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**Observational study of pregnant women
with a previous spontaneous abortion before the 10th gestation week
with and without antiphospholipid antibodies.**

Short title for the running head: Embryonic loss and antiphospholipid antibodies.

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ABSTRACT

Background: One clinical subtype of the purely obstetrical antiphospholipid antibody (aPL-Ab) syndrome (APS) requires three or more unexplained consecutive embryonic losses before the 10th week of gestation associated with persistently positive lupus anticoagulant (LAC), and/or anticardiolipin IgG or IgM, and/or anti- β 2-glycoprotein I IgG or IgM (a β 2GpI-M). This clinical APS feature appeared to be the most sensitive, associated with low evidence levels. The APS-associated serological criteria are still debated.

Patients/methods: Three episodes require a first then a second one. We prospectively observed the second pregnancy natural evolution of 284 women with a first embryonic loss, with (aPL-Ab positive) and without (aPL-Ab negative) antiphospholipid antibodies.

Results: APL-Ab positive women were more prone to pregnancy loss, embryonic loss, preeclampsia, placental abruption and intrauterine foetal growth restriction Type IIa aPL-Ab positivity (LAC present alone) is associated with the strongest risk of embryonic loss recurrence and intrauterine growth restriction, type I aPL-Ab positivities (combinations of positivities) with the strongest risks of late complications preeclampsia and placental abruption. Finally, a β 2GpI-M positivities were globally not clinically relevant.

Conclusion: Patients with a first unexplained pregnancy loss before the 10th week of gestation and positive for aPL-Abs are likely to experience a high-risk second pregnancy and a β 2GpI-M has a questionable pregnancy-related prognostic value.

Keywords:

Pregnancy

Antiphospholipid antibodies

Miscarriage

Embryo

Lupus anticoagulant

INTRODUCTION

According to the Sapporo criteria-related definition (1,2), antiphospholipid syndrome (APS) associates thrombotic and/or obstetrical events with persistently positive antiphospholipid antibodies (aPL-Ab), namely lupus anticoagulant (LAC; guidelines for the detection of LAC were recently updated (3)), and/or anticardiolipin IgG (aCL-G) or IgM (aCL-M), and/or anti- β 2-glycoprotein I IgG (a β 2GpI-G) or IgM (a β 2GpI-M) antibodies.

The following obstetrical criteria have been used to define APS: foetal loss at >10 weeks of gestation, three or more unexplained consecutive embryonic losses before the 10th week of gestation, and eclampsia or features of placental insufficiency associated with the premature birth of a morphologically normal neonate before the 34th week of gestation. Whereas the “foetal death” criterion proposed by Sapporo appeared to be the most specific during an international appraisal of the clinical and laboratory characteristics of APS (2), the “recurrent early abortion –embryonic loss” criterion was quoted as the most sensitive. There was however, only limited evidence concerning the latter criterion (2).

In women without autoimmune disease, a recent meta-analysis has shown how the strength of the association between aPL-Abs and recurrent fetal loss (RFL) varies according to the type of aPL-Ab studied (4). No data on LAC or aCL-M positivity was available for RFL occurring prior to 13 weeks' gestation. However, RFL events were significantly associated with aCL-G positivity, but not with a β 2GpI-G or a β 2GpI-M positivity (4). Despite these observations, an invitation for open debate on the serological criteria associated with APS has recently been published and suggests that the aCL-G, aCL-M and a β 2GpI-M measurements all be excluded (5).

In order to better understand the obstetrical risks related to various aPL-Abs, we used the Nîmes' HAematologists and Obstetricians (NOHA) cohort on consecutive primigravidae (6). Specifically, we performed a prospective observational study among non-APS women on the natural prognosis of a second pregnancy. One hundred forty two pairs of non-thrombotic women with a first embryonic loss, with and without aPL-Abs, were included.

MATERIALS AND METHODS.

Patients and controls.

All patients and controls were selected from the NOHA-First cohort, as previously described (6). Recruitment occurred between January 1st, 1999 and February 1st, 2004.

The patients included were primigravidae (Fig.1) selected for four characteristics. First, they all developed pregnancy loss. Second, at least 3 months after pregnancy loss, they all had at least one positive aPL-Ab among LAC, aCL-G, aCL-M, a β 2GpI-G and a β 2GpI-M (methods are

detailed further on). Third, they were negative for traditional risk factors: no evidence of maternal anatomic or hormonal abnormalities, no clinical or serological evidence of any associated inflammatory, infectious or malignant diseases, and normal maternal and paternal karyotypes. Fourth, pregnancy loss had occurred before the 10th week of gestation (gestation time was estimated using serum β HCG levels or biometry-based gestational age assessment at the time of pregnancy loss according to charts derived from published data (7, 8)).

