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Anti-Xa Assay: Therapeutic Monitoring of Heparin

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Sr. Clinical Consultant (Hemostasis)

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Learning Objectives

01

Describe the anticoagulant effects of antithrombin

02

Explain the methods used to determine heparin concentration

03

Determine the limitations of the anti-Xa assay

04

Formulate an approach for conversion to utilizing anti-Xa assay for anticoagulant monitoring

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Outline

I

What Happens Inside a Clot

II

Anti-Xa Testing & Heparin Monitoring

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What Are The Normal Stages of Coagulation?

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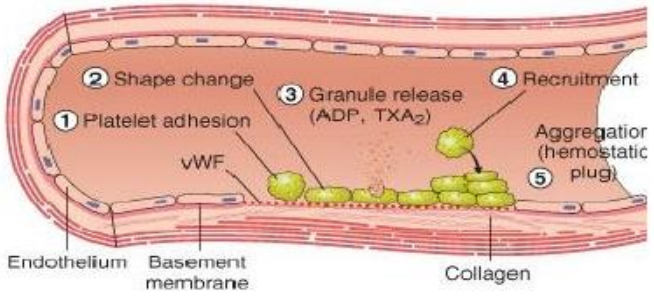
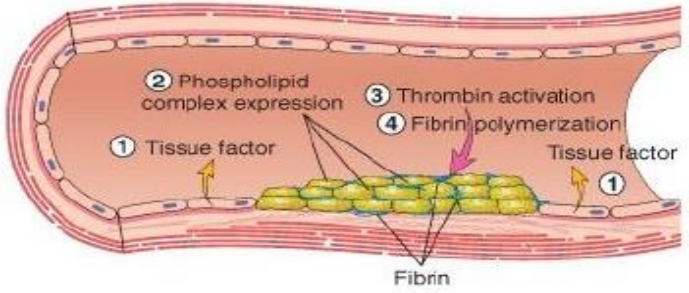
Primary Hemostasis



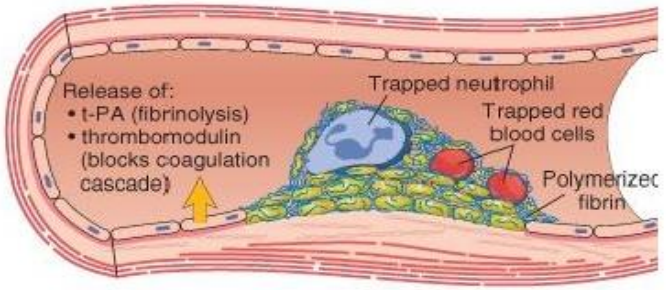
Adhesion and aggregation

Secondary Hemostasis



Activation of clotting factors and fibrin formation

Tertiary Hemostasis



Fibrin cross linking & fibrinolysis

Modified from Bhat R et al. Hematol Oncol Clin North Am. 2017;31(6):1105-1122.
Mitchell RN. Hemodynamic disorders, thromboembolic disease, and shock. In: Perkins JA, editor. Robbins and Cotran pathologic basis of disease. 8th Edition. Philadelphia: Elsevier; 2010;

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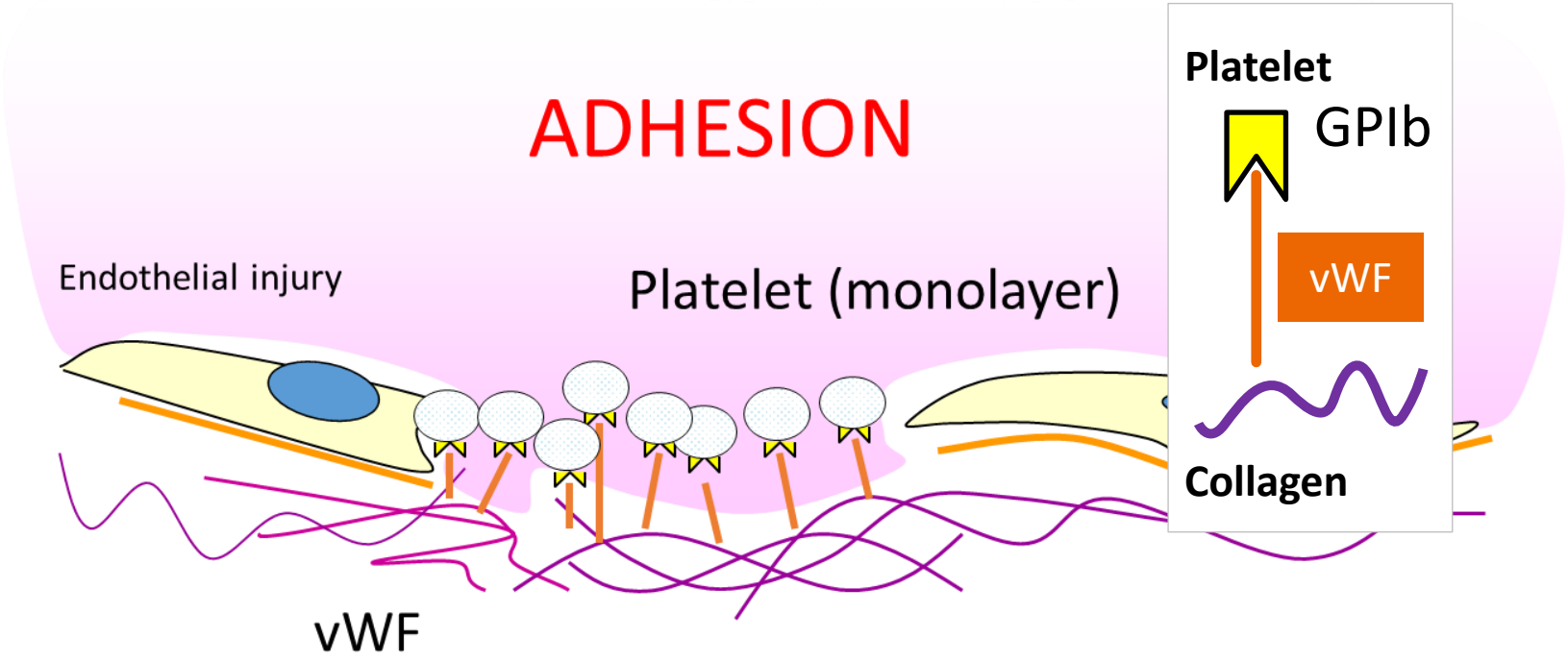
Platelet adhesion

High shear stress: requires vWF



Courtesy of Dr. William Winter

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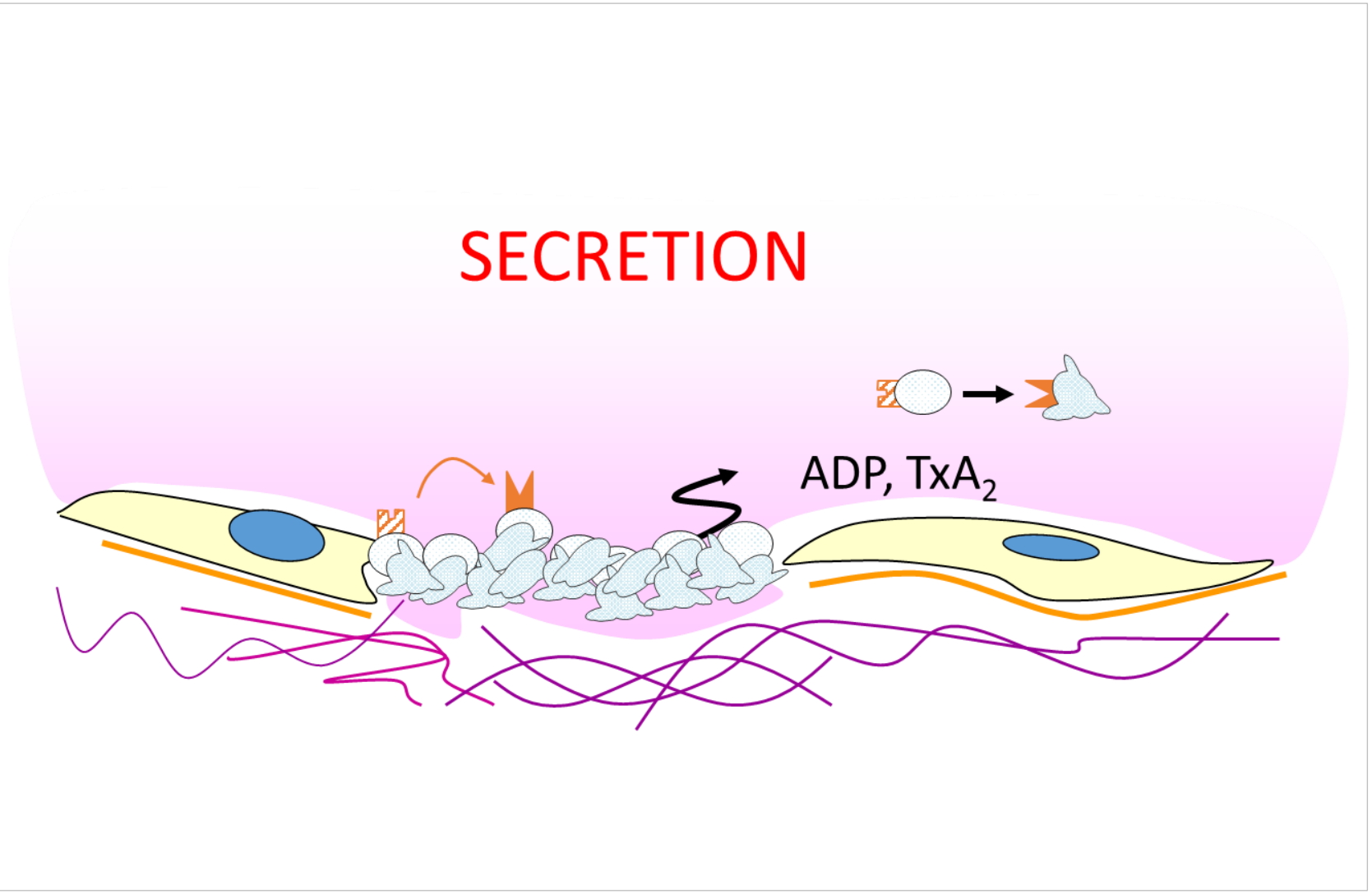
Platelet activation

