  - enhanced clot lysis (alpha-2-antiplasmin deficiency).
- Conversely, excessive production of thrombin (inherited thrombophilia) can lead

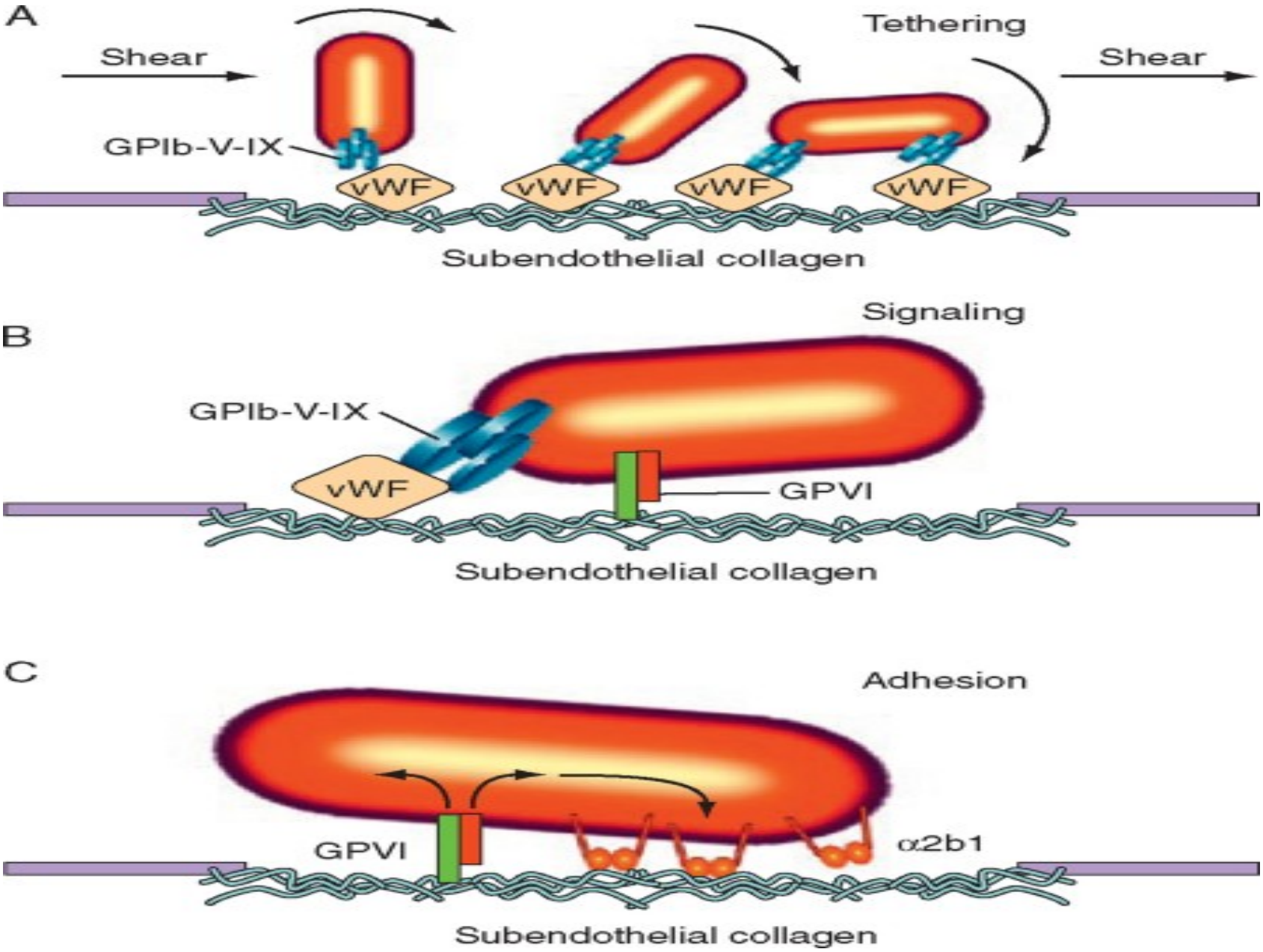
Phases of Hemostasis

- The complicated and dynamic process of hemostasis can be viewed as 4 phases.
  - Initiation and formation of the platelet plug
  - Propagation of the clotting process by the coagulation cascade
  - Termination of clotting by antithrombotic control mechanisms
  - Removal of the clot by fibrinolysis

FORMATION OF THE PLATELET PLUG

- Platelets are activated at the site of vascular injury to form a platelet plug that provides the initial hemostatic response to stop bleeding
- The functional response of activated platelets involves four different processes:
  - ***Adhesion*** — the deposition of platelets on the subendothelial matrix
  - ***Aggregation*** — platelet-platelet cohesion
  - ***Secretion*** — the release of platelet granule proteins
  - ***Procoagulant activity*** — the enhancement of thrombin generation

- Physiological stimuli for platelet activation
  - Potent stimulus
    - Collagen
    - thrombin
  - Weak stimulus
    - Epinephrine
    - ADP


A
Shear
Tethering
Shear
GPIb-V-IX
vWF
vWF
vWF
vWF
Subendothelial collagen
B
Signaling
GPIb-V-IX
vWF
GPVI
Subendothelial collagen
C
Adhesion
GPVI
α2b1
Subendothelial collagen

Some Major Subendothelial Matrix Constituents That Support Platelet Adhesion

Matrix Constituent	Comment
Collagens	Large family of proteins with certain members supporting platelet adhesion, aggregation, and secretion
von Willebrand factor	Large multimeric protein critical for hemostatic function of platelets
Fibronectin	Dimeric or multimeric protein that supports attachment and spreading of platelets
Thrombospondin-1	Trimeric protein exhibiting both adhesive and antiadhesive properties
Laminins	Proteins supporting platelet attachment
Microfibrils	Fibular bundle of protein constituents found in certain matrices

PLATELET SECRETION

- Platelets secrete a variety of substances from their granules upon cell stimulation:
  - ADP and serotonin stimulate and recruit additional platelets
  - Fibronectin and thrombospondin
  - Fibrinogen
  - Thromboxane A2
  - Growth factors, such as platelet-derived growth factor (PDGF)
  - Release of the thiol isomerase