An initial number of 173 women were thus selected and 164 were further confirmed, aPL-Ab positivity being confirmed, for participation during a second visit at last 6 weeks later (Fig. 1). This pre-inclusion visit was performed by the corresponding gynaecologist and obstetrician within the NOHA network, who then systematically pre-included a control, defined as the first consecutive primigravidae with an unexplained pregnancy loss before the 10th week of gestation and negative for the 5 aPL-Ab species. A total of 164 women pairs with and without aPL-Abs were thus pre-included.

A new pregnancy was diagnosed before January 1st, 2009 in 153 (93.3%) of the women pairs using a pregnancy blood test. A concomitant blood sample confirmed the aPL-Ab positive/negative status in 142 pairs during this second pregnancy, thus defining the definitively included patient and control women (Fig. 1).

No anti-inflammatory, immunomodulatory or antithrombotic treatments were given to these 284 women throughout their pregnancy.

Two hundred healthy age-matched women were recruited in a Public Health Centre during a systematic medical check up in order to check normal aPL-Ab values and create normal pooled plasma. Plasma from each healthy individual, as well as the pooled plasma, were divided into aliquots and frozen at -80°C.

The study was approved by the University Hospital of Nîmes institutional review board and ethics committee and by the local "Comité de Protection des Personnes soumises à la Recherche Biomédicale". This clinical investigation was performed according to the Helsinki declaration of 1975 as revised in 1996. All the women had given their informed consent to participate.

Antiphospholipid antibodies.

All tests were performed on platelet-poor plasma aliquots frozen at -80°C.

The methods used for the detection and measurement of the five aPL-Abs have previously been described (9, 10) and were carried out using home-made tests for solid phase aPL-Abs (aCL-G, aCL-M, a β 2GP1-G and a β 2GpI-M) and commercially-available reagents for LAC detection.

LAC activity was detected according to the guidelines of the International Society on Thrombosis and Haemostasis, Scientific Subcommittee on LAs/phospholipid-dependent antibodies

(11), by using 3 screening assays (LAC-sensitive activated partial thromboplastin time using the PTT LA® reagent, and tissue thromboplastin inhibition test using a single 1:500 dilution of the Neoplastin® CI Plus reagent, from Diagnostica Stago, Asnières, France; diluted Russell viper venom test: Bioclot® LA, Biopool, Umea, Sweden). LAC activity quantification using the PTT LA® reagent was arbitrarily based on the value of the clotting time ratio, between the [1:1 mixture of tested sample and healthy pooled plasma] and the healthy pooled plasma. The normal values of this ratio, evaluated by testing the 200 aliquots from healthy women, ranged from 0.78 to 1.22 with a 99th percentile value of 1.21.

The four solid-phase aPL-Abs were assayed according to recommended procedures (12, 13). Quantification was based on calibration curves performed using the Sapporo standards HCAL and EY2C9 kindly provided by The Binding Site staff, Saint Egrève, France. The normal values determined by testing the 200 aliquots from healthy women were the following: aCL-G: range 0.03-0.84 $\mu\text{g.ml}^{-1}$, 99th percentile value: 0.84 $\mu\text{g.ml}^{-1}$; aCL-M: 0.04-1.38 $\mu\text{g.ml}^{-1}$, 1.38 $\mu\text{g.ml}^{-1}$; a β 2GpI-G: 0.02-0.88 $\mu\text{g.ml}^{-1}$, 0.88 $\mu\text{g.ml}^{-1}$; and a β 2GpI-M: 0.05-0.99 $\mu\text{g.ml}^{-1}$, 0.98 $\mu\text{g.ml}^{-1}$. Following published recommendations, these four 99th percentile values were used as cut-off values to separate negative and positive results.

Positive results for aPL-Abs were categorised according to the recommendations of the ISTH subcommittee, as follows: type I, more than one laboratory criteria present (any combination); type IIa, LAC present alone; type IIb, aCL-Ab present alone; type IIc, a β 2GpI-Ab present alone.

Observational study.

Confirmation was required for all symptomatic thrombotic events during the second pregnancy and 3 months postpartum via objective tests (whole leg complete ultrasonography for deep vein thrombosis, chest computed tomography scan for pulmonary embolisms). Any positive confirmations were recorded.

