RHEUMATOLOGY

Rheumatology 2011;50:1684–1689
doi:10.1093/rheumatology/ker139
Advance Access publication 7 June 2011

Original article

Risk factors for pregnancy failure in patients with anti-phospholipid syndrome treated with conventional therapies: a multicentre, case–control study

Amelia Ruffatti[1], Marta Tonello[1], Maria S. Visentin[1], Agnese Bontadi[1], Ariela Hoxha[1], Sara De Carolis[2], Angela Botta[2], Silvia Salvi[2], Monica Nuzzo[3], Patrizia Rovere-Querini[4], Valentina Canti[4], Marta Mosca[5], Gorana Mitic[6], Maria T. Bertero[7], Vittorio Pengo[8], Marie C. Boffa[9] and Angela Tincani[3]

Abstract

Objective. To identify the risk factors associated with pregnancy failure in patients with APS treated with conventional therapy.

Methods. A multicentre, case–control study was conducted to compare APS patients with successful and unsuccessful pregnancy outcomes. We retrospectively considered 410 pregnancies of women diagnosed with primary APS. The study focused on 57 unsuccessful pregnancies (considered the study population) and 57 successful pregnancies (considered the control population) matched for age and therapy. All the patients had been treated with conventional protocol treatments including low-dose aspirin and/or heparin. The clinical and laboratory features of the two groups of women diagnosed with APS were compared.

Results. The independent risk factors for pregnancy failure were: (i) the presence of SLE or other autoimmune diseases [odds ratio (OR) 6.0; 95% CI 1.7, 20.8; $P = 0.01$]; (ii) history of both thrombosis and pregnancy morbidity (OR 12.1; 95% CI 1.3, 115.3; $P = 0.03$); and (iii) triple [Immunoglobulin (Ig) G/IgM aCLs plus IgG/IgM anti-β_2 glycoprotein I antibodies plus LA] aPL positivity (OR 4.1; 95% CI 1.0, 16.7; $P = 0.05$). APS patients diagnosed on the basis of a single positive test and/or history of pregnancy morbidity alone were generally found to have successful pregnancies.

Conclusion. It would seem from these findings that the risk of pregnancy failure in APS women planning to conceive can be stratified on the basis of some specific clinical and laboratory features.

Key words: Anti-phospholipid antibodies, Anti-phospholipid syndrome, Pregnancy, Risk factors, Heparin, Aspirin.

CLINICAL SCIENCE

Introduction

The prognosis of pregnancies in patients with APS has greatly improved over the past two decades. Providing that appropriate treatment, usually based on heparin and/or low-dose aspirin (LDA), is prescribed [1, 2], ~80% now conclude in the birth of healthy infants. Despite these encouraging statistics, many APS patients are, nonetheless, unable to give birth to healthy neonates [3].

Lima *et al.* [4] reported that history of pregnancy loss and thrombocytopenia are both predictors of pregnancy failure. According to another study [5], the use of aspirin before conception can be considered a significant,

[1]Department of Clinical and Experimental Medicine, Rheumatology Unit, University of Padua, Padua, [2]Department of Obstetrics and Gynecology, Catholic University of Sacred Hearth of Rome, Rome, [3]Rheumatology and Clinical Immunology Unit, University of Brescia, Brescia, [4]San Raffaele Scientific Institute, University of Milan, Milan, [5]Rheumatology Unit, University of Pisa, Pisa, Italy, [6]Institute of Laboratory Diagnostics, Clinical Center of Vojvodina, University Medical School of Novi Sad, Novi Sad, Serbia, [7]Clinical Immunology and Allergology Unit, Mauriziano Hospital of Turin, Turin, [8]Clinical Cardiology, Thrombosis Centre, University of Padua, Padua, Italy and [9]Laboratory of Hematology, Jean-Verdier Hospital APHP of Bondy, Bondy, France.

Submitted 30 August 2010; revised version accepted 9 March 2011.

