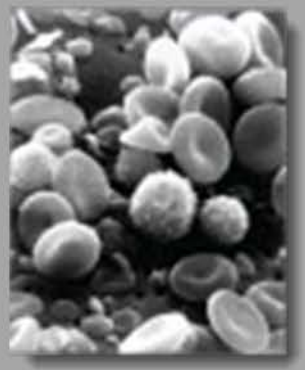

12th Annual Canadian Blood Services International Symposium

Plasma: Transfuse it, Fractionate it or Forget it?

Canadian Blood Services
it's in you to give

Plasma Testing in the Clinical Coagulation Laboratory: New drugs, new problems.

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McMaster University Health Sciences

What prevents us from bleeding?

Series of inter-related processes'…

- Primary hemostasis
  - Involves the interaction of von Willebrand factor and platelets
- Secondary hemostasis
  - End result: prothrombin is activated to thrombin, converting fibrinogen to fibrin and the formation of a fibrin clot
- Clot stabilization
  - Activation of factor XIII by thrombin, in the presence of fibrin
- Wound healing
  - Delayed activation of the fibrinolytic system

Introduction

- Laboratory tests are essential for diagnosing bleeding disorders.
- Congenital or acquired abnormalities can be found in any part of the hemostasis pathway.
- Coagulation screening tests are commonly performed as the initial investigation of a bleeding disorder.

Table 2. Differential diagnosis of abnormalities in coagulation tests

	PT/INR	APTT	TT	fibrinogen
Cause and pattern of abnormalities				
Fibrinogen deficiency (hypofibrinogenemia) or dysfunction (dysfibrinogenemia)	N – ↑	N – ↑	↑	↓
Afibrinogenemia	NC	NC	NC	ND
FVII deficiency	↑	N	N	N
FVIII, FIX, and/or FXI deficiency	N	↑	N	N
Acquired or congenital hemophilia, with an inhibitor	N	↑ †	N	N
FII, FV, and/or FX deficiency	↑	↑	N	N
Factor deficiencies not associated with bleeding (FXII, high molecular weight kininogen or prekallikrein deficiency)	N	↑	N	N
Lupus anticoagulant	N – ↑	N – ↑ ‡	N	N
Lupus anticoagulant with FII deficiency	↑	↑	N – ↑	N
Unfractionated heparin - therapy or sample contamination	N – ↑	↑	↑↑*	N
Low molecular weight heparin therapy	N	N – ↑	N – ↑	N
Direct thrombin inhibitors	N – ↑	N – ↑	↑↑	N
Direct inhibitors of FXa	N – ↑	N – ↑	N	N
Liver disease† (if early, often affects FVII, FXI and/or FXII; if late or end stage, fibrinogen is usually low; spares FVIII but can affect all other factors)	N – ↑	N – ↑	N – ↑	↓ - N – ↑
Vitamin K deficiency (or treatment with a vitamin K antagonist) which reduce levels of FVII and also FII, FIX and FX†	↑	N – ↑	N	N
Fibrinolytic therapy	↑	↑	↑	↓
Consumptive coagulopathy†	N – ↑	↑	N – ↑	N – ↓
Dilutional coagulopathy†	N – ↑	N – ↑	N – ↑	↓ - N
VWD	N	N – ↑	N	N
Preanalytical error – collected in potassium EDTA§	↑	↑	N – ↑	N
Preanalytical error – serum instead of plasma	NC	NC	NC	ND

↑, elevated levels; ↓, reduced levels, N, normal; NC, no clot; ND, not detectable.

Ref: Hayward and Moffat. Int J Lab Hematol. 35:322-33; 2013.

Test	Estimated Sensitivity (%)	Estimated Specificity (%)	Purpose of test and defects detected	Abnormalities that don't cause bleeding
APTT	2.1	98	Congenital or acquired deficiencies of intrinsic (PK, HMWK, XII, XI, IX, VIII) and common pathway (FX, FV, FII).	Contact factors deficiencies (FXII, PK, HMWK) and most lupus anticoagulants.
PT/INR	1.0	>99	Congenital or acquired deficiencies of FVII and common pathway.	
TT	1.0	96	Congenital and acquired defects in fibrinogen and fibrin polymerization, ↑ FDPs.	Valproic acid therapy and some paraproteins can prolong test.
Clauss Fibrinogen	1.0	>99	Quantitative and qualitative defects in fibrinogen.	

