The antiphospholipid syndrome

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No financial disclosure

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• 2 patients with SLE
• Peculiar hemorrhagic disorder
• Prolongation of clotting times
• No correction after mixing
• No inhibition of thrombin time
• Stable at 65 ºC
• Unstable at 80 ºC
• Not dialyzable
• False positive syphilis test

➤ Autoimmune disease

➤ Lupus anticoagulant

➤ Antibody

➤ Anti-Cardiolipin Antibodies
The first study in a larger populations showing the correlation between thrombosis and prolongation of clotting assays

(8 patients with circulating anticoagulant, of these 4 had thrombosis)

**Thrombosis in systemic lupus erythematosus** despite circulating anticoagulants

E. J. WALTER BOWIE, JOHN H. THOMPSON, JR., CHRIS A. PASCUZZI, and CHARLES A. OWEN, JR. Rochester, Minn.

Patients with antiphospholipid antibodies have often a positive VLDR tests $\rightarrow$ antibodies against cardiolipin

The possibility to measure antiphospholipid antibodies with an ELISA makes the measurement of antiphospholipid antibodies applicable in many laboratories

1985: Birth of the syndrome

A patient with thrombosis
+ Persistent LA and / or anticardiolipin antibody positive

A patient with recurrent pregnancy loss
+ Persistent LA and / or anticardiolipin antibody positive

Diagnosis

ANTI-PHOSPHOLIPID SYNDROME

Hughes GRV
Clin Exp Reumatol 1985; 26: 324
May occur in any vessel

Most frequently afflicted vessels:

- deep venous thrombosis, pulmonary emboli
- cerebral vasculature (TIA, stroke)
<table>
<thead>
<tr>
<th>Thrombotic</th>
<th>Non-thrombotic</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Thrombotic Image" /></td>
<td>Inhibition of implantation and placentation by effects on apoptosis and mitosis?</td>
</tr>
</tbody>
</table>

**Pregnancy complications in APS**
APS is a misnomer

Anti-phospholipid antibodies are not directed against phospholipids but to proteins that bind to negatively charged phospholipids.

Antibodies against $\beta_2$-glycoprotein I are generally accepted as the clinically most relevant antibodies.

*Bouma et al. EMBO J 1999; 18: 5166*
A patient with:
1. thrombosis
   recurrent pregnancy loss
   &
2. lupus anticoagulant
   anti-cardiolipin antibodies
   anti-β₂-glycoprotein I antibodies

ANTE- PHOSPHOLIPID
SYNDROME
Preliminary classification criteria

- evidence of involvement $\geq$ 3 organs, systems and/or tissues
- development of manifestations simultaneously or $< 1$ week
- confirmation by histopathology of small vessel occlusion in at least one organ or tissue
- persistent aPL

Asherson et al. Lupus 2003; 12: 530
## Non-criteria aPL associations

<table>
<thead>
<tr>
<th>manifestation</th>
<th>aPL association</th>
</tr>
</thead>
<tbody>
<tr>
<td>thrombocytopenia</td>
<td>strong</td>
</tr>
<tr>
<td>nephropathy</td>
<td>strong</td>
</tr>
<tr>
<td>heart valves</td>
<td>strong</td>
</tr>
<tr>
<td>livedo reticularis</td>
<td>strong</td>
</tr>
<tr>
<td>giant ulcers</td>
<td>strong</td>
</tr>
<tr>
<td>cognitive dysfunction</td>
<td>strong</td>
</tr>
<tr>
<td>transverse myelitis</td>
<td>strong</td>
</tr>
<tr>
<td>chorea</td>
<td>strong</td>
</tr>
<tr>
<td>hemolytic anemia</td>
<td>weak</td>
</tr>
<tr>
<td>migraine</td>
<td>very weak</td>
</tr>
<tr>
<td>multiple sclerosis</td>
<td>very weak</td>
</tr>
</tbody>
</table>
Thrombocytopenia in APS

Risk factors for thrombosis in lupus patients


Thrombocytopenia:

A serious candidate to be included in the criteria for APS

### Magnitude of the problem


LAURA ANDREOLI, CECILIA B. CHIGHIZOLA, ALESSANDRA BANZATO, GUILLERMO J. PONS-ESTEL, GUILHERME RAMIRE DE JESUS, AND DORUK ERKAN, ON BEHALF OF APS ACTION

