Hemostasis : Bleeding disorders
Diagnosis tests and case study

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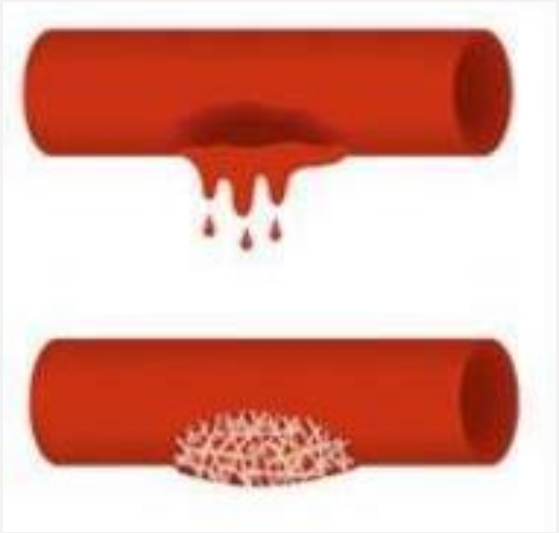
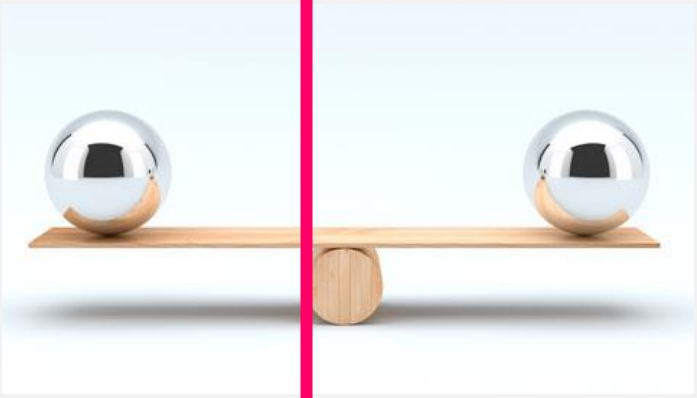
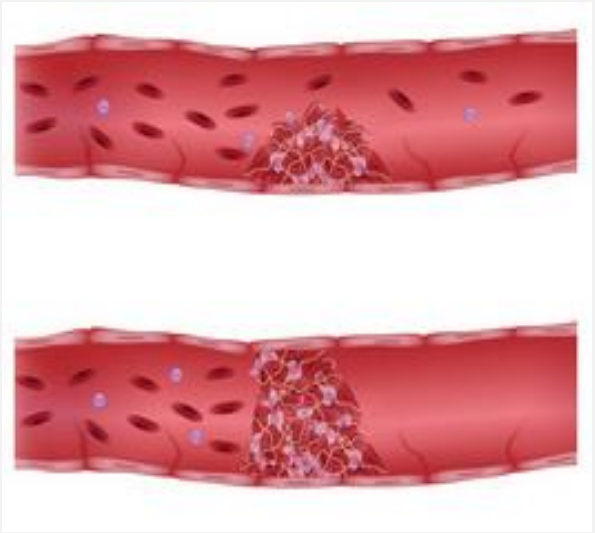
Hemostasis

Hemostasis : dysregulation results in bleeding or thrombosis !







Bleeding

Thrombosis

Either when a bleeding event occurs or to prevent bleeding event (before a surgery)

Bleeding causes

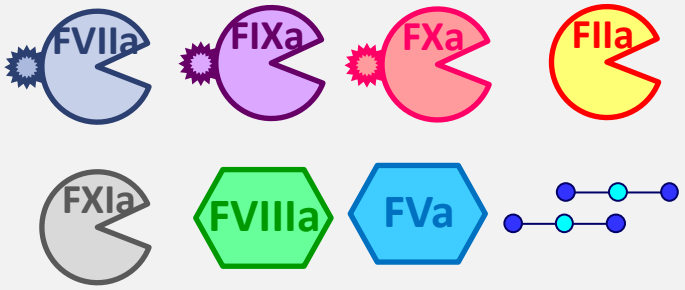
- **Defective primary hemostasis:**

Thrombocytopenia (not enough platelets) or platelet dysfunction.

Von Willebrand factor deficiency

- **Defective coagulation:**

Coagulation factor deficiency (single or multiple factor deficiency)

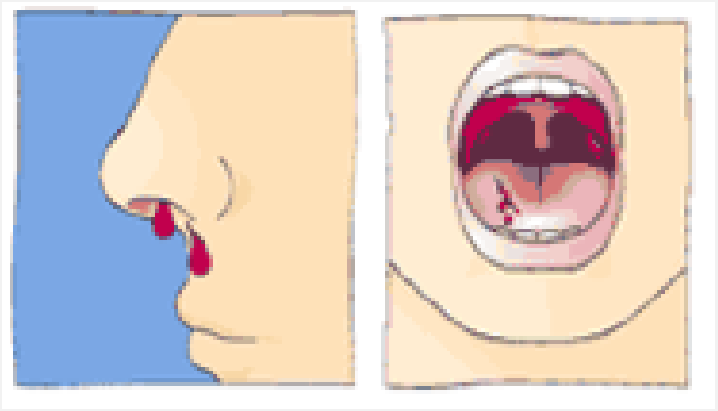


Clinical manifestations

- **Defective primary hemostasis:**

Bleedings localized in the microvasculature (not only, but essentially)

Subcutaneous bleeding
Ex: purpura/ecchymosis

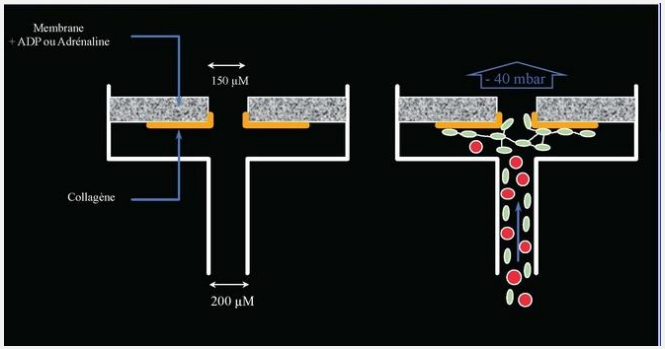


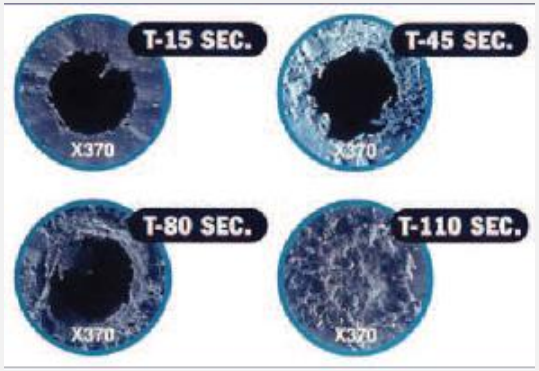
Mucosal bleedings

- **Epistaxis**
- **Menorrhagia**
- **Gingivorrhagia**

Diagnosis tests

- **Defective primary hemostasis:**





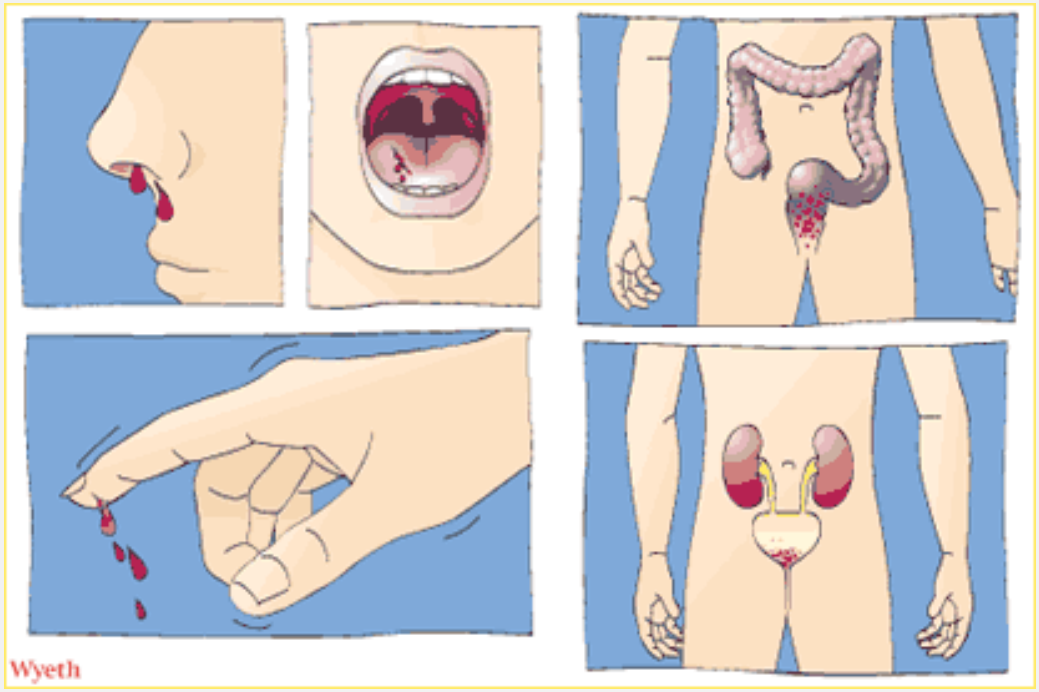
CBC (Complete Blood count)
⇨ **Platelet count** (150-400 G/L)
< 150 G/L thrombocytopenia
< 50 G/L increased bleeding risk

