RHEUMATOLOGY

Rheumatology 2020;00:1–11
doi:10.1093/rheumatology/keaa499

Original article

Maternal and foetal placental vascular malperfusion in pregnancies with anti-phospholipid antibodies

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Abstract

Objective. The objective of the study was to evaluate the rates of pathological placental lesions among pregnant subjects positive for aPL antibodies.

Methods. We performed a longitudinal case–control study including 27 subjects with primary APS, 51 with non-criteria APS, 24 with aPL antibodies associated with other well-known CTDs enrolled at the end of the first trimester of pregnancy and 107 healthy controls.

Results. Compared with controls and after correction for multiple comparisons, primary, non-criteria APS and aPL associated to CTD, subjects had lower placental weight, volume and area. After penalized logistic regression analysis to correct for potential confounders, placental lesions suggesting severe maternal vascular malperfusion (MVM) were more common among primary [odds ratio (OR) 11.7 (95% CI 1.3, 108)] and non-criteria APS [OR 8.5 (95% CI 1.6, 45.9)] compared with controls. The risk of foetal vascular malperfusion (FVM) was higher in primary APS [OR 4.5 (95% CI 1.2, 16.4)], aPL associated with CTDs [OR 3.1 (95% CI 1.5, 6.7)] and non-criteria APS [OR 5.9 (95% CI 1.7, 20.1)] compared with controls. Among clinical and laboratory criteria of APS, first trimester aCL IgG >40 UI/ml [OR 4.4 (95% CI 1.3, 14.4)], LA positivity [OR 6.5 (95% CI 1.3, 33.3)] and a history of pre-eclampsia at <34 weeks [OR 32.4 (95% CI 6.5, 161)] were the best independent first trimester predictors of severe MVM [area under the curve 0.74 (95% CI 0.6, 0.87)].

Conclusion. Compared with healthy controls, pregnant subjects with aPL antibodies have an increased risk of placental lesions, suggesting MVM and FVM. First-trimester variables such as aCL IgG >40 UI/ml and a history of pre-eclampsia were significant predictors of both severe MVM and FVM.

Key words: APS, non-criteria obstetric APS, aPL antibodies, pregnancy, placenta, maternal vascular malperfusion, foetal vascular malperfusion

Rheumatology key messages

- Anti-phospholipid antibodies exert a deleterious effect on placentation on both the maternal and foetal side.
- A history of pre-eclampsia and LA positivity predict severe placental maternal and foetal vascular malperfusion.

Introduction

aPL antibodies during pregnancy have been associated with recurrent miscarriages, increased risk of late foetal loss, pre-eclampsia, foetal growth restriction (FGR) and prematurity [1]. Major or minor reproductive failures are part of the standardized definitions of primary APS or the so-called non-criteria APS [2, 3]. Several studies have demonstrated that aPL antibodies are able to interfere with early placentation by reducing trophoblast proliferation and invasion of decidual spiral arteries but also by increasing trophoblast apoptosis and thrombosis in the intervillous space [4]. Defective placentation is considered a reliable causal factor of stillbirth, defective foetal growth and pre-eclampsia [1, 2]. A considerable amount of literature has been published on the relationship between elementary placental lesions associated with aPL antibodies [5]. The main critical issues in previous studies include the lack of a control population of healthy pregnant subjects, the heterogeneity of APS studied and the lack of a precise standardization of the

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Submitted 27 April 2020; accepted 15 July 2020

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CLINICAL SCIENCE

placental histopathological lesions [5–8]. The sampling protocol, terminology and diagnostic criteria of placental lesions have been standardized by the so-called Amsterdam criteria [9, 10]. These largely accepted standards include definite criteria to diagnose malfunctioning of the placental–foetal unit on both the maternal and foetal side and/or caused by infection/inflammation [9]. Another missing point in the previous literature is the lack of consideration of the heterogeneity of APS. Whereas classical APS is composed by well-defined clinical and laboratory criteria, most patients with aPL antibodies at the beginning of pregnancy do not fit the classical criteria of APS or aPL antibodies are associated with other CTDs [1, 2]. Several studies have suggested that the aPL antibodies profile or the subtype of APS, including the so-called non-criteria subjects, could influence both placental pathological features and obstetric outcomes [11, 12].

The purpose of this study was to evaluate placenta pathological features according to the Amsterdam classification in a group of consecutive subjects with primary APS and aPL antibodies in subjects with CTDs, in non-criteria APS and in healthy controls.

Methods

Cases and controls for this study were enrolled at our high-risk pregnancy outpatient clinic during the period 2014–2018. Cases included first-trimester pregnant women referred by the Rheumatology Department of our institute, from our general pregnancy outpatient clinic and from private practices diagnosed with persistent aPL positivity on two or more occasions 12 weeks apart. At enrolment, cases had a confirmatory screening antibody profile, including ANA, anti-dsDNA, anti-ENA, aCL antibodies (aCL IgG and aCL IgM), anti-β2-glycoprotein I antibodies (aβ2GPI IgG and aβ2GPI IgM) and LA, according to standardized methods as previously described [13]. Coexistent rheumatic diseases were classified according to widely used criteria for UCTD, RA, SLE, SS, SSc, PM/DM and mixed CTD [14–19]. In particular, primary APS was defined according to international consensus as the presence of one major clinical and one major laboratory criteria [2]. Subjects with aPL antibodies associated with CTDs, not fulfilling definitive criteria for APS, were classified as a separate category. According to Jayakody Arachchillage *et al.* [3], non-criteria APS was defined as the combination of non-criteria clinical and laboratory manifestations such as the presence of low-titre aPL antibodies with sporadic reproductive failure. Among non-criteria APS we included also asymptomatic subjects with one or more major laboratory criteria of APS. The aPL antibody risk profile was evaluated according to EULAR recommendations [20]. Antibody data used for the analysis were those obtained at enrolment. Cases were enrolled at the end of the first trimester [median gestational age at enrolment 12.5 weeks [interquartile range (IQR) 12–13.5], as confirmed by ultrasound, and underwent monthly clinical and ultrasonographic follow-up.

