Diagnostic Trends in Laboratory Evaluation of Antiphospholipid Syndrome

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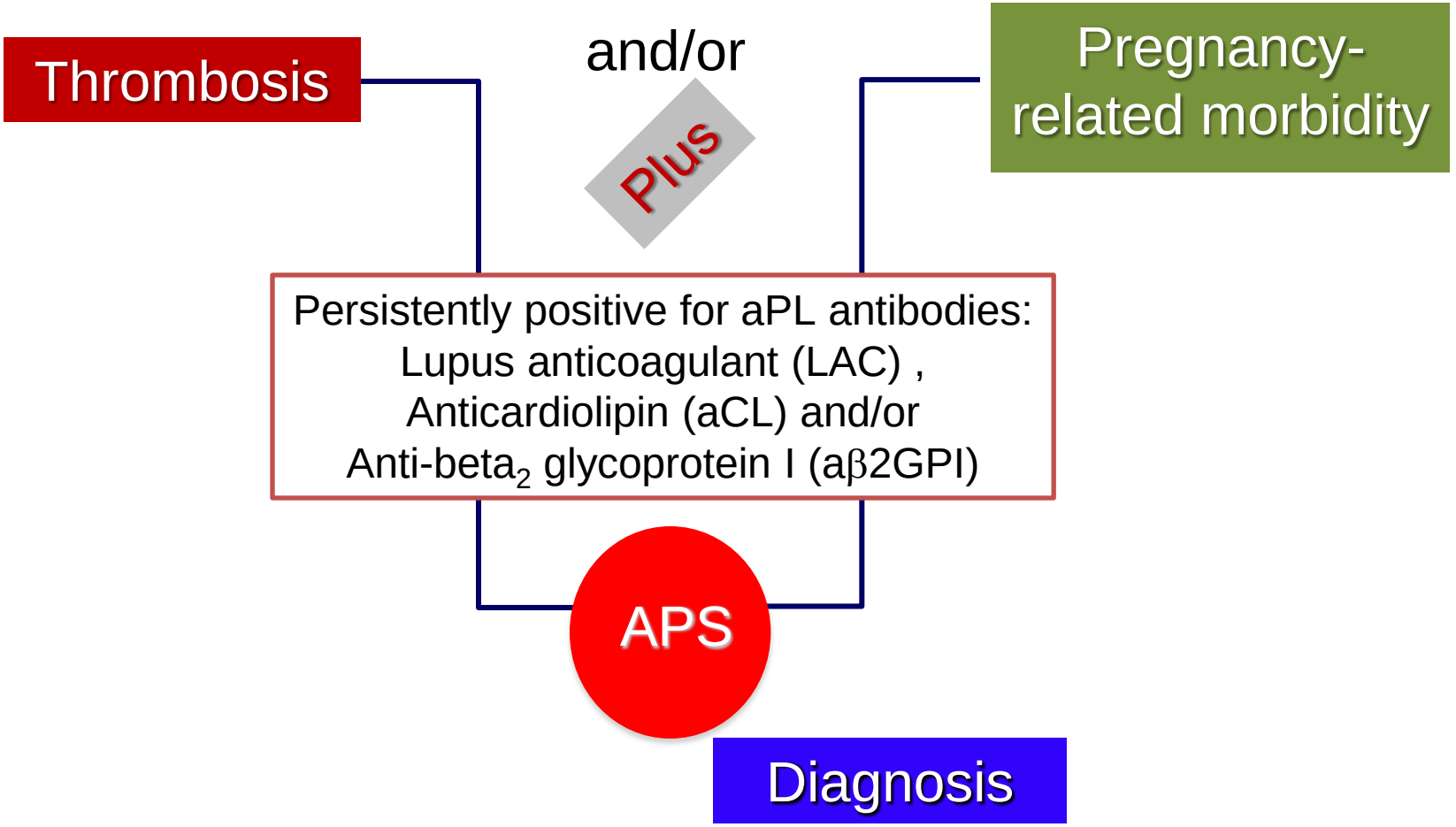
Antiphospholipid Syndrome (APS)

