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Highlights

* Women with obstetric antiphospholipid syndrome (oAPS) still develop pre-eclampsia
* Pre-eclampsia (PEcl) is associated with reduced ADAMTS13 levels
* We tested the prognostic value of ADAMTS13 in 513 oAPS women prior to treatment
* ADAMTS13-related markers predict PEcl with an excellent negative predictive value
* ADAMTS13 might help identify pregnant oAPS women at low risk of PEcl

ABSTRACT

Introduction: Women with obstetric antiphospholipid syndrome (oAPS) still develop placental diseases, mainly pre-eclampsia (PEcl), which diagnosis is associated with reduced ADAMTS13 levels. Testing ADAMTS13 in newly pregnant oAPS may provide evidence for risk stratification.

Materials and methods: We retrospectively investigated the prognostic value of ADAMTS13 activity, antigen and antibodies on stored plasma samples obtained prior to beginning low-molecular weight heparin-low dose aspirin treatment in 513 oAPS women.

Results: Some women had evidences of early positive ADAMTS13 antibodies and low ADAMTS13 activity:antigen ratio, suggestive of ADAMTS13 dysfunction. Women with a subsequent PEcl had higher ADAMTS13 antibodies ($p<0.0001$), and lower ADAMTS13 activity and activity:antigen ratios ($p<0.0001$). In multivariate analysis, these markers were significant risk factors for PEcl and for the most devastating PEcl subgroups (early-onset PEcl, severe PEcl, PEcl with no living child after 28 days). ADAMTS13-related markers showed acceptable discrimination power to predict clinical events, particularly for ADAMTS13 activity:antigen ratio in predicting PEcl cases with no living child after 28 days (AUC: 0.844 (0.712-0.974), $p<0.0001$), with excellent negative predictive value (0.990).

Conclusions: The characterization of ADAMTS13 in newly pregnant women with oAPS depicts the risk of PEcl occurrence. ADAMTS13 might help identify pregnant women with oAPS not requiring escalating treatment strategies to prevent PEcl.

Key words: antiphospholipid syndrome, pregnancy, ADAMTS13, pre-eclampsia, placenta.

1. Introduction

Purely obstetric antiphospholipid antibody syndrome (oAPS) is clinically characterized by specific morbidities occurring during pregnancy in women with no history of thrombosis [1-5]. APS is defined as repeated positive laboratory tests for IgG or IgM antiphospholipid antibodies (aPLAbs) such as lupus anticoagulant (LA), anticardiolipin antibody (aCL) and anti-β2 glycoprotein I antibody (aβ2GP1); present at moderate or high titers [2, 4, 5].

Low-dose aspirin (LDA) combined with heparin is generally used for the treatment of oAPS [6]. Among heparins, low-molecular weight heparins (LMWH) are favored for safety reasons and practical considerations. However, in oAPS, defined according to pregnancy loss criteria, the LDA-LMWH combined treatment shows some limitations, with a residual increased risk of pre-eclampsia (PEcl) development, among other placental diseases [7].

PEcl is a common placenta-mediated disease with a highly variable clinical presentation, resulting in placental hypoperfusion and ischaemia with microthrombotic lesions. The maternal systemic signs arise from soluble factors released from the placenta in response to syncytiotrophoblast stress (8, 9]. Early-onset PEcl arises from defective placentation involving inadequate cytotrophoblast invasion, whilst late-onset PEcl may center around interactions between normal placental senescence and a maternal predisposition to cardiovascular and metabolic disease [8]. PEcl is associated with early decreased levels of a disintegrin-like and metalloprotease thrombospondin type 1 motif member 13 (ADAMTS13), independently of von Willebrand factor (VWF) [10], likely contributing to the increase of circulating VWF in PEcl and possibly enhancing the placental microthrombotic risk. In parallel, ADAMTS13 dysfunction and ADAMTS autoantibodies were frequent markers in a series of patients with mainly thrombotic APS, and less frequently oAPS [11].

The Nimes Obstetricians and Hematologists – Antiphospholipid Syndrome study (NOH-APS) study [7], conducted in women with oAPS, allowed us to evaluate the prognostic value on pregnancy outcomes of early soluble factors released from the placenta, namely soluble fms-like tyrosine kinase-1 and placental growth factor (PlGF) [12], the "angiogenic factors". The biobank of plasma samples created in the NOH-APS study of women with oAPS prior to starting LMWH-LDA prophylactic treatment during a new pregnancy, allowed us to evaluate here the association between the biological markers evaluating ADAMTS13 and the occurrence of PEcl.

