

## PAPER

# Pregnancy failure in patients with obstetric antiphospholipid syndrome with conventional treatment: the influence of a triple positive antibody profile

JO Latino<sup>1,\*</sup>, S Udry<sup>1,2,\*</sup>, FM Aranda<sup>2</sup>, SDA Perés Wingeyer<sup>2</sup>, DS Fernández Romero<sup>1</sup> and GF de Larrañaga<sup>2</sup>  
<sup>1</sup>Autoimmune, Thrombophilic Diseases and Pregnancy Section, Hospital “Dr. Carlos G. Durand”, Buenos Aires, Argentina; and <sup>2</sup>Hemostasis and Thrombosis Laboratory, Hospital of Infectious Diseases “Dr. F J Muñiz”, Buenos Aires, Argentina

Conventional treatment of obstetric antiphospholipid syndrome fails in approximately 20–30% of pregnant women without any clearly identified risk factor. It is important to identify risk factors that are associated with these treatment failures. This study aimed to assess the impact of risk factors on pregnancy outcomes in women with obstetric antiphospholipid syndrome treated with conventional treatment. We carefully retrospectively selected 106 pregnancies in women with obstetric antiphospholipid syndrome treated with heparin + aspirin. Pregnancy outcomes were evaluated according to the following associated risk factors: triple positivity profile, double positivity profile, single positivity profile, history of thrombosis, autoimmune disease, more than four pregnancy losses, and high titers of anticardiolipin antibodies and/or anti- $\beta$ -2-glycoprotein-I (a $\beta$ 2GPI) antibodies. To establish the association between pregnancy outcomes and risk factors, a single binary logistic regressions analysis was performed. Risk factors associated with pregnancy loss with conventional treatment were: the presence of triple positivity (OR = 5.0, CI = 1.4–16.9,  $p = 0.01$ ), high titers of a $\beta$ 2GPI (OR = 4.4, CI = 1.2–16.1,  $p = 0.023$ ) and a history of more than four pregnancy losses (OR = 3.5, CI = 1.2–10.0,  $p = 0.018$ ). The presence of triple positivity was an independent risk factor associated with gestational complications (OR = 4.1, CI = 1.2–13.9,  $p = 0.02$ ). Our findings reinforce the idea that triple positivity is a categorical risk factor for poor response to conventional treatment. *Lupus* (2017) 0, 1–6.

**Key words:** Antiphospholipid syndrome; thrombosis; pregnancy; Hughes syndrome; anticardiolipin antibodies

## Introduction

The outcome of pregnancies in patients with obstetric antiphospholipid syndrome (APS) treated with heparin and/or aspirin has been the object of many studies.<sup>1–4</sup> In many of these investigations, patients were studied as a homogeneous group, regardless of the antiphospholipid antibody (aPL) profile or clinical features.<sup>5</sup>

Notably, conventional treatment fails in approximately 20% of pregnant APS women, without any clearly identified risk factor. Currently, new

studies have identified clinical and/or laboratory factors related to these poor obstetric outcomes.<sup>6</sup> Bramham et al.<sup>7</sup> showed that women with thrombotic APS have higher rates of pregnancy complications, such as preterm birth and small for gestational age babies, than those with obstetric APS alone. Danowski et al.<sup>8</sup> identified systemic lupus erythematosus (SLE) to be an independent risk factor associated with pregnancy loss in APS patients. Carmona et al.<sup>9</sup> reported that abnormal uterine arteries Doppler velocimetry has predictive power for adverse gestational outcomes in patients with autoimmune and thrombotic diseases. Simchen et al.<sup>10</sup> confirmed that pregnant women with APS and high positive aPL titers are a unique and extremely high-risk group for adverse fetal/neonatal outcomes. Jeremic et al.<sup>11</sup> concluded that the most important prognostic factor for pregnancy outcome in APS patients is the number of previous miscarriages. Additionally, De Carolis

