Clinical and laboratory approaches to the diagnosis of acquired bleeding disorders

Objectives

1. Discuss how to use and interpret basic coagulation testing in patients presenting with an acquired bleeding disorder
2. Use case studies to highlight key diagnostic laboratory patterns in acquired bleeding disorders
3. Discuss common questions posed to hemostasis experts regarding diagnosis of acquired bleeding disorders

Two patients with new bleeding symptoms

- Patient 1
- 80-year-old male with spontaneous thigh hematoma
- Acquired FVIII inhibitor
  - FVIII 2% (clot-based)
  - FVIII 6% (chromogenic)
  - 46 BU, high-titer inhibitor
- Normal VWF, FIX, FXI

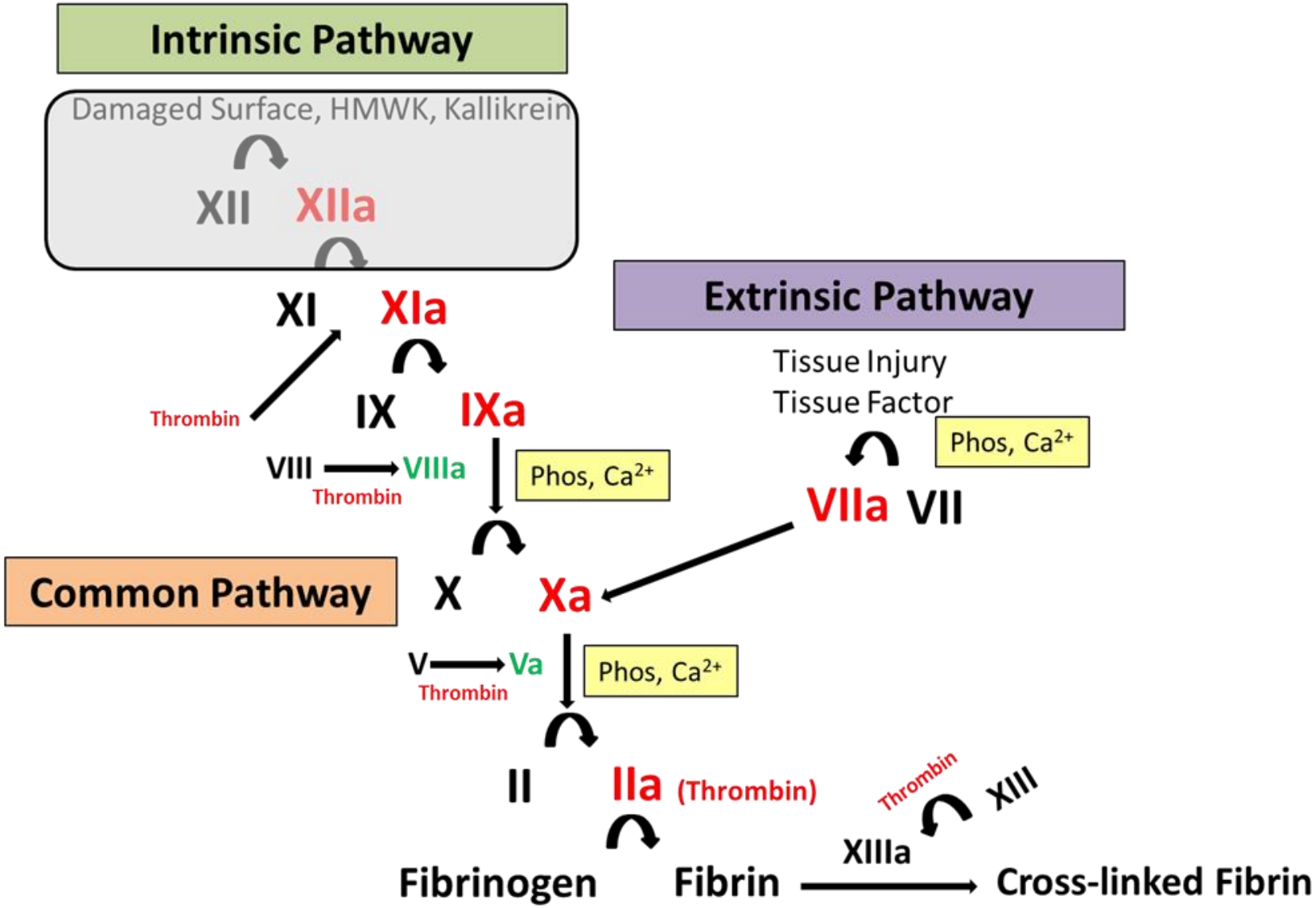
- Patient 2
- 80-year-old male with spontaneous abdominal and retroperitoneal hematomas
- Acquired FVIII inhibitor
  - FVIII 9% (clot-based)
  - At least 10 BU, high-titer inhibitor
- Normal VWF, FIX, FXI
- False-positive lupus anticoagulant testing in aPTT

Initial coagulation studies

Test	Patient 1 Results	Patient 2 Results	Reference Interval
PT	13.0 sec	14.1 sec	12.0 -15.5 sec
aPTT	92 sec	52 sec	24-35 sec
aPTT 1:1 immediate	42 sec	38 sec	24-35 sec
Platelets	210 K/µL	328 K/µL	150-400 K/µL
Fibrinogen	221mg/dL	456 mg/dL	150 -450 mg/dL
D-dimer	0.5 µg/mL FEU	0.43 µg/mL FEU	0.0-0.4 µg/mL FEU

