Optimal Utilization of Special Coagulation Testing

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DISCLOSURES:

Relevant Financial Relationship(s)
None

Off Label Usage
None

Did I change my slides?
You betcha!
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Objectives

- Recognize artifactual coagulation laboratory results
- Clinical and laboratory correlation of selected inherited and acquired bleeding disorders
- Efficient pursuit of an abnormally PT and aPTT
- Optimal utilization of thrombophilia testing
Case 1
47-Year-Old Male With Complex Cyanotic Congenital Heart Disease

- Single ventricle, D-transposition of great vessels,
  - s/p hemostatically unremarkable corrective surgery
- Medications: Antihypertensive agents
- Pre-cardiac angiography testing
  
<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb</td>
<td>23.1</td>
<td>(13.5-17.5 gm/dL)</td>
</tr>
<tr>
<td>Hct</td>
<td>72.2</td>
<td>(38.8-50%)</td>
</tr>
<tr>
<td>PT</td>
<td>22.2</td>
<td>(8-12s)</td>
</tr>
<tr>
<td>aPTT</td>
<td>47.1</td>
<td>(23-33s)</td>
</tr>
</tbody>
</table>
Normal Hematocrit  High Hematocrit

Citrate anticoagulant

Plasma

Hematocrit

1

5
# Preprocedure Coagulation Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>3.2%</th>
<th>0.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate PT (8-12s)</td>
<td>22.2</td>
<td>12.2</td>
</tr>
<tr>
<td>aPTT (23-33s)</td>
<td>47.1</td>
<td>28</td>
</tr>
</tbody>
</table>
Artifactual Causes for Coagulation Assay Abnormalities

• Hematocrit
  • High hematocrits falsely prolong clotting times

• Traumatic specimen collection/suboptimal processing
  • Clotted specimens: Falsely abnormal results

• Anticoagulants
Case 2
84-Year-Old Female With DVT

- Left thigh pain, swelling and ecchymosis
- Doppler US: Left femoral vein DVT
  - Baseline aPTT 44 s (21-33)
- Venogram
  - Consistent with presence of left femoral DVT
Venogram showing filling defect
Case 2
84-Year-Old Female

- Heparin with transition to warfarin
- Repeat aPTT off heparin 71 s
Mayo Medical Laboratories Coagulation Test Profiles

• Prolonged PT/aPTT Profile
• Bleeding Profile
• Thrombosis Profile
• Lupus Anticoagulant Profile
• Von Willebrand Disease Profile
Case 2
84-Year-Old Female: Prolong PT/aPTT Profile

- PT 35.8 s (8-12)
- 1:1 mix 11.0 s
- aPTT 112 s (21-33)
- 1:1 mix 43 (21-33)
Case 2
84-Year-Old Female: Prolonged PT/aPTT Profile

- PT 35.8 s (8-12)
- 1:1 mix 11.0 s
- aPTT 112 s (21-33)
- 1:1 mix 43 (21-33)

Consistent with presence of inhibitor

Prolonged aPTT
Inhibited on mixing study

Type of inhibitor

Drug: eg, Heparin DTI
Specific: eg, FVIII
Non-specific: eg, LAC

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Case 2
84-Year-Old Female

• aPTT 112 s (21-33)
• PNP 117 s (platelet neutralization procedure)
• DRVVT 1.1 ($\leq 1.1$)
  • Negative for lupus anticoagulant
Case 2
84-Year-Old Female

**Intrinsic**
- XII: 101%
- XI: 70%
- IX: 30%

**Extrinsic**
- VII: 7%

**aPTT**
- Bethesda titer 8 BU

**Fibrinogen**
- <1%
- V: 13%
- X: 26%
Venogram showing filling defect
Case 2
84-Year-Old Female

• Acquired hemophilia (autoimmune)
• Bleed into left thigh
  • Venogram consistent with extrinsic compression
• Transferred to Mayo Clinic
• Ultrasound
  • No DVT
Outcomes of evaluation of prolonged APTT: 100 consecutive outpatients

