The Role of Laboratory in the Management of Hemophilia

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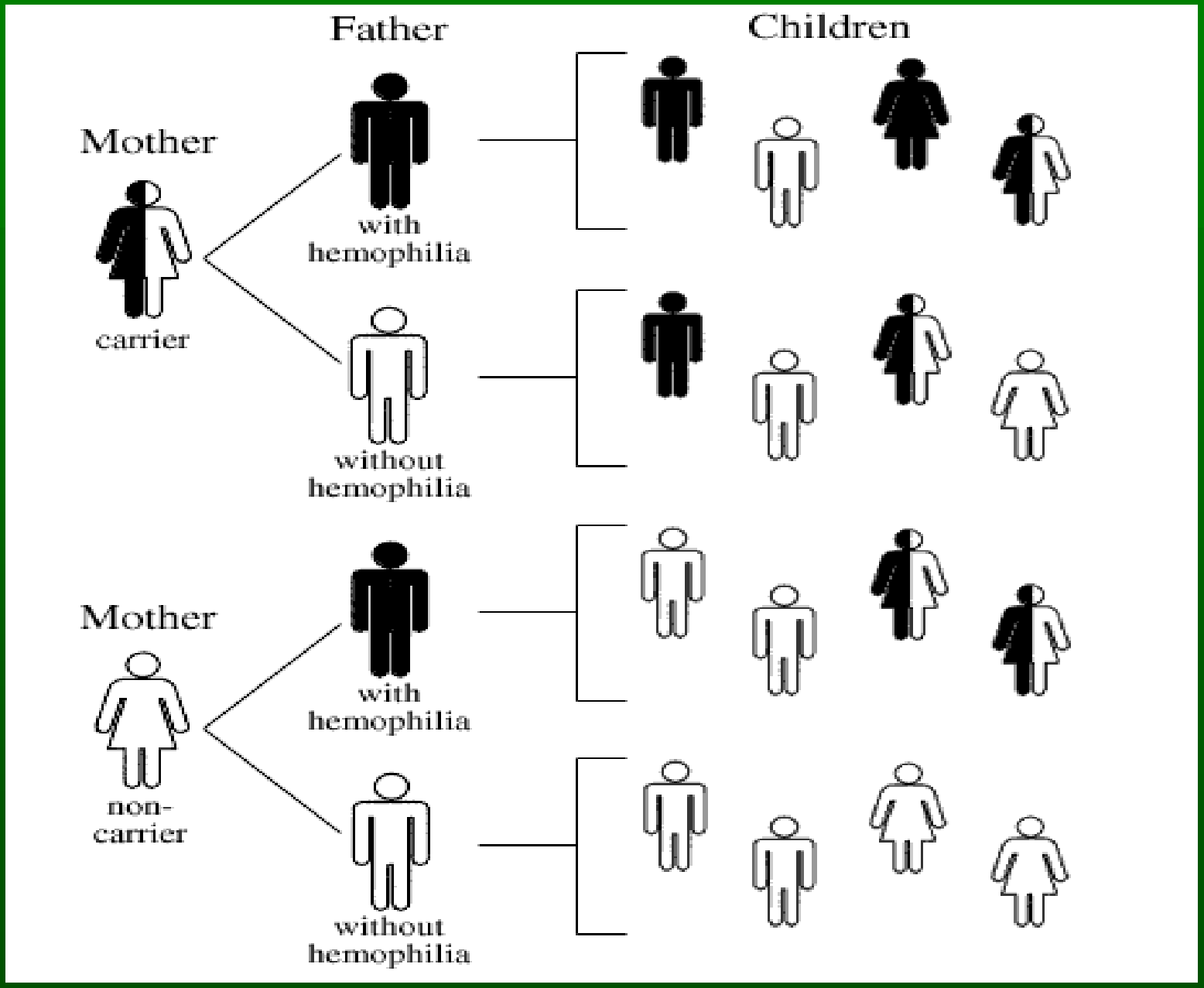
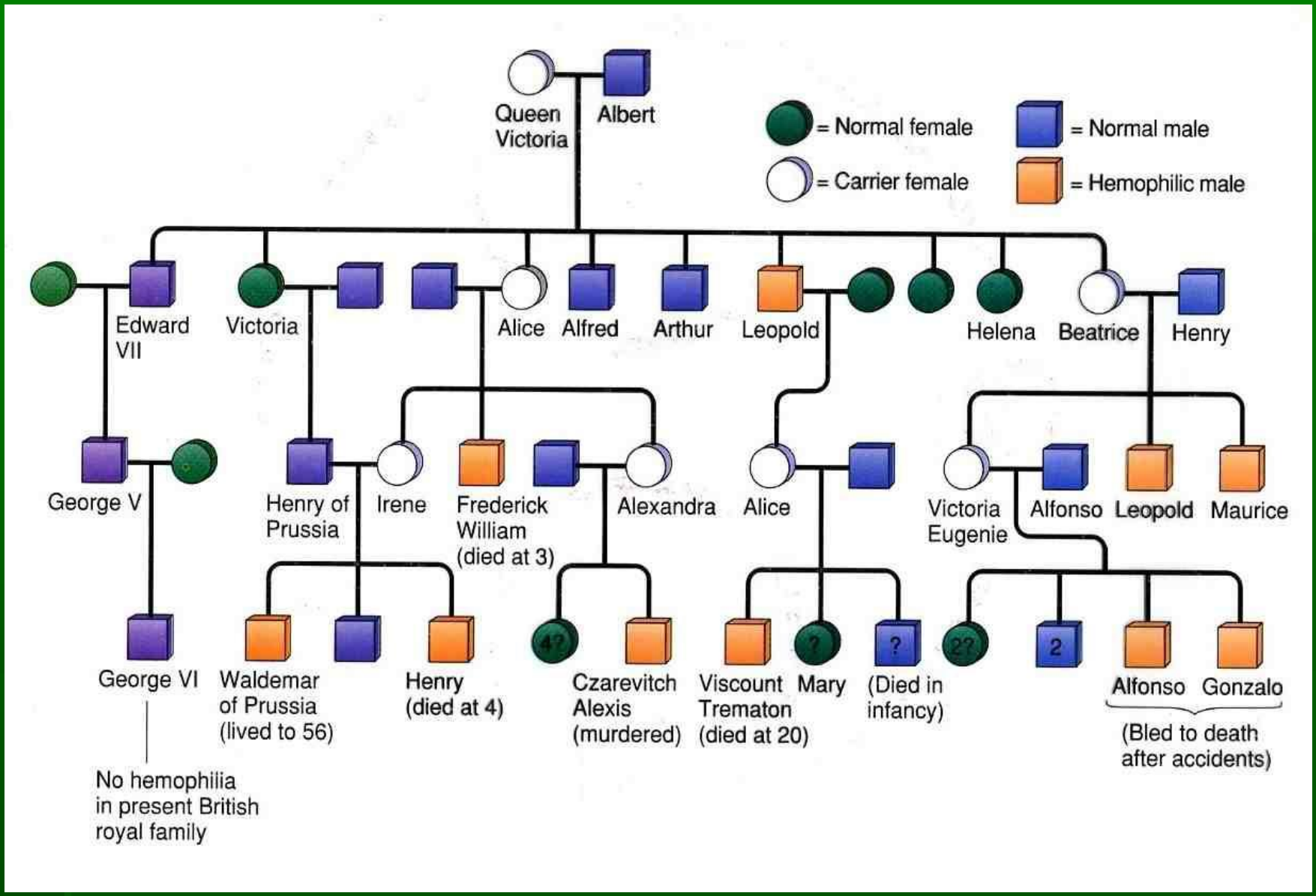
Hemophilia