Correspondence to: Gabriela de Larrañaga Hospital of Infectious Diseases “Dr. F. J. Muñiz”, Hemostasis and Thrombosis Laboratory Uspallata 2272 (1282) - Buenos Aires, Argentina.  
Email: hemostasia@gmail.com

\*These authors contributed equally to this study.  
Received 9 May 2016; accepted 5 January 2017

et al.<sup>12</sup> identified low levels of complement C3 and C4 as independent risk factors for poor pregnancy outcomes. These authors have also suggested that the presence of *Cytomegalovirus* (CMV) IgM false positivity could represent a novel prognostic factor for poor pregnancy outcome in APS patients.<sup>13</sup> More recently, Ruffatti et al.<sup>14–17</sup> evaluated the relationship between the laboratory profile and clinical features of mothers with APS, identifying an association between triple positivity for aPL, history of thrombosis and/or having an autoimmune disease with poor pregnancy outcomes and/or pregnancy complications.

Taking into account these investigations, we believe that APS patients should not be studied as a homogeneous group; rather, they should be stratified according to risk factors. We consider that identifying these risk factors associated with pregnancy failure could be used as a tool to classify these women into different risk groups and thus implement new therapeutic strategies to improve perinatal outcomes.

## Methods

### *Study population*

We carefully retrospectively selected 106 pregnancies of women with complete data who attended our center (Hospital Carlos G. Durand, Buenos Aires, Argentina) from April 2007 to December 2014 and were diagnosed and treated for APS according to updated international consensus classification criteria.<sup>18</sup> These criteria included at least one clinical criterion and the presence of at least one laboratory criterion. Clinical criteria included a history of thrombosis (venous and/or arterial thrombosis) and/or obstetric morbidity (three pregnancy losses before the 10th week and/or one pregnancy loss at or after the 10th week and/or premature delivery before the 34th week due to preeclampsia or placental insufficiency).<sup>18</sup> Laboratory criteria included positive test results for aPL, represented by a positive test for lupus anticoagulant (LA), and/or moderated or high titers for anti- $\beta_2$  glycoprotein I (a $\beta_2$ GPI) antibody IgG and/or IgM and/or moderated or high titers for anticardiolipin antibodies (aCL) IgG and/or IgM. Laboratory criteria should be positive on two or more occasions at least 12 weeks after observing positivity.<sup>18</sup> All pregnant women fulfilled the obstetric criteria for APS. These patients were only treated with conventional treatment for

obstetric APS, including low dose aspirin (LDA) 100 mg/day since the preconceptional period (at least one month before attempting conception) + a prophylactic dose of low molecular weight heparin (LMWH) 40 mg/day since the diagnosis of pregnancy. Only five pregnant women, who had a history of deep venous thrombosis, received therapeutic doses of LMWH + LDA. All SLE patients were in remission and were not receiving any specific treatment for this pathology.

Strict exclusion criteria consisted of the following: initiating conventional therapy for APS after the seventh week of gestation, having other thrombophilias (deficit of protein S, protein C and/or antithrombin, hyperhomocysteinemia, Factor V Leiden and/or prothrombin 20210), metabolic or endocrine alterations (Cushing's disease, diabetes, thyroid disease), anatomic abnormalities of the uterus, carriage of parental chromosomal abnormalities, clinical conditions that might negatively affect the pregnancy outcome (cardiovascular disease, chronic renal failure, hypercholesterolemia, etc.) or patients receiving an additional treatment for rheumatic diseases.

Pregnancy loss was defined as the loss of the product of conception during pregnancy, including fetal loss and miscarriage. Miscarriage was defined as pregnancy losses prior to 20 weeks of gestation; early and late miscarriage were defined as pregnancy losses prior to and after 10 weeks, respectively; and fetal loss was defined as pregnancy losses after 20 weeks of gestation. Pregnancy complications include: preeclampsia, fetal growth restriction and prematurity. Preeclampsia was defined as pregnancy induced hypertension after 20 weeks associated with proteinuria. Fetal growth restriction was defined as fetal weight below the 10th percentile. Low birth weight was defined as the weight of babies below the 10th percentile. Prematurity was defined as the child born before 36 completed weeks of gestation.

