Recurrent Miscarriage and Antiphospholipid Syndrome

Jana Skrzypczak

Recurrent miscarriage (RM)
three early consecutive losses or two late pregnancy losses

The definition of late fetal loss has varied from 10 weeks gestational age until later than 20 weeks

Causes of RM
• structural chromosomal abnormalities in the parents
• antiphospholipid syndrome
• uterine anomalies
• fetal chromosomal abnormalities
• endocrine and immunological factors

Antiphospholipid Syndrome (APS)

• Preventable cause of:
  - embryonic and fetal loss
  - maternal thrombotic complications

• Management of an aPL-positive patient during pregnancy should focus on the prevention of both fetal and maternal complications
Speculation on APS in the Coming Millenium
Graham Hughes J.A.I., 2000

One fetal death increases the risk of further fetal deaths 20-fold. APS is and will be in the coming millenium recognized as the major cause of this tendency.

Today’s Discussion Points

- Introduction
- aPL prevalence in women with recurrent miscarriage
- Mechanism of fetal loss in women with APS
- Management of aPL-positive patients during pregnancy and postpartum

Sydney Criteria for APS

Vascular thrombosis
- Arterial, venous, or small vessel thrombosis in any tissue or organ

Pregnancy morbidity
- ≥ 3 unexplained consecutive spontaneous abortions <10th week of gestation
- ≥ 1 unexplained deaths of a morphologically normal fetus ≥ 10th week of gestation
- ≥ 1 premature births of a morphologically normal neonate ≤ 34th week of gestation because of eclampsia or severe preeclampsia or placental insufficiency
**Laboratory criteria**

1. Lupus anticoagulant (LA) present in plasma on ≥2 occasions at least 12 weeks apart and/or
2. Anticardiolipin (aCL) antibody of IgG / IgM isotype in serum in medium or high titer (ie >40 GPL or MPL) on ≥2 occasions at least 12 weeks apart or
3. Anti-β₂ glycoprotein – 1 antibody of IgG / IgM isotype in serum (in titer > the 99th percentile) on ≥2 occasions at least 12 weeks apart

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**Etiology of APS**

APS may have multifactorial etiology

One environmental factor is infection

Series of 100 women with APS

- skin infections – 18%
- human immunodeficiency virus (HIV) – 17%
- pneumonia – 14%
- hepatitis C virus – 13%
- urinary tract infection – 10%

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**Assays for the detection of aPL**

aPL that do not prolong phospholipid - dependent clotting assays can be detected by immunoassays using phospholipid - coated surfaces

Antibodies against cardiolipin (aCL)
phosphatidylethanolamine (aPE)
phosphatidylserine (aPS)
phosphatidylcholine (aPC)
phosphatidylglycerol (aPG)
phosphatidylinositol (aPI)
β₂-glycoprotein 1 (aβ₂ GP1)

must be identified by ELISA

The results are expressed in aPL units

1 unit being equivalent to the binding capacity of 1 µg/ml pure phospholipid
Assays for the detection of aPL

aPL that prolong phospholipid-dependent clotting assays (LA) can be detected by clotting time prolongation assays.

These tests include:
- the activated partial thromboplastin time (aPTT)
- the diluted Russell’s viper venom time (dRVVT)
- the kaolin clotting time (KCT)

Types of aPL-associated abortions

LA, aCL, aβ2GP1 – are causative of pregnancy loss.

but other aPL e.g. aPC, aPG, aPA, aPJ are of diagnostic significance.

Measurement of aPS (phosphatidylserine) and aPE (phosphatidylethanolamine) is indicated in women with early recurrent pregnancy loss.

Isotype of aPL

Most aPL are the IgG or IgM class

only 10% may be IgA

Predominate role of IgG antibodies in women with RM

Titer of aPL

Medium and high titers of aCL and/or other aPL antibodies (>40 GPL units) identify the women who require pharmacological prophylaxis in the next pregnancy.
aPL prevalence

Recurrent Early Miscarriage

• 5% to 20% of women with Recurrent Early Miscarriage have been reported to have aPL, and thus „APS“
• In past studies, many of these women did not meet criteria for definite APS
  - Low titers
  - No repeated testing
  - Use of poorly standardized tests

_Branch W., 12th International Congress on Antiphospholipid Antibodies_ Florence 2007

Rai et al. 1995

500 women with three and more miscarriages (3-16)
15% were LA and/or aCL positive

Oshiro BT 1996

among 366 patients with two and more pregnancy losses
21.5% women were aPL-positive (>20 GPL)
80% patients experienced at least one stillbirth

