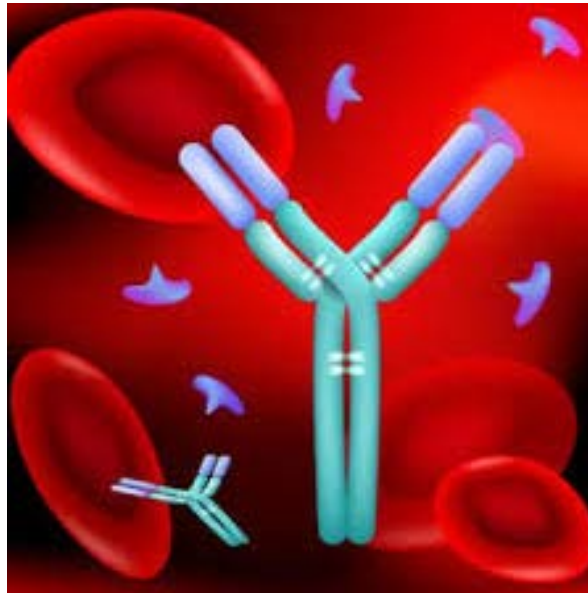


Laboratory diagnosis of antiphospholipid syndrome



The antiphospholipid syndrome

APS



- 1983 G.Hughes describes a syndrome associated with recurrent thrombotic events in patients with prolonged clotting times which did not correct in the mixing test suggesting the presence of an inhibitor
- Although the laboratory findings of the patients suggested a “bleeding profile” the presence of these antibodies were responsible for an hypercoagulable state

Antiphospholipid Syndrome

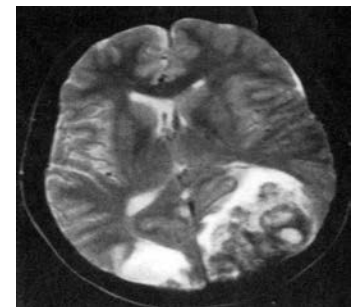
- Autoimmune disease causing a coagulation disorder with the following primary clinical manifestations:

- **Thrombosis**

- Venous thrombosis
- Arterial thrombosis

- **Obstetrical complications**

- Miscarriage
- Stillbirth
- Preterm delivery
- Preeclampsia



APS statistics



- **Estimated incidence:**
 - about 5 new cases/100,000 persons
- **Prevalence:**
 - 40-50 cases/100,000 persons
- **Prevalence higher in specific groups:**
 - SLE: 30%
 - Deep vein thrombosis: 30%
 - Stroke in < 50 yr old: 25%
 - Recurrent fetal loss: 10%
- **Primary APS** = no other autoimmune disease
- **Secondary APS** = APS associated with other autoimmune diseases (mostly SLE)

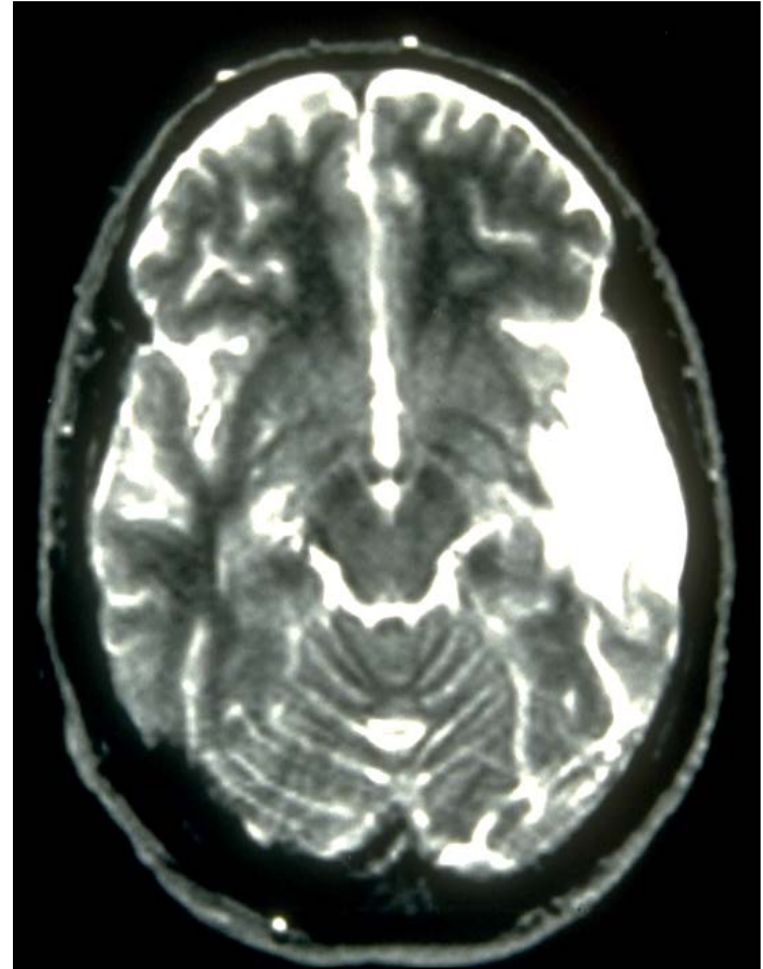
APS-associated clinical features

- Livedo reticularis
- Leg ulcers
- Migraines
- Chorea, epilepsy
- Cognitive disorders
- Heart valve lesions
- Pulmonary hypertension
- Thrombocytopenia



Stroke in APS

- Most common neurological complication
- 1/5 in young patients (<45 years)
- Recurrent events are frequent



Pregnancy loss in APS

- Three consecutive miscarriages → 15%
- Second or third trimester loss → 30%
- IUGR + late loss → 40%

With treatment 85%-90% success rate!