All pregnancy-related clinical events were recorded. Body mass index (BMI) values were calculated from data obtained during the week following the second positive pregnancy test.

Pregnancy losses were classified as embryonic before the 10th week of gestation or as foetal at 10 weeks or beyond.

Preeclampsia was defined as gravidic hypertension (systolic blood pressure (BP) > 140 mm Hg, diastolic BP > 90 mm Hg, a rise in systolic BP > 30 mm Hg, or a rise in diastolic BP > 15 mm on at least 2 occasions that were 6 hours apart) after 20 weeks and associated with a significant proteinuria (> 300 mg / 24 h).

Placental abruption was defined by clinical / ultrasonographic / pathological exam evidence of the premature separation of the placenta from the site of uterine implantation before foetus delivery.

Foetal growth was evaluated after delivery by weighing the neonate. The results were given as conceptus weight with values adjusted for the term of delivery (“adjusted conceptus weight”; percentiles were determined). Intrauterine growth restriction was defined as an “adjusted conceptus weight” lower than the 10th percentile.

Statistical analysis.

S. A. and P. F.-P. analyzed the data. All authors had full access to primary clinical and biological data. Categorical variables were expressed as frequencies and percentages; continuous variables as medians with interquartile ranges (IQR). In univariate analyses, potential risk factors for various clinical complications during the second pregnancy were estimated using a Chi-2 or a Fisher test for categorical variables and using an analysis of variance (ANOVA) or a Wilcoxon Mann-Whitney test for continuous variables. Any variable achieving a p value of less than 0.20 on univariate analysis was included in the multivariate logistic regression models. Interactions were detected using Breslow-Day tests. Step by step multivariate logistic regression models were performed to estimate the adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI) associated with each risk factor. As antiphospholipid antibodies were our variables of interest, they were deliberately kept in all multivariate models. P values were considered statistically significant at a level of 0.05 or less. Statistical analyses were performed using SAS-Windows™ software (version 9.1).

RESULTS

The main characteristics of the women with and without aPL-Abs are given in Table 1A. The positive aPL-Abs types found in the patients, their various observed associations and finally the related categories of aPL-Abs positivities according to the ISTH subcommittee recommendations (2) are given in Table 1B. Among women with aPL-Abs, 44.4% had more than one type of aPL-Ab present, 38% had only anticardiolipin antibodies, 9.2% only a lupus anticoagulant and 8.4% only anti-β2GpI antibodies. Positive IgM were found in 45 women with aPL-Abs (31.7%) and positive IgG in 111 (78.2%). An aCL-G positivity was the most prevalent marker found alone (32 women, 22.5%).

No symptomatic thrombotic event occurred during the 284 pregnancies and post partum periods (94,473 cumulated observation days).

Women with aPL-Abs experienced more clinical complications during their second pregnancy than women without aPL-Abs. A new pregnancy loss occurred in 18 women without aPL-Abs and 44 women with aPL-Abs ($p=0.0002$, $OR=3.09$, $95\%CI [1.68-5.7]$): embryonic loss accounted for all cases in women without aPL-Abs and for 40 recurrences in women with aPL-Abs and ($p=0.0012$, $OR=2.81$, $95\%CI [1.5-5.24]$). Four women without aPL-Abs and 16 women with aPL-Abs ($p=0.0007$, $OR=5.85$, $95\%CI [1.89 - 18.14]$) had preeclampsia, 3 without and 13 with aPL-Abs ($p=0.0019$, $OR=6.17$, $95\%CI [1.71-22.31]$) had placental abruption, 5 without and 18 with aPL-Abs ($p=0.0019$, $OR=4.64$, $95\%CI [1.64 -13.18]$) had an intrauterine foetal growth restriction.

The risk of various clinical complications during the second pregnancy is described in Table 2, accordingly to the categories of aPL-Ab positivity. The strongest risk of embryonic loss recurrence was associated with type IIa positivity, i.e. LAC positive alone, which was also strongly associated with intrauterine growth restriction. The strongest risk of preeclampsia was associated with type I positivity, i.e. combinations of positive aPL-Abs, which was consistently associated with placental abruption and intrauterine growth restriction, but only mildly associated with embryonic loss recurrence. Type IIb positivity, i.e. positive aCL-Abs alone, was associated with a mild risk of embryonic loss recurrence and with a stronger risk of placental abruption and intrauterine growth restriction. Type IIc positivity, characterised by the presence of positive a β 2GpI-Abs only, could not be described in the 3 late pregnancy complications and did not reach significance for embryonic loss recurrence. Increasing body mass index values were significantly protective against embryonic loss recurrence and had no detectable impact on the 3 other complications (preeclampsia: $p=0.51$, placental abruption: $p=0.21$, intrauterine growth restriction: $p=0.75$).