<table>
<thead>
<tr>
<th>Condition</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy morbidity</td>
<td>6%</td>
</tr>
<tr>
<td>Pregnancy loss</td>
<td>9%</td>
</tr>
<tr>
<td>Stroke</td>
<td>14%</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>11%</td>
</tr>
<tr>
<td>Deep venous thrombosis</td>
<td>10%</td>
</tr>
</tbody>
</table>

Systemic literature review of 120 general population studies
Limitations

- Standardization of the assays to measure the auto-antibodies.
- Cut-off (low titer antibodies).
- Presence of antibodies in healthy individuals.
- No conformation in a second sample.
- Thrombosis and pregnancy complications are multifactorial.
- Small and selected studies.
- Auto-immunity without antiphospholipid antibodies is a risk for thrombosis.
- Retrospective studies.

The true frequency of clinically significant antiphospholipid antibodies is difficult to determine.
What are clinical significant antibodies?

• Type of antiphospholipid antibody?
  • Cardiolipin $\leftrightarrow$ $\beta_2$-Glycoprotein I $\leftrightarrow$ lupus anticoagulant

• Antibody isotype?
  • IgG $\leftrightarrow$ IgM $\leftrightarrow$ IgA

• Titer of the antibodies?
  • High titer $\leftrightarrow$ low titer

• Additional risk factors.
  • Only significant in combination with other risk factors?
Relevance of antibodies

LA, anti-cardiolipin antibodies and anti-\( \beta_2 \)glycoprotein I antibodies are antibodies with overlapping specificity but they are not identical antibodies.

LA, anti-cardiolipin antibodies and anti-\( \beta_2 \)glycoprotein I antibodies are antibodies with overlapping specificity but they are not identical antibodies.
One of our own studies

RATIO study: Ischemic stroke in young women (<50 years)

Only lupus anticoagulant correlates strongly with stroke

Urbanus et al. Lancet Neurol. 2009; 8: 998
Clinical significant antibodies

- Medline searches of retrospective studies have shown that lupus anticoagulant is the assay of choice
- Additional studies have confirmed these publications
- How about prospective studies?
Antiphospholipid antibody profile thrombotic risk

- Lupus anticoagulant
- Anti-β₂-glycoprotein I antibodies
- Anticardiolipin antibodies

HR: 3.9 (1.1-14)

Antiphospholipid antibody profile adverse pregnancy outcome

Lupus anticoagulant

Anti-\(\beta_2\)-glycoprotein I antibodies

Anticardiolipin antibodies

Triple positivity

Pengo et al. Blood 2011

Lupus anticoagulant

Anti-β2-glycoprotein I antibodies

Anticardiolipin antibodies

events per 100 pt/ys

Normal subjects  Carriers single-positivity  Carriers triple positivity

Pengo et al. Blood 2011
Triple positivity?

- ELISAs are more sensitive to pick up antibodies → Only high titer antiphospholipid antibodies that also induce a lupus anticoagulant are clinically relevant antibodies?

- Only those antibodies that recognize antigens involved in all three assays are relevant?

- More than one type of antibody involved?

- ?
Risk factor for thrombosis

• Retrospective and prospective studies agreed that lupus anticoagulant correlates best with the clinical manifestations.

• The ‘ELISAs’ are often positive in healthy individuals.

• The highest correlation is found when all three assays are positive.

• Single positivity for anti-cardiolipin antibodies (as measured with the current assays) does not correlate with the clinical manifestations.

• IgG > IgM > IgA

• Titer is important.

• Higher risk in combination with other risk factors.
  • Erkan et al. Arthritis Rheum 2007; 56: 2382
Individuals at risk

- High probability
  - *Unprovoked VTE*
  - *Arterial thrombosis at the age < 50 years*
  - *Thrombosis or fetal loss in patients with autoimmune disease*
  - *Thrombosis at unusual sites*

- Moderate probability
  - *Accidentally found prolonged APTT in asymptomatic subjects*
  - *provoked VTE in young patient*
  - *recurrent spontaneous early pregnancy loss*

- Low probability
  - *Elderly patients*
One of the many challenges:

Lupus anticoagulant correlates strongly with thrombosis.

Lupus anticoagulant is caused by antibodies directed against $\beta_2$-glycoprotein I or prothrombin.