Interpretation of coagulation tests in a bleeding patient

PT	aPTT	Platelet count	Differential Diagnosis
↑	N	N	FVII deficiency versus inhibitor, some cases of warfarin/vit K, liver disease, or DIC, some cases of oral Xa inhibitors
N	↑	N	Deficiency versus inhibitor of intrinsic pathway (FVIII, FIX, FXI), vWD if FVIII low enough, heparin, some cases of oral direct thrombin inhibitor
↑	↑	N	Warfarin/vit K, liver disease, DIC, high heparin concentration, direct thrombin inhibitor, common pathway deficiency versus inhibitor, some cases of new oral anticoagulants
↑	↑	↓	DIC, severe liver disease
N	N	↓	Thrombocytopenia: ↓ production versus ↑ destruction versus sequestration
N	N	N	Vascular abnormalities, vWD, qualitative platelet disorder, mild factor deficiency, new oral anticoagulants, dysfibrinogenemia, FXIII deficiency, fibrinolytic disorders



Differential for isolated prolonged aPTT in a bleeding patient

- Factor deficiency or factor inhibitor in the intrinsic pathway
  - » Order FVIII, FIX, and FXI activities for bleeding or to assess bleeding risk
    - The affected factor will be low or undetectable and the other factors will be normal or show a pattern of an interfering substance (non-parallelism)
    - First-line factor activities are clot-based (aPTT-based), chromogenic factor VIII activity may be helpful to confirm a low/undetectable FVIII
      - › Not impacted by the same interfering substances as clot-based factor activities
    - Order von Willebrand panel if FVIII low to exclude von Willebrand disease (VWD)
    - If VWD ruled out as the cause of low FVIII, order FVIII Bethesda assay
  - » FXII and other contact factor deficiencies do not cause bleeding

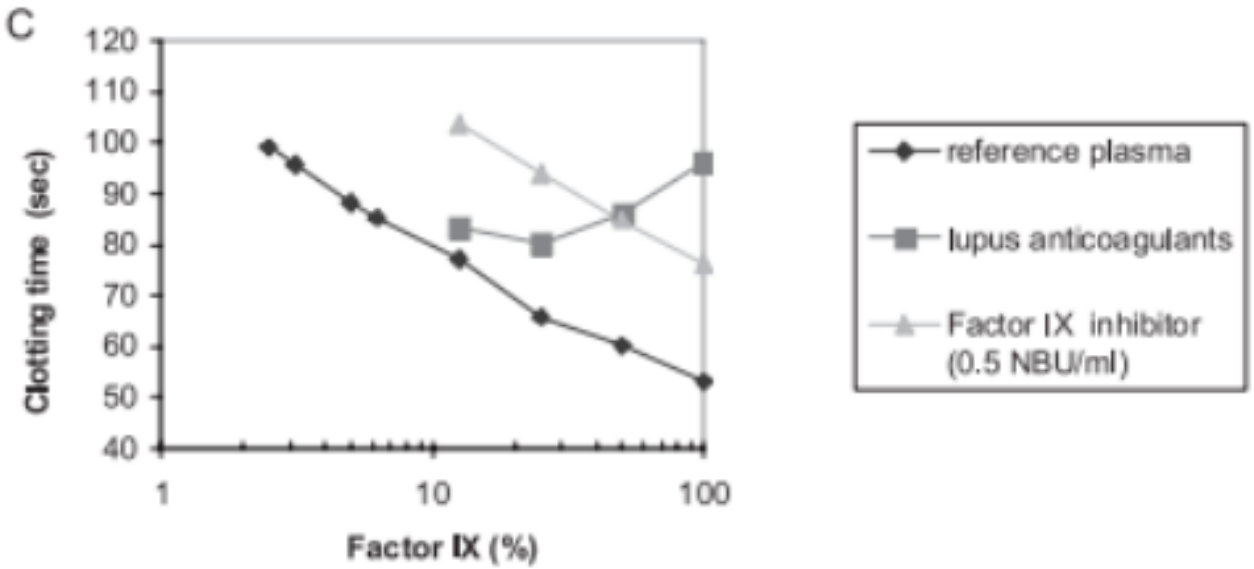
Differential for isolated prolonged aPTT in a bleeding patient, continued

- Lupus anticoagulant (LA)
  - » Does not cause bleeding except with LA hypoprothrombinemia syndrome (which also has prolonged PT)
  - » LA interference can cause falsely low aPTT-based factor activities, non-parallelism pattern, and false-positive factor inhibitor titers
- Anticoagulant medications that inhibit coagulation factors
  - » Could cause bleeding
  - » Heparin
    - PT not prolonged because reagents contain heparin neutralizers
  - » High concentration of heparin or a direct thrombin inhibitor or factor Xa inhibitor
    - **Consider anti-Xa level and thrombin time if history unclear**
    - Anticoagulant interference can cause falsely low aPTT-based factor activities and false-positive factor inhibitor titers

Factor activity patterns in patients with factor inhibitors

Test	Patient 1 Results	Patient 2 Results	Reference Interval
FVIII (aPTT-based)	<1%	9%	56 – 191%
Chromogenic FVIII	<1%	N/A	56 – 191%
FVIII Bethesda	46 BU	At least 10 BU	<=0.5 BU
FIX	107%	141%	78 - 184%
FXI	At least 66%; Non-parallelism	88%	56 – 153%

Clot-based factor assay parallelism



Marcus C. An 83-year-old man with an elevated PTT. Lablogatory. March 26,2019.

Ruinesman-Koerts J, Peterse Stienissen I, Verbruggen B. Non-parallelism in the one-stage coagulation factor assay is a phenomenon of lupus anticoagulants and not of individual factor inhibitors. Thrombosis and Hemostasis, 2010 105(5):1080-2.