Table modified from: Hayward and Moffat. Int J Lab Hematol. 35:322-33; 2013.

Case # 1

- A 36 year old female was referred to the High Risk Clinic at an acute care hospital.
- After extensive bleeding with the loss of her 3rd pregnancy she was admitted to hospital.
- On admission:
  - PT/INR 2.3 RI 0.8 – 1.2 INR
  - APTT 44 RI 22 – 35 s
  - TT 108 RI 20 – 30 s
  - Fibrinogen 0.70 RI 1.6 – 4.2 g/L

Case # 1
Additional laboratory testing

- Urea clot (FXIII) solubility test: Normal
- Factor XIII activity level:

  0.79 U/mL RI 0.60 – 1.69 U/mL

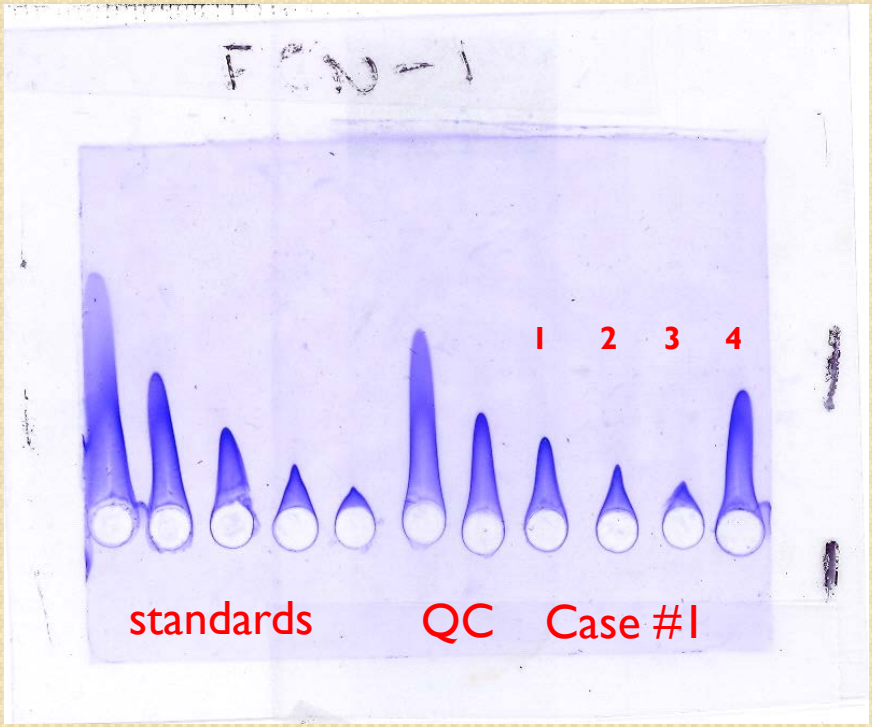
- Immunological fibrinogen (2 samples)