Antibodies against $\beta_2$-glycoprotein I or prothrombin hardly correlate with thrombotic complications.

Are anti-$\beta_2$-glycoprotein I antibodies passive bystanders or responsible for the observed clinical complications?
Anti-\(\beta_2\)Glycoprotein I antibodies and thrombosis

Thrombus formation induced by antibodies to \(\beta_2\)-glycoprotein I is complement dependent and requires a priming factor

Fabio Fischetti, Paolo Durigutto, Valentina Pellis, Alessandra Debeus, Paolo Macor, Roberta Bulla, Fleur Bossi, Federica Ziller, Daniele Sibatiero, Pierluigi Maroni and Francesco Tedesco

A

No. of thrombi

No. of vessels examined

B

% of occluded vessels

Minutes

D

- anti \(\beta_2\)GPI

+ anti \(\beta_2\)GPI

C

+ anti \(\beta_2\)GPI

- anti \(\beta_2\)GPI
Patient-derived auto-antibodies specific for β₂-glycoprotein I enhanced dose-dependently a thrombotic response in a mouse model of APS.
IgG antibodies that recognize epitope Gly40-Arg43 in domain I of beta 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis.

de Laat B, Derksen RH, Urbanus RT, de Groot PG.

Anti-domain I antibodies: a high specificity but a low sensitivity
Pathological antibodies against first domain of $\beta_2$Glycoprotein I

There is now a significant amount of evidence that auto-antibodies against β₂-glycoprotein I are important players in the increased risk of thrombotic complications in APS.

Auto-antibodies against β₂-glycoprotein I consist of various subpopulations with different domain specificity.

Antibodies directed against domain I seem to be one of the villains.
• There are no circulating antibody-β₂-glycoprotein I complexes in patients with APS.

• The levels of plasma β₂-glycoprotein I are similar in patients with APS and controls.
Auto-antibodies directed against β₂-glycoprotein I

- recognize β₂GPI when bound to a surface
- Do not recognize β₂GPI in solution

The epitope recognized by the pathological auto-antibodies is cryptic!

_Autoimmune antiphospholipid antibodies are directed against a cryptic epitope expressed when beta 2-glycoprotein I is bound to a suitable surface._

_Pengo V, Blasiolo A, Fior MG._
A hidden epitope?

Antibody binding site
Arg39-Arg43

Crystal structure of $\beta_2$-glycoprotein I
Monoclonal antibodies with specificity for different domains of β₂-glycoprotein I can induce a major conformational change.

Agar et al. Blood 2010
Can low affinity patient antibodies also open up \( \beta_2 \)-glycoprotein I?

No, there is a need for a second hit.
Exposure of anionic phospholipids can be a second hit.

We postulate that a second hit is necessary to allow recognition of $\beta_2$-Glycoprotein I by the auto-antibodies.
What are the consequences of the conformational change within $\beta_2$-glycoprotein I for the development of anti-$\beta_2$-glycoprotein I antibodies
Induction of antiphospholipid antibodies

• Secondary to auto-immunity
  • Highest frequency of thrombotic complications

• Secondary to Infections
  • Bacteria
  • Viruses

• Association with malignancies
  • Both solid tumors and hematological neoplasms.
  • May contribute to the increased thrombotic risk in these patients.

• Association with certain drugs
  • Chlorpromazine
    • IgM, no relation to thrombosis
  • Procainamide
    • IgG, higher incidence of thrombosis
  • Quinidine/quinine
Four proteins from *S. Pyogenes* were identified that binds to β2-GPI:

SclA, SclB, protein H, protein M1
Interaction of $\beta_2$-Glycoprotein I with *S*. *pyogenes* proteins

Four different *S. pyogenes* surface proteins were identified that can bind $\beta_2$-glycoprotein I.
What causes the conformational change in \( \beta_2 \)GPI?

Streptococcal surface proteins

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Van Os et al J Thromb Haemost 2011
Induction of anti-domain I antibodies by injection of protein H only!

Auto-antibodies directed against domain I of $\beta_2$GPI

In contrast to most of the identified thrombotic risk factors, the presence of antiphospholipid antibodies is a risk factor for all types of thrombosis.

