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Running title: non-conventional APL in clinical obstetrical APS

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Key words: antiphospholipid syndrome; non-conventional antiphospholipids; obstetrical complications; pregnancies treatment

Abstract

Objectives

To describe the prevalence of non-conventional APL in patients with obstetrical APS without conventional APL and the impact of treatment on pregnancy outcome.

Methods

Patients with clinical obstetrical criteria were tested for anti-phosphatidylethanolamine (aPE) IgG/M, anti-prothrombin/phosphatidylserine (anti-PS/PT) IgG/M and anti-annexin V IgG. Pregnancies losses rates were compared between APS, non-conventional APS and non-APL and in untreated pregnancies to treated ones for each group.

Results

Using the cut-offs (ROC), 65/96 (68%) patients have been considered as non-conventional APS and compared to 83 APS and 31 patients without APL. The obstetrical history in non-conventional APS did not differ in comparison to confirmed APS. The frequencies of anti-annexin V IgG antibodies tended to be more frequent in non-conventional APS (88% versus 73%; p=0.06), and those of anti-PE IgG and M were similar. The anti-PS/PT IgG and M antibodies were more frequent in confirmed APS than in non-conventional APS (63% and 37% versus 4% and 5%, p<0.0001).

Overall 261 pregnancies in patients with non-conventional APS were compared to 81 pregnancies of confirmed APS and 132 pregnancies from non-APL group. 136/474 (29%) patients have been treated during pregnancies and treatment significantly increased the rate of live birth (26% in untreated versus 72% in treated pregnancies, p<0.0001). In univariate analyses, treatment effect on pregnancies losses was similar in patients with APS and non-conventional APS, with odds ratio at 3.3 [95% CI; 1.8 to 6.1] and 6.9 [95% CI; 3.9 to 12.3] (p=0.49) and significantly more important for the 2 APS groups pooled versus non-APL group (OR at 1.9 [95% CI; 1.1 to 3.5] for non-APL group versus 5.3 [95% CI; 3.5 to 8.1] for APS groups, p=0.0025).

Conclusion

In this study 68% of patients with clinical criteria for obstetrical APS seronegative for conventional APL have non-conventional APL. These patients have a significant decrement of pregnancy losses if they receive treatment for APS during their pregnancy.

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INTRODUCTION

Antiphospholipid syndrome (APS) is characterized by the presence of thrombosis and/or obstetrical adverse events and persistent antiphospholipid antibodies (APL). The conventional APL include lupus anticoagulant (LA), Ig G/M anticardiolipin antibodies (ACL), and anti-$\beta_2$glycoprotein-I (β2GPI) antibodies[1]. A seronegative APS have been described in patients with obstetrical and thrombotic APS symptoms without detectable conventional APL [2]. Several non-conventional antiphospholipid antibodies, like anti-phosphatidylethanolamine (aPE) and anti-annexin V antibodies, have been described, but few clinical data are available with controversial results. Among 2790 patients with recurrent miscarriage and implantation failure, IgG antiphosphatidylinositol, antiphosphatidylcholine, but not antiphosphatidylserine and aPE antibodies have been more frequent than in 205 fertile women [3]. In patients with early recurrent miscarriage, only aPE IgM and anti-annexin V IgG have been found to be associated with recurrent miscarriage [4]. [5][5] Studies are lacking to determine the cut-offs of non-conventional APL in particular in obstetrical subset and demonstrate the benefit of treatment to improve the obstetrical outcome.

We aimed (1) to describe the prevalence of non-conventional APL in patients with obstetrical APS criteria without conventional APL, in comparison to healthy pregnant women and APS patients; and (2) compare the pregnancy outcome between patients with non-conventional APL antibodies, APS, and patients with clinical obstetrical criteria for APS without any APL antibody.

