Diagnostic Trends in Laboratory Evaluation of Antiphospholipid Syndrome

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Antiphospholipid Syndrome (APS)

Autoimmune disorder characterized by thrombosis, pregnancy-related morbidity or both and associated with persistent presence of antiphospholipid (aPL) antibodies
1999 Sapporo Criteria Antiphospholipid Syndrome

• Clinical
  – Thrombosis
    • Venous or arterial
  – Obstetric
    • Pre-embryonic, embryonic, fetal, or neonatal loss

• Laboratory
  – Lupus anticoagulant (LAC)
  – IgG or IgM cardiolipin antibodies (aCL)
  – Positive on 2 or more occasions at least 6 weeks apart
Classification Criteria for APS

Thrombosis

and/or

Persistently positive for aPL antibodies:
- Lupus anticoagulant (LAC)
- Anticardiolipin (aCL)
- Anti-beta_2 glycoprotein I (aβ2GPI)

Pregnancy-related morbidity

APS Diagnosis

Revised Sapporo Laboratory Criteria for APS

• LAC present detected according to ISTH SSC guidelines*
  • aCL antibodies (IgG or IgM)
    • Moderate or high titers (>40 GPL or MPL units or >99th percentile for the testing laboratory)
  • IgG or IgM β2GPI antibodies (aβ2GPI)
    • >99th percentile for the testing laboratory according to recommended procedures
• Positive on 2 or more occasions at least 12 weeks apart

Key Features of the 2006 Revised Laboratory Criteria

- IgG and IgM isotypes have equal diagnostic value, acceptable positive antibody cut-off defined
- Standardized ELISA methods to measure aCL and a$\beta_2$GPI IgG and IgM
- IgA isotype of aCL and a$\beta_2$GPI specificities, and other antibodies excluded
- Persistence of aPL antibodies must be confirmed
- Four different categories of aPL positivity defined
Clinical Indications for APS Testing

• Vascular thrombosis
  • Arterial and venous vessel thrombosis
  • Confirmed by imaging or histopathology
  • Histopathology: thrombosis without significant evidence of inflammation

• Pregnancy-related morbidity
  • ≥1 unexplained fetal deaths at or beyond 10 weeks of gestation
  • ≥1 premature birth before 34 weeks
    • Eclampsia or severe pre-eclampsia or feature of placental insufficiency
  • ≥3 unexplained consecutive spontaneous abortions before 10 weeks

Non-criteria Clinical Findings Associated with aPL Abs

- Unexplained thrombocytopenia
- Livedo reticularis
- Nephropathy
- Neurological manifestations
- Cardiac manifestations
  - Heart valve disease
  - Coronary artery disease in the young in the absence of risk factors
Epidemiology of APS

• Actual frequency unknown
  – Young to middle-aged adults
  – Apparent female predominance

• Alone or with SLE or other autoimmune disorders
  – 50% of APS patients have no other underlying condition
  – 30% of SLE patients will develop APS

Variable % of healthy individuals are aPL antibody-positive
aPL Antibodies and Autoimmune Diseases

- **SLE** 25-50%
- **Sjögrens syndrome** ~42%
- **Autoimmune thrombocytopenia** ~30%
- **Rheumatoid arthritis** ~33%
- **Vasculitis** ~20%
- **Systemic sclerosis** ~25%
- **Autoimmune hemolytic anemia** ~30%
- **Psoriatic arthritis** ~28%

Maybe induced by various infections and use of certain drugs with and without APS-specific manifestations
Testing for aPL Antibodies in the Laboratory

- Functional Assays
  - LAC
- Immunoassays
  - aCL and $\beta_2$GPI Abs

Specimen(s) ➔ Plasma*

Hemostasis ➔ Laboratories

*Platelet-poor citrate anti-coagulated plasma
Why Test for LAC, aCL and aβ₂GPI Antibodies?

- Optimal diagnostic outcome
  - aPL antibodies are heterogeneous
  - ~20% of APS patients will test negative in at least one test

- Of the three markers, aCL is the most sensitive

- LAC has the strongest predictor for thrombosis and/or obstetric APS

- Isolated IgG aCL and/or aβ₂GPI positivity associated with obstetric APS
Significance of aPL Antibodies in APS

• Risk for APS is dependent on aPL antibody characteristics
  • IgG aPL not IgM antibodies confer higher risk
  • ‘Medium-to-high’ IgG aCL antibodies associated with increased risk for thrombosis
  • Antibody ‘levels’ and types are not commutable
  • Isolated and low-positive (95th-99th percentile) IgG aPL may have clinical significance in obstetric APS
  • Role of isolated IgM aPL antibodies in APS remains unclear
Interpreting aPL Antibody Tests: Role of Autoantibody Levels and Multiple Specificity

• Level of aPL antibody
  • 10 unit increase in IgM or IgG aCL associated with a 5-7% increase in risk of thromboembolism

