# The Antiphospholipid Syndrome: A Laboratory Phenomenon?

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# Antiphospholipid Syndrome

**Antiphospholipid Antibodies**

Lupus Anticoagulants (LA)

and/or

Anticardiolipin antibodies (aCL)

and/or

Anti-β2-Glycoprotein I antibodies (aβ2GPI)

+

Arterial/Venous Thrombosis and/or

(Recurrent) Miscarriages

# APS: Vascular Thrombosis

≥ 1 episode of arterial, venous or small vessel thrombosis, in any organ or tissue.

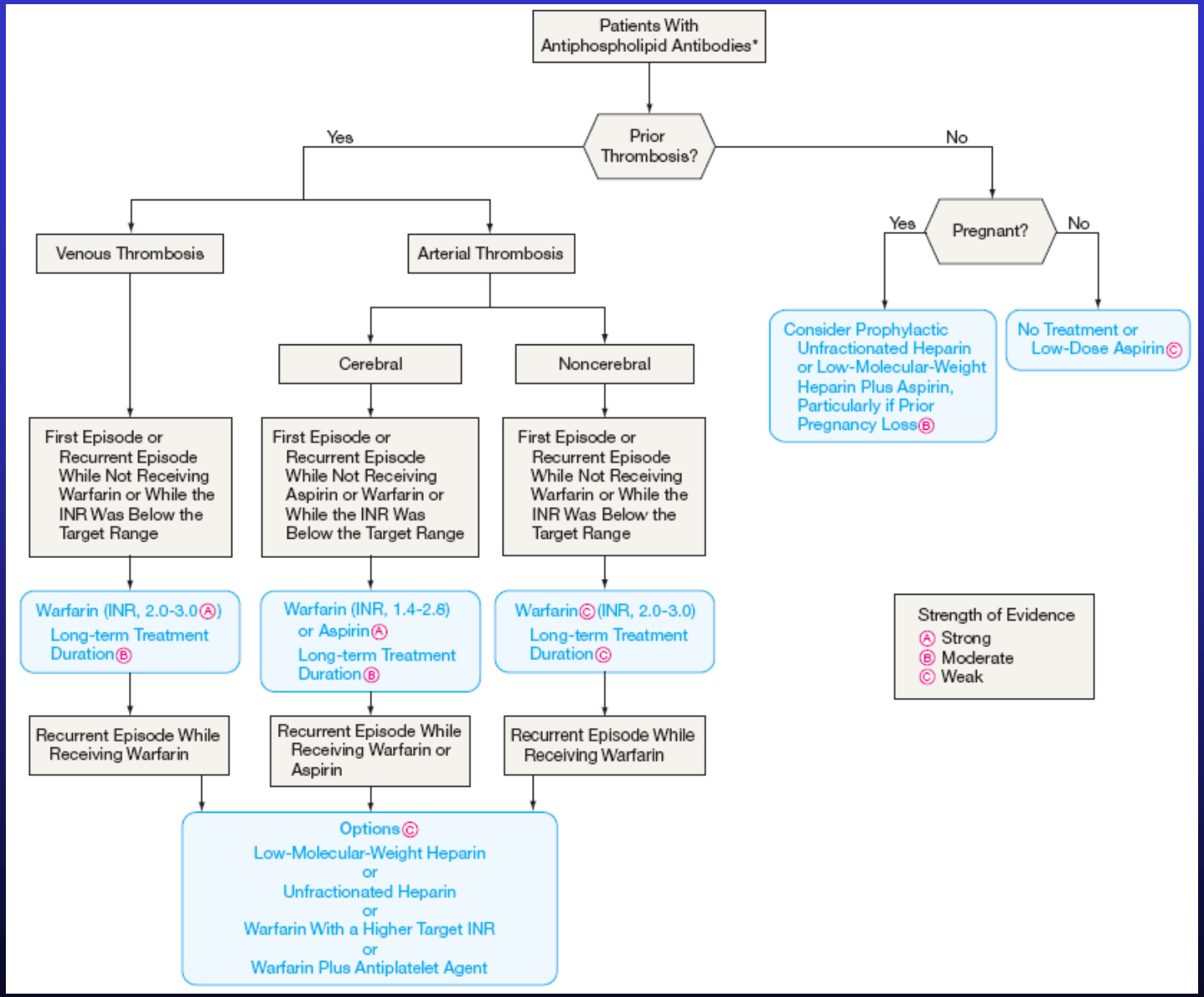
Thrombosis must be confirmed by objective validated criteria.

Thrombosis should be present without significant evidence of inflammation in the vessel wall.

Superficial venous thrombosis is not included.

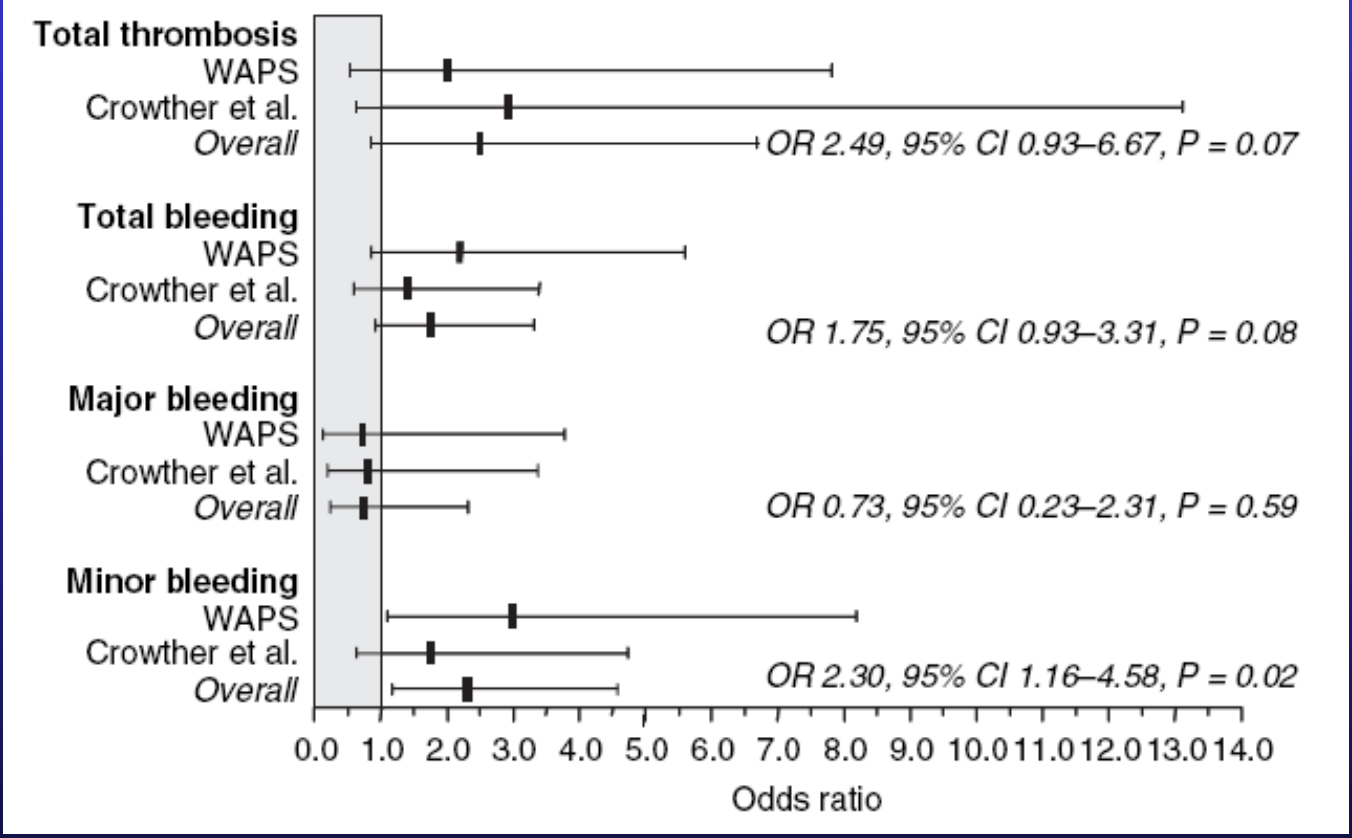
Miyakis, JTH 2006;4:295

# Antithrombotic Treatment of aPL+ Patients




Lim et al, JAMA 2006;29:1050

# Secondary Thromboprophylaxis of APS Patients: WAPS & PAPRE Studies




High-dose OAT (PT INR >3.0):

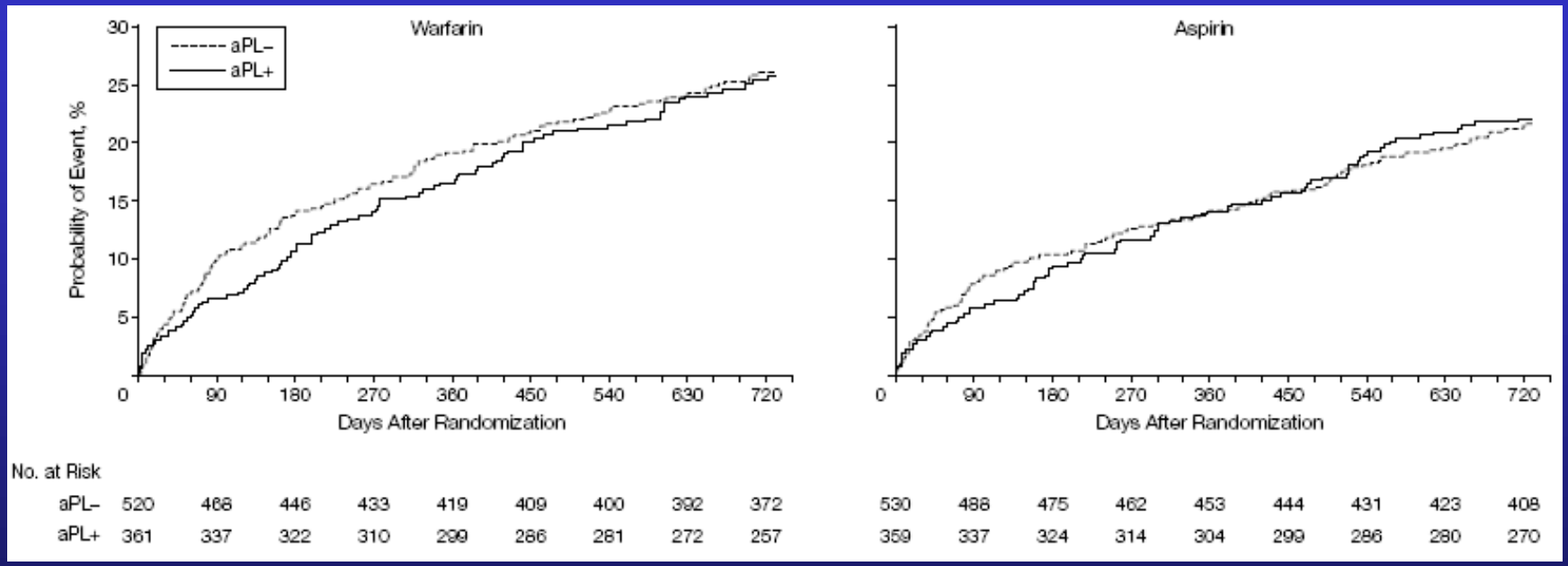
1. does not reduce the risk of re-thrombosis