GPIIb/IIIa (αIIbβ3) → conformation change

Granule secretion



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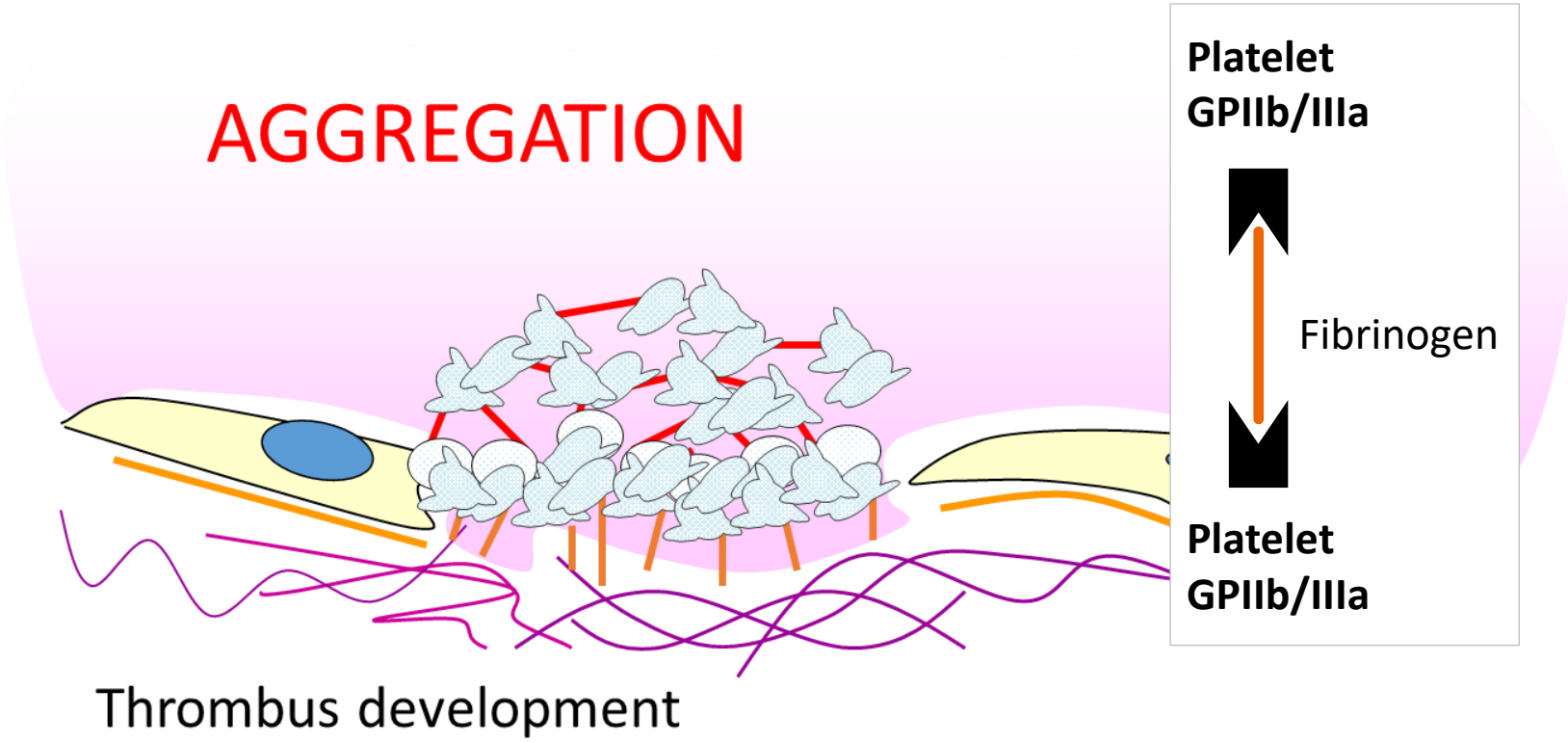
Platelet aggregation

GPIIb/IIIa → binds fibrinogen



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In Vivo Events

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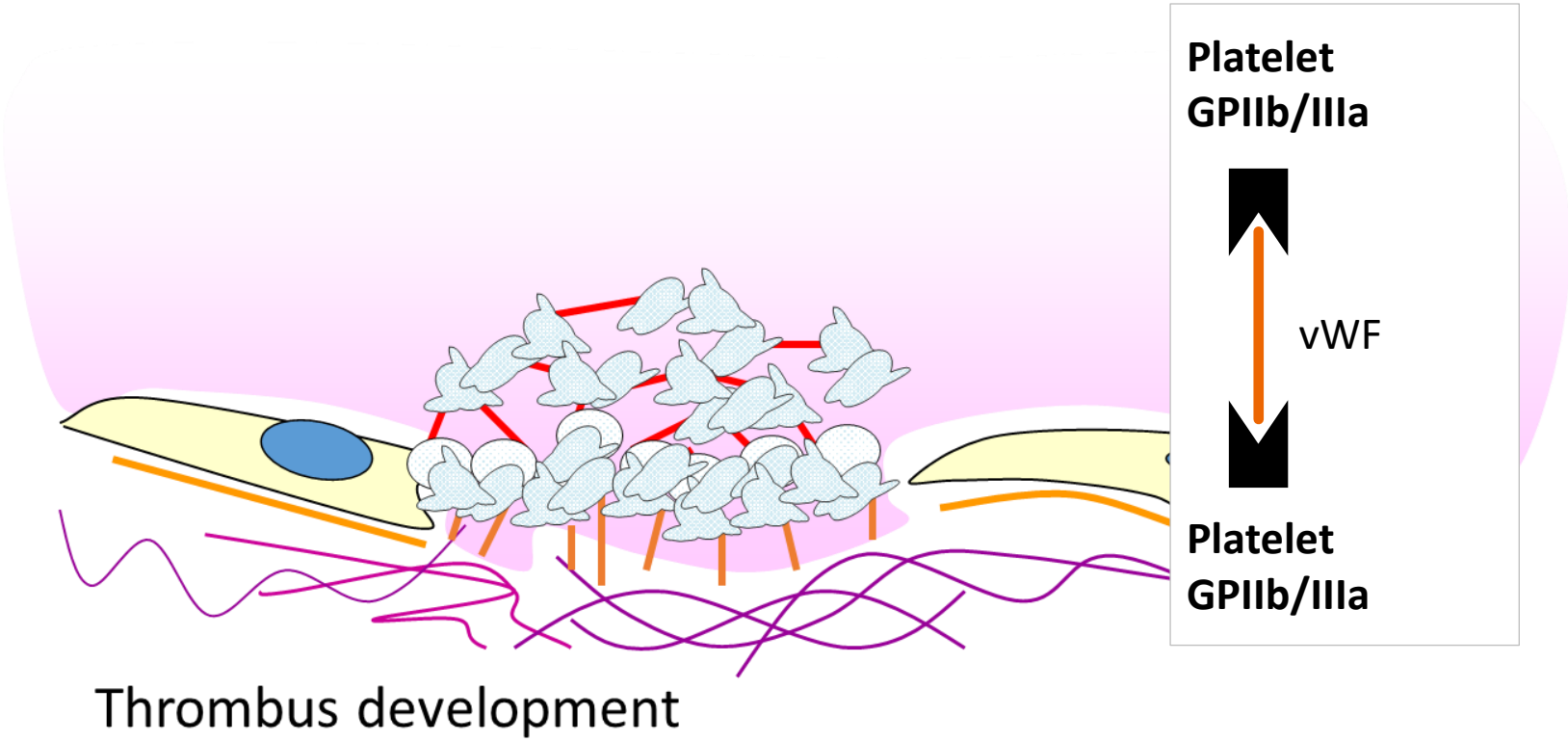
Of lesser importance in platelet aggregation

GPIIb/IIIa → binds vWF



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Interaction of Platelets & Coagulation Factors