2. Materials and methods

2.1 Cohort, treatments and definition of outcomes

We performed a retrospective study on a cohort of patients with oAPS (NOH-APS cohort [7, 12]) who initiated a hereafter referred to as the "index pregnancy" in the 18 months period after oAPS diagnosis, with a sample in the biobank of plasma samples stored at -80°C prior to starting LDA-LMWH prophylactic treatment.

Patient recruitment and group definition of the patients included into the NOH-APS study have been described in detail elsewhere [7, 12] (Fig. S1). Briefly, all women fulfilled one of the following inclusion criteria: (1) three unexplained consecutive spontaneous abortions before the 10th week of gestation that could not be accounted for by maternal anatomic or hormonal abnormalities or paternal or maternal chromosomal causes; or (2) one unexplained death of a morphologically normal fetus (fetal loss) at or after the 10th week of gestation, with the normal morphology of the fetus confirmed by ultrasound scan or direct examination of the fetus. The exclusion criteria were a history of thrombotic events (at least one clinical episode of venous, arterial, or small-vessel thrombosis in any tissue or organ other than the placenta; confirmed by objective validated criteria, *i.e.* unambiguous findings in appropriate imaging or histological studies), or any treatment given

during previous pregnancies that might have modified the natural course of the condition, such as antithrombotic agents, or immunosuppressive or immunomodulatory drugs. We excluded women whose pregnancy losses could be explained by infectious, metabolic, anatomic or hormonal factors. Women seropositive for HIV, or hepatitis B or C were also excluded.

Complete thrombophilia screening tests were systematically performed, leading to the definition of subgroups. Among the 4,801 overall screened patients for thrombophilia, this study focuses on the APS subgroup, conventionally defined as women persistently positive for LA, and/or aCL, and/or aβ2GP1 (initially: N=517) assayed as described [7, 12]. Of this subgroup, all women who initiated a new pregnancy during the 18 months observational period after oAPS diagnosis were retained here (N=513) (Fig. S1).

The study was approved by Nîmes University Hospital' Institutional Review Board and ethics committee (IRB n° 20.03.06) and performed in accordance with the policy on bioethics and human biological samples of French laws on clinical research and in accordance with the 1996 revised version of the 1975 Helsinki declaration. All the women gave their informed consent to participate. The study protocol was registered as a clinical trial (registration number NCT04319341). All authors guarantee the accuracy and completeness of the data presented in this report.

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The observed index pregnancies were treated as follows: LMWH (enoxaparin, 40 mg per day *i.e.* 4,000 U/day) was added concomitantly to LDA (100 mg/day) from the day of positive pregnancy test result until delivery. Compliance to LMWH treatment was monitored by self-declaration of the patients and their partners and systematic examination of the subcutaneous injection sites at each medical examination. Platelet counts were checked on the day before the first LMWH injection, twice a week during the first three weeks and then once a month.

Clinical outcomes were defined as follows:

the diagnosis of PEcl was the association of systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg in a woman who was normotensive before 20 weeks'gestation (WG) and a significant proteinuria defined as the presence of 0.3 g or more of protein in a 24-hour urine specimen,[13] and/or evidence of maternal acute kidney injury, liver dysfunction, neurological features, hemolysis or thrombocytopenia, or fetal growth restriction (FGR) [14, 15].

The diagnosis of severe PEcl was made according to the American College of Obstetricians and Gynecologist criteria. Briefly, PEcl was considered severe if one or more of the following criteria was present: systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg on two occasions at least 6 hours apart while the patient is on bed rest, proteinuria ≥ 5 g in a 24-hour urine specimen or ≥3 g on two random urine samples collected at least 4 hours apart, oliguria of <500 mL in 24 hours, eclamptic seizures, persistent headache or visual disturbances, abruptio placenta, pulmonary edema or cyanosis, epigastric or right upper-quadrant pain, impaired liver function (twice the normal range), and thrombocytopenia (<100,000 cells/μL), severe FGR (< 5th percentile) [16]. Chronic hypertension was defined as hypertension (blood pressure ≥140 mmHg systolic or ≥90 mmHg diastolic) present before pregnancy or diagnosed before 20 WG [13]. The diagnosis of superimposed PEcl in chronic hypertensive women needed one of the following findings: in women with hypertension and no proteinuria early in pregnancy (< 20 WG), new-onset proteinuria as defined above; in women with hypertension and proteinuria < 20 WG, sudden increase in proteinuria, sudden increase in blood pressure in a woman whose hypertension has previously been well controlled, thrombocytopenia (platelet count <100,000 cells/μL), or increase in alanine aminotransferase or aspartate aminotransferase to abnormal levels (13].