PATIENTS AND METHODS

Patients

A prospective registry of patients with clinical obstetrical criteria (Sydney) was initiated from January 2010 until March 2014. The inclusion criteria were: (1) ≥3 early miscarriages less than 10 weeks of gestation; (2) intrauterine fetal death ≥10 weeks of gestation; (3) preeclampsia, prematurity <34 weeks of gestation related to placental insufficiency; (4) absence of inherited thrombophilia (V Leiden and II homozygous mutations, proteins C, S, ATIII deficiencies) and of conventional APL (LA, ACL IgG/M, anti- β_2GPI IgG/M).

Maternal age, clinical APS features, associated autoimmune diseases, course and outcome of all pregnancies, treatments before and during all available pregnancies, and delivery mode during the last pregnancy were recorded. Neonatal outcome was assessed with following parameters for the last available pregnancy: gestational age at delivery, birth weight, 1- and 5-minute Apgar scores, and presence of neonatal complications.

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Antiphospholipid antibodies assays

All women were tested for the presence of lupus anticoagulant (LA), IgG/IgM ACL and IgG/IgM anti- β_2GPI antibodies. LA was detected using diluted Russell's viper Venom and diluted activated partial thromboplastin time as screening tests in accordance to ISTH recommendations. ACL IgG/M and anti-β_2GPI IgG/IgM were determined by two commercial ELISA from Inova Diagnostics (QUANTA Lite ACA, San Diego, CA) and ThermoFisher Scientific (Varelisa β_2GPI, Saint Quentin en Yvelines, France), respectively. ACL IgG/M and anti-β_2GPI antibodies IgG/M positivity was defined as value above 99th percentile [6]. Triple positivity was defined by the association of a positive LA test, a positive ACL (IgG and/or IgM) and a positive anti-β_2GPI (IgG and/or IgM).

Non-conventional APL were tested and consisted in anti- aPE Ig G/M, anti-prothrombin/phosphatidylserine (anti-PS/PT) IgG/M and anti-annexin V IgG APL. They were determined by commercial ELISAs using ELISIS P-Ethanolamine and ELISIS-Annexin V (Theradiag, Croissy Beaubourg, France) and QUANTA Lite aPS/PT (Inova, San Diego, CA) and expressed as U/ml. IgA anti-β2GPI antibodies and anti-domain I β2GPI antibodies were determined by QUANTA Flash β2 GPI IgA and β2GPI-Domain I (BioFlash, Inova, San Diego, CA) and expressed as chemoluminescent units (CU). All assays were performed according to the manufacturer's instructions.

This study was approved by the University Hospital of Jean Verdier Institutional Review Board and the *Comité de Protection des Personnes* (CPP, Aulnay Sous Bois, 2003).

Study groups

Women with obstetrical and/or thrombotic events fulfilling APS Sydney criteria, but seronegative for conventional APL antibodies (n=96) were evaluated for identify non-conventional APL antibodies A control group of 47 healthy pregnant patients without any pregnancy complication have been selected from Bichat Hospital, without any age difference with clinical APS and without conventional APL (31±6 versus 33±4 years; p>0.05).

A control group of patients with confirmed APS (Sydney criteria) (n=83) was also selected.

The general characteristics, pregnancies and treatment during the pregnancies of patients with non-conventional APS, non-APL group and patients with confirmed APS were compared.

Sample size

The primary objective of this study was to identify the prevalence of non-conventional APL antibodies in patients that met obstetrical APS criteria, but were seronegative for conventional APL antibodies. For this expected prevalence of 90%, 65 patients in the non-conventional APS group are necessary to estimate this proportion, with a two-sided 95% confidence interval and a precision of 7%

Data and statistical analysis

All quantitative data are expressed as means with standard deviation (SD), while qualitative data are expressed as frequencies with percentages. The Fisher's exact test was used to compare qualitative variables, while one way analysis of variance F test or Student's t-test was used for continuous variables. To determine the cut-offs of non-conventional APL, ROC analysis has been performed in patients with clinical APS without conventional APL and

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healthy pregnant controls. The cut-off which maximized sensitivity+ (1-specificity) was chosen.Univariate analyses and multivariate analyses (logistic models) were done to determine the factors associated with pregnancy losses. Odds ratios with their 95% confidence intervals were calculated. In order to take into account multiple pregnancies for the same patient, multivariate general linear models were done in addition (SAS Proc Genmod). The factors analyzed in univariate analysis included patients' age, type of obstetrical events, any treatment during the pregnancy, treatment regimen, patients' group (APS, patients with clinical APS and non-conventional APL, patients with clinical APS without any antibodies or thrombophilia), presence of previous thrombosis, number of normal and pathological pregnancies. Factors were included in multivariate analysis if p<0.05 in univariate analyses then stepwise method was used to determine the final multivariate model.

