Pregnancy Outcome in Women with Antiphospholipid Antibodies: Report on a Retrospective Study

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ABSTRACT

Antiphospholipid syndrome (APS) represents a serious risk factor in pregnancy resulting in several complications, leading to fetal loss and hemostatic complications. In this dedicated report, we describe our experiences in the treatment of pregnancies in patients with APS. The retrospective data from 140 pregnant women were investigated, and the treatment results of 121 patients were recorded. We studied two groups of patients receiving different treatment. The first group ($n = 78$) received the standard therapy with low-weight-molecular heparin (dalteparin 5000 U or certoparin 3000 U daily) and aspirin (100 mg daily) and in the second group ($n = 43$) an additional 0.2 g/kg intravenous immunoglobulin (IVIG). Outcomes were 74.3% and 83.7% live births in the first group and in the second group, respectively. The abortion rate was similar in both groups (11.5% vs. 11.6%). The late complication rate was lower in the second group (5.8% vs. 14.1%, $p < 0.05$) than in the group with standard therapy. Interestingly, we found a trend to higher percentage (> 12%) of natural killer (NK) cells in patients with pregnancy complications (60% vs. 12%, $p < 0.05$). Our retrospective data shows an improvement of late pregnancy complications by additional use of IVIG. It is possible that IVIG influences higher NK cell activity in patients with previous pregnancy complications.

KEYWORDS: Antiphospholipid syndrome, pregnancy, low-molecular-weight heparin, certoparin, dalteparin, intravenous immunoglobulins

Antiphospholipid syndrome (APS) is a prothrombotic disorder characterized by arterial and/or venous thrombosis or recurrent abortion in the presence of circulating antiphospholipid antibodies (aPL). These autoantibodies are found in up to 5% of apparently healthy controls. In contrast, anticardiolipin antibodies (aCL) are found in 5 to 51% (mean, 15.5%) and lupus anticoagulant (LA) in 0 to 14% (mean, 8.3%) of women with recurrent first-trimester abortion. aPL are also associated with preeclampsia and fetal growth retardation.[1–7]

The clinical management of pregnant woman has advanced during the past decade. Prophylactic dosage of

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A Tribute to Eberhard F. Mammen, M.D. (1930–2008); Guest Editor, Emmanuel J. Favaloro, Ph.D., M.A.I.M.S.

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aspirin and low-dose heparin use has reduced the pregnancy loss to only up to 40% of otherwise affected patients.[8] Low-molecular-weight heparin (LMWH) such as dalteparin and enoxaparin has replaced low-dose heparin and can be administered safely to pregnant women. Despite the use of these agents, many women still fail to deliver a live infant. Intravenous immunoglobulins (IVIGs) and plasma exchange have also been considered. In addition, monoclonal antibodies to B cells, B-cell growth factors, complement pathway modulation, and other plasma proteins such as antithrombin and C1 esterase inhibitors may also be useful in the management of pregnant women with APS. Aspirin and LMWH are polytherapeutic agents and can modulate the immunopathologic process in this syndrome.

IVIG has been successfully used to reduce the fetal loss in pregnant women with APS, sometimes in addition to steroids and in the presence of immunosuppressive-resistant autoimmune disease.[9] A combined regimen including IVIG, aspirin, and LMWH provides a polytherapeutic approach in the management of this catastrophic disorder. This study was to determine the safety and efficacy of IVIG in combination with LMWH.

The fetal morbidity and mortality in APS may be due not only to placental thrombosis because of direct effects of aPL on anionic phospholipids and the cofactor β2-glycoprotein I (β2GPI) on trophoblasts but also to placental inflammation due to complement activation and to impairment of trophoblast function.[7,10–12]

A well-randomized study has shown aspirin and unfractionated heparin (UFH) to have a benefit compared with aspirin alone, with a live birth rate of 78%.[13] In women with recurrent spontaneous abortion (RSA) and positive aPL, but without treatment, the spontaneous rate of live birth is below 10%.[14] This led to recommendations in the American College of Chest Physicians guidelines[15] that obstetric APS would be improved using heparin and aspirin therapy. The combination of LMWH and aspirin compared with aspirin alone did not significantly reduce pregnancy loss.[16] In contrast, the use of LMWH and aspirin showed an improvement of pregnancy outcome in comparison with UFH and aspirin in a Canadian pilot study.[17]