  0.60 g/L RI 1.6– 4.2 g/L

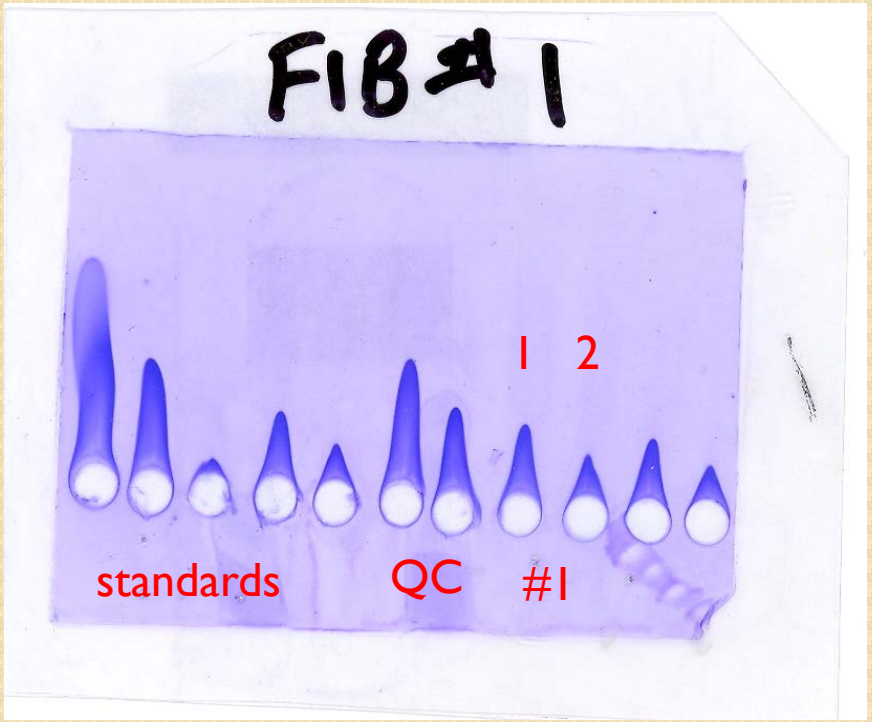
  0.80 g/L

Immunological Fibrinogen

1 = 1/10 = 0.60 g/L
2 = 1/20 = 0.65 g/L
3 = 1/40 = 0.65 g/L
4 = 1/5 = 0.60 g/L



1 = 1/10 = 0.70 g/L
2 = 1/20 = 0.80 g/L

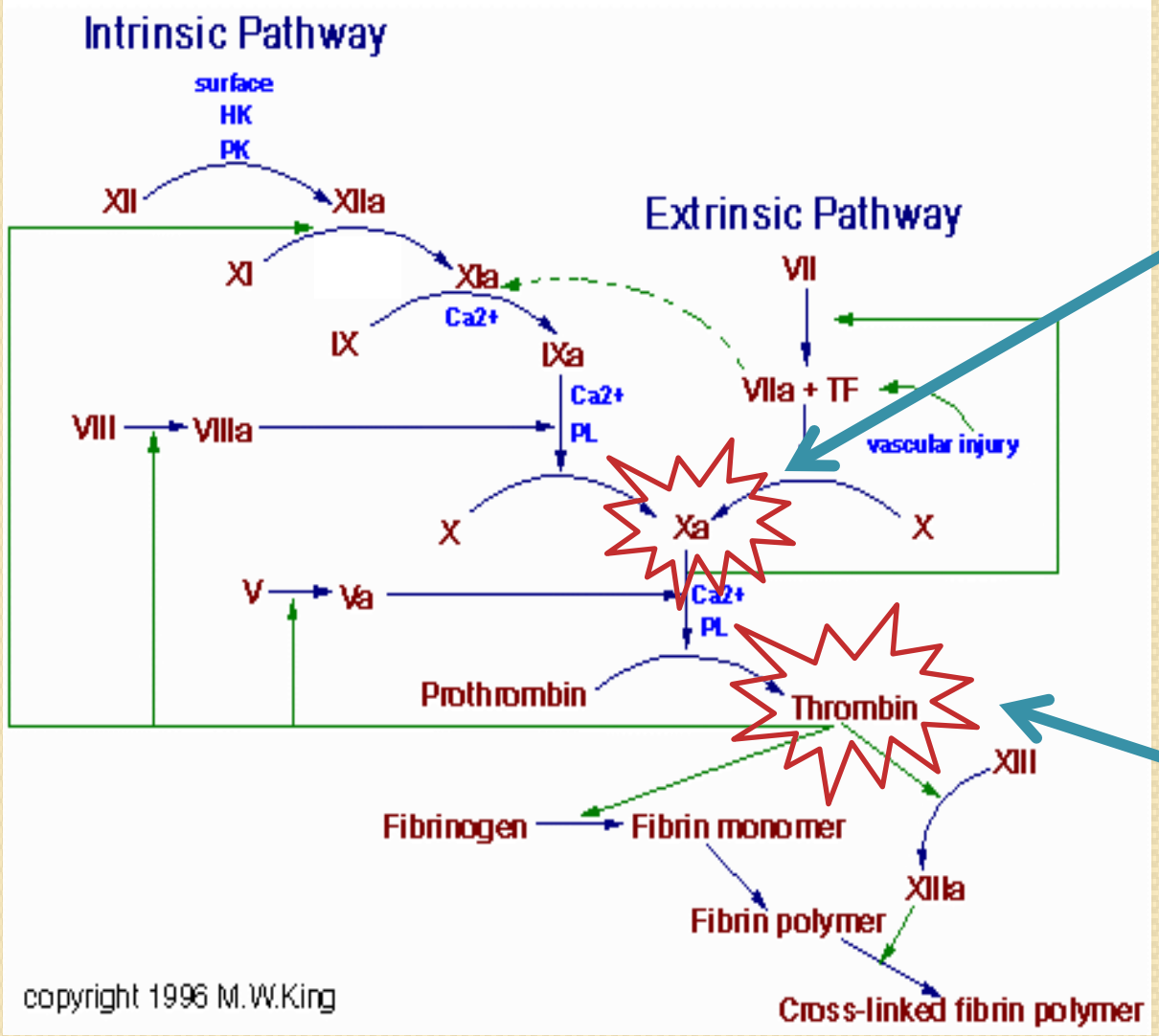


Back to Case #1

- At the time, the patient was treated with Haemocomplettan® P
  - Plasma derived fibrinogen concentrate