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artifactual</td>
<td>14%</td>
</tr>
<tr>
<td>Hemostatic abnormality</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>• No hemostatic conseq</td>
<td>36%</td>
</tr>
<tr>
<td>• Mild bleeding risk</td>
<td>18%</td>
</tr>
<tr>
<td>• Moderate bleeding risk</td>
<td>17%</td>
</tr>
<tr>
<td>• Severe bleeding risk</td>
<td>15%</td>
</tr>
</tbody>
</table>
Outcomes of evaluation of prolonged APTT: 177 consecutive inpatients

- No hemostatic consequence: 58.2%
- Mild bleeding risk: 4.5%
- Moderate bleeding risk: 1.7%
- Severe bleeding risk: 3.5%
Case 3
A MML Client Order for Multiple Factor Inhibitors

• 65-year-old male
• Order for multiple coagulation factor inhibitors
  • FXI, FIX, FVIII
  • FII, FV
• Discussion with ordering provider
  • Local aPTT prolonged and inhibited on mixing study
  • Patient not on anticoagulants
• Agreed to convert order into a Prolonged Clotting Time profile
<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>Ref range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (INR)</td>
<td>15.2 (1.4)</td>
<td>8.4-12s</td>
</tr>
<tr>
<td>PT mix</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>APTT</td>
<td>60</td>
<td>21-33s</td>
</tr>
<tr>
<td>APTT mix</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>FVIII</td>
<td>&gt;60 (8, 28, 60)</td>
<td></td>
</tr>
<tr>
<td>FIX</td>
<td>&gt;70 (20, 40, 70)</td>
<td></td>
</tr>
<tr>
<td>FIX</td>
<td>&gt;35 (8, 20, 35)</td>
<td></td>
</tr>
<tr>
<td>Assay</td>
<td>Result</td>
<td>Ref range</td>
</tr>
<tr>
<td>---------------------</td>
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</tr>
<tr>
<td>APTT</td>
<td>60</td>
<td>21-33s</td>
</tr>
<tr>
<td>APTT mix</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Thrombin time</td>
<td>&gt;240</td>
<td>16-25s</td>
</tr>
<tr>
<td>Reptilase time</td>
<td>16</td>
<td>16-22s</td>
</tr>
<tr>
<td>FVIII</td>
<td>&gt;60 (8, 28, 60)</td>
<td></td>
</tr>
<tr>
<td>FIX</td>
<td>&gt;70 (20, 40, 70)</td>
<td></td>
</tr>
<tr>
<td>FIX</td>
<td>&gt;35 (8, 20, 35)</td>
<td></td>
</tr>
</tbody>
</table>
Patient was on dabigatran (oral direct thrombin inhibitor)
How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults.

Kamal AH, Tefferi A, Pruthi RK.

• (Open online access paper)


**Actions of Protein C, S and Antithrombin**

**Intrinsic Pathway**
- Thrombin
- Surface
- Factor XI
- Factor IX
- Factor X
- Factor VIII
- Prothrombin (II)
- Factor Xa
- Factor Va
- Factor IXa
- Factor VIIIa
- Ca²⁺ PL

**Extrinsic Pathway**
- Vascular injury
- Tissue factor
- Factor VII
- Factor VIIa
- Factor IXa
- Factor Xa
- Thrombin
- Factor IX
- Factor X
- Factor VIIIa
- Ca²⁺ PL

**Protein C, Protein S**
- Protein C
- Protein S

**Antithrombin**
- Thrombin
- Factor Xa
- Factor Va
- Factor VIIIa
- Ca²⁺ PL
- Prothrombin (II)
- Fibrinogen
- Fibrin
- Fibrin (cross-linked)
- Factor XIIa
- Factor XIII
- Ca²⁺

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Activated Protein C Resistance and Factor V Leiden

- Most common congenital hereditary thrombophilia among whites

- Protein phenotype
  - Normally: Activated protein C (APC) inactivates activated factor V (fVa)
  - APC resistance: Mutated factor V resists inactivation

- Genetic basis
  - Factor V Leiden (R506Q) mutation

- Testing strategy
  - Initial APC-R assay, FV Leiden only if indicated
Review of APC-R and FV Leiden Test Ordering Patterns (MML clients)