The relationship between APS and pregnancy complications including fetal loss has been reviewed recently.[18–23] Ogasawara et al[24] found a failure rate of 19.4% after standard therapy. Tincani et al[25] found a complication rate of more than 20%, although standard therapy was associated with a premature birth rate of up to 18.8%, preeclampsia with 11.6%, and intrauterine growth retardation or restriction (IUGR) of 6%. The recurrent complications rate of patients with medium- to high-rate aPL occurred in up to 30% according to Backos et al[26] (30% were preterm, 22% were small for gestational age [SGA] babies, and 13.8% developed preeclampsia or bleeding complications). Many investigators treated such patients with prednisolone, aspirin, and IVIG[27] and others with therapeutic doses of LMWH, UFH, or LMWH and IVIG.[28] In the earlier guidelines for the treatment of APS,[18,25,29,30] the additional use of IVIG in cases with normal karyotyping and when standard therapies have failed was discussed.[31–34] It is possible that this treatment concept influenced also the elevated level of peripheral natural killer cells (pNK-cells) in women with APS and pregnancy complications.[35,36] Clinical studies[37–40] have demonstrated that increased levels of pNK cells are associated with RSA, and many investigators[41–44] have used these cells as markers for the selection of patients for IVIG therapy.

Human pNK cells are divided into two mean populations based on the type and strength of immunofluorescence staining: around 95% of pNK cells are $CD56^+$ dim $CD16^+$, in contrast with around 5% of pNK cells with $CD56^+$ bright $CD16^-$. $CD56^+$ dim $CD16^+$ pNK cells are highly cytotoxic and express members of the killer Ig-like receptor (KIR). $CD16^-$ do not express KIR and have a low toxicity function. Decidual (dNK) or uterine (uNK) NK cells are mostly $CD56^+$ bright $CD16^-$ and have a low toxicity against decidual cells, but a small proportion of the dNK cells are of the $CD56^+$ $CD16^+$ phenotype with a high cytotoxic activity. In the literature, two explanations exist for the association of pNK cells with dNK cells. First, $CD16^-$ pNK cells migrate to the uterus and undergo further differentiation by extravillous trophoblasts. Second, transforming growth factor β (TGF-β)—produced and expressed in decidual stroma—influences the conversion of $CD16^+$ pNK cells to $CD16^-$ pNK cells.[45] These findings are in agreement with the results of Sacks et al,[46] who also found in their recent study a strong correlation between pNK cells and dNK cells in women with RSA and repeated reproduction failure.

An important clinical point is the question of how dNK cells are activated and attack the trophoblast. Recent research still in progress explains this phenomena as due to a lack of inhibition of KIR[32,47–49] or an unbalance between inhibition and activation of KIR.[50–52] These experimental results are not uniform, and two working groups[53,54] have shown contrary data.

The characteristics of the obstetric APS are defined in the Sapporo classification from 1999.[55] These criteria were tested by Lockshin et al[56] to have a sensitivity and specificity of 71% and 98%, respectively. Clinical experience includes the determination of IgG and IgM-anti-β2-glycoprotein I (aβ2GPI) in the laboratory criteria of APS since 1997.[57] The results obtained from most investigators indicate that use of aβ2GPI may provide only additional information for the diagnosis of APS. Franklin et al[58] showed 22.2% positive IgG antibodies to aβ2GPI in APS. However,

Carmo-Pereira et al[59] and Chong et al[60] showed no correlation to the aCL status and aβ2GPI .

