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.AI., 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

- $\geq$ 3 unexplained consecutive spontaneous abortions $<10^{th}$ week of gestation
- $\geq$ 1 unexplained deaths of a morphologically normal fetus $\geq 10^{th}$ week of gestation
- $\geq$ 1 premature births of a morphologically normal neonate $\leq 34^{th}$ 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 et least 12 weeks apart or
3. Anti – β_2 glycoprotein – 1 antibody of IgG / IgM isotype in serum (in titer > the 99th percentile) on ≥ 2 occasions at least 12 weeks apart

Miyakiss et al.,
International consensus statement on an update of the classification criteria for define antiphospholipid syndrome.
J. Thromb. Haemost. 2005

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%

Cervera R et al.,
Ann. Rheum Dis., 2004

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)	
phosphatidyloserine	(aPS)	must be identified by
phosphatodylcholine	(aPC)	
phosphatidylglycerol	(aPG)	ELISA
phosphatidylinositol	(aPI)	
β_2-glycoprotein 1	(aβ_2 GP1)	

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 cloting assays (LA) can be detected by cloting time prolongation assays

These tests include

- the activated partial thromboplastin time (aPTT)
- the diluted Ressull's viper venom time (dRVVT)
- the kaolin cloting time (KCT)

Types of aPL – associated abortions

LA, aCL, aβ_2 GP1 – 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.,12° 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

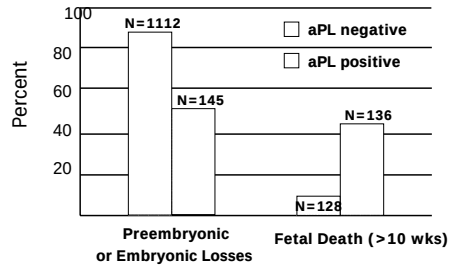
Oshiro BT 1996

among 366 patients with two and more pregnancy losses

21.5% women were aPL-positive (≥20 GPL)

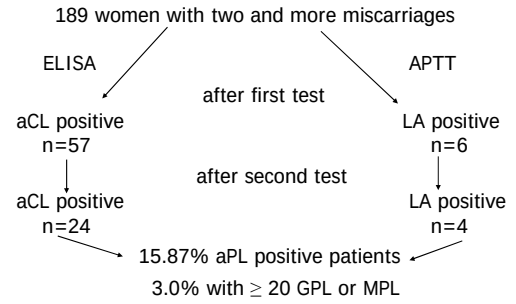
80% patients experienced at least one stillbirth

Pregnancy Outcomes in Pregnancy Loss Patients



Oshiro et al. Obstet. Gynecol, 1996

Antiphospholipid antibodies (aCL and LA)



Skrzypczak J., et al. 2006

123 patients with pregnancy loss

aCL	LA	$a\beta_2GP_1$
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 antiphospoholipid antibodies
  - Patients that initially tested positive had repeat levels performed

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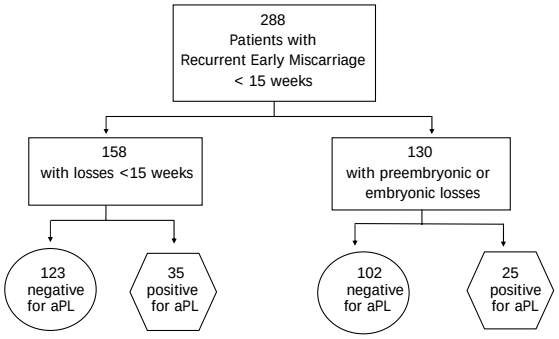
Recurrent Early Miscarriage and APS



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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

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Recurrent Early Miscarriage and APS

288
Patients with Recurrent Early Miscarriage

2.4 % met criteria for definite APS

Thus, very few women with Recurent Early Miscarriage have APS

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- 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

Stephenson M.D.,12° International Congress on Antiphospholipid Antibodies Florence 2007

Mechanisms of action

Early hypotheses

Thrombosis

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

aPL alter activity:
- platelets – aPL enhance TXA_2 production
- fibrynolysis – increase plasma PAI-1 and tPA antigens
- coagulation cascade – aPL inhibit protein C activation and the function APC