2. increases the risk of minor bleeding

compared to standard-dose OAT (PT INR 2.0-3.0)

Finazzi et al, JTH 2005;3:848

# Secondary Thromboprophylaxis of APS Patients: WARSS Study

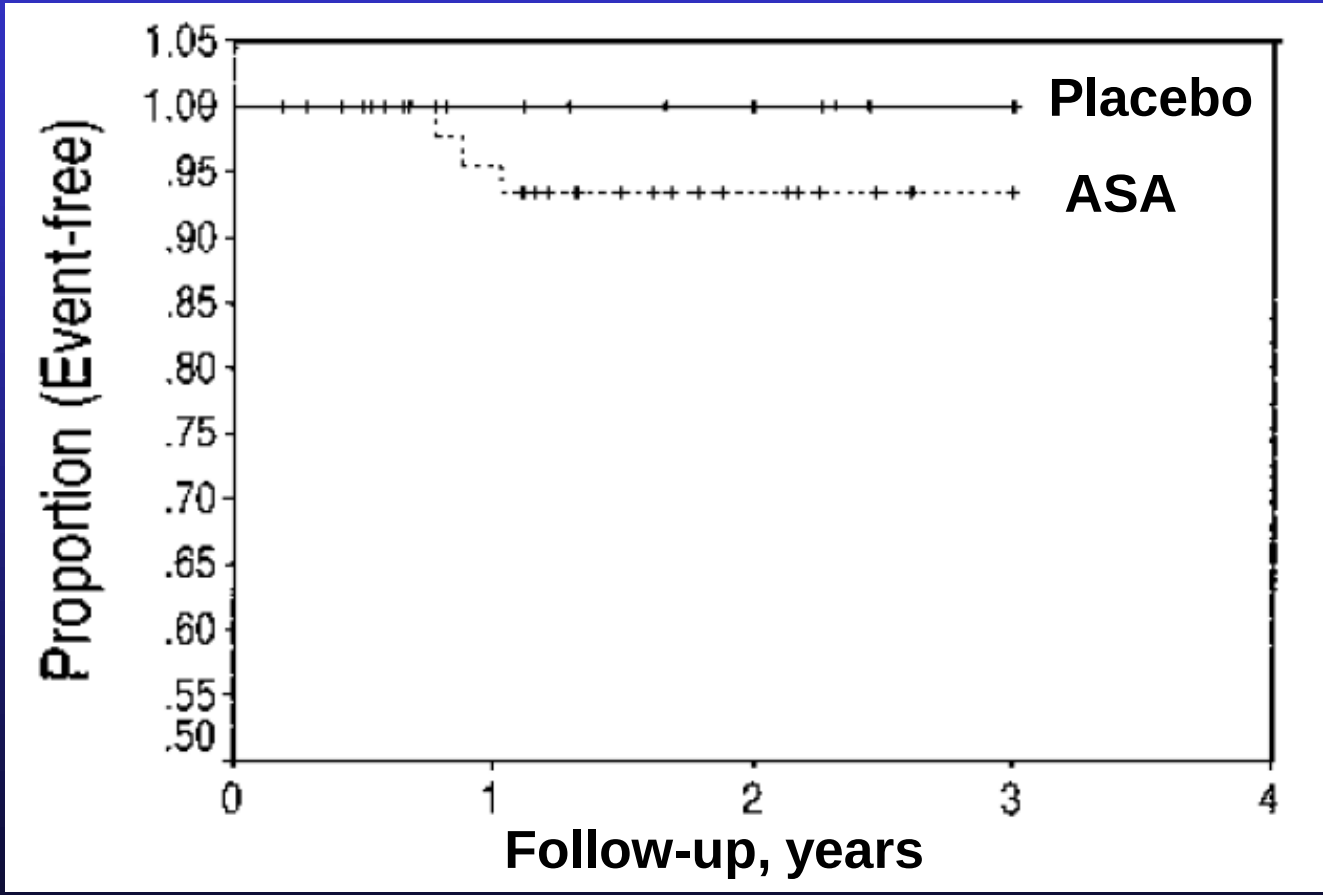



The presence of aPL (LA and/or aCL) in patients with ischemic stroke does not predict:

1. an increased risk for subsequent vascular occlusive events over 2 yr.
2. a differential response to aspirin or warfarin.

APASS Investigators, JAMA 2004;291:576

# Primary Thromboprophylaxis of aPL+ Patients: APLASA Study




Incidence rate of acute thrombosis:

2.75% pt/yr ASA

0% pt/yr Placebo

Erkan et al, Arthritis Rheum 2007;56:2382

# APS: Pregnancy Morbidity

- ≥ 1 unexplained deaths of a morphologically normal fetus at ≥ 10 w of gestation
- ≥ 1 premature births of a morphologically normal neonate < 34 w of gestation
- ≥ 3 unexplained consecutive spontaneous abortions at < 10 w of gestation

Miyakis, JTH 2006;4:295

# Treatments for Women with APS during Pregnancy

| Author, year | Treatments | Results |
|---|---|---|
| Cowchock et al, 1992 | steroid vs heparin | n.s. (75% live births) |
| Kutteh, 1996 | Heparin+low-dose ASA vs ASA | 80% viable infants vs 44% |
| Rai et al, 1997 | Heparin+ASA vs ASA | 71% live births vs 42% |
| Backos et al, 1999 | ASA+LMWH | 71% live births |
| Branch et al, 2000 | IvIg+heparin+low-dose ASA vs heparin+low-dose ASA | n.s. (100% live births) |
| Noble et al, 2005 | LMWH+low-dose ASA vs HUF+low-dose ASA | n.s. (82% live births) |

Erkan et al, Arthritis Rheum 2007;56:2382

# Mouse Models of APS

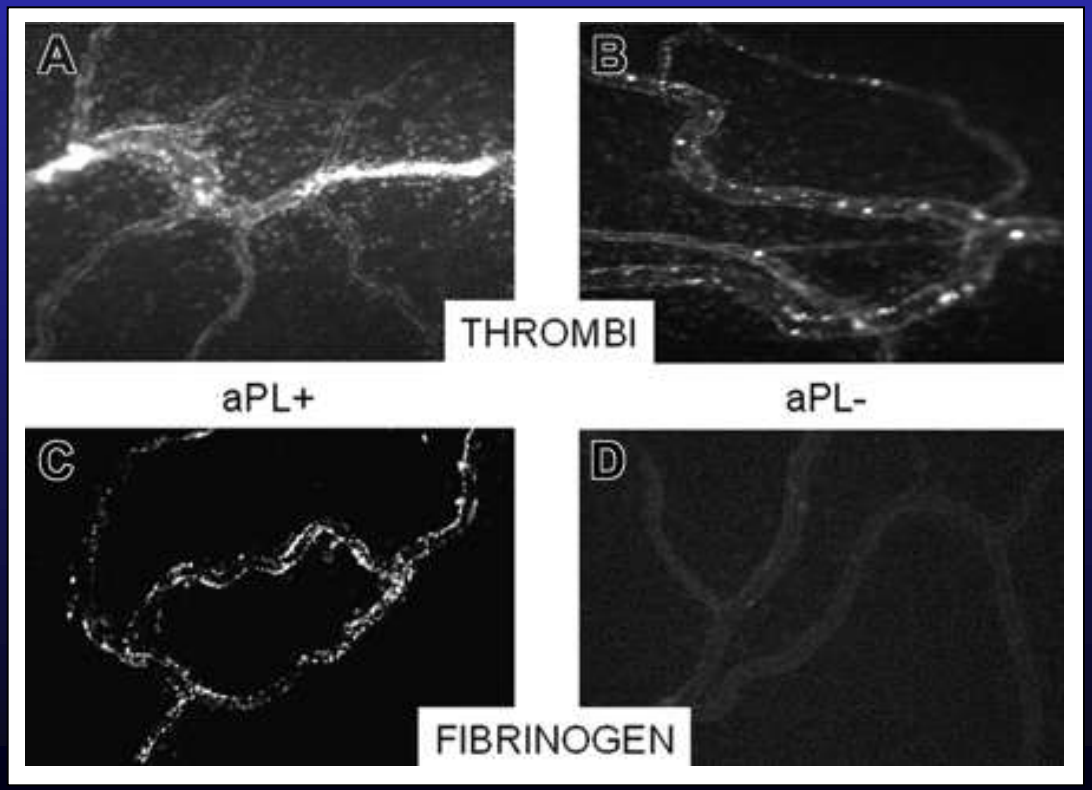
Active & passive immunization of mice induce:

- increased rate of fetal resorption
- thrombocytopenia

Thrombosis is NOT a feature of mouse APS

# aPL Are Thrombogenic in Animal Models *In Vivo*

Injection of aPL antibodies increases thrombus size in arterial & venous models of thrombosis