  - Requires SAP

- Currently RiaSTAP™ available
  - Also a plasma derived fibrinogen concentrate
  - Does not require SAP

New Oral Anticoagulants



Examples of:

Direct factor Xa inhibitors

- Rivaroxaban
- Apixaban

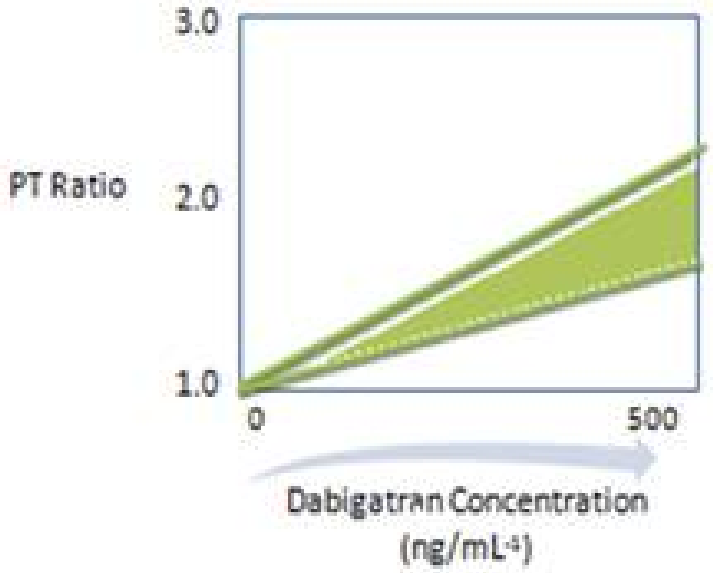
Indirect factor Xa inhibitors

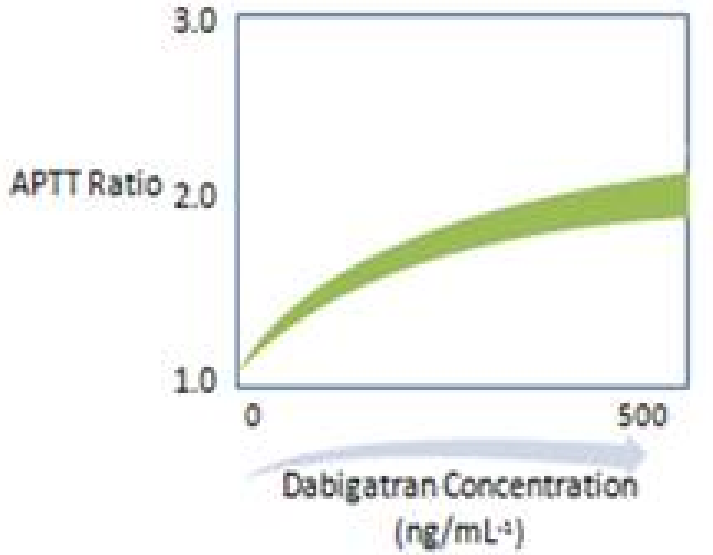
- Fondaparinux
- Idraparainux
- Oral heparins

