Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Immunoassays for the Evaluation of Antiphospholipid Syndrome

PRESENTER: Anne E. Tebo, PhD, D(ABMLI)

Slide 1:
Hello, my name is Anne Tebo. I am an Associate Professor of Pathology at University of Utah, and a Medical Director for ARUP Laboratories, in Salt Lake City, Utah. Welcome to this Pearl of Laboratory Medicine on "Immunoassays for the Evaluation of Antiphospholipid Syndrome."

Slide 2:
Antiphospholipid antibody syndrome (commonly referred to as APS) is a systemic autoimmune disease characterized by the presence of antiphospholipid (aPL) antibodies in association with thrombosis and/or specific pregnancy-related morbidities. The exact prevalence of APS is unknown; however, aPL antibodies are thought to occur in 2-5% of the general population. Individuals with this disorder may otherwise be healthy, or they also may suffer from an underlying disease, most frequently systemic lupus erythematosus (commonly called SLE).

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For a definite diagnosis of APS, the 2006 revised Sapporo criteria emphasize the presence of thrombosis and/or specific obstetric complications in the presence of at least one main laboratory detected aPL antibodies (i.e. lupus anticoagulant [LAC], IgG and/or IgM anticardiolipin [aCL], IgG and/or IgM anti-beta$_2$ glycoprotein I [aβ_2GPI]). Thrombosis and obstetric complications are relatively common clinical events in the general population and are not unique to APS; therefore, the presence of aPL antibody is an absolute requirement for the appropriate diagnosis of this disease. Although the APS diagnostic criteria were not established for routine clinical use, in the absence of formal guidelines, these have been widely adopted to assess risk for disease and the need for treatment or prophylaxis in patients. Therefore, in clinical practice, if suspicion for disease is high but laboratory studies are inconclusive or negative, deviation from the APS diagnostic criteria may be justified.

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APS is a prothrombotic condition that can affect both the venous and arterial circulations. The deep veins of the lower limbs and the cerebral arterial circulation are the most common sites of venous and arterial thrombosis, respectively. Nevertheless, tissue or organ vascular bed can be affected as is the case in catastrophic APS. It is important to confirm vascular thrombosis by imaging and histopathology.

In situations in which histopathological confirmation is sought, thrombosis should be present without evidence of inflammation in the vessel wall.

The other major clinical feature of APS is obstetrical. This includes three main categories. The first being unexplained death of one or more morphologically normal fetuses at or beyond the 10^{th} week of gestation. The second category involves premature birth of one or more morphologically normal neonates before the 34^{th} week of gestation due to either eclampsia or severe preeclampsia. The last and most challenging category is pregnancy-related morbidity associated with three or more unexplained consecutive spontaneous abortions before the 10^{th} week of gestation.

Slide 5:

The 2006 revised Sapporo laboratory criteria for APS as previously mentioned includes the lupus anticoagulant tests as well as immunoassays for the detection of IgG and IgM antibodies to cardiolipin (CL) and β_2GPI. These are generally referred to as 'criteria' aPL antibody tests. Recommendations for LAC testing include the use of at least two tests based on different principles and performed according to the International Society on Thrombosis and Hemostasis (ISTH) guidelines. The two main tests for screening LAC includes dilute Russell's viper venom time (dRVVT) and activated partial thromboplastin time (aPPT). For aCL IgG and IgM determinations, antibody cut-off values greater than 40 GPL or MPL units or more than the 99^{th} percentile for the testing laboratory's population were recommended to be positive. The GPL (IgG phospholipid) or MPL (IgM phospholipid) units are traceable to the Harris standards for calibrating IgG or IgM aCL assays, respectively. One GPL/MPL unit is defined as the cardiolipin-binding activity of 1 μg/ml of affinity-purified IgG or IgM aCL antibody. In the case of a β_2GPI IgG and IgM antibodies, cut-off values greater than 99^{th} percentile for the laboratory's population was recommended to determine positive results.

I will be addressing the rationale for these suggestions during the course of this presentation with a focus on the immunoassays for the detection of IgG and IgM antibodies to CL and β_2GPI.