- The most frequent hereditary coagulation disorders
- Frequency 1 in 10 000 births
- Not influenced by ethnicity, geographic, socio economic
- Hemophilia A is caused by deficiency of F VIII and hemophilia B by deficiency of F IX
- X linked recessive → male are more affected, female as carrier


Father
Children
Mother
carrier
with hemophilia
without hemophilia
Mother
non-carrier
with hemophilia
without hemophilia


Queen Victoria
Albert
= Normal female
= Carrier female
= Normal male
= Hemophilic male
Edward VII
Victoria
Alice
Alfred
Arthur
Leopold
Helena
Beatrice
Henry
George V
Henry of Prussia
Irene
Frederick William (died at 3)
Alexandra
Alice
Victoria Eugenie
Alfonso
Leopold
Maurice
George VI
Waldemar of Prussia (lived to 56)
Henry (died at 4)
Czarevitch Alexis (murdered)
Viscount Trematon (died at 20)
Mary
(Died in infancy)
Alfonso
Gonzalo
(Bled to death after accidents)
No hemophilia in present British royal family

Alexis

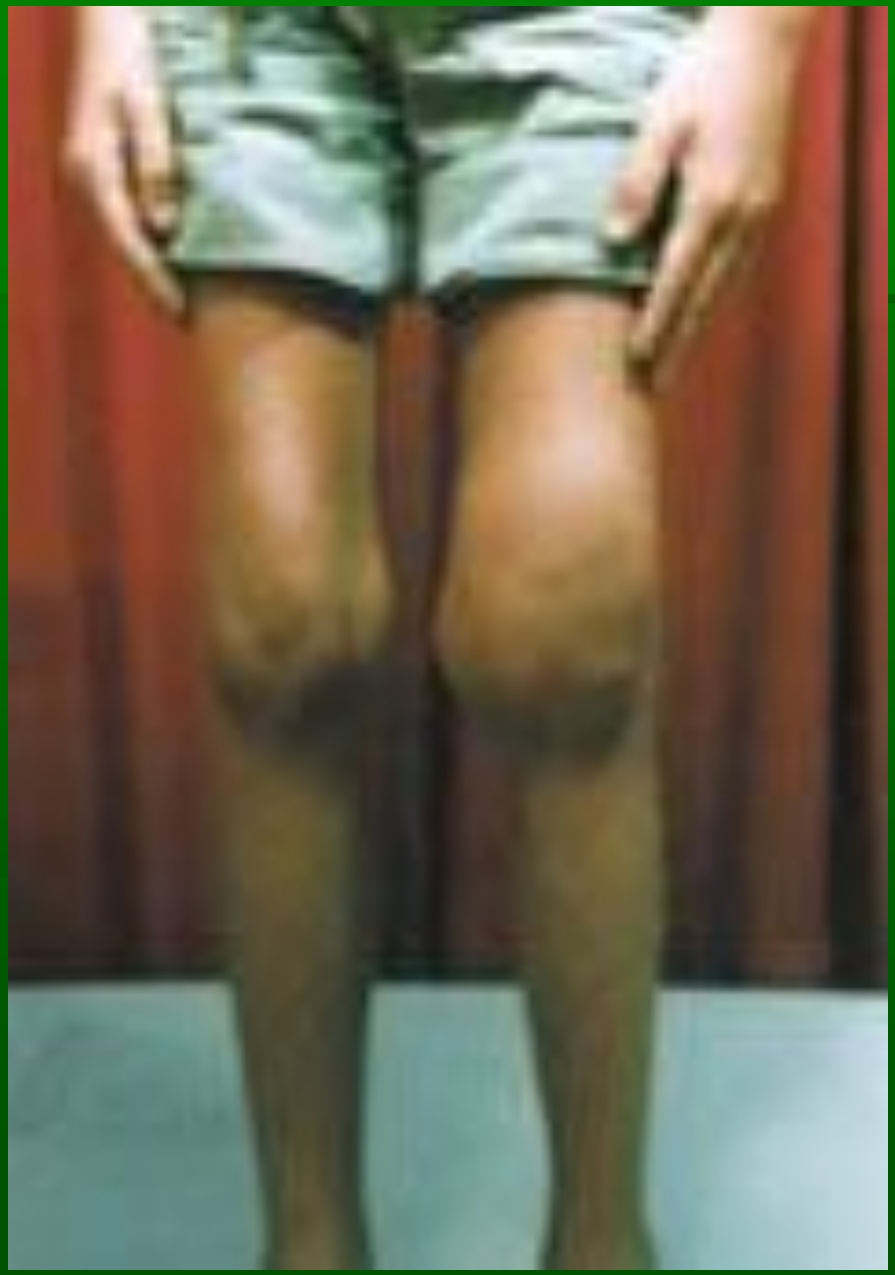
Hemophilia in Indonesia

- Based on the population of Indonesia around 200 millions it is estimated that the number of PWH in Indonesia 20 000
- In facts only around 1800 PWH have been registered
- Reasons: under diagnosed due to many PWH died before being diagnosed or moderate and mild hemophilia in the society have not been detected

Clinical manifestation

- Easy bruising in early childhood
- Spontaneous bleeding into joint and soft tissue
- Excessive bleeding following trauma or surgery
- Patients with mild hemophilia may not have excessive bleeding unless they have trauma or surgery
- Delayed bleeding