Factor IIa inhibitors

- Hirudin
- Bivalirudin
- Argatroban
- Dabigatran
- Orally available heparins

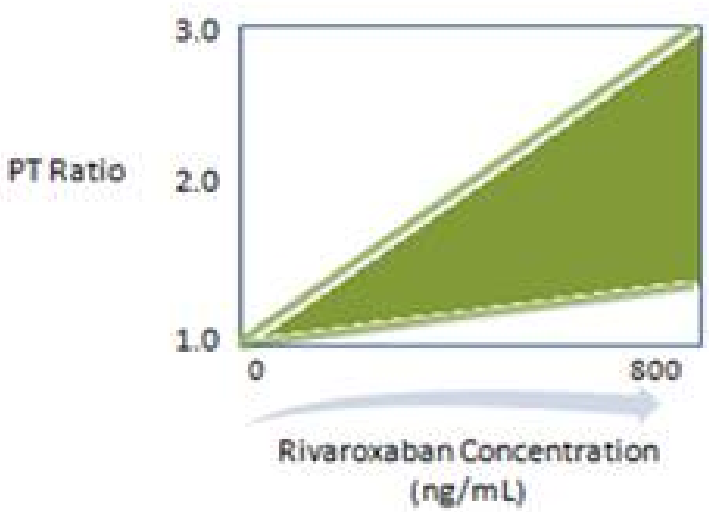
PT and APTT - Dabigatran

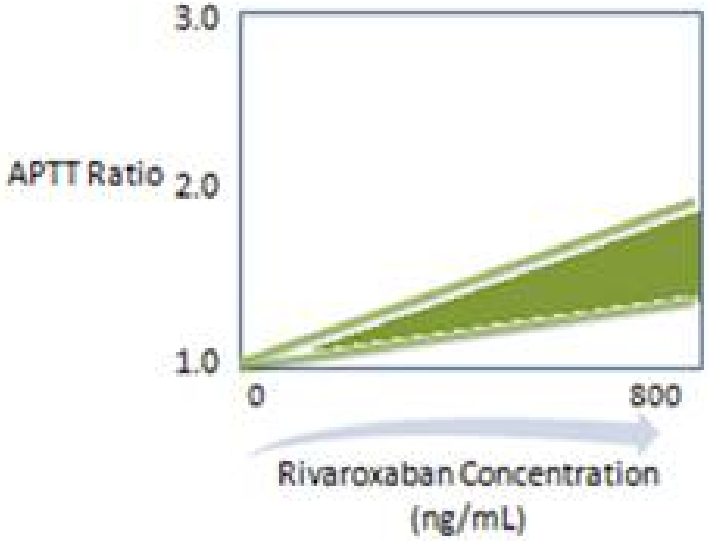




- PT reagents vary in sensitivity
- PT -13% to 43% results normal at therapeutic drug levels
- APTT prolongation is curvilinear with less reagent variation
- 11% to 26% results normal at therapeutic drug levels

