L'iter diagnostico di laboratorio nelle coagulopatie congenite emorragiche

Armando Tripodi

Angelo Bianchi Bonomi

Hemophilia and Thrombosis Center

Dept. of Clinical Sciences and Community Health

University of Milano

Congenital Hemorrhagic Coagulopathies

Aims of Laboratory Investigation

- To establish the causes of bleeding in patients who have shown evidence of abnormal bleeding
- To detect mild defects in asymptomatic patients *(pre-surgical screening)*

Congenital Hemorrhagic Coagulopathies

Most Important Screening Test

Good Collection of Clinical History

Congenital Hemorrhagic Coagulopathies

Why should clinical history be collected

- Poor sensitivity of screening tests to detect mild defects
- The type of bleeding may provide valuable clue to its etiology
- Some coagulation abnormalities are not associated with clinical bleeding *(FXII, PreKal, HMWK)*

Aims of the Clinical History

How should clinical history be collected

- Type of bleeding
- Location, frequency, duration, severity
- Whether it is spontaneous or post-traumatic
- Whether other family members have the same symptoms
- The age of appearence of the first symptoms
- Whether other diseases are present
- Whether the patient is taking drugs

Main Bleeding Symptoms

- Bleeding from mucous membranes is a typical feature of platelet disorders
- Soft-tissue bleeding is a typical feature of coagulation disorders
- Umbilical cord or delayed bleeding are typical features of factor XIII deficiency
- Simultaneous bleeding from multiple sites suggests an acute, acquired systemic coagulation or fibrinolytic disorders

Laboratory Tests

Should be aimed at investigating

- *Primary Hemostasis*
- Platelet vessel-wall interaction
- *Coagulation*
- Thrombin generation
- Fibrin formation
- *Fibrinolysis*
- Fibrin degradation

Laboratory Tests

Should be

- Sensitive
- Limited in number
- Easy to do
- Their results clinically-relevant

Two-step Laboratory Investigation

- *First Step (Simple Screening Tests)*

- To detect most frequent and well established causes of bleeding

- *Second Step (Specific Tests)*

- To detect less common causes of bleeding due to abnormalities to which the screening tests are insensitive

First Step

Laboratory Tests

First Step

- *Bleeding Time (or alternative tests)*
- Platelet Count
- Prothrombin Time (PT)
- Activated Partial Thromboplastin Time (APTT)

Bleeding Time

The time (minutes) needed to stop bleeding from a superficial incision of the skin

Laboratory Tests

Test	*Materials*	*Sensitive to:*
Bleeding Time	- Automated device - Sphygmomanometer - Filter paper - Stopwatch	- Thrombocytopenia - Thrombocytopathy

Variables affecting the Bleeding Time

- Depth of incision
- Site of incision
- Venous pressure
- End point
- Effect of drugs

The above variables and the complexity of the bleeding time made most labs to abandon this test. Alternative tests have been proposed, but not yet completely validated

Laboratory Tests

First Step

- Bleeding Time (or alternative tests)
- *Platelet Count*
- Prothrombin Time (PT)
- Activated Partial Thromboplastin Time (APTT)

Laboratory Tests

Test	*Materials*	*Sensitive to:*
Platelet count	- Electronic counter - Contrast-phase microscope - EDTA (or citrated) whole blood	- Thrombocytopenia - Thrombocytosis

Further Evaluation of Primary Hemostasis

- *Low Platelet Count*

- Investigation of thrombocytopenia

- *Prolonged Bleeding Time*

- Measurement of plasma von Willebrand factor
- Platelet aggregation studies

Laboratory Tests

First Step

- Bleeding Time (or alternative tests)
- Platelet Count
- *Prothrombin Time (PT)*
- Activated Partial Thromboplastin Time (APTT)

Laboratory Tests

Test	*Materials*	*Sensitive to:*
PT	- Thromboplastin - Calcium Chloride - Platelet Poor Plasma	- FVII - FX, FV, FII, FI - Oral anticoagulants - Unfractionated heparin *(?)*

Variables affecting PT

- Type of Thromboplastin
- Citrate concentration *(105-109 mM)*
- Calcium Chloride concentration
- Temperature
- Coagulometer

Origin of Thromboplastins

Tissue Factor & Phospholipids

- *Human*
- Placenta, recombinant relipidated tissue factor
- *Rabbit, Bovine*
- Brain