• Number of aPL antibodies present
  • Triple-positive aPL tests appear to be at high risk for a first thrombotic event and recurrence
  • aCL alone vs. aCL+LAC vs. aCL+LAC+aβ2GPI associated with 50-70% increase in the odds for thrombotic events
Challenges in Laboratory Evaluation for APS

- Clinical spectrum poorly defined
- Variability in analytes
- Variability in antibody response
- Variation in methods
- Variation in the detection systems
- Poor standardization and harmonization
  - Absence of international standards

Each contributes to significant variations in testing
Performance Characteristics of Commercial Immunoassays for the Detection of IgG and IgM Antibodies to β2 Glycoprotein I and an Initial Assessment of Newly Developed Reference Materials for Assay Calibration

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Key Words: Antiphospholipid syndrome; Immunoassays; β2 glycoprotein I; Antibodies; Standardization; Harmonization

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Objectives: To investigate the performance characteristics and impact of newly developed reference calibrators on the commutability between anti-β2 glycoprotein I (anti-β2 GPI) immunoassays in antiphospholipid syndrome (APS) and/or systemic lupus erythematosus (SLE).

- Sensitivities
  - IgG: 15.8-% 27.2%
  - IgM: 12.3% -15.8%

- Specificities
  - IgG: 79.4%- 86.5%
  - IgM: 80.6% -84.5%

- Moderate-to-almost perfect inter-assay reliability
  - Cohen kappa, 0.69-0.98

- Spearman correlation coefficients improved for IgG with reference material

- Correlations with APS clinical manifestations were kit-dependent
The Need for More Specific and Robust Markers

- Analytical challenges associated with LAC testing
- Presence of aCL does not always predict APS
  - Unreliable in the context of certain infectious diseases
- $\text{A}\beta_2\text{GPI}$ specific but lacks diagnostic sensitivity
  - Significant overlap with aCL and/or LAC antibodies
- Seronegative APS
  - Fulfill clinical criteria for APS
    - Negative for current (‘criteria’) diagnostic markers
- Need for markers with pathological relevance
  - Guide treatment or management
Classic and Seronegative APS Patients Show Similar Clinical Profiles

<table>
<thead>
<tr>
<th>Clinical Manifestations</th>
<th>Seropositive APS (n=87)</th>
<th>Seronegative APS (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep vein thrombosis</td>
<td>31.4%</td>
<td>31.0%</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>23.8%</td>
<td>28.7%</td>
</tr>
<tr>
<td>Stroke</td>
<td>14.9%</td>
<td>17.2%</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>11.9%</td>
<td>10.3%</td>
</tr>
<tr>
<td>Early spontaneous abortion</td>
<td>67.1%</td>
<td>52.1%</td>
</tr>
<tr>
<td>Stillbirths</td>
<td>62.5%</td>
<td>59.4%</td>
</tr>
<tr>
<td>Prematurity</td>
<td>28.1%</td>
<td>21.7%</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>28.1%</td>
<td>23.1%</td>
</tr>
</tbody>
</table>

‘Clinical management in patients with APS should not be based only on the presence of conventional aPL’

aPL Antibodies in APS

• Heterogeneous antibodies that recognize various
  – Phospholipids (PL)
  – PL-binding plasma proteins
  – PL-protein complexes

• Proposed plasma proteins include
  – Beta₂ glycoprotein I
  – Prothrombin
  – Protein C
  – Protein S
  – Annexin V

• Diagnostic
• Pathologic
• Treatment
APhL IgG and IgM Assays

- Detects antibodies to negatively charged phospholipids in the presence of $\beta_2$GPI
- Comparable sensitivity but higher specificity than aCL in the diagnosis of APS
  - Possible relevance in the context of infectious diseases such as syphilis
    - First line testing or alternative to aCL IgG and IgM
    - Confirmation of aCL IgM and IgG when suspicion for APS is low
APhL as an Alternative to Anti-cardiolipin for the Diagnosis of APS

Prevalence of Specific aPL antibodies

A: Manufacturers’ suggested cut-off values
B: Adjusted cut-off values

Clinical Specificity of aCL and APhL IgG/IgM Tests

Antibodies to Prothrombin

- PL-binding protein reported to be a cofactor for LAC (Loliger 1959)
- Antiprothrombin antibodies (aPT) responsible for LAC activities (Fleck et al 1988)
- Anti-β₂GPI and aPT recognized as major autoantibodies for LAC
  - aPT for prothrombin-dependent LAC
  - Anti-β₂GPI for β₂GPI-dependent LAC
- aPT antibodies are heterogeneous, clinical relevance dependent on assay principle (Galli and Barbui 1999)
- aPS/PT not aPT antibodies show correlation with disease (Atsumi et al 2000)
Table 1. The Mode of Presentation of Prothrombin Influences Its Recognition by Antiprothrombin Antibodies in ELISA Systems

<table>
<thead>
<tr>
<th>Human Prothrombin Bound to</th>
<th>Prevalence (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain polystyrene plates</td>
<td>0</td>
<td>18, 32, 33</td>
</tr>
<tr>
<td>γ-Irradiated plates</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>High-activated PVC plates</td>
<td>50-58</td>
<td>18, 33</td>
</tr>
<tr>
<td>Phosphatidylserine-coated plates</td>
<td>90</td>
<td>33</td>
</tr>
</tbody>
</table>