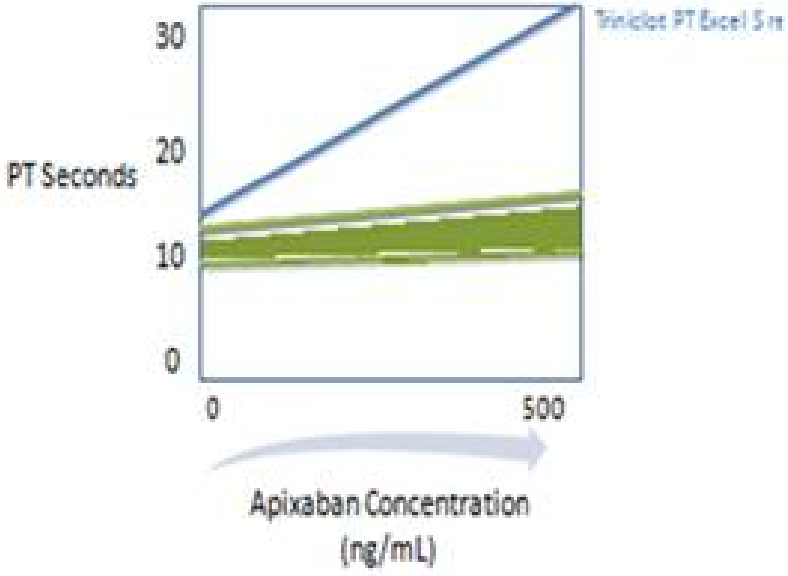
PT and APTT - Rivaroxaban

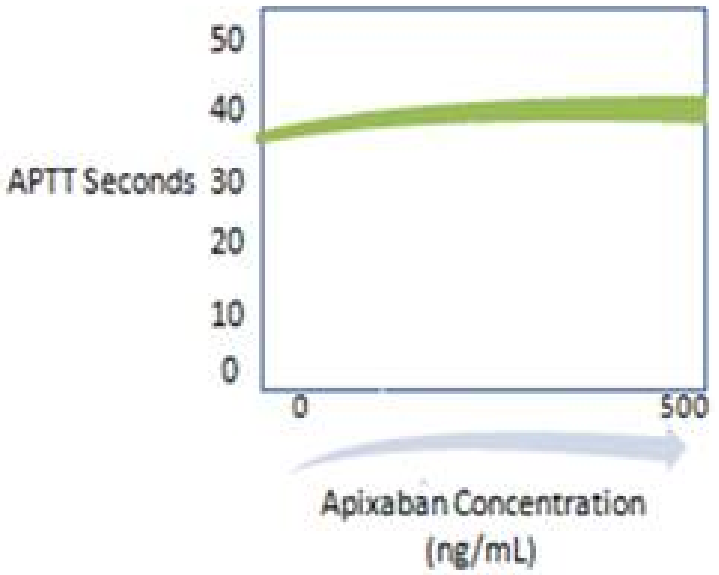




- PT and APTT – sensitivity varies between reagents
- PT - 10% to 52% results normal at therapeutic drug levels
- APTT - 31% to 59% results normal at therapeutic drug levels