The analytical study of the embryonic loss recurrence risk associated with each aPL-Ab elementary positivity is described in Table 3. LAC and aCL-M positivities independently increased the recurrence risk by a factor of roughly 3.5-2.5 on average and body mass index values were inversely correlated to this risk. There was however a strong interaction between the LAC and a β 2GpI-G positivities: LAC was a significant risk factor in a β 2GpI-G negative women but not in a β 2GpI-G positive women.

The risk of preeclampsia is analysed in Table 4: aCL-M, a β 2GpI-G and aCL-G positivities were significantly associated with a 5, 4.5 and 3 fold increased risk respectively, with no detectable interaction with LAC positivity. Body mass index values had no detectable significant impact.

The risk of placental abruption is depicted in Table 5 and is only independently related to aCL-M positivity, with a non-significant trend for a β 2GpI-G positivity.

LAC and aCL-G positivities increased the risk of intrauterine growth restriction on average by a factor of 10-3.5 (Table 6). There was a strong interaction between the LAC and aCL-G positivities since LAC was a significant risk factor in aCL-G negative women only, and a β 2GpI-G positivity was a significant risk factor in aCL-G positive women but not in aCL-G negative women.

Because the log-linearity hypothesis was not respected, we could not calculate an odds ratio quantifying the risk of placental abruption or intrauterine growth restriction associated with each unitary increase of body mass index values.

DISCUSSION

This observational study focused on non-thrombotic women positive for at least one of the five classical aPL-Abs with no evidence of infectious, inflammatory or autoimmune disease. None of the included women fulfilled the classification criteria for the antiphospholipid syndrome. They shared an unexplained embryonic loss during their first pregnancy. They are highly selected women (0.44% of the whole cohort). The natural evolution of their second non-treated pregnancy was compared to that of control aPL-Abs negative women who had developed the same isolated clinical symptoms.

The results demonstrate that positive aPL-Abs significantly increase the risk of embryonic loss (28.2% in positive women vs. 12.7% in negative women), preeclampsia (16.3% vs. 3.2%), placental abruption (13.3% vs. 2.4%) and intrauterine growth restriction (16.3% vs. 4.1%). Four cases of pregnancy loss at 10 weeks of gestation and beyond, i.e. foetal losses, were also observed in women with aPL-Abs but not in women without aPL-Abs. Sixty nine percent of the embryonic loss recurrence cases corresponded to women with aPL-Abs. The same result was found for 80% of the preeclampsia cases, 81% of the placental abruption cases and 76% of the intrauterine growth restriction cases. No pregnancy-related thrombotic events were observed. Even when disregarding the recurrence rate, this strongly suggests that women with a first embryonic loss and positive for aPL Abs will have a high risk second pregnancy.

We assume that our women with/without aPL-Abs have trustable aPL-Abs status. First, aPL-Abs testing was performed according to recommendations. Second, aPL-Abs testing was performed by a single laboratory, thus limiting the inter-laboratory variability. Third, testing was performed 3 consecutive times after the first embryonic loss, on a 23-77 week period, thus ruling out any transient positivity/negativity. Fourth, a new testing performed at least 6 months after the end of the second pregnancy gave concordant conclusions on all positivities. Fifth, some rare mild positivities were detected in women without aPL-Abs after the end of the second pregnancies but were all transient ones.

We agree that some non-significant results may be due to an insufficient statistical power. We however had to study 32,494 women with a first pregnancy to be able to individualise 142 women with a first pregnancy loss, with aPL-Abs but without the other classical risk factors. This had some significant cost: increasing this number would need huge investments. Our prospective observational study is an original one which has some limitations but gives some valuable indications.

We found LAC and aCL-M to be associated with the risk of second embryonic loss. This may be by itself a valuable result: the most recent metaanalysis (4) could not pool results for recurrent pregnancy loss prior to 13 week's gestation and failed to give any indication on the association between LAC or aCL-M and recurrent early losses.

