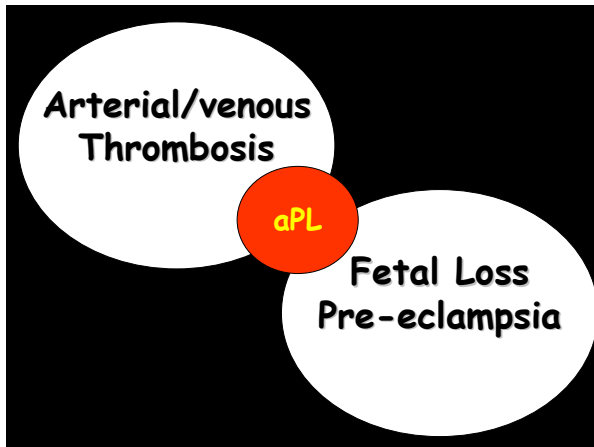


ACR/ARHP 06
Scientific Meeting
Washington, DC - November 10-15, 2006

**ACR Study Group:
Anti-phospholipid Antibodies: more
assays than autoantibodies?**

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APS: impact on real life

- aPL are strong risk factors for recurrent thrombotic events and/or fetal losses and pregnancy complications.
- aPL for 15-20% of all episodes of deep vein thrombosis and one third of strokes occurring in pts <50 ys.
- Primary versus APS associated with other systemic autoimmune diseases (mainly SLE).
- Up to 40% of SLE pts test positive for aPL: LA predicts a 50% chance of a thrombotic events over 20 ys follow-up

Antiphospholipid Syndrome: Classification Criteria

Table 1. Preliminary criteria for the classification of the antiphospholipid syndrome*

Clinical criteria	
1. Vascular thrombosis	One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by imaging or doppler studies or histopathology, with the exception of superficial venous thrombosis. For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.
2. Pregnancy morbidity	(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or (b) One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe preeclampsia or eclampsia, or severe placental insufficiency (18,19), or (c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.
In studies of populations of patients who have more than 1 type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c above.	
Laboratory criteria	
1. Anticardiolipin antibody of IgG and/or IgM isotype in blood, present in medium or high titer, on 2 or more occasions, at least 6 weeks apart, measured by a standardized enzyme-linked immunosorbent assay for β_2 -glycoprotein I-dependent anticardiolipin antibodies (7,20).	12 weeks
2. Lupus anticoagulant present in plasma, on 2 or more occasions at least 6 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis (Scientific Subcommittee on Lupus Anticoagulant/Phospholipid-Dependent Antibodies) (21), in the following steps: (a) Prolonged phospholipid-dependent coagulation demonstrated on a screening test, e.g., activated partial thromboplastin time, kaolin clotting time, dilute Russell's viper venom time, dilute prothrombin time, Testartin time. (b) Failure to correct the prolonged coagulation time on the screening test by mixing with normal platelet-poor plasma. (c) Shortening or correction of the prolonged coagulation time on the screening test by the addition of excess phospholipid. (d) Exclusion of other coagulopathies, e.g., factor VIII inhibitor or heparin, as appropriate.	
Definite antiphospholipid antibody syndrome is considered to be present if at least 1 of the clinical criteria and 1 of the laboratory criteria are met.	

Wilson W. et al, *Arthr Rheum* '99; Myakis et al *JTH* '06

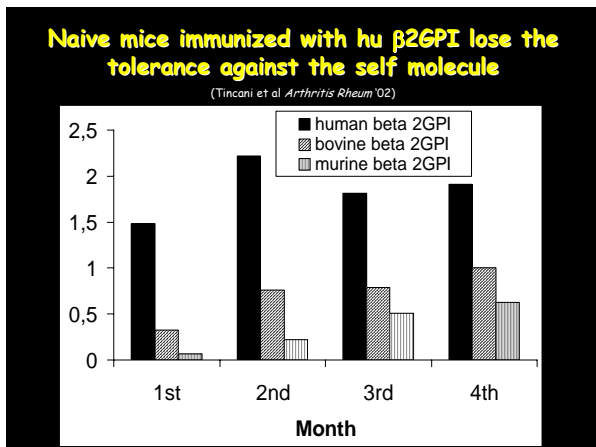
Anti-beta2GPI Ab assay is now a new formal lab classification criterium

aPL (anti- β 2GPI Abs) can be pathogenic rather than a simple serological marker for APS