Mechanisms of action

Arachidonic acid and prostacyclin

aPL inhibit arachidonic acid release; $\downarrow PGI_2$ $\uparrow TXA_2$

the alteration in the PGI_2/ TXA_2
- vasoconstriction
- platelet activation

Anticytokine effect

IL_3 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

Mechanisms of action

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

Summary of antiphospholipid antibody effects on trophoblast function

Antibody	Cell type	Binding	Trophoblast proliferation	HCG and HPL secretion	Trophoblast invasiveness	Trophoblast fusion	Reference
Polyclonal aPL (IgG)	Primary trophoblast cells	++		Reduced by 40%	Completely blocks	Completely blocks	Di Simone Et al. (1999)
Anti-β2-glycoprotein I	Choriocarcinoma cells	++	Completely blocks				Chamley et al. (1998)
Antiphosphatidylserine	Choriocarcinoma cells	++		Reduced by 40%	Completely blocks	Completely blocks	Rote et al. (1995, 1998)
Antiphosphatidylserine	Primary trophoblast cells	++		Reduced by 50%	Completely blocks		Katsuragawa et al. (1997)

HCG = human chorionic gonadotrophin;
HPL = human placental lactogen

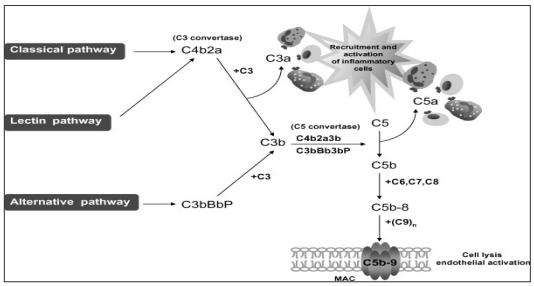
Trophoblast injuries linked to the presence of antiphospholipid (aPL) antibodies

Authors	Effects	
Peacemane et al. (1993)	Increase of placental thromboxane A2	300% versus controls
Fishmann et al. (1996)	Reduction of trophoblast interleukin -3	71% versus controls
Di Simone et al. (1997)	Reduction β-HCG secretion	40% versus controls
Di Simone et al. (2000)	Reduction of trophoblast cells invasiveness	25% versus controls
Rote et al. (1998)	Inhibition of syncytiotrophoblast formation	82% versus controls

Controls, untreated trophoblast cells

New mechanism for pregnancy loss in women with aPL

aPL mediate pregnancy complications by initiating activation of the complement cascade



Salmon J, Givardi G., J.Reprod. Immunol. 2008

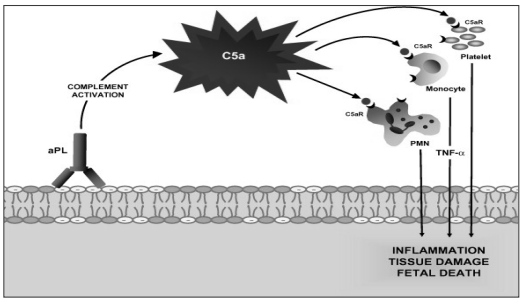
A mouse model – Salmon J., Givardi J

- 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 C_3 convertase inhibitor Cvry-Ig prevented fetal loss and growth restriction

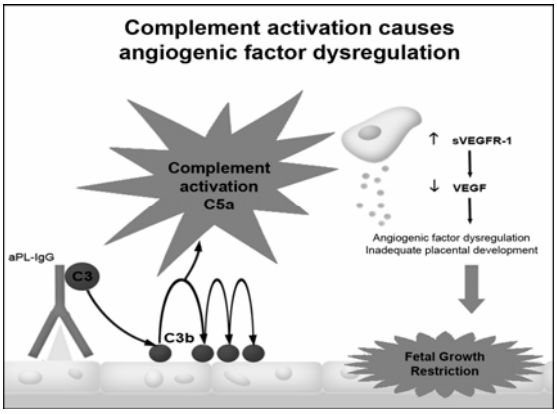
J. Reprod. Immunol., 2008, 77, 51-56

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



Salmon J., Givardi J., J. Reprod. Immunol. 2008, 77, 51-56



Lockshin M, 12 International Congress on AntiPhospholipid Antibodies, Florence 2007