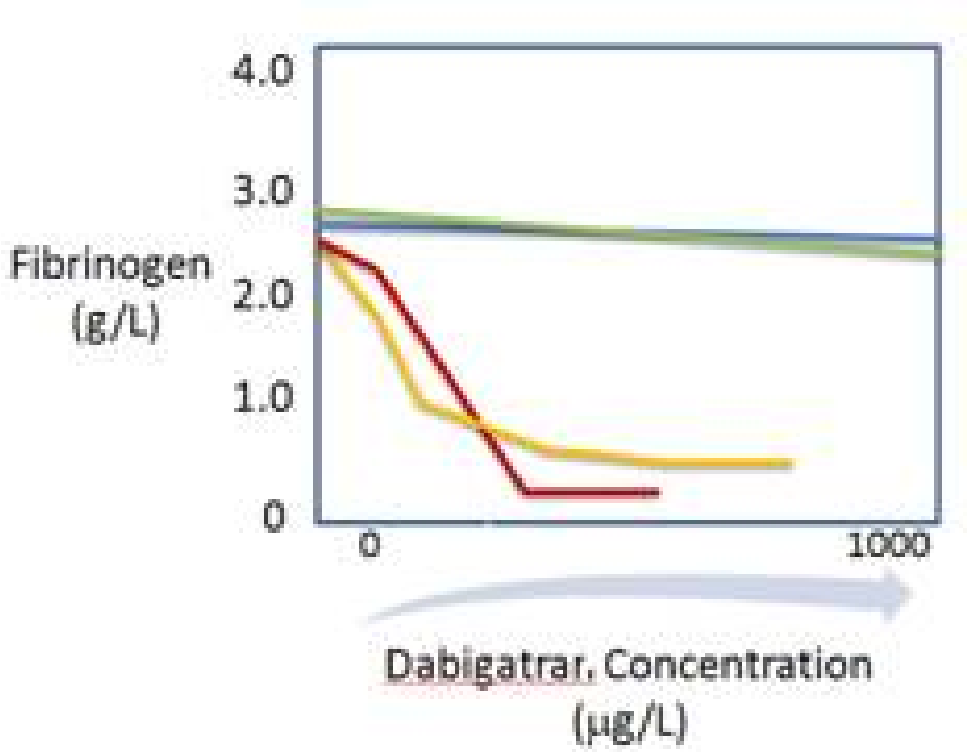
PT and APTT - Apixaban





- PT is generally normal at therapeutic drug concentrations
  - Exception: Triniclot PT Excel S reagent
- APTT prolongation is curvilinear with a concentration dependent prolongation that plateaus at 200 ng/mL

Clauss Fibrinogen - Dabigatran



Legend

Green: Fibri-Prest (Stago)
Blue: Dade Thrombin (Siemens)
Yellow: Multifibren U (Siemens)
Red: Fibrinogen C (IL)

Case # 2

- 73 year old male, retired teacher
- Right thigh bleed on warfarin
- One year later presented with left leg pain
  - Received two doses of fragmin for suspected calf vein thrombosis
  - Within 24 hours, leg became grossly swollen and discoloured due to hemorrhage
  - Hemoglobin 60 g/L, transfused 3 units red cells

Case # 2

- Lab testing initiated from community hospital
  - PT, APTT, fibrinogen, VWF levels: All normal
  - Factor assays: All normal

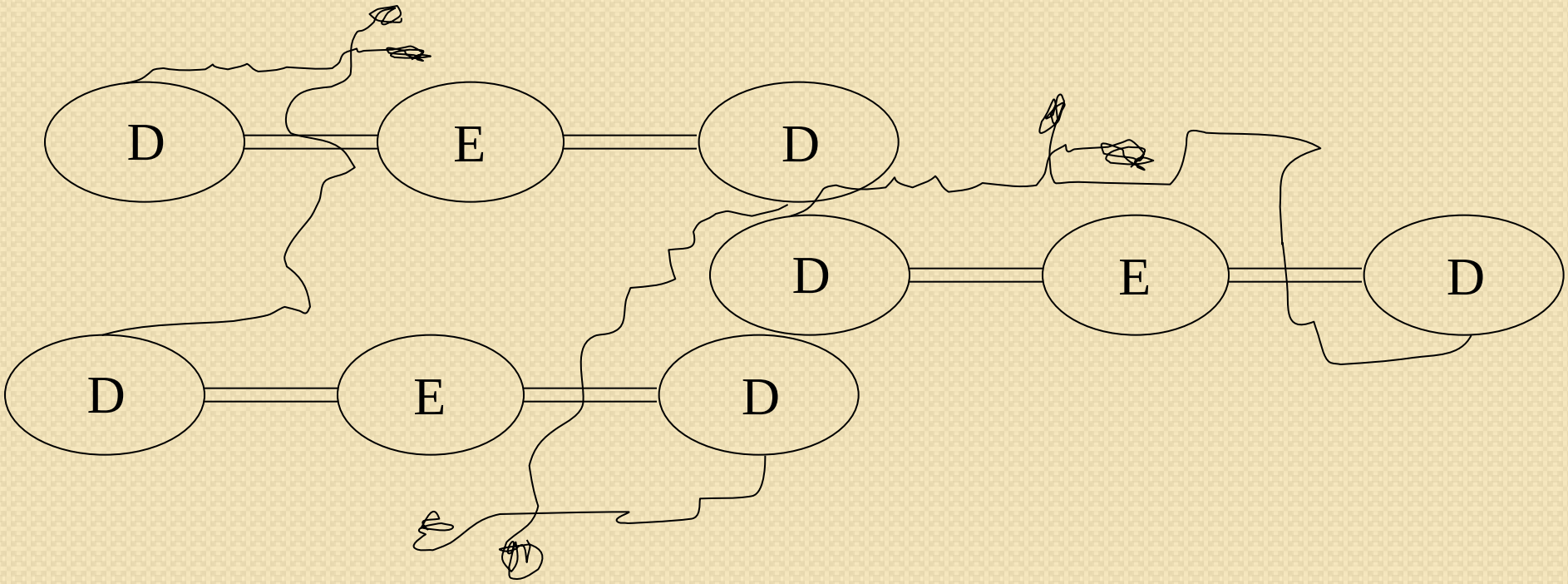
- Further specialized laboratory studies
  - Urea clot solubility test: Deficient factor XIII activity
    - No clot after 24 hours at room temperature
  - FXIII antigen assays:
    - FXIII subunit A: <0.05 U/mL RI: 0.67-1.39 U/mL
    - FXIII subunit B: 0.48 U/mL RI: 0.73-1.17 U/mL