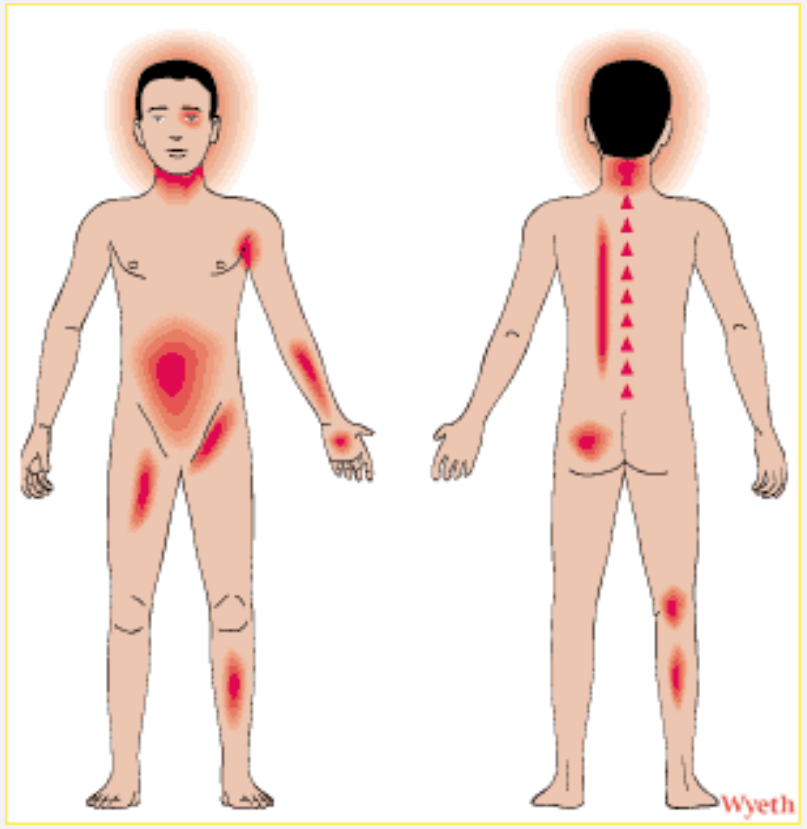
Platelet function analyzer (PFA)
Blood flow across a collagen coated membrane results in platelet activation and membrane occlusion
Time for occlusion (min)

Clinical manifestations

- **Defective coagulation:**

Any kind of bleeding



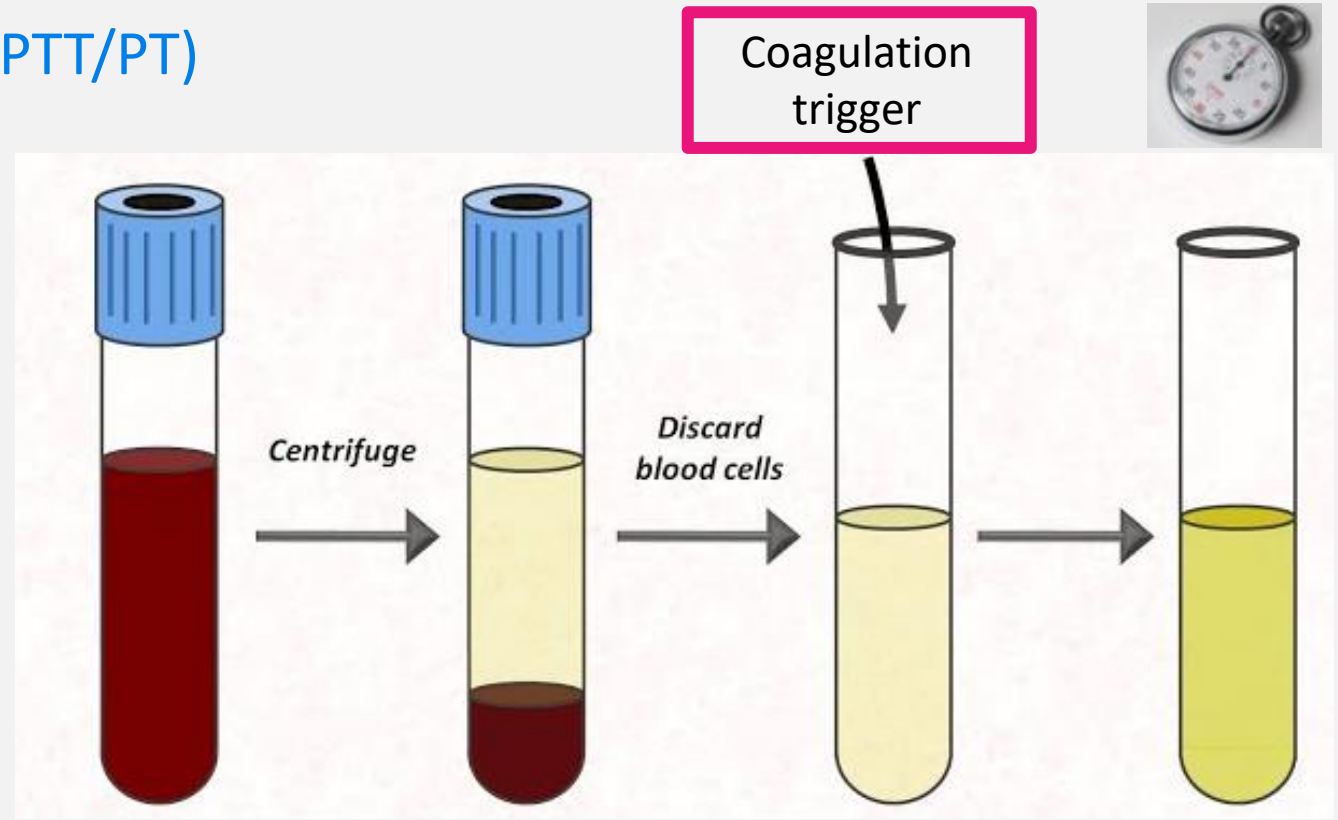


Diagnosis tests

- **Defective coagulation:**

Two different ways to activate coagulation ⇨ two different clotting times (PT and/or aPTT

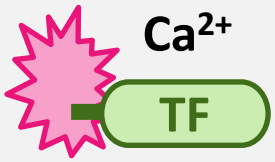
Clotting time (aPTT/PT)

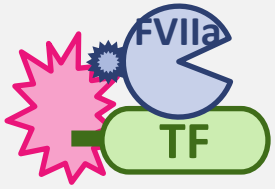


Clotting time (aPTT/PT)

1) PT (Prothrombin time)

Coagulation is activated by lipidated TF and Ca^{2+}





1) Lipidated TF binds to and activates FVIIa

2) TF/FVIIa complex activates FX

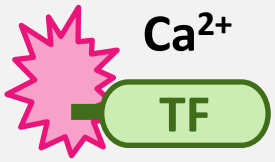
3) FXa activates FII

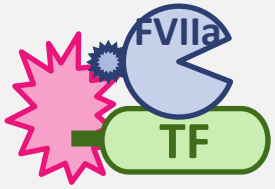
4) FIIa activates FV

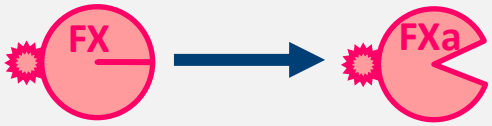
Clotting time (aPTT/PT)

1) PT (Prothrombin time)

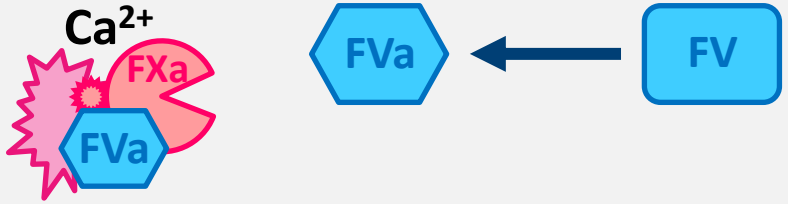
Coagulation is activated by lipidated TF and Ca^{2+}