The mean uterine artery pulsatility index in the first and second trimester was evaluated according to standard methods [21]. Pulsatility indices of uterine or umbilical arteries were considered abnormal when values were >95th percentile of reference curves. FGR was diagnosed when the foetal abdominal circumference at ultrasonographic examination was <10th percentile of our local reference curves (confirmed on at least two consecutive measurements taken 2 weeks apart after the standard 18–22 weeks ultrasound) and the pulsatility index of the umbilical artery was found to be >95th percentile of reference curves, denoting a reduced perfusion of the foetal–placental unit. Pre-eclampsia was diagnosed according to standard criteria [21]. Small for gestational age (SGA) infants were diagnosed when birthweight was <10th percentile of the Italian population [22]; preterm delivery was defined as a birth ≤37 weeks of gestational age.

Since the aim of the study was to compare histopathological placental features of APS to a normal reference, we also enrolled a control population of normal pregnancies. After each APS case we selected the subsequent healthy pregnant woman from our outpatient clinic with an uncomplicated pregnancy, adequate birthweight and no perinatal complications as a control. The study was approved by the ethical committee of our department and each subject gave informed consent.

Classification of placental lesions

Placentas from cases and controls were sent to the Department of Pathology for standard histological evaluation. At the time of sectioning, the type, number, location, size and percentage of placental involvement of all grossly visible lesions were recorded. At least one sample was taken for each type of lesion. Additionally, at least three full-thickness blocks of normal-appearing parenchyma, two blocks containing cross sections of umbilical cord and two rolls of the extraplacental membranes were submitted for examination. For each case, all the slides were reviewed by two physicians experienced in placental pathology using a predefined set of variables. Pathologists were blinded to APS diagnosis and gestational age was the only data given at the time of revision. Pathological variables assessed and classified according to the Amsterdam Placental Workshop Group Consensus Statement [9] were placental inflammatory-immune processes including maternal and foetal acute inflammatory responses (stage and grade); non-infectious chronic villitis, distinguishing between low grade (more than one focus, each involving <10 contiguous villi, either focal if seen on only one slide or multifocal if seen on two or more slides) and high grade (patchy in case of multiple foci, at least one affecting >10 contiguous villi, diffuse when >30% distal villi were involved); foetal–stromal vascular lesions including obstructive lesions of the umbilical cord and proximal (chorionic or stem vessels) villous thrombosis; avascular

villi, reserving the term 'severe foetal vascular malperfusion' for severe cases (≥45 avascular villi in three disc sections, two or more thrombi in the proximal foetal vessels or multiple non-occlusive thrombi); maternal stromal–vascular lesions, including accelerated villous maturation and distal villous hypoplasia, infarcts, abruptio placentae (arterial), marginal abruptio (acute *vs* chronic) and decidual vasculopathy (persistence of arterial smooth muscle, fibrinoid necrosis, atherosis, lymphocytic/plasmacytic vasculitis or thrombosis in any location). Maternal vascular malperfusion (MVM) was defined as severe when multiple marginal infarcts and/or multiple lesions involving >30% of the placental parenchyma were found in the sample [9, 10].

Gross data were verified by microscopy when the nature of the lesions was not clear after macroscopic examination (e.g. haemorrhagic infarcts *vs* intervillous thrombi, peripheral atrophy *vs* infarction). For microscopic lesions, quantification was by visual inspection of the slides by trained pathologists. Histological lesions associated with FGR were subsequently grouped according to the Amsterdam Placental Workshop Group Consensus Statement [9]. According to this classification system, lesions were divided into two categories: MVM (no, mild, moderate/severe), including early and late infarctions (yes, no), retroplacental haemorrhage (no, mild, severe), massive perivillous fibrinoid deposition (none, 25–50% of villi, >50% of villi), distal villous hypoplasia (yes, no), accelerated villous maturation (yes, no), decidual arteriopathy as evidenced by the presence of mural hypertrophy (yes, no), muscularized arteries (yes, no) and acute atherosis (yes, no); and foetal vascular malperfusion (FVM), including vascular lesions/abnormalities of cord (yes, no), foetal vascular thrombosis (no, mild, moderate/severe), intramural fibrin deposition (yes, no) and the presence of avascular villi (no, mild, moderate/severe) and karyorrhexis (yes, no) [9, 10]. According to Katzman and Genest [23], massive perivillous fibrin deposition (MPFD) was defined as severe if it met the criteria of classic maternal floor infarction (basal villi embedded in fibrin ≥3mm in thickness, on at least one slide) and/or of transmural MPFD (≥50% of villi encased by fibrin from the maternal to foetal surface, on at least one slide).