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



Miyakis et al. J Thromb Haemost. 2006;4:295-306

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 β_2GPI antibodies ($a\beta_2$GPI)
  - >99th percentile for the testing laboratory according to recommended procedures
- Positive on 2 or more occasions at least 12 weeks apart

Miyakis et al. J Thromb Haemost. 2006;4:295-306
***Pengo et al. J Thromb Haemost. 2009;7:1737-40**

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 aβ_2GPI IgG and IgM
- IgA isotype of aCL and aβ_2GPI 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

Miyakis et al. J Thromb Haemost. 2006;4:295-306

Non-criteria Clinical Findings Associated with aPL Abs

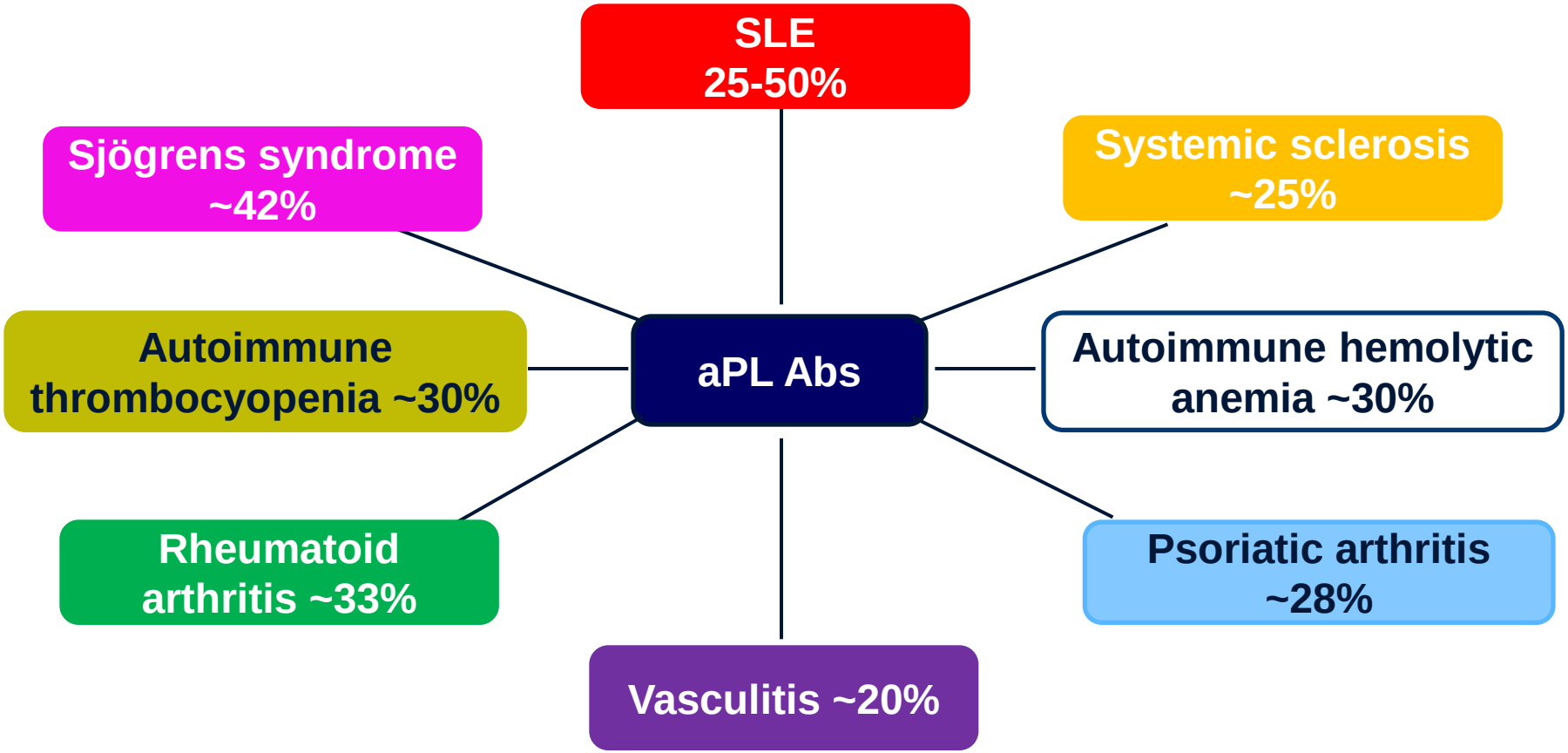
- Unexplained thrombocytopenia
- Livedo reticularis
- Nephropathy
- Neurological manifestations
- Cardiac manifestations
  - Heart valve disease
  - Coronary artery disease in the young in the absence of risk factors