Correspondence to: Amelia Ruffatti, Reumatologia, Policlinico Universitario, Via Giustiniani, 2 - 35128 Padova, Italy.
E-mail: amelia.ruffatti@unipd.it

independent, prognostic factor associated with a favourable outcome, while an abnormal umbilical artery Doppler velocimetry in foetuses between 23 and 26 weeks of gestation appears to be predictive of pregnancy failure. Danowski *et al*. [6] recently demonstrated that pregnancy loss is more frequent in APS patients also affected with SLE than in women with APS alone. We have observed [7, 8] that different subsets of APS patients with history of pregnancy morbidity can be identified on the basis of their clinical and laboratory characteristics. In particular, women with previous thromboembolism and/or triple aPL positivity [LA plus Immunoglobulin (Ig) G aCL plus IgG anti-β_2 glycoprotein I (anti-β_2GPI) antibodies] have been found to have a higher risk of unsuccessful pregnancy, whereas those with single (LA or aCL or anti-β_2GPI) or double positivity (aCL plus anti-β_2GPI) without thrombosis have a lower risk of complications during pregnancies managed with conventional treatment [8]. The persistence of uterine artery notching and lower complement C3 and C4 component levels have, moreover, been found to be independent predictors of lower neonatal birth weight and of birth at an earlier gestational age, whereas a false-positive IgM TORCH (*Toxoplasma,* rubella, CMV and herpes simplex virus tests) appears to be an independent predictor of lower neonatal birth weight [9].

Identifying the risk factors associated with pregnancy failure could be an important step in aiding clinicians to manage pregnancies in these patients. Some patients, in fact, may require a personalized therapy strategy, in addition to standard protocols, to improve their chances for a successful outcome [10, 11]. As the studies carried out until now [4–9] have referred to small cohorts of APS patients, a case–control study drawing on a large multicentre cohort of conventionally treated pregnancies was designed and conducted to verify if specific laboratory profiles and/or clinical characteristics are predictive of unsuccessful pregnancy outcome.

Methods

Patients

We retrospectively considered 410 pregnancies of women attending six Italian Centres (Brescia, Milan, Padua, Pisa, Rome and Turin) and one Serbian Centre (Novi Sad), all being members of the European Forum on aPLs. All the women had been diagnosed with primary APS on the basis of the International Consensus Statement classification criteria [12]. During their pregnancies the women had been treated with conventional protocol treatments including LDA (100 mg) or heparin, alone or together. The study population consisted in those APS subjects who had a pregnancy failure, defined in accordance with the Sydney classification criteria [12] and thus with one or more of the following characteristics: (i) one or more unexplained fetal deaths of a morphologically normal foetus at or beyond the 10th week of gestation; (ii) one or more premature births of a morphologically normal neonate before the 34th week of gestation due to eclampsia or severe pre-eclampsia or signs of placental insufficiency; and (iii) three or more unexplained, consecutive, spontaneous abortions before the 10th week of gestation excluding causes due to maternal anatomical or hormonal abnormalities and paternal/maternal chromosomal abnormalities.

The control population consisted of APS women with successful pregnancies matched for age, anti-thrombotic therapy (LDA or heparin, alone or together; the drug dosage utilized; and the gestational age when therapy was begun) and additional treatment with steroids. The period examined was between 1988 and 2009 (median year 2003) for the APS patients with pregnancy failure and between 1986 and 2009 (median year 2005) for the patients with successful pregnancies. Pregnancies occurred in these APS patients before the studied pregnancy were untreated. The study was approved by the local ethics committee (ethics committee of University Hospital of Padua, Padua, Italy) and, in accordance with the principles laid down by the Declaration of Helsinki, the participants' medical records were reviewed after written informed consent was obtained. The following baseline data were registered and compared in the study and control populations: APS associated with SLE or other autoimmune diseases, mean disease duration of APS, mean disease duration of SLE or other autoimmune diseases, history of pregnancy morbidity or of thrombosis or of the two together, previous late fetal loss or premature birth or recurrent early abortion, more than one type of pregnancy morbidity, other APS-related manifestations (thrombocytopenia, epilepsy, headache, livedo reticularis, heart valve lesions and haemolytic anaemia), genetic risk factors for thrombosis (factor V Leiden, prothrombin G20210A mutation, decrease in C and S protein antigens and activities), acquired risk factors for thrombosis (hypertension, BMI $\geqslant 30\,kg/m^2$), aPL profile, other autoantibodies (ANAs, anti-dsDNA, anti-ENAs and anti-thyroid antibodies), low C3 and/or C4 levels (<80 and 10 mg/dl, respectively), thrombocytopenia (platelet count $<100\,000/mm^3$). The following data were registered at the end of pregnancies: demographic findings; treatment; type of pregnancy outcome; and maternal/fetal/neonatal complications. Since we were interested in verifying if a decrease in C3 and/or C4 levels, a platelet count $>20\%$ lower than basal values or signs of placental thrombosis at the end of the pregnancies were associated with failure, we compared these parameters in the two study populations.