Aside from the embryo loss risk associated with LAC and aCL-M positivities, we observed an inverse effect concerning body mass index values. Only 4 women with and 4 women without aPL-Abs had BMI values lower than 18.5 kg.m^{-2} , while only 11 patients and 10 controls had BMI values higher than 30 kg.m^{-2} : a shift in the distribution of extreme weights, with their related metabolic consequences, is thus not a valid explanation for our data, as recently described (14). Low body mass index values and serum progesterone concentrations in pregnant women have previously been described to increase the risk of miscarriage (15), and oestradiol levels to be inversely associated with central fat accumulation in young women (16). A relation between the body mass index, the female sex hormones crucial to the evolution of pregnancy and the risk of miscarriage is thus currently suspected. Another hypothesis may be that some species of antiphospholipid / anticofactor antibodies able to impair pregnancy viability may be partly neutralized in women with progressive fat accumulation.

We could not find any obvious clinical value associated with a β 2GpI-M. Thus, in the very strict framework defined by our patients, we agree with the proposal of Galli, Reber, de Moerloose and De Groot (5) to limit the measurement of anti- β 2-glycoprotein I antibodies to IgG. A European cohort of 109 pregnant women fulfilling the criteria of the antiphospholipid syndrome, including 73 with a purely obstetrical syndrome, has recently been published (17): 3 women were positive for a β 2GpI-M but their precise obstetrical morbid antecedents were not given, thus impairing any analytical comparison. None of our patients had isolated positive a β 2GpI-M.

Galli's *et al.* recent proposal also suggests withdrawing anticardiolipin antibodies. However, these antibodies provide useful clinical information for our cohort of women. We confirm their high frequency in obstetrical patients (17). In our precise clinical setting, we cannot withdraw (5) the anticardiolipin IgM test, whose positivity was independently associated with the risk of preeclampsia, placental abruption and embryonic loss recurrence in a β 2GpI-G negative women.

Nor can we withdraw the anticardiolipin IgG test, which was independently associated with the risk of preeclampsia.

A strong interaction between LAC and a β 2GpI-G was evidenced about the risk of embryo loss recurrence, which was increased in LAC positive/a β 2GpI-G negative women but not in LAC positive/a β 2GpI-G positive women. This statistical fact is not so easy to understand. It should basically imply that a β 2GpI-G positivity counteracts the pathogenic effect of LAC on early pregnancy. Recent studies have shown that angiogenic factors trigger endometrial vasculogenesis and angiogenesis during the initiation steps of embryo implantation, which is crucial to initial pregnancy. Plasmin-cleaved β 2GpI is an *in vitro* inhibitor of angiogenesis (18). β 2GpI has antiangiogenic functions which depend on the presence of domain I (19) and plasmin-cleaved β 2GpI in its domain V promotes angiogenesis by attenuating the anti-angiogenic property of angiostatin4.5 (20). Some a β 2GpI-G may thus modify the global effect of β 2GpI/ β 2GpI-derived fragments on angiogenesis, which may be promoted. Some a β 2GpI-G may finally counteract the pejorative impact of LAC on embryonic development. However, this purely speculative hypothesis is not so easy to reconcile with the growing strong association of LAC activity with a β 2GpI-G autoantibodies in patients with the APS, and our results may be due to the small number of patients with aPL-Abs that we analysed. Another strong and complex interaction between LAC and aPL-Ab positivities was evidenced for the risk of intrauterine growth restriction. LAC positivity increased the risk in aCL-G negative but not positive women, the unique subgroup in which a β 2GpI-G positivity had some clinical impact. No explanation is available.

Our aPL-Abs positive patients do not have an obstetrical antiphospholipid syndrome in its clinical subtype characterised by recurrent unexplained early pregnancy wastage. Two consecutive unexplained embryonic losses are however necessary before the third one. Recurrent unexplained embryonic loss is frequent (1.5% of all women) and the level of medical evidence linking it to aPL-Abs is low (2). An overestimation of such an entity is thus likely. Our study shows that roughly 1 out of 8 women with a first embryonic loss but without aPL-Abs will spontaneously experience a second one: a hazardous association with aPL-Abs is thus a real risk. It also shows that a β 2GpI-G, a cornerstone to diagnose APS (21), do not strikingly increase the risk of a second embryonic loss, may be due to non-thrombotic mechanisms in such a clinical setting (22). This may explain why other less specific antibodies can have some clinical importance: their heterogeneity has been clearly demonstrated (23, 24), with pathogenic opportunities for some minor components (24).