MATERIALS AND METHODS

Patients

Between 1988 and 2008, 156 patients were diagnosed with positive aCL or LA with known outcome of pregnancy. After repeated measurement after 6 weeks, 140 women were considered to have a true APS. Sixty-two women were given standard treatment in their previous pregnancies with LMWH (dalteparin 2500 to 5000 U or certoparin 3000 U daily) or UFH (2 × 5000 U calciparin) together with 100 mg aspirin. Three women received additional cortisone (20 mg daily until 12th week of gestation), and 78 women had not received therapy for a previous pregnancy (Table 1). Sixty-three women had ongoing recurrent abortion (21 women with 3 abortions, 20 women with 4 abortions, and 22 women with 5 or more abortions), and 35 women had severe preeclampsia < 34th week of gestation or a hemolytic anemia, elevated liver enzymes, and low platelet count (HELLP)-syndrome identified within their clinical history. In 21 patients, there was a late abortion after the 12th week of gestation, and 19 women had an uncomplicated pregnancy with a live-born baby. The nomenclature for description of recurrent abortion according to Farquharson et al was used.[33] No additional triggering factors, such as hereditary thrombophilias or anatomic or genetic abnormalities, occurred during this study.

At the time of diagnosis of a new pregnancy, all women were started with LMWH (3000 anti-Xa U certoparin or 5000 U dalteparin daily) together with 100 mg aspirin daily. In the group previously treated with standard therapy but with treatment failure, additional 0.2 g/kg IVIG was given until 30 to 32 weeks of gestation. The dosages were in agreement with the recommendations of Kiprov et al[61] and Stojanovich et al.[62]

The aim of this retrospective analysis was to describe our experiences in the treatment of APS pregnancies. We included all information about pregnancy outcome, such as recurrent abortion, preeclampsia, and fetal death. Additionally, classic side effects of all drugs used was documented. All patients gave written informed consent for inclusion in this study.

Blood Sampling

Blood samples were collected from patients during their first visit after identification of a history of recurrent spontaneous abortion (3 abortions or more), severe preeclampsia, and/or treatment failure after standard therapy of APS. Plasma samples were anticoagulated in 1/10 volume with 3.2% sodium citrate and stored at room temperature not more than 1 hour prior to double centrifugation at 3000 × *g* for 15 minutes. The resulting platelet-poor plasma was stored at −70°C until analysis.

Laboratory Methods

IgG (positive titers > 20 IgG phospholipids (GPL) U/mL) and IgM (positive titers > 20 IgM phospholipids (MPL) U/ml) aCL assay was performed using ELISA assays (Pharmacia Diagnostics, Freiburg, Germany). The LA was assayed according to the phospholipid-dependent method (using "Screen" and "Confirm" simplified dilute Russell's viper venom test [DRVVT]; Instrumentation Laboratories, Munich, Germany). The test was measured on fresh plasma samples and was considered positive when LA Screen/LA Confirm was more than 1.20 (using data from Brandt et al[19] and Bertolaccini et al[57]).

aβ2GPI was determined by ELISA assay from Pharmacia Diagnostics (positive values > 20 U/mL). Measurements of aPL were repeated after 6 weeks.

NK cells $CD56^+CD16^+$ were determined by flow cytometry after separation of blood using Ficoll-Paque gradient at 4°C and the mononuclear fraction, which was aspirated off the gradient and then washed twice with phosphate-buffered saline before staining. NK cells are determined using color flourescence-activated cell sorting (FACS) scan after staining with monoclonal antibodies

Table 1 Pregnancy Outcome Dependence on Treatment (Basic Data)

	Outcome with Treatment (*N* = 62)	Outcome without Treatment (*N* = 78)	*p* Value
Age (years)	30 ± 5	28 ± 4	NS
Diagnosis			
RSA, *n*	11	52	< 0.02
Late abortion, *n*	7	14	< 0.05
Severe preeclampsia, *n*	23	12	< 0.05
Uncomplicated pregnancies, *n*	19	0	
Live birth, *n* (%)	42 (67.7)	12 (15.4)	< 0.05

NS, not significant.

Table 2 Pregnancy Outcome after Two Different Treatment Regimens

Diagnosis	Treatment		*p* Value
	LMWH + Aspirin (*N* = 78)	LMWH + Aspirin + IVIG (*N* = 43)	
Abortion early, *n* (%)	5 (6.4)	3 (6.9)	NS
Abortion late, *n* (%)	4 (5.1)	2 (4.7)	NS
Preeclampsia, *n* (%)	9 (11.5)	1 (2.3)	< 0.05
Fetal death, *n* (%)	2 (2.6)	1 (2.3)	NS
Live birth (uncomplicated delivery), *n* (%)	59 (74.3)	36 (83.7)	NS

NS, not significant.

to CD16, CD56, and CD8. The normal values were 3 to 12% according to Beer et al.[41]

Statistical Analysis

The statistical analysis was performed by means of a computer-assisted statistical program. Differences between groups were calculated with the Mann-Whitney test. p values < 0.05 were reported to be statistically significant.