Antibody detection

aCL and anti-β_2GPI of IgG and IgM isotypes were determined by three of the centres using a home-made ELISA following the minimal requirements proposed by the European Forum on aPLs [13, 14]. The results of aCL testing were expressed as IgG phospholipid (GPL) or IgM phospholipid (MPL) using international reference material [15]. The results of anti-β_2GPI assays were calculated as arbitrary units using a standard curve obtained from a pool of positive samples calibrated to Koike's monoclonal antibodies (HCAL for the IgG and EY2C9 for the IgM anti-β_2GPI) [16]. In

accordance with the Sydney updated classification criteria [12], the cut-off values for medium/high titres for both aCL and anti-β_2GPI antibodies were calculated using the 99th percentile obtained by testing 80 age-matched healthy women. The other four centres assessed IgG and IgM aCL and anti-β_2GPI antibodies using commercial kits following the manufacturers' instructions. LA was assessed by multiple coagulation tests using platelet-poor plasma samples following internationally accepted guidelines [17]. IgG and/or IgM aCL as well as IgG and/or IgM anti-β_2GPI were considered as a single antibody. Single aPL positivity was considered as LA or IgG/IgM aCL or IgG/IgM anti-β_2GPI, double positivity was considered as IgG/IgM aCL plus IgG/IgM anti-β_2GPI or IgG/IgM aCL plus LA or IgG/IgM anti-β_2GPI plus LA and triple positivity was considered as IgG/IgM aCL plus IgG/IgM anti-β_2GPI plus LA. Other autoantibodies including ANAs, anti-dsDNA, anti-ENAs and anti-thyroid antibodies were determined using routine methods. Diagnosis of APS was made on the basis of at least two consecutive positive test results for LA and/or IgG/IgM aCL and/or IgG/IgM anti-β_2GPI antibodies carried out >12 weeks apart; then aPLs were determined at the beginning of the studied pregnancy.

Statistical methods

The statistical comparison between features pertaining to the successful and unsuccessful pregnancies was made using the chi-square, Fisher's exact and the Mann–Whitney tests. A stepwise forward conditional procedure including all the significant risk factors obtained from the univariate analysis was used for the logistic regression analysis to identify significant independent risk factors. The results are expressed as odds ratio (OR) with 95% CI.

Results

Clinical characteristics of the unsuccessful pregnancies

There were 57 pregnancy failures (13.9%) between 1986 and 2009 out of 410 pregnancies in APS patients. Their clinical characteristics are outlined in Table 1. The mean (S.D.) age of these patients was 31 (3.9) years (range 24–41 years). Nine (15.8%) of these women were treated during their pregnancies with LDA, 32 (56.1%) with prophylactic doses of heparin with or without LDA and 16 (28.1%) with therapeutic doses of heparin with or without LDA. Fixed rather than adjusted heparin doses were used preferentially (77.1%) and treatment was started at the beginning of pregnancy in most cases [77.1%, at a mean (S.D.) gestational age of 7.3 (4.8) weeks (range 4–22 weeks)] and before conception in 22.9% of cases. Additional steroid treatment was also prescribed to women affected with associated autoimmune diseases (36.8%).

In accordance with the Sydney classification criteria [12], pregnancy failure was defined as fetal death [25 (43.9%) women], premature birth [22 (38.6%)] and spontaneous abortion [10 (17.5%)]. The maternal complications observed during the pregnancies were pre-eclampsia [7 (12.3%) women], the Haemolytic anaemia Elevated Liver enzymes and Low Platelet count (HELLP) syndrome [5 (8.8%)] and thrombosis [3 (5.3%)]. There were one or more fetal complications in 30 (52.6%) cases: intrauterine growth restriction ($<$10th percentile) in 20 (35.1%), Doppler abnormalities in 23 (40.3%), oligohydramnios in 8 (14.0%) and abnormal heart rate in 4 (7.1%). Six premature neonates died (10.5%) and 13 were underweight (22.8%). Neonatal malformations or thrombosis were not noted.