Eclampsia was defined as PEcl resulting in seizures.

HELLP syndrome was defined by the presence of all three of the following criteria: hemolysis (characteristic peripheral blood smear and serum lactate dehydrogenase [LDH] ≥ 600 U/L or serum

total bilirubin ≥ 1.2 mg/dL), elevated liver enzymes (serum aspartate aminotransferase [AST] ≥ 70 U/L), and low platelet counts (< 100,000 cells/µL) (17].

Birthweights were assessed by birthweight percentile charts stratified for maternal age, pre-pregnancy body mass index (BMI), parity, gestational age at delivery, and sex [18]. FGR was defined as birthweight < 10^{th} percentile, and severe when < 5^{th} percentile.

Abruptio placenta was defined according to classical clinical prenatal signs and symptoms: vaginal bleeding accompanied by nonreassuring fetal status or uterine hypertonicity, or sonographic visualization of abruption, and evidence of retroplacental clots during examination of the delivered placenta. Cases were confirmed by histopathological diagnosis.

Fetal death was defined before 20 WG, stillbirths from 20 WG to delivery, and neonatal death before reaching 28 days of age.

2.2 Laboratory methods

We obtained blood samples collected for standard monitoring before LDA-LMWH treatment. Citrate-anticoagulated blood samples were taken by clean venipuncture the day before starting treatment, then immediately centrifuged twice at 4000 g for 20 minutes, and aliquots of platelet-poor plasma stored at -80°C until testing.

Stored plasma samples were assayed, blinded and in duplicate, for ADAMTS13-related markers, activity (chromogenic assay based on reference [19]), antigen and IgG autoantibodies, and VWF antigen (VWF-Ag) using commercially-available Technozym® kits from Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Vienna, Austria. Briefly, to assay ADAMTS13 activity (ADAMTS13-Act), VWF73, a region from D1596 to R1668 of VWF, which produces a minimal substrate for ADAMTS13, is coupled to glutathione s-transferase (GST), insolubilized by anti-GST antibodies to a microplate and exposed to diluted patient plasma. The active ADAMTS13-mediated VWF73 cleavage site is revealed by conjugated specific antibodies. ADAMTS13 antigen (ADAMTS13-

Ag) was quantified using an enzyme-linked immunosorbent assay (ELISA) principle via a monoclonal antibody directed against the CUB domain of ADAMTS13. Anti-ADAMTS13 IgG autoantibodies (aADAMTS13-IgG) were quantified by ELISA using insolubilized recombinant ADAMTS13 incubated with diluted patient plasma: ADAMTS13-bound patient IgG is revealed using anti-human IgG conjugated antibodies. ADAMTS13 deficiency was defined as activity or antigen levels lower than 0.40 IU/ml. aADAMTS13-IgG was considered positive if higher than 15 $U.ml^{-1}$: data from the manufacturer, locally checked on 300 controls, corresponding to the 99^{th} percentile of the observed distribution.

Plasma levels of sFlt1 and PlGF were measured using ELISA kits (R&D Systems Europe, Lille, France) as previously described [12].

LA, aCL of IgG isotype (aCL-G) and of IgM isotype (aCL-M), aβ2GP1 of IgG isotype (aβ2GP1-G) and of IgM isotype (aβ2GP1-M) have been previously tested as described [7, 12]. All plasma samples had been assayed for the 4 solid-phase aPLAbs by a method based on calibration curves established using the Sapporo standards HCAL and EY2C9 kindly provided by The Binding Site staff, Saint Egrève, France (normal values: aCL-G, 0.85 mg/mL, corresponding to 42.1 GPL units; aCL-M, 1.39 mg/mL, corresponding to 42.1 MPL units; aβ2GP1-G, 0.89 mg/mL, corresponding to 42.1 GPL units; and aβ2GP1-M, 0.99 mg/mL, corresponding to 42.1 MPL units). LA activity was quantified using the PTT-LA® reagent from Stago, Asnières, France and 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 alone.

