University of Connecticut School of Pharmacy

Laboratory Monitoring of Anticoagulation

Michael Smith, Pharm. D., BCPS, CACP
East Region Pharmacy Clinical Manager
Hartford HealthCare

Laboratory Monitoring of Anticoagulation

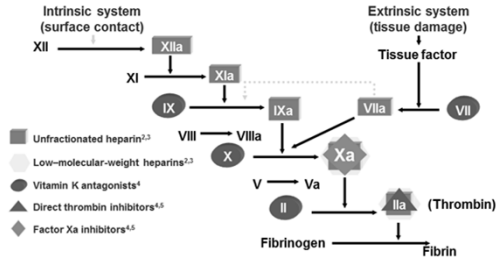
Learning Objectives

- Explain the role of common laboratory tests used in monitoring of anticoagulation therapy.
- Identify an alternative to INR monitoring for warfarin therapy.
- Identify the clinical situations requiring Activated whole blood Clotting Time (ACT) and Anti-factor Xa activity monitoring for unfractionated heparin.
- Discuss the technical differences between point of care testing and laboratory testing and the influence on patient care.

Laboratory Monitoring of Anticoagulation

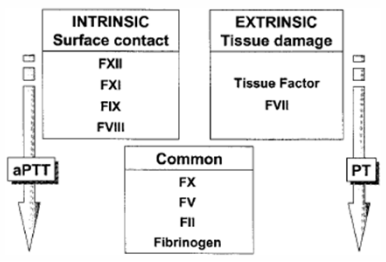
- Clotting times:
  - Prothrombin Time (PT)
    - International Normalized Ratio (INR)
  - Activated Partial Thromboplastin Time (aPTT)
  - Thrombin Time (TT)
  - Activated whole blood Clotting Time (ACT)
  - Anti-factor Xa activity (Anti-Xa)
- Coagulation Factor Activity
- Fibrin D-dimer (D-dimer)

Laboratory Monitoring of Anticoagulation



1. Adapted from Petitou M et al. *Nature*. 1991;350(6319 suppl):30-33. 2. Hirsh J, Fuster V. *Circulation*. 1994;89:1449-1468. 3. Hirsh J et al. *Chest*. 2001;119(1 suppl):64S-94S. 4. Nutescu EA et al. *Pharmacotherapy*. 2004;24(7 Pt 2):82S-87S. 5. Bauer KA. *J Thromb Thrombolysis*. 2006;21:67-72.

Laboratory Monitoring of Anticoagulation



Francis. *Pharmacotherapy* 2004;24(8):108S-119S

Prothrombin Time

- The time it takes plasma to clot after exposure to a tissue factor reagent.
- Assess both the extrinsic and common pathways of coagulation.
- The clot is detected by visual, optical or electromechanical methods.
- Normal range and result is highly dependant on the laboratory equipment and reagent in use; generally 11-13 seconds.

Allen SM. 2009. Hematology: blood coagulation tests. In: M. Lee, editor. Basic skills in interpreting lab reports. 4th ed. Bethesda (MD): ASHP. P 363-390
DeMott WR. 1994. Coagulation. In: Jacobs et al, editor. Laboratory test handbook. 3rd ed. Hudson (OH): Lexi-Comp. P398-480

Prothrombin Time

International Normalized Ratio

- INR= (patient PT/control PT) ^{ISI}
- ISI- international sensitivity index of the reagent
- Control PT= the mean normal PT for the lab using that particular reagent.
- Developed by the WHO to standardize warfarin monitoring

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Prothrombin Time

Table 1. Basic statistics of INR values obtained with different methods.[a]

Calibrator	Innovin Dade Behring	PT-Fibrinogen Recombinant Etaloquick	PT-Fibrinogen HS Plus Etaloquick	Neoplastine CL Plus Etaloquick	Owen PT Bioclin	Nycotest PT Axis-Shield	SPA 50 Etaloquick
Individual calibration[b]							
Mean	2.27	2.53	2.45	2.06	2.40	2.73	2.26
SD	0.72	1.00	0.81	0.66	0.70	1.07	0.62
Median	2.22	2.31	2.42	2.04	2.44	2.71	2.32
Bioclin calibration[c]							
Mean	2.36	2.18	2.61	1.97	2.40	2.44	2.53
SD	0.87	0.71	0.93	0.54	0.70	0.72	0.76
Median	2.28	2.04	2.56	1.97	2.43	2.47	2.59

[a] PT results, in INR units, obtained for 149 blood samples by use of seven different commercial PT determination methods and four calibrators.
[b] Statistics (mean, SD, median) for the data obtained by use of individual calibrators for each of the seven methods. The Dade Behring calibrator was used to calibrate Innovin, Etaloquick calibrator was used to calibrate PT-Fibrinogen Recombinant, and so forth. The original data are illustrated in Fig. 1A.
[c] Statistics (mean, SD, median) for the data in Fig. 1B; a universal calibrator (Bioclin) was used for calibration of all seven methods.