Catastrophic APS

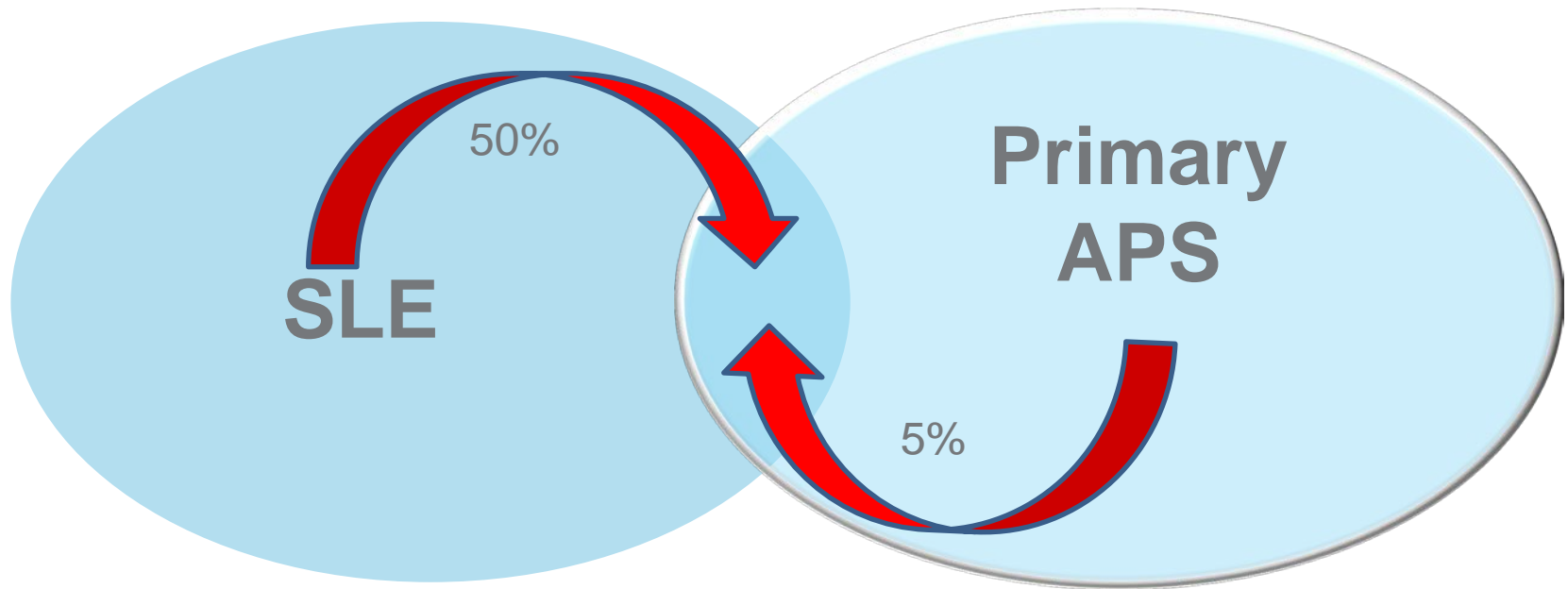
- Term proposed in 1992
- Accelerated form of APS with multiorgan thrombotic failure
- Around 50% mortality, it may show up 'ex novo'
- Trigger: infection in many cases
- 1% prevalence in APS

CATASTROPHIC APS

International consensus for classification criteria

- 1. Clinical evidence of vessel occlusions affecting 3 or more organs or systems
- 2. Development of the manifestations simultaneously or in less than a week
- 3. Confirmation by histopathology of small vessel occlusion in at least one organ.
- 4. Laboratory confirmation of the presence of aPL (LA, ACL, anti- β 2GPI)

APS and SLE relationship



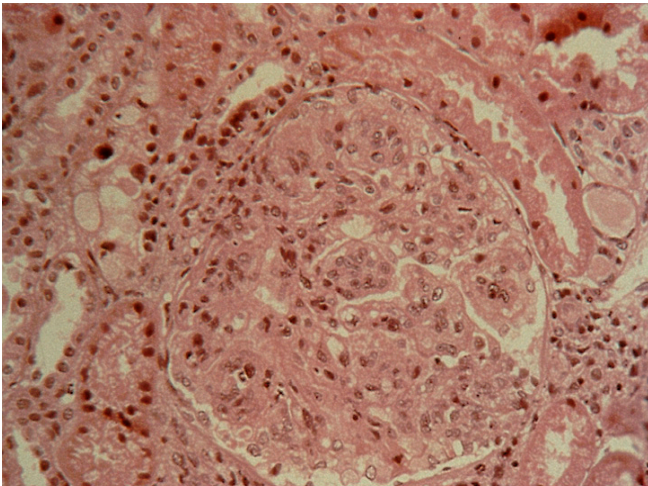
50% of SLE developed
APS after 10 years
Shah et al, Lupus 1993

5% of APS evolved into
SLE after 10 years
Gomex-Puerta et al, Medicine 2005

SLE vs. APS

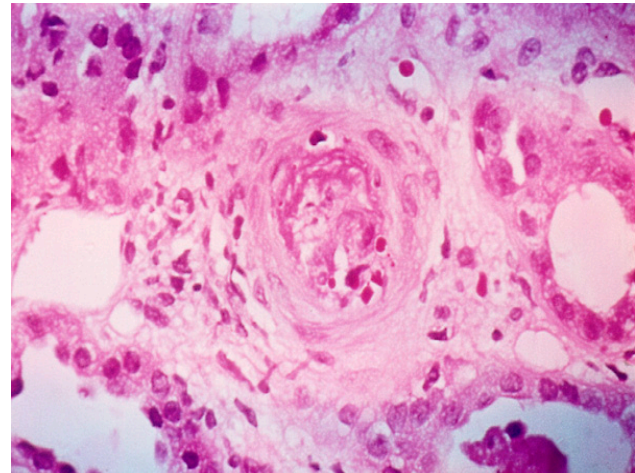
SLE

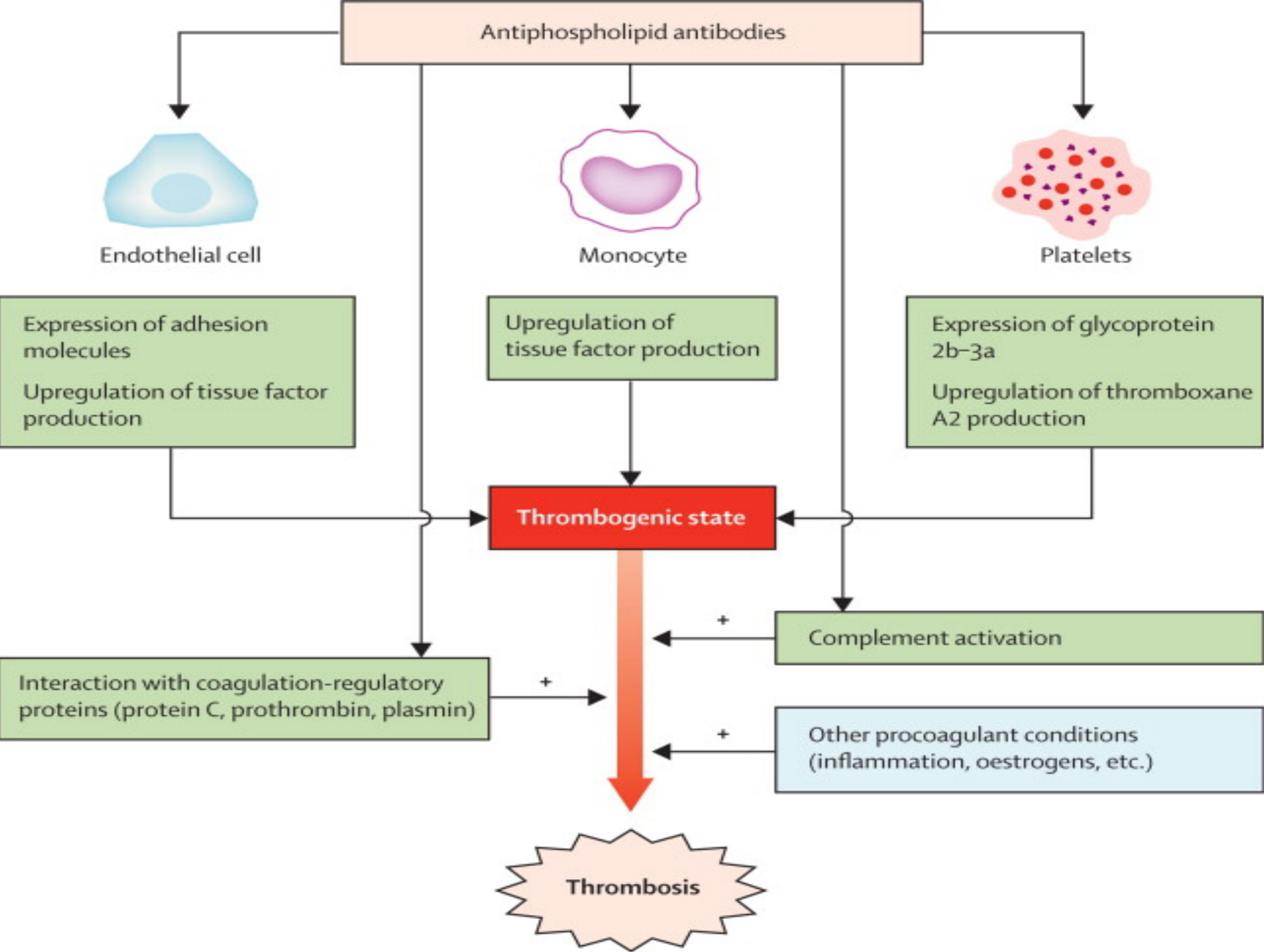
- Affects small vessels
- Vasculitic disease which is immune complex mediated
- Treatment is lifelong immunosuppression