Pregnancy Outcomes in Pregnancy Loss Patients

Antiphospholipid antibodies (aCL and LA)

189 women with two and more miscarriages

ELISA
aCL positive
n=57

after first test
APTT
LA positive
n=6

aCL positive
n=24

after second test
LA positive
n=6

15.87% aPL positive patients
3.0% with ≥20 GPL or MPL

Skrzypczak J., et al. 2006

123 patients with pregnancy loss

aCL LA aβ₂GP₁
3 2 14
2.4% 1.6% 11.4%

Skrzypczak J., et al. 2008

Recurrent Early Miscarriage

- Design
  - Retrospectively analyzed population presenting with Recurrent Early Pregnancy Miscarriage
    * All patients evaluated in similar fashion with particular attention to establishing the timing of each pregnancy loss
  - All were tested for antiphospholipid antibodies
  - Patients that initially tested positive had repeat levels performed

Branch W., 12th International Congress on Antiphospholipid Antibodies Florence 2007
Recurrent Early Miscarriage and APS

Repeat Results

Losses < 15 weeks

- 35 positive for aPL

- 5 patients (3.1%) had positive repeat testing

Preembryonic / embryonic losses

- 25 positive for aPL

- 2 patients (1.5%) had positive repeat testing

Thus, very few women with Recurrent Early Miscarriage have APS

288 Patients with Recurrent Early Miscarriage < 15 weeks

158 with losses <15 weeks

130 with preembryonic or embryonic losses

123 negative for aPL

35 positive for aPL

102 negative for aPL

25 positive for aPL
• APS/RM is over diagnosed if miscarriage are not sent for cytogenetic analyses
  - 29 couples were excluded when 40% of their miscarriages were tested
  - 164 couples were included when only 6% of their miscarriages were tested
• Including cytogenetic analyses of ≥1 miscarriages in the APS/RM criteria would result in a more homogeneous cohort

Mechanisms of action

Early hypotheses

**Thrombosis**

- aPL might induce thrombosis in the uteroplacental circulation, particularly in the spiral arteries

- platelets - aPL enhance TXA₂ production

- fibrinolysis - increase plasma PAI-1 and tPA antigens

- coagulation cascade - aPL inhibit protein C activation and the function APC

**Arachidonic acid and prostacyclin**

- aPL inhibit arachidonic acid release; ↓ PGI₂ ↑ TXA₂
  - the alteration in the PGI₂/TXA₂ ratio
    - vasoconstriction
    - platelet activation

**Anticytokine effect**

- IL₁ serum level is lower in pregnant women with APS
- TNFα serum level is higher in patients with APS

**Induction of placental cell apoptosis**
Mechanisms of action

Recent data

**Inhibition of trophoblast invasiveness**

PL antibodies recognize as antigens some membrane PL like PS and react with PS

they would exert inhibitory effect on

1. trophoblast intercellular fusion
2. chorionic gonadotropin secretion
3. trophoblast invasiveness

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In vitro studies

- aPL can bind to human trophoblast cells
- Circulating aPL might interact with endothelium of maternal vessels (which prevent the correct endothelial-trophoblast interaction)

Another hypothesis

- aPL may directly bind to the endovascular trophoblast populations
dissolution or abnormal formation of endovascular trophoblast phenotype

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Summary of antiphospholipid antibody effects on trophoblast function

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cell type</th>
<th>HCG and HPL secretion</th>
<th>Trophoblast invasiveness</th>
<th>Trophoblast fusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyclonal aPL (IgG)</td>
<td>Primary trophoblast cells</td>
<td>-</td>
<td>Reduced by 40%</td>
<td>Completely blocks</td>
<td>[Narva et al. (1993)]</td>
</tr>
<tr>
<td>anti-β2-glycoprotein</td>
<td>Choriocarcinoma cells</td>
<td>-</td>
<td>Completely blocks</td>
<td>Complete blocks</td>
<td>[Chamley et al. (1998)]</td>
</tr>
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<td>anti-phosphatidylserine</td>
<td>Choriocarcinoma cells</td>
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<td>[Rote et al. (1995, 1998)]</td>
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</table>

HCG = human chorionic gonadotrophin;
HPL = human placental lactogen
**Trophoblast injuries linked to the presence of antiphospholipid (aPL) antibodies**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Effects</th>
<th>Controls, untreated trophoblast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peacemane et al. (1993)</td>
<td>Increase of placental thromboxane A2</td>
<td>300% versus controls</td>
</tr>
<tr>
<td>Fishmann et al. (1996)</td>
<td>Reduction of trophoblast interleukin -3</td>
<td>71% versus controls</td>
</tr>
<tr>
<td>Di Simone et al. (1997)</td>
<td>Reduction of HCG secretion</td>
<td>40% versus controls</td>
</tr>
<tr>
<td>Di Simone et al. (2000)</td>
<td>Reduction of trophoblast cells invasiveness</td>
<td>25% versus controls</td>
</tr>
<tr>
<td>Rote et al. (1998)</td>
<td>Inhibition of syncytiotrophoblast formation</td>
<td>82% versus controls</td>
</tr>
</tbody>
</table>