Placental volumes were calculated from measurements of major and minor diameters and thickness (largest diameter × smallest diameter × thickness). Placental area was calculated by assuming an elliptical surface (largest diameter × smallest diameter × π/4). Since we were mainly interested in the APS-related features in viable pregnancies, we excluded from the analysis placentas from pregnancies <24 weeks.

Statistical analysis

Comparisons of continuous variables between multiple groups were performed by Kruskal–Wallis analysis of variance with Bonferroni correction for pairwise comparisons. Chi-squared analysis was used to compare categorical variables. Partitioning of chi-squared statistics with Bonferroni correction was used to compare categories in multiway contingency tables. Multinomial penalized logistic and ordinal logistic regression analyses were used to test the association of maternal and foetal vascular malperfusion with the type and aPL antibody risk profile. Penalized maximum likelihood estimation has been proposed as a suitable method of regression analysis for uncommon events [24]. In logistic models, MVM and FVM (no, moderate, severe) were inserted as three-level nominal or ordinal outcome measures, whereas the type of APS (primary, non-criteria and aPL antibodies associated with CTDs) or severity of aPL profile (no, low risk, high risk), maternal age, weeks of delivery, parity (nulliparous *vs* multiparous), smoking during pregnancy (yes, no) and type of treatment [none, corticosteroids, low-molecular weight heparin (LMWH), low-dose aspirin] were inserted as explanatory variables.

Finally, we used stepwise logistic regression models to find the most predictive first-trimester classical clinical (previous two or more cases of unexplained abortions, previous foetal death >10 weeks, previous thrombosis, previous FGR or pre-eclampsia at <34 weeks, treatment with corticosteroids, LMWH or low-dose aspirin) and APS laboratory findings of severe MVM or FVM. In these last models, initial exposure variables included previously described clinical variables and LA (yes, no), aCL IgG and IgM (negative, 10–40 U/ml, >40 U/ml) and aβ2GPI IgG and IgM (negative, 7–10 U/ml, >10 U/ml), whereas severe MVM and FVM were inserted as binary outcome variables. The predictive value of the models obtained was checked by receiver operating characteristics curve analysis.

Results

Laboratory and clinical characteristics and pregnancy outcomes

During the period of the study, 112 pregnant subjects with various types of APS were seen at our high-risk pregnancy unit and were initially enrolled in the study. Five patients were excluded because of unconfirmed positivity of aPL at enrolment and an additional 5 subjects were excluded because of late miscarriage (13–20 weeks), leaving 102 subjects for analysis.

The laboratory and clinical characteristics of cases are reported in Table 1. Non-criteria APS included 11 subjects with both minor laboratory and major/minor clinical criteria and 40 subjects without any major clinical criteria but with one or more persistently positive major laboratory finding. CTDs associated with aPL antibodies included 4 subjects with SLE, 3 with RA, 1 with SSc and 16 with UCTD. None of these subjects had a complete, classical APS. A triple positivity of antibody tests was recorded in 10 (9.8%) cases (7 among primary APS and 3 in aPL antibodies related to other CTDs). As for the clinical characteristics, primary APS included 7 subjects (25.9%) with a prevalent thrombotic phenotype and 20 (74.1%) with an obstetric phenotype. Of the previous

Table 1 Laboratory and clinical characteristics of subjects enrolled in the study

Characteristics	Primary APS ($N = 27$), *n* (%)	Non -criteria APS ($N = 51$), *n* (%)	aPL associated with CTD ($N = 24$), *n* (%)	Overall cases ($N = 102$), *n* (%)
ANA	21 (77.8)	16 (31.4)	21 (87.5)	58 (56.9)
ANA titre >1/160	12 (44.4)	5 (9.8)	15 (62.5)	32 (31.4)
Anti-dsDNA	1 (3.7)	—	2 (8.3)	3 (2.9)
ENA	4 (14.8)	—	6 (25)	10 (9.8)
Anti Ro-SSA	3 (11.1)	—	5 (20.8)	8 (7.8)
Anti-Scl-70	1 (3.7)	—	—	1 (1)
Anti RNP	—	—	1 (4.2)	1 (1)
aCL IgG	14 (51.9)	4 (7.8)	10 (41.6)	28 (27.4)
10–40 U/ml	4 (14.8)	4 (7.8)	6 (25)	14 (13.7)
>40 U/ml	13 (48.1)	—	4 (16.7)	17 (16.7)
aCL IgM	11 (40.7)	33 (64.7)	16 (66.7)	60 (58.8)
10–40 U/ml	5 (18.5)	28 (54.9)	15 (62.5)	48 (47.1)
>40 U/ml	6 (22.2)	5 (9.8)	1 (4.2)	12 (11.8)
aβ2GPI IgG	10 (37)	11 (21.6)	5 (20.8)	26 (25.5)
7–10 U/ml	—	—	—	—
>10 U/ml	10 (37)	11 (21.6)	5 (20.8)	26 (25.5)
aβ2GPI IgM	8 (29.6)	12 (23.5)	4 (16.7)	24 (23.5)
7–10 U/ml	—	1 (2)	—	1 (1)
>10 U/ml	8 (29.6)	11 (21.6)	4 (16.7)	23 (22.5)
LA	6 (22.2)	—	1 (4.2)	7 (6.9)
Previous miscarriage	25 (92.6)	21 (41.2)	6 (25)	52 (51)
1	7 (25.9)	14 (27.5)	2 (8.3)	23 (22.5)
2	3 (11.1)	1 (2)	1 (4.2)	5 (4.8)
≥3	15 (55.6)	6 (11.8)	3 (12.5)	24 (23.5)
Previous thrombosis	7 (25.9)	2 (3.9)	—	9 (8.8)
Previous pre-eclampsia	3 (11.1)	2 (3.9)	2 (8.3)	7 (6.9)
Previous FGR	3 (11.1)	1 (2)	3 (12.5)	7 (6.9)
Previous preterm birth (≤37 weeks)	7 (25.9)	2 (3.9)	2 (8.3)	11 (10.8)
Previous stillbirth	—	1 (2)	—	—
Double/triple positivity	9 (33.3)	6 (11.8)	4 (16.7)	19 (18.6)
Severity of aPL antibodies profile				
Low risk	18 (66.7)	45 (88.2)	20 (83.3)	83 (81.4)
High risk	9 (33.3)	6 (11.8)	4 (16.7)	19 (18.6)