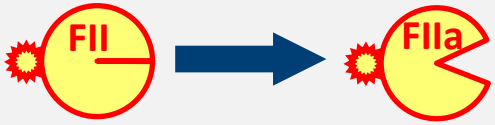




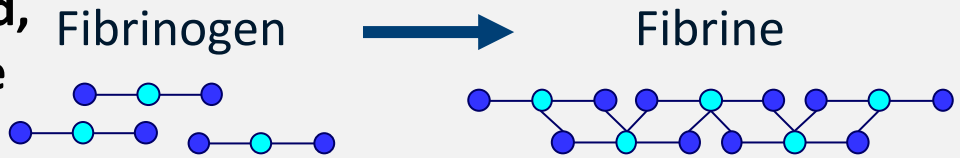


5) FXa and FVa form the "prothrombinase" complex





6) When enough FIIa is generated, it converts fibrinogen into fibrine to form the fibrin clot

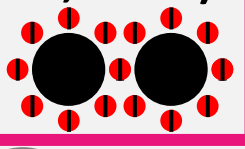


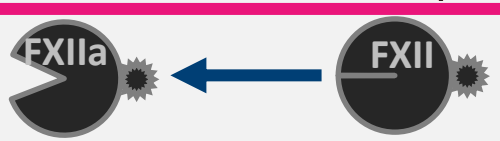
Clot formation

Clotting time (aPTT/PT)

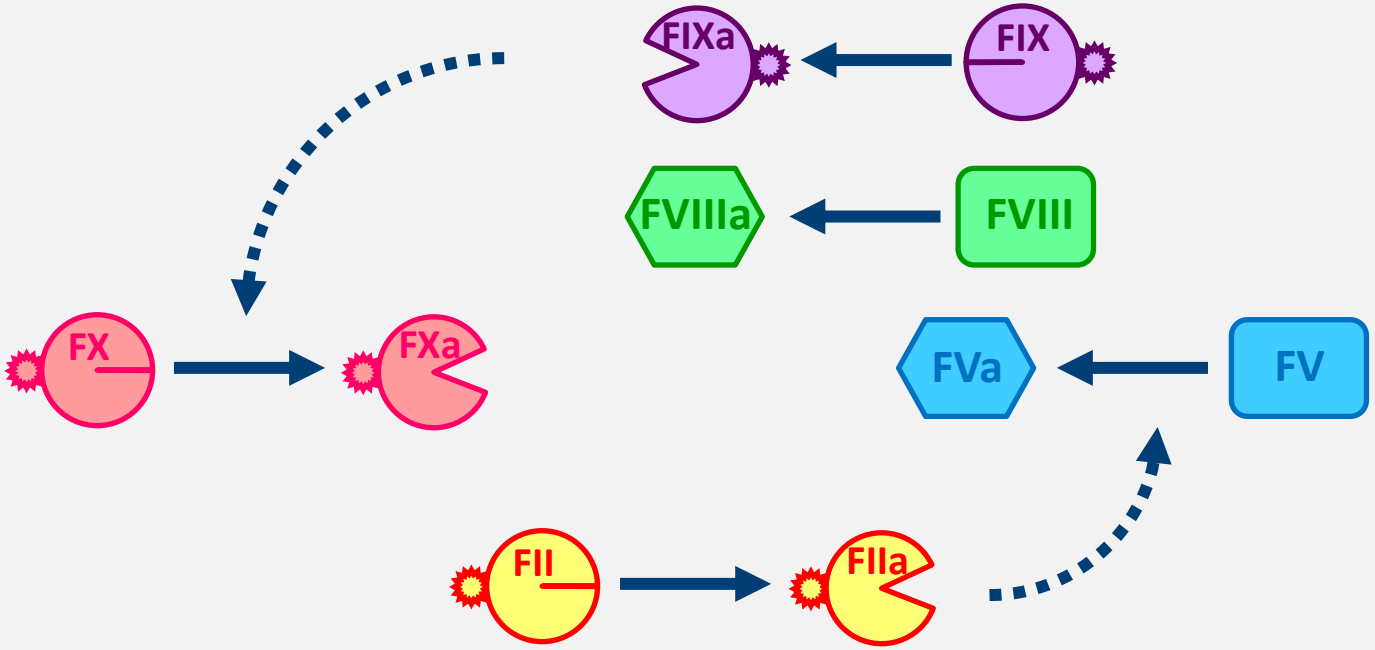
2) aPTT (activated partial thromboplastin time)

FXII activator (kaolin, silica)



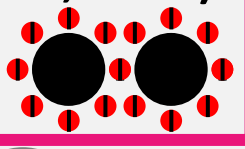


1) Cascade activation of FXI, FIX, FX, FII, FV, and FVIII



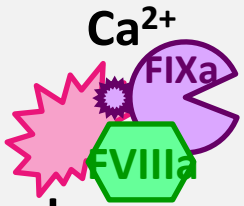
Clotting time (aPTT/PT)

FXII activator (kaolin, silica)

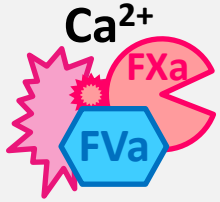


2) aPTT (activated partial thromboplastin time)

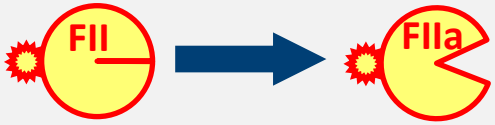
2) After few minutes, Ca^{2+} and PL vesicles are added



3) To form "Tenase" and "prothrombinase" complexes



4) These complexes increase the rate of FIIa generation



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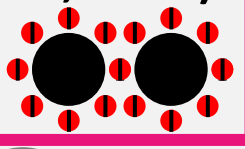

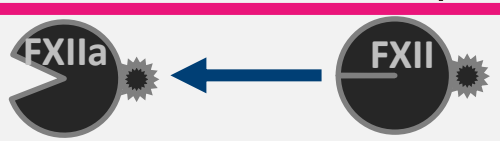

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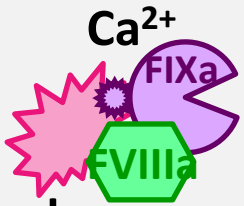

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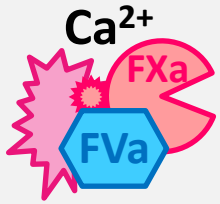

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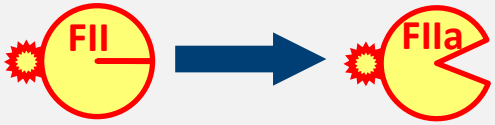
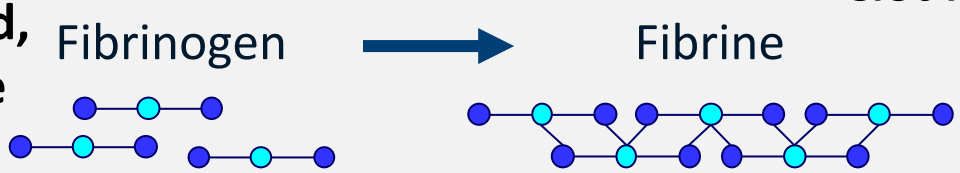


5) When enough FIIa is generated, it converts fibrinogen into fibrine to form the fibrin clot

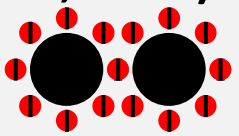
Clot formation



Clotting time (aPTT/PT)

PT

FXII activator (kaolin, silica)