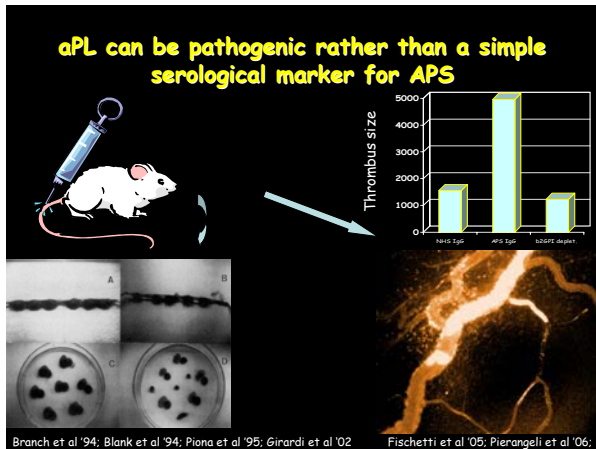
APS as an autoantibody-mediated disease

- A defined circulating Ab or cell-mediated immunity to autoAgs
- The ability to generate the autoAb or self-reacting cells following the immunization with the self-Ag (with complete Freund's adjuvant)
- The ability to produce the disease in an experimental animal by passive transfer of the Ab or the self-reacting cells
- The ability to produce the disease in an experimental animal by immunization with the self- Ag (with complete Freund's adjuvant)
- Definition of a specific autoAg

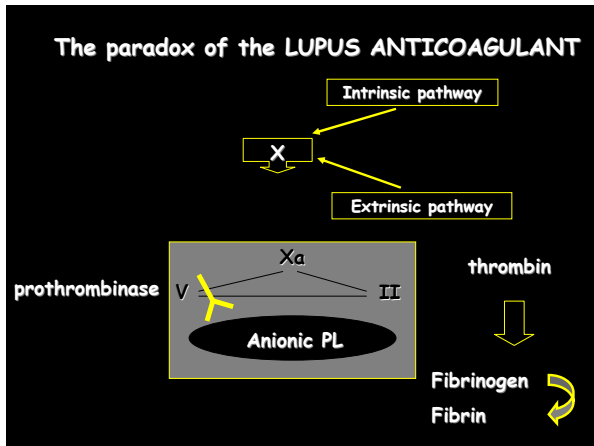
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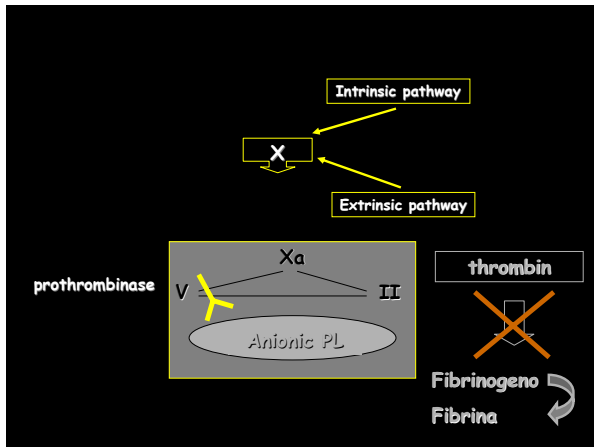


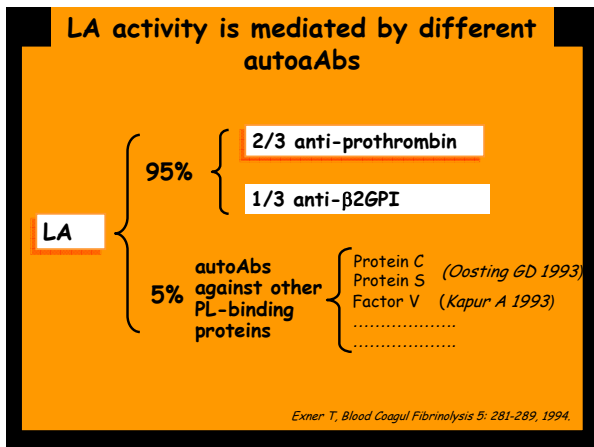
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- Definition of a specific autoAg