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

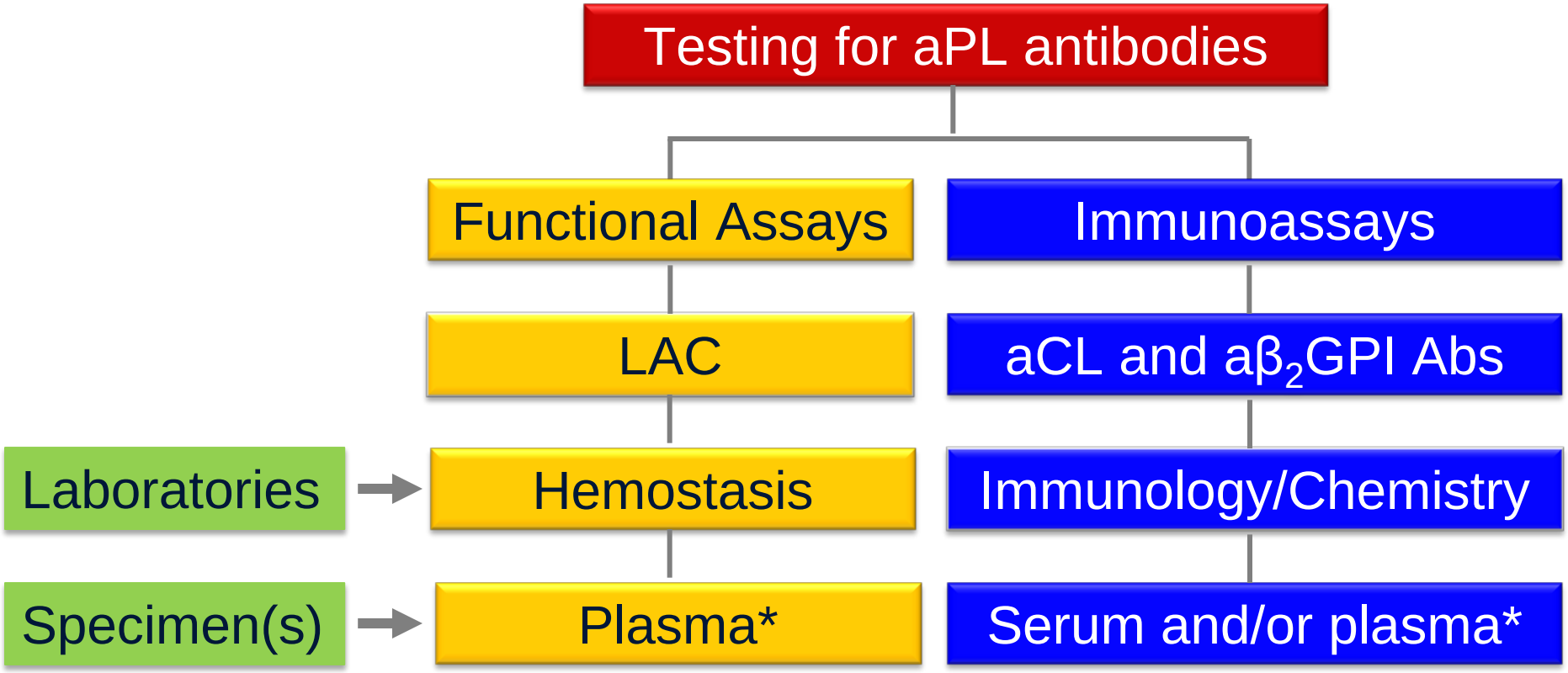
Variable % of healthy individuals are aPL antibody-positive

aPL Antibodies and Autoimmune Diseases



Maybe induced by various infections and use of certain drugs with and without APS-specific manifestations