FXIIa ← FXII

FXIa ← FXI

FIXa ← FIX

FVIIIa ← FVIII

Ca^{2+} TF

FVIIa TF

Ca^{2+} FIXa FVIIIa

FX → FXa

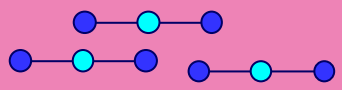
Ca^{2+} FXa FVa

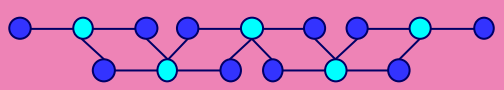
FVa ← FV

FII → FIIa

Clot formation

Fibrinogen → Fibrine





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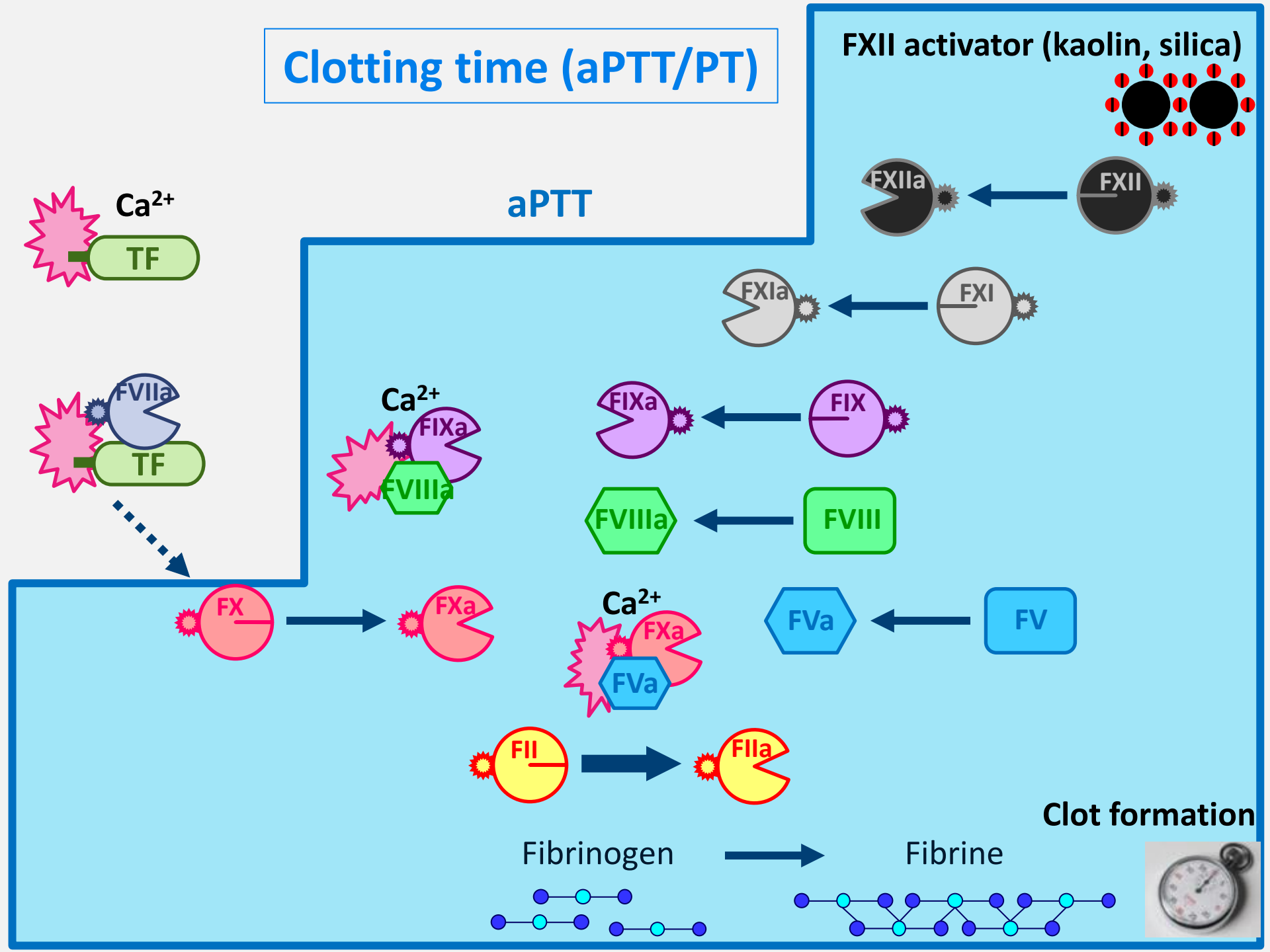
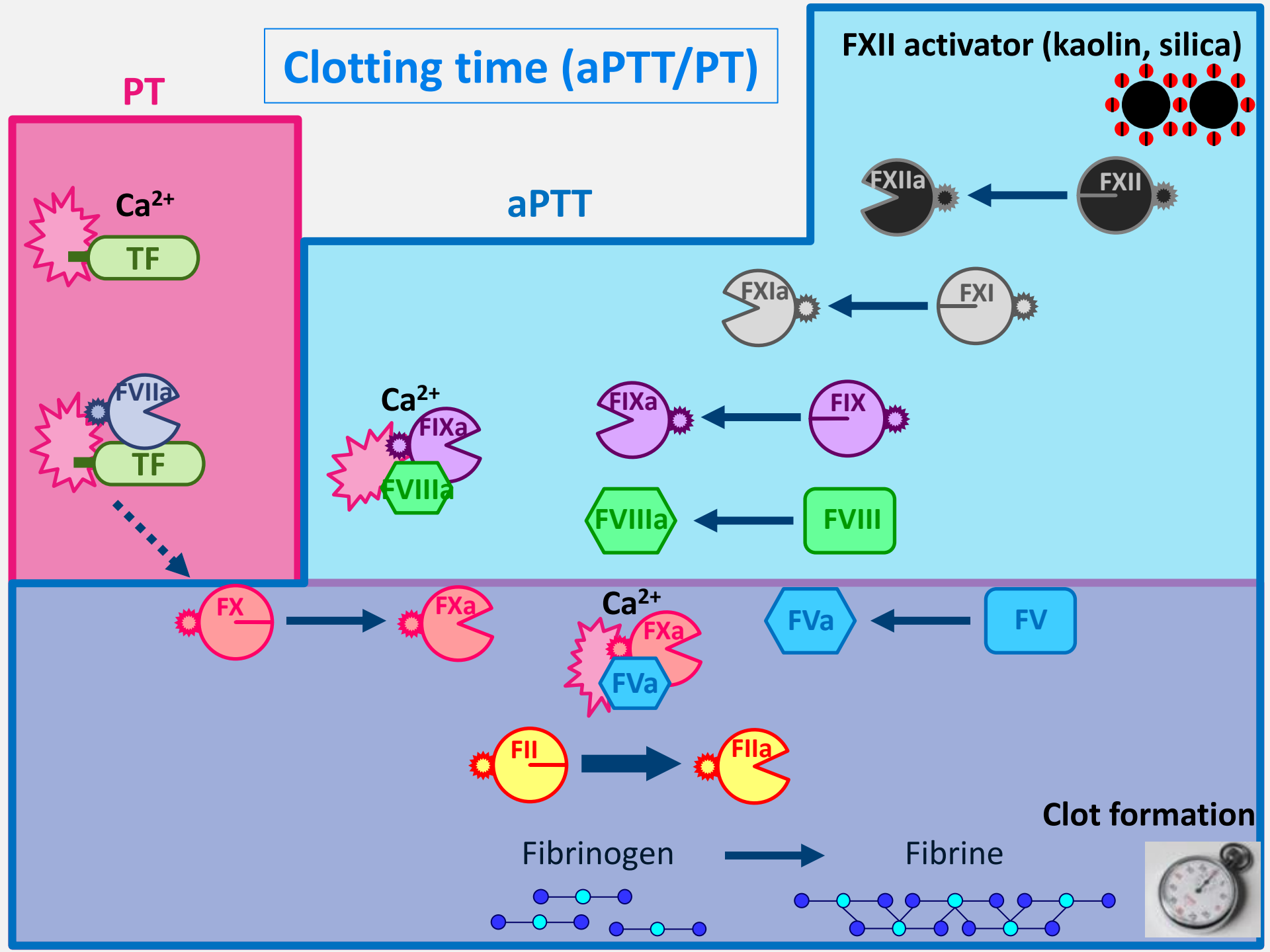
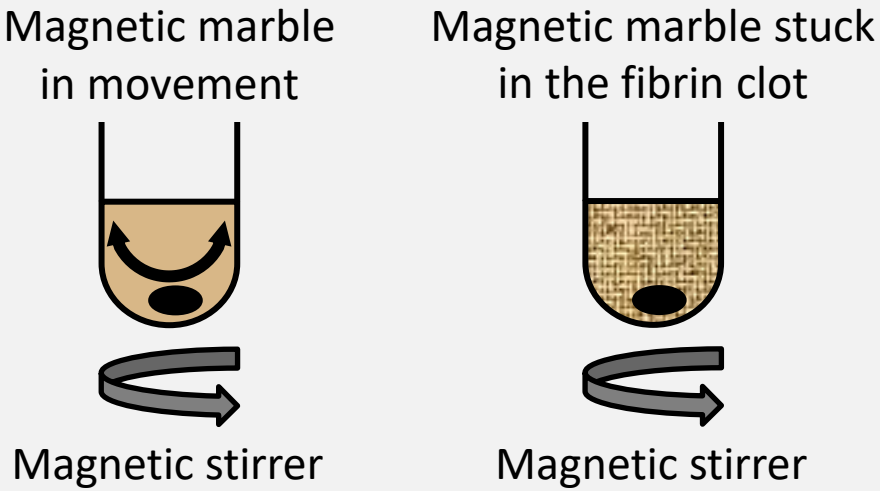