THE DIAGNOSTIC TESTS



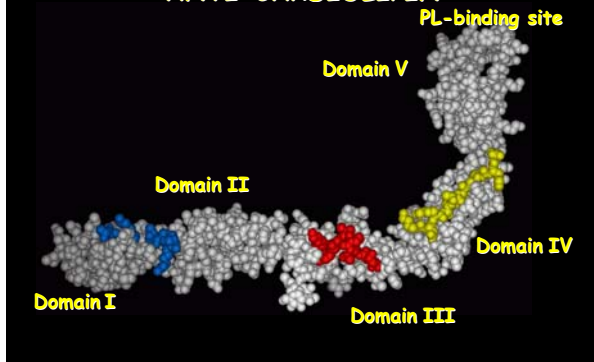




LA

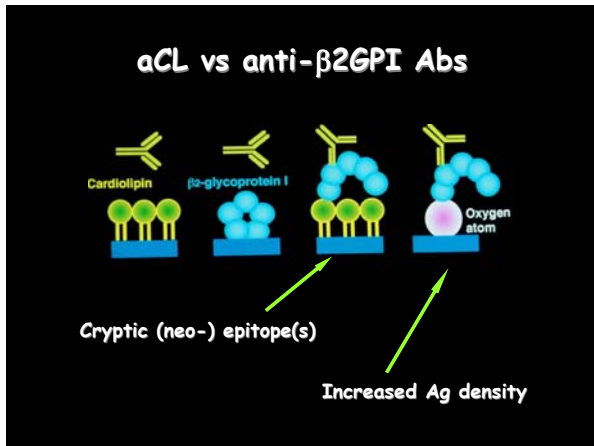
- No single test is 100% sensitive
- At least 2 tests (aPTT, dRVVT, KCT)
- Heparin/OAT may affect the results
- Good reproducibility for strong +ve
(Jennings et al JTH 04; Pengo et al Thromb Res 06)
- Normalization ratio, cut off, control +ve sample

SOLID PHASE ASSAYS: ANTI-CARDIOLIPIN



Anti- β 2GPI assay



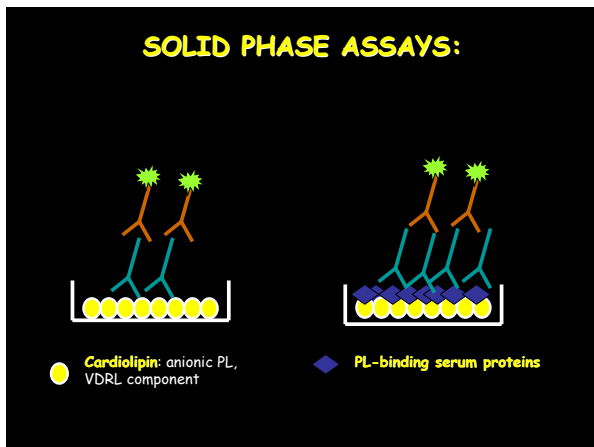


aCL & anti- β 2GPI standardization

(Tincani et al *Thromb Haemost*'01; Tincani et al *Thromb Res*'04; Reber et al *Thromb Haemost*'05)

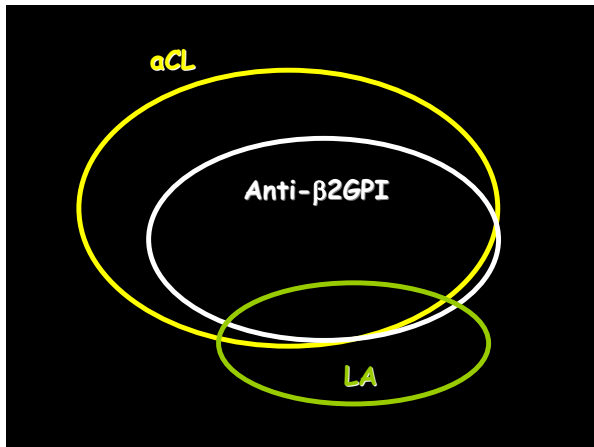
Minimal requirements

- Samples in duplicate
- Cut off levels for each lab.
- Cut off level in percentile
- Stable external controls
- Range of positivity