PROCOAGULANT ACTIVITY

- Platelet procoagulant activity is an important aspect of platelet activation and involves
  - exposure of procoagulant phospholipid
  - assembly of the enzyme complexes in the clotting cascade on the platelet surface

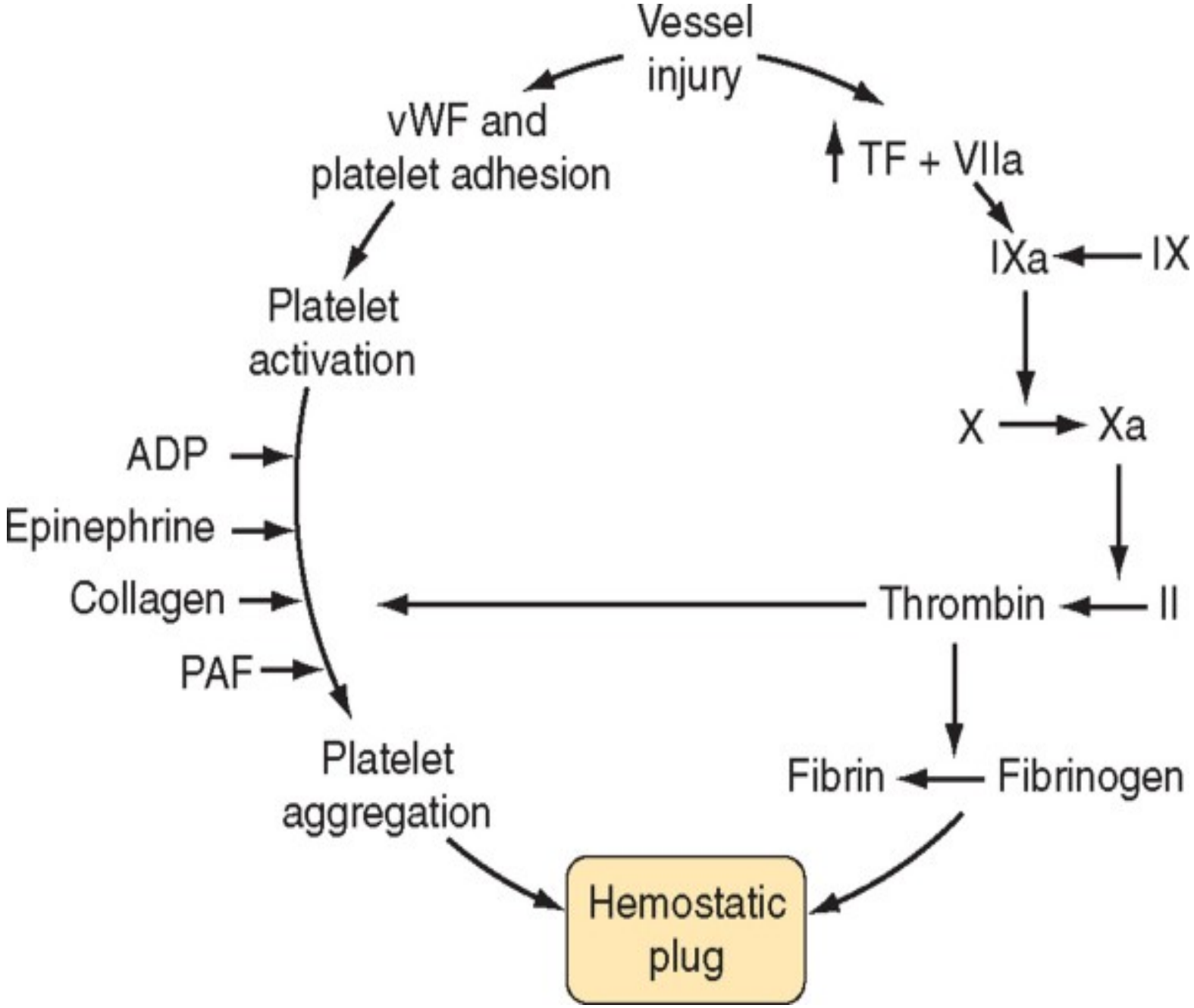
- These complexes are an important example of the close interrelationship between platelet activation and activation of the clotting cascade