  Factor VIII and F IX are not required for platelet plug formation



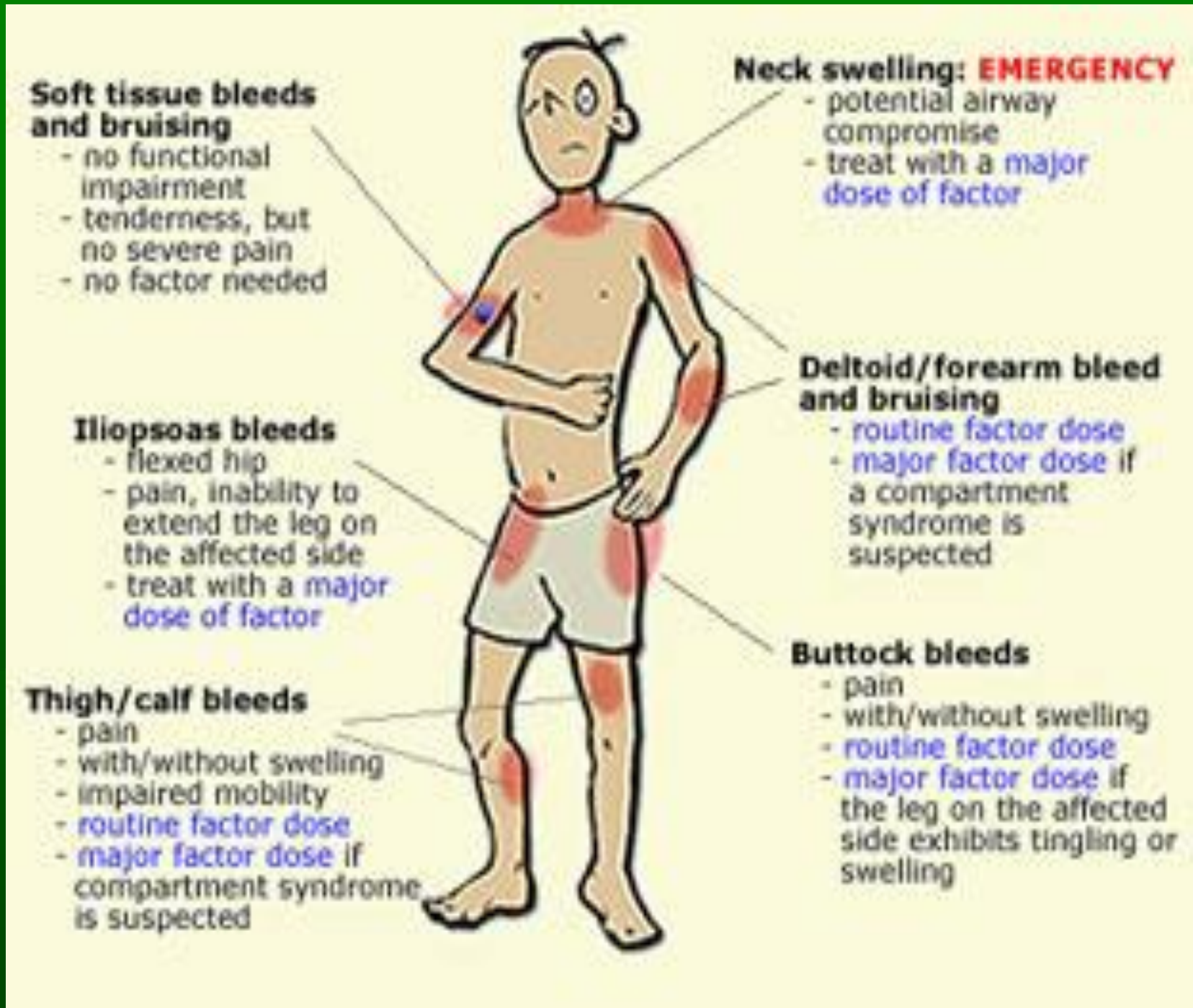
Site of bleeding

Serious

- Joints (Hemarthrosis)
- Muscle/soft tissue
- Mouth, nose, gum
- Hematuria

Life threatening

- CNS/ Intracranial
- Gastro intestinal
- Neck/throat
- Severe trauma


Soft tissue bleeds and bruising
- no functional impairment
- tenderness, but no severe pain
- no factor needed
Neck swelling: EMERGENCY
- potential airway compromise
- treat with a major dose of factor
Deltoid/forearm bleed and bruising
- routine factor dose
- major factor dose if a compartment syndrome is suspected
Iliopsoas bleeds
- flexed hip
- pain, inability to extend the leg on the affected side
- treat with a major dose of factor
Buttock bleeds
- pain
- with/without swelling
- routine factor dose
- major factor dose if the leg on the affected side exhibits tingling or swelling
Thigh/calf bleeds
- pain
- with/without swelling
- impaired mobility
- routine factor dose
- major factor dose if compartment syndrome is suspected

Severity of bleeding in hemophilia

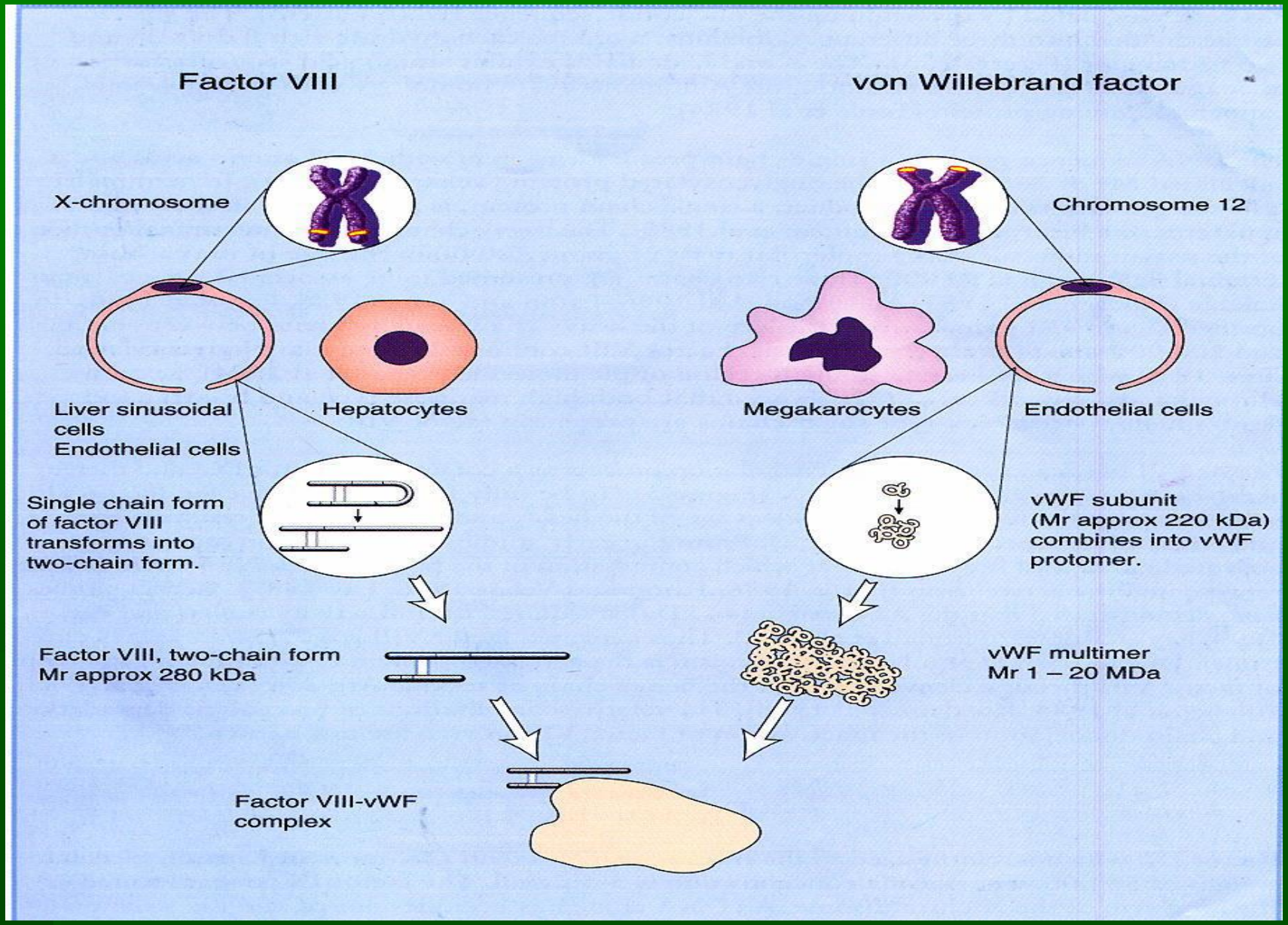
Severity	Clotting factor level % activity (IU/mL)	Bleeding episodes
Severe	1% (<0.001)	Spontaneous bleeding predominantly in joints and muscles
Moderate	1% – 5% (0.01 -0.05)	Occasional spontaneous bleeding. Severe bleeding after trauma or surgery
Mild	5% – 40%(0.05- 0.40)	Severe bleeding after major trauma or surgery