We do not imagine testing each woman with a first embryonic loss for aPL-Abs: in this very frequent situation, patients with aPL-Abs are a minority, good percentages of secondary

spontaneous successful births are observed (70%) and no treatment is evaluated. The medical and economical assessment of testing after one embryonic loss is thus uncertain. Women, however, currently have their first pregnancy later and later on, sometimes as each attempt can generate a risky pregnancy, with limited tries before physiology censorship. We thus think that an evaluation should be first performed in women at 35 years or beyond, as reproductive performances decrease. And testing first LAC, which is associated with the highest risks, but not aβ2GpI-M.

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AUTHORSHIP CONTRIBUTIONS.

Dr. Gris, Dr. Quéré, and Dr. Marès designed the study.

Dr. Gris, Dr. Quéré and Dr. Balducchi co-ordinated and participated to the patients follow up.

Dr. Chauleur and Dr. Galanaud gathered the data.

Dr. Alonso and Dr. Fabbro-Peray analyzed the data.

Dr. Cochery-Nouvellon and Dr. Mercier co-ordinated and performed laboratory investigations.

Dr. Gris and Dr. Alonso drafted the manuscript.

All authors critically reviewed the manuscript.

DISCLOSURE OF CONFLICT OF INTEREST.

None of the authors declared any conflict of interest.

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Table 1. Primigravidae with a first embryonic loss.

1A: main characteristics of the women with and without aPL-Abs. Quantitative data are described as median; interquartile range [range] values.

1B: aPL-Abs types found positive in the women with aPL-Abs (grey squares), their various observed associations and finally the related categories of aPL-Abs positivities according to the ISTH subcommittee recommendations. Lupus anticoagulant (LAC), anticardiolipin IgG (aCL-G) or IgM (aCL-M), anti-β2-glycoprotein I (aβ2GpI-G) or IgM (aβ2GpI-M).

1A.	Women without aPL-Abs	Women with aPL-Abs	P
N	142	142	
First pregnancy loss:			
Age (years)	28; 6 [23-34]	28; 6 [20-37]	0.87
Gestational age (days)	37; 15 [10-68]	37; 16 [9-69]	0.79
Observation length (days)	2079; 663 [533-3276]	2077; 654 [544-3289]	0.96
Body mass index (kg.m ⁻²)	25.8; 4.4 [16.4-32.7]	25.9; 4.1 [16.4-32.5]	0.86
LAC activity	0.97; 0.20 [0.78-1.18]	1.03; 0.77 [0.73-2.71]	<10 ⁻⁴
aCL-G, μg.ml ⁻¹	0.48; 0.42 [0.06-0.83]	2.13; 2.12 [0.07-4.67]	<10 ⁻⁴
aCL-M, μg.ml ⁻¹	0.77; 0.69 [0.07-1.38]	0.91; 0.80 [0.05-2.62]	0.005
aβ2GpI-G, μg.ml ⁻¹	0.43; 0.36 [0.04-0.90]	0.64; 1.25 [0.01-2.79]	<10 ⁻⁴
aβ2GpI-M, μg.ml ⁻¹	0.47; 0.51 [0.05-1]	0.62; 0.57 [0.01-2.91]	0.018

1B.

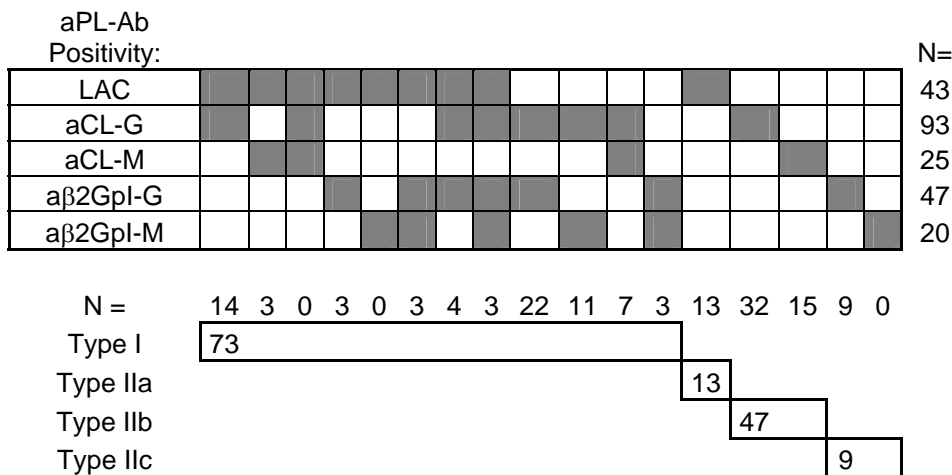


Table2. Risk of various clinical complications during the second pregnancy in women with aPL-Abs and a previous embryonic loss during their first pregnancy, as a function of the type of aPL-Ab positivity according to the ISTH subcommittee classification (2). No/Yes: absence/presence of the clinical complication under investigation during the second pregnancy.