preterm births recorded in the history of subjects, 3 of 11 (27.3%) were spontaneous (1 in primary APS and 2 in aPL associated with CTDs), whereas the others were associated with indicated preterm delivery because of FGR or pre-eclampsia.

Table 2 reports the main sociodemographic and pregnancy characteristics of the cases and controls. In a four-group non-parametric analysis of variance, controls had higher gestational age and birthweight compared with cases. In addition, there was a significantly higher gestational age among non-criteria subjects compared with primary APS or aPL associated with CTD. There were no differences in maternal age or BMI between the groups studied. Hydroxychloroquine and steroid treatment were more common among women with CTDs. The rates of LMVH and low-dose aspirin use and of severe (≤32 weeks of pregnancy) prematurity were more common among subjects with primary APS. All the cases of severe prematurity involved a medically indicated preterm delivery.

Placenta pathologic features

Table 3 reports a comparison of macroscopic placental characteristics between APS cases and healthy pregnant controls. After correction for multiple comparisons, primary APS, aPL associated to CTDs and non-criteria APS subjects had lower placental weight, volume and area compared with controls. Among subjects with primary APS there was an excess rate of retroplacental haematoma >2 cm ($P < 0.001$) (Table 4). Overall, among cases there was an increased rate of moderate/severe retroplacental haematomas ($P = 0013$), muscularized arteries ($P < 0.001$) and accelerated villous maturation ($P < 0.001$) compared with controls.

Table 2 Pregnancy characteristics of subjects with aPL antibodies and controls

Characteristics	Controls ($n = 107$), median (IQR)	Primary APS $n = 27$), median (IQR)	Non-criteria APS ($n = 51$), median (IQR)	aPL associated with CTD ($n = 24$), median (IQR)
Maternal age, years	33 (29–36)	35 (32.5–37.2)	32 (29–36)	34 (31–37)
Maternal BMI, kg/m^2	21.4 (19.5–24.8)	21.7 (20.2–26.3)	23.7 (21.6–26.3)	21.9 (19.8–23)
Mean uterine artery pulsatility index				
First trimester	1.57 (1.3–2.05)	1.56 (1.4–2.01)	1.4 (1.28–1.86)	1.47 (1.17–1.62)
Second trimester	0.7 (0.59–0.96)*	1.08 (0.91–1.26)	1.05 (0.85–1.15)	1.16 (0.86–1.22)
Delivery, weeks	40 (38.6–41)*	38.1 (35.3–39)	39 (38–40.5)**	38.2 (36.2–40)
Neonatal weight, g	3320 (3075–3655)*	3040 (2372–3508)	3100 (2650–3345)	2700 (2500–3140)
Birthweight centile	44 (28–73)	39 (14.2–78)	35 (15–54)	26 (10–49)***
		n (%)	*n* (%)	*n* (%)
Nulliparous	29 (27.1)	19 (70.4)[a]	19 (37.3)	6 (25)
Umbilical artery pulsatility index				
Normal	107 (100)	21 (77.8)	44 (86.3)	16 (66.7)
>95th percentile	—	3 (14.3)	6 (11.8)	8 (33.3)
Absent/reverse	—	3 (14.3)	2 (3.9)	—
Smoking during pregnancy	4 (3.8)	4 (14.8)	7 (13.7)	5 (20.8)
Therapy		27 (100)	14 (27.5)	17 (70.8)
Steroid	—	5 (18.5)	—	7 (29.2)[a]
HCQ	—	5 (18.5)	—	7 (29.2)[a]
ASA	—	22 (81.5)[a]	13 (25.5)	14 (58.3)
LMWH	—	27 (100)[a]	5 (9.8)	4 (16.7)
Pre-eclampsia	—	6 (22.2)	5 (9.8)	3 (12.5)
FGR	—	6 (22.2)	8 (15.7)	8 (33.3)
SGA	—	4 (14.8)	7 (13.7)	7 (29.2)
Severe (≤32 weeks) prematurity	—	4 (14.8)[a]	—	—
Delivery				
Vaginal	72 (67.3)	11 (40.7)	32 (62.7)	10 (41.7)
Caesarean section	28 (26.2)	16 (59.3)[a]	16 (31.4)	14 (58.3)[a]
Operative vaginal	7 (6.5)	—	3 (5.9)	—
Neonate sex (male)	61 (57)	17 (63)	26 (51)	10 (41.7)

[a]Rates higher than expected ($P < 0.05$) by partitioning chi-squared analysis and Bonferroni correction for multiple comparisons (analysis restricted to the three groups of cases with aPL).
*$P < 0.05$ compared with cases; **$P < 0.05$ compared with primary APS; ***$P < 0.05$ compared with controls by Kruskal–Wallis analysis of variance and Bonferroni correction for multiple comparisons.