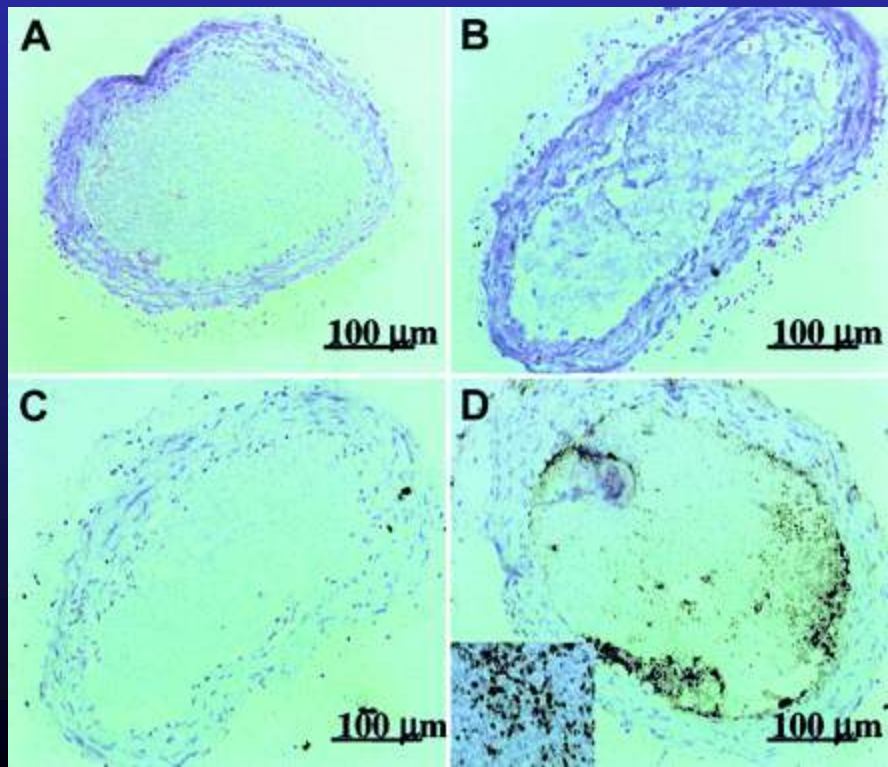



Fischetti F et al, Blood 2005;106:2340

# aPL Are Thrombogenic in Animal Models *In Vivo*

- Vessel injury is a pre-requisite
- Complement activation is needed
- F(ab)2 fragments of IgG are thrombogenic
  (i.e., engagement with Fc receptor NOT necessary)

Control IgG aPL IgG




Emathoxylin-eosin staining

Immunoistochemical staining

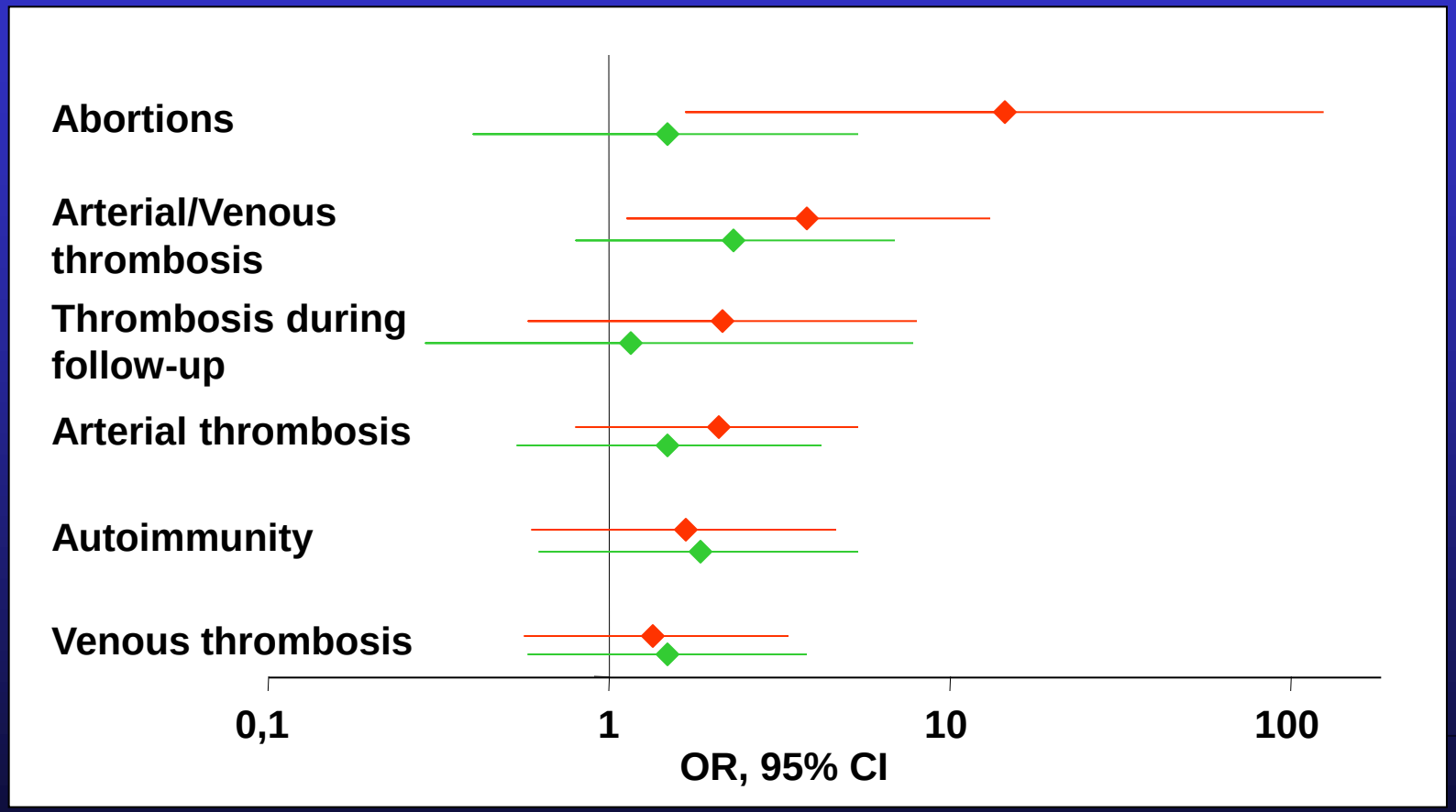
Jankowski M et al, Blood 2003;101:157

# aPL antibodies & Thrombosis:
## Strength of Association, Odds Ratio

| | Cerebral Stroke | Deep Vein Thrombosis | Any Thrombosis |
|---|---|---|---|
| LA | 8.6-10.8 | 4.1-16.2 | 5.7-7.3 |
| IgG ELISAs | | | |
| aCL | n.s.-18 | n.s.-2.5 | n.s.-3.66 |
| aβ2GPI | n.s.-8.3 | n.s.-19 | n.s.-27.1 |

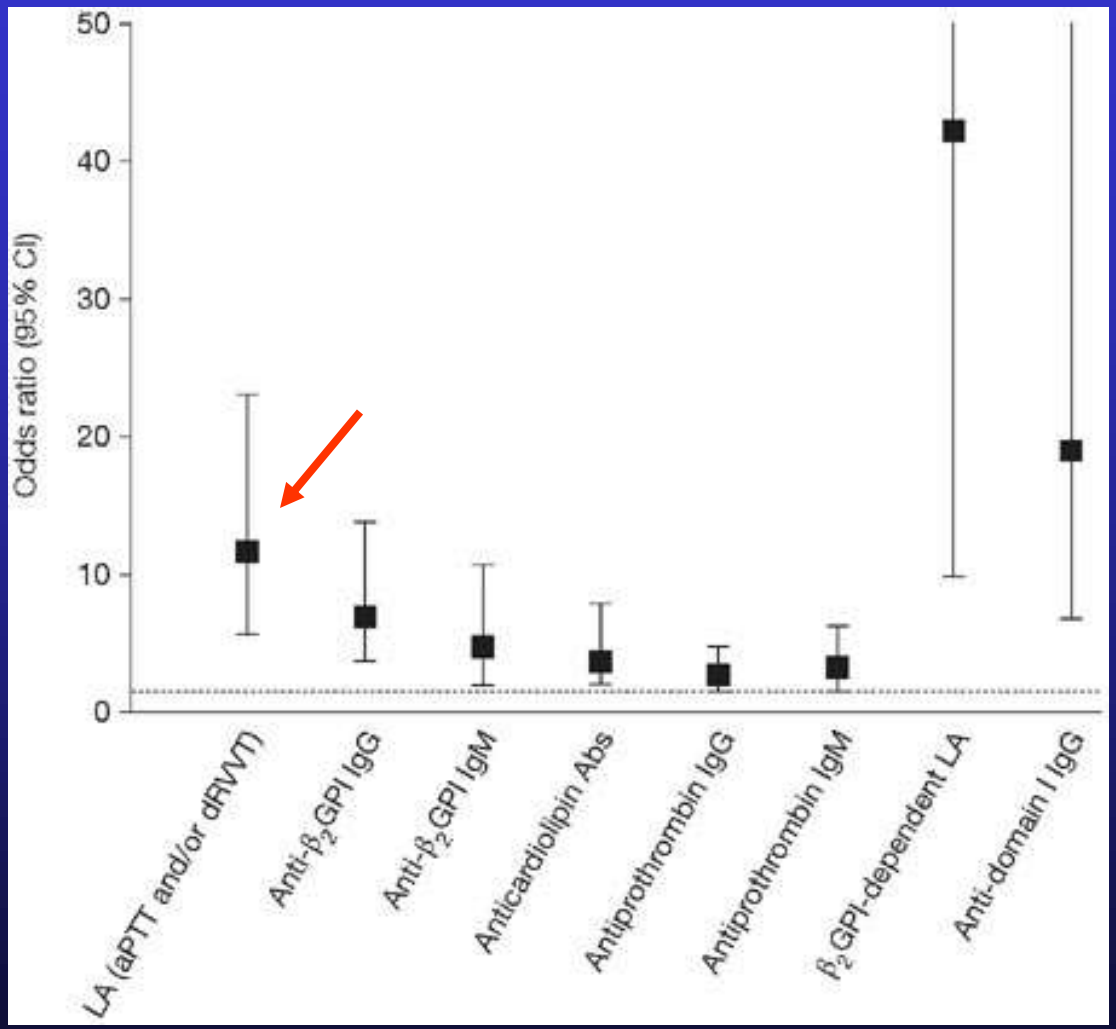
Galli et al, Blood 2003;101:1827 & 102:2717