Factor VIII (antihemophilic factor)

- glycoprotein , not stable
- Synthesized : liver, kidney, and spleen
- Gene of F VIII located at chromosome X
- In circulation : complex with von Willebrand factor, protects from proteolytic degradation
- Function : intrinsic pathway as cofactor of F IXa in the activation of F X

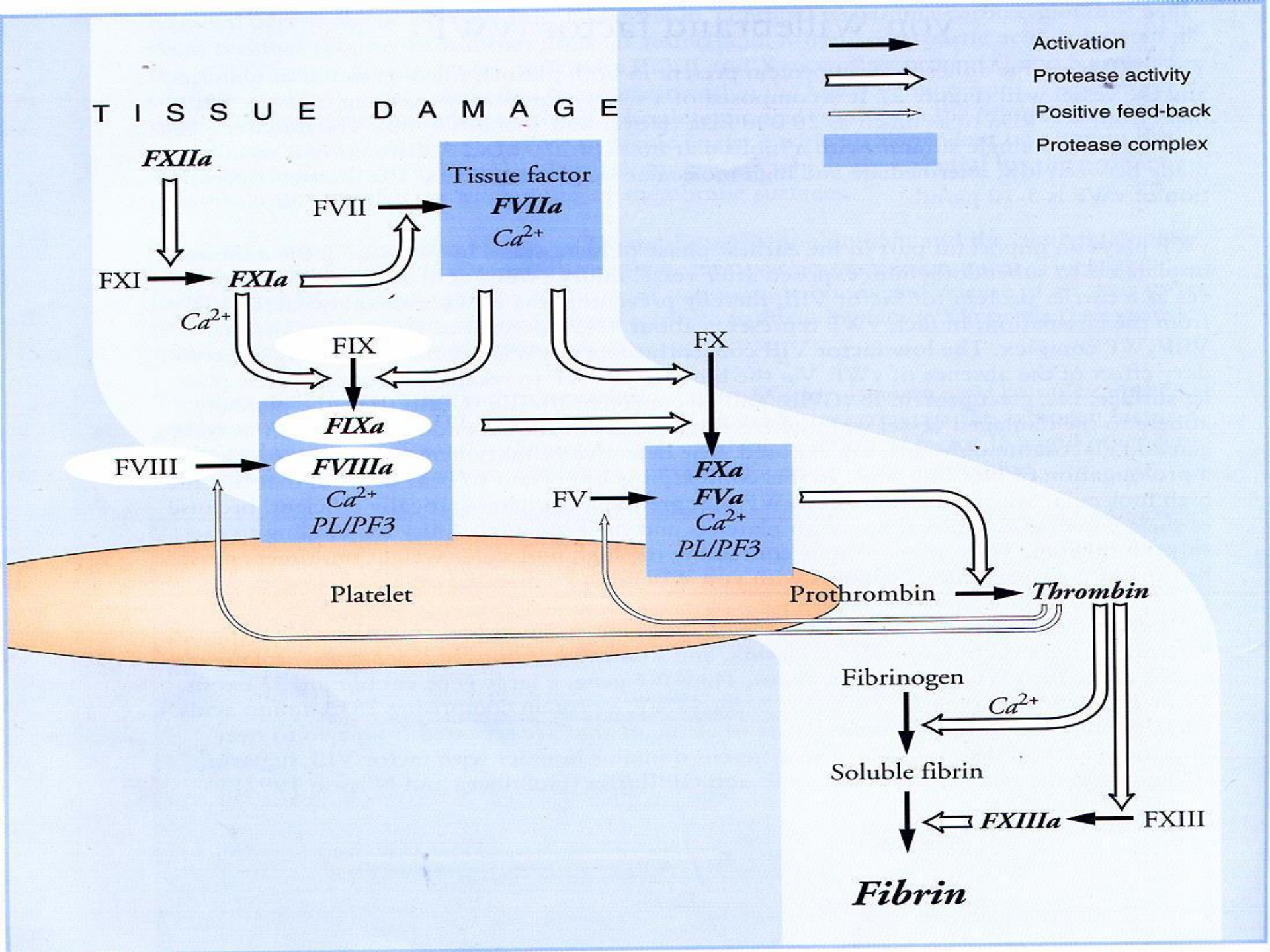
von Willebrand's factor

- multimer BM 1 - 20 x 10^6 dalton
- synthesized : endothelial cell and megakariocyte
- function :
  - carrier of F VIII, protects from proteolytic degradation
  - Adhesion and aggregation of platelet, as a bridge between platelet and subendothelial tissue