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**

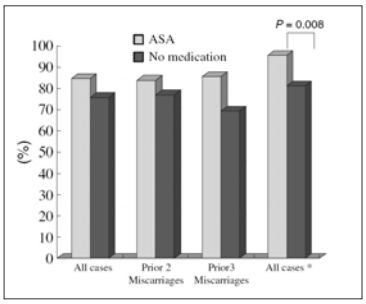
Bates S., et al. Chest 2004 sup 126, 627S-644S

Pabinger I., Vormittag R., J. Thromb. Haemost 2005, 3, 1603-1610

Why aspirin ?

- Improves placental blood flow
  - decreases thromboxane A_2 / 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



Live birth rate in patients with occasional antiphospholipid antibodies treated with aspirin and unexplained patients with nomedication

Sugiuva-Ogasawara M., et al.
Am. J. Reprod.I Immunol., 2008, 59, 235-241

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

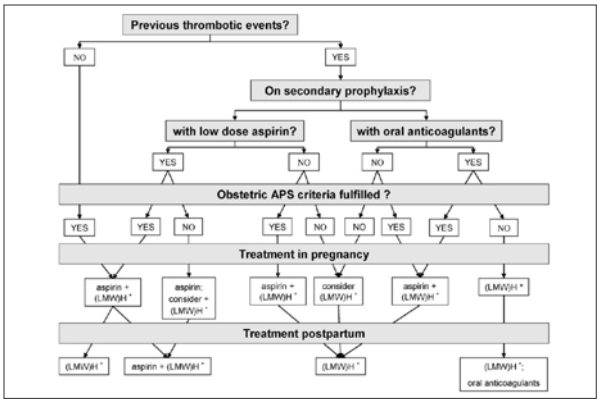
Reference	Years of publications	Study type	No. of women	Live – birth (%) according to pharmacological treatment				
				None	P+A	A	A+H	IvIG
Cowchock et al.	1992	Rando	20		75		75	
Balasch	1993	Pro, O	18		100	91		
Silver et al.	1993	Rando	34			100		
Rai et al	1995	Pro, O	20	10				
Kutteh	1996	C, NR	50			44	80	
Kutteh and Ermel	1996	C,NR	50				80/76	
Tuppala et al.	1997	Rando	12	17		17		
Laskin et al.	1997	Rando	88	52	60			
Rai et al.	1997	Rando	90			42	71	
Backos et al.	1999	Pro, O	150				71	
Pattison et al.	2000	Rando	40	85		80		
Farquharson et al.	2002	Rando	98			72	78	
Triolo et al.	2003	Rando	40				84	57
Noble et al.	2005	C, NR	50				84/80	

Derksen R.H, Groot Ph.G., J. Reprod. Immunol., 2008, 77, 41-50

Comparision of three trials comparing pregnancy outcome for treatment with low dose aspirin (LDA) and LDA plus heparin

	Trial		
	Rai et al. (1997), randomized	Kutteh (1996), prospective, controlled (non-randomized)	Farquharson et al. (2002), randomized
Live-birth rate (%)			
LDA	45	44	72
LDA + H	71	80	78
Patients (n)	90	50	98
LAC positive (%)	91	0	42
Cut-off IgG-aCL (U)	5	27	9
Cut-off IgM-aCL (U)	3	23	5
Start LDA	Positive pregnancy test	Preconception	Before 12 weeks
Start heparin	Positive fetal heart activity	Positive pregnancy test	Positive fetal heart activity
Heparin type	Unfractionated	Unfractionated	LMWH
Heparin dosage	Fixed	adjusted	Fixed

Derksen R.H, Groot Ph.G., J. Reprod. Immunol., 2008, 77, 41-50



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

Shoenefeld et al. Ann. Rheum. Dis. 2008, 67, 438-441