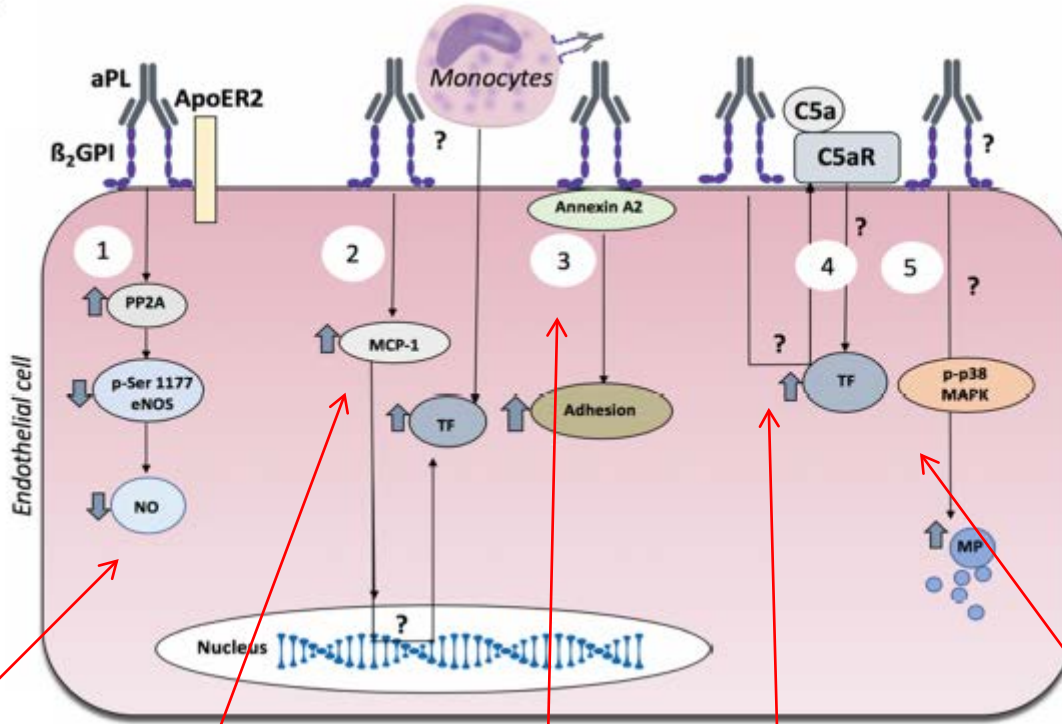
APS

- Affects large and small vessels
- Thrombotic disease which is mediated by a coagulation disorder
- Treatment is lifelong anticoagulation





A



Reduced NO generation

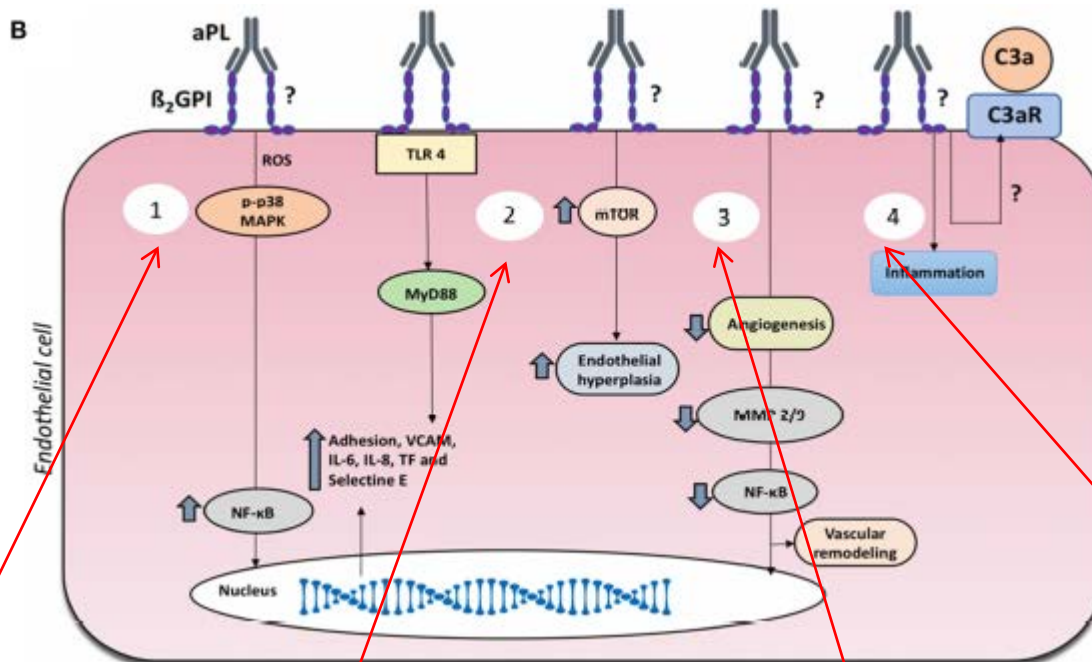
Elevated adhesion of monocytes, increased TF

Elevated expression of adhesion molecules

Complement generation, TF expression

Increased production of MPs

Endothelial dysfunction associated with thrombosis in APS



Induction of inflammation

mTOR-mediated Cell proliferation

Reduced maternal vascular remodeling

Inflammation and placental damage by C activation

Endothelial dysfunction associated with obstetric complications in APS

Antiphospholipid Antibodies Promote the Release of Neutrophil Extracellular Traps: A New Mechanism of Thrombosis in the Antiphospholipid Syndrome

Srilakshmi Yalavarthi¹, Travis J. Gould², Ashish N. Rao¹, Levi F. Mazza¹, Alexandra E. Morris¹, Carlos Núñez-Álvarez³, Diego Hernández-Ramírez³, Paula L. Bockenstedt⁴, Patricia C. Liaw², Antonio R. Cabral³, and Jason S. Knight¹

experimental models of APS. For example, aPL have been shown to indirectly activate neutrophils through the complement cascade and the well-recognized neutrophil stimulator C5a (14, 15). Here, we believe that *in vitro* aPL-mediated NET release is independent of C5a, as neither total IgG fractions, nor anti- β_2 GPI monoclonals, were dependent on the presence of serum (and exogenous complement) for neutrophil activation. Further, APS patient sera promoted NET release even when heat-inactivated (data not shown). However,