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For the laboratory evaluation of aPL antibodies, important characteristics considered to improve the diagnostic outcome in APS have been defined. Notable amongst these is the recommendation to test for IgG and IgM antibody of CL and β_2GPI specificities, documentation of aPL antibody persistence at least 12 weeks apart from initial testing, and how to establish cut-off level for positivity as well as categorizing aPL antibodies based on the type and number positive. Antibodies to prothrombin, phosphatidylserine, phosphatidylserine/prothrombin complex, as well as aCL and aβ_2GPI IgA isotype were excluded.

A major challenge for these guidelines was the specific reference to standardized enzyme-linked immunosorbent-assay (ELISA) methods to measure aCL and aβ_2GPI IgG and IgM antibodies. Immunoassays other than ELISA are currently approved by regulatory authorities and in use in some clinical laboratories in the United States (US) and elsewhere. In addition, the use of cut-off values greater than 40 GPL or MPL units to define positivity may not be commutable to all aCL assays, as the threshold used to distinguish moderate-to-high positive from low positive results has no acceptable standard.

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aPL antibodies are heterogeneous and are generally characterized based on either functional or immunoassays. The functional or coagulation-based assays designed to detect the so-called lupus anticoagulants (which have stronger associations with thrombosis) require specialized instrumentations and expertise. LAC testing is usually performed in the hemostasis or coagulation laboratory. In the immunoassays, targets are immobilized on a solid surface that may be amenable for manual, semi-automated or automated platforms. The most common type of immunoassay is the ELISA. However, newer automated technologies based on similar or different analytical principles to ELISA are currently being used in clinical laboratories. Immunoassays are usually performed in immunology or chemistry laboratory. While the clot-based assays and some of the immunoassays require platelet-poor plasma, most assays for the detection of aCL and aβ_2GPI antibodies utilize serum.

Immunoassays to detect aCL and aβ_2GPI antibodies provide clinicians with additional information that is not obtainable with the LAC test. This information includes determination of the titers of these antibodies as well as their isotype. More important, the immunoassays are not influenced if the patient is on anticoagulation (oral or subcutaneous) or anti-platelet therapy, and may be more sensitive than the functional LAC assay. In the next few slides, I will be addressing issues relating to the performance of immunoassays for the detection of aCL and aβ_2GPI IgG and IgM antibodies.

Slide 8:

It is widely recognized that immunoassays for the detection of aCL and aβ_2GPI IgG and IgM antibodies lack harmonization and standardization. A number of APS experts have published recommendations on best practices as well as issues relating to standardization of immunoassays used in the detection of aPL antibodies. Diversity in analytical or manufacturing processes, absence of standards, different methods of calibrating, performing and reporting results for aCL and aβ_2GPI IgG and IgM antibodies have been identified as factors responsible for the heterogeneity in tests performance. Suggestions on how kit manufacturers and clinical laboratories can reduce assay variability have also been addressed in a number of studies with emphasis on the need for international calibration materials, particularly for aβ_2GPI IgG and IgM assays.

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For the detection of aPL antibodies in immunoassays, two main pre-analytical issues need to be considered in the clinical laboratory. These include the specimen type and aPL antibody tests offered. While serum is usually the preferred assay for most aPL immunoassays, platelet-poor plasma is the required specimen for LAC testing. Specimens should be processed, handled, and stored following manufacturer's recommendations for the specific test as well as following good laboratory practice. The need to streamline specimen collection for APS evaluation has led to validation of plasma specimen in the more recently developed and validated immunoassays. Prior to adopting plasma in aPL antibody immunoassays, it is important for laboratories to verify the commutability between serum and plasma specimens as the use of citrate as anticoagulant may lead to an approximate 10% dilution compared to serum. With respect to aPL test panels and recommendations for testing, it is likely that not all laboratories offer the full test menu required for evaluation of APS; this information should be communicated to the attending clinician with specific recommended follow-up action.

Slide 10:
Intra- and inter-laboratory variability in aPL antibody testing is a known diagnostic challenge even amongst laboratories using kits from the same manufacturer. It is important for laboratories to recognize that analytical variability occurs at all steps in assay development, validation, and testing. Laboratories are strongly recommended to verify the reference intervals as well as the diagnostic and analytical performance of aPL antibody tests.