Statistical analysis was performed using SAS (version 9.4; SAS Institute, Cary, NC), and significance was defined as $p < 0.05$.

RESULTS

Non-conventional APL cut-offs in patients with pregnancy morbidity without conventional APL

During the study, 96 patients with clinical APS (Sydney criteria) without any detectable thrombophilia (proteins C, S ATIII, factors II and V homozygous mutations, conventional APL), have been tested for non-conventional APL. Among them, recurrent miscarriage was noted in 44 (46%) cases, intrauterine fetal deaths in 38 (40%) cases, premature term<34 weeks of gestation in 25 (27%) cases, preeclampsia and/or HELLP syndrome in 25 (26%) cases and thrombosis in 14 (15%) cases. Mixed thrombotic and obstetrical features were present in 15 patients (15%) and isolated obstetrical features in remaining patients (85%). Associated auto-immune disease was noted in 10 cases (10%), mainly Sjogren's syndrome (n=4) and thyroiditis (n=4).

The mean levels of anti-Annexin IgG levels were at 7.7±6.1 U/ml in patients with clinical APS versus 2.2±1.9 U/ml in controls (p<.0001), anti-PE IgG at 7.3±7.1 U/ml versus 2.5±2.2 U/ml (p<0.0001), anti-PE IgM at 8.8±11.9 U/ml versus 5.5±5.8 U/ml (p=0.07), without any significant difference for anti-PS/PT IgG and M mean levels between the groups.

As there is no established cut-offs for non-conventional APL, ROC analysis has been performed to determine the cut-offs for each non-conventional APL (**Table 1, Figure 1**). The cut-offs of non-conventional antibodies were determined using ROC analysis for each antibody in comparison to healthy pregnant women without any obstetrical adverse event and without any detectable thrombophilia (n=47). Among these 96 patients with clinical APS without conventional APL, using the established cut-offs (ROC analysis), 65/96 (68%) patients have been considered as non-conventional APS (p.e clinical Sydney APS criteria with at least one non-conventional APL) and 31/96 remaining patients fulfilled clinical Sydney criteria without detectable conventional and non-conventional APL (non-APL group). Anti-annexin V IgG and anti-PE IgG were sufficient to discriminate patients with clinical APS from controls with the cut-off at 6 U/ml (**Figure 1**). With this cut-off, when anti-annexin V IgG ≤ 6 U/ml and anti-PE IgG ≤ 6 U/ml, then 43/69 (62% corresponding to a percentage of 38% of false negative) were patients without clinical APS. If Anti-annexin V IgG > 6 U/ml or

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anti-PE IgG > 6 U/ml, then 62/66 (94% corresponding to a percentage of 6% of false positive) were patients with clinical APS. Sensitivity of the test was 74% and specificity 91%, with Yule's coefficient of colligation at 0.92 indicating a strong relationship between the level of anti-annexin V IgG and anti-PE IgG and the presence of clinical APS.

Comparison of non-conventional APS to confirmed APS and non-APL group

Patients Characteristics

The 65 patients with non-conventional APS have been compared to 83 patients with confirmed APS and 31 non-APL patients (**Table 2).** The conventional APL in patients with confirmed APS were lupus anticoagulant in 18 (36%) cases, ACL IgG/M in 62 (79%) and 13 (23%) cases and anti-β2GPI IgG/M in 30 (46%) and 4 (9%), respectively.