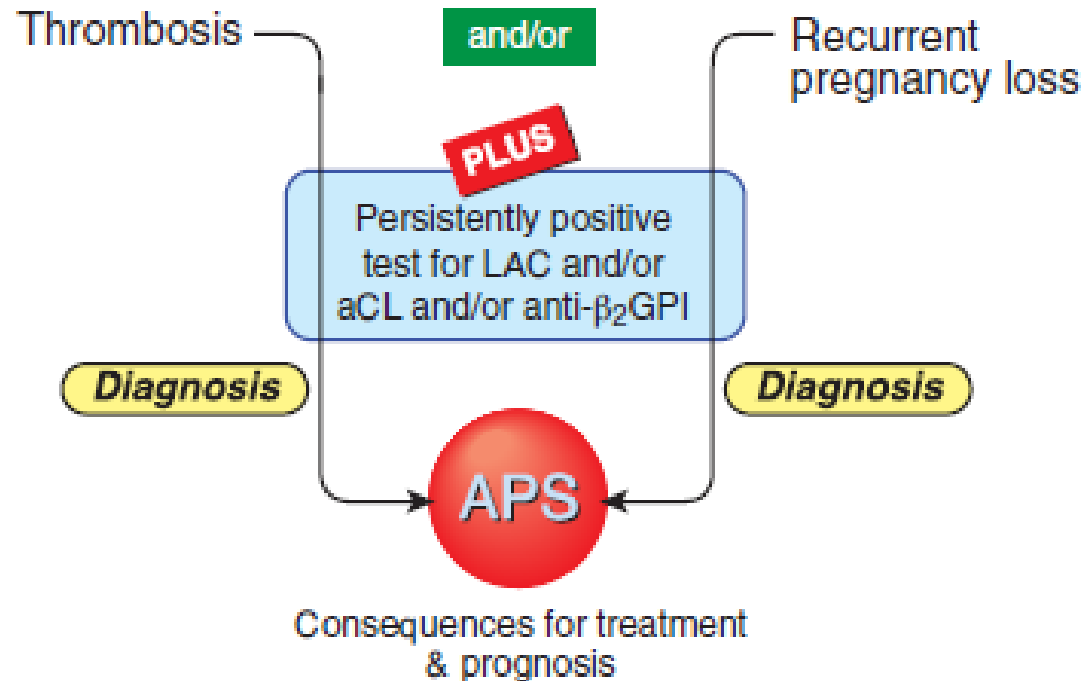


Primary laboratory tests

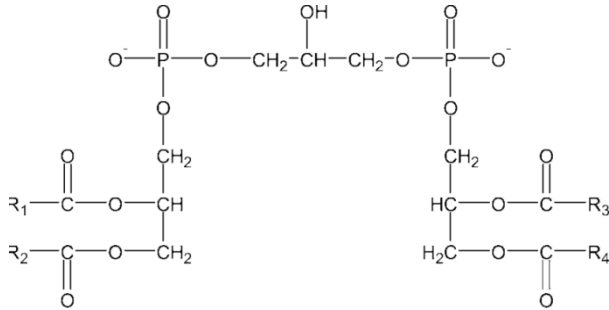
Assays included in the current classification criteria

- Lupus anticoagulant
- Anti-cardiolipin IgG, IgM
- Anti- β_2 GPI IgG, IgM
- Confirmation of the tests is mandatory in 12 weeks interval in order to exclude transient antibodies

The updated classification criteria for APS



anti-Cardiolipin antibodies



- Cardiolipin is a negatively charged phospholipid (*diphosphatidylglycerol*) in the membrane of mitochondria and in bacterial membranes
- Plays important roles in cellular and mitochondrial function as a signaling platform
- Serves as a proton trap
- Triggers apoptosis
- Abnormal synthesis or remodeling of CL leads to rare genetic disorders (Barth syndrome)

anti-Cardiolipin antibodies

- Cardiolipin is the major constituent of the syphilis assay developed in the early 1900's by Wasserman and Neisser (extract of heart tissue)
- “False positive” syphilis serological reaction
- In the 1980s first assays for detection of aCL antibodies were developed



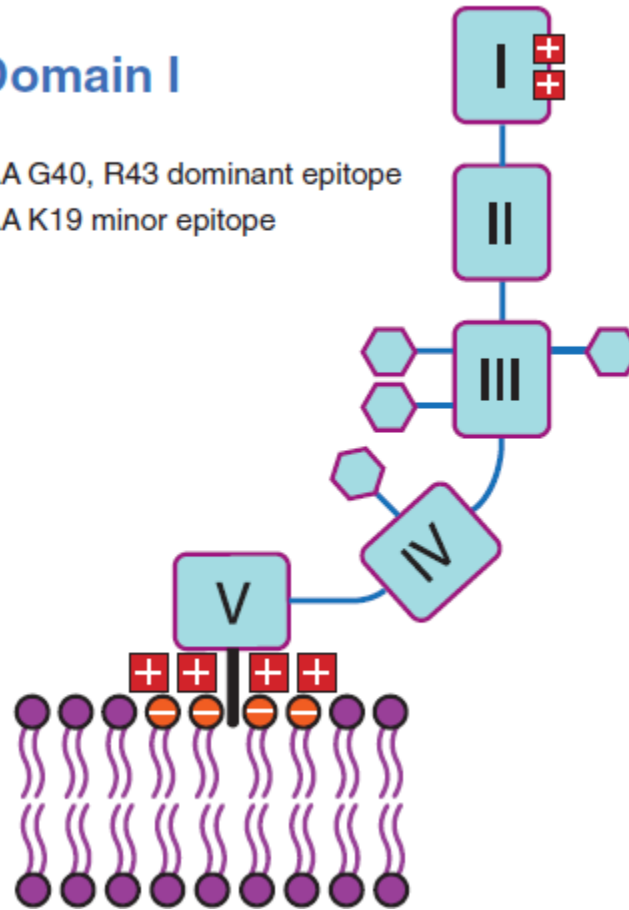
β 2-glycoprotein antibodies

- β 2-GPI is an anionic phospholipid-binding glycoprotein, normal component of human serum
- also known as Apo-H
- The homology with other proteins of the immune system suggest a function in innate immunity
- Suggested roles in haemostasis
 - Inhibition of ADP-mediated PLT aggregation
 - Regulation of contact activation
 - Binding to vWF (A1 domain)
- Antibodies against purified β 2GPI have been described by several groups in the '90s
- In the 1990s it was shown that binding of aCL antibodies to CL often requires a protein co-factor: β 2GPI
- Assays measuring antibodies against β 2GPI were developed in the late '90s - early 2000s