# PS/PT Antibodies as Diagnostic Markers for APS

<table>
<thead>
<tr>
<th>References</th>
<th>Key Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnalato et al. 2013</td>
<td>Strong correlation with LAC; aPS/PT IgG associated with venous thrombosis and not pregnancy-related APS manifestations</td>
</tr>
<tr>
<td>Vlagea et al. 2013</td>
<td>aPS/PT IgG associated with venous thrombosis and obstetric complication</td>
</tr>
<tr>
<td>Sanfelippo et al. 2013</td>
<td>aPS/PT can contribute to identification of APS</td>
</tr>
<tr>
<td>Fabris et al. 2014</td>
<td>Additional diagnostic value for APS; relevant for difficult to interpret LAC results</td>
</tr>
<tr>
<td>Nojima et al. 2014</td>
<td>aPS/PT associated with arterial thrombosis; aCL and aPS/PT independently associated with RPL; and aPS/PT had the highest association with the presence of LAC activity</td>
</tr>
<tr>
<td>Heikal et al. 2015</td>
<td>Good correlation with LAC; relevant for difficult to interpret LAC results</td>
</tr>
<tr>
<td>Žigon et al. 2015</td>
<td>aPS/PT associated with recurrent early or late abortions and premature delivery irrespective of other aPL</td>
</tr>
<tr>
<td>Kitaori et al. 2015</td>
<td>LA-aPTT StaClot and aPS/PT IgG might be suitable for use in routine practice for patients with RPL</td>
</tr>
<tr>
<td>Hoxha et al. 2015</td>
<td>IgG and/or IgM aPS/PT independent risk factors for LAC; present in 9.4% of the APS-negative patients compared to 2% of healthy controls (p=0.043); significantly more frequent in thrombosis than pregnancy morbidity subset (p=0.01)</td>
</tr>
</tbody>
</table>
State-of-the-art for aPS/PT IgG and IgM Assays

• Significance
  • Can contribute to assess the risk of thrombosis
  • Can contribute to a better identification of patients
  • Multivariate analysis: independent risk factor for disease
  • Results appear to be consistent between groups

• Challenges
  • Correlation with LAC is variable and isotype-dependent
  • Performance characteristics inadequately defined
  • No consensus on the relevance of IgM isotype

Anti-Domain I (DI) of $\beta_2$GPI (aDI)

- Cryptic epitope, binding with oxidation
  - Circular to fish-hook configuration

- aDI associated with symptomatic APS
  - vs. symptomatic aPL carriers or infection-related aPL

- Some APS patients develop antibodies reacting against $\beta_2$GPI epitopes other than DI
  - Other anti-$\beta_2$GPI antibody subsets may be clinically relevant
Role of aD1 Antibodies in Diagnosis and Stratification of APS

• Classifies patients at-risk for thrombosis (triple aPL)
• APS diagnosis and stratification dependent on solid phase assays used for aCL and aβ2GPI detection

aDI: State-of-the-art

- aDI antibodies are positively correlated with
  - ‘Medium to high’ titers of aPL
  - Presence of LAC
  - Thrombotic and pregnancy manifestations
    - Enabling identification of patients at higher risk of clinical events
- Preliminary results suggest that assays to detect aDI antibodies are comparable
- Prospective studies are needed to support their use in clinical setting

Meroni PL. Lupus. 2016;25: 905-10
# APL Profiles as Risk Factors in APS

<table>
<thead>
<tr>
<th>Types of profile</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td># of positive aPL antibody types*</td>
<td>Triple positivity (aCL, LAC, and aβ&lt;sub&gt;2&lt;/sub&gt;GPI): greater risk of thrombotic events than single or double positivity</td>
</tr>
<tr>
<td>Types of aPL antibody**</td>
<td>&gt;1 aPL positive (I), LAC alone (IIa), aCL alone (IIb) and aβ&lt;sub&gt;2&lt;/sub&gt;GPI alone (IIc)</td>
</tr>
<tr>
<td>Antiphospholipid score (aPL-S)***</td>
<td>Quantitative marker based on relative risks for APS events for each aPL test</td>
</tr>
<tr>
<td>Global APS score (GAPSS)****</td>
<td>Combination of independent risk for APS, aPL profile, conventional cardiovascular risk factors and autoimmune disease serology</td>
</tr>
</tbody>
</table>

***Otomo et al. Arthritis & Rheumatism. 2012;64:504-12  
****Sciascia et al. Rheumatology. 2013;52:1397-403
Conclusion

• Laboratory testing method-dependent
  – Integrate testing and reporting of all aPL assays
  – Interpretative comments should reflect the analytes in panel, reference ranges, units, clinical significance and recommended follow-up
  – Evidence of persistence

• Low positive results should be interpreted with caution
  – Analytical impression
  – Biologic variability

• No consensus for routine use of other aPL antibodies
Thank You

Department of Pathology