RESULTS

To date, a total of 78 untreated patients have been reported with 15.4% live births (Table 1) and 67.7% live births after standard therapy in 62 patients. In addition, 43 women treated with LMWH and aspirin and IVIG (second treatment) delivered viable infants in 83.7% of cases compared with 78.0% after standard therapy (first treatment, Table 2), and 86.4% versus 86.0% after inclusion of preeclampsia. The abortion rate in the treatment group was 29.0%, and 54.7% of the women with live-born babies had a diagnosis of preeclampsia. Interestingly, the group without previous treatment had a higher abortion rate of 84.6%, and all live-born babies resulted from women with severe preeclampsia.

The values of aCL, aβ2GPI, and LA showed no differences between different groups (Table 3). Positive aβ2GPI were low in all groups. The percentage of pNK cells > 12 were between 55% and 65% in both groups with pregnancy complications. These values were statistically higher ($p < 0.05$) than the rate of pNK cells of $> 12\%$ in uncomplicated pregnancies.

The distribution of $CD56^+$ $CD16^+$ cells in peripheral blood showed a trend to more live-born cases in association with low levels of NK cells and higher concentrations in patients with pregnancy complications (Fig. 1, Table 4). The number of women in each group was too small for a strong statistical analysis. LMWH and IVIG were both well tolerated. A typical infusion reaction characterized by flushing and headache was seen in one patient. This reaction could be avoided by stopping the infusion. In 18 patients, we observed bleeding in early pregnancy, and the bleeding stopped after removing aspirin or LMWH. We observed one skin reaction after dalteparin injection of 5000 U per day, but without thrombocytopenia or other signs of heparin-induced side effects (i.e., HIT II) (Table 5).

DISCUSSION

In our retrospective study, the pregnancy outcome in APS patients with failure after standard therapy was compared with patients after first and standard therapy with LMWH and aspirin. The optimal therapy of pregnant women with fetal loss and moderate and high titers of aPL is controversial. Recommendations for use

Table 3 Distribution of aCL, aβ2GPI, LA, and NK Cells in Different Patient Groups after First Diagnosis

aPL	Abortions (*N* = 84)	Preeclampsia/ HELLP (*N* = 35)	Uncomplicated Pregnancies (*N* = 19)	*p* Values
aCL-IgG and IgM, *n* (%)	17 (20.2)	5 (14.2)	5 (26.3)	NS
aCL-IgG alone, *n* (%)	50 (59.5)	22 (62.9)	11 (57.8)	NS
aCL-IgM alone, *n* (%)	7 (8.3)	3 (8.5)	0	NS
LA alone, *n* (%)	6 (7.1)	1 (2.8)	2 (10.5)	NS
LA with aCL, *n* (%)	1 (1.2)	3 (8.5)	1 (5.8)	NS
aβ2GPI alone, *n* (%)	3 (3.6)	1 (2.8)	0	NS
NK cells $CD56^+$ $CD16^+$, %	65	55	12	< 0.05

NS, not significant.