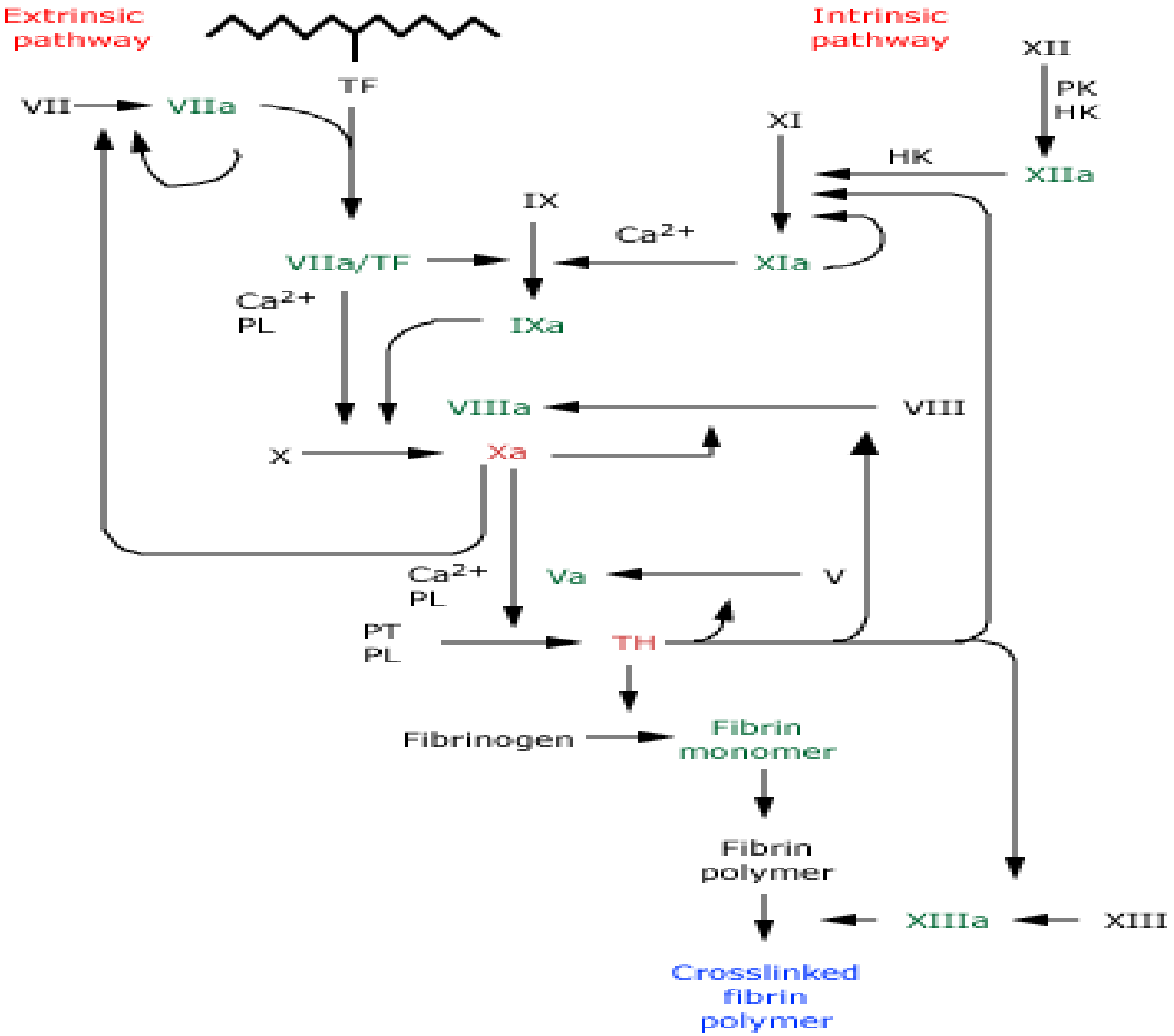
COAGULATION CASCADE

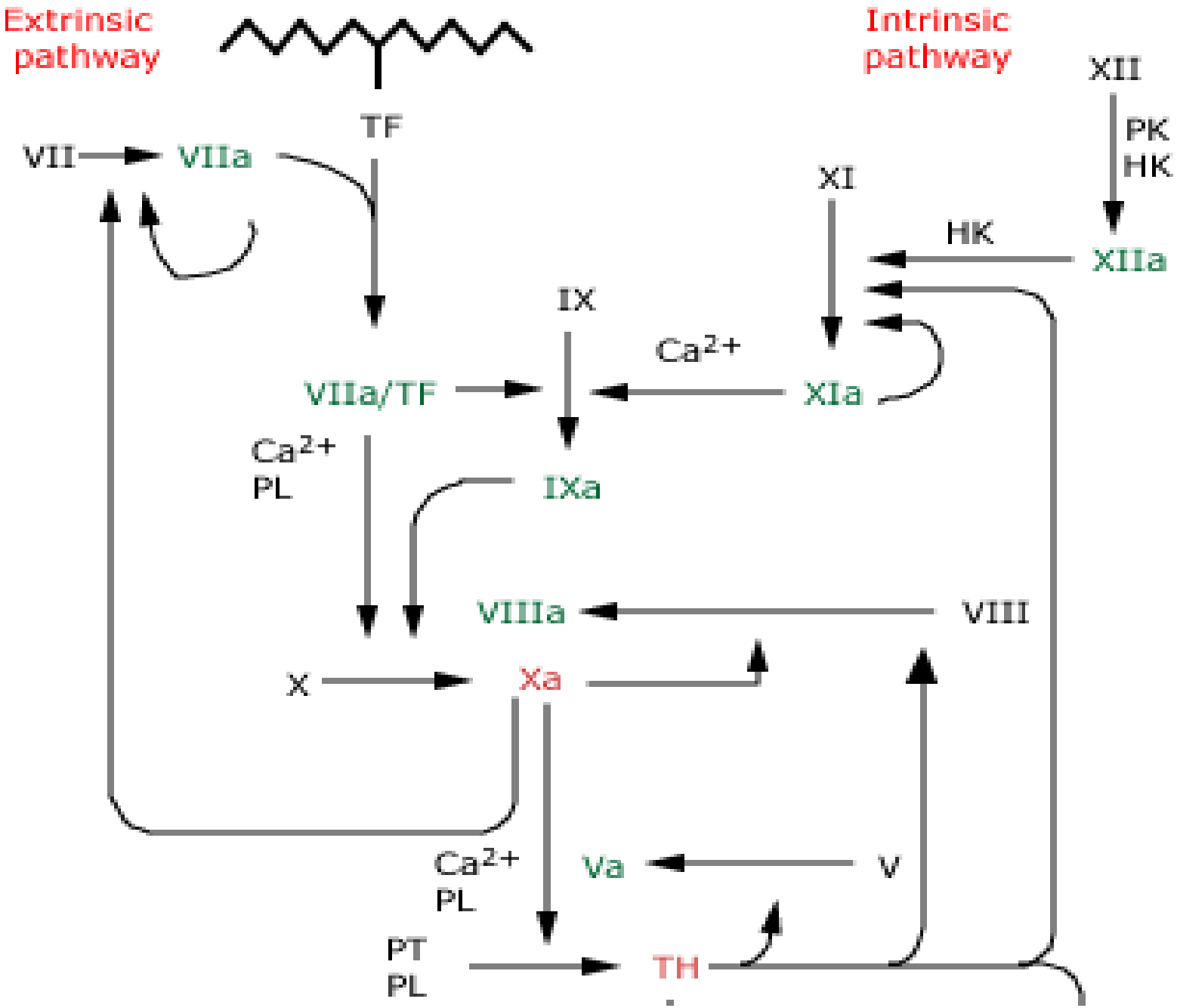
- Traditionally, the clotting cascade is depicted as consisting of an intrinsic and extrinsic pathway
- The intrinsic pathway is initiated by the exposure of blood to a negatively charged surface (such as celite, kaolin, or silica in the in vitro activated partial thromboplastin clotting time [aPTT])
- The extrinsic pathway is activated by tissue factor exposed at the site of injury or tissue factor-like material (thromboplastin, TPL in the in vitro prothrombin clotting time [PT]).