	No	Yes	OR	95%CI	p
Embryonic loss < 10 weeks of gestation					
*aPL-Ab status:					0.0045
Negative for all aPL-Abs	124	18	1		
Type I aPL-Ab positivity	55	18	2.38	1.14-4.99	
Type IIa aPL-Ab positivity	6	7	8.81	2.59-29.9	
Type IIb aPL-Ab positivity	35	12	2.44	1.06-5.60	
Type IIc aPL-Ab positivity	6	3	3.05	0.66-14.1	
<i>Total</i>	226	58			
* Body mass index:			0.871	0.791-0.961	0.0056
Preeclampsia					
*aPL-Ab status:					0.0016
Negative for all aPL-Abs	120	4	1		
Type I aPL-Ab positivity	41	12	8.78	2.68-28.74	
Type IIa aPL-Ab positivity	6	0	Not assessable		
Type IIb aPL-Ab positivity	29	4	4.14	0.98-17.54	
Type IIc aPL-Ab positivity	6	0	Not assessable		
<i>Total</i>	202	20			
Placental abruption					
*aPL-Ab status:					0.0089
Negative for all aPL-Abs	121	3	1		
Type I aPL-Ab positivity	45	8	7.17	1.82-28.23	
Type IIa aPL-Ab positivity	5	1	8.07	0.71-91.95	
Type IIb aPL-Ab positivity	29	4	5.56	1.18-26.23	
Type IIc aPL-Ab positivity	6	0	Not assessable		
<i>Total</i>	206	16			
Intra-uterine growth restriction					
*aPL-Ab status:					0.0062
Negative for all aPL-Abs	119	5	1		
Type I aPL-Ab positivity	44	9	4.87	1.55-15.32	
Type IIa aPL-Ab positivity	4	2	11.9	1.75-81.07	
Type IIb aPL-Ab positivity	28	5	4.25	1.15-15.69	
Type IIc aPL-Ab positivity	6	0	Not assessable		
<i>Total</i>	201	21			

Table 3. Risk of embryonic loss recurrence during the second pregnancy according to the various aPL-Abs present. **3A:** univariate regression analysis; **3B:** multivariate regression analysis models. cOR: crude odds ratios, aOR: adjusted odds ratios. LAC* a β 2GpI-G: interaction factor between LAC positivity and a β 2GpI-G positivity. No / Yes: absence (n=226) / presence (n=58) of embryonic loss recurrence during the second pregnancy.

3A

	No	Yes	cOR	95%CI	P
LAC positivity	29	14	2.16	1.06-4.43	0.033
aCL-G positivity	69	24	1.61	0.89-2.91	0.116
aCL-M positivity	16	9	2.41	1.01-5.78	0.043
a β 2GpI-G positivity	37	10	1.06	0.49-2.29	0.874
a β 2GpI-M positivity	17	3	0.67	0.19-2.37	0.774
Body mass index (kg.m ⁻²)			0.88	0.802-0.966	0.007

3B

	aOR	95%CI	P
LAC positivity	3.69	1.57-8.68	0.003
aCL-G positivity	1.54	0.79-3.01	0.20
aCL-M positivity	2.66	1.06-6.71	0.038
a β 2GpI-G positivity	1.69	0.68-4.24	0.26
a β 2GpI-M positivity	0.99	0.26-3.87	0.99
LAC* a β 2GpI-G	0.06	0.006-0.73	0.027
Body mass index (kg.m ⁻²)	0.87	0.79-0.96	0.006

In a β 2GpI-G negative women:

	aOR	95%CI	P
LAC positivity	3.62	1.55-8.42	0.003
aCL-G positivity	1.42	0.67-3.02	0.37
aCL-M positivity	2.65	1.06-6.63	0.037
a β 2GpI-M positivity	0.97	0.18-5.22	0.97

In a β 2GpI-G positive women:

	aOR	95%CI	P
LAC positivity	0.26	0.025-2.65	0.26
aCL-G positivity	1.40	0.276-7.13	0.68
aCL-M positivity	Not assessable		
a β 2GpI-M positivity	0.83	0.065-10.7	0.89

Table 4. Risk of preeclampsia during the second pregnancy according to the various aPL-Abs present. **3A:** univariate regression analysis; **3B:** multivariate regression analysis models. cOR: crude odds ratios, aOR: adjusted odds ratios. No / Yes: absence (n=202) / presence (n=20) of preeclampsia during the second pregnancy.