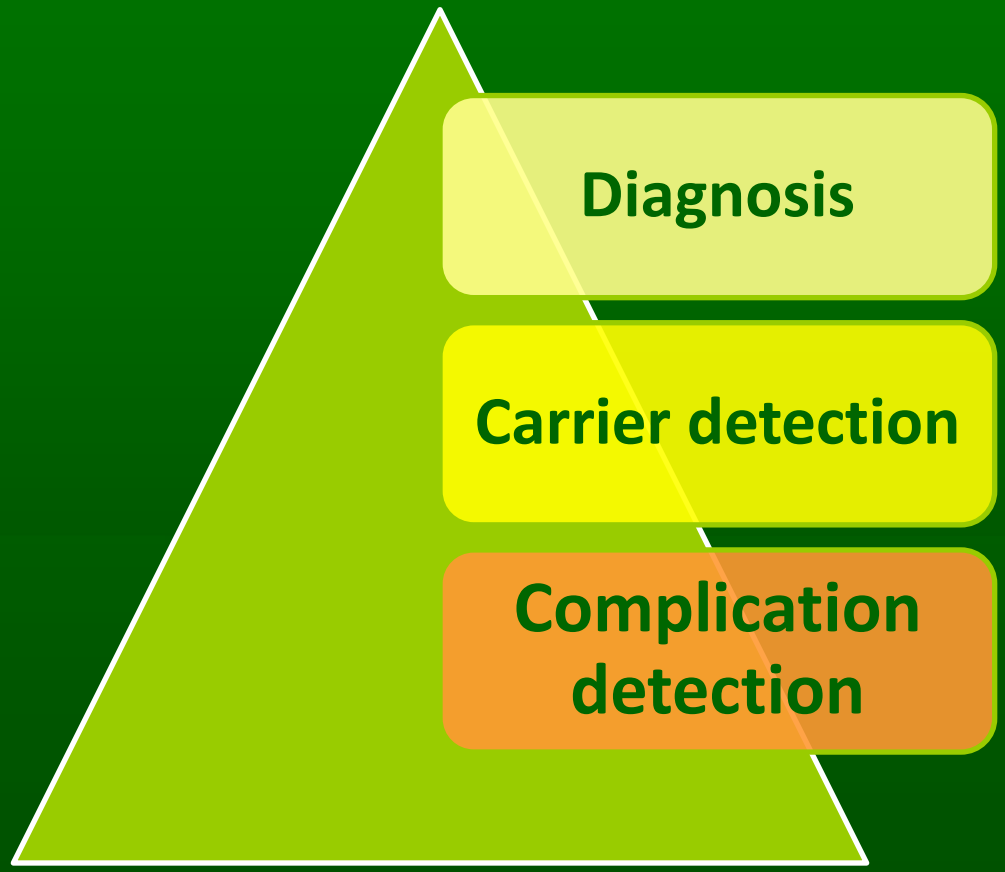

Factor VIII
von Willebrand factor
X-chromosome
Chromosome 12
Liver sinusoidal cells
Endothelial cells
Hepatocytes
Megakarocytes
Endothelial cells
Single chain form of factor VIII transforms into two-chain form.
vWF subunit (Mr approx 220 kDa) combines into vWF protomer.
Factor VIII, two-chain form Mr approx 280 kDa
vWF multimer Mr 1 – 20 MDa
Factor VIII-vWF complex

Factor IX

- Synthesized: liver
- Vitamin K dependent
- Function : in the intrinsic pathway, activate F X $\rightarrow$ F Xa


Activation
Protease activity
Positive feed-back
Protease complex
TISSUE DAMAGE
FXIIa
FXI
FXIa
FVII
Tissue factor
FVIIa
Ca2+
Ca2+
FIX
FX
FIXa
FVIII
FVIIIa
Ca2+
PL/PF3
FV
FXa
FVa
Ca2+
PL/PF3
Platelet
Prothrombin
Thrombin
Fibrinogen
Ca2+
Soluble fibrin
FXIIIa
FXIII
Fibrin

The role of Laboratory in the Management of Hemophilia



Diagnosis of hemophilia

Anamnesis : bleeding history, family history

Physical examination: hematoma, hemarthrosis

Laboratory diagnosis : ??

Laboratory diagnosis of hemophilia

- **Platelet count : normal**

  no alteration in the production and consumption of platelet

- **Bleeding time : normal**

  F VIII and IX are not required in the hemostatic plug formation

Laboratory diagnosis of hemophilia

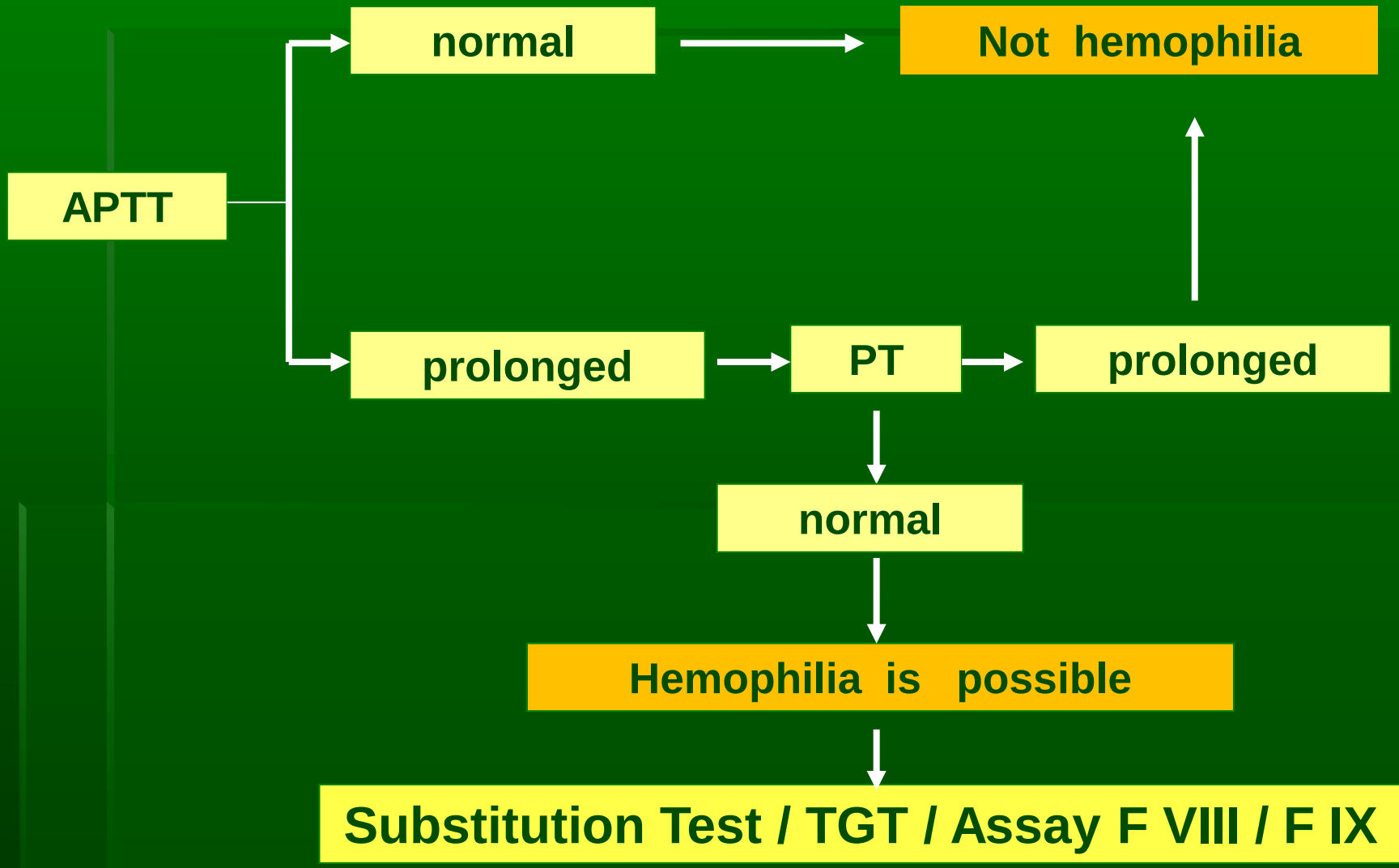
- **PT normal since PT assess the extrinsic and common pathways.**
- **APTT prolonged because APTT assess the intrinsic and common pathways.**