Prothrombin Time (PT)

Results expression

- Time (seconds)
- % Activity
- Ratio (PTpatient/PTnormal)
- INR (International Normalized Ratio)

Usefulness of the Prothrombin Time (PT)

- Diagnosis and management of
  - Congenital hemorrhagic coagulopathies
  - Disseminated intravascular coagulation
- Prognosis of liver cirrhosis
  - Model of end stage liver disease (MELD)
- Dose-adjustment of VKA

Laboratory Tests

First Step

- Bleeding Time (or alternative tests)
- Platelet Count
- Prothrombin Time (PT)
- *Activated Partial Thromboplastin Time (APTT)*

Laboratory Tests

Test	*Materials*	*Sensitive to:*
APTT	- Activator - Phospholipids - Calcium Chloride - Platelet Poor Plasma	- PK, FXII, HMWK, FXI - FIX, FVIII - FX, FV, FII, FI - Oral anticoagulants - Unfractionated heparin, *LMWH* - Lupus Anticoagulants

Variables affecting APTT

- Type/Concentration of Activator
- Type/Concentration of Phospholipids
- Activation time
- Citrate concentration *(105-109 mM)*
- Calcium chloride concentration
- Temperature
- Coagulometer

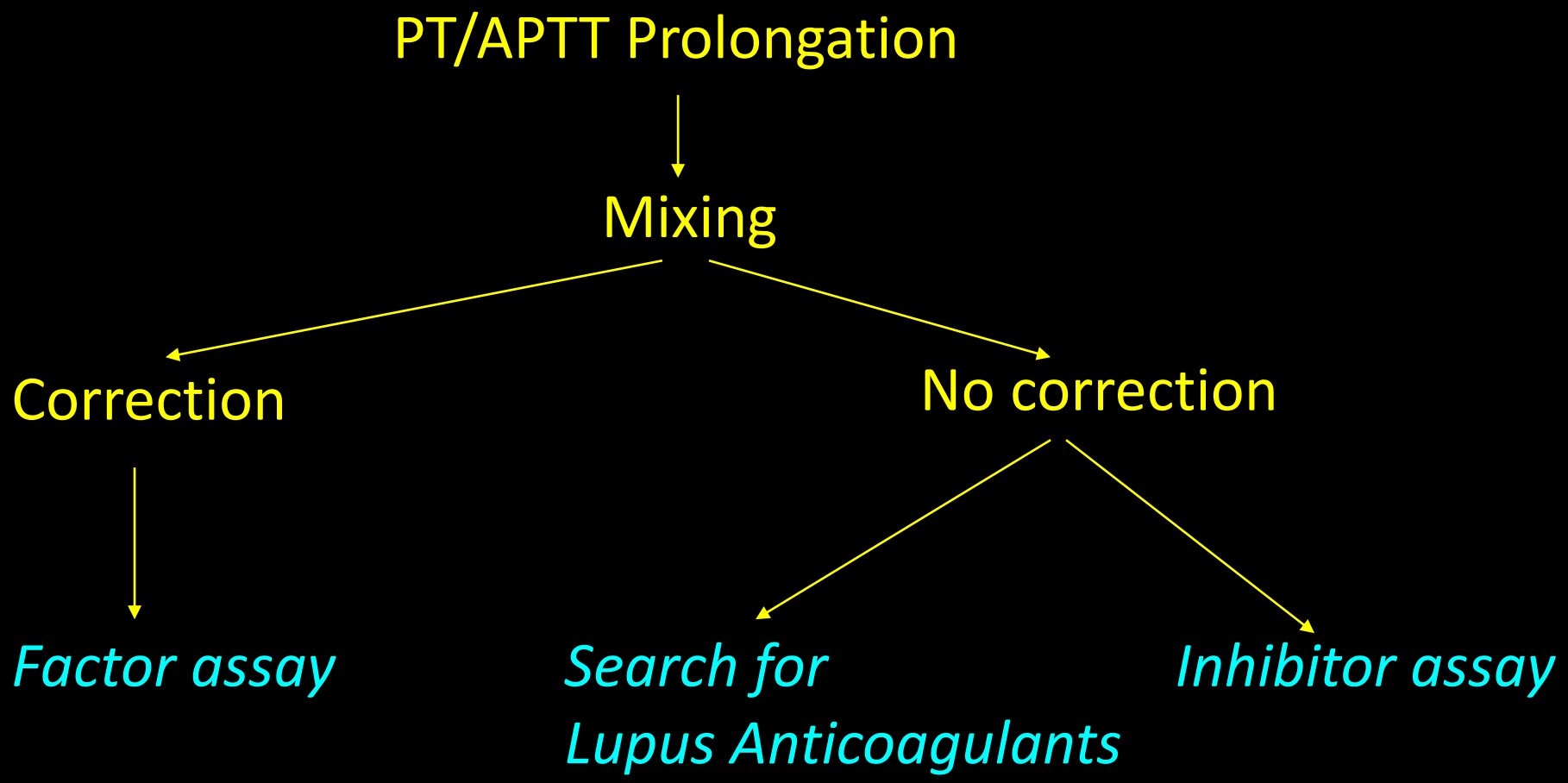
Types of APTT Activators

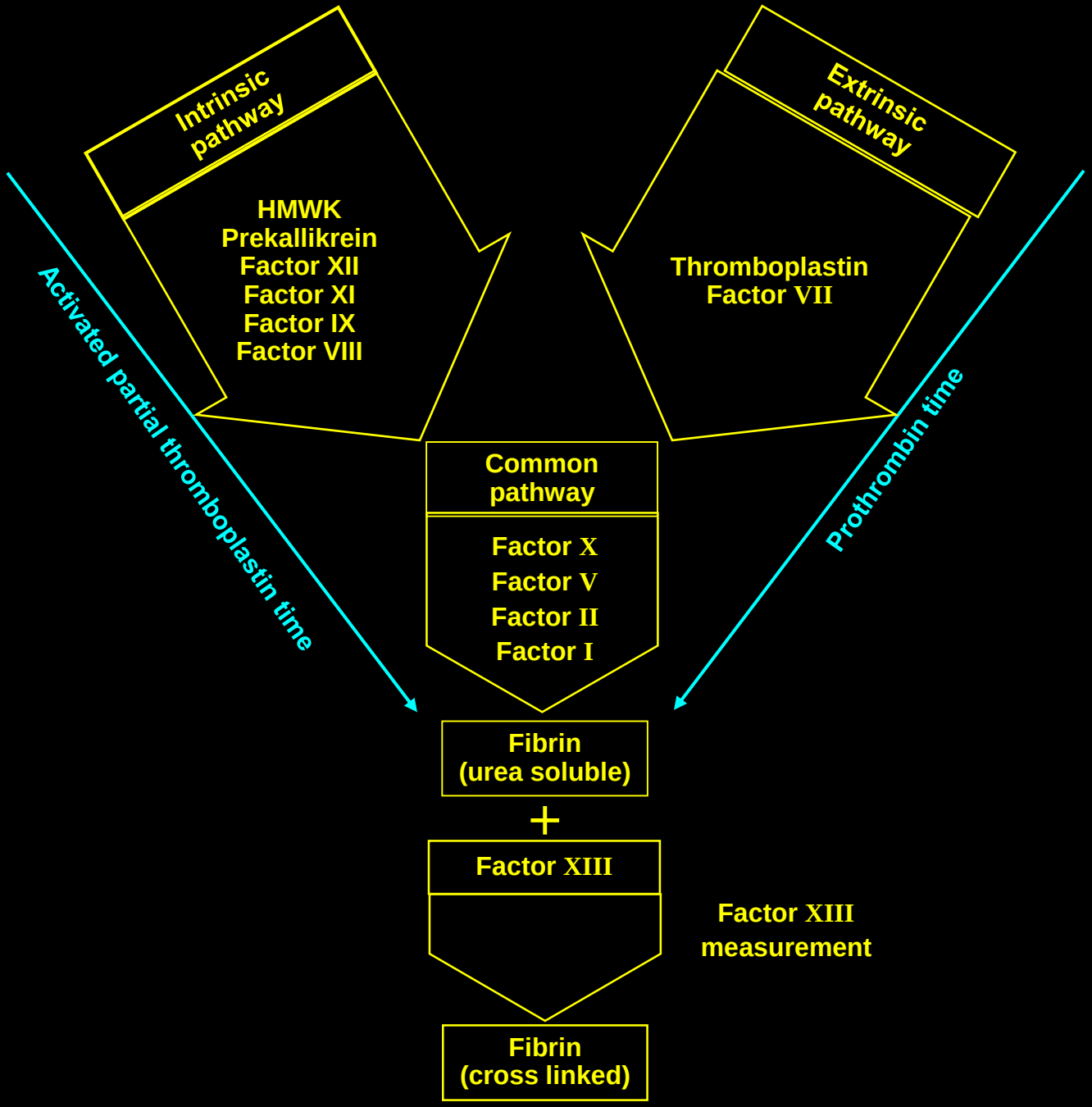
- *Particulate*
- Kaolin (sensitive, but unpractical)
- Silica (sensitive and practical)
- *Soluble*
- Ellagic acid (practical, but rather insensitive)