1. Antiphospholipid antibodies disturb the hemostatic balance in more than one way

2. The consequences of the presence of these antibodies for the hemostatic balance are unique

3. A general consensus is that the presence of these antibodies resulted in cell activation (e.g. tissue factor expression)
Pathophysiology

Anti-β₂-glycoprotein I antibodies

Endothelial cells
Upregulation
Tissue factor

Monocytes
Upregulation
Tissue factor

Platelets
Thromboxane synthesis

Prothrombotic state

Second hit

Other procoagulant conditions
(inflammation, smoking etc)

Thrombosis

Adapted from Ruiz-Irastorza et al. 2010
Mechanism of disease

Activation of monocytes, endothelial cells and platelets
Induction of a proinflammatory and prothrombotic phenotype
Many competing mechanisms have been proposed to explain the pathologic mechanism.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Target cell</th>
<th>In vitro experimental evidence</th>
<th>In vivo experimental evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin A2</td>
<td>Endothelial cells, monocytes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>GPIbα</td>
<td>Platelets</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>LRP8</td>
<td>Endothelial cells, monocytes, platelets</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>TLR2</td>
<td>Endothelial cells and monocytes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>TLR4</td>
<td>Endothelial cells, monocytes, trophoblasts</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>TLR8</td>
<td>Monocytes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
A model for antiphospholipid antibody mediated cell activation

anti-β₂-glycoprotein I antibody

stabilization by antibody

β₂-glycoprotein I

phospholipid binding and conformational change

GPIIbα

LRP8

Annexin A2

TLR2/4/8

Activation of endothelial cells, monocytes, platelets
Induction of a proinflammatory and prothrombotic phenotype

de Groot P G , and Urbanus R T Blood 2012;120:266-274
Cellular activation by anti-\(\beta_2\)Glycoprotein I antibodies
• The presence of lupus anticoagulant is strongly associated with a history of thrombo-embolic complications and fetal losses.

• Is there a need for more intensive treatment compared to thrombotic complications causes by other risk factors or idiopathic?

• The treatment of APS patients with LMWH +/- aspirin is successful in >70% of the pregnancies.
• In the last decade more reviews on treatment of APS were published than original papers.

• It is essential that studies on thrombotic risk factors of aPL take other risk factors into account.

• In presence of multiple thrombotic risk factors it is difficult to discern the one that “caused” thrombosis.

• Data on factor V Leiden indicate that a risk factor for a first venous thrombosis is NOT necessarily a risk factor for recurrence!
The strongest risk factor for recurrence is the prior VTE event itself, particularly if idiopathic


5 years risk for recurrent thrombosis after withdrawal of anticoagulants:
about 20%


Pengo et al. Blood 2011
secondary prophylaxis

- INR 2.0-3.0 as effective as INR 3.1-4.0
- VKA for “prolonged period or for life” is current practice
- Long turn LMWH can be an alternative

primary prophylaxis

- asymptomatic carriers of aPL have a low thrombotic risk
- prophylaxis in high risk situations important
- serologic profile important
Treat additional risk factors

- LAC-positive smokers have a double risk for stroke compared to non-smokers
- LAC positive users of oral contraceptives have a > 7 times increased risk for stroke
  (Urbanus et al. Ratio study. Lancet Neurol 2009; 8: 998)

- SLE by itself is a thrombotic risk
- aPL appear more thrombogenic in SLE compared to non-SLE patients
54 SLE patients with thrombosis during 2 year follow-up vs. 108 SLE patients without thrombosis during 2 years follow-up

Table 3. Risk factors identified by multivariate analysis as showing a significant association with thrombovascular events during the 2-year followup in patients with systemic lupus erythematosus*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever use of antimalarial drugs</td>
<td>0.32 (0.14–0.74)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Older age</td>
<td>1.04 (1.01–1.07)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* 95% CI = 95% confidence interval.

Conclusion. The results from this nested case-control study demonstrate that, after accounting for the effects of disease severity, disease duration, and calendar year, antimalarial drugs were found to be thromboprotective, being associated with a 68% reduction in the risk of all TEs, with a range of risk reduction of at least 26% up to as high as 86%.