Clotting time (aPTT/PT)
PT
aPTT
FXII activator (kaolin, silica)
Ca2+
TF
FXIIa
FXII
FXIa
FXI
FVIIa
TF
Ca2+
FIXa
FVIIIa
FIXa
FIX
FVIIIa
FVIII
FX
FXa
Ca2+
FXa
FVa
FVa
FV
FII
FIIa
Clot formation
Fibrinogen
Fibrine

Chronometric assay

Different methods for clotting time measurement

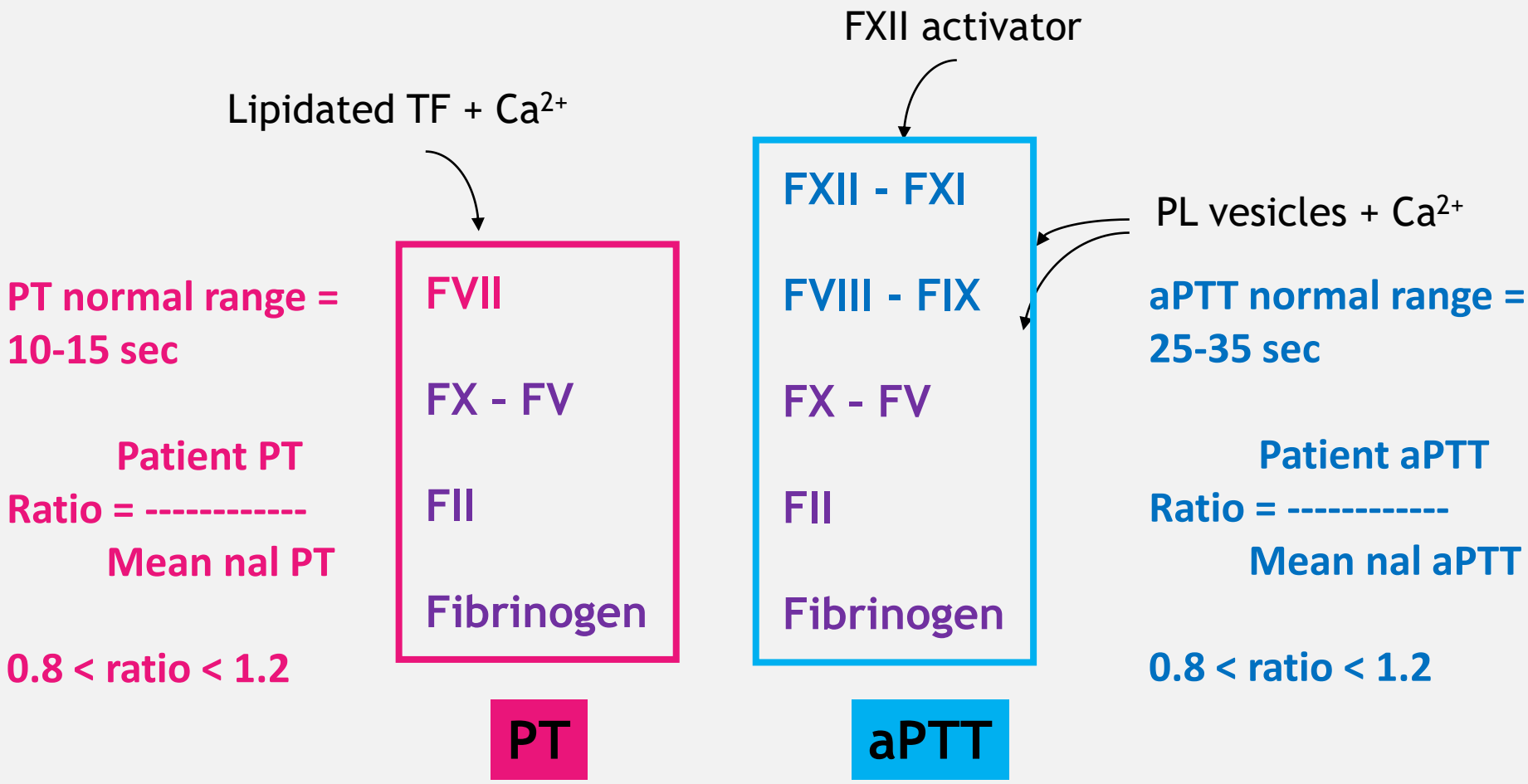
- **Mechanical**



- **Photo-optical**

Fibrine formation ⇨
Photo-dispersion

Coagulation monitoring



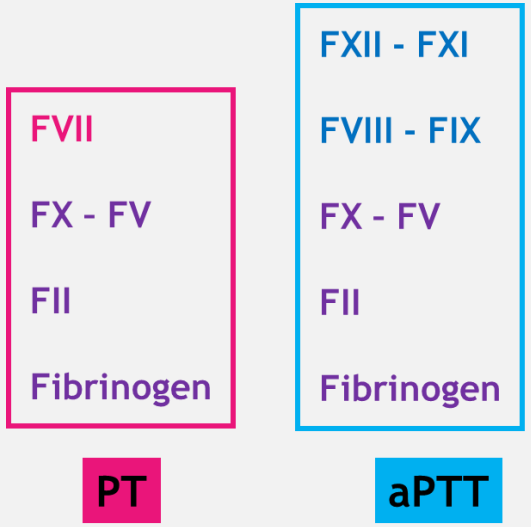
Thrombin clotting time (Fibrinogen)

Case study # 1

A 65 years old women hospitalized for suspicion of liver disease (swelling of the abdomen, yellowing of the skin and eyes)

Coagulation assays
PT = 22 sec (r = 1.7)
aPTT = 43 sec (r = 1,5)
Fibrinogen 1.5 g/L (Nal range = 2 – 4 g/L)

CBC
Platelets = 110 G/L



Bleeding risk ? How to prevent/treat it ?

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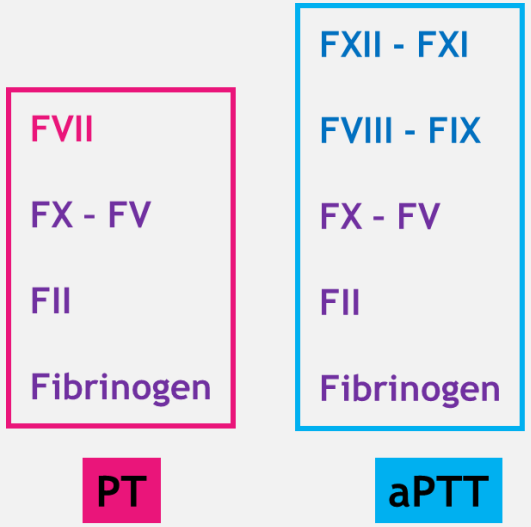

We need to know which factor(s) is/are missing
Common pathway factor assay (individual measurement of each factor activity)

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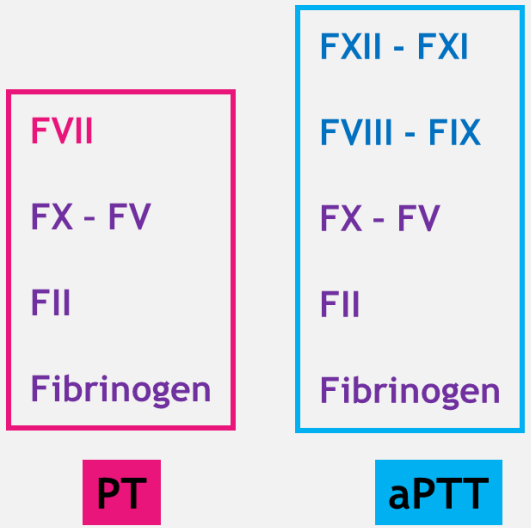

Mixing study

Plasma from patient + factor-deficient plasma = MIX

Repeat clotting assay on the mix
Determination of plasma factor level according to a std curve

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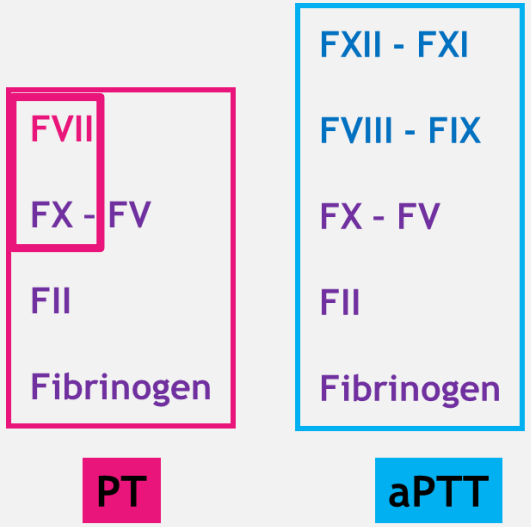

For technical reasons, it is much easier to determine (FVII + FX) residual activity than individually (much less expensive !)