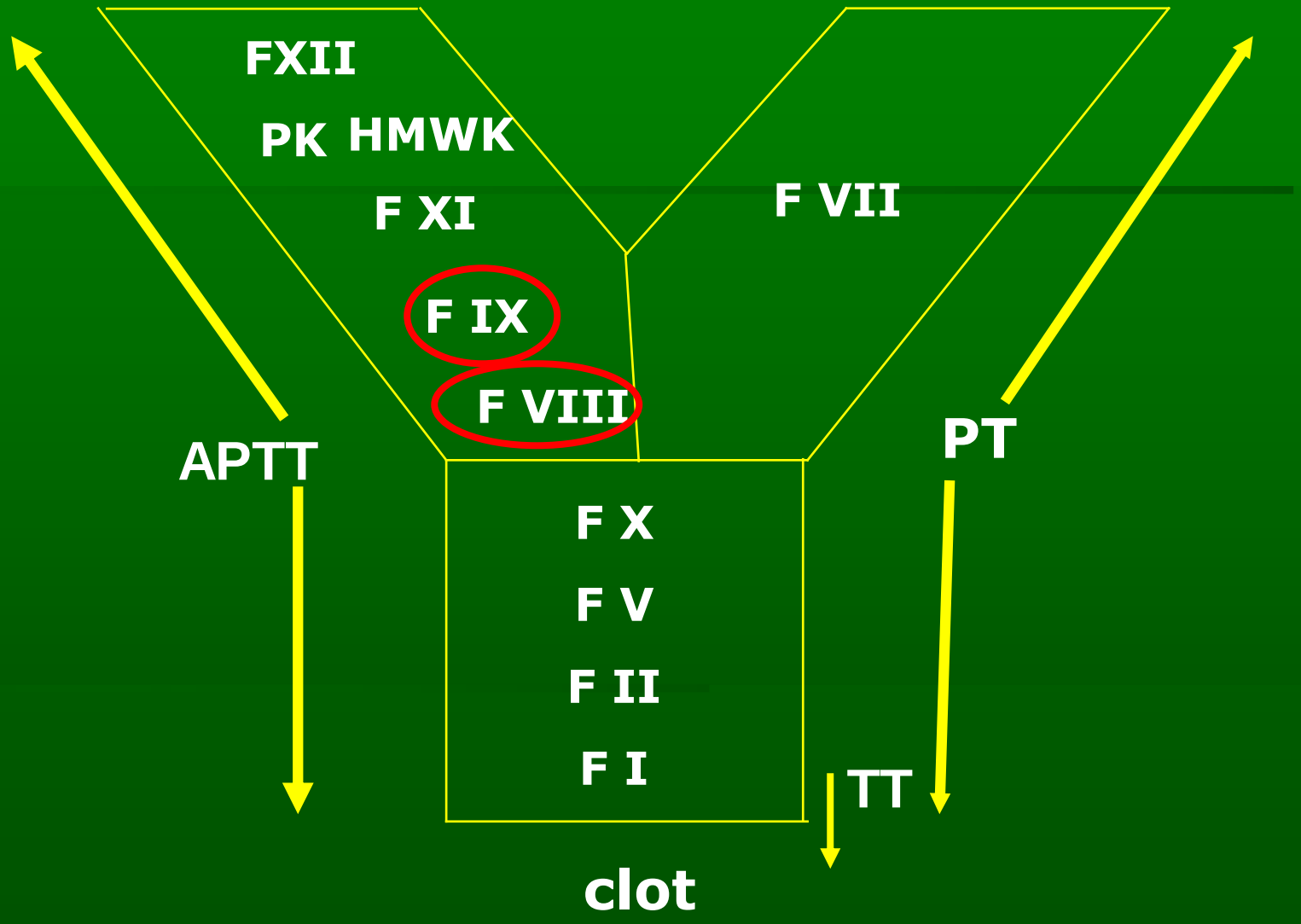
  F VIII and IX function in the intrinsic pathway
- **TT normal because TT only assess the changes of fibrinogen to fibrin**

Screening for diagnosis

Condition	PT	APTT	BT	Platelet count
Hemophilia A or B	normal	**prolonged**	normal	normal
Von Willebrand disease	normal	Normal or prolonged	prolonged	Normal or reduced
Platelet defect	normal	normal	prolonged	Normal or reduced

Screening and diagnosis of hemophilia




FXII
PK HMWK
F XI
F IX
F VIII
F VII
APTT
PT
F X
F V
F II
F I
TT
clot

Diagnosis of Hemophilia and DD/ hemophilia A or B

- Thromboplastin Generation Time
- APTT substitution test

  Based on the difference between F VIII and F IX properties.

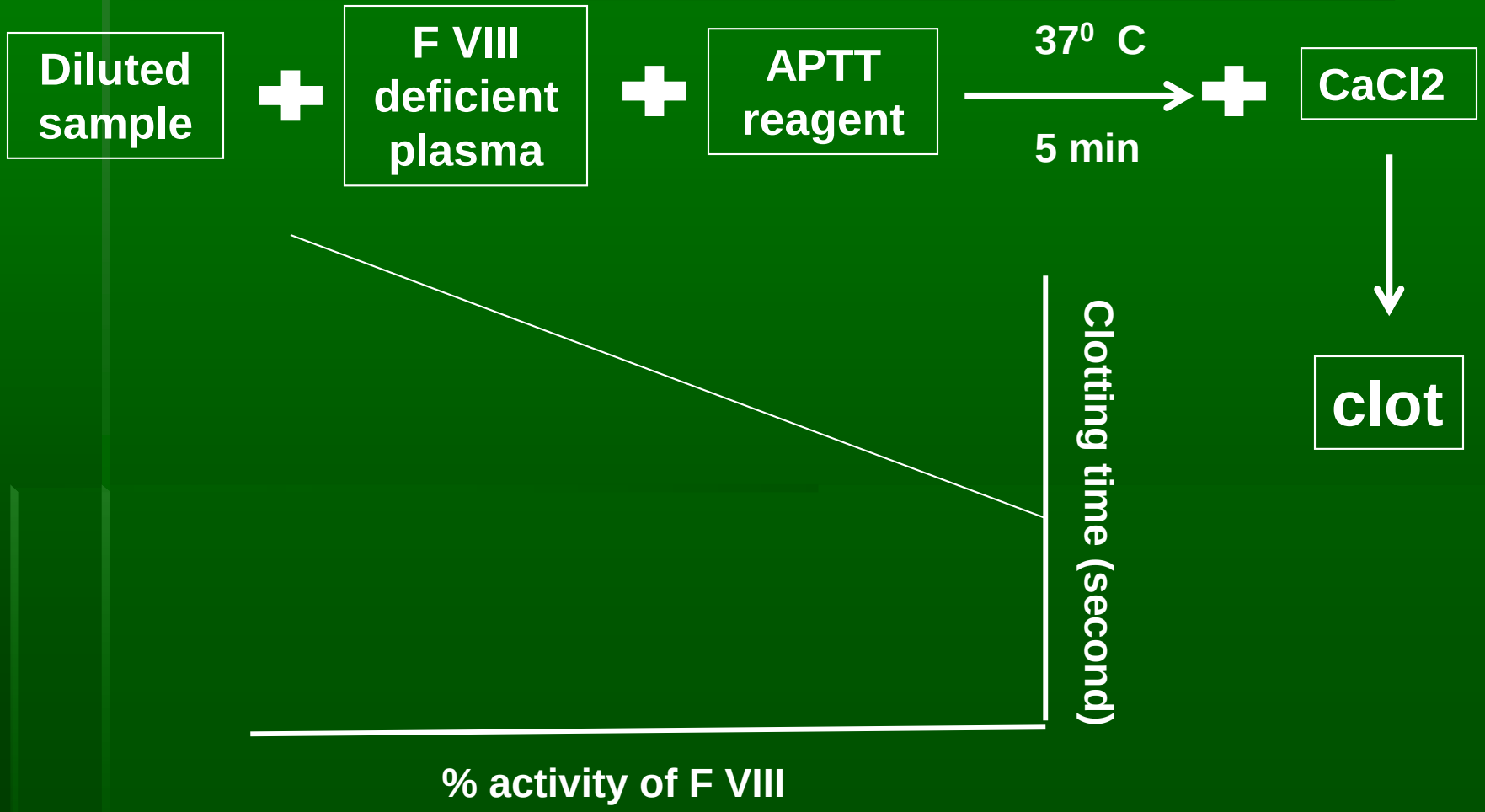
  F VIII is consumed during coagulation process $\rightarrow$ F VIII is absent in serum.