- January 2010 to October 2010
- 917 APC-R test orders
- APC-R normal: 471/971 (51%)
  - Factor V Leiden ordered separately by providers (not indicated)
  - All 471 FV Leiden results were negative
- Recommended testing strategy:
  - Initial APC-R assay, FV Leiden only APC-R ratio abnormally low
Testing Strategy for APC-R and FV Leiden

Screening With the Activated Protein C Resistance Assay Yields Significant Savings in a Patient Population With Low Prevalence of Factor V Leiden

Laura J. Taylor, MT(ASCP), Robert A. Oster, PhD, George A. Fritsma, MS, MT(ASCP), Patricia H. Tichenor, MT(ASCP), Cari E. Reed, MT(ASCP), Barbara M. Eiland, MT(ASCP), Christine L. Hudson, MT(ASCP), and Marisa B. Marques, MD

Key Words: Factor V Leiden; Activated protein C resistance; Cost savings

DOI: 10.1309/4370VLY9P8DEWF6
## Cost Savings by Screening With APCR in a Population With Low Prevalence of FVL

<table>
<thead>
<tr>
<th>Test ordered</th>
<th>Tests (no.)</th>
<th>Total cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline (2 yr)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVL</td>
<td>299 × $187 = $55,913</td>
<td></td>
</tr>
<tr>
<td>APCR</td>
<td>75 × $45 = $3,375</td>
<td>374</td>
</tr>
<tr>
<td><strong>Theoretical cost without interventions for subsequent 6 yr</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVL</td>
<td>1,980 × $187 = $370,260</td>
<td></td>
</tr>
<tr>
<td>APCR</td>
<td>495 × $45 = $22,275</td>
<td>2,475</td>
</tr>
<tr>
<td><strong>Actual cost with interventions for subsequent 6 yr</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVL</td>
<td>154* × $80 = $12,320</td>
<td></td>
</tr>
<tr>
<td>APCR</td>
<td>710 × $4.50 (2 yr) + 1,765 × $5.36 (4 yr) = $12,655</td>
<td>2,475*</td>
</tr>
</tbody>
</table>

* All 154 patients also had an APCR assay
† Impact of interventions after 6 years: $392,535 – $24,975 = $367,560 or $61,260/year

Optimal Recommended Testing Strategy

• Initial APC-R
• Reflexive FV Leiden (if indicated)
Thrombophilia Profile

**THRMP/83093 Thrombophilia Profile**

*Testing begins with*
- Prothrombin Time (PT), Plasma
- Activated Partial Thromboplastin Time (APTT), Plasma
- Dilute Russell's Viper Venom Time (DRVVT), Plasma
- Thrombin Time (Bovine), Plasma
- Fibrinogen, Plasma
- D-Dimer, Plasma
- Soluble Fibrin Monomer
- Antithrombin Activity, Plasma
- Protein C Activity, Plasma
- Protein S Antigen, Free, Plasma
- Prothrombin G20210A A Mutation, Blood
- Activated Protein C Resistance V (APCRV, Plasma)
- Special Coagulation Interpretation

**APTT:** >36 sec

**Thrombin Time (Bovine)**

**DRVVT:** ≥ 1.2 sec

**APCRV**
- <2.3
- or
- Prolonged baseline APTT

**Protein C Activity:**
- Males <65%
- Females <50 years: <50%
- ≥ 50 years: <65%

**Protein S Antigen, Free**
- Males <65%
- Females <50 years: <50%
- ≥ 50 years: <65%

**Antithrombin Activity:**
- <80%
- No evidence of an acquired deficiency

**PT Mix 1:1**
- PT >14.0 seconds

**APTT Mix 1:1**
- APTT >36 sec

**DRVVT Mix**
- DRVVT >1.2 sec

**Thrombin Time (Bovine)**
- 15-23 sec
- >23 sec

**Factor V Leiden (R506Q) Mutation**
- Protein C Antigen
- Protein S Antigen, Total
- Antithrombin Activity:
  - <60%
  - No evidence of an acquired deficiency

**Reptilase Time**
- Evidence of lupus-like anticoagulant
- Not diagnostic of lupus-like anticoagulant

**DRVVT confirmation**
- Evidence of lupus-like anticoagulant
- No evidence of heparin in sample

**Evidence of inhibition**
- Evidence of coagulation factor deficiency

**All initial testing within reference ranges for age and gender**
- No further testing is performed

*Additional assays may be performed if further clarification or confirmation is necessary. These may include:
- Coagulation Factor Assays
- Staclot Lupus Anticoagulant
- Protein S Activity

**Unfractionated/low-molecular weight heparin or direct thrombin inhibitor (eg, dabigatran, lepirudin or argatroban)**

An interpretive report is provided that includes all profile tests (always performed) and any reflex tests performed (if appropriate)
Does knowledge of presence of thrombophilia affect acute management of VTE?