Testing for aPL Antibodies in the Laboratory



*Platelet-poor citrate anti-coagulated plasma

Why Test for LAC, aCL and aβ_2GPI 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 aβ_2GPI 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+aβ_2GPI 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 β_2 Glycoprotein I and an Initial Assessment of Newly Developed Reference Materials for Assay Calibration

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Key Words: Antiphospholipid syndrome; Immunoassays; β_2 glycoprotein I; Antibodies; Standardization; Harmonization

Am J Clin Pathol June 2016;145:796-805

Objectives: To investigate the performance characteristics and impact of newly developed reference calibrators on the commutability between anti–β_2 glycoprotein I (anti–β_2 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
- $A\beta_2$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 Profile

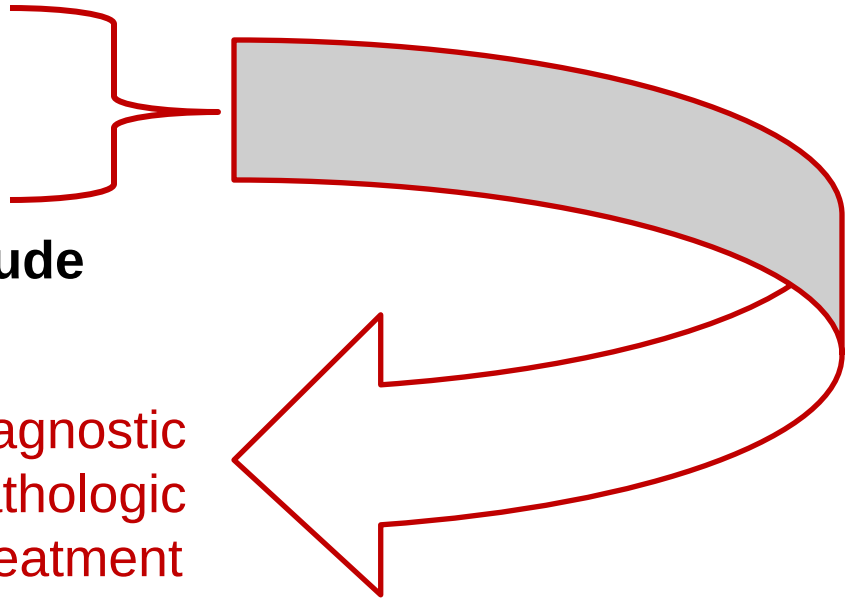
Clinical Manifestations	Seropositive APS (n=87)	Seronegative APS (n=67)
Deep vein thrombosis	31.4%	31.0%
Pulmonary embolism	23.8%	28.7%
Stroke	14.9%	17.2%
Transient ischemic attack	11.9%	10.3%
Early spontaneous abortion	67.1%	52.1%
Stillbirths	62.5%	59.4%
Prematurity	28.1	21.7%
Pre-eclampsia	28.1%	23.1%

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

Rodriguez-Garcia et al. Ann Rheum Dis. 2012;71:242-4.

aPL Antibodies in APS

- **Heterogeneous antibodies that recognize various**
  - Phospholipids (PL)
  - PL-binding plasma proteins
  - PL-protein complexes
- **Proposed plasma proteins include**
  - $\underline{\text{Beta}_2 \text{ glycoprotein I}}$
  - Prothrombin
  - Protein C
  - Protein S
  - Annexin V