Factor XIII

- Plasma FXIII is a transglutaminase
- Consists of:
  - 2 - subunit A
    - Catalytic subunit
    - Calcium binding site
  - 2 - subunit B (formerly subunit S)
    - Carrier subunit
- Zymogen activated by thrombin and calcium, in the presence of fibrin.

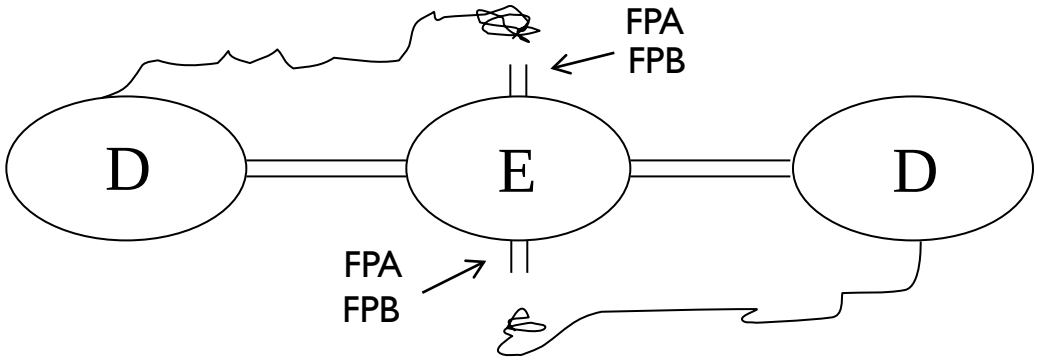
Factor XIII Function

- Stabilizes initial fibrin clot
  - Forms covalent bonds which
    - cross-link fibrin monomers
    - cross-links α_2-antiplasmin into fibrin clot



Factor XIII Deficiency – Why is the PT and APTT normal?

- Thrombin cleaves FPA and FPB from fibrinogen to form fibrin monomers.
- Monomers polymerize to form soluble fibrin.
- FXIII is activated and forms insoluble fibrin.
- In-vitro clot detection systems detect soluble (non-cross linked) fibrin.



Factor XIII Clot Solubility Assays

- Qualitative screening test
- abnormal when factor XIII is reduced to ≤ 0.02 – 0.03 U/mL
- Principle:
  - Non-crosslinked fibrin can be dissolved by adding denaturing agents (i.e. 5 M urea, 2% monochloroacetic acid) whereas cross-linked fibrin do not dissolve.

Normal
Factor XIII

No
Factor XIII

FXIII - Sensitivity and Specificity

- Not reported
- Clot solubility assays only detect severe deficiencies
- FXIII activity assays are useful for monitoring therapy
  - Able to quantitate a wider range of levels
- Most congenital deficiencies are severe subunit A deficiencies

Ref: Hayward and Moffat. Int J Lab Hematol. 35:322-33; 2013.

Back to Case #2

- Patient was treated with Fibrogammin® P
  - human plasma derived FXIII product
- Regular laboratory monitoring of patient's samples
  - FXIII clot solubility test
  - FXIII activity levels

Conclusions

- PT and APTT:
  - Screen for numerous coagulopathies.
  - Demonstrate excellent specificity but poor sensitivity.
  - Not suitable for monitoring new oral anticoagulants due to the wide variability in sensitivity between reagents.
  - normal with a factor XIII deficiency, requiring specialized testing.
- Dabigatran may cause false low Clauss fibrinogen levels, dependent on thrombin reagent used.

Thank you for your attention.