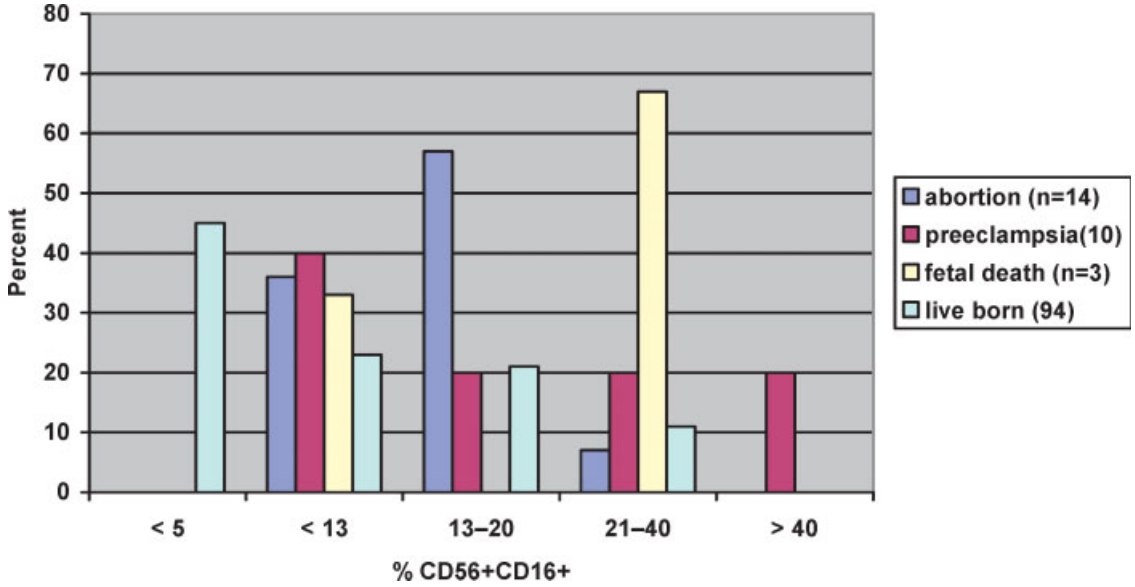

Figure 1 Distribution of NK cells ($CD56^+$ $CD16^+$) in treated patients ($N = 121$).

of LMWH and aspirin to treat APS in pregnancy were examined in only two trials involving 140 patients.[13,63] The treatment was started with aspirin (100 mg daily) if a positive pregnancy test occurred and with LMWH when a viable intrauterine pregnancy was documented and continued until delivery and 6 weeks postpartum.

The problems in most of the studies relate to the inclusion criteria. We included only patients with moderate and high titers of aCL and with specificity to aβ2GPI.[49] Lynch et al[63] found for patients with fetal loss an incidence of 27.7% positivity for IgG (aCL), 5.0% positivity for IgM (aCL), and 15.5% positivity for LA. In contrast with a previous investigation by Kaira et al,[64] Lockwood et al[65] showed an increased level of aCL-IgM between 18% and 30%. In a normal pregnant population, Fialova et al[66] indicated an incidence of 5.1% positivity (> 20.0 GPL) for IgG antibodies and 7.3% positivity for IgM (> 10 MPL) antibodies (aCL). Aoki et al[37] found IgG/IgM antibodies (aCL) in 14% and IgG antibodies in 12% of patients with a strong association of IgG antibodies to fetal loss. Branch et al[67] found in severe preeclampsia 16% positive for IgG antibodies and in fetal loss 16% positive for IgG antibodies and 5% positive for IgM antibodies. In another article published in 2000,[66] the incidence of aCL-IgG and IgM in pregnant women was very low (between 2.5% and 5%)

Table 4 Pregnancy Outcome and the Influence of the NK Cells

	Percentage of NK Cells (%)					
	0–5	**6–12**	**> 12**	**13–20**	**21–40**	**> 40%**
Diagnosis	**Percentage of Patients Yielding Each Level of NK Cells (%)**					
Abortion ($N = 14$)	0	5	65	8	4	0
Preeclampsia ($N = 10$)	0	4	55	2	2	2
Fetal death ($N = 3$)	1	0	67	2	0	0
Live birth ($N = 94$)	42	22	32	20	10	0