Clot based factor assay parallelism

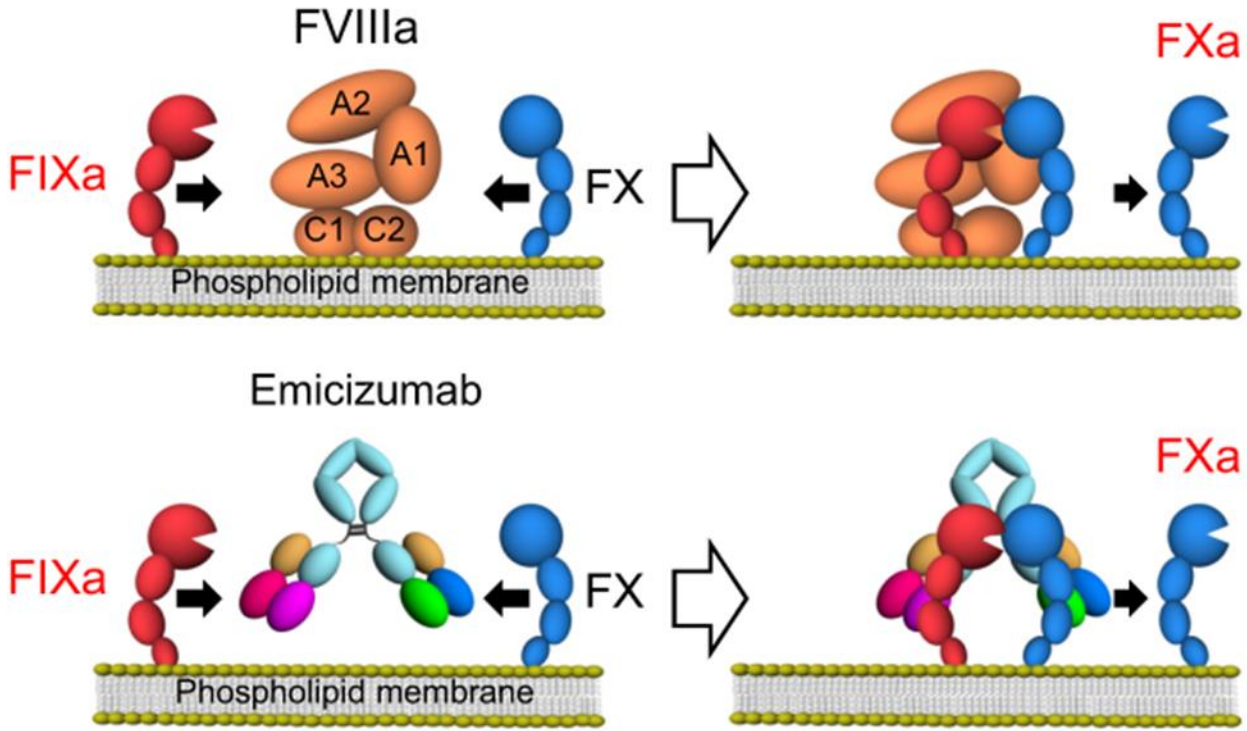
Dilution	Calculated activity (%) based on PTT	Correct for dilution	Activity (%)
1:10	98.0%	None (x 1)	98.0%
1:20	48.2%	X 2	96.4%
1:40	25.5%	X 4	102.0%

Dilution	Calculated activity (%) based on PTT	Correct for dilution	Activity (%)
1:10	0%	None (x 1)	0%
1:20	20%	X 2	40%
1:40	27.5%	X 4	110%

Patient 2, clot-based versus chromogenic FVIII assays

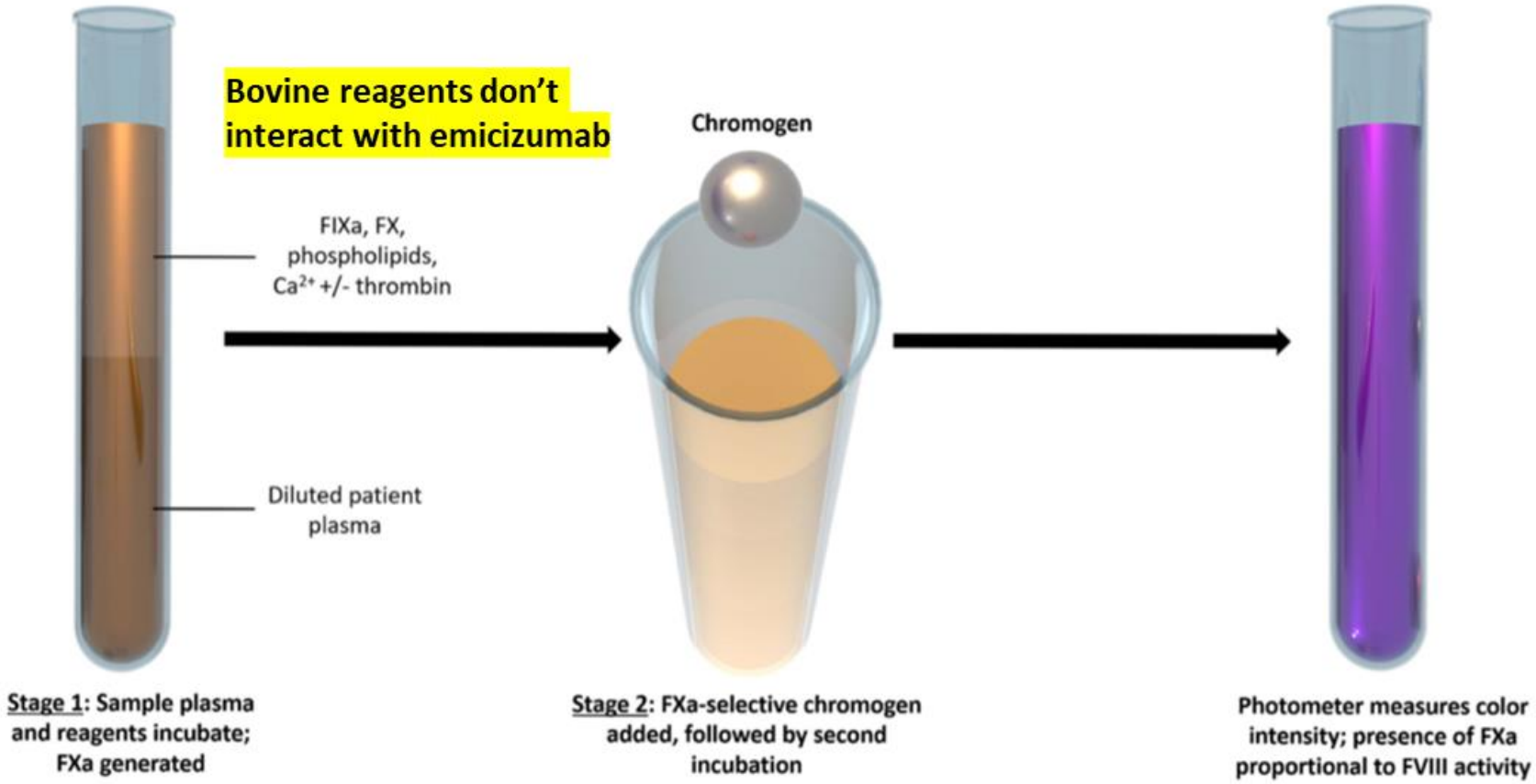
Date(s)	FVIII Clot-based (RI 56 – 191%)	FVIII Chromogenic (RI 56 – 191%)	Notes
Sept 10 - 19	• aPTTs ~50 sec (RI 24 – 35) • FVIII activity values ranging from 9% to 16% • Bethesda steady in 10 BU range	25% (on 9/15)	• Differences in clot-based and chromogenic assays due to autoantibody kinetics and assay conditions
October 1 – Emicizumab	• aPTT ~22 sec • FVIII activity 443%	56%	• Patient endogenous FVIII starting to normalize, based on chromogenic assay • aPTT-based FVIII not reliable when emicizumab present