The age of APS patients was higher than in patients with non-conventional APS (40±9 versus 32±5 years; p<0.0001). The obstetrical history, the number of uncomplicated pregnancies and live births, as well as the frequencies of recurrent miscarriages, intrauterine fetal deaths and premature deliveries did not differed between the 3 groups. The frequency of associated thrombosis was also similar (**Table 2).** The frequencies of anti-annexin V IgG antibodies tended to be more frequent in non-conventional APS with a 15% difference (88% versus 73%; p=0.06), and those of anti-PE IgG and M were similar in comparison with confirmed APS. The anti-PS/PT IgG and M antibodies were significantly more frequent in confirmed APS than in non-conventional APS (63% and 37% versus 4% and 5%, respectively, p<0.0001). Anti-domain I β2GPI antibodies were showed in 50% of confirmed APS and only 4% of non-conventional APS (p<0.0001). IgA anti-β2GPI antibodies were exclusively present in patients with confirmed APS in 9 (41%) out of the 22 available cases.

There was no association between the type of non-conventional APL and any type of obstetrical events (recurrent miscarriages, prematurity, intrauterine fetal death or pre-eclampsia) or thrombosis in non-conventional APS and APS groups.

Pregnancies outcome and treatment

Overall 261 pregnancies which occurred in 65 patients with non-conventional APS were compared to 81 pregnancies of confirmed APS patients and 132 pregnancies of 31 patients from non-APL group.

Considering 474 pregnancies from all 3 groups (**Table 3**), the rate of pregnancies losses without any treatment was quite similar in all groups, with 64% in non-APL group, 74% in APS and 75% in non-APL group. 136/474 (29%) patients have been treated during pregnancies and treatment significantly increased the rate of live birth (26% in untreated versus 72% in treated pregnancies, p<0.0001). The number of treated pregnancies tended to be more frequent in APS patients (n=33; 41%) in comparison to non-conventional APS (n=67; 26%) and non-APL group (n=36; 27%) (p=0.05). The aspirin-low-molecular weight heparin (LMWH) combination was used more frequently in APS patients (n=19/33; 58%) in comparison to non-conventional APS (n=19/67; 28%) and non-APL group (n=17/36; 47%)

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(p<0.05). In univariate analyses, treatment effect on pregnancies losses was similar in patients with APS and non-conventional APS, with odds ratio at 3.3 [95% CI; 1.8 to 6.1] and 6.9 [95% CI; 3.9 to 12.3] (p=0.49) and significantly more important for the 2 APS groups pooled versus non-APL group (OR at 1.9 [95% CI; 1.1 to 3.5] for non-APL group versus 5.3 [95% CI; 3.5 to 8.1] for APS groups, p=0.0025).

In univariate analysis, the number of complicated pregnancies, the treatment during pregnancies in particular in APS and non-conventional APS groups, was significantly associated with live births (**Table 3**). In multivariate analysis, the pregnancy treatment and less previous complicated pregnancies were independently associated with live births (**Table 3**).

Discussion

In the present study, we show that the non-conventional antiphospholipid antibodies could be detected in patients with APS-defined obstetrical adverse events without any conventional APL. Among the non-conventional antibodies, mainly anti-annexin V IgG and anti-PE antibodies were significantly associated with adverse obstetrical events. Conventional APS treatment (aspirin and LMWH alone and/or in combination) dramatically improved the obstetrical outcome in patients with non-conventional APL. Non-conventional APL displayed different profiles, with anti-annexin V IgG and anti-PE in non-conventional APS and anti-PS/PT IgG/M and IgA anti-β2GPI antibodies in patients with confirmed APS.

In the absence of standardized method for non-conventional APL, the cut-offs need to be established, as they could vary in different clinical subsets and conditions. We have previously demonstrated that IgG/M ACL and anti-β2GPI levels are lower in healthy newborns than in healthy pregnant and non-pregnant women [6]. Low levels of APL have been included in several studies of obstetrical APS, in patients with thrombosis and aspirin-LMWH combination could improve the pregnancy outcome in patients with low APL titers [7] [8] [9]. Only one study has previously determined the non-conventional anti-PE and anti-annexin V cut-offs in comparison to healthy controls [5]. We used ROC analysis to determine the cut-off of each APL. Anti-annexin V IgG and anti-PE IgG antibodies were sufficient to discriminate patients with clinical APS from healthy controls with AUC at 0.93 and 0.82 (Odds ratio at 0.482 [0.343 to 0.625] and 0.84 [0.64 to 1.076], respectively).