Among elementary lesions describing FVM, in primary APS there was an excess rate of intramural fibrin deposition ($P = 0.05$) and villous stromal/vascular karyorrhexis ($P = 0.014$). Overall, compared with healthy controls, cases had increased rates of foetal vascular thrombosis ($P = 0.009$) and intramural fibrin deposition ($P < 0.001$). The rate of overall MPFD (>25% of villi) was higher in primary APS ($P < 0.001$) and in overall cases than in controls ($P < 0.001$). The median gestational age, birthweight and centile of birthweight were lower among subjects with severe MVM or FVM than in subjects with mild–moderate MVM/FVM or negative for these lesions (Table 5). Overall, MVM lesions were more common among pregnancies complicated by FGR ($P = 0.008$) or SGA ($P = 0.02$).

Multivariate studies of the association between placental pathology and type of APS

Table 6 reports the results of penalized logistic regression analysis. After adjustment for the confounding effect of maternal age and BMI, smoking, gestational age and type of treatment at the beginning of pregnancy, severe MVM was found to be more common among primary APS, non-criteria APS and overall cases compared with controls (Table 6). When MVM was modelled as an ordinal variable (no, mild/moderate, severe), the results were similar and the odds ratios (ORs) of MVM were 4.1 (95% CI 1.2, 14.6), 3 (95% CI 1.4, 6.5) and 2.8 (95% CI 1.3, 5.7) in primary APS, non-criteria APS and overall cases, respectively, compared with controls. The risk profile based on aPL antibodies was only weakly associated with severe MVM.

Compared with healthy subjects, overall FVM was more common in cases compared with controls (Table 5). The modelling of FVM as an ordinal variable indicated an increased risk of FVM for primary APS [OR 3.7 (95% CI 1.1, 12.2)], aPL antibodies associated to CTDs [OR 2.8 (95% CI 1.4, 5.7)] and non-criteria APS [OR 4.2 (95% CI 1.4, 12.3)] compared with healthy

Table 3 Placental and cord macroscopic characteristics in cases and healthy controls

Characteristics	Controls ($n = 107$), median (IQR)	Primary APS ($n = 27$), median (IQR)	Non-criteria APS ($n = 51$), median (IQR)	aPL associated with CTD ($n = 24$), median (IQR)	Overall cases ($n = 102$), median (IQR)
Placental weight, g	476 (418–560)	410 (378–451.8)*	420 (358–470)*	390 (347–420)*	409 (358–475)*
Placental diameter, cm					
Maximum	19 (17.5–20.5)	17 (16–18.5)*	18 (16–19)*	17 (16–18.5)*	17.3 (16–19)*
Minimum	16 (14.7–17.5)	14 (13.5–15)*	15 (14.5–16.5)	15 (13.8–16)	15 (14–16.5)
Placental thickness, cm					
Maximum	3 (2.5–3.5)	2.7 (2.5–3)*	3 (2.5–3.4)	2.5 (2.5–2.7)*	2.7 (2.5–3)*
Minimum	2 (1.5–2)	1.6 (1.45–1.82)	1.5 (1.3–2)	1.6 (1.5–1.7)	1.55 (1.4–1.76.5)
Placental:foetal weight ratio	6.9 (5.9–7.7)	7.5 (5.6–8.5)	7.1 (6.2–8.2)	6.9 (6.2–7.8)	7.1 (6–8.3)
Placental area, cm^2	389.8 (325.9–441.08)	303.2 (267.5–343.9)*	346.5 (295. 5–399.4)*,**	324.8 (280.2–367.5)*	332 (289–382)*
Placental volume, cm^3	720 (574.6–868.8)	528 (462–604.8)*	594 (519.8–735)*,**	548 (474.3–644.6)*	577 (501–666)*
Cord diameter					
Maximum	1.2 (1–1.5)	1.1 (1–1.3)	1.2 (1–1.3)	1.2 (1–1.25)	1.2 (1–1.3)
Minimum	1 (1–1.2)	1 (1–1)*	1 (0.8–1.1)*	1 (1–1.2)	1 (0.9–1.1)
	n (%)	*n* (%)	*n* (%)	*n* (%)	
Umbilical cord morphology					
Hypercoiled cord	13 (12.1)	6 (22.2)	6 (11.8)	4 (16.7)	16 (15.7)
Hypocoiled cord	1 (0.9)	7 (25.9)[a]	18 (35.3)[a]	8 (33.3)[a]	33 (32.4)*
Single umbilical artery	1 (0.9)	1 (3.7)	1 (1.9)	0 (0)	2 (2)
Cord knots	2 (1.9)	0 (0)	0 (0)	0 (0)	0 (0)

[a]Rates higher than expected ($P < 0.05$) by partitioning chi-squared analysis and Bonferroni correction for multiple comparisons.

*$P < 0.05$ compared with controls; **$P < 0.05$ compared with primary APS by Kruskal–Wallis analysis of variance or chi-squared analysis and Bonferroni correction for multiple comparisons.

controls. Finally, the aPL antibody risk profile was positively associated with overall FVM.