Clin Chem. 2005;51(3):553-560

Prothrombin Time

Clinical uses of PT/INR

- Warfarin monitoring
- General test for state of anticoagulation
- Assessment of liver disease, synthetic function
- Diagnosing disseminated intravascular coagulation

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Prothrombin Time

Factors that prolong the PT/INR

- Warfarin!
- Other anticoagulants
  - Direct oral anticoagulants
  - Argatroban
  - Heparin, LMWH (the lab will correct for this)
- Liver disease
- Vitamin k deficiency
- Coagulation factor deficiency
- Antiphospholipid Antibodies

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Prothrombin Time

Warfarin inhibits the production of vitamin K dependent coagulation factors:

- II
- VII
- IX
- X

Protein C
Protein S

- PT/INR measures factors II, VII, and X

Ansell *Chest*. 2008;133(6);160S-198S

Prothrombin Time

Anticoagulation begins not when adequate serum levels of warfarin are attained, but when serum levels of the coagulation factors decrease.

- Short half-lives: factor VII (6 hrs)
- Long half-lives: factor X (35 hrs)
  factor II (60 hrs)

Ansell *Chest*. 2008;133(6);160S-198S

Prothrombin Time

When and how often should it be monitored?

- Baseline?
  - May discover underlying deficiency
  - Define "baseline" today? last week?
- Day 1? 2? 3?
  - Therapeutic anticoagulation occurs when factors II and X are adequately decreased (5 days)
- Then every week, 4 wks, 12 wks?

Prothrombin Time

What about patients with Antiphospholipid Antibodies Syndrome?

- Autoimmune disease with a persistent presence of antibodies against specific phospholipid-binding proteins.
  - Anticardiolipon antibody
  - Lupus anticoagulant
  - Anti-β_2-glycoprotein I antibody
- May interfere with PT/INR measurements

Prothrombin Time

- May interfere with PT/INR measurements in patients receiving warfarin!
- What can we do?

Prothrombin Time

- Use alternative test method (factor II or X) in patients with APLA on warfarin: obtain a correlation of factor II or X and INR at some point.
- INR 2-3 correlates with
  - Factor II 15-40% of normal = INR 1.8-3.3
  - Factor X 24-45% of normal = INR 2-3
  - these values may differ at your lab

Moll, S Antiphospholipid Antibody Syndrome and Thrombosis. Webinar archived at http://www.standingstoneinc.com/Webinars/WebinarArchive.aspx Accessed 1/2/13

Activated Partial Thromboplastin Time

- The time it takes plasma to clot after exposure to a reagent without tissue factor.
  - initiates the intrinsic pathway
- Normal range and result is highly dependant on the laboratory equipment and reagent in use; generally 25-35 seconds.
- Unlike the INR, there is no standardization for the aPTT

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Activated Partial Thromboplastin Time

- Universal but not Standard!

Year	Reagent	aPTT(sec)	aPTT Ratio
1989	Actin FS	60-85	1.8-2.5
1991	Actin FS	79-105	2.3-3.0
2001	Actin	49-92 to 49-109	1.9-3.7 to 2.1-4.6
2001	Actin FS	72-119 to 98-165	2.6-4.3 to 3.7-6.2
2001	Actin FSL	57-98 to 84-124	2.1-3.5 to 2.5-3.8
2001	IL Test	49-109 to 63-101	1.7-3.8 to 1.9-3.3
2001	Thrombosil I	44-75 to 58-112	1.6-2.7 to 2.4-4.5

- Lab must calibrate a new therapeutic range with every reagent lot change

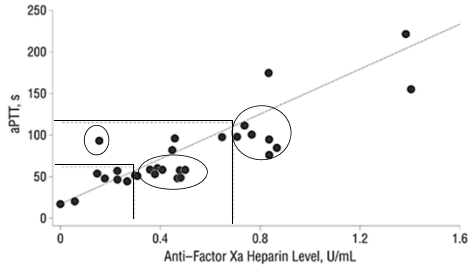
Raschke *Ann Inter Med*. 2003;138:720-723

Activated Partial Thromboplastin Time

- "…the therapeutic aPTT range should be adapted to the responsiveness of the reagent and coagulometer used."
- "…select an aPTT range that correlates with a heparin level of 0.3-0.7 units."
- How to: collect patient samples that span the therapeutic range and plot aPTT vs anti-factor Xa, perform a regression analysis.