Clinical characteristics of the successful pregnancies

The control population was made up of 57 APS patients [mean (S.D.) age of 31.5 (3.6) years (range 24–40 years)]

Table 1 Statistical comparison of clinical features in successful and unsuccessful pregnancy groups

Clinical and laboratory features	Unsuccessful pregnancies $n=57$	Successful pregnancies $n=57$	*P*-value	OR (95% CI)
APS associated with SLE or other autoimmune diseases[a]	28/57 (49.1)	7/57 (12.3)	0.000	6.9 (2.7, 17.8)
Disease duration of APS, mean (S.D.)	31.9 (50.1)	25.9 (27.6)	0.90[e]	–
Disease duration of SLE and other autoimmune diseases, months, mean (S.D.)	74.5 (90.9)	32.2 (22.1)	0.97[e]	–
History of pregnancy morbidity alone	29/57 (50.9)	45/57 (78.9)	0.003	0.3 (0.1, 0.6)
Previous late fetal loss	29/47 (61.7)	33//47 (70.2)	0.79[e]	–
Previous premature birth	2/47 (4.3)	6/47 (12.8)	0.79[e]	–
Previous early abortions	7/47 (14.9)	6/47 (12.8)	0.79[e]	–
More than one type of pregnancy morbidity[b]	9/47 (19.1)	2/47 (4.3)	0.79[e]	–
History of thrombosis	28/57 (49.1)	12/57 (21.1)	0.003	3.6 (1.6, 8.2)
History of both pregnancy morbidity and thrombosis	18/57 (31.6)	2/57 (3.5)	0.000	12.7 (2.8, 57.9)
Other APS-related manifestations[c]	19/57 (34.5)	7/57 (12.3)	0.01	3.8 (1.4, 9.9)
Acquired risk factors for thrombosis[d]	16/56 (28.6)	12/55 (21.8)	0.548[e]	–

Values are represented as *n* (%), unless otherwise mentioned. [a]UCTD, SS and type I diabetes. [b]Previous late fetal loss plus premature birth or previous late fetal loss plus early abortion. [c]Thrombocytopenia, epilepsy, headache, livedo reticularis, heart valve lesions and haemolytic anaemia. [d]Hypertension and BMI $\geqslant 30$ kg/m. [e]NS: not statistically significant.

whose pregnancies ended successfully. Their clinical characteristics are summarized in Table 1. Five (8.8%) were treated during their pregnancies with LDA, 34 (59.6%) with prophylactic doses of heparin with or without LDA and 18 (31.6%) with therapeutic doses of heparin with or without LDA. Fixed rather than adjusted heparin doses were used preferentially (63.2%) and treatment was started at the beginning of pregnancy in most cases [72%, at a mean (S.D.) gestational age of 7.5 weeks (3.9) (range 4–22 weeks)] and before conception in 28% of cases. Additional steroid treatment was also prescribed to women affected with associated autoimmune diseases (22.8%). All gave birth to live infants between the 36th and 41st week of gestation [mean (S.D.) 38.2 (1.2)]. No maternal or fetal complications were noted.

Laboratory characteristics

The laboratory features of all the women studied are reported in Table 2. In particular, single aPL positivity was found in 14 APS patients with unsuccessful pregnancies (6 IgG/IgM anti-β_2GPI, 5 LA and 3 IgG/IgM aCL) and in 28 with successful pregnancies (13 IgG/IgM anti-β_2GPI, 8 IgG/IgM aCL and 7 LA); double aPL positivity was detected in 6 APS patients with unsuccessful pregnancies (4 IgG/IgM anti-β_2GPI plus LA, 1 IgG/IgM aCL plus LA and 1 IgG/IgM anti-β_2GPI plus IgG/IgM aCL) and in 13 with successful pregnancies (6 IgG/IgM anti-β_2GPI plus IgG/IgM aCL, 5 IgG/IgM anti-β_2GPI plus LA and 2 IgG/IgM aCL plus LA). Eighteen APS patients with unsuccessful pregnancies and four with successful ones had triple aPL positivity (IgG/IgM aCL plus IgG/IgM anti-β_2GPI plus LA).

