Lupus Anticoagulant: Overview of Laboratory Diagnosis & Case Studies

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Disclosures

Relevant Financial Relationship
- NONE

Off-Label and/or Investigational Uses
- NONE
What is a Lupus Anticoagulant?

• Term coined in 1972 by Feinstein & Rapaport.

• Autoantibodies that inhibit phospholipid (PL)-dependent coagulation \textit{in vitro}.
  • Directed against a phospholipid-binding protein (β2-GPI).
  • Enhanced PL binding by β2-GPI \(\rightarrow\) prolongation of \textit{in vitro} clotting times.

Coagulation in vitro

Intrinsic
- XII
- XI
- IX
- VIII
- aPTT

Fibrinogen

Extrinsic
- VII

PT
- X
- V
- II

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Antiphospholipid Antibody Syndrome (APS)

Clinical Criteria

1. Objectively confirmed vascular thrombosis.
2. Pregnancy morbidity.
   - Late pregnancy loss.
   - Repeated early pregnancy loss.
   - Premature birth related to severe preeclampsia or placental insufficiency.

Laboratory Criteria (persistent ≥12 weeks)

1. Lupus anticoagulant (LA)
   - ISTH criteria
2. aCL &/or β2-GPI
   - IgG, IgM
   - Moderate-high titer.

1 clinical + 1 laboratory criterion fulfills diagnosis.

Epidemiology of LA

- **APS***
  - 53.6% LA
  - 12.1% LA without aCL

- **SLE**
  - 31% LA
  - 50% chance of thrombosis at 20 years.

- **Asymptomatic individuals**
  - Generally low incidence/prevalence.
  - Depends on how and who you test.

LA Diagnosis

• EQA results reveal diagnostic challenges.
• Lack of conformity…
  • No gold standard.
  • Sensitivity and selection of assays.
  • Variable adherence to guidelines.
  • Variable recommendations within guidelines.
  • New challenge of DOACs.

LA Diagnostic Guidelines

- ISTH
- BCSH
- CLSI/NCCLS
  - H60-A. **2014** (www.clsi.org)
Variations Between Guidelines

**Sequence of Testing:**
Screen $\rightarrow$ Confirm $\rightarrow$ Mix (CLSI 2014)

...or
Screen $\rightarrow$ Mix $\rightarrow$ Confirm (ISTH 2009, BCSH 2012)

**Others:**
- Mixing step.
- Ratios, reference intervals.
Criteria for Lupus Anticoagulant Diagnosis

- **Procure**
  - Patient considerations.
  - Specimen collection, transport & processing.

- **Screen**
  - Prolongation of at least one of two phospholipid-dependent clotting times.

- **Confirm**
  - Demonstrate phospholipid-dependence of clotting time prolongation.

- **Mix**
  - Show that prolongation is due to an inhibitor on mixing 1:1 with NPP.

- **Exclude**
  - Exclude/evaluate for other potential causes of the prolongation.

- **Interpret & Report**
  - Report interpretative comment with numerical results.
Procurement – Patient Considerations

Test the right patient…

• Is testing appropriate?
  • What is patient probability of LA?
  • See ISTH 2009 guidelines.

…At the right time

• Anticoagulation therapy.
  • Before initiation or after cessation.

• Acute phase.
  • Elevated FVIII, FDPs, microparticles.
Procurement – Sample Considerations

• Strict adherence to standards for proper specimen collection, handling, and processing.

• Prompt processing to platelet-poor plasma via double centrifugation.
  • PLT <10 x 10^9/L
  • NPP must also meet this target.
Case 1

- 35-year-old female. Referred in sample.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT</td>
<td>35s</td>
<td>&lt;36s</td>
</tr>
<tr>
<td>INR</td>
<td>1.0</td>
<td>0.8-1.1</td>
</tr>
<tr>
<td>DRVVT Screen</td>
<td>1.1</td>
<td>&lt;1.2</td>
</tr>
</tbody>
</table>

Lupus anticoagulant not detected?
The phone call....

- “I don’t buy the result.”
  - Hx unexplained ischemic stroke.
  - Previous LA+ 13 weeks ago.
  - aCL IgG >99th percentile.
  - PTT persistently elevated measured at local institution.

- “What gives?”

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Some digging reveals…

- Outside institution used platelet *rich* plasma procedure instead of platelet poor.
- Sample then frozen, transported, thawed & tested as usual.
- False negative due to excess phospholipid from platelets.
- Recollection with proper procedures proved persistence of LA.
Procurement – Sample Considerations

Preanalytical issues matter!
Criteria for Lupus Anticoagulant Diagnosis

- **Procure**
  - Patient considerations.
  - Specimen collection, transport & processing.

- **Screen**
  - Prolongation of at least one of two phospholipid-dependent clotting times.

- **Confirm**
  - Demonstrate phospholipid-dependence of clotting time prolongation.

- **Mix**
  - Show that prolongation is due to an inhibitor on mixing 1:1 with NPP.