# Clinical Associations of IgG aβ2-GPI & aCL Antibodies in 112 APS Patients



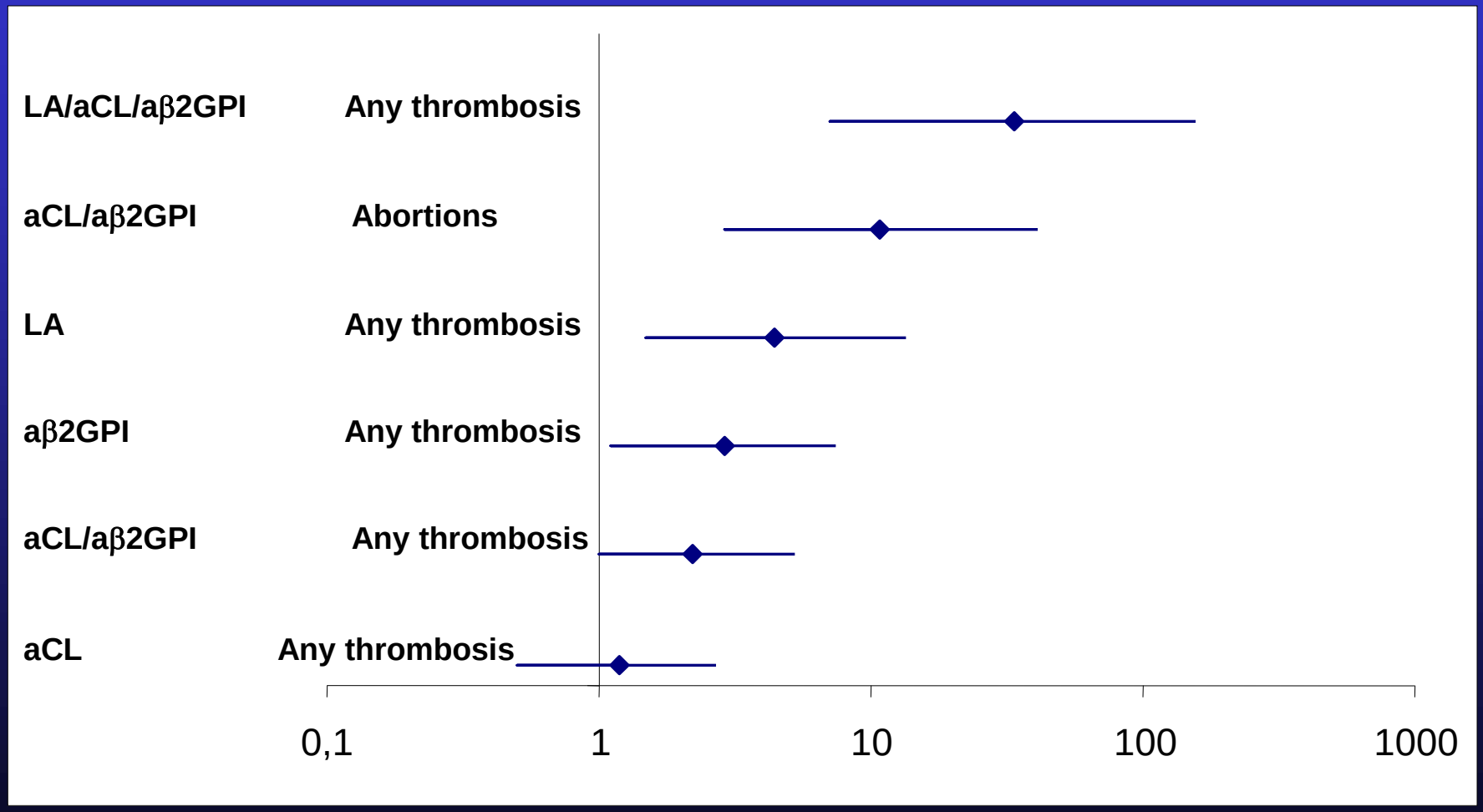

Galli et al, Blood 2007;110:1178

# Correlation between Antibody Specificity & Thrombosis




De Laat et al. Nat Clin Pract Rheumatol. 2008;4:192

# Antiphospholipid Profile & APS: Multivariate Analysis of 100 Patients




Pengo et al, TH 2005;93:1147

# Update on the Classification Criteria of APS: Laboratory

1. LA present in plasma on two or more occasions, at least 12 weeks apart, detected according to the SSC-ISTH guidelines

2. aCL antibodies of IgG and/or IgM isotype, at medium or high titer, on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA for β2GPI-dependent antibodies

3. aβ2GPI antibodies of IgG and/or IgM isotype, in titer > 99th percentile, on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA

Miyakis, JTH 2006;4:295

# Update on the Classification Criteria of APS: Laboratory (Miyakis et al, 2006)

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# SSC Criteria & British Guidelines For LA Testing

1. Prolongation of at least one phospholipid dependent coagulation test.

2. Evidence of inhibitory activity shown by the effect of patient plasma on pooled normal plasma.

3. Evidence that the inhibitory activity is dependent on phospholipids.

4. LA must be carefully distinguished from other coagulopathies that may give similar results or may occur concurrently with LA.

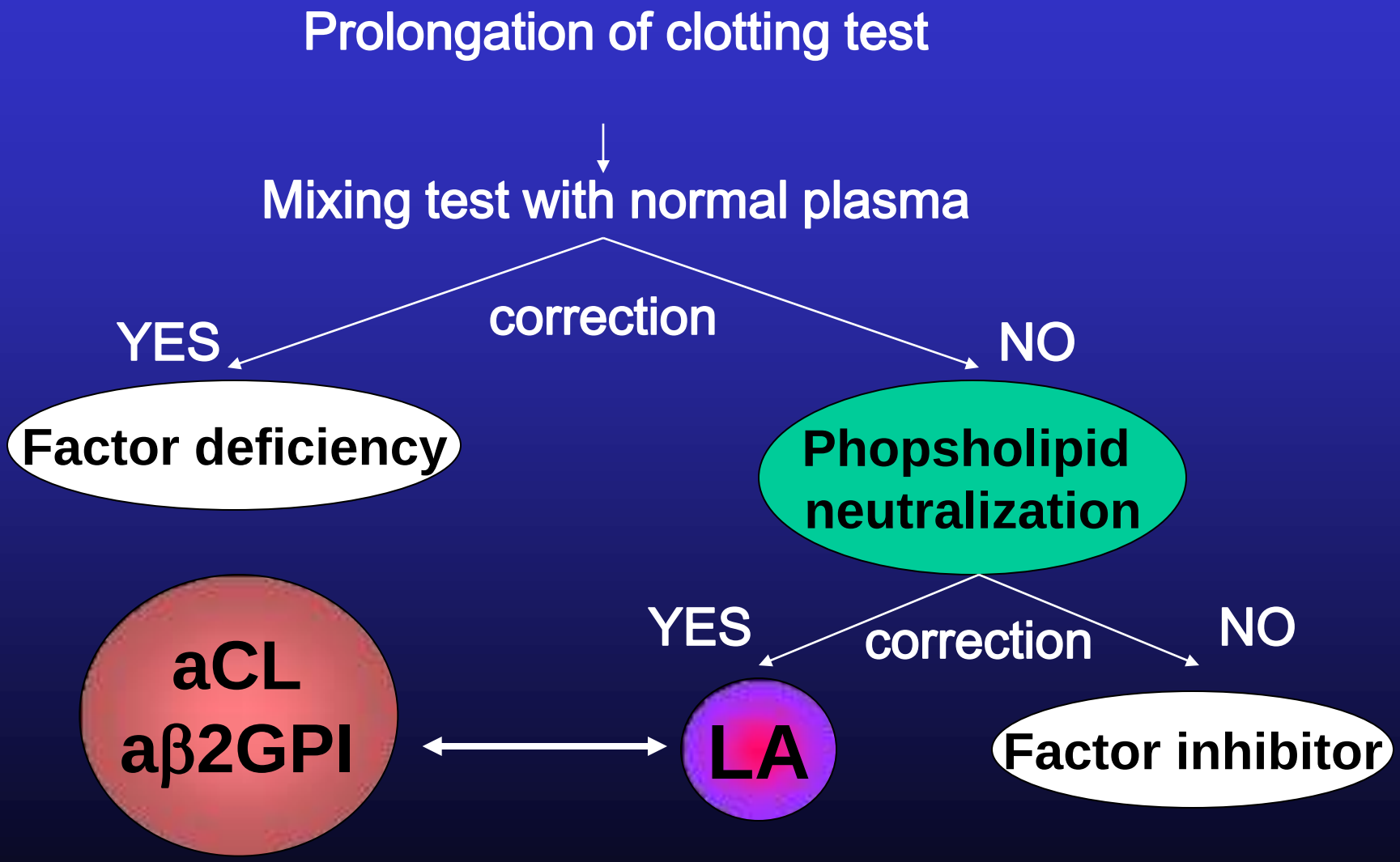
Brandt et al, Thromb Haemost 1995;74:1185; Greaves et al, BJH 2000;109:704

# SSC Recommendation For LA Testing (Brandt et al, 1995)

1. Residual platelet count in plasma <10000/mmc
2. Use the same assay principle for screening & confirmatory test
3. Use routine PT & aPTT to rule out other coagulopathies
4. Solid-phase assays should not be used as confirmatory procedures for LA

Thromb Haemost 1995;74:1185

# Flow Chart for the Diagnosis of LA



# Forthcoming Guidelines For LA Testing
## (Pengo et al, 2009)

1. Patient selection
2. Blood collection
3. Choice of test
4. Mixing test
5. Confirmatory test
6. Expression of results
7. Interpretation of results
8. Transmission of results

JTH 2009;in press

# Forthcoming Guidelines For LA Testing
## (Pengo et al, 2009)

1. Patient selection
   a. unexplained prolonged aPTT
   b. significant probability of APS

      Low: VTE or arterial thrombosis in elderly pts.