Emicizumab (Hemlibra)



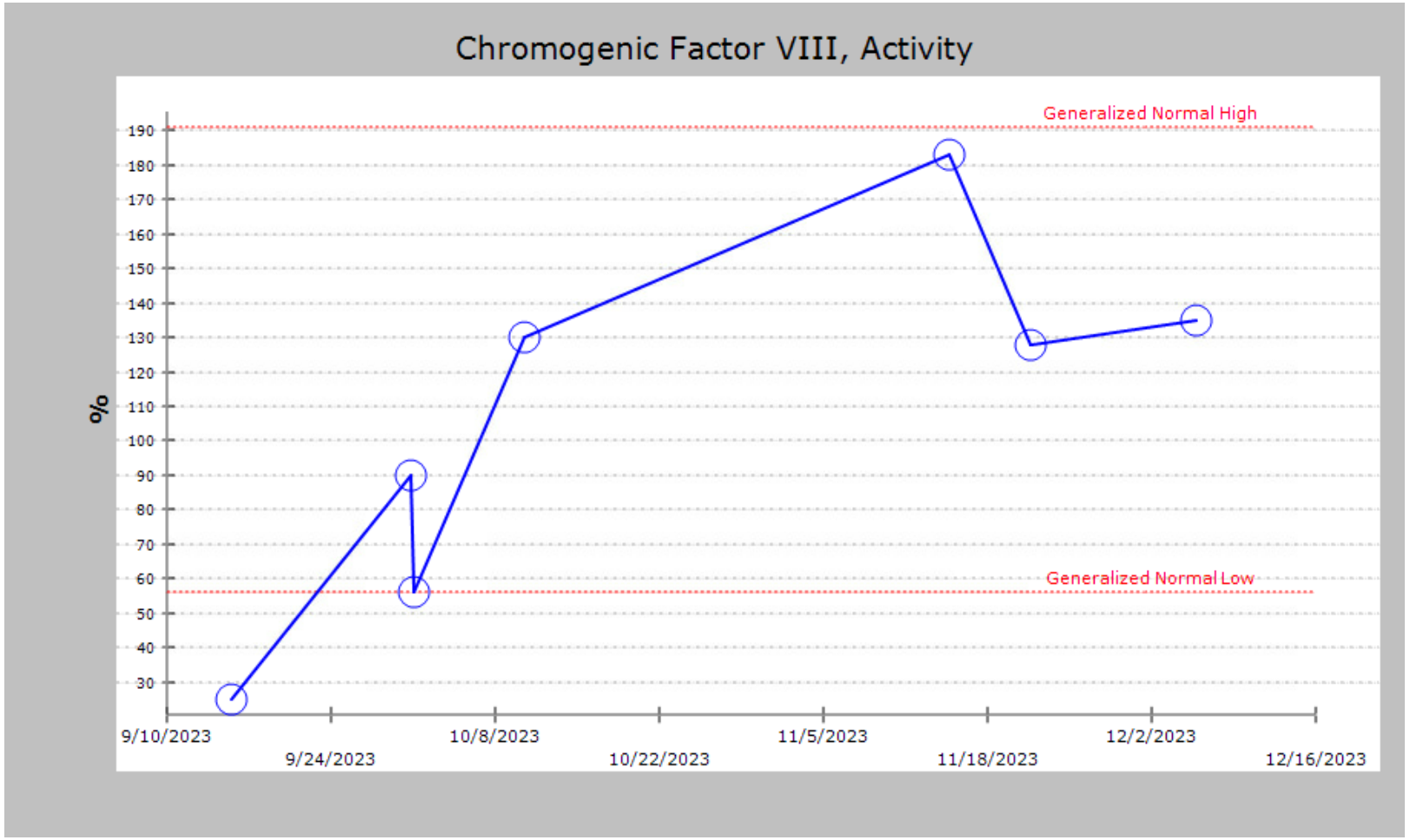
Source: LaboratoryHaemostasis.com

Chromogenic FVIII assay

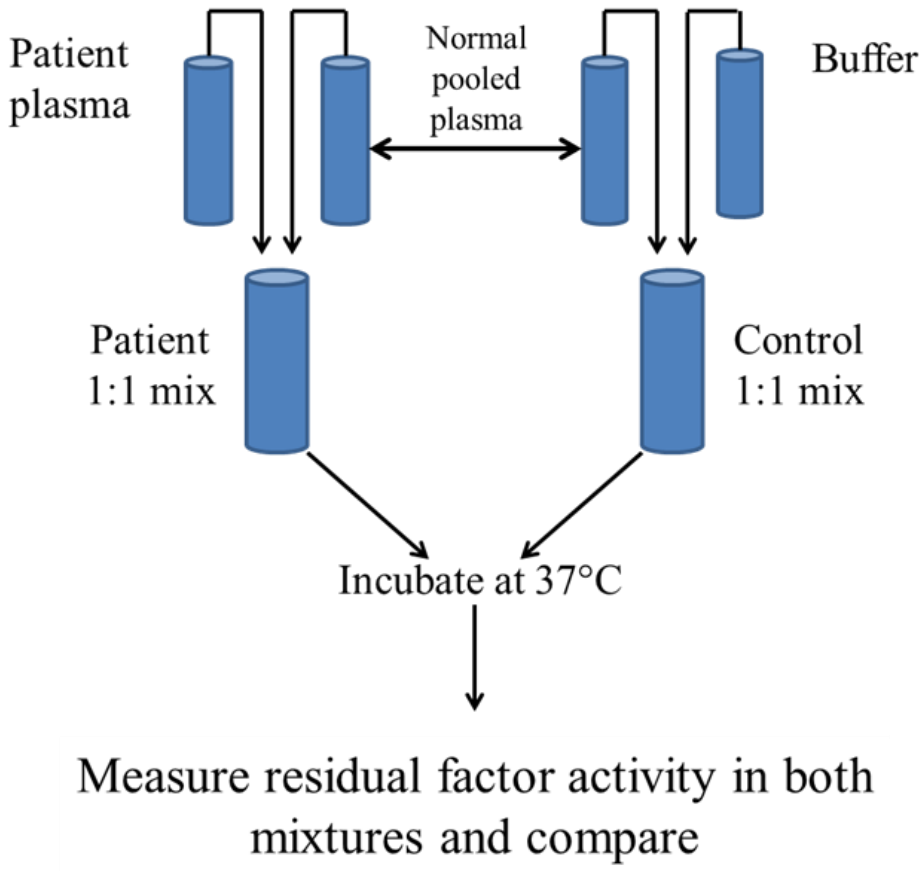


Al-samkari H, Croteau SE. Shifting Landscape of Hemophilia Therapy: Implications for Current Clinical Laboratory Coagulation Assays. Am J Hematol. 2018;93:1082-1090.

Patient 2, recovery of FVIII activity with immunosuppression



Bethesda method for quantifying factor inhibitors



- 1 Bethesda unit is the inhibitor titer that decreases residual factor activity to 50% of expected
- "No inhibitor" control has 50% activity (recover everything)
- Assay is performed at multiple dilutions of patient plasma

Factor VIII/IX Bethesda Units Calculation

Result of Factor Beth CT:	48
	46
Average:	47

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Accession number [redacted] **Name:** [redacted]

Dilution Factor	STA-R Beth Result	%Residual Activity	Bethesda Units	Factor Inhibitor Bethesda Units (BU)
1:3	12	26	1.94	5.8
1:4	11	23	N/A	N/A
1:5	11	23	N/A	N/A
1:10	14	30	1.74	17.4
1:20	16	34	1.56	31.2
1:30	19	40	1.32	39.6
1:40	21	45	1.15	46
1:50	25	53	0.92	46
1:60	30	64	0.64	38.4
1:70	28	60	0.74	51.8
1:80	33	70	0.51	40.8
1:90	35	74	0.43	38.7
1:100	35	74	0.43	43
1:200	39	83	N/A	N/A
1:300	42	89	N/A	N/A
1:400	43	91	N/A	N/A
1:500	45	96	N/A	N/A
1:600	42	89	N/A	N/A
1:700	44	94	N/A	N/A
1:800	49	104	N/A	N/A
1:900	50	106	N/A	N/A
1:1000	51	109	N/A	N/A

- Patient 1 has straightforward Bethesda kinetics, despite being an autoantibody
  - More similar to Type I (typical alloantibody) kinetics
- FVIII recovery increases as free antibody is diluted
- Titer obtained from recovery closest to 50%, corrected for dilution
  - 46 BU