3A

	No	Yes	cOR	95%CI	P
LAC positivity	23	5	2.6	0.86-7.81	0.15
aCL-G positivity	53	12	4.22	1.64-10.88	0.002
aCL-M positivity	13	3	2.57	0.67-9.9	0.16
a β 2GpI-G positivity	26	9	5.54	2.09-14.65	0.001
a β 2GpI-M positivity	14	3	2.37	0.62-9.07	0.19
Body mass index (kg.m ⁻²)			1.038	0.89-1.21	0.42

3B

	aOR	95%CI	P
LAC positivity	1.49	0.45-5	0.52
aCL-G positivity	3.09	1.13-8.48	0.03
aCL-M positivity	5.06	1.15-22.3	0.03
a β 2GpI-G positivity	4.61	1.53-13.88	0.007
a β 2GpI-M positivity	1.3	0.3-5.59	0.73

Table 5. Risk of placental abruption during the second pregnancy according to the various aPL-Abs present. **3A:** univariate regression analysis; **3B:** multivariate regression analysis models. cOR: crude odds ratios, aOR: adjusted odds ratios. No / Yes: absence (n=206) / presence (n=16) of placental abruption during the second pregnancy.

3A

	No	Yes	cOR	95%CI	P
LAC positivity	23	5	2.62	1.15-11.34	0.036
aCL-G positivity	57	8	2.61	0.94-7.3	0.084
aCL-M positivity	13	3	3.43	0.87-13.55	0.096
a β 2GpI-G positivity	29	6	3.66	1.24-10.84	0.025
a β 2GpI-M positivity	15	2	1.82	0.38-8.76	0.35

3B

	aOR	95%CI	P
LAC positivity	2.54	0.74-8.66	0.14
aCL-G positivity	1.99	0.66-5.95	0.22
aCL-M positivity	5.53	1.24-24.55	0.02
a β 2GpI-G positivity	3.21	0.91-11.3	0.07
a β 2GpI-M positivity	0.99	0.18-5.64	0.99

Table 6. Risk of intrauterine foetal growth restriction during the second pregnancy according to the various aPL-Abs present. **3A:** univariate regression analysis; **3B:** multivariate regression analysis models. LAC*aCL-G: interaction factor between LAC positivity and aCL-G positivity.

cOR: crude odds ratios, aOR: adjusted odds ratios. No / Yes: absence (n=201) / presence (n=21) of intrauterine foetal growth restriction during the second pregnancy.

3A

	No	Yes	cOR	95%CI	P
LAC positivity	21	7	4.29	1.56-11.81	0.008
aCL-G positivity	55	10	2.41	0.97-6	0.052
aCL-M positivity	14	2	1.41	0.3-6.66	0.653
a β 2GpI-G positivity	27	8	3.97	1.5-10.46	0.008
a β 2GpI-M positivity	15	2	1.31	0.28-6.15	0.67

3B

	aOR	95%CI	P
LAC positivity	10.27	2.37-44.52	0.002
aCL-G positivity	3.55	1.09-11.58	0.036
aCL-M positivity	1.47	0.26-8.16	0.66
a β 2GpI-G positivity	2.43	0.8-7.43	0.12
a β 2GpI-M positivity	0.5	0.09-2.75	0.42
LAC*aCL-G	0.09	0.009-0.83	0.03

In a CL-G negative women:

	aOR	95%CI	P
LAC positivity	13.5	2.83-64.4	0.0011
aCL-M positivity	2.34	0.34-15.92	0.3814
a β 2GpI-G positivity	0.7	0.06-8.82	0.7810
a β 2GpI-M positivity	1.13	0.05-28.14	0.9426

In a CL-G positive women:

	aOR	95%CI	P
LAC positivity	0.84	0.15-4.84	0.8452
a β 2GpI-G positivity	4.26	1.03-17.6	0.0451
a β 2GpI-M positivity	0.65	0.07-6.23	0.7095

Figure 1. The selection of the patients.