      Moderate: recurrent spontaneous pregnancy loss, provoked VTE in young pts.

      High: unprovoked VTE or unexplained arterial thrombosis in young pts.; thrombosis at unusual site; thrombosis or pregnancy morbidity in autoimmune disease; late pregnancy loss.

JTH;in press

# Forthcoming Guidelines For LA Testing
(Pengo et al, 2009)

3. Choice of test
   a. 2 tests based on different principles
   b. dRVVT first test to be used
   c. sensitive aPTT second test to be used
   d. LA present when either test is positive
   e. A screening test is considered positive when the clotting time is above the local cut-off

JTH;in press

# Lupus Anticoagulants & Thrombosis:
## Analysis of coagulation tests in 72 Patients

| Coagulation test | Total | Venous | Arterial |
|---|---|---|---|
| | | Thrombosis | |
| In-house dRVVT | .05 | n.s. | n.s. |
| LA screen | .002 | .03 | n.s. |
| DVV test | .004 | .018 | n.s. |
| Bioclot LA | .025 | n.s. | n.s. |
| In-house KCT | n.s. | n.s. | n.s. |
| Kaoclot | n.s. | n.s. | n.s. |
| CSCT | n.s. | n.s. | n.s. |

Galli et al, TH 2000;84:1012

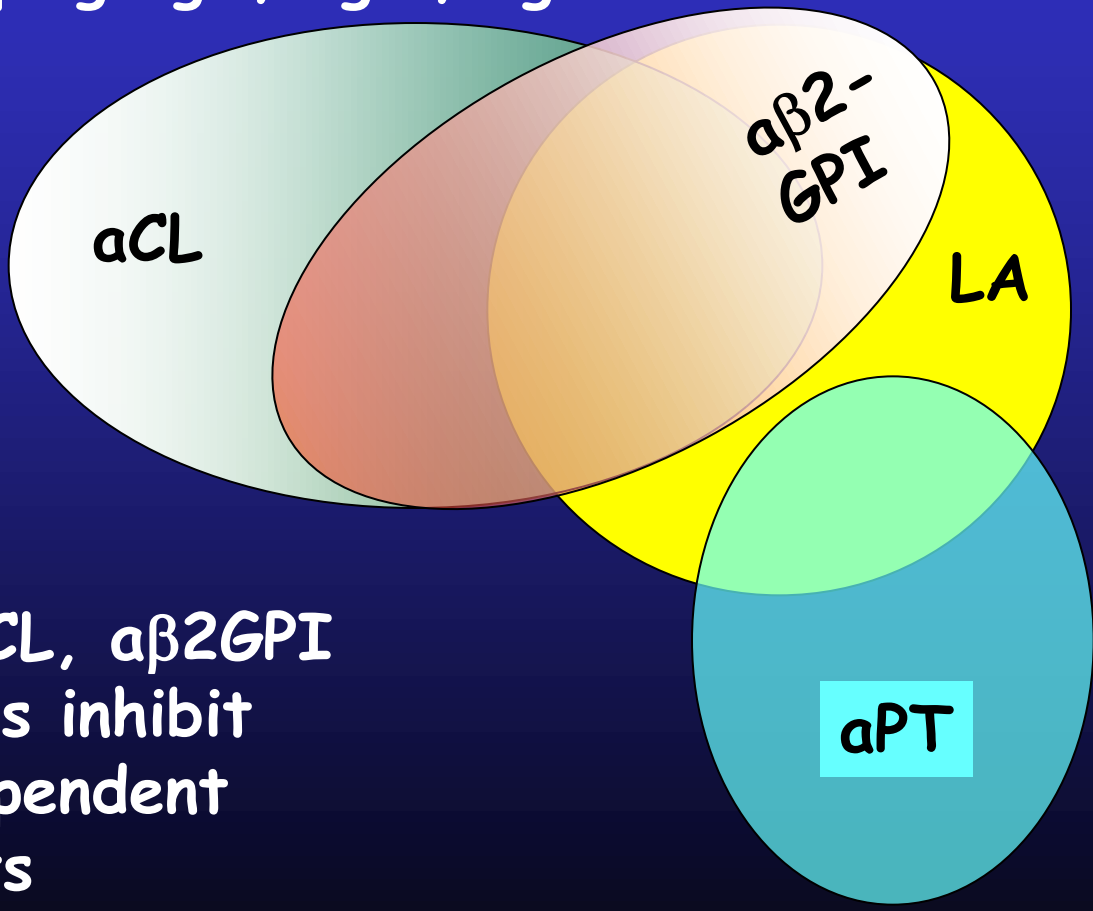
# Antiphospholipid Antibodies

β2-Glycoprotein I
Prothrombin
} Lupus Anticoagulants

High- and low-molecular weight kininogens
Factor XII
Annexin V
(activated) Protein C
Protein S
Thrombomodulin
Tissue plasminogen activator
Oxidised-LDL
Factor VII/VIIa
Complement components H and C4b
EPCR
Anti-tissue protein antibodies

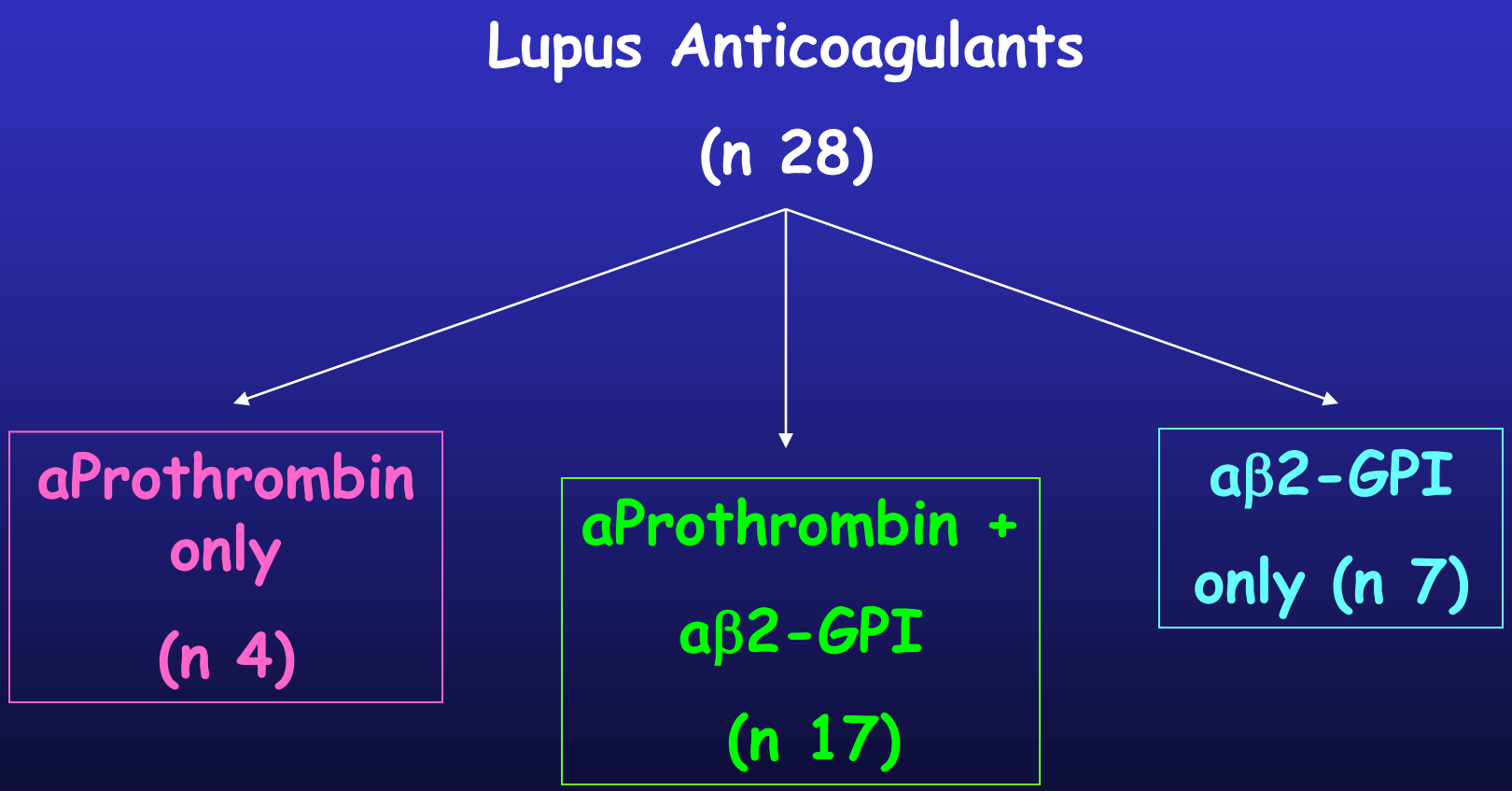
# aPL Antibodies

aCL and aβ2GPI antibodies are strictly linked & partially overlapping IgG, IgM, IgA



