Laboratory Monitoring of Anticoagulation

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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.

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)

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.
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

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

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

Prothrombin Time

Warfarin inhibits the production of vitamin K dependent coagulation factors:

- II Protein C
- VII Protein S
- IX
- X
- PT/INR measures factors II, VII, and X

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)
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.
  – Anticardiolipin antibody
  – Lupus anticoagulant
  – Anti-β-glycoprotein I antibody
• May interfere with PT/INR measurements

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

Activated Partial Thromboplastin Time

• Universal but not Standard!

<table>
<thead>
<tr>
<th>Year</th>
<th>Reagent</th>
<th>aPTT (sec)</th>
<th>aPTT Ratio</th>
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<tbody>
<tr>
<td>1990</td>
<td>Actin FS</td>
<td>65-94</td>
<td>1.5-2.5</td>
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<tr>
<td>1991</td>
<td>Actin FS</td>
<td>70-105</td>
<td>1.8-2.5</td>
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<tr>
<td>2001</td>
<td>Actin FS</td>
<td>67-110 to 86-180</td>
<td>2.6-4.3 to 3.7-4.2</td>
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<tr>
<td>2001</td>
<td>Actin FS</td>
<td>72-119 to 98-165</td>
<td>2.6-4.3 to 3.7-4.2</td>
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<tr>
<td>2001</td>
<td>IL Test</td>
<td>49-109 to 63-101</td>
<td>1.7-3.8 to 1.9-3.3</td>
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<td>2001</td>
<td>Thrombosil I</td>
<td>44-75 to 58-112</td>
<td>1.6-2.7 to 2.4-4.5</td>
</tr>
</tbody>
</table>

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

Accessed 1/2/13

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.

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

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

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.

Thrombin Time

Clinical uses for the TT

• Evaluation of an inherited fibrinogen disorder
• Detection of heparin
• Diagnoses of DIC
**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)

**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**

“Heparin Resistance”

- Increased clearance, protein binding, change in volume of distribution
- Falsely shortened aPTT due to elevated coagulation factors, most commonly factor VIII

**Anticoagulation Monitoring**

**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
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!


Improved accuracy means:
- Less dosage adjustments
- Less work
- Less lab tests
- Less chance for error
- Better treatment


Dose Changes/24 hours:
- Anti-factor Xa: 0.46
  - aPTT: 0.84 p<.0001
  - Nearly twice as many dose changes!


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:
  - <200 ng/ml by ELISA
  - <500 ng/ml by cold latex agglutination
- Elevated levels indicate recent or ongoing thrombosis and fibrinolysis.


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


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.

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

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
- Difference of 2.2-2.7 as good as 2.9-3.4?

Bussey H, Walker B. Lab or POC INR Results – Which are more reliable?

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?

Point of Care Testing

- Clinical outcomes are more important than 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

- 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

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