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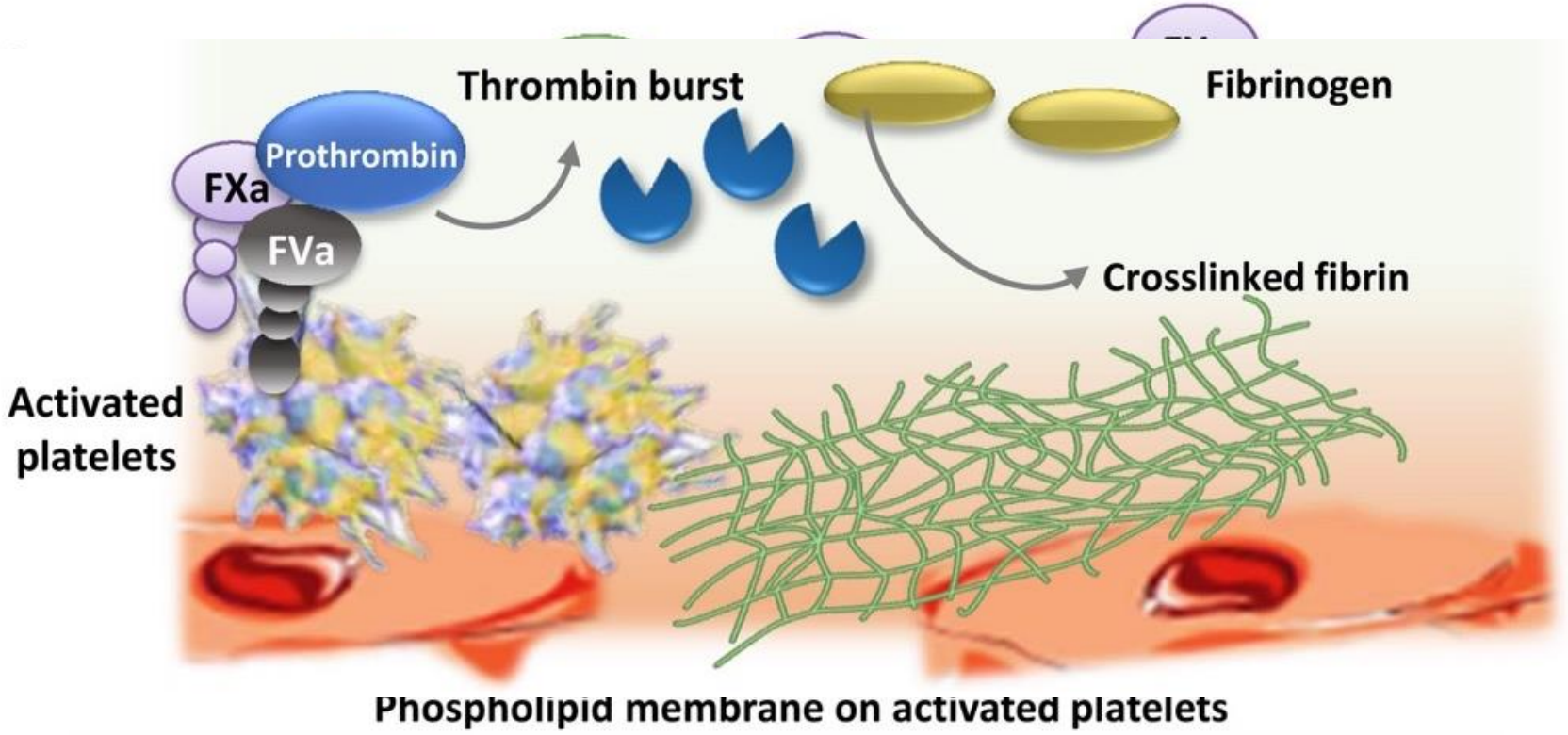
Propagation



O'Donnell JS et al. Br J Haematol 2019;186:24-36

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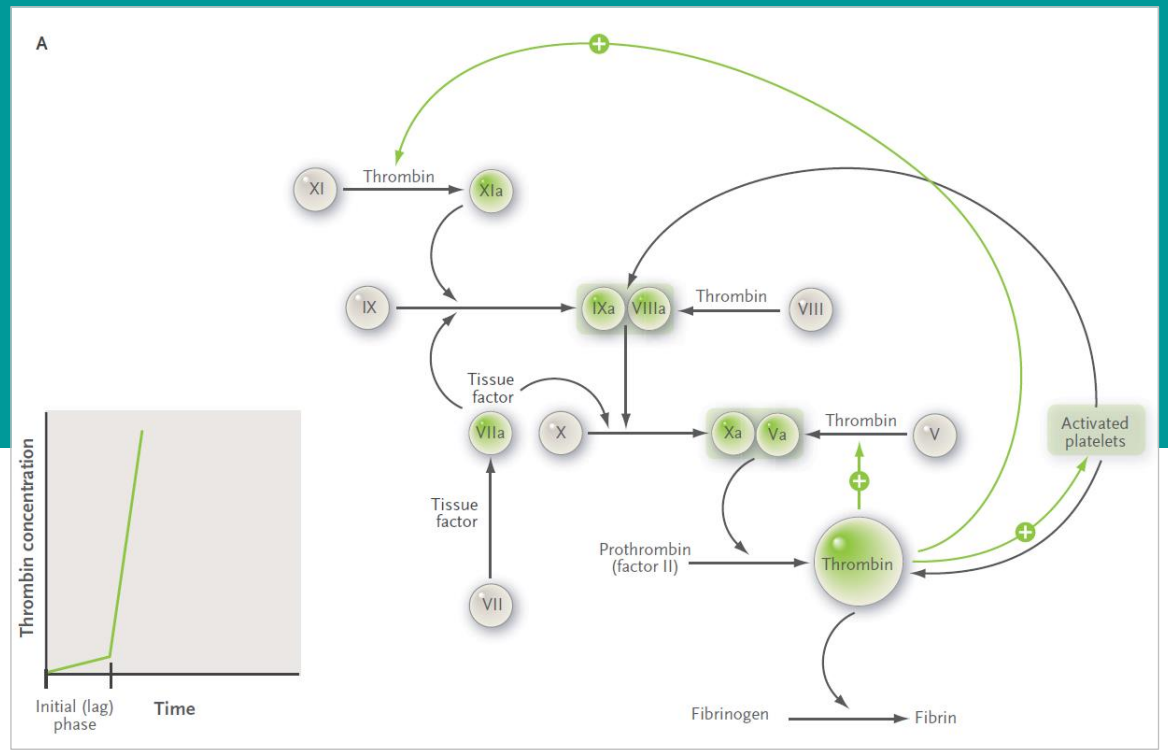
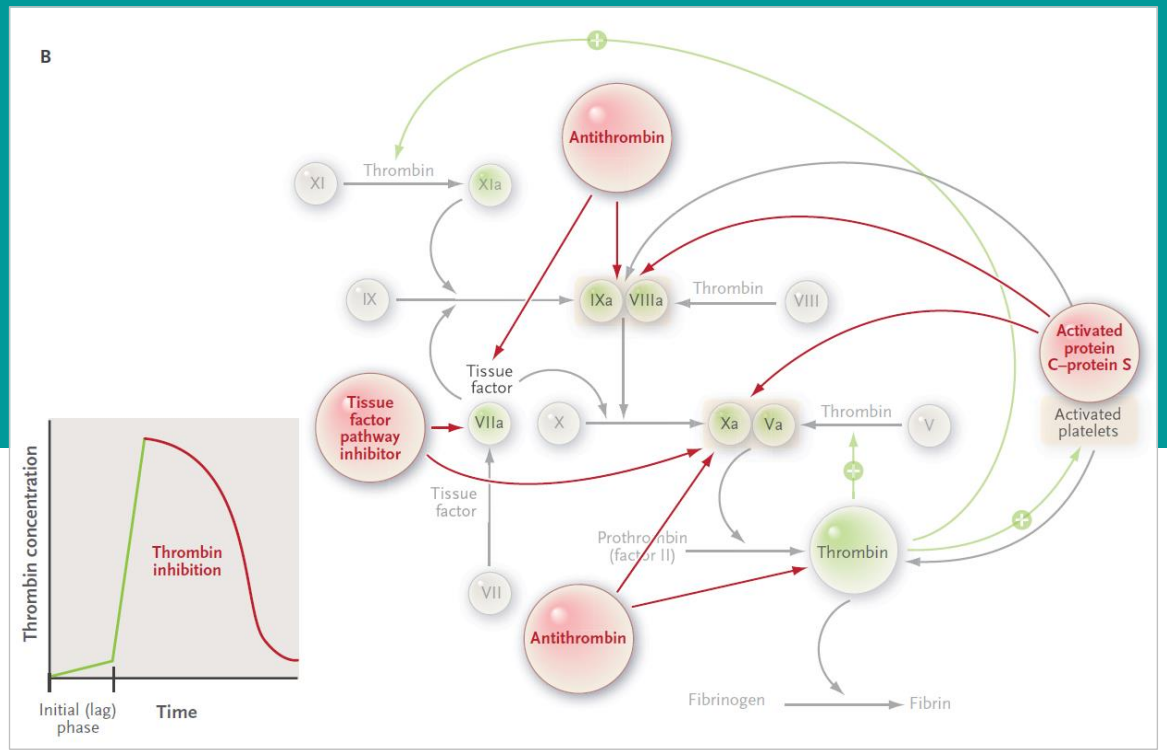
Thrombin Generation & Inhibition





Tripodi A et al. N Engl J Med 2011;365:147-56.