In stepwise predictive models, first-trimester aCL IgG >40 UI/ml [OR 4.4 (95% CI 1.3, 14.4)], LA positivity [OR 6.5 (95% CI 1.3, 33.3)] and history of pre-eclampsia at <34 weeks [OR 32.4 (95% CI 6.5, 161)] were the best independent first-trimester clinical and laboratory predictors of severe MVM and the predictive value of the logistic model was fair [area under the curve (AUC) 0.74 (95% CI 0.6, 0.87)]. On the other hand, the best independent predictors of severe FVM were aCL IgG >40 UI/ml [OR 3.2 (95% CI 1, 9.9)], aCL IgM >40 UI/ml [OR 5.5 (95% CI 1.5, 19.3)] and a history of pre-eclampsia <34 weeks [OR 14 (95% CI 3.3, 60.1)], but the predictive value of this model was poor [AUC 0.69 (95% CI 0.57, 0.82)].

Discussion

The results of this study suggest that compared with healthy controls, pregnant subjects with aPL antibodies during the first trimester of pregnancy had lower morphometric placental measures (weight, volume, area) and higher rates of placental lesions, suggesting severe MVM or FVM. Among MVM elementary placental lesions, retroplacental haematoma, muscularized arteries and accelerated villous maturation were more common among APS cases than in controls. Finally, among APS subjects there was an excess in the rate of typical FVM lesions such as foetal vascular thrombosis and intramural fibrin deposition. The predictive values of the type and titre of aPL antibodies at the first trimester were good for severe MVM but poor for FVM.

aPL antibodies interfere negatively with trophoblast activity in both the early and late stages of gestation. During the early phases, aPL antibodies exert an inhibitory effect on syncytiotrophoblast, increasing apoptosis and reducing proliferation [4]. Late effects include a failed and/or defective invasion of spiral arteries, a diffuse prothrombotic placental effect with formation of thrombi in the intervillous space, infarction and fibrin deposition around the placental villi [7, 8]. The final effects of these modifications include increased placental blood flow resistance and placental insufficiency leading to adverse pregnancy outcomes such as stillbirth,

Table 4 Placental microscopic characteristics of cases and healthy controls

Characteristics	Controls (*N* = 107), *n* (%)	Primary APS (*N* = 27), *n* (%)	Non-criteria APS (*N* = 51), *n* (%)	aPL associated with CTD (*N* = 24), *n* (%)	Overall cases (*N* = 102), *n* (%)
MVM					
Recent infarct	7 (6.5)	1 (3.7)	3 (5.9)	—	4 (3.9)
Recent infarct >2 cm	3 (2.8)	1 (3.7)	1 (1.9)	—	2 (2)
Remote infarct	6 (5.6)	3 (11.1)	8 (15.7)	2 (8.3)	13 (12.7)
Remote infarct >2 cm	3 (2.8)	2 (7.4)	8 (15.7)	—	10 (9.8)
Retroplacental haemorrhage/haematoma					
Mild	3 (2.8)	—	1 (1.9)	3 (12.5)	4 (3.9)
Moderate–severe	—	6 (22.2)	—	—	6 (5.9)*
Haematoma >2 cm	1 (0.9)	6 (22.2)[a]	—	—	6 (5.9)
Mural hypertrophy	10 (9.3)	3 (11.1)	1 (1.9)	—	4 (3.9)
Muscularized arteries	18 (16.8)	12 (44.4)	25 (49)[a]	9 (37.5)	46 (45.1)*
Acute atherosis	1 (0.9)	—	—	—	—
Accelerated villous maturation	39 (36.5)	21 (77.8)	29 (56.9)	12 (50)	62 (60.8)*
Distal villous hypoplasia	1 (0.9)	3 (11.1)	1 (1.9)	—	4 (3.9)
FVM					
Foetal vascular thrombosis	2 (1.9)	2 (22.2)	6 (11.8)	3 (12.5)	11 (10.8)*
Intramural fibrin deposition	15 (14)	17 (63)[a]	22 (43.1)	12 (50)	51 (50)*
Avascular villi					
Small foci	9 (8.4)	6 (22.2)	10 (19.6)	2 (8.3)	18 (17.6)
Large foci	2 (1.9)	1 (3.7)	—	—	1 (1)
Villous stromal-vascular karyorrhexis	3 (2.8)	7 (25.9)[a]	2 (3.2)	—	9 (8.8)
High-grade villitis of unknown aetiology					
Patchy	3 (2.8)	1 (3.7)	7 (13.7)	2 (8.3)	10 (9.8)
Diffuse	3 (2.8)	1 (3.7)	5 (9.8)	—	6 (5.9)
Massive perivillous fibrin deposition					
25–50% of villi	1 (0.9)	7 (25.9)[a]	4 (7.8)	2 (8.3)	13 (12.7)*
>50% of villi	—	4 (14.8)	2 (3.9)	2 (8.3)	8 (7.8)

[a]Rates higher than expected ($P < 0.05$) by partitioning chi-squared analysis and Bonferroni correction for multiple comparisons. *$P < 0.05$ compared with controls by chi-squared analysis.