Hirsh. *Chest*. 2008;133(6):141S-159S

Regression line for Thrombosil I on ACL 3000 coagulometer



Bates. *Arch Intern Med* 2001;161:385-391.

Activated Partial Thromboplastin Time

Clinical uses of the aPTT

- Monitoring therapy with unfractionated heparin (1.5-2.5 X control)
- Monitoring therapy with injectable direct thrombin inhibitors: argatroban
- General test for state of anticoagulation
- Diagnosing disseminated intravascular coagulation

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Activated Partial Thromboplastin Time

Factors that prolong the aPTT

- Heparin/Direct thrombin inhibitors
- DOACs- No reliable correlation with oral agents.
- Liver disease
- Coagulation factor deficiency
  - Hemophilia A or B, von Willebrand disease, factor VIII deficiency.
- Antiphospholipid Antibodies

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Thrombin Time

- Measures the time it takes for the final step of coagulation, the conversion of fibrinogen to fibrin.
- Normal range and result is highly dependant on the laboratory equipment and reagent in use; generally 14-19 seconds.
- Not useful as a screening test for coagulation abnormalities.

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Thrombin Time

Clinical uses for the TT

- Evaluation of an inherited fibrinogen disorder
- Detection of heparin
- Diagnoses of DIC

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Activated whole blood Clotting Time

- Measures the time it takes whole blood to clot when exposed to a reagent that activates the intrinsic pathway.
- Normal range and result is highly dependant on the laboratory equipment and reagent in use; generally 70-120 seconds.
- Used to monitor heparin when large doses are administered. (above the reliability of the aPTT)
  - Cardiopulmonary bypass (>480 seconds)
  - Cardiac catheterization (300-350 seconds)

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Anticoagulation Monitoring

41yo F pregnant with twins admitted for DVT

- Began on Heparin 80 unit/kg bolus; 18 unit/kg/hr infusion. Eventually stabilized on 2,000 units/hr = 25.7 units/kg/hr
- Develops persistent gross hematuria, Hgb falls from 10.4 to 7.9 g/dL over 4 days

Anticoagulation Monitoring

MD wants to know why so much heparin? Should we decrease the rate?

- Questions to ask:
  - Patient weight correct?
  - Pump programmed correctly?
  - Correct patient's lab work?
- What else can be checked?

Anticoagulation Monitoring

- Anti-factor Xa levels were markedly elevated.
- Heparin infusion reduced to 1,500 units/hr
- Hematuria resolved, no further thromboembolic or hemorrhagic sequelae.
- Diagnosed with "heparin resistance"

Anticoagulation Monitoring

"Heparin Resistance"

- Increased clearance, protein binding, change in volume of distribution

Or

- Falsely shortened aPTT due to elevated coagulation factors, most commonly factor VIII

Hirsh. *Chest*. 2008;133(6):141S-159S

Anti-factor Xa

- Measures in units of enzymatic activity, a functional test that measures the level of activity, chromogenic test.
- Calibrated to measure a specific level of an anticoagulant medication. i.e units of heparin/ml
- Normal range is zero!
- No reagent/lab variability

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Anti-factor Xa

Clinical uses of Anti-Xa
- Monitoring heparin infusion: 0.3-0.7 units/ml
- Monitoring LMWH/Fondaparinux
  - Enoxaparin: 0.6-1.0 units/ml
  - Dalteparin: 1.05 units/ml
- Fondaparinux
  - 2.5mg dose: 0.2-0.4 mcg/ml
  - 7.5mg dose: 0.5-1.5 mcg/ml

"Established" ranges
- Monitoring oral agents? Not yet!

Hirsh. *Chest*. 2008;133(6):141S-159S

Anti-factor Xa

- Measures a single enzyme- Factor Xa
- Free from interference from warfarin, sample tube fill, storage, enzyme levels.
- Improved accuracy means:
  - Less dosage adjustments
  - Less work -Less lab tests
  - Less chance for error - Better treatment

Francis. *Pharmacotherapy* 2004;24(8):108S-119S

Anti-factor Xa

268 pts randomized to two monitoring methods- aPTT or Anti-factor Xa

Dose Changes/24 hours:

Anti-factor Xa : 0.46

aPTT : 0.84 p<.0001

Nearly twice as many dose changes!

Rosborough *Pharmacotherapy.* 1999;19(6):760-766

Anti-factor Xa

LMWH + Fondaparinux testing
- Who, When, Why?
- Obese – maybe
- Renal dysfunction - ok
- Pregnancy - yes
- Small or elderly - maybe
- Pre-surgery- someday?