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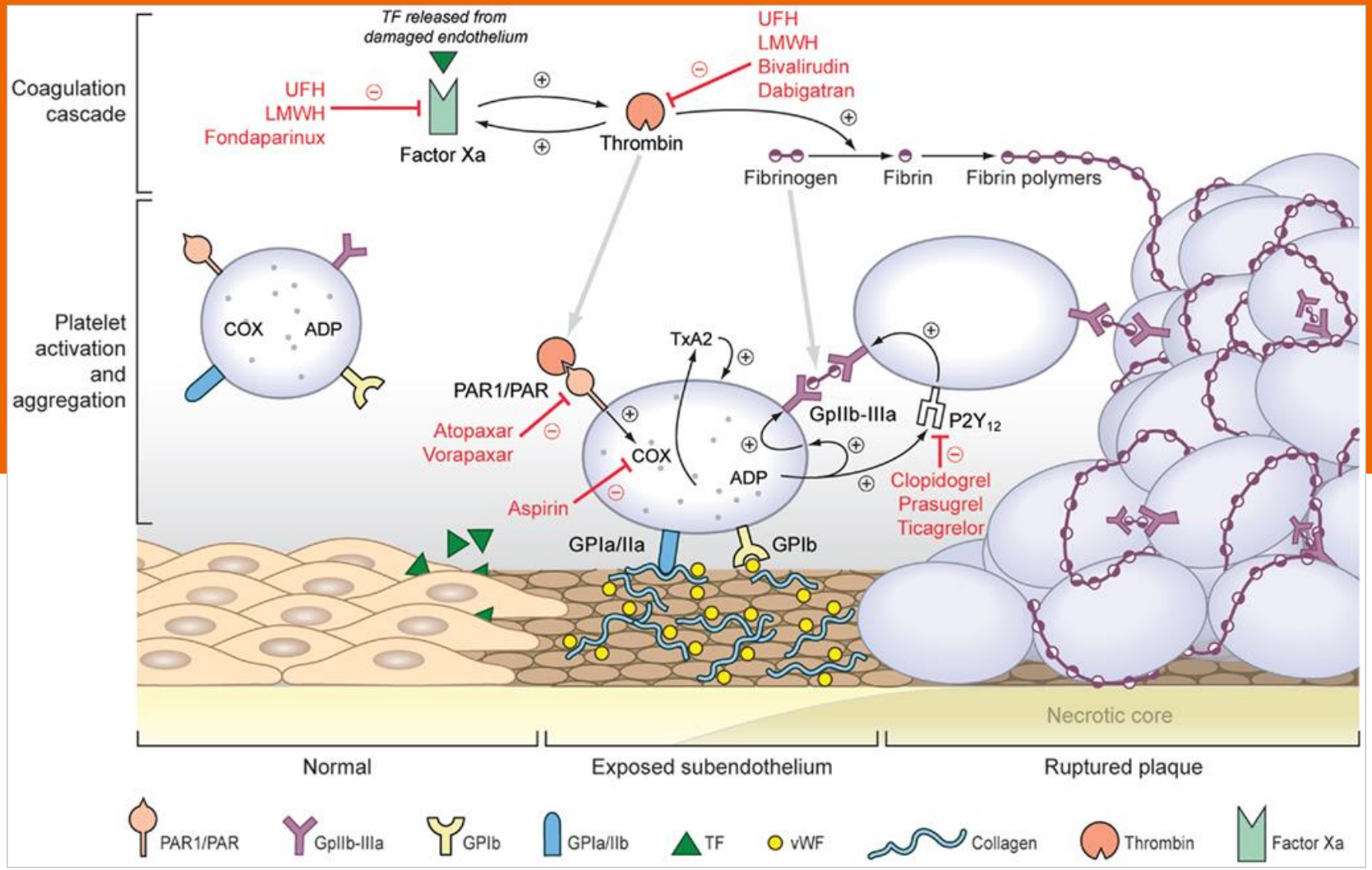
Anticoagulant and Antiplatelet Effects



Lilly SM et al. Front Pharmacol 2011;1-13

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Outline

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What Happens Inside a Clot

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Anti-Xa Testing & Heparin Monitoring

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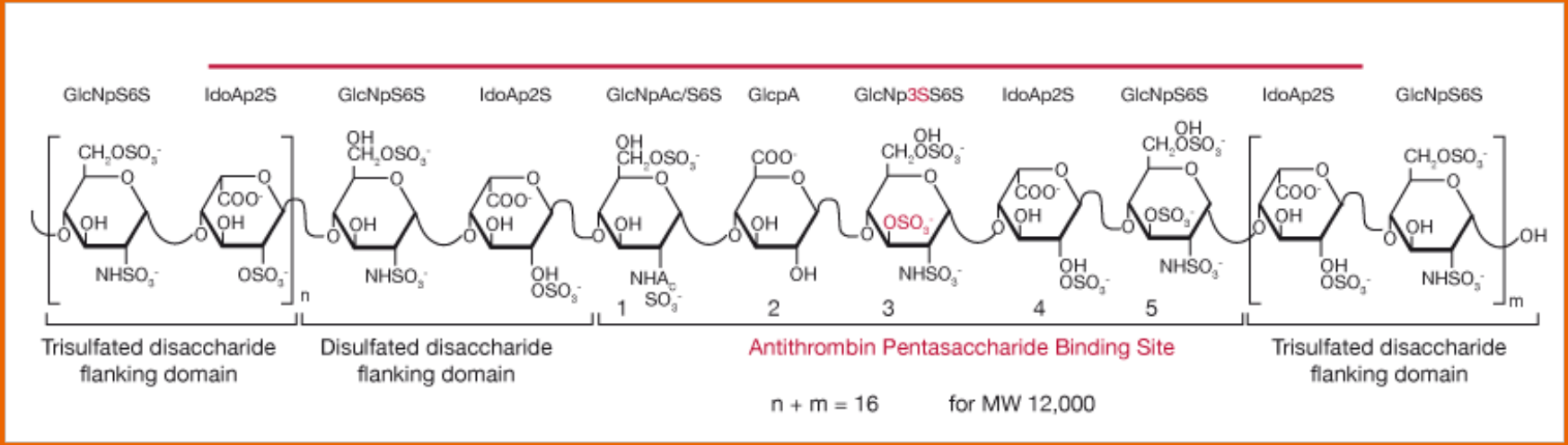
Heparin's Antithrombin (AT) Binding Site and its Structural Variants



- **Unfractionated Heparin (UFH): 12k-16kD**
- **Pentasaccharide motif binds antithrombin**
- **Elimination via-**
  1. depolymerization/cleavage in macrophages and endothelial cells
  2. Renal clearance of lower molecular weight forms

Adapted from Linhardt RJ et al. Semin Thromb Hemost 2014:33(5):453-465

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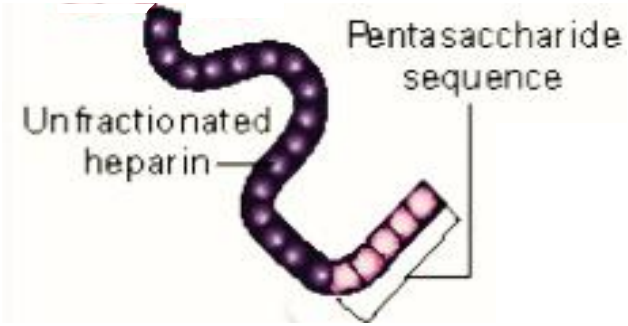
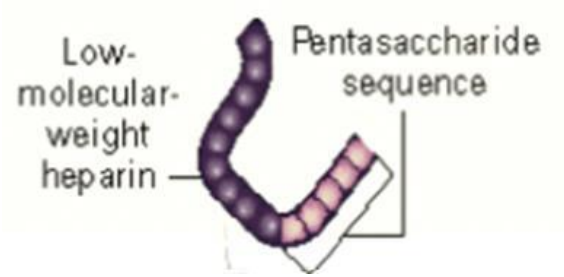
Differences in UFH and LMWH

	UFH	LMWH
Monitoring	Requires monitoring	No routine monitoring
	Variable dose response	Predictable dose response
	All patients	Select patients monitored
Assay	aPTT	Heparin anti-Xa assay only
	Heparin anti-Xa assay	





UFH = Unfractionated Heparin
LMWH = low-molecular-weight heparin

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How Heparin Works

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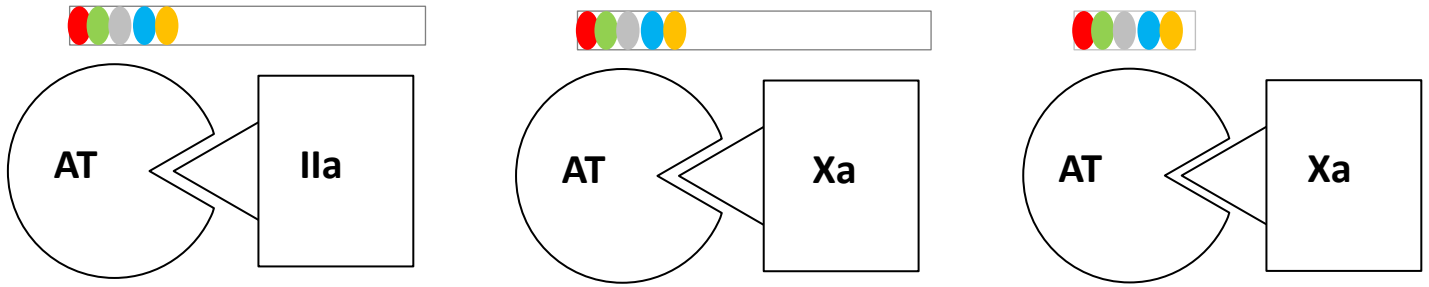
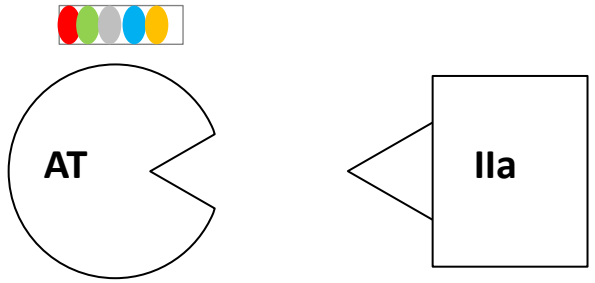
Accelerated Inhibition



No Acceleration of IIa inhibition



- Binds AT, inhibits Xa and IIa
- LMWH anti-Xa ->IIa (Xa >> IIa)
- Fondaparinux has only anti-Xa activity, not IIa

Legend

UFH

LMWH

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Why Monitor Heparin?