Factor VIII/IX Bethesda Units Calculation

Result of Factor Beth CT:	51
	54
Average:	52.5

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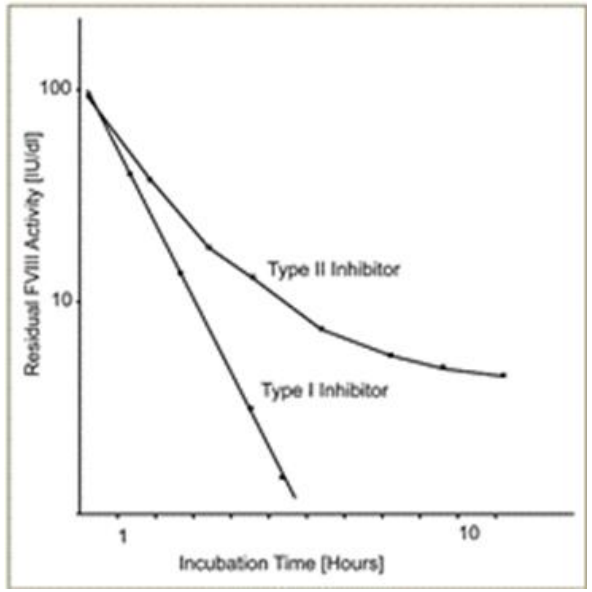
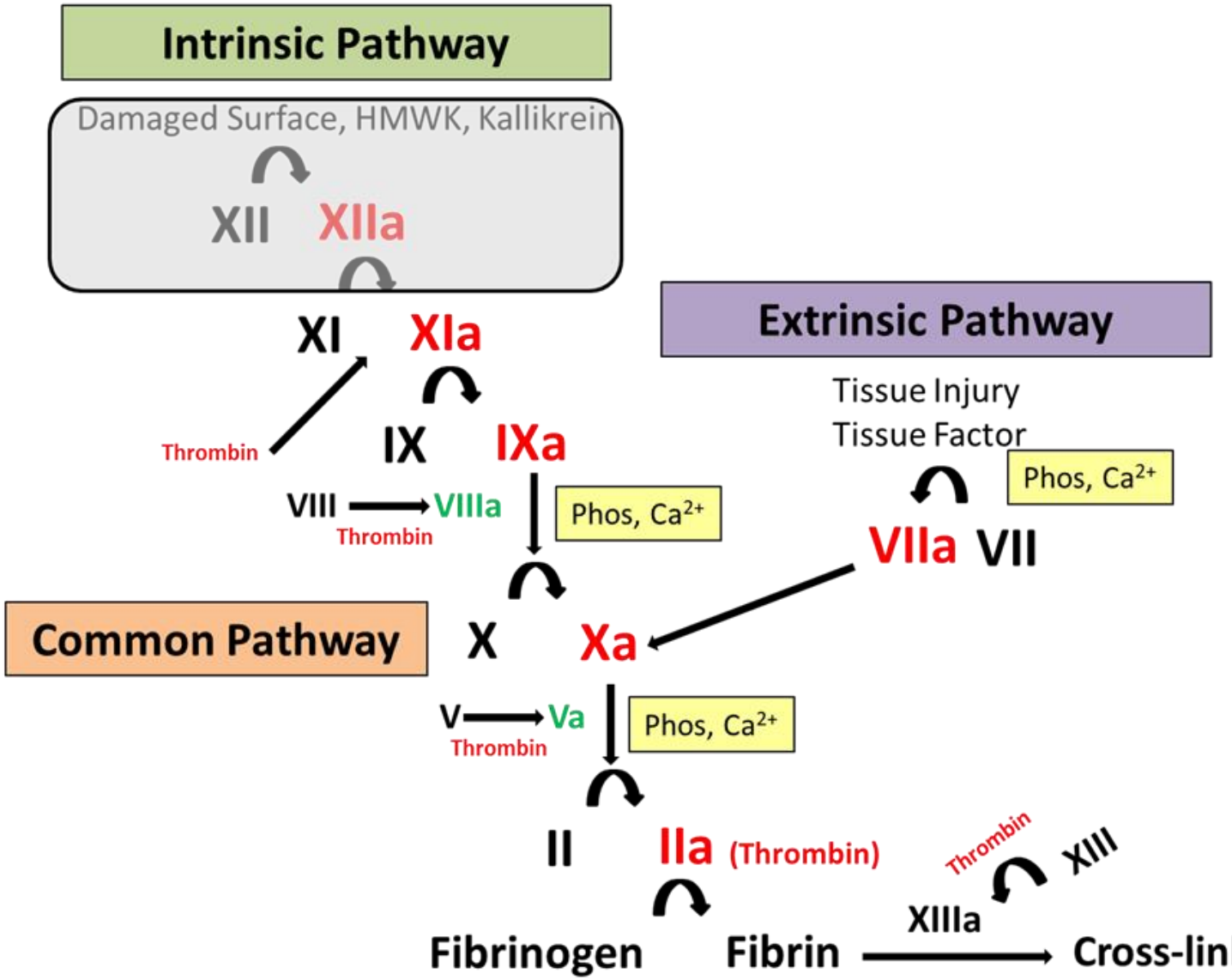
Accession number: [redacted] Name [redacted]

Dilution Factor	STA-R Beth Result	%Residual Activity	Bethesda Units	Factor Inhibitor Bethesda Units (BU)
1+1	12	23	N/A	N/A
1:2	17	32	1.64	3.2
1:3	19	36	1.47	4.4
1:4	23	44	1.18	4.7
1:5	23	44	1.18	5.9
1:10	26	50	1	10
1:20	25	48	1.06	21.2
1:30	23	44	1.18	35.4
1:40	23	44	1.18	47.2
1:50	23	44	1.18	59
1:60	23	44	1.18	70.8
1:70	21	40	1.32	92.4
1:80	23	44	1.18	94.4
1:90	26	50	1	90
1:100	22	42	1.25	125
1:200	29	55	0.86	172
1:300	38	72	0.47	141
1:400	42	80	N/A	N/A
1:500	43	82	N/A	N/A
1:600	46	88	N/A	N/A
1:700	48	91	N/A	N/A
1:800	50	95	N/A	N/A
1:900	50	95	N/A	N/A
1:1000	51	97	N/A	N/A

- Patient 2 has Type II inhibitor kinetics (complex, non-linear), often seen with autoantibodies
- Incomplete inactivation of FVIII by the antibody even at high antibody titers
- Results in less accurate inhibitor titer
  - At least 10 BU, may be higher





Lupus Anticoagulants

- The coagulation cascade includes phospholipid-dependent reactions
- LAs are autoantibodies that antagonize phospholipid-protein complexes, resulting in prolonged laboratory clotting times
- Laboratory diagnosis of LAs includes comparison of 2 clotting times, (low phos and high phos conditions)
- Factor inhibitors can cause false-positive LA testing
- For example, FVIII inhibitors cause false-positive results in aPTT-based LA testing such as the hexagonal phospholipid neutralization assay