  F IX is vitamin K dependent factors, is adsorbed by Ba(SO4) or Al $(OH)_3$ $\rightarrow$ F IX is absent in adsorbed plasma
- Factor VIII/IX Assay

Methods for Factor VIII assay

- Clotting Assay for Factor VIII :
  - One stage
  - Two stage
- Chromogenic substrate

One-stage Clotting Assay for F VIII



Two-stage Clotting Assay for Factor VIII

1st stage Conversion F X to activated F X

Adsorbed patient plasma or standard

+

F X, activated F IX, Phospholipid, Ca, F V in excess

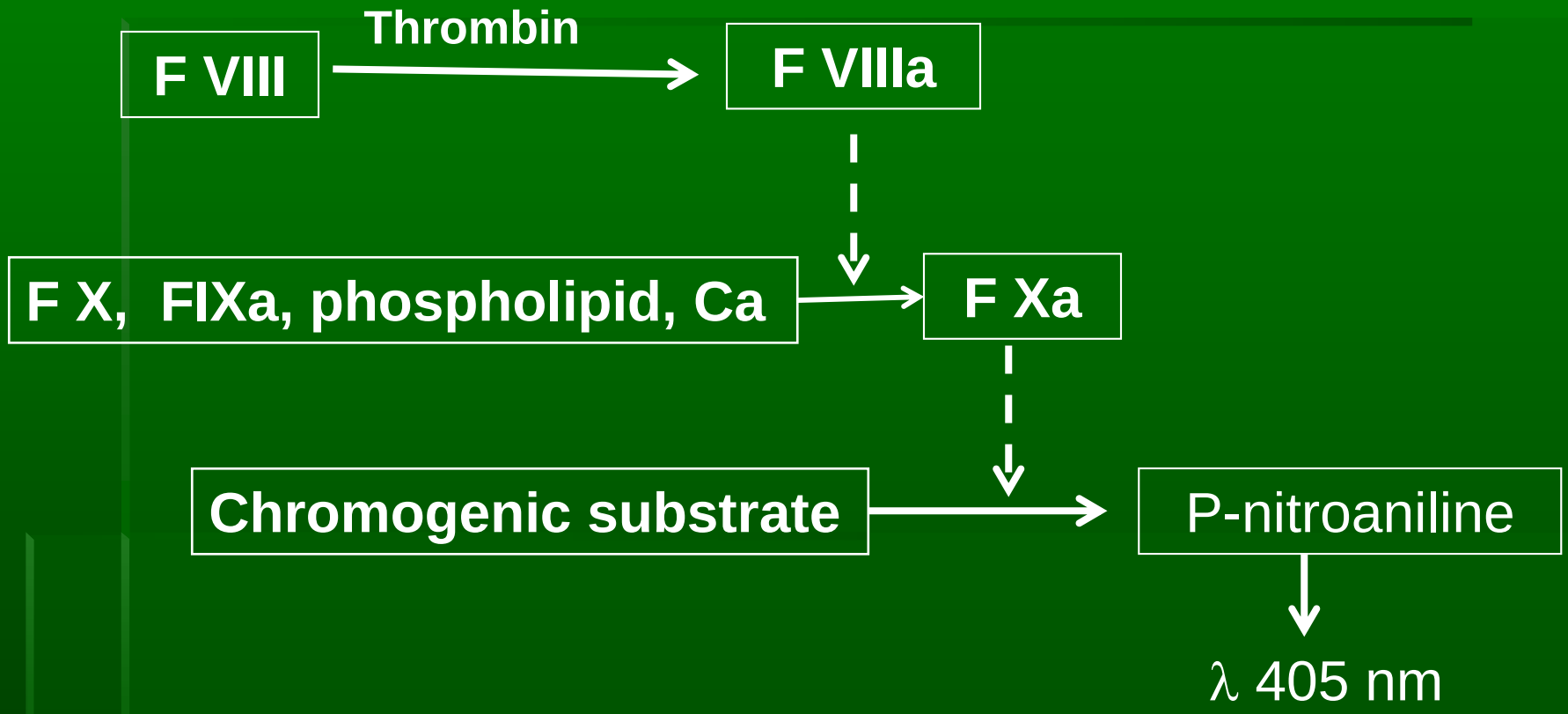
2nd stage Clot formation

+

Source of prothrombin and fibrinogen

→ Clot

Factor VIII assay by Chromogenic substrate



Indication of doing factor VIII assay

- To determine diagnosis of hemophilia
- To monitor the activity of F VIII before and after surgery
- To test the quality of cryoprecipitate. AABB guidelines recommend F VIII content in cryoprecipitate of 80 Unit per bag
- To detect carrier by phenotypic method.

  Ratio F VIII/vWF < 0.7 gives 80% chance being carrier

Activity of F VIII

- Normal range : 50 - 150 %
- Severe Hemophilia A : < 1 %
- Moderate Hemophilia A : 1 - 5 %
- Mild Hemophilia A : >5 - 40 %
- von Willebrand disease: normal or low
- F VIII ⬆ acute phase reactant

  risk factor of thrombosis

Activity of F IX

- Normal range : 50 - 150 %
- Severe Hemophilia B : < 1 %
- Moderate Hemophilia B : 1 - 5 %
- Mild Hemophilia B : >5 - 40 %

Low :

deficiency of vit. K

Vitamin K antagonist

liver disease

bile obstruction

DD/ Hemophilia A and hemophilia B

Hemophilia A

- APTT prolonged
- F VIII ⬇
- F IX normal
- TGT/diff. APTT using plasma : abnormal

Hemophilia B

- APTT prolonged
- F VIII normal
- F IX ⬇
- TGT/diff. APTT using serum : abnormal

DD/ Hemofilia A dan von Willebrand's d.

Hemofilia A

- F VIII ↓
- Bleeding time : **N**
- vWF level : **N**
- vWF : Ristosetin cofactor : **N**

Von Willebrand's d.