prematurity, pre-eclampsia and FGR [1, 11, 12]. In the past, numerous, mostly uncontrolled studies have evaluated the effects of aPL antibodies on several elementary placental lesions such as infarctions, decidual arteriopathy, vascular thrombosis or villous abnormalities [6–8]. The main drawbacks of these studies are the lack of appropriate controls, the heterogeneity of the APS definition and, most importantly, the lack of consensus in the diagnosis and definition of placental lesions [5]. In 2016, an international workshop issued a consensus statement on the diagnosis of placental lesions in pregnancies complicated by pre-eclampsia, FGR and infection/inflammation [9]. Placental lesions associated with FGR and/or pre-eclampsia have been defined as MVM or FVM according to the side of the lesions [9, 10]. Currently there are no data among APS pregnant subjects on the distribution of these lesions and their relationship with the type of APS group or aPL antibody risk profile. High rates of retroplacental haematoma, defective spiral artery remodelling or accelerated villous maturation have been described among women with APS with and without SLE [7, 8]. On the other hand, the association between aPL antibodies and increased rates of placental decidual arteriopathy appears to be less certain [5]. Our study confirms these data, also suggesting that the rates and severity of several types of MVM lesions are higher in primary APS compared with both negative controls and non-criteria APS.

We also found that among APS pregnant subjects there was an excess of MPFD, a lesion caused by extensive fibrinoid deposition encasing villi [23]. The association between this type of placental lesion and APS has been reported by others and is considered a consequence of an extensive hypercoagulable state of the trophoblast vessels induced by aPL antibodies [6, 7].

Among the lesions suggesting FVM, we found that foetal vessel thrombosis and intramural fibrin deposition were more common among APS than controls. Placental lesions suggesting foetal thrombotic vasculopathy, a synonym of FVM, have been associated with APS, thrombophilia and APS-related intrauterine foetal death or FGR [25]. Finally, we failed to confirm an association between the so-called villitis of unknown origin and APS, suggesting that although villitis of unknown origin is associated with an increased risk of FGR, its association with APS is weak [5].

A proposed strategy to choose the appropriate treatment to prevent APS-related complications is to stratify

Table 5 Obstetric correlates of placental lesions suggesting MVM and FVM

Characteristics	Negative ($n = 66$), median (IQR)	Mild/moderate ($n = 121$), median (IQR)	Severe ($n = 22$), median (IQR)	Overall ($n = 143$), median (IQR)
MVM				
Gestational age, weeks	40 (38.6–4.7)	39.2 (38–4.6)	37.2 (35.1–39)*	39 (38.6–40.7)*
Birthweight, g	3290 (3040–3640)	3230 (2810–3460)	2662 (2175–3180)*	3180 (2695–3437)*
Birthweight centile	40 (24.6–75.3)	43 (23–67)	20.5 (10.8–45.5)*	40 (18.5–88.8)
	n (%)	*n* (%)	*n* (%)	*n* (%)
Pre-eclampsia	1 (1.5)	8 (6.6)	5 (22.7)[a]	13 (9.1)
FGR	1 (1.5)	15 (12.4)	6 (27.3)[a]	21 (14.7)*
SGA	1 (1.5)	13 (10.7)	5 (22.7)[a]	18 (12.6)*
First trimester uterine artery pulsatility index >95th percentile	6 (9.1)	12 (9.9)	1 (4.5)	13 (9.1)
Second trimester uterine artery pulsatility index >95the percentile	2 (3)	6 (5)	2 (9.1)	7 (4.9)
Umbilical artery pulsatility index >95the percentile	3 (4.5)	13 (10.7)	7 (31.8)	20 (14)*

	Negative ($n = 119$), median (IQR)	Mild/moderate ($n = 64$), median (IQR)	Severe ($n = 26$), median (IQR)	Overall ($n = 90$), median (IQR)
FVM				
Gestational age, weeks	39.4 (38.1–40.6)	40 (38.3–41)	37.7 (36.3–39)*	39 (38–40.4)*
Birthweight, g	3250 (2897–3530)	3267.5 (2925–3592.5)	2815 (2502.5–3180.5)*	3155 (2775–3461)*
Birthweight centile	43 (25–70)	42.5 (15–74)	23.5 (11.8–47)*	38.5 (14.2–66.7)*
	n (%)	*n* (%)	*n* (%)	*n* (%)
Pre-eclampsia	7 (5.8)	4 (6.3)	3 (11.5)	7 (7.8)
FGR	10 (8.4)	7 (10.9)	5 (19.2)	12 (13.3)
Small for gestational age	8 (6.7)	6 (9.4)	19 (73.1)	25 (27.8)
First trimester uterine artery pulsatility index >95the percentile	13 (10.9)	4 (6.3)	2 (7.7)	6 (6.7)
Second trimester uterine artery pulsatility index >95th percentile	4 (3.4)	3 (4.7)	3 (11.5)	6 (6.7)
Umbilical artery pulsatility index >95the percentile	10 (8.4)	7 (10.9)	6 (23.1)	13 (14.4)

[a]Rates higher than expected ($P < 0.05$) by partitioning chi-squared analysis and Bonferroni correction for multiple comparisons. *$P < 0.05$ compared with controls by chi-squared or Kruskal–Wallis analysis of variance.

subjects with aPL antibodies according to their laboratory profiles or by combining laboratory and clinical features [26]. Although several studies have attempted to define the best clinical and laboratory predictors of subsequent maternal thrombosis or pregnancy complications, data on the relationship between classical clinical and laboratory features of APS and placental findings are still lacking. In our study, a history of a previous (<34 weeks) severe pre-eclampsia was the best clinical predictor of subsequent severe placental MVM and FVM, confirming that among women with APS, reproductive history plays a key role in abnormal placentation. LA is considered the best predictor of thrombosis in non-pregnant subjects and of pregnancy complications [1, 2]. Our study suggests that LA positivity at the beginning of pregnancy is associated with pathological, mainly thrombotic, features on the maternal side of the placenta. Although statistically significant, the relationship between placental features of FVM and aCL IgG and/or aCL IgM positivity was weak, probably suggesting that the pathogenesis of foetal vascular lesions is heterogeneous.