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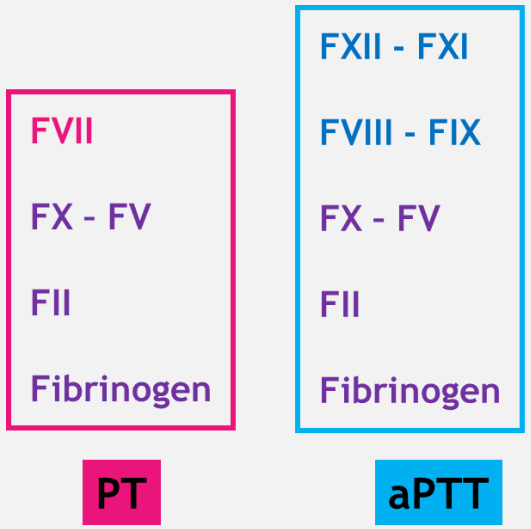
CBC
Platelets = 110 G/L

FII = 45% (Nal range = 70 – 140 %)
FV = 41% (Nal range = 70 – 140 %)
FVII + FX = 43% (Nal range = 70 – 140 %)



What can you conclude ? Where are clotting factors from ? What would you expect for FXII, FXI, FIX, and FVIII ?

Case study # 1

A 65 years old women hospitalized for suspicion of liver disease (swelling of the abdomen, yellowing of the skin and eyes)

Global deficiency! All clotting factors are missing !

Two hypothesis: defect in factor production OR excessive consumption!

What is the best treatment strategy if she experiences a severe bleeding event or if a surgery is planned ?

Case study # 1

A 65 years old women hospitalized for suspicion of liver disease (swelling of the abdomen, yellowing of the skin and eyes)

Global deficiency! All clotting factors are missing !

Two hypothesis: defect in factor production OR excessive consumption!

What is the best treatment strategy if she experiences a severe bleeding event or if a surgery is planned ?

Plasma infusion (Fresh Frozen Plasma – FFP)

Case study # 2

A pregnant women (33rd week of amenorrhea).
Coagulation tests are required before anesthesia

Coagulation assays
PT = 18 sec (r = 1.4)
aPTT = 30 sec (r = 1,0)
Fibrinogen 3 g/L (Nal range = 2 – 4 g/L)

CBC
Platelets = 220 G/L

Bleeding risk ? How to prevent/treat it ?

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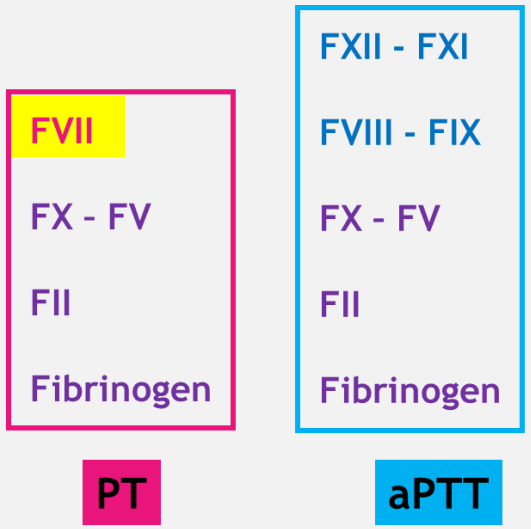
Fibrinogen 3 g/L (Nal range = 2 – 4 g/L)

CBC
Platelets = 220 G/L

Because automated procedure:
FII = 90% (Nal range = 70 – 140 %)
FV = 95% (Nal range = 70 – 140 %)
FVII + FX = 24% (Nal range = 70 – 140 %)
FX = 92% (Nal range = 70 – 140 %)
FVII = 15% (Nal range = 70 – 140 %)



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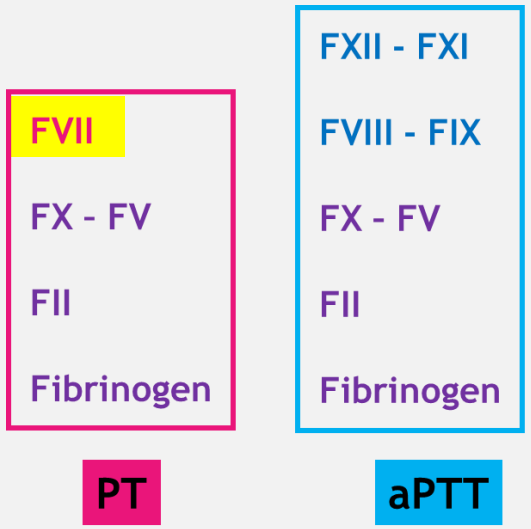
FII = 90% (Nal range = 70 – 140 %)
FV = 95% (Nal range = 70 – 140 %)
FVII + FX = 24% (Nal range = 70 – 140 %)
FX = 92% (Nal range = 70 – 140 %)
FVII = 15% (Nal range = 70 – 140 %)



Mild FVII deficiency
Associated with mild bleeding risk
Substitutive therapy “on-demand”

Case study # 3

A 4 years old boy with acute bleeding (blood in urine and stools)

Coagulation assays
PT = 32 sec (r = 2.5)
aPTT = 62 sec (r = 2.1)
Fibrinogen 2.5 g/L (Nal range = 2 – 4 g/L)

CBC
Platelets = 350 G/L

How to treat efficiently and specifically ?

Case study # 3

A 4 years old boy with acute bleedings (blood in urine and stools)

Coagulation assays
PT = 32 sec (r = 2.5)
aPTT = 62 sec (r = 2.1)
Fibrinogen 2.5 g/L (Nal range = 2 – 4 g/L)

CBC
Platelets = 350 G/L

FII = 10% (Nal range = 70 – 140 %)
FV = 85% (Nal range = 70 – 140 %)
FVII + FX = 6% (Nal range = 70 – 140 %)

Global deficiency ? What is the common point between FX, FVII, and FII ?
What would you expect for FIX ?

Case study # 3

A 4 years old boy with acute bleedings (blood in urine and stools)

FVII, FIX, FX, and FII are vitamin K-dependent factors

CL°) Vit K-dependent factors deficiency!

How to treat efficiently and specifically?

Case study # 3

A 4 years old boy with acute bleedings (blood in urine and stools)

FVII, FIX, FX, and FII are vitamin K-dependent factors

CL°) Vit K-dependent factors deficiency!

How to treat efficiently and specifically?

Vit K-dependent factors concentrates (prothrombin complex concentrate)

Vit K (delay of action since involved in clotting factors synthesis)

Case study # 3

A 4 years old boy with acute bleedings (blood in urine and stools)

Clinical setting leading to vitamin K-dependent factor deficiency ?

Case study # 3

A 4 years old boy with acute bleedings (blood in urine and stools)

Clinical setting leading to vitamin K-dependent factor deficiency ?

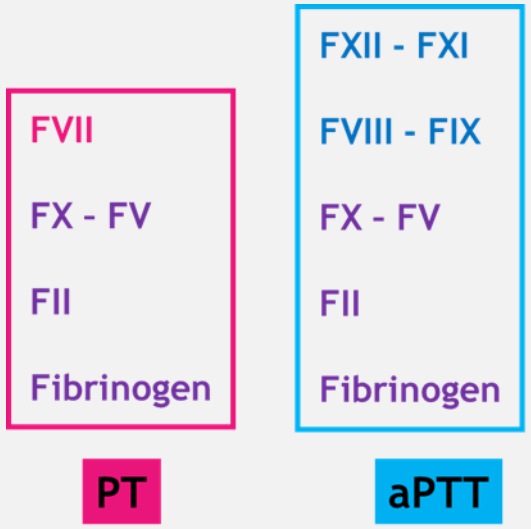
- **Therapeutic use of vitamin K antagonists**
- **Poisoning with vitamin K antagonists**
- **Alteration of the intestinal microbiota (antibiotic therapy)**
- **Nutritional deficiency (severe malnutrition)**

Case study # 4

A 76 years old man with bleeding after total hip arthroplasty (in the outflow from the drain)

Coagulation assays
PT = 15 sec (r = 1.2)
aPTT = 60 sec (r = 2.1)
Fibrinogen 2.1 g/L (Nal range = 2 – 4 g/L)

CBC: normal platelet count



Case study # 4

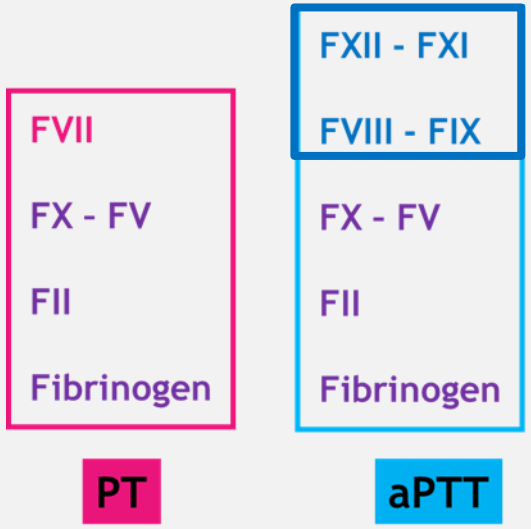
A 76 years old man with bleeding after total hip arthroplasty (in outflow from the drain)

Coagulation assays
PT = 15 sec (r = 1.2)
aPTT = 60 sec (r = 2.1)
Fibrinogen 2.1 g/L (Nal range = 2 – 4 g/L)

CBC: normal platelet count

FVIII = 75% (Nal range = 50 – 150 %)
FIX = 85% (Nal range = 60 – 120 %)
FXI = 78% (Nal range = 60 – 120 %)
FXII = 80%

No coagulation factor deficiency !!!