Risk factors for pregnancy failure

As indicated in Tables 1 and 2, at univariate analysis, the APS women with unsuccessful pregnancies had a significant prevalence of the following: SLE or other autoimmune diseases, thrombotic events, history of both pregnancy morbidity and vascular thrombosis, other APS-related manifestations, IgG anti-β_2GPI antibodies, LA activity, triple aPL positivity, other autoantibodies, lower C3 and/or C4 levels both at baseline and at the time of pregnancy failure, a decreased platelet count (>20% with respect to the basal value) detected at the time of pregnancy failure and placental thrombosis. There was a significant prevalence of single aPL positivity and history of pregnancy morbidity alone, instead, in women with successful pregnancies. The independent features related, at multivariate analysis, to pregnancy failure were: the presence of SLE or other autoimmune diseases (OR 6.0; 95% CI 1.7, 20.8; $P = 0.01$), history of both thrombosis and pregnancy morbidity (OR 12.1; 95% CI 1.3, 115.3; $P = 0.03$) and triple aPL positivity (OR 4.1; 95% CI 1.0, 16.7; $P = 0.05$). The prevalence of the three risk factors in the two groups is summarized in Table 3.

Discussion

This is the first case–control study, to our knowledge, aiming to investigate the risk factors for pregnancy failure in women diagnosed with definite APS treated with conventional therapy protocols. The clinical and laboratory features of APS patients attending seven centres were assessed.

The study's most important finding was that there are three major independent risk factors for pregnancy failure in APS: (i) SLE or other autoimmune diseases; (ii) a history

Table 2 Statistical comparison of laboratory features in successful and unsuccessful pregnancy groups

Laboratory features	Unsuccessful pregnancies ($n = 57$), n (%)	Successful pregnancies ($n = 57$), n (%)	P-value	OR (95% CI)
IgG aCL	36/56 (64.3)	27/57 (47.4)	0.157[c]	–
IgM aCL	16/56 (28.6)	7/57 (12.3)	0.055[c]	–
IgG anti-β_2GPI	24/39 (61.5)	16/45 (35.6)	0.03	2.9 (1.2, 7.0)
IgM anti-β_2GPI	16/39 (41.0)	14/45 (31.1)	0.473[c]	–
LA	41/54 (75.9)	25/57 (43.9)	0.001	4.0 (1.8, 9.1)
Single aPL positivity	14/38 (36.8)	28/45 (62.2)	0.04	0.4 (0.1, 0.9)
Double aPL positivity	6/38 (15.8)	13/45 (28.9)	0.249[c]	–
Triple aPL positivity	18/38 (47.4)	4/45 (8.9)	0.000	9.2 (2.8, 30.9)
Other autoantibodies[a]	42/53 (79.2)	24/53 (45.2)	0.001	4.6 (2.0, 10.9)
Decrease in C3 and/or C4 at baseline	17/46 (37.0)	4/44 (9.1)	0.004	5.9 (1.8, 19.3)
Decrease in C3 and/or C4 at the end of pregnancy	6/40 (15.0)	1/46 (2.7)	0.046	7.9 (0.9, 69.1)
Decrease in platelets at baseline	4/55 (7.3)	5/54 (9.3)	0.742[c]	–
Decrease in platelets at the end of pregnancy	20/53 (37.7)	5/54 (9.3)	0.001	11.5 (2.0, 17.4)
Genetic risk factors for thrombosis[b]	8/56 (14.3)	3/55 (5.5)	0.215[c]	–
Placental thrombosis	17/20 (85.0)	5/33 (15.2)	0.000	31.7 (6.7, 150.0)

[a]ANAs, anti-dsDNA, anti-ENAs and anti-thyroid antibodies. [b]Factor V Leiden, prothrombin G20210A mutation and decrease in C and S protein antigens and activities. [c]NS: not statistically significant.