The strengths of this study were the prospective and longitudinal approach, the use of placentas from a healthy population as controls, the criteria used to define APS groups, the use of well-defined strict pathological criteria to define placental lesions and the use of an international nomenclature to report elementary and global maternal and foetal lesions.

The main limitations include the relatively small number of cases, which restricts further inferences, a single recruiting centre and the inclusion criteria of the study. In fact, since we included only pregnant subjects at the end of first trimester of pregnancy, we missed all the early placental lesions that are typical of APS [1, 11, 12].

Table 6 Association between type and severity of APS and placental lesions suggesting MVM and FVM

	MVM negative (n = 66), OR (95% CI)	MVM (mild–moderate) (n = 121), OR (95% CI)	MVM severe (n = 22), OR (95% CI)	Overall MVM (n = 143), OR (95% CI)
Characteristics of population under study				
Controls	46 (69.7)	58 (47.9)	3 (13.6)	61 (42.7)
	Reference	Reference	Reference	Reference
Primary APS	5 (7.6)	13 (10.7)	9 (40.9)	22 (15.4)
	Reference	2 (0.4, 9.6)	11.7 (1.3, 108)	2.6 (0.6, 12)
Non-criteria APS	8 (12.1)	37 (30.6)	6 (27.3)	43 (30)
	Reference	3.2 (1.3, 8.2)	8.5 (1.6, 45.9)	3.6 (1.4, 8.9)
aPL associated with CTD	7 (10.6)	13 (10.7)	4 (18.2)	17 (39.5)
	Reference	1.2 (0.3, 4.8)	4.8 (0.5, 42.2)	1.3 (0.34, 5.3)
Overall cases	20 (30.3)	63 (52.1)	19 (86.4)	82 (57.3)
	Reference	2.6 (1.1, 6.2)	8 (1.6, 38.6)	2.9 (1.3, 6.7)
Severity of aPL antibodies profile				
Controls	46 (69.7)	58 (47.9)	3 (13.6)	61 (42.7)
	Reference	Reference	Reference	Reference
Low risk	17 (25.8)	46 (38)	15 (68.2)	61 (42.7)
	Reference	2.4 (1, 5.8)	8.3 (1.7, 40.6)	2.7 (1.2, 6.4)
High risk	3 (4.5)	17 (14)	4 (18.2)	21 (14.7)
	Reference	6 (1, 36.8)	5.5 (0.4, 77)	5.4 (0.9, 31)

	FVM negative (n = 119), OR (95% CI)	FVM (mild–moderate) (n = 64), OR (95% CI)	FVM (severe) (n = 26), OR (95% CI)	Overall FVM (n = 90), OR (95% CI)
Characteristics of population under study				
Controls	77 (64.7)	25 (39.1)	5 (19.2)	30 (33.3)
	Reference	Reference	Reference	Reference
Primary APS	10 (8.4)	9 (14.1)	8 (30.8)	17 (18.9)
	Reference	4.1 (0.9, 17.8)	4.5 (0.7, 28.5)	4.5 (1.2, 16.4)
Non-criteria APS	23 (19.3)	19 (29.7)	9 (34.6)	28 (31.1)
	Reference	2.8 (1.2, 6.5)	4.5 (1.2, 16.6)	3.1 (1.5, 6.7)
aPL associated with CTD	9 (7.6)	11 (17.2)	4 (15.4)	15 (16.7)
	Reference	6.5 (1.7, 25.4)	6.7 (0.9, 47)	5.9 (1.7, 20.1)
Overall cases	42 (35.3)	39 (60.9)	21 (80.8)	60 (66.7)
	Reference	3.3 (1.5, 7.3)	4.7 (1.3, 16.9)	3.5 (1.7, 7.3)
Severity of aPL antibodies profile				
Controls	77 (64.7)	25 (39.1)	5 (19.2)	30 (30.3)
	Reference	Reference	Reference	Reference
Low risk	34 (28.6)	27 (42.2)	17 (65.4)	44 (48.9)
	Reference	3 (1.4, 6.9)	5 (1.4, 17.8)	3.4 (1.6, 7.2)
High risk	8 (6.7)	12 (18.8)	4 (15.4)	16 (17.8)
	Reference	5.6 (1.4, 22.8)	3.3 (0.5, 23.7)	4.5 (1.3, 16)

ORs and 95% CIs were obtained by multinomial or binomial penalized logistic regression including MVM or FVM as outcome variables and characteristics or severity of APS, maternal age, smoking, nulliparity, gestational age and type of treatment (none, corticosteroids, LMWH, low-dose aspirin) as explanatory variables.

In conclusion, the results of this study showed that in subjects with APS with a viable pregnancy at the end of first trimester there was a subsequent excess of both MVM and FVM placental lesions compared with healthy controls. Both first-trimester clinical and laboratory variables, such as a previous history of pre-eclampsia or aCL IgG/IgM are associated with subsequent severe MVM or FVM.

Funding: No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article.

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

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