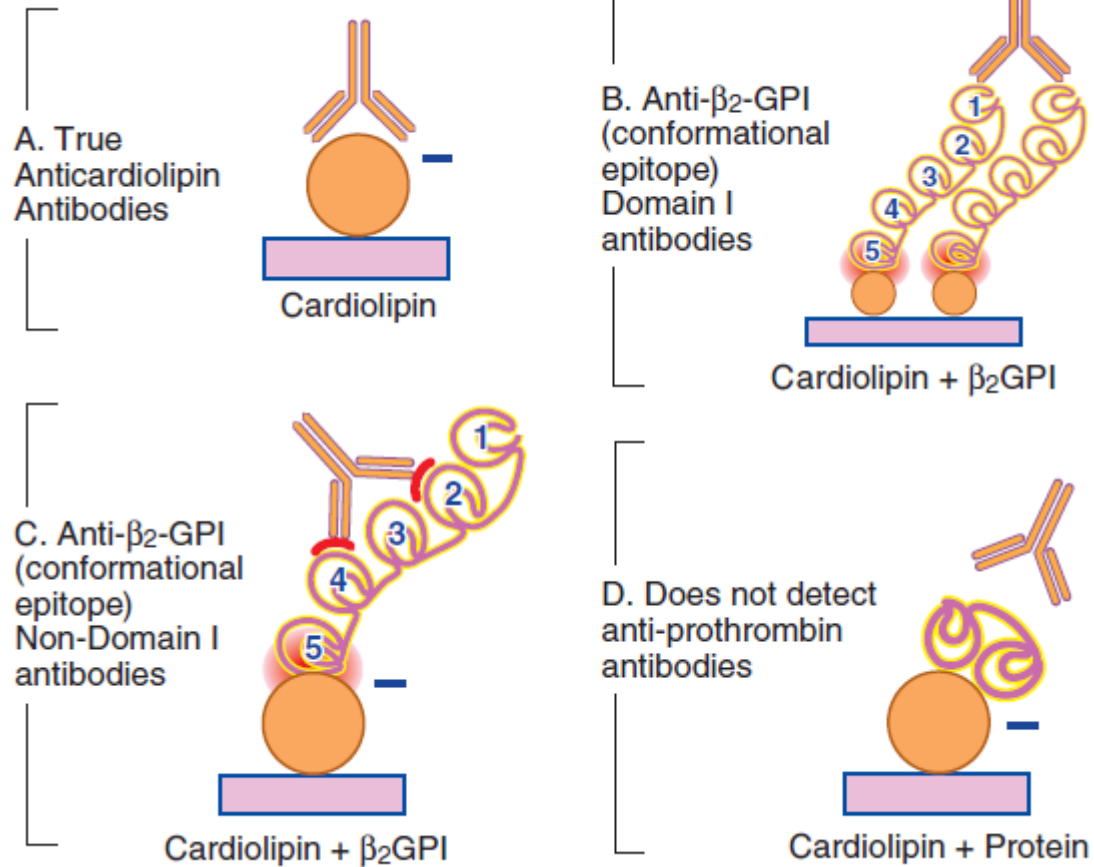
The β 2GPI structure

Domain I

- AA G40, R43 dominant epitope
- AA K19 minor epitope



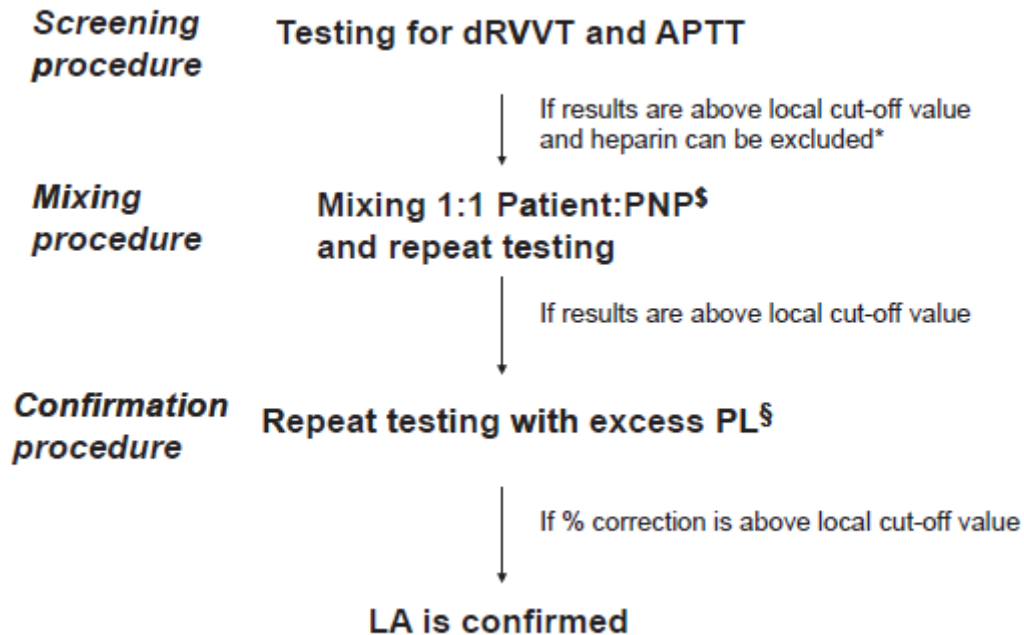
aCL Elisa



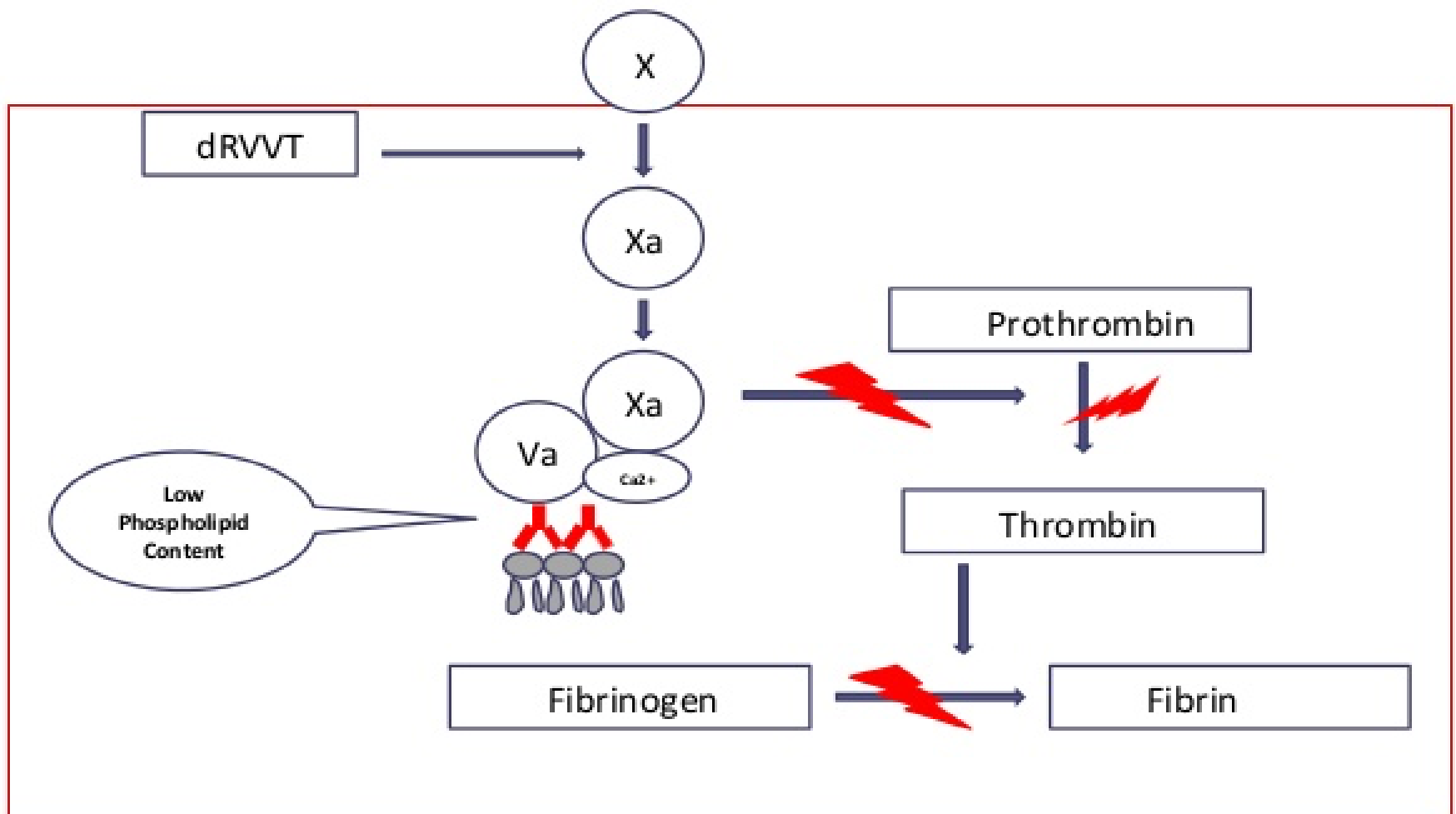
Lupus anticoagulant (LA or LAC)