Pharmacokinetics vary widely

Pharmacodynamics vary widely—effective anticoagulation at a given concentration

Options for Therapeutic Monitoring

- ✓ PTT
- ✓ Anti-Xa assay
- Whole blood/activated clotting time
- Protamine titration
- Ion-specific electrode

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Current Status
Unfractionated Heparin Monitoring

aPTT Primary Assay for Monitoring

- ~15M - 26M test per year (~30 – 50% of total aPTT orders)
- Limitations for predicting adequacy of anticoagulation
- Cannot assay LMWH levels
- Difficult for laboratory to validate aPTT therapeutic range with each reagent lot / new analyzer

Low Molecular Weight replacing Unfractionated Heparin

- No routine monitoring
- Select patients may require monitoring
- Cannot be monitored with aPTT
- Use of UFH will not go away entirely

Heparin Anti-Xa Assay Alternative

- ~3.4M test per year
- Interest in adding Heparin anti-Xa growing
- Better measurement of UFH concentration
- Better workflow and outcomes
- Required for LMW Heparin
- Can be used for new oral Heparin anti-Xa anticoagulants

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324 THE NEW ENGLAND JOURNAL OF MEDICINE Aug. 17, 1972

A PROSPECTIVE STUDY OF THE VALUE OF MONITORING HEPARIN TREATMENT WITH THE ACTIVATED PARTIAL THROMBOPLASTIN TIME

DILIP BASU, M.D., ALEXANDER GALLUS, M.B., M.R.A.C.P., JACK HIRSH, M.D., F.R.A.C.P., AND JOHN CADE, M.D., PH.D., M.R.A.C.P.

Abstract Two hundred and thirty-four patients treated with continuous intravenous infusions of heparin were studied prospectively to seek a relation between the activated partial thromboplastin time (APTT) and recurrent venous thromboembolism or bleeding during treatment. One hundred and sixty-two patients were treated for venous thromboembolism and the remaining 72 for other diseases. The heparin dose was adjusted to keep the APTT between 1½ and 2½ times control levels. The five patients with venous thromboembolism in whom recurrence developed had a significantly lower APTT than patients without recurrence even though they received similar amounts of heparin. No recurrence developed in any patient with a mean APTT within the therapeutic range. Bleeding occurred in 19 patients whose mean heparin dose and APTT were similar to those of patients without bleeding. Thus, recurrence of venous thromboembolism during heparin treatment appears to be rare if the APTT is prolonged to 1½ times or more control values at all times.

Prospective study in patients with venous thromboembolism who were treated with heparin and found that the risk for recurrent thromboembolic disease is associated with failure to obtain aPTT ratio of approximately **1.5 to 2.5** times the control value

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aPTT Monitoring of Heparin

LMWH and Fondaparinux do not generally prolong aPTT-so can only be used for unfractionated heparin

Is similar to using INR for warfarin, can be informative about adequacy of therapy but does not return a concentration

Generally, therapeutic levels are 1.5-2.5x normal (ie ~45-75 seconds)

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Did You Know?
Disadvantages of aPTT Monitoring of UFH

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More frequent monitoring required

Does not measure heparin concentration

Often only 50% aPTT change due to heparin dose

Pre-analytical Variables effect results

No standardization of aPTT reagents between vendors or reagents

Cannot be used to measure LMW Heparin, Fondaparinux, Heparin anti-Xa inhibitor drugs

Yearly aPTT reagent lot establishment of UFH therapeutic range:

- Lot to lot changes can result in therapeutic range change
- Time, labor for laboratory staff
- Samples difficult to obtain over full range

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Pre-analytical Variables effect aPTT

Platelet Factor 4 neutralizes heparin

- Traumatic venipuncture
- Time plasma on cells > 1 hour
- Time to assay > 4 hours
- Improper centrifugation, plasma not platelet poor
- Frozen sample not platelet poor plasma

Tube under fill prolongs clot assays

- Falsely elevated aPTT result

Line draw contaminated with heparin

- Falsely elevated aPTT result

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Interference with aPTT Assessment of Heparin Effect

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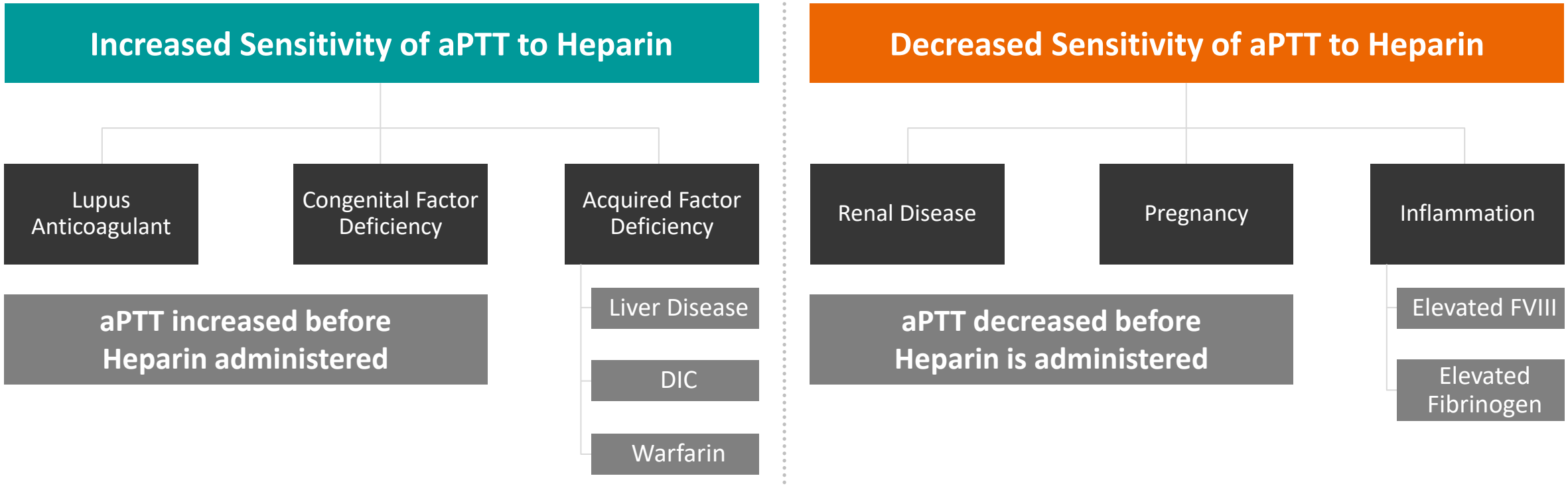

None of these interfere with Heparin anti-Xa monitoring of Heparin

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Laboratory & Physician Comfort Zone
Advantages of aPTT Monitoring of UFH

- Widely used global marker for clotting
- Physician familiarity with results
- Inexpensive
- Readily available in all size laboratories
- Highly automated

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Anti-Xa Assay

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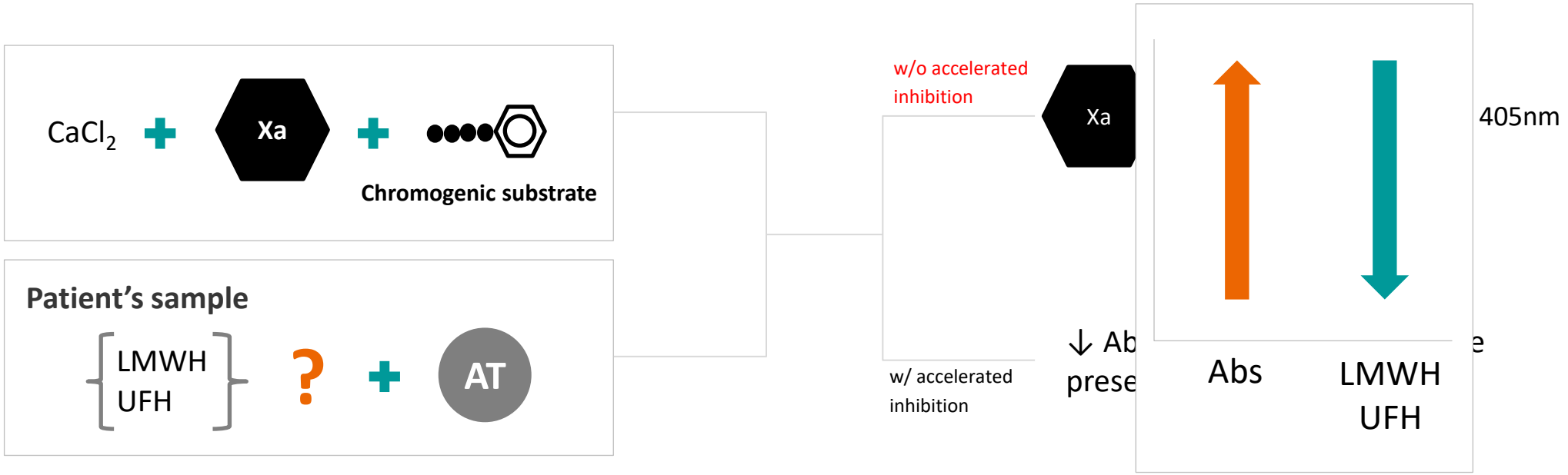
Anti-Xa Assay