TABLE 3 Prevalence of the independent risk factors for pregnancy failure in successful and unsuccessful pregnancy groups

APS associated with SLE or other AD	History of both pregnancy morbidity and thrombosis	Triple aPL positivity	Patients with pregnancy failure, %	Patients with successful pregnancies, %
1	1	1	13.16	0
1	1	0	2.63	0
0	1	1	13.16	2.2
1	0	1	10.53	2.2
0	1	0	5.26	0
1	0	0	21.05	8.8
0	0	1	10.53	4.4
0	0	0	23.68	82.2

AD: autoimmune diseases.

of both thromboembolism and pregnancy morbidity; and (iii) full (triple) aPL antibody positivity. SLE and history of thromboembolism are not novel risk factors for pregnancy morbidity, as some authors [6] have reported that the frequency of pregnancy loss is greater in APS associated with SLE than in primary APS. Others [7] have demonstrated that a history of thromboembolism predicts new thromboembolic events and new unsuccessful pregnancies. Yet others [8] have shown that triple aPL positivity is a marker of poor pregnancy outcome. What is novel about the present study is that all three parameters were found to be independent risk factors for poor pregnancy outcome in APS women with pregnancy failure, despite conventional treatment. These results could be clinically useful for pregnancy counselling and for treatment planning of these APS patients.

As far as aPL profiles are concerned, we confirm that single positivity is not a risk factor for pregnancy failure in treated patients [8]. We have found, in fact, that APS patients diagnosed on the basis of a single positive test generally have successful pregnancies when they are treated with conventional therapy. A history of pregnancy morbidity alone is, likewise, predictive of successful outcome provided that the patients are treated with conventional therapy. The type of previous pregnancy morbidity does not apparently affect the outcome of a new pregnancy. Congenital thrombophilia was, likewise, not correlated with pregnancy failure, although the number of positive cases may be too small for statistical analysis.

Other risk factors for pregnancy failure assessed by us were identified at univariate analysis. The first was lower C3 and C4 levels at baseline and at the time of pregnancy failure, both found to be significantly correlated with poor outcome. The role of complement activation in the pathogenesis of APS pregnancy morbidity is an intriguing question. Some authors [18, 19] have, in fact, demonstrated that activation of complement components C3, C4 and C5 increases the risk of injury or death of the embryo or foetus in animal models injected with aPL. Other investigators [20] have, moreover, found increased complement deposition (C4d and C3b) in placentas from aPL-positive women. Our results, in accordance with those of a recent, prospective cohort study [9], seem to further contribute to defining the important role of the complement system in the pathogenesis of pregnancy failure in APS patients.

A platelet decrease (>20% of the baseline value) immediately preceding pregnancy failure is also significantly associated with pregnancy morbidity. As hypothesized in previous reports [10, 21], it seems to be a very early sign of pregnancy complications, especially when it takes place during the first or second trimester.

Finally, placental intervillous thrombosis and infarction were significantly correlated with pregnancy failure in these patients. However, as reported in a recent review [22], not all placentas from women with aPL-associated pregnancy failure show signs of infarction or placental vasculopathy. Some authors [23] have demonstrated, in fact, that placentas from aPL-positive women have histopathological signs of inflammation, supporting the hypothesis that thrombotic events are not the only pathogenic mechanism in APS patients with recurrent fetal loss.

Conclusion

The major independent risk factors for poor pregnancy outcome in pregnant APS women being treated with conventional therapy protocols are SLE or other autoimmune diseases, a history of both thrombosis and pregnancy morbidity, and triple aPL antibody positivity. APS patients with a history of pregnancy morbidity alone and/or a single aPL antibody positivity generally have successful pregnancies. Our study may, however, contain selection and information biases due to its retrospective and multicentric nature. If these findings are confirmed by large-scale prospective studies, prenatal counselling and therapeutic recommendations for APS patients planning to conceive will need to be differentiated on the basis of the clinical and laboratory features.

Rheumatology key messages

- There are three independent factors for poor outcome in conventionally treated pregnant APS women.
- Previous pregnancy morbidity alone and/or a single aPL positivity are associated with successful pregnancies.

Acknowledgements

The authors are grateful to Dr Elisa Salvan for the statistical calculations and to Mrs Linda Inverso Moretti for editing the English version of this manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

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