The presence of obstetrical and/or thrombotic features in the absence of conventional APL has previously been termed as "seronegative APS". Rodriguez-Garcia et al. described patients with non-criteria APS without APL which have similar thrombotic and pregnancy morbidity in comparison to confirmed APS [10]. In our study, patients with and without conventional APL have similar frequencies of thrombotic and pregnancy complications, as well the number of live births. In patients with APS-like features, it was thus hypothesized that antibodies against other phospholipids or cofactors, such as prothrombin, phosphatidylethanolamine or annexin V, could be detected [10]. Several studies reported various non-conventional APL in patients with thrombosis and pregnancy morbidity, but relatively few data are yet available. Significantly more anti-PE APL have been found in 98 patients with unexplained thrombosis,

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with mean levels similar to APS patients [11]. Among 743 women with recurrent early spontaneous abortion, higher levels of anti-PE IgM, anti-annexin V IgG have been found in comparison to matched controls [5]. Considering APL levels by percentiles, anti-PE IgG and M, anti-annexin V IgG but not IgM were significantly associated with spontaneous abortion at titers > 80e percentiles with increasing odds ratio with percentiles [5]. Anti-annexin V antibodies have been found in 19% of patients with recurrent early miscarriages with similar frequency in healthy controls [12]. Anti-PS/PT APL seem to be rare in patients with non-conventional APS, and were found in 12/169 (6.5%) patients in previous study and 4-5% in our cohort [13]. Isolated IgA anti- β2GPI antibodies have been found in 22.4% of clinical APS, even more frequently than 15% of IgG/M anti-β2GPI conventional antibodies, but only APS patients have detectable IgA anti-β2GPI APL in our study [14]. Anti-β2GPI domain I antibodies have been found in 3/40 (7.5%) of patients with seronegative APS in a previous report, similarly to 4.1% of non-conventional APS in our report [15].

No studies previously assessed the possible benefit of treatment during the pregnancy in patients with non-conventional APL. In our study, pregnancy treatment significantly increased the live birth rate in non-conventional APS, with similar effect to confirmed APS, and significantly more than in patients without any APL. Aspirin-LMWH combination was significantly less frequent in non-conventional APS, whereas the number of pregnancies with favorable outcome was similar. Even this point could suggest that monotherapy with aspirin or LMWH could be sufficient in non-conventional APS, studies are needed to determine the best regimen in these patients. In univariate analysis, pregnancy loss in patients under hydroxychloroquine was dropped to 17% from 60% without hydroxychloroquine. Even not significant in multivariate analysis, the number of patients under hydroxychloroquine was small. Recent preliminary studies in obstetrical APS showed that hydroxychloroquine could be effective drug in addition to aspirin-LMWH combination for obstetrical outcome [16].

Even if non-conventional APL could be detected, one should try to demonstrate its pathogenic link with the underlying clinical features. Only one experimental study actually demonstrated that passive immunization of aPE in mice resulted in increase of fetal resorption, placental thrombosis and apoptosis with decreased trophoblastic cell invasion [17].

Our study has several limitations which could strain the definite conclusions. First, non-conventional APL have not been systematically controlled, or were controlled under aspirin and heparin combination and we could not confirm their persistence in all positive cases. Nevertheless, precise clinical history allows us to exclude other situations which could be associated with transitory autoantibodies, as infectious and neoplastic conditions. Most of the previous studies which assessed the non-conventional APL also failed to analyze the persistence of these APL. The use of various treatment regimens did not allow us to determine the impact of such drug, as steroids and hydroxychloroquine, and the best regimen for patients with non-conventional APS remain to be determined.