- Provides a functional concentration of heparin (IU/mL)
- Can be used to calculate protamine dose
- Values are compared to standard curve of known heparin spiked into control plasma
- Can also be used for LMWH, fondaparinux, but each needs a separate standard curve and a separate reference range
- Lab must know what anticoagulant the patient is being monitored
- Requires prompt sample processing-platelets can neutralize heparin

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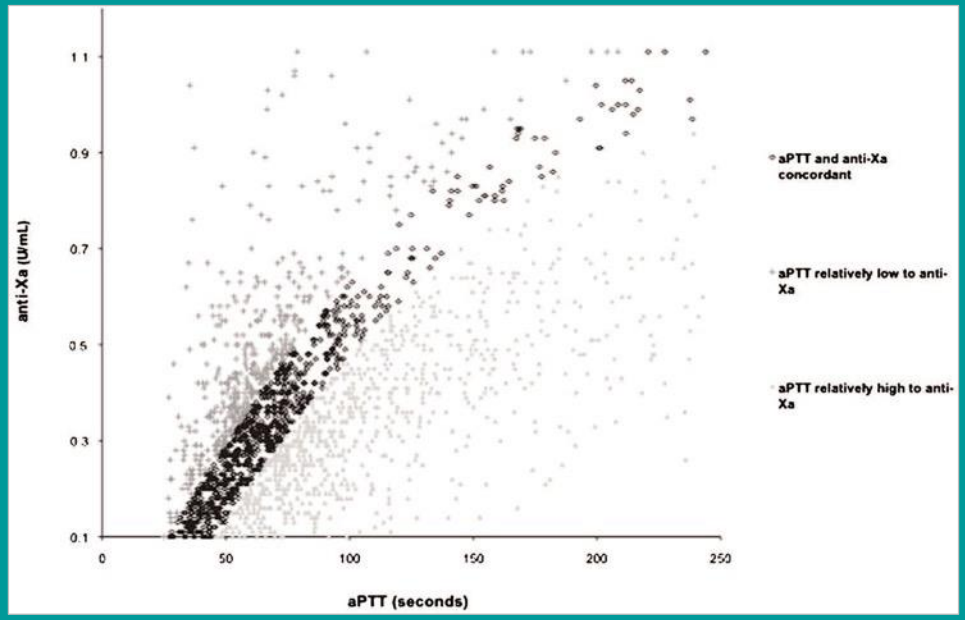
RESEARCH REPORTS

Anticoagulation

Discordant aPTT and Anti-Xa Values and Outcomes in Hospitalized Patients Treated with Intravenous Unfractionated Heparin

Elizabeth A Price, Jing Jin, Huong (Marie) Nguyen, Gomathi Krishnan, Raffick Bowen, James L Zehnder



CONCLUSIONS: aPTT and anti-Xa values are frequently discordant when used to measure UFH in hospitalized patients. A disproportionate prolongation of the aPTT relative to the anti-Xa was the most common discordant pattern in our study. Patients with relatively high aPTT to anti-Xa values appear to be at increased risk of adverse outcomes. Monitoring both aPTT and Xa values may have utility in managing such patients.

Price EA et al. Ann Pharmacother 2013;47:151-158

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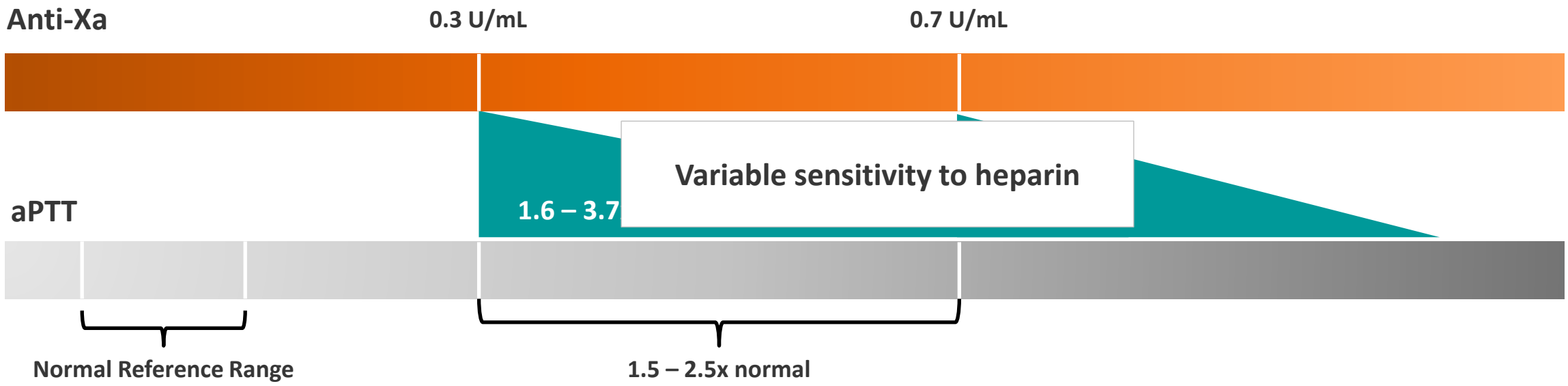
aPTT – Therapeutic Range

- 1.5 – 2.5x normal control
- Calibrated by:
  - Protamine titration (0.2 – 0.4 U/mL)
  - Anti-Xa (0.3 - 0.7 U/mL)



Modified from https://www.medpagetoday.com/mayo/article/500315 (Dr. Kinard)
Kearon C et al. Chest. 2012;141:e419S–e494S

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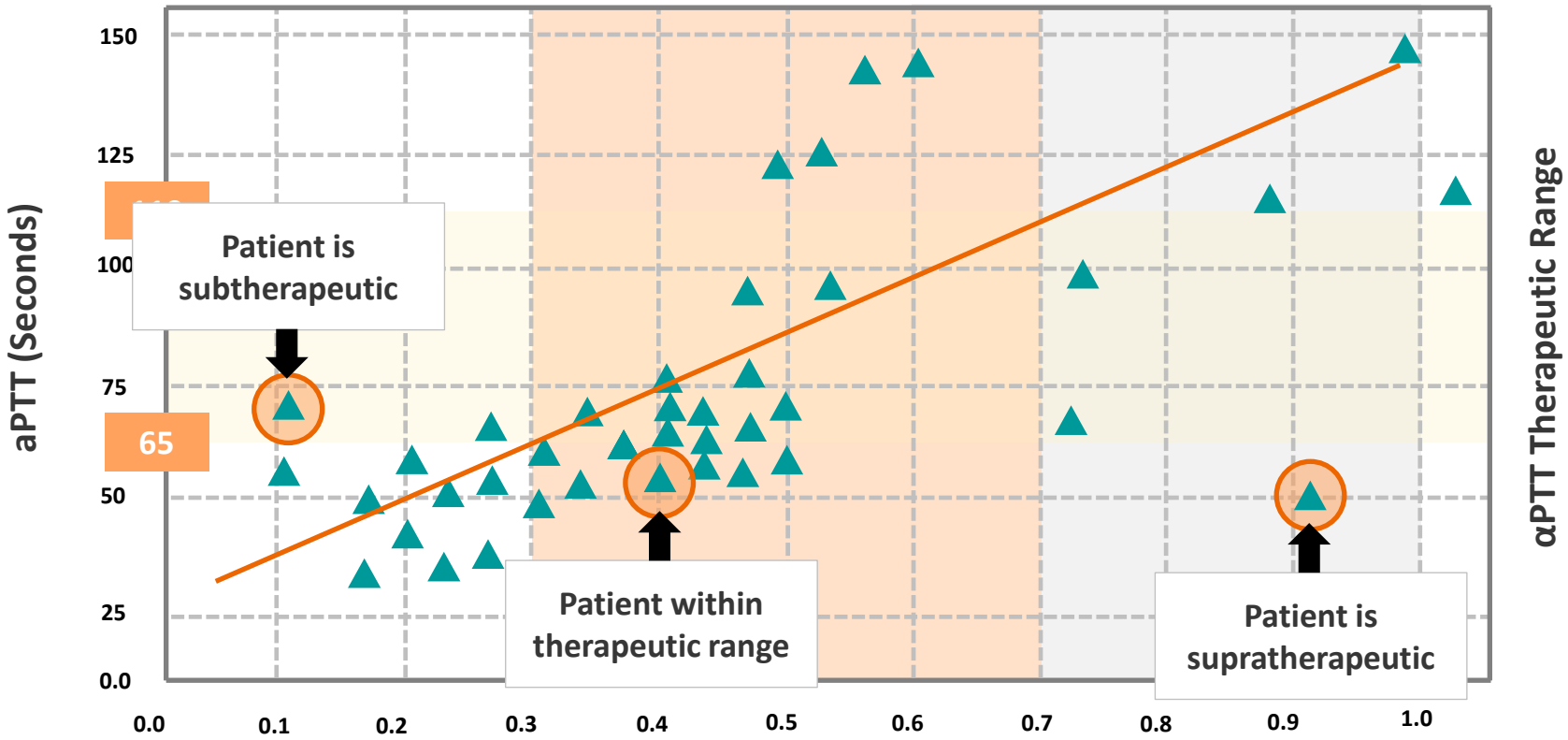
Brill Edwards Curve -> Heparin Response Curve



http://laboratory-manager.advanceweb.com/Archives/Article-Archives/Using-the-Anti-Xa-Heparin-Assay-to-Monitor-Heparin.aspx