2.3 End points and statistical analysis

The primary endpoint was the occurrence of a clinical event occurring after 19 completed weeks' gestation (WG) during the observed pregnancy, and diagnosed as a PEcl. The secondary

outcome was the prevalence of the main PEcl subtypes, chosen for their clinical consequences: early-onset PEcl before 34 WG; severe PEcl; and PEcl associated with fetal death *or* stillbirth *or* neonatal death by day 28.

Quantitative data are presented as the median, interquartile range (IQR) and range values. Qualitative data are presented as values and percentages. Mann-Whitney test, Kruskal-Wallis test, Chi^2 tests and Fisher's exact tests were used, as appropriate, for comparisons between baseline characteristics. Statistical dependence between two quantitative data was investigated using the non-parametric Spearmans' ρ rank correlation coefficient.

All analyses were based on pregnancy outcomes that occurred during the index pregnancy. Analyses of PEcl, which by definition occurs after 19 completed WG, were restricted to women with an ongoing viable pregnancy. The ADAMTS13 related markers were analyzed as continuous variables and after categorization into tertiles.

Putative predictors of the various outcomes among the clinical predictors (age, body mass index, thrombotic and atherothrombotic familial antecedents, antecedent of PEcl in the mother, smoking history, varicose veins, preexisting hypertension, preexisting embryonic/fetal pregnancy loss, primary/secondary pregnancy loss and initial inflammatory disease), the metabolic markers at inclusion (hypercholesterolemia defined as a fasting cholesterol concentration above 5.2 mmol/L, hypertriglyceridemia as a fasting triglyceride concentration above 1.7 mmol/L and diabetes mellitus), the biological predictors at inclusion (positive LA, positive aCL-G, positive aCL-M, positive aβ2GP1-G, positive aβ2GP1-M, triple positivity, the *F5* rs6025 or *F2* rs1799963 polymorphisms, sFlt1, PlGF, ADAMTS13-Act, ADAMTS13-Ag, aADAMTS13-IgG, VWF-Ag, ABO blood group) were evaluated first by univariate analysis then by multivariate analysis. For multivariate models, a stepwise variable selection was performed, starting with all the variables from the univariate models with a $p < 0.25$ as potential predictors, with adjustment finally performed for all variables with $p < 0.25$ in the univariate models. The first model was mainly fed by quantitative data, and the second by categorized data. The final model included only main effects with $p < 0.10$.

Discrimination was assessed by computing receiver operating characteristic (ROC) curves and calculating the area under the curve (AUC: C statistic). The best cut-off value was identified as the point maximizing the computed Youden index (sensitivity Se + specificity Sp -1).

All tests were two-sided and assessed at the 5% significance level. The study design was based on our recruitment capacities assessed over a 10-years period, thus no sample size calculation was performed. Statistical analyses were performed using StatView®-windows software version 5.0 (SAS Institute Inc., Cary, NC, USA) and XLSTAT® software version 2015.4.01.20116 (Addinsoft SARL, Paris, France).

3 Results

3.1 Patient characteristics and index pregnancy outcomes (Table 1).

N	513
Age, years	29 (4) [18-41]
>35 years, n (%)	15 (2.9%)
Body mass index, kg/m^2	26.0 (4.6) [15.3-37.0]
>30 kg/m^2	60 (11.7%)
<18.5 kg/m^2	5 (1%)
Ethnicity:	
Caucasian	489 (95.3%)
From Europe	418 (81.5%)
From North Africa	67 (13.1%)

Black African	22 (4.2%)
Asian	6 (1.2%)
MEDICAL HISTORY:	
Positive history in a first-degree relative:	
Venous thromboembolism	12 (2.3%)
Atherothrombosis	53 (10.3%)
Recurrent abortions	14 (2.7%)
Fetal loss	6 (1.2%)
Positive history of maternal PEcl:	22 (4.3%)
Previous pregnancy loss (PL) subtypes:	
Embryonic PL < 10 WG Fetal PL $\geq$ 10 WG	204 (39.8%) 309 (60.2%)
Primary PL Secondary PL	341 (66.5%) 172 (33.5%)
Inflammatory disease	32 (6.2%)
Current tobacco users	50 (9.7%)
Hypertension	17 (3.3%)
Hyperlipidemia	
Hypercholesterolemia	31 (6.0%)
Hypertriglyceridemia	27 (5.3%)
Interval inclusion/new pregnancy, days:	143 (33) [91-378]
ANTIPHOSPHOLIPID ANTIBODIES:	