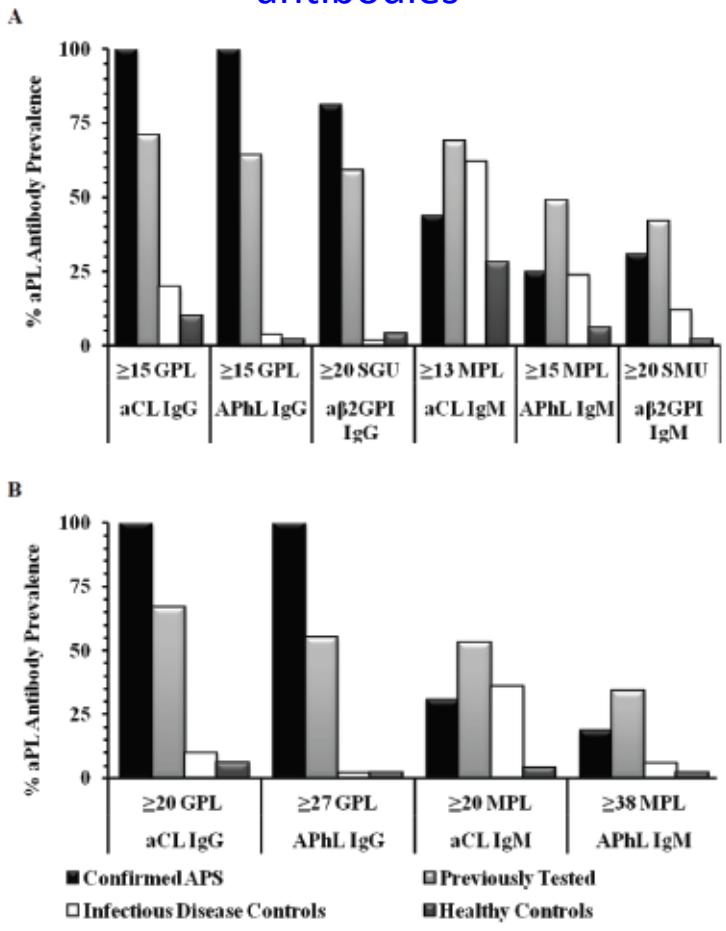


APhL IgG and IgM Assays

- Detects antibodies to negatively charged phospholipids in the presence of β_2GPI
- 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

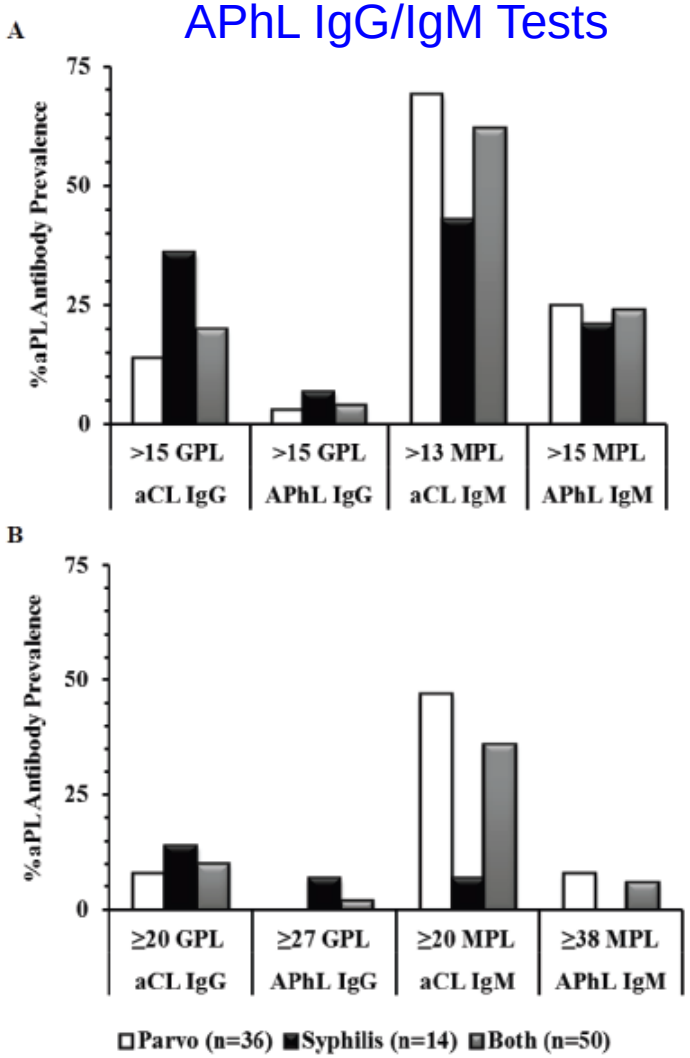
APhL as an Alternative to Anti-cardiolipin for the Diagnosis of APS

