Establishing new positive ranges for anti-phospholipid antibody tests based on the local population

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Disclosures

• Dr. Sosnovske has no conflicts of interest to disclose
Learning Objective

- Understand how the initial verification of a lab instrument can affect the results of a test, and how it can impact a patient.
Brief outline

• Part 1: Instrument validation and verification.
• Part 2: Local population study, and establishing new cutoff values.
• Part 3: Look back to determine how new cutoff values would have affected 1 year of test results.
Antiphospholipid antibody syndrome (APAS) is an autoimmune disorder that is caused by a person making antibodies to phospholipids that are found on their own cell membranes. Results in an abnormally long aPTT. Clinical consequences of this can range from no symptoms to spontaneous venous thromboembolism (VTE), or spontaneous pregnancy loss.
Consequences of APAS

• Many people with symptomatic APAS need to have anticoagulation and/or antiplatelet therapy for life.
  – Increase risk of bleeding
Part 1: The Setup

- TriCore uses the BioPlex platform (Bio-Rad Laboratories, Hercules, CA) for testing antiphospholipid antibody syndrome (APAS), which is an FDA approved test.

- ELISA test for:
  - anti-cardiolipin (aCL) IgA, IgG, and IgM
  - anti-beta2-glycoprotein 1 (aB2GP1) IgA, IgG, and IgM

- At the time of instillation, Bio-Rad gave a positive cut off value of 20 U/mL.

- Bio-Rad recommended that cut off values be established based on a 99th percentile of the local population.
Where did the 20 U/mL cutoff value come from?

- FDA validation study (from the machine documentation)
- Set-up verification (available samples)
- It is unclear why the manufacturer recommended a cutoff value of 20 U/mL

<table>
<thead>
<tr>
<th>Antibody</th>
<th>BioRad validation (n=300)</th>
<th>Initial verification (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2-glycoprotein-1 IgA (U/mL)</td>
<td>12.1</td>
<td>42</td>
</tr>
<tr>
<td>B2-glycoprotein-1 IgG (U/mLI)</td>
<td>6</td>
<td>23.8</td>
</tr>
<tr>
<td>B2-glycoprotein-1 IgM (U/mL)</td>
<td>19.4</td>
<td>22.9</td>
</tr>
<tr>
<td>Anti-cardiolipin IgA (APL-U/mL)</td>
<td>14.5</td>
<td>45.5</td>
</tr>
<tr>
<td>Anti-cardiolipin IgG (GPL-U/mL)</td>
<td>8.5</td>
<td>27.6</td>
</tr>
<tr>
<td>Anti-cardiolipin IgM (MPL-U/mL)</td>
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</table>
It becomes much less clear.

- There are no international standards in place for the detection APAS antibodies.
- Different manufacturers use different monoclonal antibodies for detection.
- Leads to a high degree of variability between commercially available tests for APAS.
- Increases the importance of establishing a local population norm for the tests.
Part 2: The study

• Introduction:
• We wanted to establish a local population cutoff for the APAS tests.
• To do this we proposed collecting 120 samples from a normal local population.
Methods

• Whole blood samples in sodium citrate were collected from 120 healthy donors.
• Stored at -70 degrees C for up to 12 months.
• Concentrations of aCL and aB2GP1 were determined using the BioPlex 2200 System.

• The 99th percentile for each part of the assay was determined, and implemented as new cut off value (starting 1/15/2020)
Results

Table. 99\textsuperscript{th} percentile determinations from validation study, verification study and local population study

<table>
<thead>
<tr>
<th>Antibody</th>
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<th>Local population (n=120)</th>
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<td>9.6</td>
</tr>
<tr>
<td>Anti-cardiolipin IgM MPL-U/mL</td>
<td>27.9</td>
<td>19.6</td>
<td>25.9</td>
</tr>
</tbody>
</table>
Part 3: look back for impact

- Newly derived cutoffs were applied to 1,118 aCL and 1,140 aB2GP1 results retrieved from the TriCore data warehouse over a 12-month period (1/1/2018 to 12/31/2018).
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<th>Antibody</th>
<th>BioRad validation (n=300)</th>
<th>Initial verification (n=37)</th>
<th>Local population (n=120)</th>
<th>Number positive (% of total) with manufacturer’s proposed cutoff</th>
<th>Number positive (% of total) with NM 99th percentile cut-off</th>
</tr>
</thead>
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<tr>
<td>B2-glycoprotein-1 IgA (U/mL)</td>
<td>12.1</td>
<td>42</td>
<td>10.6</td>
<td>11/1,140 (1.0)</td>
<td>26/1,140 (2.3)</td>
</tr>
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<td>B2-glycoprotein-1 IgG (U/MI)</td>
<td>6</td>
<td>23.8</td>
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<td>35/1,140 (3.1)</td>
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<td>10</td>
<td>14/1,118 (1.3)</td>
<td>25/1,118 (2.2)</td>
</tr>
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<td>8/1,118 (0.7)</td>
</tr>
</tbody>
</table>
Results

• Based on our population’s 99\textsuperscript{th} percentile cut-off values, 27 previously negative individuals would now be labeled positive, whereas only 3 previously positive individuals would now be labeled as negative; the majority of patient results (97.4% of tests) did not change.

• Note: this is a battery of tests, and a result is dependent on the overall pattern of testing, as well as a second test at least twelve weeks apart.
Conclusion

• Given guideline recommendations that a local population be used to establish cut-off values, TriCore Reference Laboratories have changed the cut-off values to the 99\textsuperscript{th} percentile of the local population.

• The 99\textsuperscript{th} percentile results from this study were similar to those established by Bio-Rad Laboratories during their validation.

• It is unclear why a uniform value of greater than or equal to 20 units was applied as the FDA-cleared cut-off.
Further work

• It would be interesting to send the samples to a lab that assays the APAS with a different method, and compare the results.
• Request IRB approval for evaluate the clinical significance for the changes in reference ranges.
Thanks to:

- TriCore special coagulation group
- Dr. Grenache
- Dr. Marlar
- Dr. Rollins-Raval