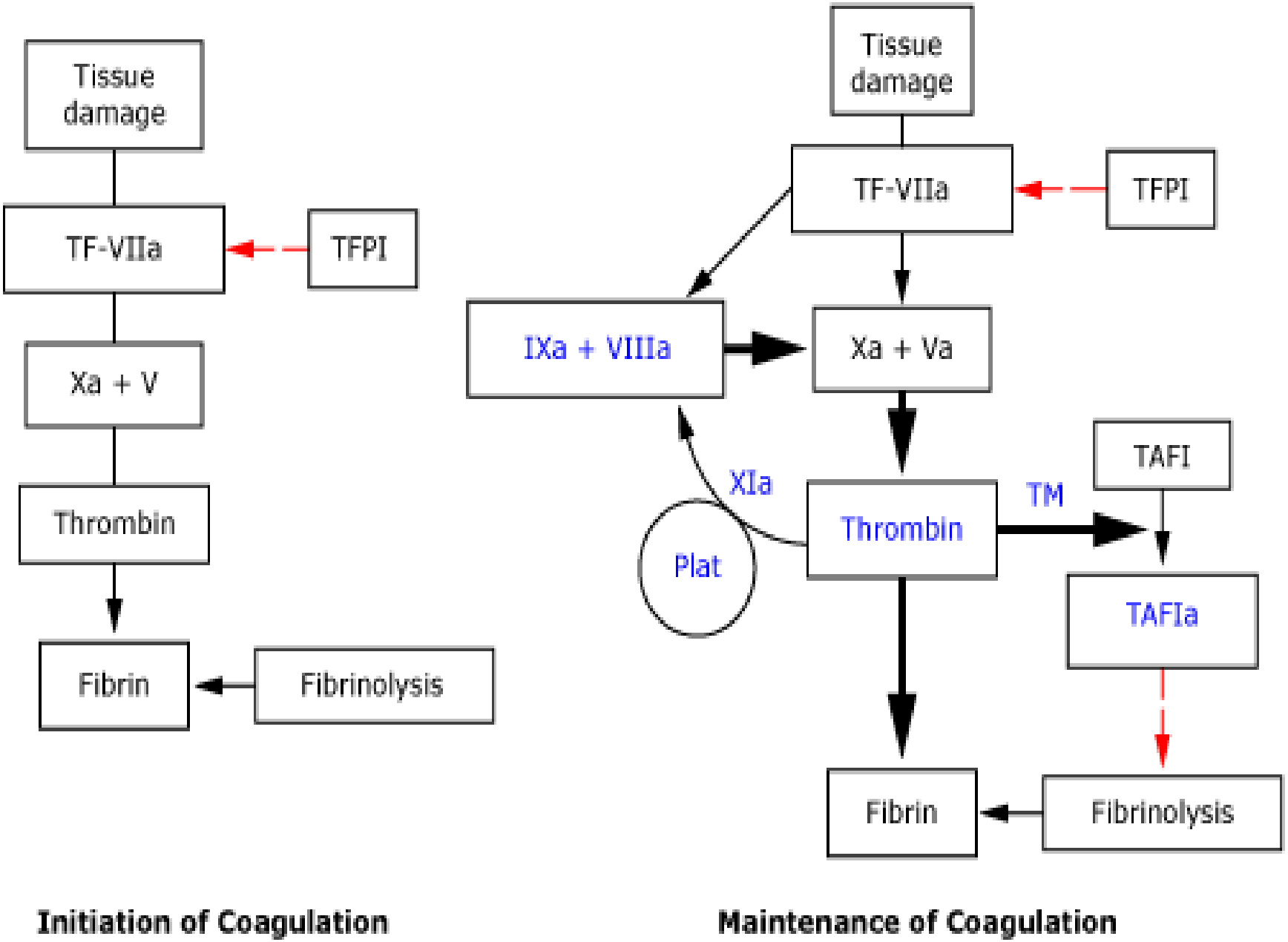
COAGULATION CASCADE

- Common pathway, both pathways converge on the activation of factor X which, as a component of prothrombinase, converts prothrombin to thrombin
- Thrombin converts fibrinogen from a soluble plasma protein into an insoluble fibrin clot.

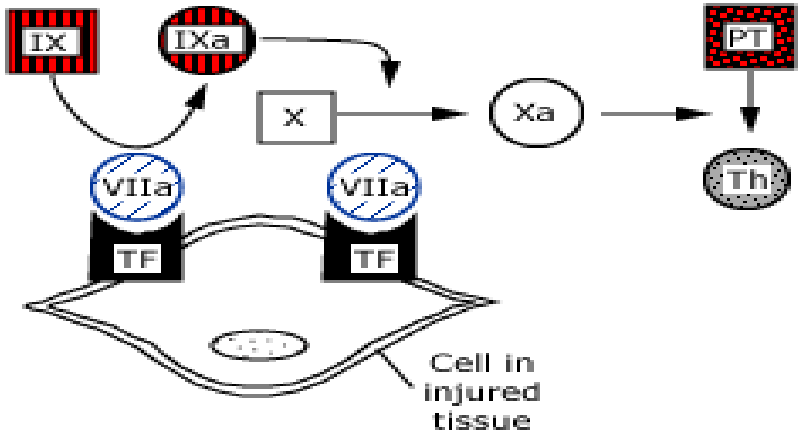

Vessel injury
vWF and platelet adhesion
TF + VIIa
IXa
IX
Platelet activation
X
Xa
ADP
Epinephrine
Collagen
PAF
Thrombin
II
Platelet aggregation
Fibrin
Fibrinogen
Hemostatic plug


Extrinsic pathway
Intrinsic pathway
TF
VII
VIIa
XII
PK
HK
XI
HK
XIIa
IX
Ca2+
VIIa/TF
XIa
Ca2+
PL
IXa
VIIIa
VIII
X
Xa
Ca2+
PL
Va
V
PT
PL
TH
Fibrinogen
Fibrin monomer
Fibrin polymer
XIIIa
XIII
Crosslinked fibrin polymer