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*CLSI. Lab Testing for LAC. Approved Guideline CLSI H60-A. 2014*
Test Considerations

Screening
- Maximum of two screening assays.
- Different principles & coagulation pathways.
- First line:
  - DRVVT
  - Lupus-sensitive aPTT (usually)

Confirming & Mixing
- Confirm performed with same assay as screen with abnormality.
- To mix…or not to mix?
Major LA Assays

Intrinsic
- aPTT (SCT)
- XII
- XI
- IX
- VIII
- DRVVT
- X
- V
- II
- Fibrinogen

Extrinsic
- VII
# Testing Stages Applied

<table>
<thead>
<tr>
<th></th>
<th>Screening step (Patient plasma)</th>
<th>Confirmatory step (Patient plasma + PL excess)</th>
<th>Mixing step (Mix 1:1 with NPP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>aPTT</strong></td>
<td>Lupus sensitive aPTT</td>
<td>Platelet neutralization procedure (PNP)</td>
<td>aPTT 1:1 mix</td>
</tr>
<tr>
<td><strong>DRVVT</strong></td>
<td>DRVVT Screen</td>
<td>DRVVT Confirm</td>
<td>DRVVT Mix</td>
</tr>
</tbody>
</table>
aPTT

Advantages

• Cheap, fast, familiar.
• aPTT mixing studies routine.
• Can select sensitive reagents according to LA detection goals.

Limitations

• Sensitivity variable.
• No built-in confirmatory step.
  • Platelet neutralization procedure.
  • SCT kits, others.
• Interference by anything that affects the aPTT.
dRVVT

Advantages

• Lengthy experience.
• Commercial kits.
• Heparin neutralizers.
• Reactions independent of factor VIII.

Limitations

• Reagent & procedural variations.
• Interferences:
  • Heparin excess.
  • DTIs, DXa inhibitors.
  • Warfarin, factor deficiencies.
• Other challenges.
What about other tests?

- **Intrinsic**
  - KCT
  - XII
  - XI
  - IX
  - VIII
  - Textarin
  - Taipan venom

- **Extrinsic**
  - dPT
  - VII
  - X
  - V
  - II

Fibrinogen
Criteria for Lupus Anticoagulant Diagnosis

**Procure**
- Patient considerations.
- Specimen collection, transport & processing.

**Screen**
- Prolongation of at least one of two phospholipid-dependent clotting times.

**Confirm**
- Demonstrate phospholipid-dependence of clotting time prolongation.

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CLSI. Lab Testing for LAC. Approved Guideline CLSI H60-A. 2014
Exclude & Evaluate: Can I trust this result?

Know your tests!

• What are its limitations?
• What patterns are seen with specific interferences?
• What other tests are available to help sort out the results?
  • TT, reptilase, factor assays, inhibitor screens, fibrinogen, D-Dimer, etc.
  • Additional LA assays.

Know your patient!

• Do the results make sense?
• Clinical history?
• Presentation?
• Anticoagulation?
Lupus Anticoagulant & Anticoagulants

- **Drug**
  - Heparins
  - Warfarin
  - DTIs
  - DXai

- **Effect**
  - Depends
  - False+/-
  - False+
  - False+

- **Testing?**
  - Caution/Avoid
  - Depends/?Modify
  - Avoid
  - Avoid
Case 2

- 55-year-old female with recent clot.

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</tr>
<tr>
<td>INR</td>
<td>1.5</td>
<td>0.8-1.1</td>
</tr>
<tr>
<td>TT</td>
<td>15</td>
<td>&lt;18s</td>
</tr>
<tr>
<td>DRVVT Screen</td>
<td>1.4</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>DRVVT Mix</td>
<td>1.4</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>DRVVT Confirm</td>
<td>1.3</td>
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Criteria for Lupus Anticoagulant Diagnosis

- **Procure**
  - Patient considerations.
  - Specimen collection, transport & processing.

- **Screen**
  - Prolongation of at least one of two phospholipid-dependent clotting times.

- **Confirm**
  - Demonstrate phospholipid-dependence of clotting time prolongation.

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Case 3

- 45-year-old male. Jotted down LA testing results from institutional patient portal.

<table>
<thead>
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<th>Test</th>
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</thead>
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<td>DVVC</td>
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<tr>
<td>DVVM</td>
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</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>SLCT</td>
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</tr>
<tr>
<td>SLPL</td>
<td>41.5</td>
</tr>
<tr>
<td>SLRAT</td>
<td>1.07</td>
</tr>
</tbody>
</table>

“You know what these mean, right??”
Interpretation & Reporting

• Interpretation of entire panel of raw data by experienced & qualified individual.

• Report:
  • Final LA status:
    • Present, not detected, indeterminate.
  • Additional tests &/or follow-up
  • Repeat testing after 12+ weeks if positive.
  • Reference intervals/cut-offs with numerical data.
Case 3

A copy of the full report is found in his chart...

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<tr>
<td>SLRAT</td>
<td>1.07</td>
<td>&lt;1.21</td>
</tr>
</tbody>
</table>

“Lupus anticoagulant NOT DETECTED by both the DRVVT and Silica Clotting Time.”
Criteria for Lupus Anticoagulant Diagnosis

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Summary

• LA diagnosis is important, but often challenging.
  • No gold standard test.
  • Guidelines
    • Good results start at the source…
      • Patient
      • Specimen
    • ...and continue with the lab
      • Adherence to guidelines & good approach
      • Appropriate interpretative reporting.
      • Overall quality.

• Awareness of issues essential for appropriate clinical application and follow-up of results.
Questions & Discussion