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



Suh-Lailam et al. Int J Clin Exp Pathol. 2012;5:210-5

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-β_2GPI and aPT recognized as major autoantibodies for LAC
  - aPT for prothrombin-dependent LAC
  - Anti-β_2GPI for β_2GPI-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

Human Prothrombin Bound to	Prevalence (%)	References
Plain polystyrene plates	0	18, 32, 33
γ-Irradiated plates	55	32
High-activated PVC plates	50-58	18, 33
Phosphatidylserine-coated plates	90	33

Galli and Barbui. Blood. 1999;93:2149-57

PS/PT Antibodies as Diagnostic Markers for APS

References	Key Findings
Pregnalato et al. 2013	Strong correlation with LAC; aPS/PT IgG associated with venous thrombosis and not pregnancy-related APS manifestations
Vlagea et al. 2013	aPS/PT IgG associated with venous thrombosis and obstetric complication
Sanfelippo et al. 2013	aPS/PT can contribute to identification of APS
Fabris et al. 2014	Additional diagnostic value for APS; relevant for difficult to interpret LAC results
Nojima et al. 2014	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
Heikal et al. 2015	Good correlation with LAC; relevant for difficult to interpret LAC results
Žigon et al. 2015	aPS/PT associated with recurrent early or late abortions and premature delivery irrespective of other aPL
Kitaori et al. 2015	LA-aPTT StaClot and aPS/PT IgG might be suitable for use in routine practice for patients with RPL
Hoxha et al. 2015	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)

State-of-the-art for aPS/PT IgG and IgM Assays

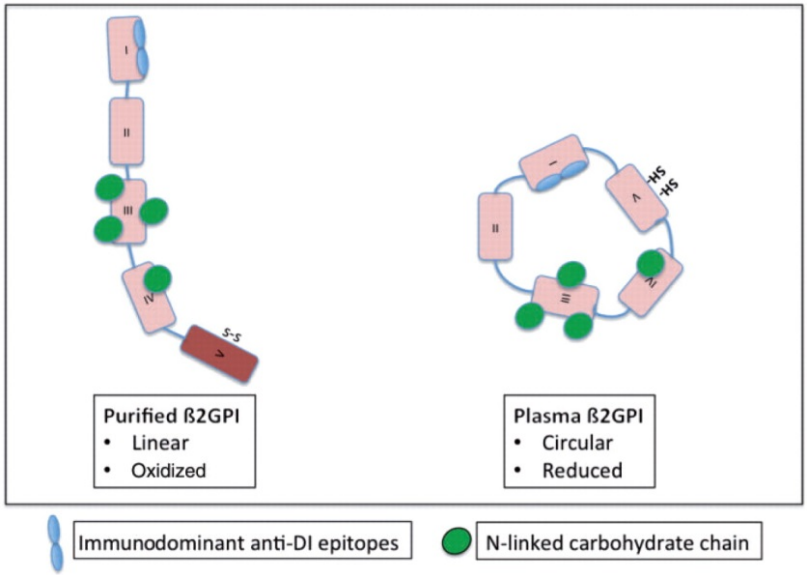
- Significance
  - Can contribute to assess the risk of thrombosis
  - Can contribute to a better identification of patients
  - Multivariate analysis: independent risk factor for disease
  - Results appear to be consistent between groups
- Challenges
  - Correlation with LAC is variable and isotype-dependent
  - Performance characteristics inadequately defined
  - No consensus on the relevance of IgM isotype

Peterson et al. Adv Clin Chem. 2016;73:1-28.