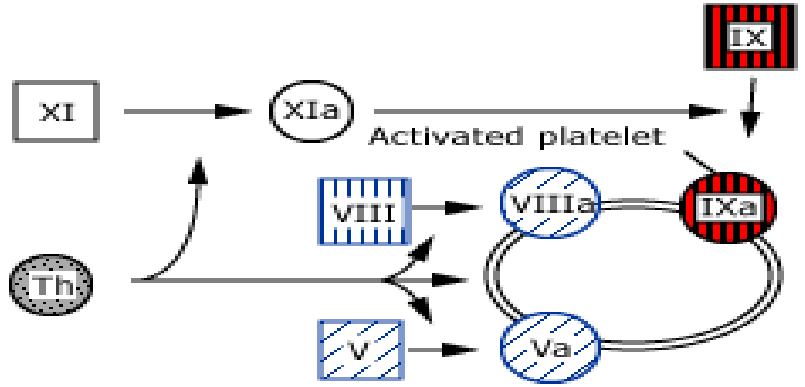

Extrinsic pathway
Intrinsic pathway
TF
VII
VIIa
XII
PK
HK
XI
HK
XIIa
IX
Ca2+
VIIa/TF
XIa
Ca2+
PL
IXa
VIIIa
VIII
X
Xa
Ca2+
PL
Va
V
PT
PL
TH


Tissue damage
TF-VIIa
TFPI
Xa + V
Thrombin
Fibrin
Fibrinolysis
Initiation of Coagulation
Tissue damage
TF-VIIa
TFPI
IXa + VIIIa
Xa + Va
XIa
Plat
Thrombin
TM
TAFI
TAFIa
Fibrin
Fibrinolysis
Maintenance of Coagulation

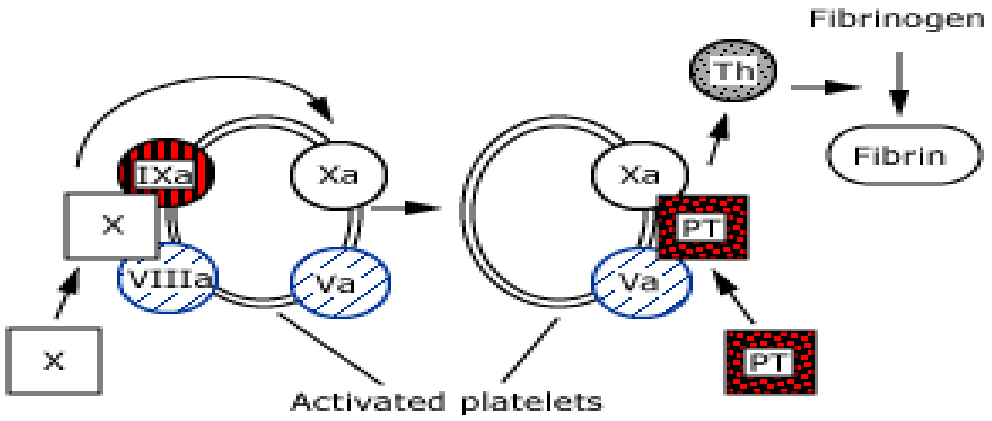
A Initiation
TF complexes with factor VIIa formed at the site of tissue injury; the subsequent activation of factor X and factor IX generate small amounts of thrombin



B Amplification
Thrombin activates platelets and cofactors (V, VII); coagulation factors and cofactors assemble on surface of activated platelets (VIIIa, Va, IXa); multiple feedback loops amplify the process



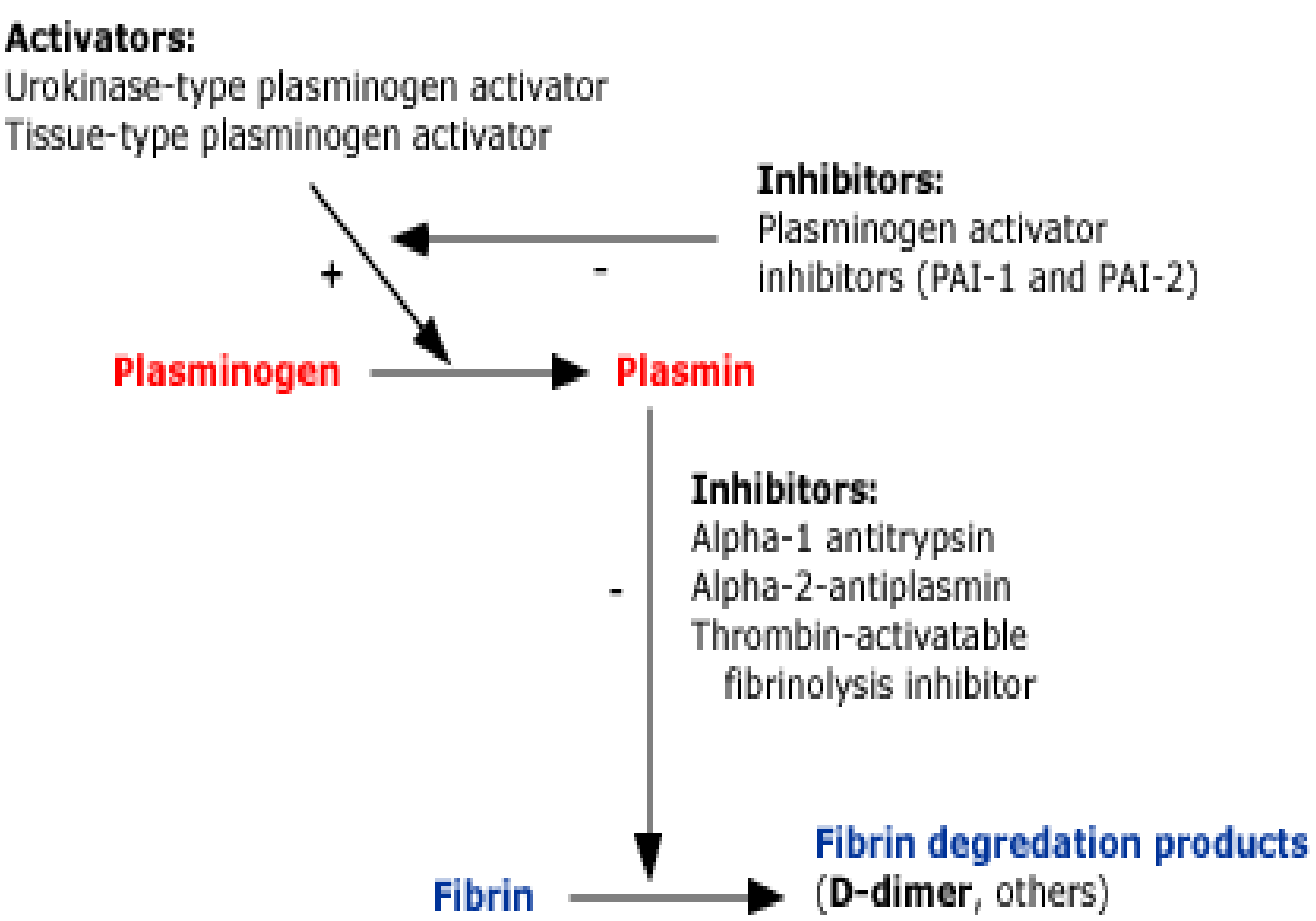
C Propagation
Assembled complexes continue cascade on surface of activated platelets; the prothrombinase complex converts prothrombin to thrombin which then converts fibrinogen to fibrin; this is followed by clot stabilization