- Lupus anticoagulant: Immunglobulin causing prolongation of *in vitro* coagulation assays, but causing thrombosis *in vivo*
- History: Observation of *in vitro* inhibitor of coagulation in SLE patients that was associated with increased frequency of thrombosis
- Detected by functional (coagulation) assays
 - aPTT
 - Diluted Russell's viper venom time (dRVVT)
 - Kaolin clotting time

Flow chart for the laboratory detection of lupus anticoagulant



dRVVT **Screen** (Lupus Anticoagulant)



Lupus anticoagulant assay

- Interlaboratory variation of LA
 - False positive detection 24%
 - False negative detection 18,5%
- Heparin contamination
- Preanalytical variables
 - PLT contamination
 - Improper plasma preparation
- Difficult during acute thrombosis and pregnancy

Other “non-criteria” antiphospholipid antibodies

Antibodies against other phospholipids and phospholipid binding proteins:

- Phosphatidylserine (PS)
- Phosphatidyl-ethanolamine (PE)
- Prothrombin (PT)
- Phosphatidylserine/prothrombin (PS/PT)
- Annexin V

Box 1 Definitions of medium-high antiphospholipid antibody (aPL) titres, and of high-risk and low-risk aPL profile

High-risk aPL profile.

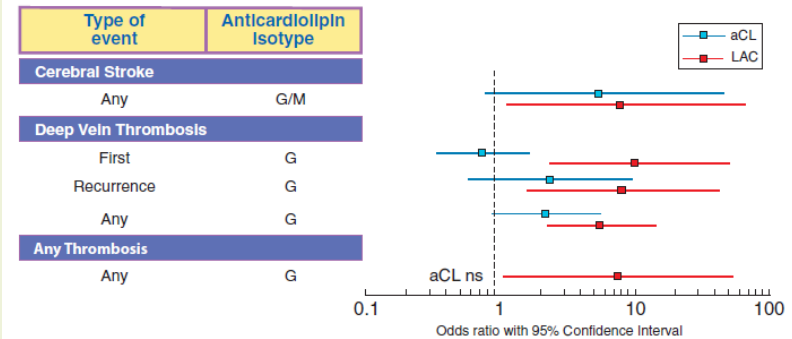
- ▶ The presence (in 2 or more occasions at least 12 weeks apart) of **lupus anticoagulant** (measured according to ISTH guidelines), or of **double** (any combination of lupus anticoagulant, aCL antibodies or antibeta2 glycoprotein I antibodies) or **triple** (all three subtypes) aPL positivity, or the presence of persistently high aPL titres.

Medium-high aPL titres.

- ▶ Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma present in titres >40 IgG phospholipid (GPL) units or >40 IgM phospholipid (MPL) units, or >the 99th percentile, measured by a standardised ELISA. Antibeta2 glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma in titre >the 99th percentile, measured by a standardised ELISA.¹

Low-risk aPL profile.

- ▶ Isolated aCL or antibeta2 glycoprotein I antibodies at low-medium titres, particularly if transiently positive.³



ORIGINAL ARTICLE

Confirmation of initial antiphospholipid antibody positivity depends on the antiphospholipid antibody profile

V. PENGO,* A. RUFFATTI,† T. DEL ROSS,† M. TONELLO,† S. CUFFARO,† A. HOXHA,† A. BANZATO,* E. BISON,* G. DENAS,* A. BRACCO* and S. PADAYATTIL JOSE*

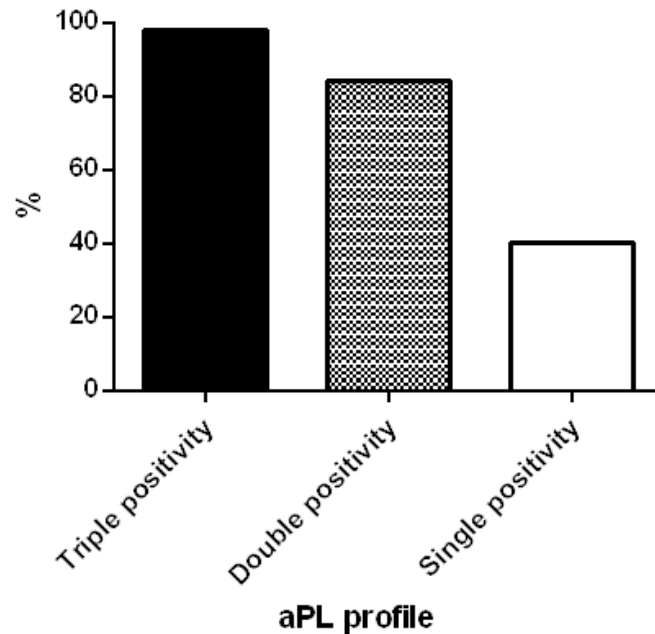
*Clinical Cardiology, Thrombosis Center, Department of Cardiac Thoracic and Vascular Sciences, University of Padua; and †Rheumatology Unit, Department of Medicine, University of Padua, Padua, Italy

During a four-years period 225 patients were initially positive to one or more test and 161 were available for confirmation after 3 months.