**New mechanism for pregnancy loss in women with aPL**

aPL mediate pregnancy complications by initiating activation of the complement cascade

![Complement Cascade Diagram](image)


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**A mouse model – Salmon J., Givardi J.**

- Passive transfer of IgG from women with RM and aPL antibodies results in 40% frequency fetal resorption compared to < 10% in mice treated with IgG from healthy individuals
- Inhibition of the complement cascade in vivo using the C3 convertase inhibitor Covy-Ig prevented fetal loss and growth restriction

Proposed mechanism for pregnancy complications associated with aPL antibodies

aPL antibodies are preferentially targeted to the placenta, where they activate complement via the classical pathway.


Management

Current recommendations for women with antiphospholipid syndrome and recurrent miscarriage include treatment with a combination of

- **low dose aspirin** and
- a low dose of either unfractionated or low molecular weight **heparin**


Why aspirin?

- Improves placental blood flow
  - decreases thromboxane A2 / prostacyclin ratio
- Stimulates IL-3 production
  - IL-3 is a growth factor for the trophoblast
  - promotes invasion and expansion

Aspirin is also useful in patients with occasional aPL but not APS

Livelihood patients with occasional antiphospholipid antibodies treated with aspirin and unexplained patients with no medication

Why heparins?

- inhibit coagulation
- have anti-inflammatory effects
- prevent leukocyte adhesion to vascular endothelial cells and transmigration
- block activation of complement
  - at multiple levels of the cascade
- limit antibody targeting to trophoblast
- enhance trophoblast invasiveness
Studies on live-birth rates to pharmacological treatment in women with aPL and recurrent early pregnancy loss or least one fetal in absence of SLE or previous thrombosis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Years of publications</th>
<th>Type</th>
<th>Study</th>
<th>Live-birth (%) according to pharmacological treatment</th>
<th>No. of women</th>
<th>Type of treatment</th>
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<tbody>
<tr>
<td>Groot et al.</td>
<td>1996</td>
<td>Randon</td>
<td></td>
<td>80/76</td>
<td>71</td>
<td>LMWH</td>
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<td>Kutcher et al.</td>
<td>1998</td>
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<td>LMWH</td>
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<tr>
<td>Farquharson et al.</td>
<td>2002</td>
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<td></td>
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<tr>
<td>Trillos et al.</td>
<td>2003</td>
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<tr>
<td>Noble et al.</td>
<td>2005</td>
<td>Randon</td>
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Comparison of three trials comparing pregnancy outcome for treatment with low dose aspirin (LDA) and LDA plus heparin

<table>
<thead>
<tr>
<th>Trial</th>
<th>Live-birth rate (%)</th>
<th>Patients (n)</th>
<th>LAC positive (%)</th>
<th>Cryx (IgM-IgG, U)</th>
<th>Start LDA</th>
<th>Heparin type</th>
<th>Heparin dosage</th>
<th>Positive fetal heart activity</th>
<th>Positive pregnancy test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rai et al. (1997)</td>
<td>45</td>
<td>90</td>
<td>92</td>
<td>5</td>
<td>Preconception</td>
<td>Unfractionated</td>
<td>Fixed</td>
<td>Positive</td>
<td>Before 12 weeks</td>
</tr>
<tr>
<td>Kutcher (1996)</td>
<td>44</td>
<td>50</td>
<td>62</td>
<td>3</td>
<td>Preconception</td>
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Previous treatment events

1. On secondary prophylaxis?
   - with low dose aspirin?
     - yes
     - no
   - with potent anticoagulants?
     - yes
     - no

Gestotic APS criteria fulfilled?

1. APS criteria fulfilled
   - yes
   - no

Treatment in pregnancy

1. Treatment in pregnancy
   - yes
   - no
2. Treatment subsequent
   - yes
   - no
Antiphospholipid syndrome dilemmas
still to be solved: 2008 status

Future directions for APS research
- Aetiology role of infection, drugs, tumours
- Mechanisms complement role, cytokines/chemokines role
- Diagnostics additional autoantibodies (Multiplex)
- Therapy anticoagulation resistant cases
  primary prophylaxis


Thank you