Most commercial kits include positive and negative controls with predefined acceptable ranges. The use of an external (non-kit) quality control reagent, close to the assay cut-off, to assess and monitor test performance is critical. This reagent can be obtained commercially or generated by the laboratory. In addition to the afore-mentioned analytical variables, appropriate quantification of aCL and aβ_2GPI antibody concentrations in immunoassays requires multipoint calibration as well as the use of statistically correct curve-fitting and calculation methods. Single point calibration is generally not suitable for aPL antibody testing.

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Effective reporting and communication of aPL antibody evaluation remains a diagnostic challenge. It is recommended for the aPL antibody report to include the antibodies for aCL and aβ_2GPI IgG and IgM antibodies in numerical values and indicate if the results are negative, low-positive, medium/moderate positive, or high-positive based on percentile values of the analytical reportable range of the assay.

With respect to the interpretative comments, further diagnostic actions such as repeat testing to document persistence or recommending LAC or aβ_2GPI antibodies if only aCL was ordered should be included in the reports. Transient positivity of aPL antibodies is associated with a number of infectious diseases. Therefore, documentation of aPL antibody persistence is important in establishing a diagnosis of APS. In addition, aPL antibodies are thought to occur in 2-5% of the general population. This prevalence may vary in certain demographics, geographic regions, as well as kits used in evaluation.

Laboratories may also want to consider providing estimates of risk for specific APS clinical manifestations based on the specific positive aPL antibody test, the relative antibody concentration, isotype, and/or the number aPL antibody tests positive.

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The clinical manifestations associated with APS are relatively common in the general population; thus, the presence of aPL antibodies is an absolute requirement for the diagnosis of definite APS. Although these antibodies may overlap, their heterogeneity and lack of standardization requires the use of all three tests for optimal diagnostic outcome. It is likely that as more becomes known about the disease, specific tests will become available. Of the 3 recommended aPL antibody tests for evaluating APS, aCL is the most sensitive while the LAC has been reported in several studies to have the best predictive value for both thrombosis and adverse pregnancy outcome. In obstetric APS, there are reports of isolated aCL and/or aβ_2GPI positivity. Nevertheless, these results should be interpreted with a degree of caution.

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While the laboratory criteria give equal diagnostic relevance for IgG and IgM antibodies to CL and β_2GPI specificities, it is recognized that IgG and not the IgM isotype of both aPLs confer higher risk for thrombosis. Furthermore, increasing concentrations of aCL IgG antibodies (medium or higher titers) correlate with high odds ratio for thrombosis. In obstetric APS, however, low-positive aCL antibodies (based on the 95^{th}-99^{th} percentile) have been reported to be of some clinical relevance, as mentioned earlier. In the absence of acceptable calibrators for aβ_2GPI assays, the role of 'low-positive' antibodies, particularly of IgM isotype, remain unclear.

Slide 14:
The recognition that certain aPL antibody types, the number of positive aPL antibodies, or combination of aPL antibodies may be useful in stratifying risk for disease has recently received a lot of interest. In this regard, several approaches to profile aPL antibodies and assess risk for APS have been reported. Regardless of the approach to estimate risk, APS patients positive for all three markers, carry a much higher risk of thrombosis and/or pregnancy loss than individuals with positivity for only one of these tests. Of all three APS tests, LAC has the strongest prediction for disease.

Slide 15:
In closing, the presence of at least one aPL antibody test in the context of thrombosis or pregnancy-related morbidity is required for the diagnosis of definite APS. Of these, LAC has the strongest prediction for disease. The presence of medium or high positive IgG and not IgM aCL antibodies constitute a higher risk for APS. IgG isotype antibodies have higher risk for APS compared to IgM.

While laboratory investigation of aPL antibodies is method-dependent, integration of testing and reporting is important. Interpretative comments should reflect the tests performed, reference intervals, units, clinical significance of positive results based on the characteristics of the specific assay(s), as well as recommended follow-up by the clinician.

Slide 16: References

Slide 17: Disclosures

Slide 18: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on "Immunoassays for the Evaluation of Antiphospholipid Syndrome." My name is Anne Tebo.