MULTICOMPONENT COMPLEX

- Four multicomponent macromolecular complexes play a major role in the coagulation pathways:
  - three procoagulant complexes
  - one anticoagulant complex
- These complexes consist of the enzyme, cofactor proteins, calcium ions, and cellular membrane components

- Extrinsic X-ase (ten-ase) — consists of activated factor VII (factor VIIa) as the protease, tissue factor on phospholipid membranes as the cofactor, calcium ions, and factor X as the substrate
- Intrinsic X-ase (ten-ase) — consists of factor IXa as the protease, activated factor VIII (factor VIIIa), calcium, and phospholipids as the cofactors, and factor X as the substrate
- Prothrombinase — consists of factor Xa as the protease, factor Va as the cofactor, calcium, phospholipids, and prothrombin (factor II) as the substrate.
- The protein C anticoagulant complex — consists of thrombin (factor IIa) as the enzyme, thrombomodulin as the cofactor, and protein C as the substrate.


Activators:
Urokinase-type plasminogen activator
Tissue-type plasminogen activator
Inhibitors:
Plasminogen activator
inhibitors (PAI-1 and PAI-2)
+
-
Plasminogen
Plasmin
Inhibitors:
Alpha-1 antitrypsin
Alpha-2-antiplasmin
Thrombin-activatable
fibrinolysis inhibitor
-
Fibrin
Fibrin degredation products
(D-dimer, others)

APPROACH TO BLEEDING PATIENT

- The clinical approach in a patient with abnormal hemostasis includes:
  - The bleeding history
    - Medication use
    - comorbidity
  - Physical Examination
    - Description of hemostatic lesion
  - Laboratory evaluation of Hemostasis
    - Screening for primary & secondary hemostatic disorders
    - Special studies
    - Evaluation of PLT function
  - DDx for the abnormal test results

THE BLEEDING Hx

- Mode of onset&duration of bleeding tendency
- Frequency & duration of episodes
- Triggering events
- Site of bleeding
- Comorbid conditions
- Medication hx
- Hx of bleeding during procedures or stress
- Hx of delayed wound healing
- Family hx of bleeding
- Personal habits

Clinical Characteristics	BLEEDING DISORDER	
	Platelet Defect	***Clot Factor Deficiency***
Site of Bleeding	**Skin. Mucous Membrane**	**Deep in soft tissue**
Bleeding after minor cuts	**Yes**	**Not usual**
Petechiae	**Present**	**Absent**
Ecchymosis	**Small & Superficial**	**Large & Palpable**
Hemarthrosis, Hematomas	**Rare**	**Common**

PHYSICAL EXAMINATION

- The extent of bleeding
  - Vital signs
  - Transfusion & other intervention
- The pattern & site of bleeding
  - Distribution
  - IV sites
- Physical findings from comorbid conditions
  - Splenomegaly
  - Liver disease
  - Joint conditions (deformity, degree of mobility)
  - Peripheral nerves & conditions of the muscle

DESCRIPTION OF HEMOSTATIC LESION

- Petechiae
  - Pinpoint, red lesions, <2-3mm, do not blanch
  - Platelet defect or vessel wall abnormality
- Purpura
  - Large skin hemorrhage, 3-10mm
  - PLT defect or coagulation defect(less common)
- Ecchymosis (COMMON BRUISES)
  - Confluent purpuric lesions, venules & arterioles
  - Both primary & secondary hemostatic abnormalities
- Hematoma
- telangiectasias

PETECHIAE





PURPURA





ECCHYMOSIS





LABORATORY EVALUATION OF HEMOSTASIS

- Clues from Hx & P/E about the choice of tests
- 4 groups of tests
  - Screening tests for primary hemostasis
  - Screening tests for secondary hemostasis
  - Evaluation of platelet function
  - Special studies

- Screening tests for primary hemostasis
  - CBC & DIFFERENTIAL
  - PLATELET COUNT
  - PERIPHERAL BLOOD SMEAR
  - BLEEDING TIME (BT)

- Screening tests for secondary hemostasis
  - Prothrombin Time (PT)
  - Activated Partial Thromboplastin Time(aPTT)
  - Whole Blood Clotting Time
  - Thrombin Time (TT)
  - 1:1 mix (normal plasma with the patient plasma)

- Special Studies
  - Platelet aggregometry (functional studies)
  - Factor Assay
  - Clot solubility in 5M urea
  - Tests of fibrinolysis
    - Rapid clot lysis test
    - Euglobin lysis test
  - Fibrin Degradation Products(FDPs) & D-dimer test
  - Screening for thrombophilic disorders
  - Rumpel Leede Vascular fragility test
  - Other tests