Table 5 Side Effects after Different Treatment Regimens

	Treatment Arm	
Side Effects	**LMWH + Aspirin**	**LMWH + Aspirin + IVIG**
Bleeding in early pregnancy	$n = 10$ No further bleeding after cessation of aspirin	$n = 8$ No bleeding after cessation of aspirin or LMWH
Skin reaction	$n = 1$ (Recurrence after cessation of treatment)	0
Flush	0	$n = 1$ (Late reaction and spontaneous recurrence)

and showed no association with the severity of disease. An investigation of Barbui et al[68] indicated a high incidence of LA (positive in 14% of women with abortions). Ogasawara et al[24] found the prevalence of antibodies to be 5.4% IgG and IgM antibodies and 11.3% for LA. Similar results were reported by Soltesz et al[69] with 14.5% LA and 26.1% IgG antibodies alone and 39.0% IgM antibodies alone, respectively, and both antibodies together in 20.3%. In these previously reported studies, no investigators have described an increase in NK cells in women with APS.[17,66] Konova et al[70] showed that 64.3% of women with recurrent fetal loss and APS had elevated NK cells of $> 12\%$.

Lynch et al[63] also found more antibodies between 20 and 30 U IgG than in higher concentrations reported by Konova et al.[70] The identification of LA or LA and aCL together was higher in preeclamptic patients and lower in the abortion group and also lower in women with uncomplicated pregnancies. The main problem with these findings is the fluctuations in antibodies during pregnancy,[21,48,63,69] and it is possible that persistent high levels of aCL or LA in women with APS are associated with higher pregnancy complications. Unfortunately, we could not repeat all measurements of aCL during pregnancy. We found antibodies directly against β2GPI, but the clinical relevance of this finding is uncertain.[71] In the patient group with the additional therapeutic use of IVIG, the late pregnancy complications were lower (statistically significant) than in the LMWH and aspirin population. These findings are in agreement with the results of Branch et al,[67,71] Vaquero et al,[72] and Valensie et al.[73] A difference in the abortion rate was not demonstrated. These results are in agreement with Carp et al[8] that IVIG did not influence the abortion rate in comparison with LMWH, but the incidence of preeclampsia was lower than that during LMWH and aspirin treatment. Heparin appears to protect pregnancies and prevented abortions by inhibition of the complement system rather than by the anticoagulation system.[74] Expression of different cytokines leads to an increase of activated killer cells, which attack the trophoblast. It is possible that IVIG had a stronger influence of cytokine-activated killer cells in late pregnancy than did LMWH, because anticoagulation is not sufficient to prevent pregnancy complications.[71,72] Recent data have shown that NK cells have a direct cytotoxic effect against trophoblast cells by interaction with the activating KIR receptor.[75] Another mechanism of action for IVIG is that anti-idiotypic antibodies, which may be present in the IVIG preparation, bind autoantibodies or downregulate B-cell receptors or bind receptors of regulatory T cells resulting in suppression of cytokine production and NK cell activity.[9,76] We could demonstrate a subgroup of APS women with RSA and high percentage of NK cells. This finding is in agreement with Perricone et al,[77] which demonstrated that 52% of APS patients had increased levels of NK cells of $CD56^+$ $CD16^+$ type. The association of high pNK cells and poor pregnancy outcome (preeclampsia) was reviewed by Sargent et al.[78] The authors summarized that aberrant NK cells activation locally or systemically (peripheral blood) may be the cause of pregnancy complications.

In conclusion, the additional use of IVIG in women with treatment failure in our retrospective observational study indicates a decrease of pregnancy complications but no differences in the abortion rate. Because there are only rare side effects of IVIG in combination with LMWH and aspirin, IVIG may be a suitable alternative therapy for women with repeated late pregnancy complications, early pregnancy failure, and APS. Despite the important fact that IVIG can cross the placenta, no fetal adverse effects have been reported in studies performed on patients suffering hematologic and autoimmune disorders.[79]

There are no clear recommendations regarding optimal therapy in failure of treatment or refractory cases; however, additional IVIG with clinical judgment will be important to manage such pregnancies. Further studies with NK cells are needed for the evaluation of a strong recommendation in the treatment of refractory cases.

IN APPRECIATION

Both Dr. Eberhard Mammen and Dr. Rodger Bick had a strong interest in the diagnosis and management of APS in pregnant women. They covered this topic together in many educational workshops and provided an authoritative account of this syndrome. Through their effort, there was an increased awareness and development of standard-of-care approaches around the globe. The authors are strongly influenced by their teachings, and the current work is because of the leadership and directions of these two wonderful physicians. Their teaching will continue to impact this area in years to come.

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