Pregnancy outcomes were assessed on the basis of the following parameters: pregnancy loss, fetal loss, and miscarriage and pregnancy complications.

All subjects were of Argentine descent and had the same ethno-geographic and social origin. The Argentinean population is the result of genetic admixture processes involving three continental contributors: Native Americans, Western Africans and Europeans (mainly Spaniards and Italians), although the African component detected in different studies was very low (less than 4%).<sup>19,20</sup> We

selected a representative sample of the admixed, urban Argentinean population.

## Laboratory tests

### *Blood samples*

Blood was obtained by clean venipuncture (12-hour fast) and collected into plastic tubes containing sodium citrate (ratio 9:1). After double centrifugation at 2500g for 15 minutes, platelet-poor plasma was immediately assayed for LA tests and then stored at  $-40^{\circ}\text{C}$ . Blood was collected into tubes and allowed to clot at  $37^{\circ}\text{C}$  and then centrifuged at 1500g for serum preparation. Serum was stored at  $-40^{\circ}\text{C}$  until use.

### *Serum*

aCL was measured by homemade standardized enzyme immunoassays of IgG and IgM isotypes as previously reported.<sup>21</sup>  $\text{a}\beta_2\text{GPI}$  was measured by a commercial enzyme immunoassay kit of IgG and IgM isotypes (BioSystems S.A., Barcelona, Spain). We considered a positive result of serum levels of aCL or  $\text{a}\beta_2\text{GPI}$  in medium or high titers on two occasions at least 12 weeks apart. We excluded weak or positive results on only one occasion.

A positive result of aCL IgG and IgM isotypes or  $\text{a}\beta_2\text{GPI}$  IgG and IgM isotypes was defined as  $>95\text{th}$  percentile = 15 GPL or MPL or 10 UL, respectively. A medium titer was defined as  $>99\text{th}$  percentile = 40 GPL or MPL or 40 UL, respectively, according to the international criteria.<sup>18</sup> A high titer was arbitrarily defined as 80 GPL or MPL or 80 UL, respectively.<sup>10</sup>

### *Plasma citrate*

Plasma samples were evaluated for the presence of LA activity using two tests: dilute Russell viper venom time (TriniCLOT Lupus Screen and/or Confirm, Tcoag, France) and Silica Clotting Time (Hemosil, Instrumentation Laboratory, USA). To identify the inhibitor, these clotting tests were carried out on 1:1 mixtures of patient and normal plasma when they corresponded. As confirmatory tests, we used a phospholipid neutralization procedure. LA was diagnosed when at least one of the screening and one of the confirmatory procedures were positive, according to the international criteria.<sup>18</sup> We considered a patient to have LA if the test was positive on two occasions at least 12 weeks apart.

## Risk factors

We classified patients according to positivity on laboratory tests for aPL: triple positive for aPL (LA+, aCL+,  $\text{a}\beta_2\text{GPI}$ +), double positive for aPL (LA+ and aCL+ or  $\text{a}\beta_2\text{GPI}$ +; or aCL+ and  $\text{a}\beta_2\text{GPI}$ +) and single positive for aPL (LA+ or aCL+ or  $\text{a}\beta_2\text{GPI}$ ).

We also considered the following as risk factors: having autoimmune disease (SLE), history of thrombosis, number of pregnancy losses, and having high levels of aCL and/or  $\text{a}\beta_2\text{GPI}$  titers (80 GPL or MPL and/or 80 UL, respectively).<sup>10</sup>

Other risk factors were not taken into account because of the limited number of cases.

## Ethics

The study was approved by the ethical committee of the respective medical centers and was performed according to the principles of the Declaration of Helsinki. Informed consent was obtained from all of the participants.