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Preanalytical, Analytic, and Biologic Factors Known to Influence aPTT and anti-Xa Levels

Factor	aPTT	Antifactor Xa
Preanalytic		
Blood sampling in the evening (due to diurnal variation)[13–15]	↑	↔
Blood sampling in the morning (due to diurnal variation)[13–15]	↓	↔
High concentration of citrate in collection tube (3.2% is standard)[4, 16, 17]	↑	↔
Improper blood sampling (obtaining sample too close to heparin administration site without proper flushing)[4]	↑	↑
Underfilled sample tubes[4, 10, 16]	↑	↔
Delay in sample analysis (> 2 hrs)[4, 17, 18]	↓	↓
Analytic		
Reagent used (change in lot numbers can also affect results)[4, 10]	↕	↕
Coagulometer used[4, 10]	↕	↕
Blood sampling in the morning (due to diurnal variation)[13–15]	↓	↔
High concentration of citrate in collection tube (3.2% is standard)[4, 16, 17]	↑	↔
Biologic		
Antithrombin deficiency[8, 10]	↓	↓
Increased levels of acute phase reactants (factor VIII or fibrinogen)[8, 10, 17]	↓	↔
Increased heparin-binding proteins (inflammation, infection, malignancy)[4, 8, 10]	↓	↓
Obesity (increased volume of distribution)[4, 10]	↓	↓
Impaired renal function (decreased UFH elimination)[4]	↑	↑
Liver disease (decreased clotting factor production)[8, 10]	↑	↔
Consumptive coagulopathy[8, 10]	↑	↔
Lupus anticoagulant[8, 10]	↑	↔
Deficiencies of specific clotting factors (preallikrein and factors IX, XI, and XII)[8, 10]	↑	↔
Elderly[4, 8, 10]	↑	↔
Recent use of low-molecular-weight heparins or fondaparinux (particularly in setting of impaired renal function)[7]	↔	↑
Hypertriglyceridemia (triglyceride level > 360 mg/dl)[18]	↔	↑
Hyperbilirubinemia (total bilirubin level > 6.6 mg/dl)[18]	↔	↓

aPTT = activated partial thromboplastin time; ↑ = increase in laboratory result; ↓ = decrease in laboratory result; ↔ = little to no effect; ↕ = variable response; UFH = unfractionated heparin.

Vandiver JW, et al. Pharmacotherapy. 2012;32;546-558.

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Limitations of Chromogenic Anti-Xa Assay

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Normal Plasma

Hemolysis

Icterus

Hypertriglyceridemia

Antithrombin Deficiency

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General Recommendations on When to Choose anti-Xa

- Anyone with baseline alterations in aPTT
- Anyone taking drugs altering heparin protein binding
- Anyone where protamine reversal is planned
- Potentially in patients with altered heparin clearance (i.e., renal patients)

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Different Approach to Monitor UFH
Why the Heparin anti-Xa assay?

Better Patient Care

- Smoother Dose Response
- Patient Therapeutic Faster
- Stable Heparin Levels
- Improved Outcomes

Improve Workflow

- Fewer Lab Tests
- Fewer Dose Changes

Efficient

- Overall Minimal Cost to Change
- Lab and Nursing Labor Savings

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Advantage of Heparin anti-Xa Monitoring for Heparin

- Direct measure of heparin's functional activity
- Little or no effect based on variables of patient clinical status
- Limited effect due to pre-analytical variables
- Literature based therapeutic range, no need for laboratory to establish range

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Anti-Xa vs. aPTT
-> Heparin Monitoring

Study Design and Intervention	Study Outcomes
Design: nonblinded, randomized, controlled trial (n=131)[44] Inclusion criteria: patients with heparin resistance who had active VTE requiring > 35,000 units of i.v. UFH in previous 24 hrs Intervention: randomized to UFH monitoring with either antifactor Xa (target range 0.35–0.67 unit/ml) (n=65) or aPTT (target range 60–85 sec) (n=66); both equivalent to 0.2–0.4 unit/ml by protamine titration	Primary outcomes: Recurrent VTE: 4.6% in antifactor Xa group vs 6.1% in aPTT group (p=0.7) Bleeding: 1.5% in antifactor Xa group vs 6.1% in aPTT group (p=0.4) Secondary outcome: Mean heparin dose required: 1884 units/hr in aPTT group vs 1690 units/hr in antifactor Xa group (p<0.001)
Design: prospective, nonblinded, randomized, controlled trial (n=268)[45] Inclusion criterion: i.v. UFH for any indication Intervention: randomized to UFH monitoring with either antifactor Xa (target range 0.35–0.7 unit/ml) (n=137) or aPTT (target range 60–95 sec) (n=131); both equivalent to 0.2–0.4 unit/ml by protamine titration	Primary outcomes: Mean no. of monitoring tests/24 hrs: 1.46 in antifactor Xa group vs 1.68 in aPTT group (p<0.0001) Mean no. of dosing changes/24 hrs: 0.46 in antifactor Xa group vs 0.84 in aPTT group (p<0.0001) Secondary outcome: Mean total cost/96 hrs of treatment: $31.46 in antifactor Xa group vs $27.10 in aPTT group
Design: prospective, cohort study (n=197) Inclusion criterion: i.v. UFH for any indication for ≥ 16 hrs[46] Intervention: newly designed i.v. UFH protocol by antifactor Xa levels, but also based on patient's sex, age, height, and weight	Primary outcome: Percentage of levels in goal range: At 7–9 hrs: 62% At 16–24 hrs: 87% At 24–36 hrs: 86%
Design: prospective, observational, cohort study (n=50)[47] Inclusion criterion: i.v. UFH for any indication for > 24 hrs Intervention: newly designed i.v. UFH protocol based on antifactor Xa level (target range 0.3–0.7 unit/ml)	Primary outcomes: Percentage of patients with their first antifactor Xa level in goal range: 52% (26 patients) Percentage of patients who achieved an antifactor Xa level in goal range within the first 24 hrs: 92% (46 patients)
Design: prospective, observational, cohort study (n=119)[48] Inclusion criterion: i.v. UFH for any indication for ≥ 48 hrs Intervention: newly designed i.v. UFH nomogram based on HCV (n=60) and previously used aPTT-based i.v. UFH nomogram (n=59)	Primary outcomes: Percentage of levels measured at the appropriate time: 92.9% in HCV group vs 80.1% in aPTT group (p<0.0001) Percentage of dosage adjustments made correctly: 94.7% in HCV group vs 89.3% in aPTT group (p=0.01) Secondary outcome: Mean therapeutic heparin infusion rate: no significant difference between groups
Design: retrospective, observational, cohort study (n=100)[49] Inclusion criterion: i.v. UFH for any indication for ≥ 24 hrs, with appropriate protocol compliance Intervention: aPTT-based nomogram (aPTT target 60–90 sec, equivalent to 0.3–0.7 unit/ml by antifactor Xa assay) (n=50) and antifactor Xa–based nomogram (target 0.3–0.7 unit/ml) (n=50)	Primary outcome: Mean time to achieve level in target range: 28 hrs in antifactor Xa group vs 48 hrs in aPTT group (p<0.0001) Secondary outcomes: Percentage of levels in goal range: 66% in antifactor Xa group vs 42% in aPTT group (p<0.0001) Mean no. of monitoring tests/24 hrs: 2.5 in antifactor Xa group vs 2.8 in aPTT group (p<0.01) Mean no. of dosage adjustments/24 hrs: 0.8 in antifactor Xa group vs 1.6 in aPTT group (p<0.0001) Length of stay, mortality rate, and rate of major bleeding: no significant difference between groups

aPTT = activated partial thromboplastin time; HCV = heparin correlation value; UFH = unfractionated heparin; VTE = venous thromboembolism.