APTT result expression

- Clotting time (seconds)
- Ratio (patient-to-normal clotting time)

Further Evaluation of Coagulation




Intrinsic pathway
HMWK
Prekallikrein
Factor XII
Factor XI
Factor IX
Factor VIII
Extrinsic pathway
Thromboplastin
Factor VII
Activated partial thromboplastin time
Prothrombin time
Common pathway
Factor X
Factor V
Factor II
Factor I
Fibrin (urea soluble)
+
Factor XIII
Factor XIII measurement
Fibrin (cross linked)

A. TRIPODI

Need for differential diagnosis when APTT is prolonged owing to coag factors deficiency

- FIX, FVIII or FXI deficiency
- *Hemorrhagic risk*
- FXII, Pre-kallicrein or HMWK deficiency
- *No hemorrhagic risk*

Usefulness of the APTT

- Diagnosis and management of
  - Congenital hemorrhagic coagulopathies
  - Disseminated intravascular coagulation
- Dose-adjustment of unfractionated heparin therapy
  - Therapeutic interval: 1.5-2.5 times prolongation over the baseline value
- Search for circulating anticoagulants

Second Step

Clinical history of bleeding, but
normal first step laboratory tests

Laboratory Tests

Second Step

- *FXIII*
- Platelet Factor 3 (phosphatidylserine)
- Hyperfibrinolysis
- Von Willebrand Factor
- Dysfibrinogenemia

Factor XIII Assay

- Clot solubility in 5M urea
- Functional
- Immunochemical

Laboratory Tests

Second Step

- FXIII
- *Platelet Factor 3 (phosphatidylserine)*
- Hyperfibrinolysis
- Von Willebrand Factor
- Dysfibrinogenemia

Isolated Defect of PF3 Procoagulant Activity (Scott syndrome)

- Weiss HJ et al
  Isolated deficiency of platelet procoagulant activity
  Am J Med 1979; 67: 206

- Toti F et al
  Scott syndrome, characterized by impaired transmembrane migration of procoagulant phosphatidylserine and hemorrhagic complications, is an inherited disorder
  Blood 1996; 87: 1409