Lupus anticoagulant (LA)	
Positive for LA	319 (61.7%)
Anticardiolipin IgG	
Positive for aCL-G	244 (47.2%)
aCL-G titer, µg/ml	0.70 (1.37) [0.05-7.1]
Anticardiolipin IgM	
Positive for aCL-M	369 (71.9%)
aCL-M titer, µg/ml	1.60 (1.55) [0.05-18.1]
Anti-β2-glycoprotein I IgG	
Positive for aβ2GP1-G	114 (22.1%)
aβ2GP1-G titer, µg/ml	0.57 (0.55) [0.03-6.9]
Anti-β2-glycoprotein I IgM	
Positive for aβ2GP1-M	209 (40.7%)
aβ2GP1-M titer, µg/ml	0.72 (0.85) [0.05-19.4]
Categories of positive aPlAb	
I	383 (74.7%)
IIa	31 (6%)
IIb	98 (19.1%)
IIc	0
LA + aCL + aβ2GP1	149 (28.8%)
CONSTITUTIONAL THROMBOPHILIAS:	
F5 rs6025 or *F2* rs1799963	17 (3.3%)

INDEX PREGNANCY OUTCOMES:	
Ongoing pregnancies at 20 WG	383 (74.7%)
Stillbirths ≥ 20 WG to delivery	29 (5.7% - 7.6%)
Perinatal death > 22 WG and < 8 days	43 (8.4% - 11.2%)
Neonatal death < 28 days	18 (3.5% - 4.7%)
PEcl	57 (11.1% - 14.9%)
Severe PEcl	40 (7.8% - 10.4%)
Early-onset PEcl < 34 WG	38 (7.4% - 9.9%)
Eclampsia	11 (2.1% - 2.9%)
HELLP syndrome	16 (3.1% - 4.2%)
Placental abruption	11 (2.1% - 2.9%)
FGR < p10	73 (14.2% - 19.1%)
Severe FGR < p5	26 (5.1% - 6.8%)

Table 1. Clinical and biological characteristics of the APS women who initiated a new pregnancy (index pregnancy) during the 18 months' observational period after obstetric APS diagnosis, at baseline and at follow-up.
Quantitative data are given as median (interquartile range) [range] values, qualitative data as number (percentage) values. When two percentages are given, the first is calculated on the whole number of pregnancies, the second is restricted to ongoing pregnancies at 20 weeks of gestation (WG).PEcl: preeclampsia. FGR: fetal growth restriction; p10: 10th percentile; p5: 5th percentile. PMC: PEcl and/or abruptio placenta and/or FGR.Type I, more than one aPlAb present; type IIa, LA present alone; type IIb, aCL-Ab present alone; type IIc, aβ2GP1-Ab present alone. Triple positivity: positive LA test, CL-Ab test and aβ2GP1-Ab test

The women in our oAPS cohort were mainly non-obese Caucasian Europeans under 35 year' old, with no thrombotic family history. The main clinical presentation and the main criterion at inclusion were primary oAPS and fetal death, respectively. A minority had pre-existing hypertension or/and hyperlipidemia, 4% had a maternal history of PEcl, and around 10% were current smokers.

The most frequent aPlAbs detected were ACL-M or LA. About 20% of the women were positive only for aCL-Ab (8.5% had only aCL-M antibodies). Three-quarters of the patients were positive for more than one APS marker. More than one-quarter of the patients exhibited triple positivity. Only 3.3% of the participants had superimposed positivity for the *F5* rs6025 or *F2* rs1799963 polymorphisms.

Only three-quarters of the women had an ongoing viable pregnancy after 19 completed WG, despite LMWH-LDA treatment, and a further 5.7% experienced stillbirth. Neonatal death before 28 days was reported in 3.5% of the women. Placental diseases were diagnosed in 17.7% of the women, the most frequent being PEcl (11.1%; severe PECl: 7.8%; early-onset PECl: 7.4%), followed by FGR (14.2%; severe FGR: 5.1%) and abruptio placenta (2.1%).