Case study # 4

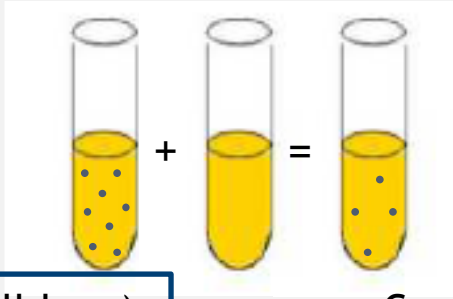
A 76 years old man with bleeding after total hip arthroplasty (in outflow from the drain)

aPTT is sensitive to anticoagulant therapy (can be used to monitor anticoagulant)

Mixing studies help to distinguish between a clotting factor deficiency and an inhibitor (to be performed before clotting factor assay !)

Mix patient plasma + Nal plasma (1:1)
Repeat aPTT measurement

Here mixing study
aPTT = 55sec (r = 1.9)



Uncorrected aPTT (still long)
Presence of an inhibitor
(here anticoagulant heparin)

Corrected aPTT (nal range)
Absence of clotting factor
Clotting factors assay required !

Case study # 5

An 18 months old child with signs of bleeding (lingual frenulum, elbow and knee hematoma, easy bruising)

Coagulation assays
PT = 12 sec (r = 1)
aPTT = 100 sec (r = 3.3)
Fibrinogen 3.2 g/L (Nal range = 2 – 4 g/L)

CBC: normal platelet count

Case study # 5

An 18 months old child with signs of bleeding (lingual frenulum, elbow and knee hematoma, easy bruising)

Coagulation assays
PT = 12 sec (r = 1)
aPTT = 100 sec (r = 3.3)
Mixing study aPTT = 35 sec (r = 1.2)
Fibrinogen 3.2 g/L (Nal range = 2 – 4 g/L)

Correction ⇨ clotting factor assay

CBC: normal platelet count

Case study # 5

An 18 months old child with signs of bleeding (lingual frenulum, elbow and knee hematoma, easy bruising)

Coagulation assays
PT = 12 sec (r = 1)
aPTT = 100 sec (r = 3.3)
Fibrinogen 3.2 g/L (Nal range = 2 – 4 g/L)

CBC: normal platelet count

FVIII < 1% (Nal range = 50 – 150 %)
FIX = 110% (Nal range = 60 – 120 %)
FXI = 95% (Nal range = 60 – 120 %)
FXII = 80%

Severe FVIII deficiency !
Hight bleeding risk

Case study # 5

An 18 months old child with signs of bleeding (lingual frenulum, elbow and knee hematoma, easy bruising)

What are the possible causes of FVIII deficiency

Differential diagnosis with von Willebrand disease !

vWF assays (activity and plasma level) = 98%

Severe hemophilia A

Treatment: substitutive therapy (FVIII supplementation) or Emicizumab (Emlibra® FDA approved 2018)

See Dr CASARI's lecture on Thursday for the treatment of hemophilia and vWF disease.

Case study # 6

A young girl (13 years old) with recurrent nosebleeds (bilateral) and menorrhagia (heavy periods) = Mucocutaneous bleedings

Coagulation assays
PT = 14 sec (r = 1.1)
aPTT = 60 sec (r = 2)
Fibrinogen 4 g/L (Nal range = 2 – 4 g/L)

CBC:
Platelets = 350 G/L

PFA-100 lengthened

Case study # 6

A young girl (13 years old) with recurrent nosebleeds (bilateral) and menorrhagia (heavy periods) = Mucocutaneous bleedings

Coagulation assays
PT = 14 sec (r = 1.1)
aPTT = 60 sec (r = 2)
Fibrinogen 4 g/L (Nal range = 2 – 4 g/L)

CBC:
Platelets = 350 G/L

PFA-100 lengthened

Mixing study aPTT = 32 sec (r = 1.2)
Correction ⇨ clotting factor assay

FVIII < 35% (Nal range = 50 – 150 %)
FIX = 100% (Nal range = 60 – 120 %)
FXI = 80% (Nal range = 60 – 120 %)
FXII = 78%

Case study # 6

A young girl (13 years old) with recurrent nosebleeds (bilateral) and menorrhagia (heavy periods) = Mucocutaneous bleedings

Coagulation assays
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Fibrinogen 4 g/L (Nal range = 2 – 4 g/L)

CBC:
Platelets = 350 G/L

PFA-100 lengthened

Mixing study aPTT = 32 sec (r = 1.2)
Correction ⇨ clotting factor assay

FVIII < 35% (Nal range = 50 – 150 %)
FIX = 100% (Nal range = 60 – 120 %)
FXI = 80% (Nal range = 60 – 120 %)
FXII = 78%

Mild FVIII deficiency
Associated with primary hemostasis dysfunction
??

Case study # 6

A young girl (13 years old) with recurrent nosebleeds (bilateral) and menorrhagia (heavy periods) = Mucocutaneous bleedings

Von Willebrand factor assay required !

Activity: 21% (Nal range = 50 – 150 %)
Ag: 25% (Nal range = 50 – 150 %)

Quantitative or qualitative defect ?

Case study # 6

A young girl (13 years old) with recurrent nosebleeds (bilateral) and menorrhagia (heavy periods) = Mucocutaneous bleedings

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Quantitative!

vWF functions ?

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Von Willebrand factor assay required !

Activity: 21% (Nal range = 50 – 150 %)
Ag: 25% (Nal range = 50 – 150 %)

Quantitative or qualitative defect ?
Quantitative!

vWF functions ?
Chaperon of FVIII and platelet adhesion to wound site

Synthetized by endothelial cells and megakaryocytes

See Dr CASARI's lecture on Thursday for the treatment of hemophilia and vWF disease.

Case study # 7

A man (65 years old) with metastatic prostate cancer

Coagulation assays
PT = 30 sec (r = 2.1)
aPTT = 55 sec (r = 1.4)
Fibrinogen 0.5 g/L (Nal range = 2 – 4 g/L)

CBC:
Platelets = 80 G/L

Case study # 7

A man (65 years old) with metastatic prostate cancer

Coagulation assays
PT = 30 sec (r = 2.1)
aPTT = 55 sec (r = 1.4)
Fibrinogen 0.5 g/L (Nal range = 2 – 4 g/L)

CBC:
Platelets = 80 G/L

FII = 65% (Nal range = 70 – 140 %)
FV = 40% (Nal range = 70 – 140 %)
FVII + FX = 53% (Nal range = 70 – 140 %)

Case study # 7

A man (65 years old) with metastatic prostate cancer

Global deficiency

Two hypothesis: defect in factor production OR excessive consumption!

Case study # 7

A man (65 years old) with metastatic prostate cancer

Global deficiency

Two hypothesis: defect in factor production OR excessive consumption!

D-Dimers (Degradation fibrin products)
= 8 µg/mL (Nal < 1 µg/mL)

What does that tell you?