## Statistical analysis

Statistical analysis was performed using the SPSS statistical software package (version 15.0 for window SPSS, Chicago, IL, USA). Because the data were not normally distributed, they were presented as medians and percentiles (25 and 75) or percentages. Nonparametric tests (Mann–Whitney U) were used to compare quantitative data, and the Chi-squared test or Fisher's exact test was used to compare proportions. A simple binary logistic regression analysis was performed to test the association between pregnancy failure and the studied variables (multivariable logistic regression was not performed). The odds ratio (OR) was estimated to assess the strength of this association.  $p < .05$  was considered statistically significant (bold values).

## Results

This study included 106 pregnancies of diagnosed obstetric APS women (median age: 32 years; interquartile range: 28–37) treated with conventional therapy (LDA + prophylactic or therapeutic LMWH) between 2007 and 2014 at our clinical

center. All patients had a history of pregnancy morbidity: 49 (46.2%) with fetal loss, 75 (70.8%) with early miscarriage, 36 (33.9%) with late miscarriage, 45 (42.4%) with premature birth, 17 (16.0%) with a history of preeclampsia and 17 (16.0%) with a low weight birth.

Of these patients, 18 (16.9%) had APS associated with SLE, five (4.7%) had previously suffered from venous thrombosis and 33 (28%) patients presented more than four pregnancy losses.

aCL was positive in 46 cases (43.4%) and presented with high titers ( $X > 80$  GPL and/or MPL) in 11 (10.4%) cases; a $\beta$ 2GPI was positive in 35 (33.0%) cases and presented with high titers ( $X > 80$  UL, IgG and/or IgM) in 12 (11.3%) cases. LA was positive in 64 cases (60.4%). High aPL titers (aCL and/or a $\beta$ 2GPI) were present in 19 cases (17.9%).

A triple positivity profile for aPL was detected in 14 (13.2%) patients, a double positivity profile for aPL was detected in 11 (10.4%) patients and single positivity for aPL was detected in 81 (76.4%) patients.

Notably, the patients with triple positivity for aPL showed a high prevalence of other risk factors, as presented in Table 1. We show that the high aPL titer was more prevalent among patients with triple positivity for aPL in comparison with double or single positivity for aPL (71.4% vs 18.2% vs 8.6%, respectively); this distribution was statistically significant ( $p < .01$ ).

We further analyzed the group of APS patients according to pregnancy outcomes after treatment (Table 2). Among them, 18 (16.9%) had pregnancy loss: seven (6.6%) had fetal loss and 11 (10.4%) exhibited miscarriage. Live birth was present in 88 (83%) cases and gestational complications in 19 cases (17.9%) (six fetal growth restriction, five preeclampsia and 11 premature). We analyzed pregnancy outcome and different independent risk factors.

To establish the association between pregnancy outcomes and risk factors, a single binary logistic regression analysis was performed.

Risk factors associated with pregnancy loss with conventional treatment were: the presence of triple positivity for aPL (OR = 5.0, CI = 1.4–16.9,  $p = 0.01$ ), high a $\beta$ 2GPI titers (OR = 4.4, CI = 1.2–16.1,  $p = 0.023$ ) and history of more than four pregnancy losses (OR = 3.5, CI = 1.2–10.0,  $p = 0.018$ ).

The presence of triple positivity for aPL was an independent risk factor associated with gestational complications (OR = 4.2, CI = 1.2–13.9,  $p = 0.02$ ). Moreover, the presence of single positivity for aPL also turned out to be associated with a good response to treatment (OR = 0.3, CI = 0.1–0.9,  $p = 0.03$ ).

## Discussion

When we considered conventional treatment with LMWH and LDA established throughout all patients without discriminating risk factors, the results were similar to those reported by other groups; in fact, live birth occurred in 83.0%, pregnancy loss occurred in 16.9% and pregnancy complications occurred in 17.9%.<sup>2,22</sup> Although we could interpret these results as representing a good response to treatment, we need to consider that obstetric APS is an entity that includes wide variability in the response to treatment according to some risk factors. For this reason, it is essential to analyze our patients considering specific risk factors to understand the impact of these factors on the response to treatment.