### Table 5  Effects of antimalarials in patients with systemic lupus erythematosus (SLE) graded according to the quality of evidence

<table>
<thead>
<tr>
<th>Quality of evidence</th>
<th>AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>High:</td>
<td></td>
</tr>
<tr>
<td>Reduction of SLE activity (also in pregnancy)</td>
<td>CQ/HCQ</td>
</tr>
<tr>
<td>Reduction of mortality</td>
<td>CQ/HCQ</td>
</tr>
<tr>
<td>Moderate:</td>
<td></td>
</tr>
<tr>
<td>Increase in BMD</td>
<td>HCQ</td>
</tr>
<tr>
<td>Protective effect on thrombotic events</td>
<td>CQ/HCQ</td>
</tr>
<tr>
<td>Protective effect on irreversible organ damage</td>
<td>HCQ</td>
</tr>
<tr>
<td>Low:</td>
<td></td>
</tr>
<tr>
<td>Reduction of severe flares</td>
<td>HCQ</td>
</tr>
<tr>
<td>Adjuvant effect for achieving LN remission</td>
<td>HCQ</td>
</tr>
<tr>
<td>Beneficial effect on serum lipid levels</td>
<td>CQ/HCQ</td>
</tr>
<tr>
<td>Protective effect on osteonecrosis</td>
<td>HCQ</td>
</tr>
<tr>
<td>Delaying the evolution to SLE</td>
<td>HCQ</td>
</tr>
<tr>
<td>Protective effect on cancer</td>
<td>CQ/HCQ</td>
</tr>
<tr>
<td>Very low:</td>
<td></td>
</tr>
<tr>
<td>Reduction of 1–25 (OH)(_2) vitamin D levels</td>
<td>HCQ</td>
</tr>
<tr>
<td>Reduction of atherosclerosis</td>
<td>CQ/HCQ</td>
</tr>
</tbody>
</table>

AM, antimalarial; BMD, bone mineral density; CQ, chloroquine; HCQ, hydroxychloroquine; LN, lupus nephritis.

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**Author’s recommendation**

Almost any patient should be treated with HCQ once the diagnosis is certain.

**No consensus on optimal dose (400, 200 mg/dag or even less)**
HCQ reduces thrombotic risk in SLE irrespective of aPL

Case-control study

- 144 SLE patients with aPL and no prior thrombosis (follow-up 75 months)
- 144 matched aPL-negative SLE patients (follow-up 113 months)

Figure 3. Kaplan-Meier survival curves for thrombotic events in patients treated (dashed line) and not treated (solid line) with hydroxychloroquine, among A, antiphospholipid antibody (aPL)-positive patients (log rank $P = 0.30$, unadjusted analysis) and among B, aPL-negative patients (log rank $P = 0.001$, unadjusted analysis). mo = months.

442 SLE patients (LUMINA cohort)

Investigate relation between thrombosis, aPL and HCQ use

**Results**

- Univariate analysis: EVER USE of HCQ reduces thrombotic risk
- **Multivariate analysis: No protective effect of HCQ**

of note: in both analysis aPL was NOT associated with thrombosis
in both analysis smoking and disease activity were associated with thrombosis

Mo et al. Rheumatology 2005; 44: 1303
STATINS
• cholesterol-lowering agents with anti-inflammatory properties

• direct effect on the endothelial expression of adhesion molecules on plaque formation on thromboxane synthesis

• aPL antibodies induce increased expression, function and transcription of tissue factor on Ecs
• In animal models activation of ECs and thrombogenicity of aPL can be reversed by treatment with statins

• One month 20 mg/day fluvastatin in APS patients significantly inhibits p38 MAP kinase and NFκB with downregulation of tissue factor and other prothrombotic biomarkers

• Three months 40 mg/day fluvastatin in persistently aPL-positive patients, with or without SLE significantly reduced pro-inflammatory and prothrombotic biomarkers

These findings provide further support for the potential beneficial effects of statins in aPL-positive patients, justifying further controlled clinical studies.

Conclusions

• The presence of antiphospholipid antibodies is one of the most common acquired risk factors for thrombosis and fetal loss.

• Antiphospholipid antibodies are a heterogeneous family of autoantibodies, antibodies against domain I of $\beta_2$-glycoprotein I seem to be the relevant antibodies.

• Lupus anticoagulant is the assay of choice for detection.

• Infections are the major cause of antiphospholipid antibodies.

• $\beta_2$-glycoprotein I is a biosensor in our circulation for LPS and other unwanted compounds.

• Secondary prophylaxis: INR 2.0-3.0 prolonged

• Primary prophylaxis: Only in high risk situations
Thanks to the team:

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Bas de Laat

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