Coagulation Factor Activity

- The levels of various individual coagulation factors can be measured to diagnose specific factor deficiencies.
- Useful for diagnosing hemophilia A (factor VIII), hemophilia B (factor IX), and other more rare deficiencies.
- Alternative monitoring for warfarin
  - Factor II 15-40% of normal = INR 1.8-3.3
  - Factor X 24-45% of normal = INR 2-3

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Fibrin D-dimer

- D-dimer is a major product of the breakdown of fibrin.
- It is formed when plasmin cleaves the cross-linked fibrin of a clot.
- Normal levels are:
  - <200ng/ml by ELISA
  - <500ng/ml by cold latex agglutination
- Elevated levels indicate recent or ongoing thrombosis and fibrinolysis.

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Fibrin D-dimer

Clinical uses of D-dimer

- Rule out active DVT/PE
- Evaluate the need for continuing anticoagulation treatment/prophylaxis after an acute event.

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Anticoagulation Monitoring

JL has been in the therapeutic range for 6 straight weeks. Today she notes no changes in diet, exercise, health, or other medications. Her POC result is 4.7 and she is sent to the lab for venipuncture confirmation. The venipuncture result came back at 3.2. She asks you…

- Why the discrepancy, what do I believe, should I ever trust the POC result?

Point of Care Testing

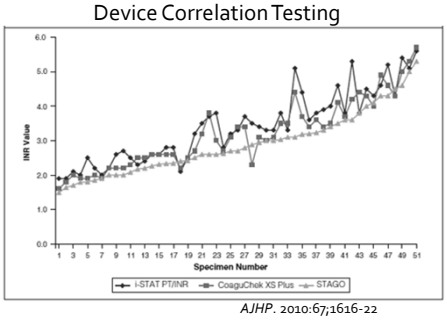
- Point of Care testing refers to the use of small, hand-held devices to perform testing at or near the patient rather than in a central laboratory.
- Advantages include rapid turn around, ability for home testing, convenience
- Disadvantages include cost, technical limitations of the devices

Point of Care Testing

- Coagulation testing is a complicated interplay of different coagulation factors and thromboplastins, and technological steps to measure these effects.
- Measuring a process vs an amount/concentration.
- Long ago it was recognized that different labs gave different results for the PT
  - WHO standardized thromboplastins and instituted the INR
  - Thromboplastins are only standardized to an INR of 4-4.5
  - Original WHO standard thromboplastins are long gone.

Point of Care Testing

Device Correlation Testing



AJHP. 2010:67;1616-22

Point of Care Testing

What does correlation testing tell us?

- That POC and venous lab testing methods provide unequal results.
- (what about lab vs lab correlation studies)

Point of Care Testing

- There is always a difference in testing:
  - One method to another
  - One lab to another
- Can not determine a true "accurate" method
- Discordance doesn't mean better or worse
- We substitute variability and repeatable results as markers of accuracy.
- Difference of <0.5 considered acceptable.
  - Mathematical difference vs dosing decision difference
  - 2.2-2.7 as good as 2.9-3.4?

Bussey H, Walker B. Lab or POC INR Results - Which are more reliable? http://www.clotcare.com/faq_inrreliability.aspx. Accessed 1/3/13

Point of Care Testing

POC Error

- Squeezing finger too hard
- Too much time between lancing and applying blood to the test strip
- Improper storage of test strip

Venipuncture Error

- Under/over-filling the collecting tube
- Low HGB/HCT
- Device not calibrated appropriately

Bussey H, Walker B. Lab or POC INR Results - Which are more reliable? http://www.clotcare.com/faq_inrreliability.aspx. Accessed 1/3/13

Point of Care Testing

- Clinical outcomes are more important then scientific correlations!
- Many trials demonstrating good clinical outcomes using POC devices- only.
  - ARISTOTLE, ROCKET-AF, many self testing studies.
- Bottom line, treat the patient not the number.
  - Do we expect an out of range result?
  - Retest in a short amount of time.

Laboratory Monitoring of Anticoagulation

Assay	Dabigatran	Oral Xa inhibitors
PT	Not Reliably	Sensitive
aPTT	Sensitive	Not reliably
Anti Xa	No	Sensitive
TT	Sensitive	No

- PT and aPTT do not respond in a linear or predictable fashion.
  - They are useful for testing for the presence of the drug in the plasma.
  - Not for determination of the extent of anticoagulation.
- Caution when trying to test INR with concurrent warfarin + DOAC use.

Miyares MA. *AJHP* 2012;69:1473-84-356

Laboratory Monitoring of Anticoagulation

- Specialty reference labs can determine serum levels for the new oral agents
- Therapeutic ranges are loosely defined
- E-carin- can directly correlate to dabigatran
- Anti factor IIa testing is potentially available to monitor all oral agents in the future.

Miyares MA. *AJHP* 2012;69:1473-84-356