3 months
Acute management

Long term
Secondary prophylaxis
<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin (prolonged thrombin time, normal reptilase time)</td>
<td>53/167</td>
<td>32%</td>
</tr>
<tr>
<td>Heparin + Warfarin</td>
<td>27/167</td>
<td>16%</td>
</tr>
<tr>
<td>Warfarin (decreased vit K dependent factors)</td>
<td>6/167</td>
<td>4%</td>
</tr>
<tr>
<td>Neither</td>
<td>81/167</td>
<td>49%</td>
</tr>
<tr>
<td><strong>Antithrombin activity levels:</strong> 62/167 below reference range (&gt;80%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Timing of Thrombophilia Testing: Does Knowledge of Thrombophilia Affect Acute Management of VTE? No except…

- Lupus anticoagulant
  - Prolonged baseline aPTT
  - Monitoring of UFH not feasible
    - Monitor anti-Xa (heparin) levels
    - Use LMWH

- Congenital protein C deficiency
  - Premature discontinuation of heparin
    - Warfarin skin necrosis

- Future state
  - Use of novel agents (rivaroxaban/apixaban)
Take Home Messages
When to Test

Testing is best accomplished after completion of appropriate duration of anticoagulation…

**IF** it will change clinical management
Does knowledge of presence of thrombophilia affect chronic management of VTE?

- 3 months
  - Acute management
- Long term
  - Secondary prophylaxis
## Recurrent VTE

<table>
<thead>
<tr>
<th>Factor</th>
<th>Incidence* (95% CI)</th>
<th>Relative risk (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td>3,500 (1,900-6,100)</td>
<td>1.3 (1.0-3.3)</td>
</tr>
<tr>
<td>PTG20210A</td>
<td></td>
<td>1.4 (0.9-2.0)</td>
</tr>
<tr>
<td>AT def</td>
<td>10,500 (3,800-23,000)</td>
<td>2.5</td>
</tr>
<tr>
<td>PC def</td>
<td>5,100 (2,500-9,400)</td>
<td>2.5</td>
</tr>
<tr>
<td>PS def</td>
<td>6,500 (2,800-11,800)</td>
<td>2.5</td>
</tr>
<tr>
<td>Antiphospholipid antibody</td>
<td></td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Per 100,000 person-years
### Case 4: MML Sample submitted for lupus anticoagulant testing

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>Ref range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (INR)</td>
<td>15.2 (1.4)</td>
<td>8.4-12s</td>
</tr>
<tr>
<td>PT mix</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>APTTT</td>
<td>42</td>
<td>21-33s</td>
</tr>
<tr>
<td>APTTT mix</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Thrombin time</td>
<td>16</td>
<td>16-25s</td>
</tr>
<tr>
<td>DRVVT Screen</td>
<td>2.2</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>DRVVT Mix</td>
<td>1.6</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>DRVVT Confirm</td>
<td>1.3</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>Staclot aPTT</td>
<td>68</td>
<td>&lt;55s</td>
</tr>
<tr>
<td>Staclot Delta</td>
<td>14</td>
<td>&lt;8s</td>
</tr>
</tbody>
</table>
Lupus anticoagulant (LAC) testing

- Data diagnostic of LAC but
- Clinical information obtained...
- ....patient was on direct Xa inhibitor (rivaroxaban [Xarelto]).....
- ..which results in a false positive LAC!
Conclusions

• Cost-effective strategies to Special Coagulation Testing
  • Be aware of artifactual causes
  • Avoid testing patients on anticoagulants
  • Determine if results will change patient management

• A profile approach is convenient for physicians and patients
  • Cost effectiveness not formally studied