Key laboratory points for acquired FVIII inhibitors

- FVIII inhibitors demonstrate an isolated prolonged aPTT in a bleeding patient
- FVIII inhibitors show time dependence in mixing studies and may require an incubated aPTT mixing study to demonstrate an inhibitor pattern
- Lupus anticoagulants and anticoagulant medications can interfere with factor assays (erroneously low results and/or non-parallelism) and cause false-positive inhibitor titers in Bethesda assays
- Factor VIII inhibitors can cause false-positive lupus anticoagulant testing in aPTT-based testing such as the hexagonal phospholipid neutralization assay
- Perform VWF testing to rule out VWD in patients with low FVIII activity to avoid misdiagnosis of acquired hemophilia A
- Chromogenic FVIII activity may be useful to confirm low FVIII activity and exclude low clot-based activity due to an interfering substance

Key laboratory points for acquired FVIII inhibitors, continued

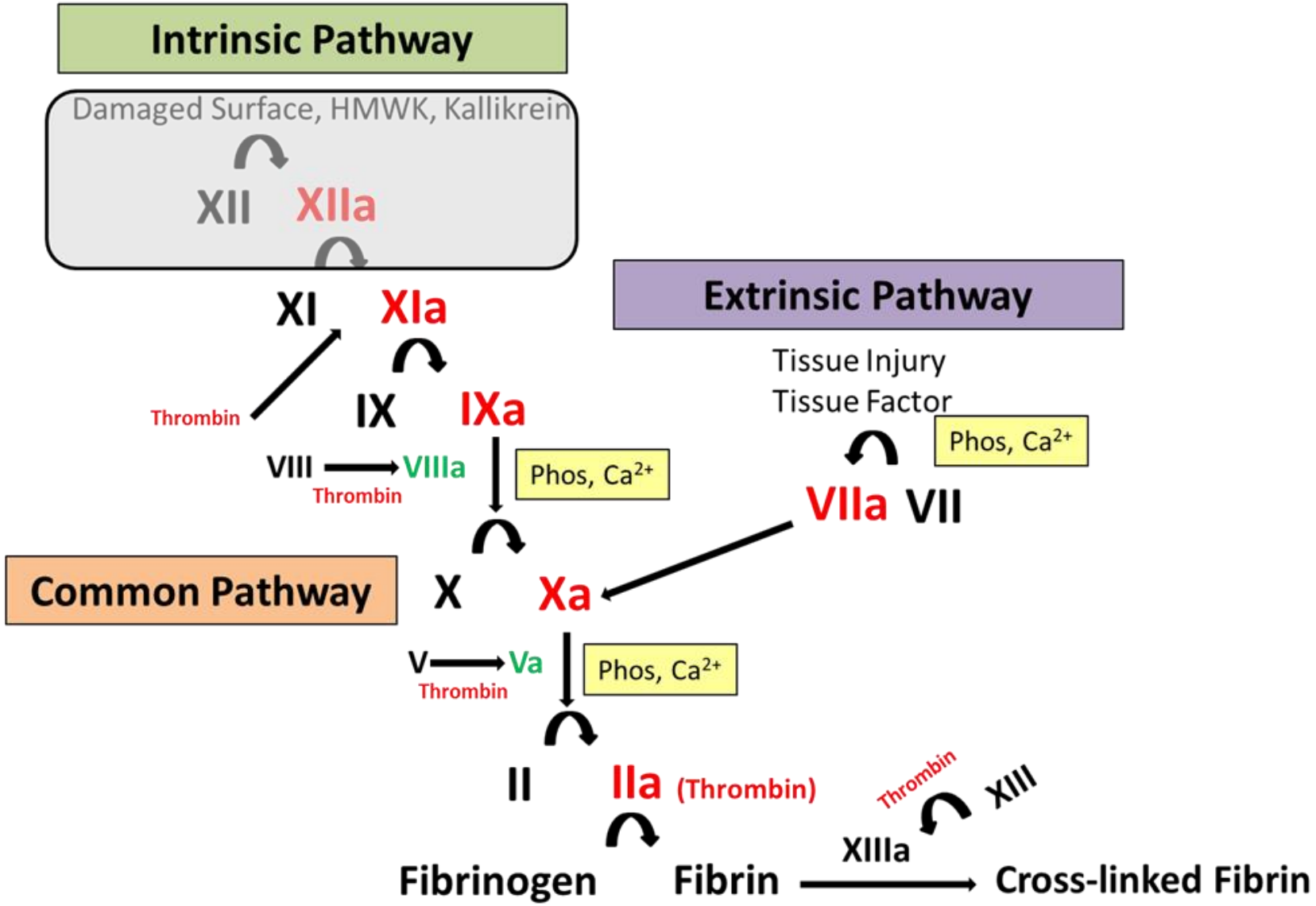
- FVIII activity and inhibitor quantification may be less accurate in acquired factor VIII deficiency due to unusual antibody kinetics
  - » Trends useful in individual patients
- aPTT-based tests are unreliable in the presence of emicizumab
  - » Includes aPTT, clot-based FVIII activity and Bethesda, other clot-based factor activities
  - » Patient FVIII and many FVIII products can be monitored using chromogenic assays that utilize bovine reagents

Ketteler C, Hoffmann I, Davidson S, et al. Impact of different factor VIII inhibitor kinetic profiles on the inhibitor titer quantification using the Nijmegen-Bethesda assay. RPTH. 2022;6:e:12799.