Conclusion

Our study shows the interest to detect the non-conventional APL in patients with APS-like obstetrical features. Treatment during pregnancy in these patients dramatically improves the pregnancy outcome. Prospective studies are warranted to confirm the benefit of treatment for patients with non-conventional APL and determine the best regimen.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article. Arsene Mekinian and Pascale Nicaise-Roland have full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis.

Study conception and design. AM, LC, OF, PNR.

Acquisition of data. AM, BMC, FM, LC, AM, AT, SCM, OF, PNR.

Analysis and interpretation of data. AM, FM, OF, PNR.

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Table 1. Non-conventional APL: cut-offs from 96 patients with clinical APS in comparison to 47 healthy pregnant controls (ROC analysis).

APL	Cut-off U/ml	Sensibility	Specificity	AUC	Odds Ratio Estimates*	95% CI	
Anti-Annexin V IgG	6	98%	60%	0.93	0.48	0.34	0.63
Anti-PE IgG	6	94%	46%	0.82	0.85	0.65	1.08
Anti-PE IgM	6	72%	39%	0.64	0.97	0.87	1.07
Anti-PS/PT IgG	15	9%	93%	0.54	1.01	0.92	1.09
Anti-PS/PT IgM	13	40%	74%	0.62	1.03	0.94	1.14

* OR for an increase of 1 unit of the Non-conventional APL variable

Table 2. Characteristics of patients with confirmed APS, non-conventional APS and non-APL group.

	Non-conventional APS N=65	Confirmed APS N=83	Non-APL group N=31
Obstetrical history			
Age (years)	32±5 (n=64)	40±9* (n=78)	35±6*** (n=29)
Normal pregnancies	39/64 (60%)	30/46 (65%)	20/31 (65%)
Number of normal pregnancies	1.0 ±1.0	1.2±1.4	1.0±1.3
Recurrent Miscarriages	28/65 (43%)	18/45 (38%)	16/31 (52%)
Intrauterine fetal deaths	29/65 (45%)	19/47 (40%)	9/30 (30%)
Prematurity <34 wg	14/64 (22%)	11/41 (27%)	11/30 (37%)
Preeclampsia/ HELLP	12/65 (18%) / 3/65 (5%)	8/46 (17%) / 1/18 (6%)	8/30 (27%) / 2/31 (7%)
Live-born babies	1.4±1.0 (n=64)	1.6±0.9 (n=16)	1.6±1.4 (n=31)
Thrombosis history	10/65 (15%)	12/48 (25%)	4/31 (13%)
Thrombosis number	0.2±0.4	0.3±0.5	0.2±0.5
Anti-Annexin V IgG	57/65 (88%)	38/52 (73%) (p=0.057)	0
Anti-PE IgG	39/61 (60%)	26/50 (52%)	0
Anti-PE IgM	8/61 (12%)	4/50 (8%)	0
Anti-PS/PT IgG	2/56 (3%)	31/49 (63%)*	0
Anti-PS/PT IgM	3/56 (5%)	18/49 (37%)*	0
Anti-domain I β2 GPI	2/49 (3%)	12/24 (50%)*	0
Anti-β2 GPI IgA	0	9/22 (41%)*	0

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Pregnancies treatment	N=261	N=81	N=132
Treated pregnancies	67 (26%)	33 (41%)*	36 (27%)
Aspirin /LMWH alone	35 (13%)	11 (14%)	14 (11%)
Aspirin-LMWH	19 (7%)	19 (24%)*	17 (13%)***
Prednisone	12 (5%)	3 (4%)	5 (4%)
Hydroxychloroquine	7 (3%)	3 (4%)	2 (2%)
Neonates characteristics (last pregnancy)	N=64	N=18	N=31
Birth weight (gr)	3823±4238 (n=26)	3064±722 (n=9)	2330±724*** (n=10)
SGA (<10e percentile)	10/27 (37%)	12/18 (67%)	6/11 (54%)
Apgar 10 / IU admission	26/28 (93%) / 1 (3%)	10/10 (100%) /1 (9%)	11/11 (100%) / 2(14%)

Values are means with SD and numbers with frequencies and data are expressed considering the missing data.