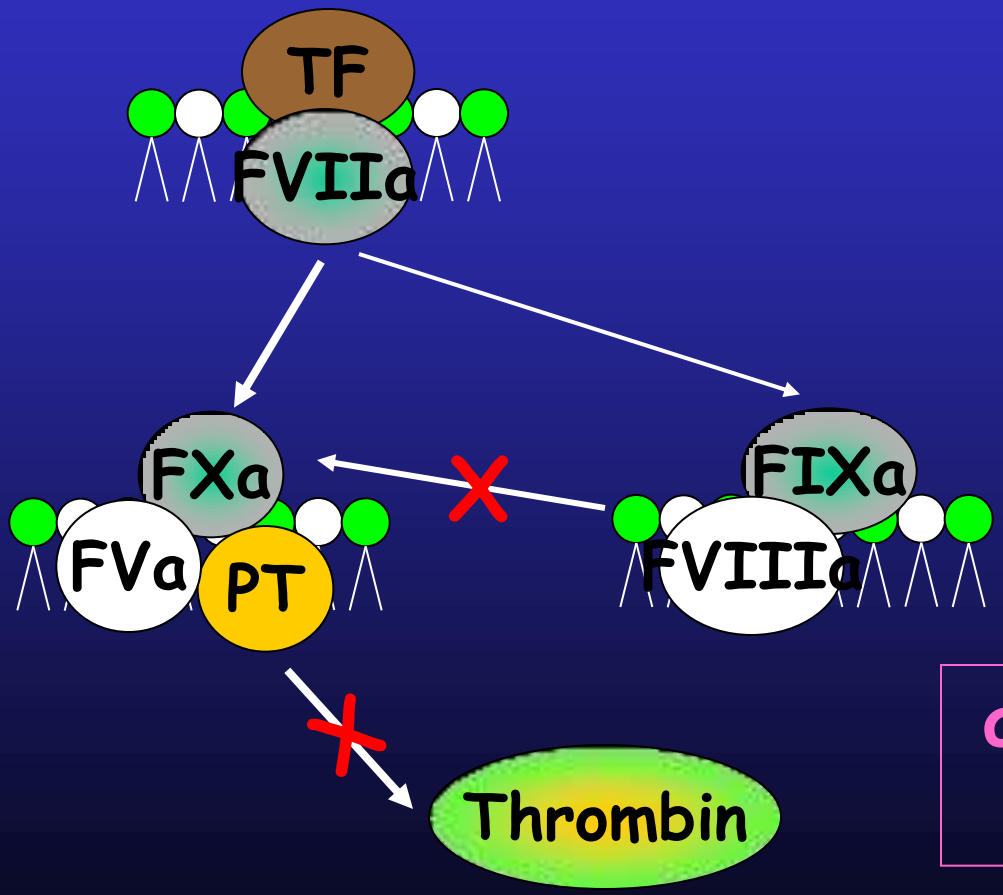

Subgroups of aCL, aβ2GPI & aPT antibodies inhibit phospholipid-dependent coagulation tests