PL-binding proteins bound by autoAbs in the solid-phase assays

Bovine β 2GPI	Anionic PL	aCL assay
Human β 2GPI	γ -irr. plates	anti- β 2GPI assay
Human prothrombin	Anionic PL (PS)	Anti-PT assay
Protein C, Protein S and C4b-binding protein Activated Protein C	Anionic PL	aCL assay +/-
Thrombomodulin	Anionic PL	aCL assay +/-
Annexin V	Anionic PL	aCL assay +/-
High molecular weight kininogen	Neutral PL (PE)	anti-PE assay



Correlation among assays

- Persistent +ve aCL: high chance for LA and/or anti- β 2GPI Abs (Nash et al *JTH*'04; Neville et al *J Rheum*'06)
- 26% of APS pts positive for aCL only (Nash et al *JTH*'04)
- Higher sensitivity for aCL

aPL assays: predictive value

LA

Strong risk factor for thrombosis (irrespective of the site, the assay used, the presence of SLE) (Galli et al *Blood* '03)

Strong risk factor for recurrent fetal loss (Opatrný et al *J Rheum* '06)

aCL:

Risk factor for thrombosis (IgG, >40 GPL) (Galli et al *Blood* '03) **and recurrent fetal loss** (Opatrný et al *J Rheum* '06)

aPL assays: predictive value

Anti-β2GPI assay

Risk factor for thrombosis (Galli et al *Blood* '03)

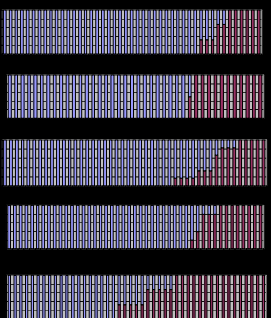
Risk factor for recurrent fetal loss (Opatrný et al *J Rheum* '06)

Anti-PT assay:

No clear correlation with thrombosis and recurrent fetal loss (Galli et al *Blood* '03)

Heterogeneity

Single bars on X axis represent the single samples evaluated by the 5 different methods. Y axis shows the percentage of concordance for negative (blue) or positive sera (red).



Tincani et al *CER* in press

β2GPI-dependent LA

(Simmlink et al *JTH*'03, Pengo et al *JTH*'04)

Table 4. Association between type A or type B anti-β₂GPI antibodies and (β₂GPI-dependent) LAC activity and thrombosis

	Total population, N = 198		
	Anti-β ₂ GPI IgG present, n = 52		Anti-β ₂ GPI IgG not present, n = 146
	Type A, n = 30	Type B, n = 22	
LAC, n = 63	28	6	29
β ₂ GPI-dependent LAC, n = 25	23	2	0
Thrombosis, n = 60	25	7	28
Odds ratio for thrombosis	18.9	1.1	NA
95% CI	5.3-6.8	2.8-0.4	NA

Numbers are depicted as absolute number of patients.
NA indicates not applicable.

(Bas de Laat et al. *Blood*'05)

International consensus statement on an update of the preliminary classification criteria of the antiphospholipid syndrome



XI Int. Congress on Antiphospholipid Antibodies - Sidney 12-'04
Myakis et al *J Thromb Haemost* 2006;4:295-306

Investigators are strongly advised to classify APS patients in studies into one of the following categories:

- Ia: Anti-cardiolipin antibody present alone
- Ib: Lupus Anticoagulant present alone
- Ic: Anti-Beta-2 glycoprotein-I antibody present alone
- II: More than one Laboratory criteria present (any combination).

•More positive tests more risk !!!

LUPUS ANTICOAGULANT: the paradox

- *In vitro* prolongs the PL-dependent coagulation assays
- *In vivo* is a strong thrombophilic risk factor
- It's only "partially" associated to lupus