*p<0.05 Non-conventional APS vs APS

**p<0.05 Non-conventional APS vs non-APL group

***p<0.05 Non-APL group vs APS

Table 3. Factors associated with pregnancies losses of 474 pregnancies of patients with confirmed APS, non-conventional APS and non-APL group (univariate and multivariate analysis).

Parameter	%Pregnancy losses	OR univariate [95% CI]	p-value multivariate	OR multivariate [95% CI]
Group APS (at risk) Non-conventional APS Non-APL group	158/261: 61% 48/81: 59% 75/132: 57%	0.93 [0.56 to 1.55] 0.87 [0.57 to 1.34]	0.56	1.16 [0.63 to 2.14] 0.85 [0.52 to 1.41]
Pregnancy Treatment Treated Untreated	36/136: 26% 245/338: 72%	0.14 [0.09 to 0.22]	<0.0001	0.14 [0.08 to 0.22]
Type of Pregnancy Treatment None (at risk) Aspirin or LMWH alone Aspirin and LMWH Other	245/338: 72% 22/60: 37% 9/55: 16% 5/21:24%	0.23 [0.13 to 0.40] 0.08 [0.04 to 0.17] 0.13 [0.04 to 0.36]	Not included	
Type of Pregnancy Treatment by Group APS untreated (at risk) APS treated • Aspirin or LMWH alone • Aspirin and LMWH • Other	146/194: 75% 10/33: 30% 7/34: 21% 3/18: 17% 2/13: 15%	3.35 [1.84 to 6.07]	0.0125 <0.0001	9.91 [3.38 to 29.07]

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Non-conventional APS untreated (at risk)	38/48: 79%	6.91 [3.89 to 12.27]	<0.0001	13.05 [6.16 to 24.54]
Non-conventional APS treated	12/65: 18%			
• Aspirin or LMWH alone	6/11: 55%			
• Aspirin and LMWH	3/19: 16%			
• Other	1/3: 33%			
Non-APL group untreated (at risk)	61/96: 64%	1.95 [1.08 to 3.51]	0.04	2.47 [1.06 to 5.79]
Non-APL group treated	14/34: 41%			
• Aspirin or LMWH alone	9/14: 64%			
• Aspirin and LMWH	3/16: 19%			
• Other	2/4:50%			
Aspirin			Not included	
Yes	32/118: 27%	0.16 [0.10 to 0.26]		
No	249/356: 70%			
LMWH			Not included	
Yes	15/81: 19%	0.12 [0.06 to 0.21]		
No	266/393: 68%			
Steroids			Not included	
Yes	5/20: 25%	0.23 [0.08 to 0.64]		
No	276/454: 61%			
Hydroxychloroquine			Not included	
Yes	2/12: 17%	0.13 [0.03 to 0.59]		
No	279/462: 60%			
Age			Not included	
> 30 years	176/316: 56%			
≤30 years	87/132: 66%	1.49 [0.98 to 2.28]		
Uncomplicated pregnancies				
None	170/318: 55%			
At least one or more	110/155: 71%	2.20 [1.45 to 3.33]	0.0002	2.06 [1.27 to 3.36]
Complicated pregnancies				
≤2	34/107: 32%	0.22 [0.14 to 0.35]		0.30 [0.17 to 0.89]
>2	246/366: 67%		<0.0001	
Thrombosis				
Yes	32/67: 48%	0.59 [0.35 to 0.99]		
No	249/407: 61%		NS	
Isolated early recurrent miscarriages				
Yes	137/277: 49%			
No	249/407: 61%	2.92 [1.96 to 4.35]	NS (p=0.054)	1.66 [0.99 to 2.77]

NS: non-significant; APS: antiphospholipid syndrome; LMWH: low-molecular weighted heparin.

Figure 1. ROC analysis of non-conventional APL in patients with clinical APS without conventional APL antibodies: anti-annexin V IgG and anti-PE IgG are sufficient to discriminate patients with clinical APS from healthy controls.