Patients were classified as

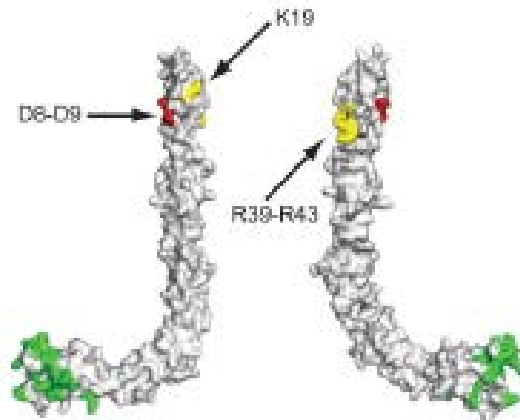
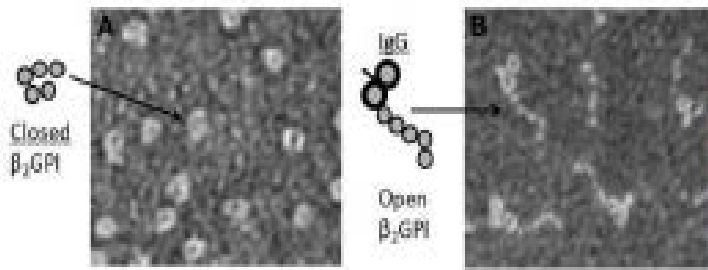
- **triple positive** (n=54: LAC+, aCL+, a β 2GPI+, same isotype),
- **double positive** (n=50: LAC-, aCL+, a β 2GPI+, same isotype)
- **single positive** (n=53: LAC or aCL or a β 2GPI antibodies as the sole positive test).

aPL profile confirmation after 3 months



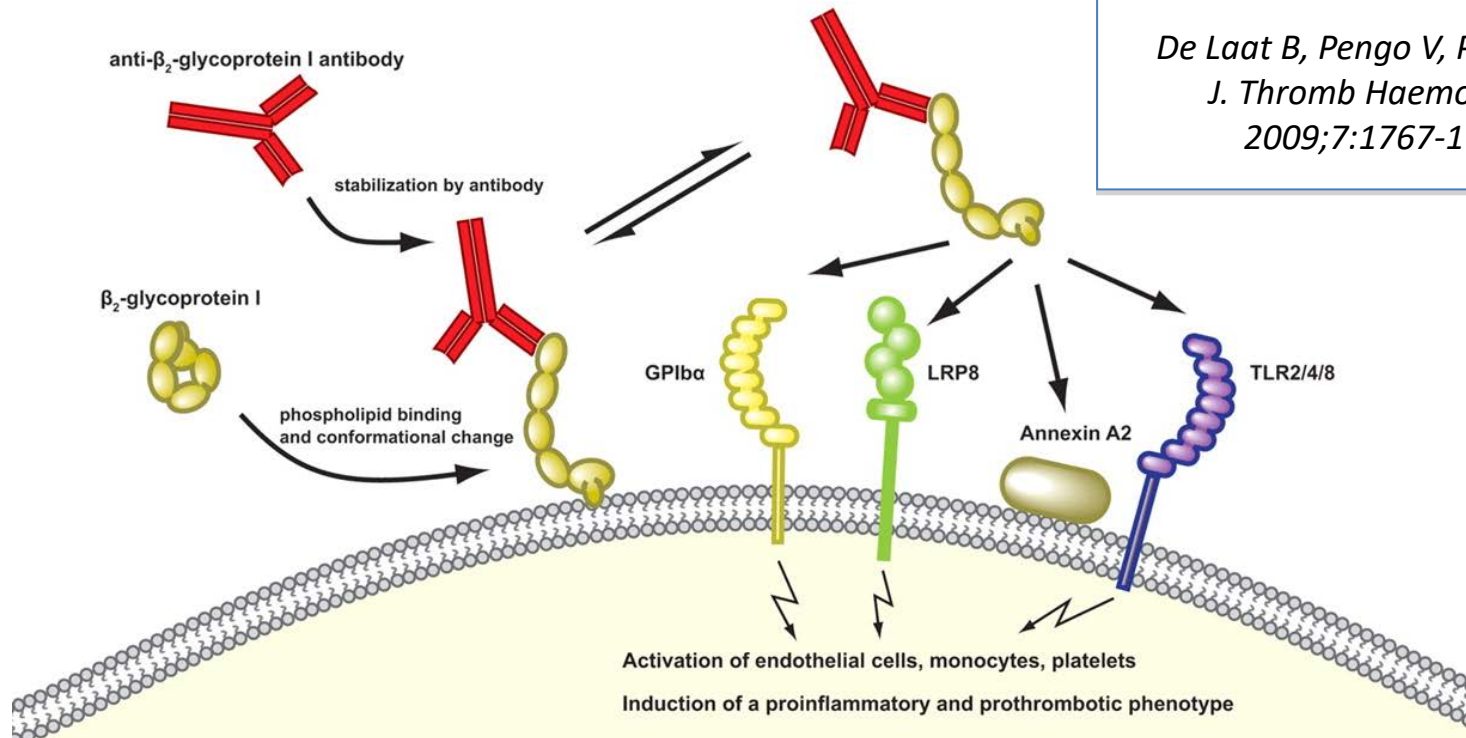
Why triple positivity is unique?

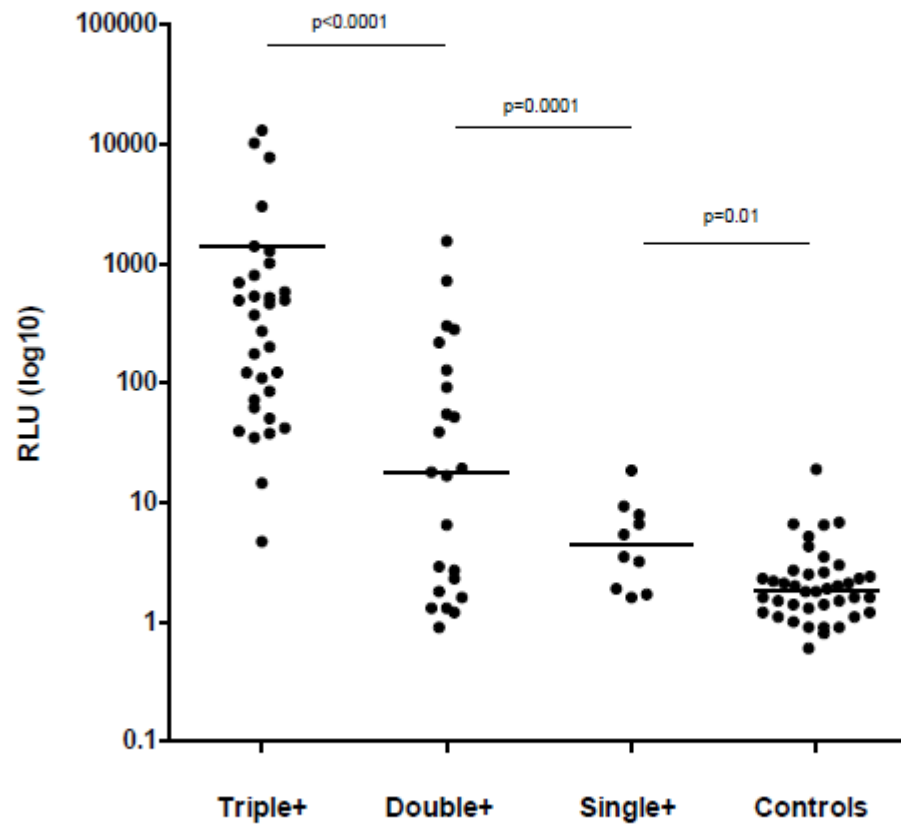
Answer: In contrast to single test positivity, triple positivity arises from the presence of a single (possible pathogenic) antibody