When we analyzed the relationship between pregnancy outcomes with conventional treatment and the studied risk factors, we found that there was a strong association between triple positivity for aPL and/or the presence of high a $\beta$ 2GPI titers, especially with pregnancy loss. Moreover, it

**Table 1** Prevalence of risk factors for pregnancy failure among obstetric APS patients divided according to their aPL profile

| Risk factor                        | All APS patients | Single positivity<br>81/106 (76%) | Double positivity<br>11/106 (10%) | Triple positivity<br>14/106 (13%) |
|------------------------------------|------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| History of thrombosis              | 5/106 (4.7%)     | 2/81 (2.5%)                       | 1/11 (9.1%)                       | 2/14 (14.3%)                      |
| APS associated with SLE            | 18/106 (16.9%)   | 9/81 (11.1%)                      | 2/11 (18.2%)                      | 7/14 (50.0%)                      |
| More than four pregnancy losses    | 31/106 (29.2%)   | 23/81 (28.4%)                     | 1/11 (9.1%)                       | 7/14 (50.0%)                      |
| aCL $X > 80$                       | 11/106 (10.4%)   | 5/81 (6.2%)                       | 1/11 (9.1%)                       | 5/14 (35.7%)                      |
| a $\beta$ 2GPI $X > 80$            | 12/106 (11.3%)   | 2/81 (2.5%)                       | 1/11 (9.1%)                       | 9/14 (64.2%)                      |
| aCL and/or a $\beta$ 2GPI $X > 80$ | 19/106 (17.9%)   | 7/81 (8.6%)                       | 2/11 (18.2%)                      | 10/14 (71.4%)                     |

APS: antiphospholipid syndrome; aPL: antiphospholipid antibodies; SLE: systemic lupus erythematosus, aCL: anticardiolipin antibodies; a $\beta$ 2GPI: anti- $\beta$ 2 glycoprotein I antibodies.

**Table 2** Association between major risk factors for pregnancy failure among obstetric APS patients after conventional treatment

| Pregnancy outcomes | Global APS patients | SLE N = 18    | Single positivity N = 81 | Double positivity N = 11 | Triple positivity N = 14 | History of thrombosis N = 5 | More than four pregnancy losses N = 31 | High aCL titers N = 11 | High aβ2GPI titers N = 12 |
|--------------------|---------------------|---------------|--------------------------|--------------------------|--------------------------|-----------------------------|--|------------------------|---------------------------|
| Pregnancy losses   | 18/106 (16.9%)      | 3/18 (16.7%)  | 10/81 (12.3%)            | 2/11 (18.2%)             | 6/14 (42.8%)             | 2/5 (40.0%)                 | 9/31 (29.0%)                           | 3/11 (27.3%)           | 5/12 (41.7%)              |
| Complications      | 19/106 (17.9%)      | 6/18 (33.3%)  | 12/81 (14.8%)            | 1/11 (9.1%)              | 6/14 (42.8%)             | 0/5 (0.0%)                  | 5/31 (16.1%)                           | 3/11 (27.3%)           | 3/12 (25.0%)              |
| Live birth         | 88/106 (83.0%)      | 15/18 (83.3%) | 71/81 (87.6%)            | 9/11 (81.8%)             | 8/14 (57.1%)             | 3/5 (60.0%)                 | 22/31 (70.9%)                          | 8/11 (72.7%)           | 7/12 (58.3%)              |
| Fetal losses       | 7/106 (6.6%)        | 3/18 (16.7%)  | 3/81 (3.7%)              | 1/11 (9.1%)              | 3/14 (21.4%)             | 0/5 (0.0%)                  | 2/31 (6.4%)                            | 2/11 (18.2%)           | 1/12 (8.3%)               |
| Miscarriage        | 11/106 (10.4%)      | 0/18 (0.0%)   | 7/81 (8.6%)              | 1/11 (9.1%)              | 3/14 (21.4%)             | 2/5 (40.0%)                 | 7/31 (22.6%)                           | 1/11 (9.1%)            | 4/12 (33.3%)              |

APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus; aCL: anticardiolipin antibodies; aβ2GPI: anti-β2 glycoprotein I antibodies.

is notable that the other risk factors analyzed were more prevalent in the triple positive aPL group. We hypothesized that these other risk factors are relevant, but not independent from exhibiting triple positivity for aPL. These findings are in agreement with those of previous studies from several groups worldwide who reported that patients with triple positivity for aPL and higher aβ2GPI titers have a worse prognosis and a poorer response to treatment.<sup>14,15,23,24</sup> Despite the relationship between some risk factors mentioned in other studies, we consider that the main strength of our work is that we performed a comprehensive analysis of the distribution of these factors according to aPL profile.

Notwithstanding, we acknowledge that our work has some limitations. We did not include all described risk factors because we did not have the results for all patients with regard to uterine arteries Doppler velocimetry, serum C3 and C4 levels and CMV IgM false positivity results.

There is a growing body of published evidence that the presence of triple positivity for aPL is due to antibodies directed to the first domain of β2GPI.<sup>23–25</sup> These pathogenic antibodies are more frequent in patients having higher antibody titers and triple positivity for aPL. This seems to make sense because these pathogenic antibodies have LA activity.<sup>26</sup> On the other hand, single positivity for the aPL profile seems to be associated with positive tests for anti-domain 4/5, considering these are non-pathogenic antibodies.<sup>27</sup> In fact, we found that single positivity in aPL patients have successful pregnancies when they are treated with conventional therapy.

In conclusion, our findings reinforce the idea that triple positivity for aPL is a categorical risk factor for poor response to conventional treatment. To prevent future pregnancy losses, we propose adding further treatment in patients with triple antibody positivity for aPL, especially when they have high antibody titers.

## Acknowledgments

We would like to thank Mrs Analía Lucero for her excellent technical support.

## Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Ministry of the Government of the Autonomous City of Buenos Aires, GCABA Grant 2014.