Anti-Domain I (DI) of β_2GPI (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 β_2GPI epitopes other than DI
  - Other anti-β_2GPI antibody subsets may be clinically relevant

Schematic of oxidized and reduced ß2GPI



Ioannou Y. Rheumatology. 2012;51:32-36

Role of aD1 Antibodies in Diagnosis and Stratification of APS

***Table 4a: Additional diagnostic value of the aD1 IgG assay**, presented by contingency tables. Figures indicate the number of patients testing positive or negative for aD1 IgG or minimal one of the formal aPL.*

		Overall population (a) (n = 426)		APS (b) (n = 101)		AID (c) (n = 123)		DC (d) (n = 82)		HC (e) (n = 120)	
		aPL		aPL		aPL		aPL		aPL	
		+	-	+	-	+	-	+	-	+	-
aD1 IgG	+	59	2	54	0	3	0	1	0	1	2
	-	144	221	47	0	36	84	29	52	33	84
Total		203	223	101	0	39	84	30	52	34	86

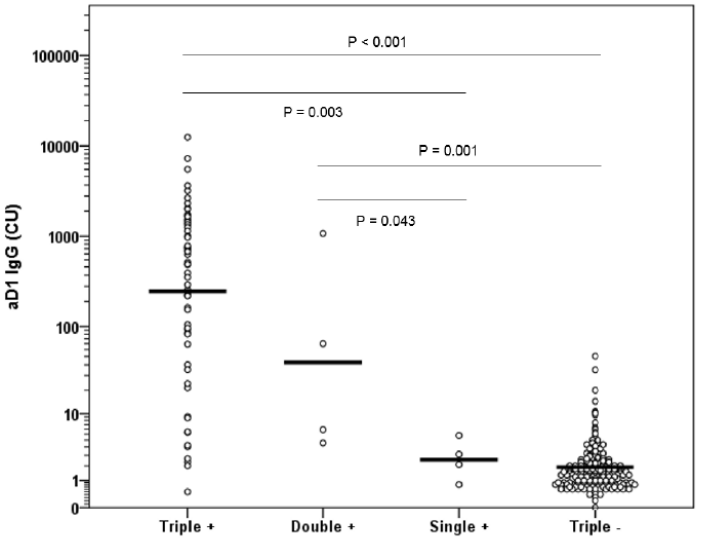
APS = antiphospholipid syndrome, AID = autoimmune disease, DC = diseased control, HC = healthy control. aPL = antiphospholipid antibody.

***Table 4b: additional value of aD1 IgG in risk stratification** using a multivariable logistic regression model. The additional value of the covariates aβ₂GPI and/or aD1 IgG is expressed as p-value and OR; c-statistics of each model are presented.*

Covariates		p-value	OR	95% CI	AUC predicted probability
LAC + aCL IgG/IgM	+ aβ₂GPI IgG	0.005	12.2	(2.2 – 69.0)	0.77
LAC + aCL IgG/IgM	+ aD1 IgG	0.13	2.6	(0.7 – 9.2)	0.76
LAC + aCL IgG/IgM + aβ₂GPI IgG	+ aD1 IgG	0.79	0.8	(0.2 – 4.1)	0.77

LAC = lupus anticoagulant, aCL = anticardiolipin antibody, aβ₂GPI = anti-β₂-glycoprotein I antibody. OR = odds ratio, 95% CI = 95% confidence interval, AUC = area under the curve.

Distribution of the aD1 IgG Titers Based on aPL Profile



- Classifies patients at-risk for thrombosis (triple aPL)
- APS diagnosis and stratification dependent on solid phase assays used for aCL and $a\beta_2$GPI detection

De Craemer et al. J Thromb Haemost. 2016 Jun 17

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

Types of profile	Characteristics
# of positive aPL antibody types*	Triple positivity (aCL, LAC, and aβ_2GPI): greater risk of thrombotic events than single or double positivity
Types of aPL antibody**	>1 aPL positive (I), LAC alone (IIa), aCL alone (IIb) and aβ_2GPI alone (IIc)
Antiphospholipid score (aPL-S)***	Quantitative marker based on relative risks for APS events for each aPL test
Global APS score (GAPSS)****	Combination of independent risk for APS, aPL profile, conventional cardiovascular risk factors and autoimmune disease serology

*Galli et al. Blood. 2003;101:1827-32
**Miyakis et al. J Thromb Haemost. 2006;4:295-306
***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