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.

Introduction to APAS

- 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 99^{th} 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 cut off value of 20 U/mL

Table 1: APAS 99th percentiles from validation and verification studies

Antibody	BioRad validation (n=300)	Initial verification (n=37)
B2-glycoprotein-1 IgA (U/mL)	12.1	42
B2-glycoprotein-1 IgG (U/Ml)	6	23.8
B2-glycoprotein-1 IgM (U/mL)	19.4	22.9
Anti-cardiolipin IgA (APL-U/mL)	14.5	45.5
Anti-cardiolipin IgG (GPL-U/mL)	8.5	27.6
Anti-cardiolipin IgM (MPL-U/mL)	27.9	19.6

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. 99th percentile determinations from validation study, verification study and local population study

Antibody	BioRad validation (n=300)	Initial verification (n=37)	Local population (n=120)
B2-glycoprotein-1 IgA (U/mL)	12.1	42	10.6
B2-glycoprotein-1 IgG (U/Ml)	6	23.8	6.3
B2-glycoprotein-1 IgM (U/mL)	19.4	22.9	20.1
Anti-cardiolipin IgA (APL-U/mL)	14.5	45.5	10
Anti-cardiolipin IgG GPL-U/mL	8.5	27.6	9.6
Anti-cardiolipin IgM MPL-U/mL	27.9	19.6	25.9

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).

Results

Antibody	BioRad validation (n=300)	Initial verification (n=37)	Local population (n=120)	Number positive (% of total) with manufacturer's proposed cutoff	Number positive (% of total) with NM 99th percentile cut-off
B2-glycoprotein-1 IgA (U/mL)	12.1	42	10.6	11/1,140 (1.0)	26/1,140 (2.3)
B2-glycoprotein-1 IgG (U/Ml)	6	23.8	6.3	12/1,140 (1.1)	35/1,140 (3.1)
B2-glycoprotein-1 IgM (U/mL)	19.4	22.9	20.1	11/1,140 (1.0)	11/1,140 (1.0)
Anti-cardiolipin IgA (APL-U/mL)	14.5	45.5	10	14/1,118 (1.3)	25/1,118 (2.2)
Anti-cardiolipin IgG GPL-U/mL	8.5	27.6	9.6	12/1,118 (1.1)	23/1,118 (2.1)
Anti-cardiolipin IgM MPL-U/mL	27.9	19.6	25.9	15/1,118 (1.3)	8/1,118 (0.7)

Results

- Based on our population's 99th 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 99th percentile of the local population.
- The 99th 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.

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