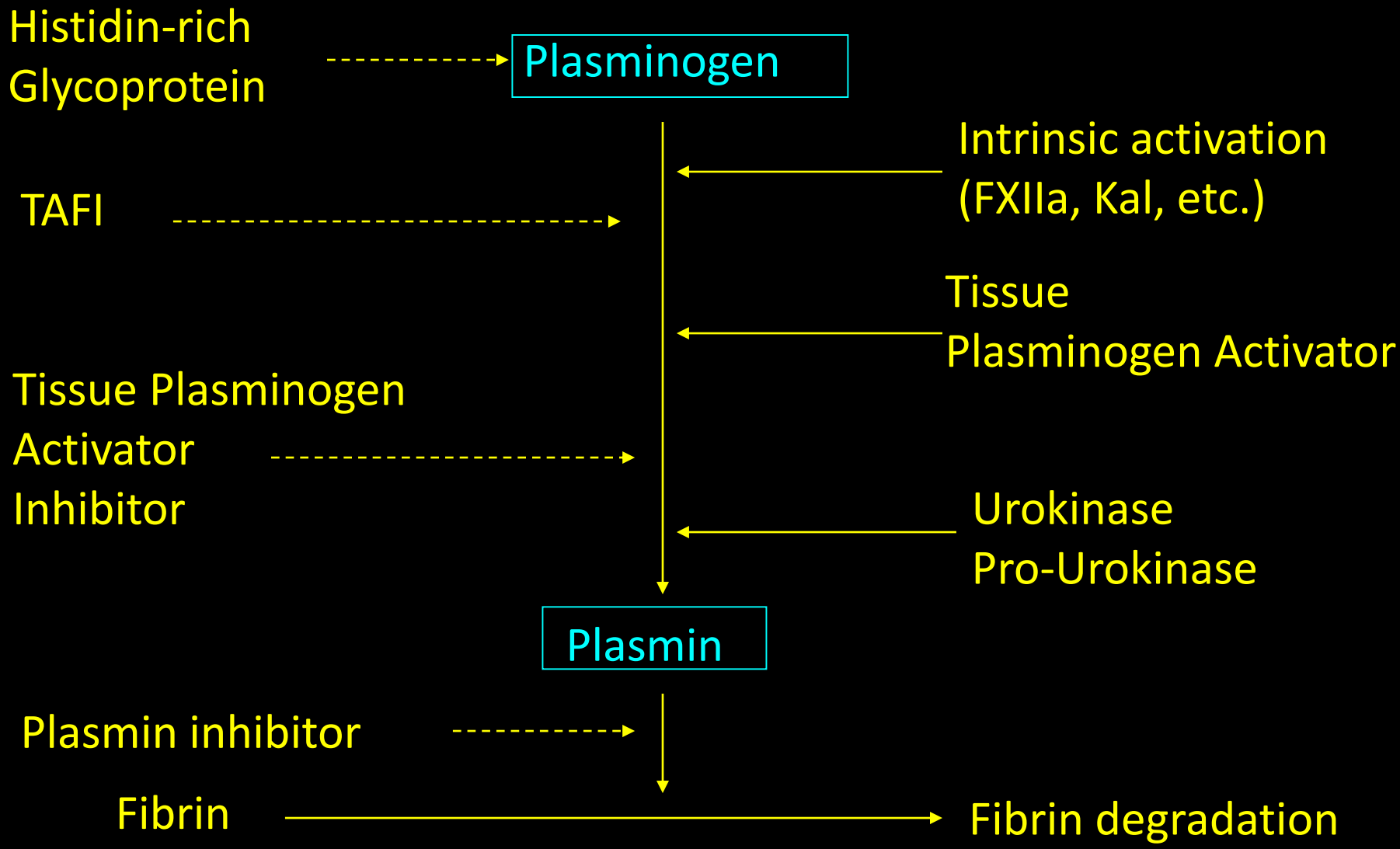
- Charles L. Percy CL et al
  Laboratory monitoring of Scott Syndrome
  Br J Haematol 2009; 149: 803

Laboratory Tests

Second Step

- FXIII
- Platelet Factor 3 (phosphatidylserine)
- *Hyperfibrinolysis*
- Von Willebrand Factor
- Dysfibrinogenemia

Fibrinolysis



Congenital Deficiency of Plasmin Inhibitor

- *Homozygotes*

- Severe hemophilia-like bleeding tendency since childhood
- Rebleeding from wounds

- *Heterozygotes*

- About 20% of patients present with mild bleeding tendency (easy bruising, oozing from dental extractions)

Laboratory Tests

Second Step

- FXIII
- Platelet Factor 3 (phosphatidylserine)
- Hyperfibrinolysis
- *Von Willebrand Factor*
- Dysfibrinogenemia

The APTT & Willebrand disease

Mild forms of Willebrand disease may present with (near) normal APTT

Laboratory Tests

Second Step

- FXIII
- Platelet Factor 3 (phosphatidylserine)
- Hyperfibrinolysis
- Von Willebrand Factor
- *Dysfibrinogenemia*

Dysfibrinogenemia
Main Characteristics

- *Abnormal fibrinogen in plasma*

- Low functional fibrinogen (Clauss method)
- Normal or high immunochemical fibrinogen
- Prolonged thrombin clotting time

Dysfibrinogenemia

Symptoms

- None
- Hemorrhage
- Arterial and/or Venous Thromboembolism

Conclusions

- A rational two-step approach combining
- *Clinical data*
- *Laboratory data*
- Helps identifying the majority of congenital hemorrhagic coagulopathies