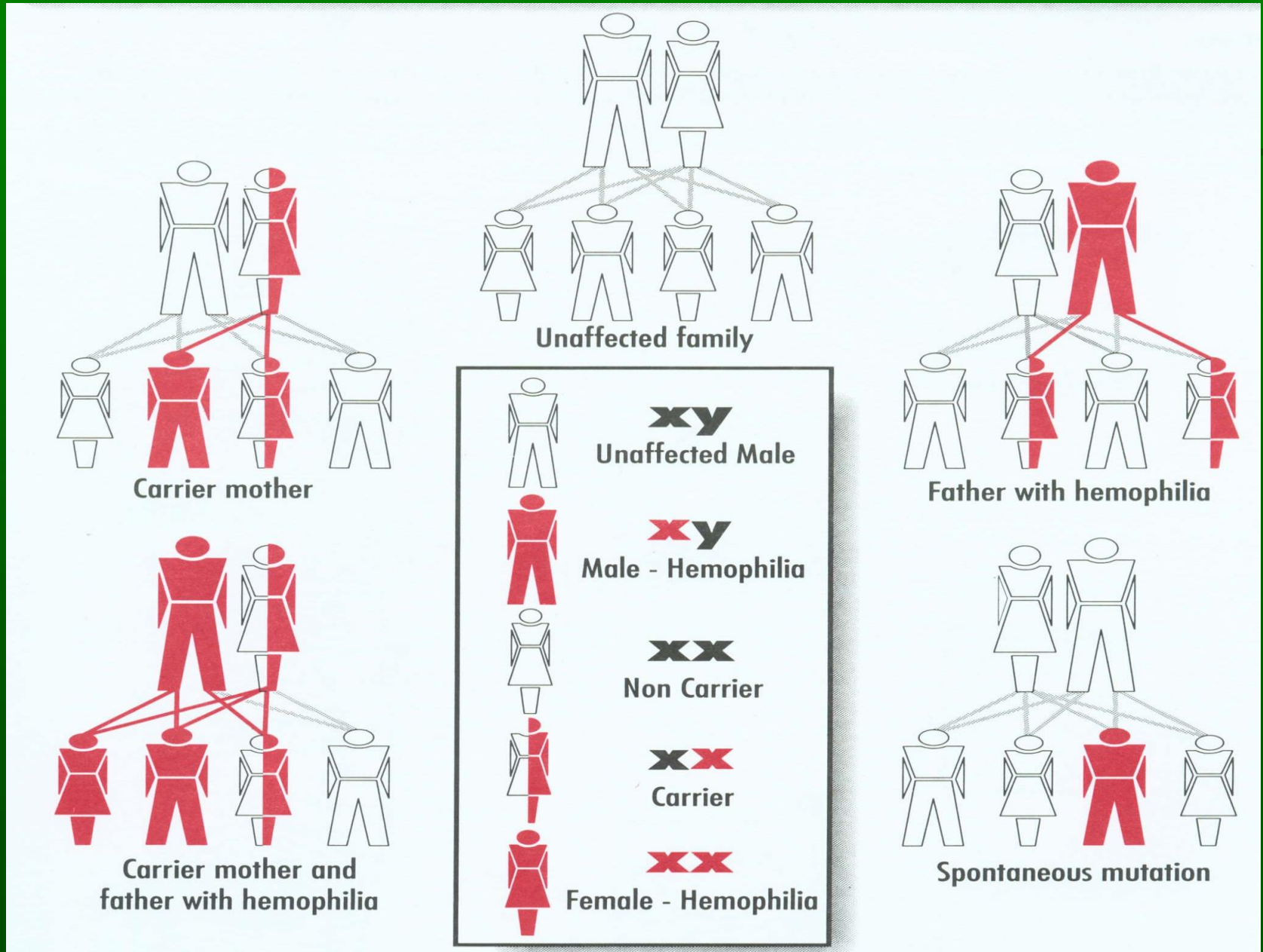
- F VIII N / ↓
- Bleeding time prolonged
- vWF level ↓
- vWF : Ristosetin cofactor ↓

If laboratory facility is not available

	Clotting time	Bleeding time
Hemophilia	Prolonged	normal
von Willebrand disease	normal	Prolonged

Carrier detection

Pattern of inheritance of X linked



Status of women in hemophilia family

- Obligat carrier
- Non carrier
- Possible carrier

Carrier of hemophilia

- Daughters of PWH are obligate carrier
- Most carrier are asymptomatic
- A few carrier may have F VIII/IX level in the hemophilia range (mostly mild), very rare in moderate or severe (Lyonisation)
- Carrier with low level of F VIII/IX $\rightarrow$ bleeding manifestation$\rightarrow$ treatment
- Menorrhagia is common manifestation
- Immediate female relative of PWH, should have F VIII/IX checked

Carrier Detection

Phenotypic

- Pedigree analysis
- F VIII/vWF Ratio
- Bivariate Linier Discriminant Analysis

Genotypic

- Direct detection of mutation
- Polymorphism gene tracking

Bivariate Linear Discriminant Analysis

Input data from possible carrier

α = age in year

β = blood group group O = 0 Non O = 1

γ = vWF Ag in IU/mL

δ = VIII in IU/mL

π = genetic probability of carriership

Input data from non carrier reference group

μ_x = mean of Ln of vWF:Ag level in IU/mL

μ_y = mean of Ln F VIII activity in IU/mL

Calculate for possible carrier

$X = \ln(\gamma) - \mu_x$

$Y = \ln(\delta) - \mu_y$

Bivariate linear discriminant analysis (cont)

Calculate the coefficient for modified discriminant:

$a = - 0{,}0955 – 0{,}0156\alpha + 0{,}000196\alpha^2 + 0{,}0298\beta$

$b = 0{,}649 – 0{,}00184\alpha + 0{,}000314\alpha^2 + 0{,}117\beta$

Calculate predicted means of the discriminants for carrier and non carrier:

$c = - 0{,}391 – 0{,}00571\alpha + 0{,}0001\alpha^2 – 0{,}0648\beta$

$d = -0{,}347 – 0{,}00171\alpha + 0{,}0000473\ \alpha^2 + 0{,}0754\beta$

Calculate the Odds ratio:

$e = ax + by$

$= 4{,}28\ (e-c)$

$g = 7{,}97\ (e-d)$

$h = 0{,}623 + 0{,}5(f+g)\ (f – g)$

LR = exp (-h) , the Odds ratio favouring carriership

Calculate final probability of carriership

$Pc = \pi LR/(\pi LR + 1 – \pi)$

Complication Detection

Chronic Complication of Hemophilia

- Musculoskeletal:
  - Chronic hemophilic arthropathy
  - Contractures
  - Pseudotumor formation (soft tissue and bone)
  - Fracture
- Inhibitor of F VIII/IX
- Transfusion-related infection : HIV, HBV, HCV, parvovirus B19

Inhibitor in hemophilia

- Incidence in severe hemophilia A 20 % - 33%
- Incidence in hemophilia B 1% - 4%
- Plasma derived products: 80% high responding type, very few temporary
- Recombinant F VIII : < 50% high responding type, 1/3 temporary
- Laboratory test : Bethesda inhibitor assay

Inhibitor F VIII

Patient Plasma + Pool normal plasma

F VIII def.plasma + pool normal plasma

Incubate for 2 hours

Perform F VIII assay

Perform F VIII assay

Determine residual F VIII
Convert to Bethesda unit

Comprehensive Care Team for Hemophilia

- Team comprise: hematologist (pediatriciant and adult) clinical pathologist, specialist in physical medicine and rehabilitation, orthopedic surgeon, dentist, psychiatrist, nurse
- Function of the team:
  - Coordinate care and service to patients
  - Provide education to patient and family members
  - Documentation of treatment and measurement of long-term outcome especially musculoskeletal function
  - Conduct research to improve management

Management for Hemophilia in Indonesia

- Since 1997 Comprehensive Care Team for Hemophilia was established in Dr. Cipto Mangunkusumo Hospital, Jakarta
- Meeting to solve the problem in the patient management, donation from WFH
- Surgery: TKR, implant in hip surgery
- We also have Indonesian Hemophilia Society: PWH, family, doctors, nurse, social workers.


Thank you