Patient 3

- 75-year-old female who underwent total knee arthroplasty 1 month prior to admission at a small local hospital
  - » Epistaxis and GI bleeding
  - » Pre-operative PT and aPTT were normal 1 month prior

Test	Result	Reference Interval
PT	61.8 sec	12.0 -15.5 sec
INR	13.1	0.9 – 1.1
aPTT	> 150 sec	24-35 sec
Platelets	430 K/µL	150-400 K/µL
Fibrinogen	535 mg/dL	150 -450 mg/dL
D-dimer	0.8 µg/mL FEU	0.0-0.4 µg/mL FEU


Intrinsic Pathway
Damaged Surface, HMWK, Kallikrein
XII
XIIa
XI
XIa
Thrombin
IX
IXa
VIII
VIIIa
Thrombin
Phos, Ca2+
Extrinsic Pathway
Tissue Injury
Tissue Factor
Phos, Ca2+
VIIa
VII
Common Pathway
X
Xa
V
Va
Thrombin
Phos, Ca2+
II
IIa (Thrombin)
Thrombin
XIII
XIIIa
Fibrinogen
Fibrin
Cross-linked Fibrin

Differential for prolonged PT and aPTT

- Multiple factor deficiencies
  - » Warfarin/vitamin K deficiency
  - » Liver disease
  - » Disseminated intravascular coagulation (DIC)
- Factor deficiency or factor inhibitor in the common pathway
  - » Including lupus anticoagulant hypoprothrombinemia syndrome
- Fibrinogen abnormality
- Anticoagulants
  - » Warfarin – decreases in vitamin K-dependent factors
  - » High concentration of heparin or a direct thrombin inhibitor or factor Xa inhibitor

Patient 3, Additional studies

Test	Result	Reference Interval
PT	61.8 sec	12.0 -15.5 sec
PT 1:1 mix	56.0 sec	12.0 -15.5 sec
PTT	> 150 sec	24-35 sec
PTT 1:1 mix, immediate	>150 sec	24-35 sec
Anti-Xa Assay	Not detected	0.35 – 0.70 U/mL
Thrombin Time (human thrombin)	17 sec	14.7 – 19.5 sec

Patient 3; Factor assays (common pathway)

Dilution	Factor II	Factor V	Factor X
1:2	No clot	No clot	3%
1:5	No clot	No clot	5%
1:10	No clot	No clot	8%
1:20	22%	No clot	No clot
1:40	43%	No clot	No clot
1:80	57%	No clot	No clot
1:160	No clot	No clot	No clot
1:320	No clot	No clot	No clot
Interpretation	Low FII activity with non-parallelism pattern Ref (86 – 150%)	<5% FV activity Ref (62 - 140%)	Low FX activity with non-parallelism pattern? Ref (81 – 157%)

Patient 3: Bethesda assays

Test	Result	Reference Interval
Factor II Bethesda	No inhibitor detected	No inhibitor detected
Factor V Bethesda	80.7 BU; High-titer FV inhibitor; Risk factor: Fibrin glue exposure	No inhibitor detected
Factor X Bethesda	No inhibitor detected	No inhibitor detected

Key laboratory points for factor inhibitors

- Panels of factor assays allow identification of patterns and definitive diagnosis
- Non-parallelism in clot-based factor assays signals assay interference (lupus anticoagulants, anticoagulants, inhibitors against other factors) rather than a factor inhibitor against the factor being assayed
- Non-parallelism will typically interfere with multiple assays based on the same parent assay (such as all aPTT-based factor assays)

Patient 4

- 35-year-old woman with developmental delay living with her parents in a rural area
- Presented to the ED of a local hospital with prolonged PT and aPTT, a GI bleed, and hematuria
  - » Clotting times only responded transiently to FFP
  - » Clotting times did not improve with vitamin K administration
  - » Transferred to our hospital
- No previous bleeding history
- No current medications
- No previous coagulation testing

Patient 4; Initial lab results

Test	Result	Reference Interval
PT	60 sec	12.0 -15.5 sec
INR	6.7	0.9 – 1.1
PT 1:1	17 sec	12.0 -15.5 sec
aPTT	75 sec	24-35 sec
aPTT 1:1, imm	36 sec	24-35 sec
Platelets	265 K/µL	150-400 K/µL
Fibrinogen	400 mg/dL	150 -450 mg/dL
D-dimer	0.2 µg/mL	0.0-0.4 µg/mL
LFTs	Normal	Normal

Patient 4; Factor assays

Test	Result	Reference Interval
Factor II	52%	(86 – 150%)
Factor V	101%	(62 – 140%)
Factor VII	17%	(80 – 181%)
Factor VIII	180%	(56 – 191%)
Factor IX	40%	(78 – 184%)
Factor X	42%	(81 – 157%)
Factor XI	93%	(56 – 153%)

Patient 4; Diagnosis

- Deficiencies of vitamin K-dependent factors
  - » Vitamin K failed to correct the clotting times
  - » Combined inherited deficiency of vitamin K-dependent clotting factors unlikely
  - » Inhibitor of multiple factors unlikely (mixing studies did not correct)
  - » Superwarfarin ingestion?
    - Positive for brodifacoum by LC-MS/MS
- Patient lived in a rural area and was inadvertently exposed to a rodenticide used on her parent's farm
- Coagulopathy resolved after treatment with high-dose vitamin K

Key laboratory points

- Potential limitations of mixing studies
  - » Interpretation and criteria for correction poorly standardized between labs
  - » Multiple or severe factor deficiencies may demonstrate incomplete correction
  - » Weak inhibitors (low concentration/low titer) may correct due to dilution
  - » Some inhibitors demonstrate time- and temperature-dependent prolongation and require incubation to be identified as inhibitors (FVIII inhibitors)

Adcock DM, Moore GW, del Lima Montalvao S, et al. Activated partial thromboplastin time and prothrombin time mixing studies: Current state of the art. Semin Thromb Hemost. 2023;49(6):571-579.

Favaloro E. Coagulation mixing studies: Utility, algorithmic strategies and limitations for lupus anticoagulant testing or follow up of abnormal coagulation tests. Am J Hematol. 2020;95(1):117-128.

Conclusions

1. Basic coagulation testing helps to guide more specialized testing when working up acquired bleeding disorders
2. Recognizing patterns is critical for accurate diagnosis when evaluating a panel of factor assays
3. Multidisciplinary collaboration is helpful when evaluating patients with complex bleeding disorders, including acquired bleeding disorders

ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.