# Contribution of aβ2-GPI and aPT Antibodies to Lupus Anticoagulant Activity




Horbach et al, TH 1998;79:790

# Lupus Anticoagulant Effect of aPT Antibodies




inhibit

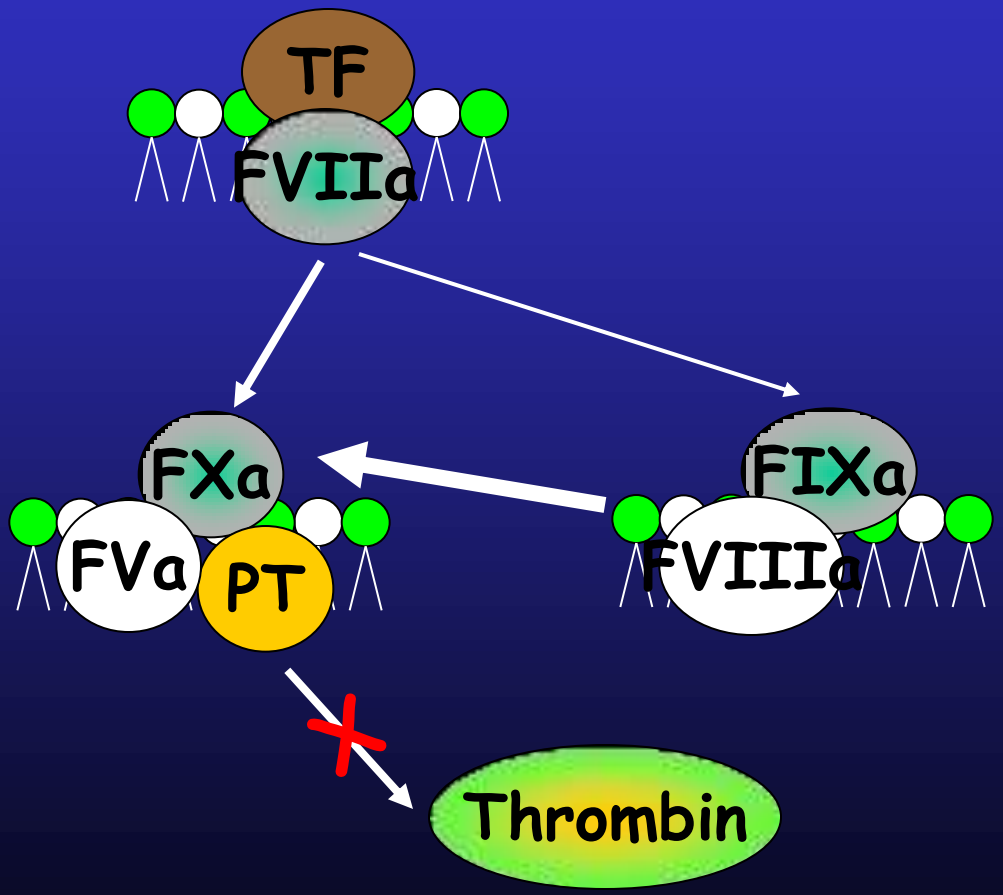
Factor X activation

&

PT activation

aPTT, KCT, CSCT Best

# Lupus Anticoagulant Effect of aβ2-GPI Antibodies




inhibit PT activation

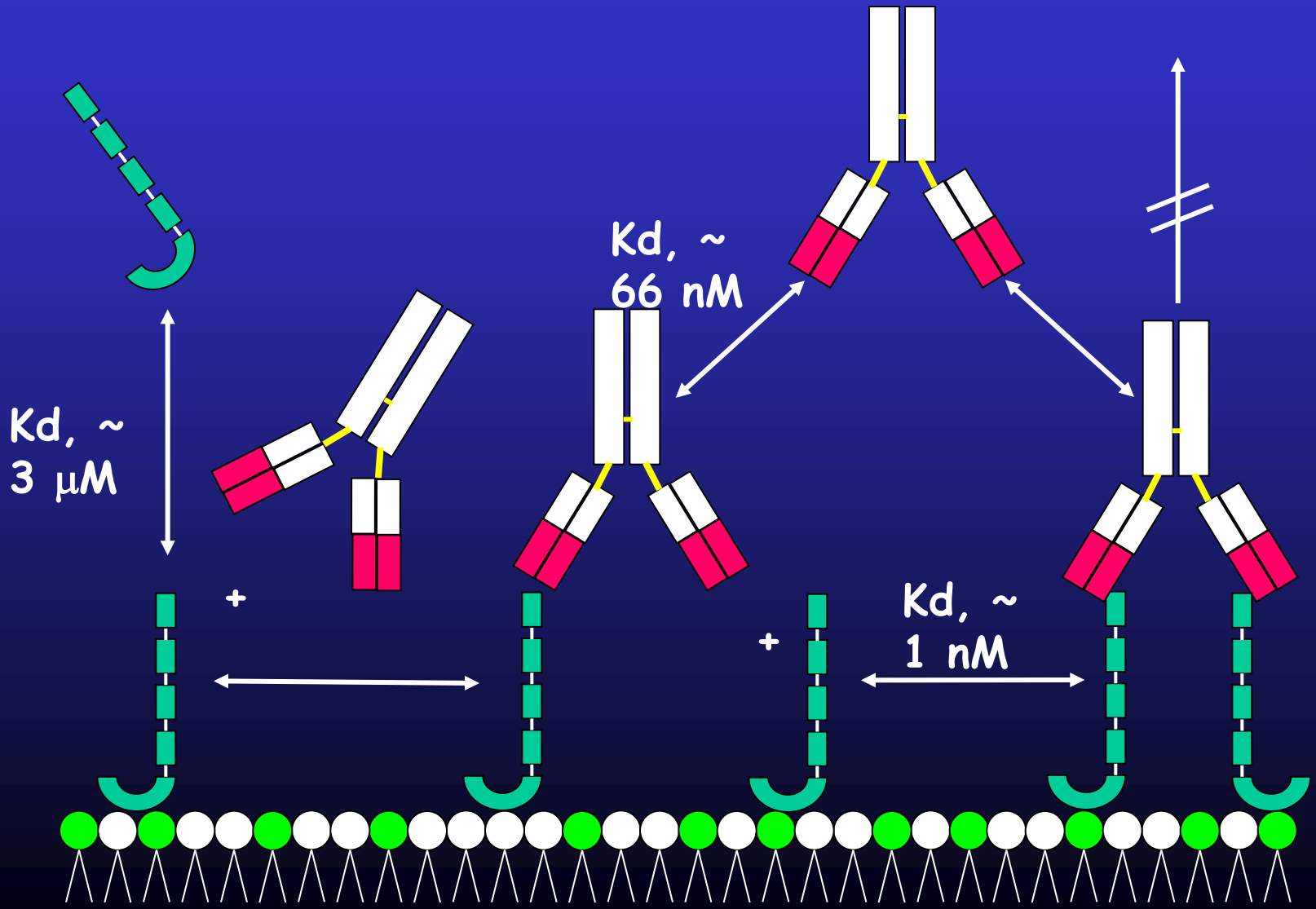
&

stimulate Factor X activation

dRVVT Best

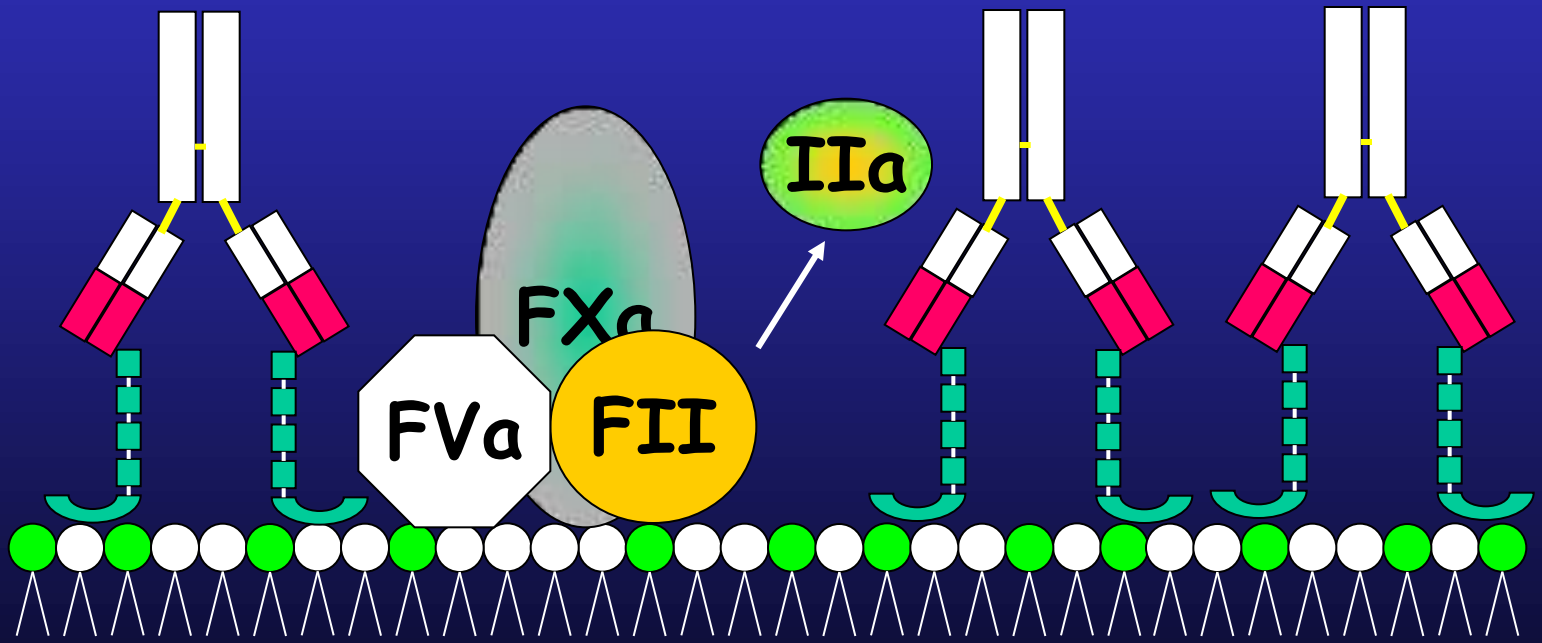
# aβ2-Glycoprotein I Antibodies

Immune recognition upon binding of β2-GPI to a suitable anionic (phospholipid) surface (Willems et al, 1996)



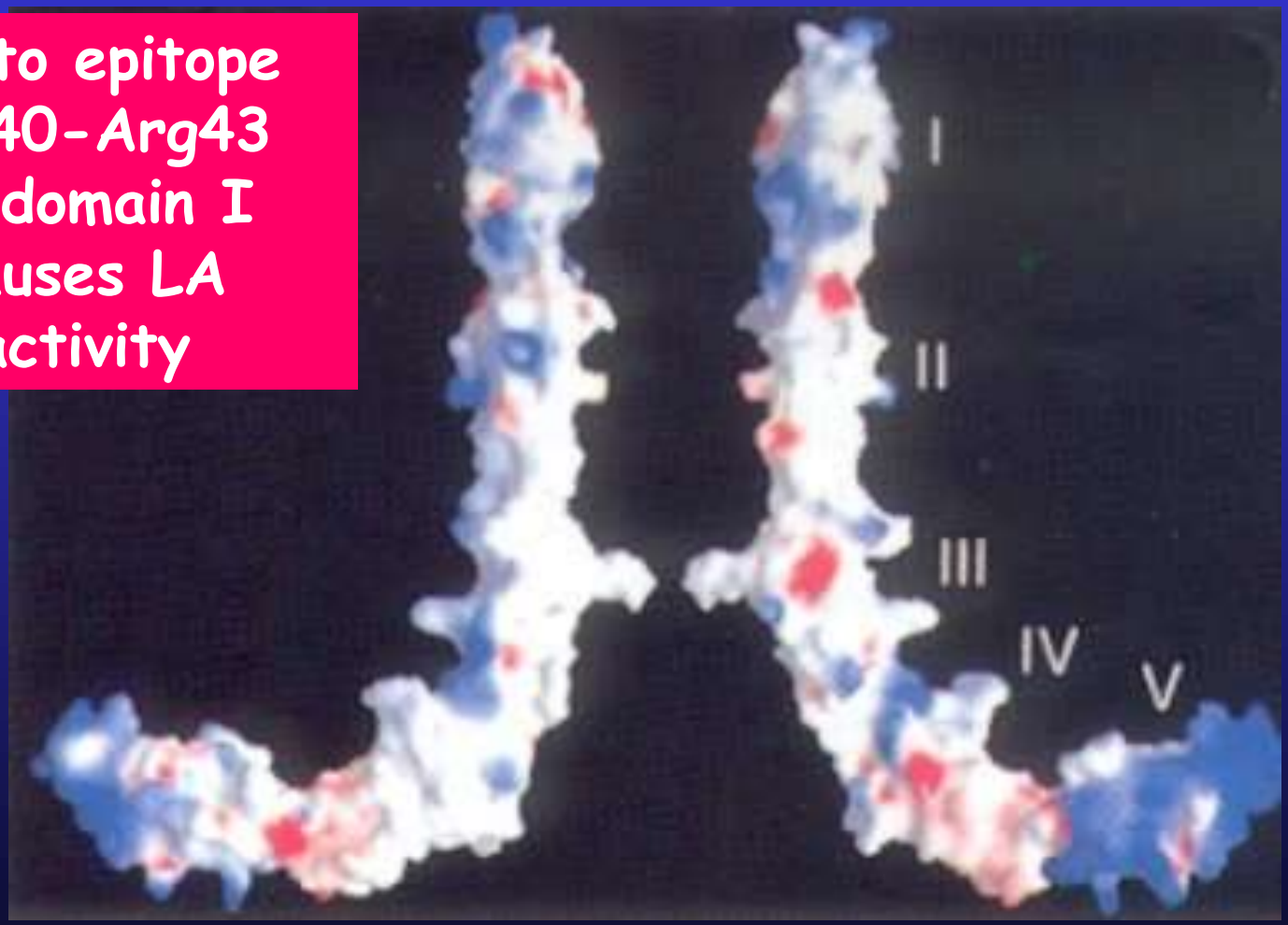
# aβ2-Glycoprotein I Antibodies

Compete with coagulation factors for the anionic phospholipid surface



# β2-Glycoprotein I

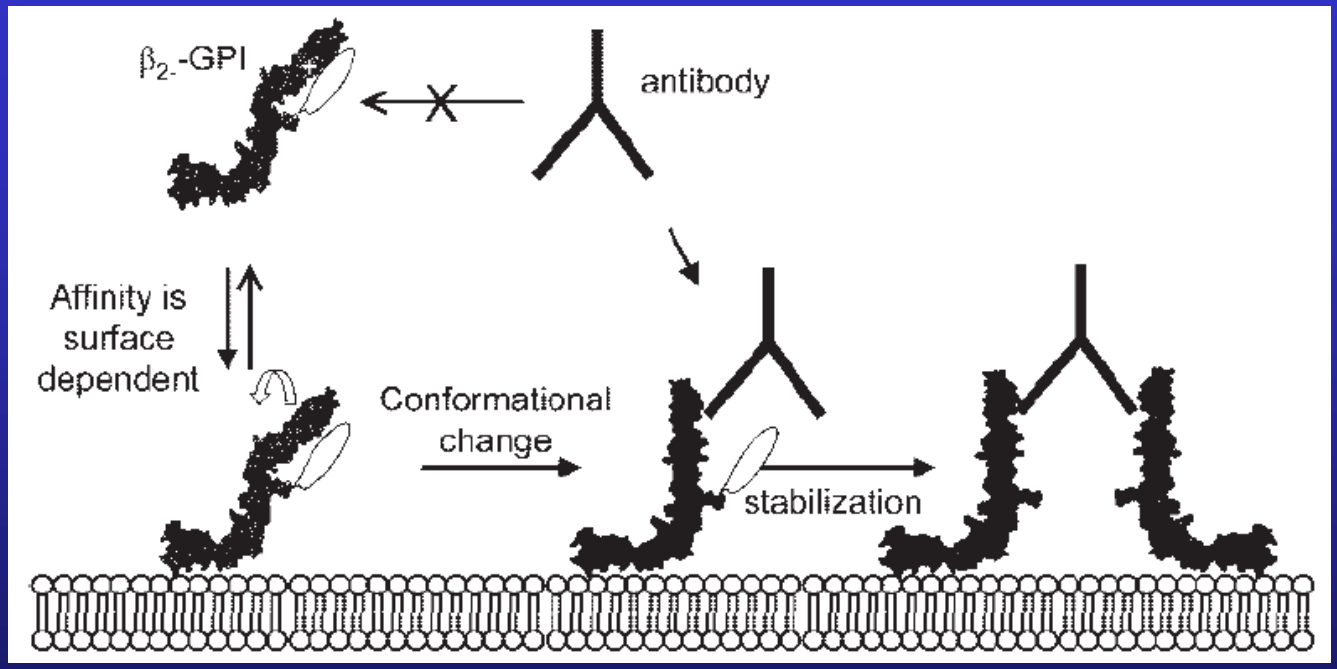
Ab to epitope Gly40-Arg43 on domain I causes LA activity