Vandiver JW, et al. Pharmacotherapy. 2012;32;546-558

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Two Studies: aPTT versus Heparin anti-Xa Monitoring for UFH

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852-bed medical center; IV UFH infusion[1]

May 1, 2005–April 31,2007 (aPTT); 50 patients
June 1, 2007–Sept 1, 2009 (Heparin anti-Xa); 50 patients

Outcome	aPTT	Heparin anti-Xa
Mean time therapeutic (hours)	48	28

371-bed medical center; IV UFH infusion for DVT/PE[2]

March 1, 2009–May 31, 2010 (aPTT); 98 patients
Aug 1, 2010–Oct 31, 2010 (Heparin anti-Xa); 88 patients

Outcome	aPTT	Heparin anti-Xa
Mean time therapeutic (hours)	39.8	22.2

1Guervil D. et al, The Annals of Pharmacotherapy. 2011 Jul/Aug;45.
2Vandiver J, et al. Hospital Practice. 2013 Apr;41(2).

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Reduction of RBC Transfusions Venous Thrombombolism (VTE), Stroke, Acute Coronary Syndrome (ACS)

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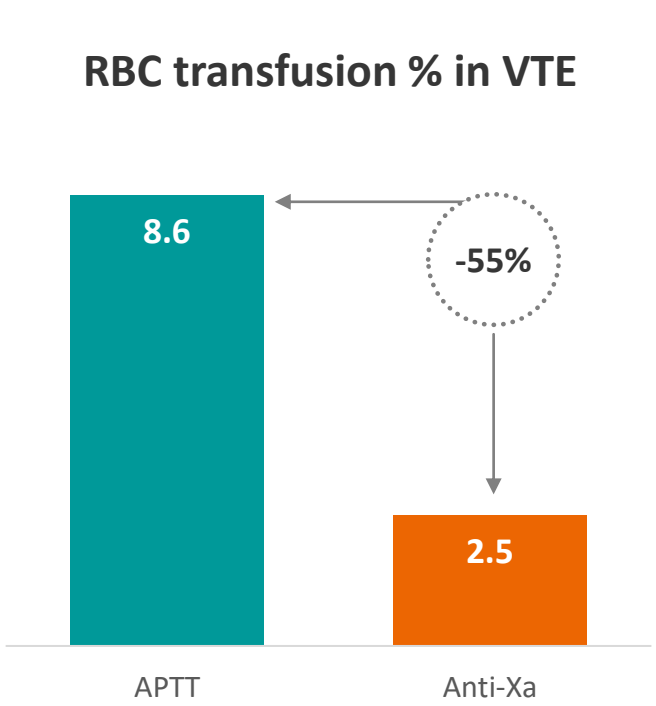

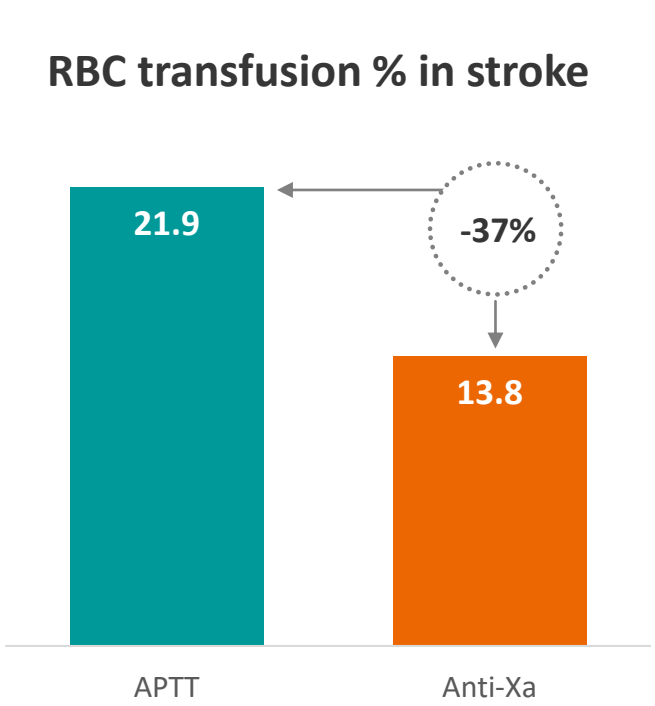

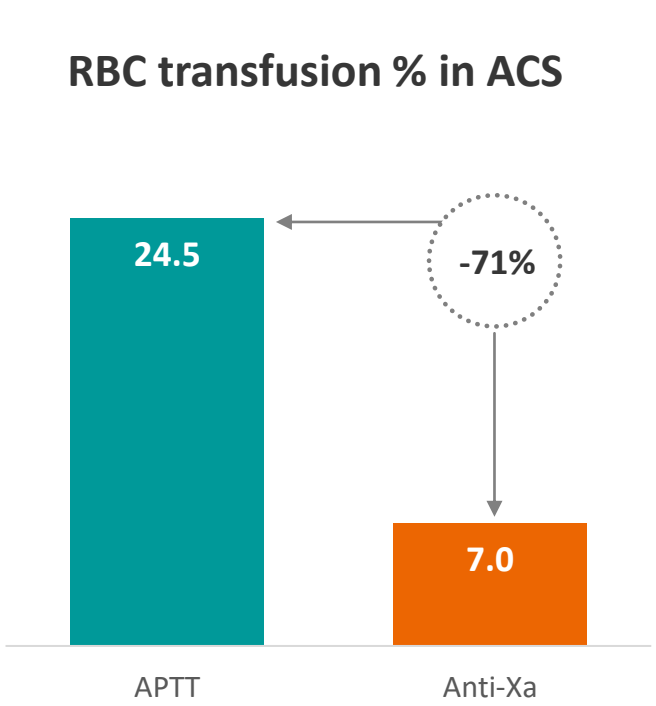

Belk KW, et al. J Thromb Haemost. 2016;14:2148-57

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Reduced Need for Blood Transfusions: Impact on Triple Aim Goals

Quality of care

↓ risk of complications among hospitalized UFH treated patients

↓ intensity and duration of UFH treatment

Patient experience

↓ length of stay in hospital

↓ dependance on mechanical ventilation

Cost reduction

Estimated incremental hospitalization cost associated with RBC transfusions range from $4408 for intraoperative transfusion to over $10,000 for postoperative transfusions.

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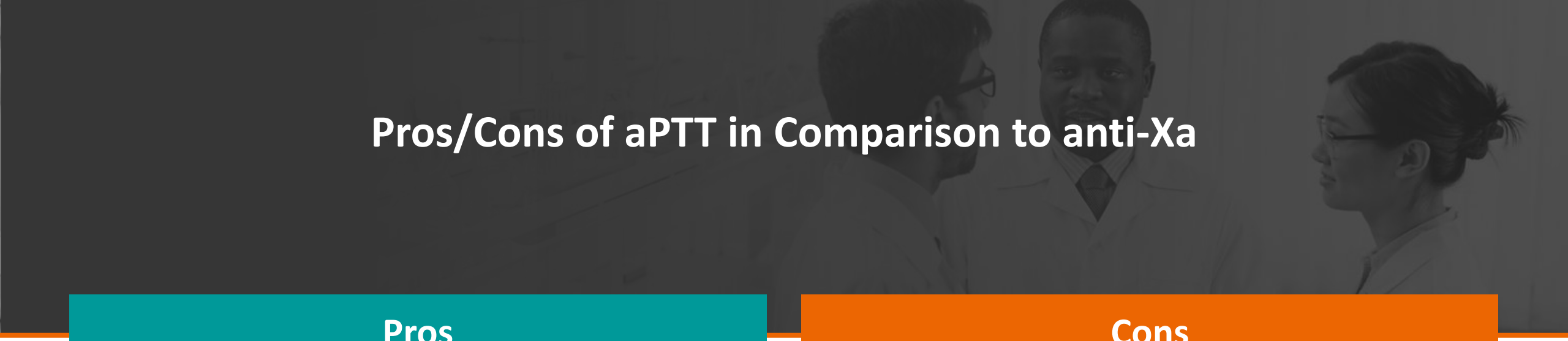

Pros/Cons of aPTT in Comparison to anti-Xa

Pros

- Cheaper
- More extensive literature, provider experience
- Available 24/7 in every lab

Cons

- Greater number of interferences
- Less reliable correlation with heparin level and therapeutic effect
- Therapeutic window less well defined-requires correlation to secondary method
- More lot to lot, lab to lab variation, requiring additional correlation with another method

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What Does anti-Xa Heparin Monitoring Improve Patient Care & Hospital Cost?

Time

Heparin anti-Xa achieves goal range 4 hours sooner than aPTT

Accuracy

Heparin anti-Xa assay provides more accurate monitoring of heparin therapy

Clinical Outcomes

Heparin anti-Xa higher % within therapeutic range

Heparin anti-Xa fewer monitoring tests and dose adjustments

Labor

Decreased tech, phlebotomy, nurse time with Heparin anti-Xa monitoring improves workflow

Patient Safety

Mortality and bleeding rates decrease with Heparin anti-Xa monitoring

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Things to consider when transitioning from aPTT to anti-Xa

01

Pharmacy/Clinician -> Input

02

Laboratory -> Communication

03

Education

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Conclusions

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01 Clinicians are more familiar with aPTT

02 Monitoring UFH/LMWH with anti-Xa is the method of choice

03 aPTT is more readily available in **MOST** hospital labs

04 Education!!!!

Final Thought

"…the uncomfortable marriage of convenience that is represented by UFH and the APTT will continue to endure and is unlikely to be terminated in the near future."

Baluwala I et al. Expert Review in Hematalogy. 2017;7:595-605

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Questions?