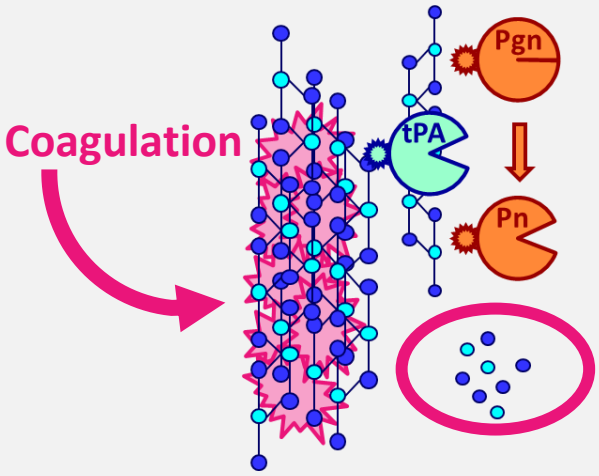
Case study # 7

A man (65 years old) with metastatic prostate cancer

Global deficiency

Two hypothesis: defect in factor production OR excessive consumption!

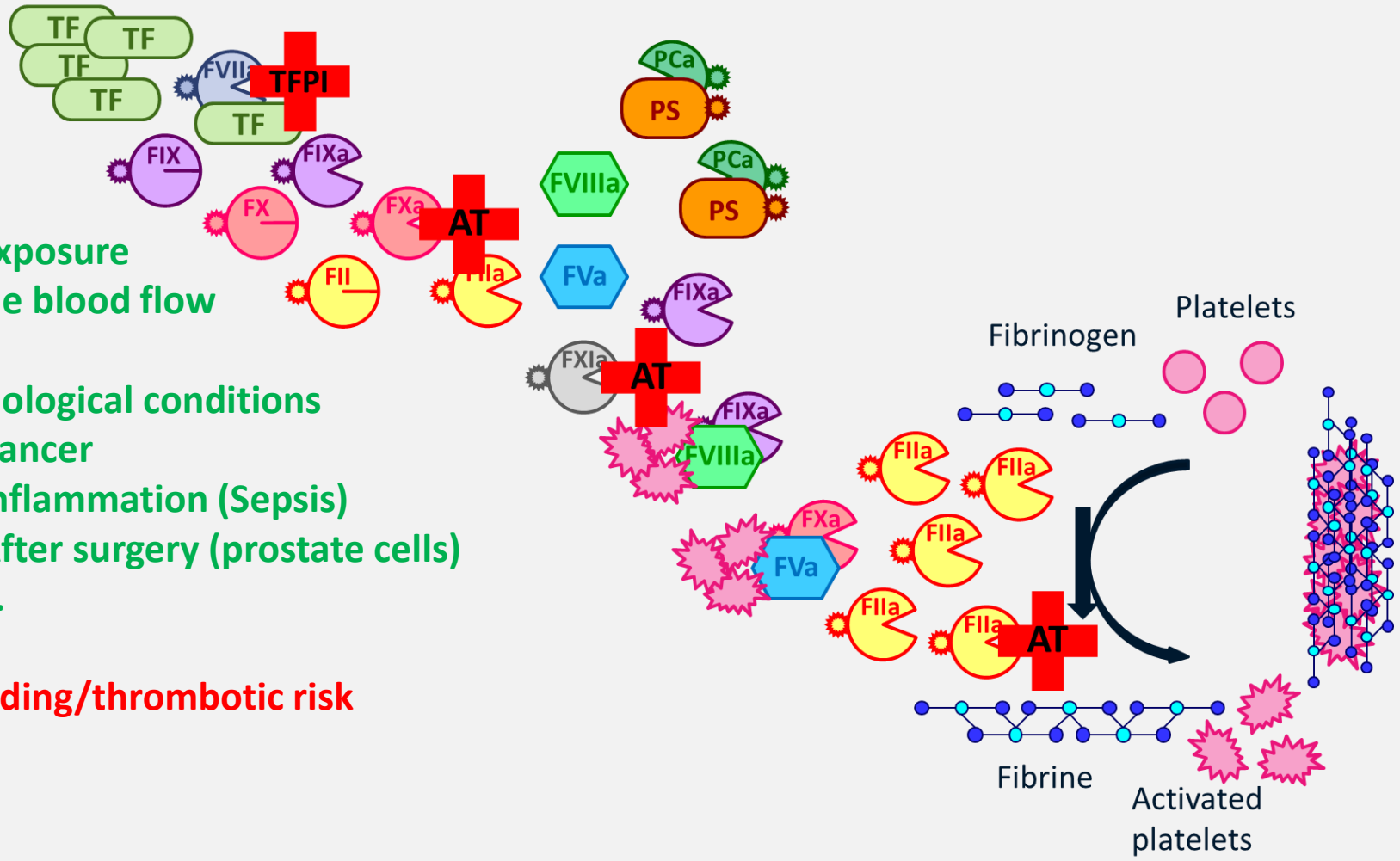
D-Dimers (Degradation fibrin products)
= 8 µg/mL (Nal < 1 µg/mL)



What does that tell you?

⇨ **Excessive consumption… DIC**

Disseminated Intravascular Coagulation



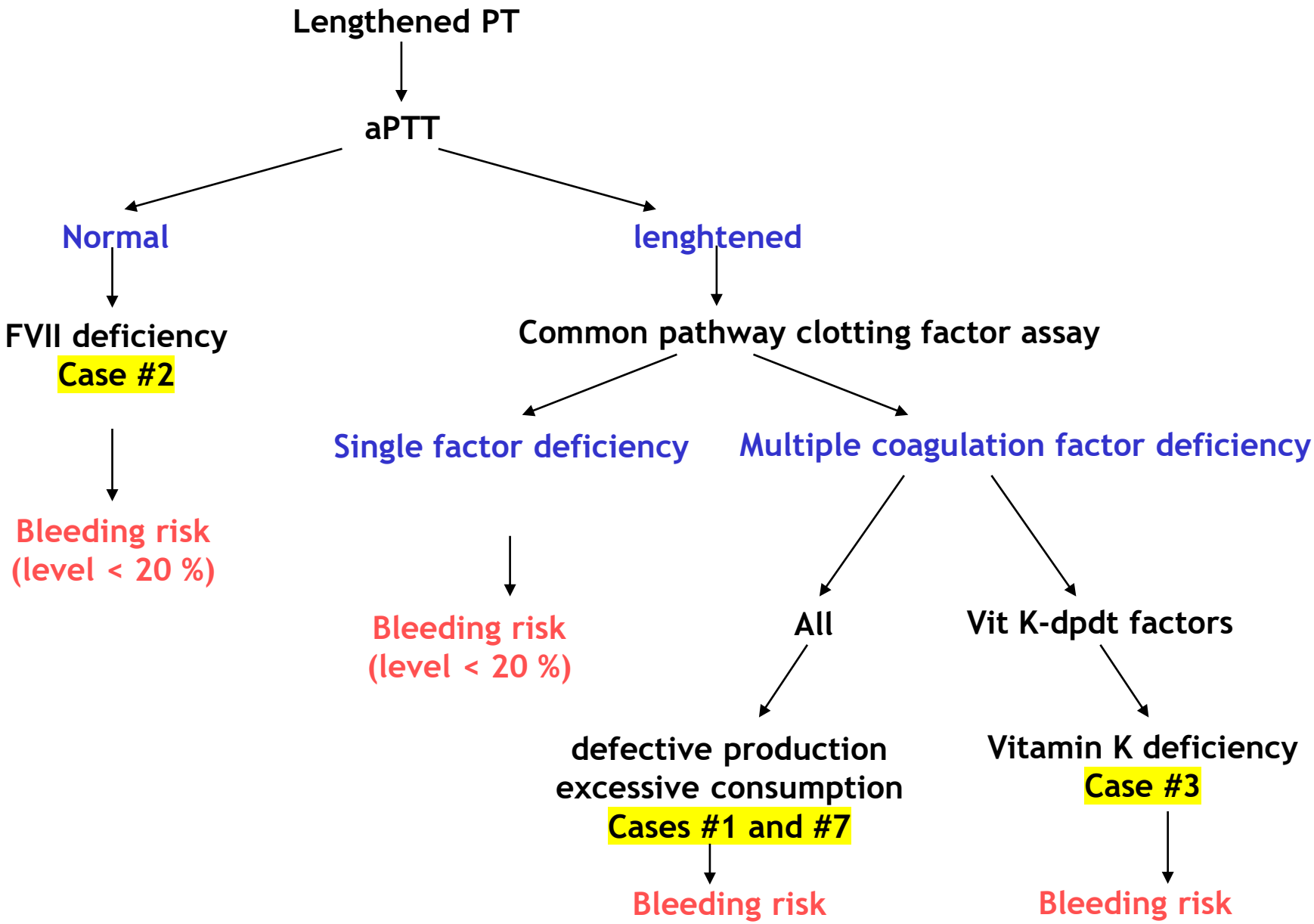
TF exposure
in the blood flow

Pathological conditions

- **Cancer**
- **Inflammation (Sepsis)**
- **After surgery (prostate cells)**
- **…**

Bleeding/thrombotic risk

PT elongation



aPTT elongation