## References

- 1 Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. *Am J Obstet Gynecol* 1996; 174: 1584–1589.
- 2 Rai R, Cohen H, Dave M, *et al.* Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *BMJ* 1997; 314: 253–257.
- 3 Franklin RD, Kutteh WH. Antiphospholipid antibodies (APA) and recurrent pregnancy loss: treating a unique APA positive population. *Hum Reprod* 2002; 17: 2981–2985.
- 4 Mak A, Cheung MW, Cheak AA, *et al.* Combination of heparin and aspirin is superior to aspirin alone in enhancing live births in patients with recurrent pregnancy loss and positive anti-phospholipid antibodies: a meta-analysis of randomized controlled trials and meta-regression. *Rheumatology (Oxford)* 2010; 49: 281–288.
- 5 Empson M, Lassere M, Craig JC, *et al.* Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of therapeutic trials. *Obstet Gynecol* 2002; 99: 135–144.
- 6 Ruffatti A, Tonello M, Visentin MS, *et al.* Risk factors for pregnancy failure in patients with anti-phospholipid syndrome treated with conventional therapies: a multicentre, case-control study. *Rheumatology (Oxford)* 2011; 50: 1684–1689.
- 7 Bramham K, Thomas M, Nelson-Piercy C, *et al.* First-trimester low-dose prednisolone in refractory antiphospholipid antibody-related pregnancy loss. *Blood* 2011; 117: 6948–6951.
- 8 Danowski A, de Azevedo MN, de Souza Papi JA, *et al.* Determinants of risk for venous and arterial thrombosis in primary antiphospholipid syndrome and in antiphospholipid syndrome with systemic lupus erythematosus. *J Rheumatol* 2009; 36: 1195–1199.
- 9 Carmona F, Font J, Azulay M, *et al.* Risk factors associated with fetal losses in treated antiphospholipid syndrome pregnancies: a multivariate analysis. *Am J Reprod Immunol* 2001; 46: 274–279.
- 10 Simchen MJ, Dulitzki M, Rofe G, *et al.* High positive antibody titers and adverse pregnancy outcome in women with antiphospholipid syndrome. *Acta Obstet Gynecol Scand* 2011; 90: 1428–1433.
- 11 Jeremic K, Stefanovic A, Dotlic J, *et al.* Neonatal outcome in pregnant patients with antiphospholipid syndrome. *J Perinat Med* 2015; 43: 761–768.
- 12 De Carolis S, Botta A, Santucci S, *et al.* Predictors of pregnancy outcome in antiphospholipid syndrome: a review. *Clin Rev Allergy Immunol* 2010; 38: 116–124.
- 13 De Carolis S, Santucci S, Botta A, *et al.* The relationship between TORCH complex false positivity and obstetric outcome in patients with antiphospholipid syndrome. *Lupus* 2012; 21: 773–775.
- 14 Ruffatti A, Tonello M, Cavazzana A, *et al.* Laboratory classification categories and pregnancy outcome in patients with primary antiphospholipid syndrome prescribed antithrombotic therapy. *Thromb Res* 2009; 123: 482–487.
- 15 Ruffatti A, Calligaro A, Hoxha A, *et al.* Laboratory and clinical features of pregnant women with antiphospholipid syndrome and neonatal outcome. *Arthritis Care Res (Hoboken)* 2010; 62: 302–307.
- 16 Ruffatti A, Calligaro A, Del Ross T, *et al.* Risk-based secondary prevention of obstetric antiphospholipid syndrome. *Lupus* 2012; 21: 741–743.
- 17 Ruffatti A, Salvan E, Del Ross T, *et al.* Treatment strategies and pregnancy outcomes in antiphospholipid syndrome patients with thrombosis and triple antiphospholipid positivity. A European multicentre retrospective study. *Thromb Haemostasis* 2014; 112: 727–735.
- 18 Miyakis S, Lockshin MD, Atsumi T, *et al.* International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemostasis* 2006; 4: 295–306.
- 19 Catelli ML, Alvarez-Iglesias V, Gomez-Carballa A, *et al.* The impact of modern migrations on present-day multi-ethnic Argentina as recorded on the mitochondrial DNA genome. *BMC Genet* 2011; 12: 77.
- 20 Corach D, Lao O, Bobillo C, *et al.* Inferring continental ancestry of argentineans from Autosomal, Y-chromosomal and mitochondrial DNA. *Ann Hum Genet* 2010; 74: 65–76.
- 21 De Larranaga GF, Forastiero RR, Carreras LO, *et al.* Different types of antiphospholipid antibodies in AIDS: a comparison with syphilis and the antiphospholipid syndrome. *Thromb Res* 1999; 96: 19–25.
- 22 Erkan D, Patel S, Nuzzo M, *et al.* Management of the controversial aspects of the antiphospholipid syndrome pregnancies: a guide for clinicians and researchers. *Rheumatology (Oxford)* 2008; 47(Suppl 3): iii23–iii27.
- 23 Forastiero R, Martinuzzo M. The emerging role of multiple antiphospholipid antibodies positivity in patients with antiphospholipid syndrome. *Expert Rev Clin Immunol* 2015; 11: 1255–1263.
- 24 Pengo V, Banzato A, Bison E, *et al.* Antiphospholipid syndrome: critical analysis of the diagnostic path. *Lupus* 2010; 19: 428–431.
- 25 Banzato A, Pozzi N, Frasson R, *et al.* Antibodies to Domain I of beta(2)Glycoprotein I are in close relation to patients risk categories in Antiphospholipid Syndrome (APS). *Thromb Res* 2011; 128: 583–586.
- 26 De Laat B, Derksen RH, Urbanus RT, *et al.* IgG antibodies that recognize epitope Gly40-Arg43 in domain I of beta 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis. *Blood* 2005; 105: 1540–1545.
- 27 Pengo V, Ruffatti A, Tonello M, *et al.* Antibodies to Domain 4/5 (Dm4/5) of beta2-Glycoprotein 1 (beta2GP1) in different antiphospholipid (aPL) antibody profiles. *Thromb Res* 2015; 136: 161–163.