- Evaluation of Platelet Function
  - BT
  - VWF assay
    - VWF Ag
    - VWF R:CoF - Ristocetin
    - FVIII- Coagulation assay (carried by VWF)
    - VWF multimer study
  - Platelet aggregometry
  - Membrane Glycoproteins(GP)
    - GP Ib/IX & GP IIa/IIIb
    - Using monoclonal Abs & Flowcytometry
  - Platelet granule content (Electron Microscope)
    - Dense granules
    - α - granules

Test	Normal Rangea (±2 SD) and Reference	Common Causes of Abnormalities
Platelet count		
Phase microscopy	**140,000–440,000/μl**	**Thrombocytopenia, thrombocytosis**
Automated	**177,000–406,000/μl**	
PTT	**26–37 sec; (51)c**	**Deficiencies or inhibitors of prekallikrein; high-molecular-weight kininogen; factors XII, XI, IX, VIII, X, and V; prothrombin or fibrinogen; lupus inhibitors; heparin; warfarin**
PT	**12.0–15.5 sec; (62)c**	**Deficiencies or inhibitors of factors VII, X, and V; prothrombin or fibrinogen; dysfibrinogenemia; lupus inhibitors; heparin; warfarin**

Thrombin timeb	**14.7–19.5 sec**	**Afibrinogenemia, dysfibrinogenemia, hypofibrinogenemia, and hyperfibrinogenemia; inhibitors of thrombin (heparin) or fibrin polymerization (fibrin degradation products, paraproteins)**
Fibrinogen assayb	**150–430 mg/dl; (66)**	**Afibrinogenemia, dysfibrinogenemia, and hypofibrinogenemia; inhibitors of thrombin or fibrin polymerization**
Factor VIII assayb	**50–150 U/dl**	**Hemophilia A and von Willebrand disease; acquired antibodies to factor VIII**

Factor VIII assayb	**50–150 U/dl**	**Hemophilia A and von Willebrand disease; acquired antibodies to factor VIII**
Fibrin degradation product assay	**0–5 µg/ml; (77)**	**Disseminated intravascular coagulation; fibrinogenolysis; thrombolytic drugs, liver disease; dysfibrinogenemia**
D-dimer assay	**0–0.4 µg/ml**	**Disseminated intravascular coagulation; recent surgery; pregnancy**

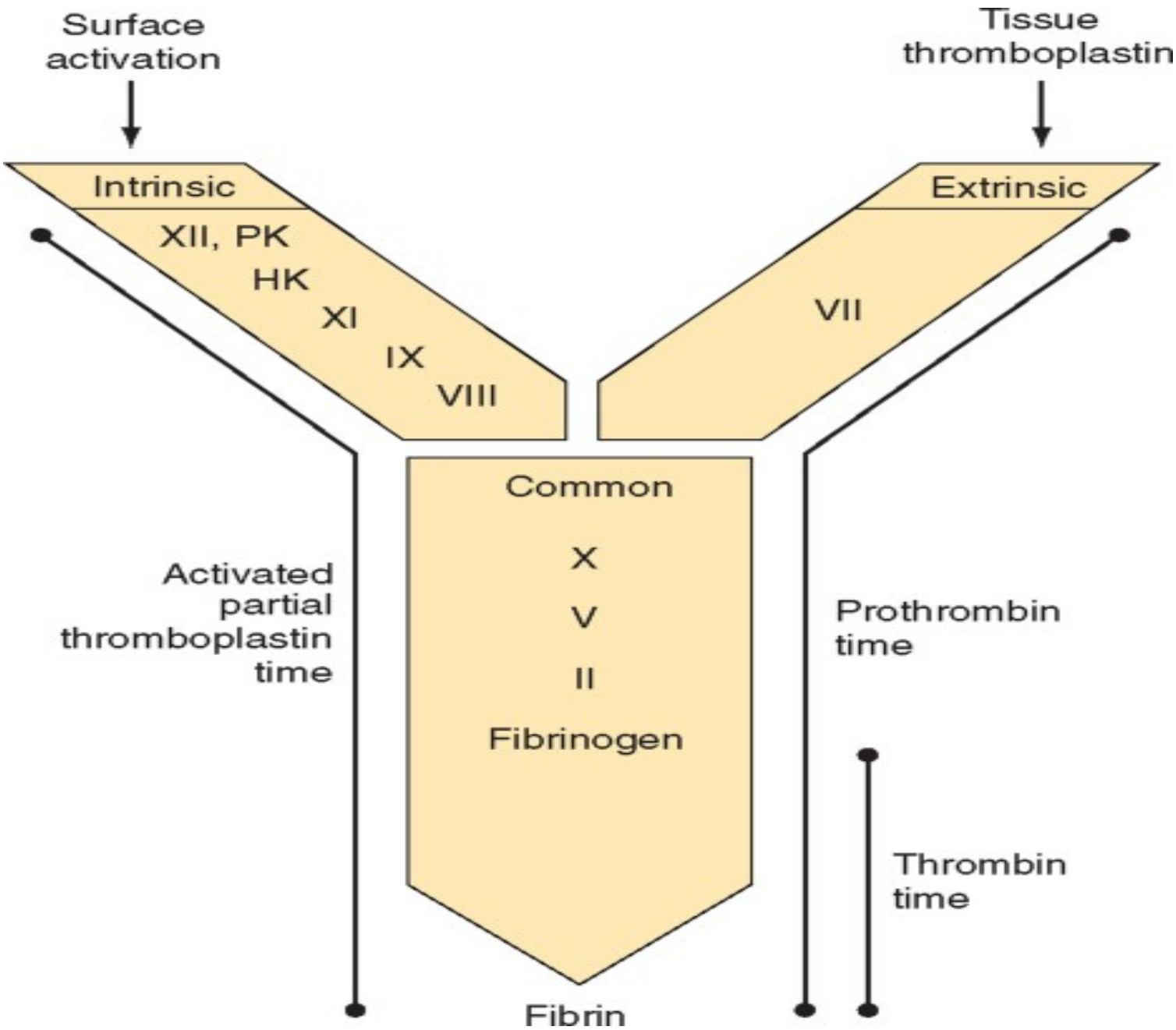
PT	aPTT	PLT	Differential Diagnosis
↑	—	—	**Common** **Acquired factor VII deficiency (early liver disease; early vitamin K deficiency; early warfarin therapy)** **Rare** **Factor VII inhibitor; dysfibrinogenemia; some cases of DIC; inherited factor VII deficiency; certain factor X variants; superwarfarin ingestion**
—	↑	—	**Common** **Deficiency or inhibitor of factors VIII, IX, or XI; vWD; heparin** **Rare** **Lupus inhibitor with qualitative platelet defect; certain factor X variants**