Bouma et al, 1999; de Laat et al, 2005

# Antibodies to Domain I of β2-GPI

## Immune recognition requires a conformational change




Bind to domain I coated on hydrophobic plates

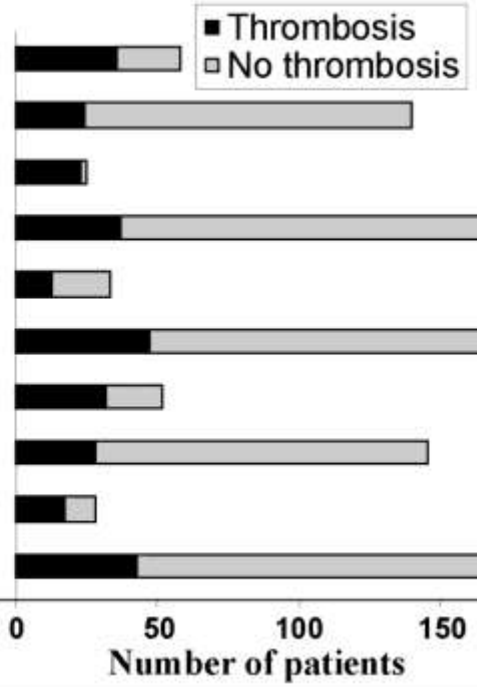
Have β2-GPI-dependent LA activity

Correlate with thrombosis, OR 18.9 (95% CI 6.8-53.2)

de Laat et al, Blood 2005;105:1540 & 2006;107:1916

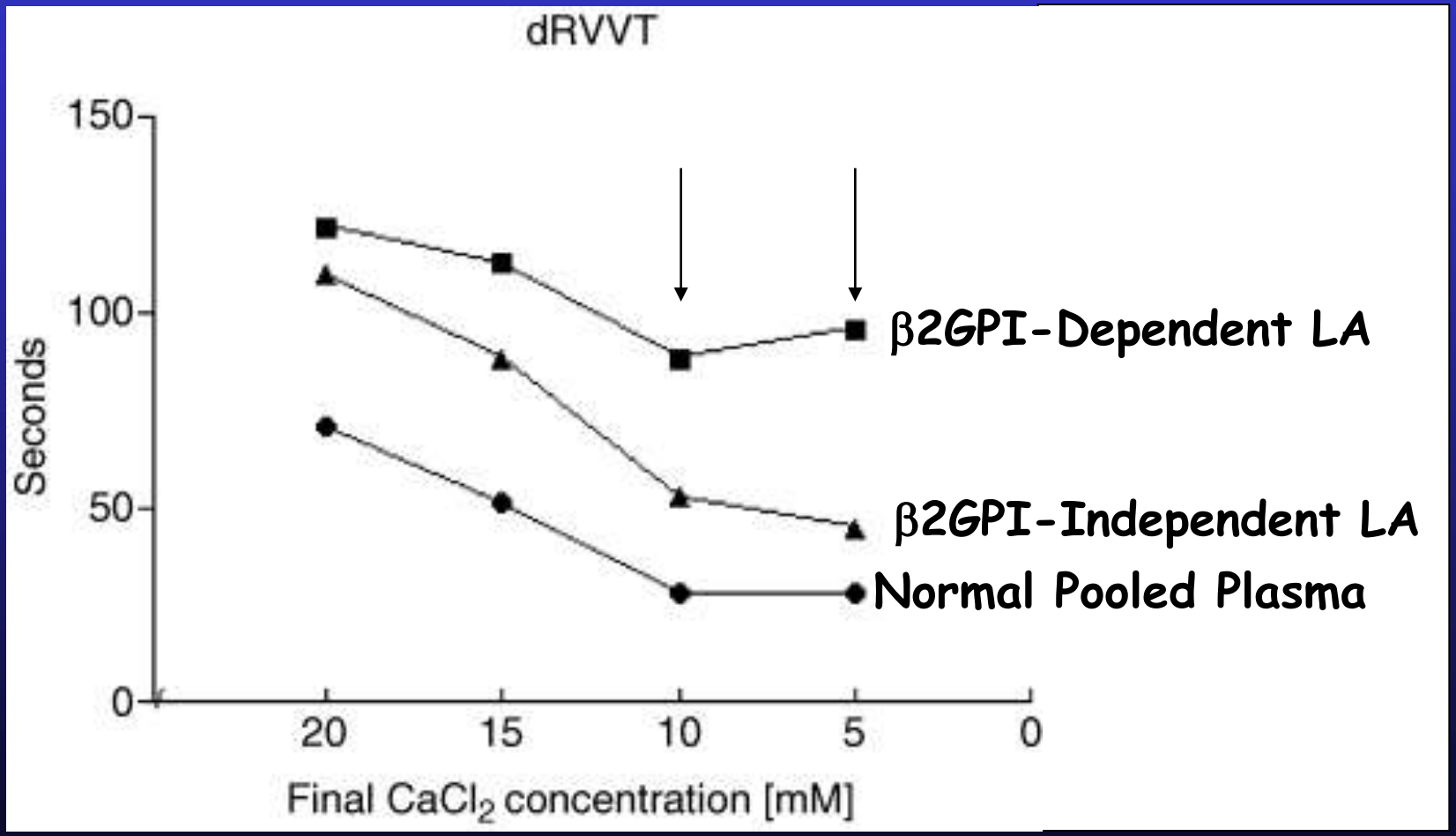
# aβ2-GPI-Dependent LA Activty is Associated with Thrombosis

| Test | result | n | Thrombosis (n) | No thrombosis (n) | Pos. predictive value | Neg. predictive value |
|---|---|---|---|---|---|---|
| *Original PTT-LA* | + | *58* | 36 | 22 | 0.62 | |
| | - | *140* | 24 | 116 | | 0.83 |
| *$\beta_2$ - GPI dependent LAC* | + | *25* | 23 | 2 | 0.92 | |
| | - | *173* | 37 | 136 | | 0.79 |
| *$\beta_2$ - GPI independent LAC* | + | *33* | 13 | 20 | 0.39 | |
| | - | *166* | 47 | 119 | | 0.72 |
| *Anti-$\beta_2$ -GPI IgG antibodies* | + | *52* | 32 | 20 | 0.61 | |
| | - | *146* | 28 | 118 | | 0.81 |
| *Anti-$\beta_2$ -GPI IgM antibodies* | + | *28* | 17 | 11 | 0.61 | |
| | - | *170* | 43 | 127 | | 0.75 |



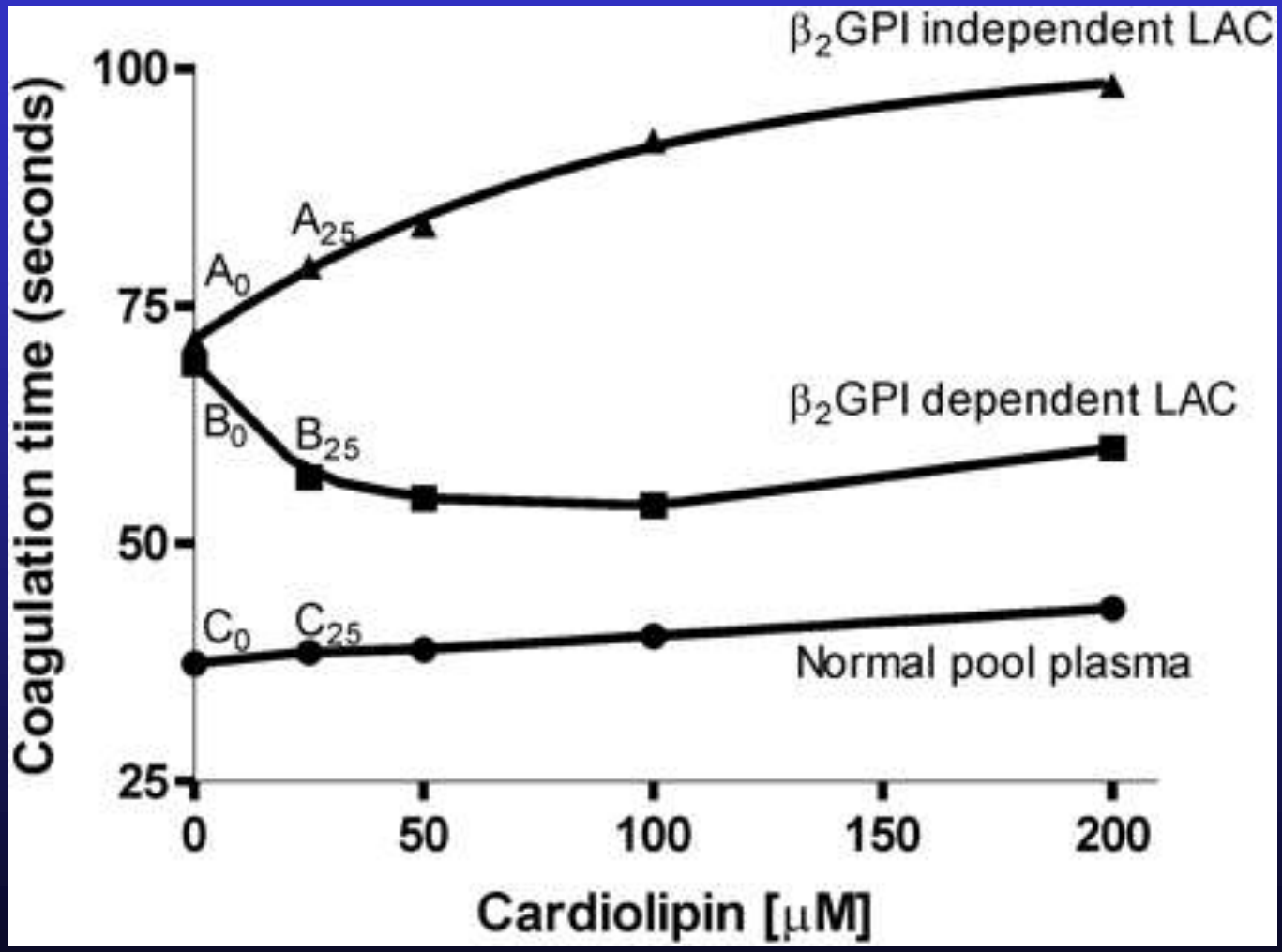

De Laat et al, 2004

# Effect of Calcium on LA Activty of aβ2-GPI antibodies




Pengo et al, 2003

# Effect of Cardiolipin on LA Activty of aβ2-GPI antibodies




De Laat et al, 2004

# Conclusions - Future Perspectives

The diagnosis and treatment of APS requires a thorough laboratory effort to identify the various aPL antibodies.

Such a correct identification is relevant to establish the risk of first thrombosis or recurrence of (thrombotic/obstetrical) event.

In the coming years newer assays will help to reduce the presently cumbersome laboratory patient's workout.