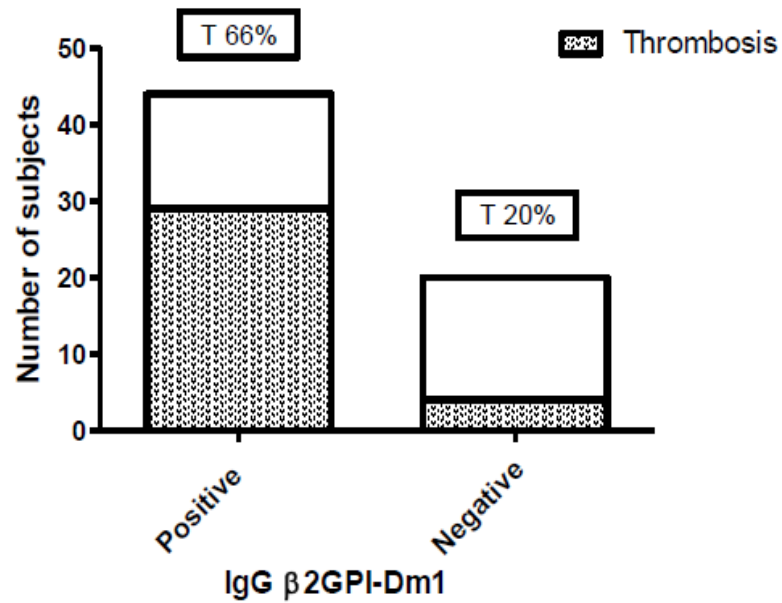
In a double-blinded multicenter study slightly more than half of the patients (55%) had antidomain I antibodies and an odds ratio of 3.5 for thrombosis compared to those patients without antidomain I antibodies

De Laat B, Pengo V, Pabinger J
J. Thromb Haemostasis
 2009;7:1767-1773

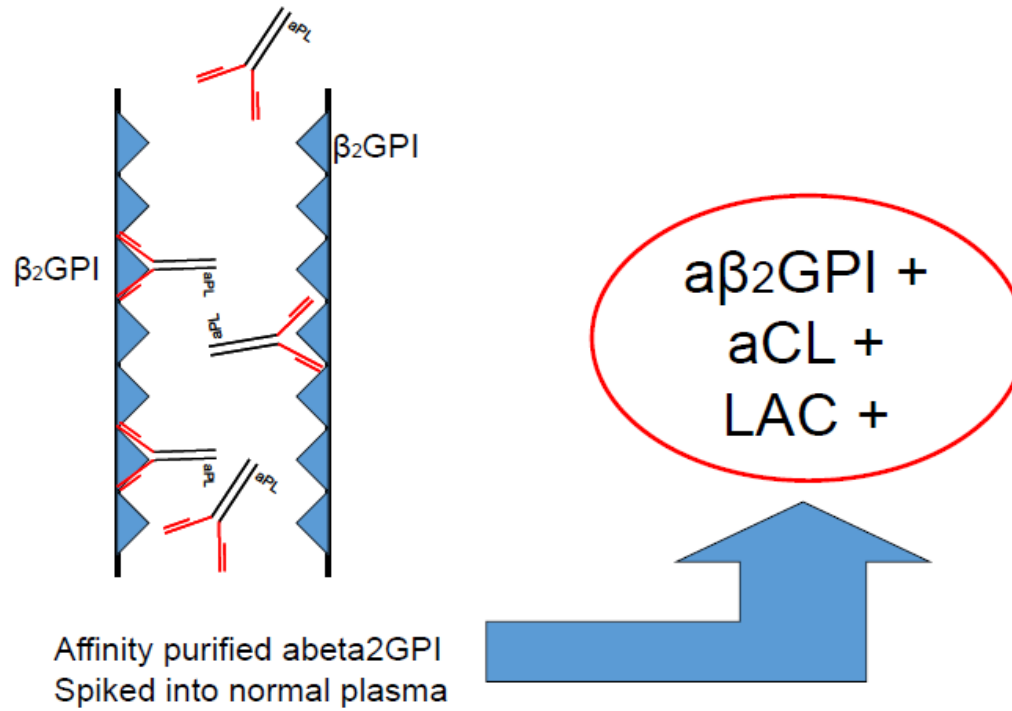




Association of positive IgG $\alpha\beta 2\text{GPI-Dm1}$ with thrombosis



Triple positive plasma passed through a beta2GPI affinity column



aCL ELISA and LAC activity of affinity-purified anti human β 2-GPI autoantibodies from triple positive patients

Affinity purified anti β 2-glycoprotein I antibody preparations						
	Control IgG	1	2	3	4	5
Protein [μ g/ml]	75	61	39	49	68	34
β 2GPI ELISA	0.015	2.378	2.658	2.297	2.242	2.255
aCL ELISA	0.005	2.081	2.037	1.645	1.806	1.648
dRVVT ratio	0.95	1.6	1.3	1.6	1.4	1.5

EDITORIAL

Four good reasons to appreciate triple positivity

Vittorio Pengo

Clinical Cardiology, Thrombosis Centre, Department of Cardiac Thoracic and Vascular Sciences, University of Padua, Padua, Italy

- High association with thromboembolic events and CAPS
- No need to confirm 12 weeks later
- Strong association with a single pathogenic antibody
- Independent of the method and the platform of detection

- The LA test plays a key role in the diagnosis of the APS but several parameters interfere with the performance of the assay
- Epidemiologic analyses examining the antibody profile in risk stratification will be of great value in prognosis and decision making for treatment
- The presence of anti-domain I β 2GPI antibodies is of great importance and the specific detection and measurement will be important

Closing the serological gap

- The **seronegative-APS patients**: Individuals who present a clinical picture of APS but are persistently negative for “criteria” APS assays
- Need further investigation with additional assays and follow up
- Reconsider the existing classification criteria

Closing the Serological Gap in the Antiphospholipid Syndrome: The Value of “Non-criteria” Antiphospholipid Antibodies

Navid Zohoury, Maria Laura Bertolaccini, Jose Luis Rodriguez-Garcia, Zakera Shums, Oier Ateka-Barrutia, Maurizio Sorice, Gary L. Norman, and Munther Khamashta

Results. Using 4 of 11 non-criteria tests, a cumulative 30.9% of SN-APS patients were detected. Combining results of all 11 non-criteria tests, 25 SN-APS (36.8%) and 89 SP-APS (83.2%) were positive for 1 or more non-criteria antibodies.

Conclusion. Failure to diagnose APS can result in severe clinical consequences. Patients displaying clinical features of APS, but negative for conventional criteria markers, should undergo additional testing for non-criteria biomarkers. In our cohort, around one-third of SN-APS patients showed reactivity to 1 or more non-criteria markers. An update to the current classification criteria incorporating new serological markers should be considered to identify and stratify patients with APS for more effective treatment and management. (First Release September 1 2017; J Rheumatol 2017;44:1597–602; doi:10.3899/jrheum.170044)