PT	aPTT	PLT	Differential Diagnosis
↑	↑	—	**Common** **Vitamin K deficiency; liver disease; warfarin; heparin; superwarfarin** **Rare** **Deficiency or inhibitor of factors X or V, prothrombin, or fibrinogen; lupus inhibitor with hypoprothrombinemia; DIC; dysfibrinogenemia; primary fibrinolysis**
—	—	↑	**Myeloproliferative disorders**

PT	aPTT	PLT	Differential Diagnosis
↑	↑	↓	**Common** **DIC; liver disease** **Rare** **Heparin therapy with associated thrombocytopenia**
—	—	↓	**Common** **Increased platelet destruction; decreased platelet production; hypersplenism; hemodilution** **Rare** **Certain inherited platelet disorders (Wiskott- Aldrich syndrome, Bernard-Soulier syndrome)**

PT	aPTT	PLT	Differential Diagnosis
—	—	—	**Common** **Mild vWD; acquired qualitative platelet disorders (uremia, antiplatelet medications)** **Rare** **Inherited qualitative platelet disorders; vascular disorders; fibrinolytic disorders; factor XIII deficiency; autoerythrocyte sensitization; dysfibrinogenemia; mild factor deficiency (VIII, IX, XI); disorders of platelet procoagulant activity**

DISORDER	BT	PLT	PT	aPTT	TT	FIB
Vasculopathies, CTD, disorders of collagen	Long	N	N	N	N	N or I
Thrombocytopenia	Long	Low	N	N	N	N
Qualitative PLT abnormality	Long	N	N	N	N	N
Hemophilia A	N	N	N	Long	N	N
VWD	Long	N	N	Long	N	N
DIC	Long	Low	Long	Long	Long	Low


Surface activation
Tissue thromboplastin
Intrinsic
XII, PK
HK
XI
IX
VIII
Extrinsic
VII
Common
X
V
II
Fibrinogen
Activated partial thromboplastin time
Prothrombin time
Thrombin time
Fibrin

Von Willebrand Disease

- Erik von Willebrand in 1926
- Impaired synthesis or function of vWF
- The most common inherited bleeding disorder
- Less commonly acquired forms can occur
- Functions VWF
  - Binding platelets
  - Carrier of F VIII

- Clinical features
  - Mucocutaneous bleeding , easy bruising
  - Type 2N -different
- Types
  - Type 1
    - Quantitative abnormality
  - Type 2
    - Qualitative abnormality
    - Types- 2A, 2B, 2N, 2M
  - Type 3
    - Total absence of VWF

- Diagnosis of vWF
  - History & physical exam
  - Laboratory
    - BT & platelet function analysis
    - VWF
    - VWF
    - Factor VIII level
    - VWF multimer analysis
- Treatment
  - Desmopressin
  - Replacement of VWF
  - Others (antifibrinolytic, estrogen, recombinant F VIIa

ITP

- Immune (Idiopathic) Thrombocytopenic Purpura
- Acquired condition caused by increased destruction of plts along with inhibition of megakaryocytes
- Acute (childhood) vs Chronic (adult) types
- Associated with antecedent viral infection or underlying immune conditions

- Common acquired bleeding condition which occurs more commonly in children & women
- Mucocutaneous bleeding as well as intracranial bleeding in severe cases
- Diagnosis
  - Dx of exclusion using diagnostic criteria
  - CBC & Peripheral smear
  - Other serological tests
  - BM study
- Treatment
  - Conservative
  - Corticosteriods
  - Other immune suppressive agents
  - Splenectomy

Hemophilia

- Definition & types
- Genetics
- Clinical Features
- Treatment

Introduction

- Hemophilia
  - Inherited bleeding disorders
  - Deficiency of F VIII (Hemophilia A) & Deficiency of F IX (Hemophilia B
- Hemophilia A
  - 1 in 5,000 to 10,000 males
  - 60% severe
- Hemophilia B
  - 1 in 25,000 to 30,000 males
  - 50% severe

- Hemophilia A & B combined
  - 1 in 5,000 male birth & 80% of them are A
- In Ethiopia
  - Expected 15,000
  - 120 detected & identified

Genetics

- X- linked recessive
  - All daughters of a hemophiliac father are obligate carrier & none of his sons would be affected
  - 50% of the daughters of a carrier mother are carriers and 50% are normal.
  - 50% of the sons of a carrier mother are hemophiliac and 50% are normal
- ?Female hemophiliac

- Family History

- Symptomatic female hemophiliac
  - Lyonization
  - Mating between a hemophiliac male and a carrier
  - Abnormalities in X-chromosome (like XO in Turner’s syndrome)
- Bleeding in carriers
  - Reduced activity
  - Behave like a mild hemophilia

Diagnosis

- Family history
  - Vital
  - Positive in 2/3rd of the cases
  - Negative family history
    - Sporadic case
    - Successive generation of female offspring & early neonatal death
- Screening
  - Platelet count, PTT, PT
  - Isolated prolonged PTT
- Factor Assay & Activity

- Specific assay
- Differentiate from VWD
  - Pattern of bleeding
  - Type 2N
- Severity
  - > 5% factor activity = mild
  - 1-5% factor activity = moderate
  - < 1% factor activity = severe

- Treatment
  - Replacement of the deficient factor
    - Plasma
    - Cryoprecipitate (for Hemophilia A)
    - Factor concentrates
    - Others
      - Adjuvant
      - By pass agents

Thank you!!!