3.2 ADAMTS13 markers, VWF-Ag and angiogenic factors (Table 2)

Globally, we did not find any severe ADAMTS13 deficiency. A total of 61 patients (11.8%) were positive for ADAMTS13-IgG, 28 patients had ADAMTS13-related activity:antigen (Act:Ag) ratio below 0.7, 11 below 0.6 and 2 below 0.5.

Participants with an ongoing viable pregnancy after 19 completed WG who developed a PEcl syndrome had lower ADAMTS13-Act values, higher amounts of aADAMTS13-IgG and lower values of the ADAMTS13 Act:Ag ratio than those who did not. They also had higher sFlt1 values and lower PlGF:sFlt1 ratio values. Positivity for aADAMTS13-IgG was observed in 33/57 (58%) women who developed PEcl and in 16/326 women who did not (4.9%) ($p < .0001$), with many of the positive patients showing values ranging between 20 and 30 $U.ml^{-1}$ (20 in the PEcl group and 3 in the no-PEcl group).

	oAPS group (N=513)				
		oAPS-20WG (N=383)	**PEcl-Neg (N=326)**	**PEcl-Pos (N=57)**	**P**
ADAMTS13-Act, IU.ml^{-1}	0.904 [0.26] (0.426-1.437)	0.904 [0.26] (0.452-1.437)	0.932 [0.247] (0.5-1.437)	0.785 [0.190] (0.452-1.130)	<.0001
ADAMTS13-Ag, IU.ml^{-1}	1.007 {0.312] (0.532-1.680)	1.004 [0.325] (0.533-1.674)	1.007 [0.325] (0.533-1.674)	0.990 [0.311] (0.581-1.640)	0.99
aADAMTS13-IgG, U.ml^{-1}	10.37 [5.33] (0.66-31.2)	10.43 [5.71] (0.66-31.2)	10.13 [5.15] (0.66-19.69)	16.26 [14.79] (3.57-31.2)	<.0001
ADAMTS13, Act/Ag	0.894 [0.193] (0.361-1.251)	0.894 [1.197] (0.361-1.174)	0.905 [0.185] (0.653-1.167)	0.770 [0.239] (0.361-1.174)	<.0001
VWF-Ag, IU.ml^{-1}	1.20 [0.81] (0.34-2.38)	1.19 [0.80] (0.35-2.38)	1.23 [0.80] (0.35-2.38)	1.15 [0.73] (0.35-2.36)	0.199
PlGF, ng.L^{-1}	9.3 [1.70] (5.59-12.62)	9.29 {1.67] (5.59-12.41)	9.34 [1.71] (5.59-12.41)	8.94 {1.35] (5.96-10.97)	0.111
sFlt1, ng.L^{-1}	32 [20] (1-81)	32 [20] (2-81)	31 [20] (5-81)	38 [21.3] (2-59)	0.0384
PlFGF/sFlt1	0.295 [0.232] (0.07-11.33)	0.291 [0.228] (0.07-5.42)	0.299 [0.239] (0.07-2.26)	0.240 [0.204] (0.12-5.42)	0.0353

Table 2. Levels of ADAMTS13 markers, von Willebrand factor (VWF) and of angiogenic factors in newly pregnant women with obstetric APS (oAPS) before receiving the LMWH-LDA treatment: in all patients, in the group of women with an ongoing viable pregnancy after 19 completed weeks of gestation (oAPS-20WG) and according to the subsequent development of a pre-eclampsia (PEcl; negative: PEcl-Neg; positive: PEcl-Pos).
Results are given as median, [interquartile range] and (range values). P applies to the comparison of values between the PEcl-Neg and PEcl-Pos groups.
ADAMTS13-Act: ADAMTS13 activity; ADAMTS13-Ag: aADAMTS13 antigen; aADAMTS13-IgG: anti-ADAMTS13 autoantibodies, IgG isotype; VWF-Ag: von Willebrand factor antigen; sFlt1: soluble splice variant of the vascular endothelial growth factor VEGF receptor; PlGF: placental growth factor.

Women with positive aADAMTS13-IgG had lower ADAMTS13-Act values than women with negative aADAMTS13-IgG (0.833 [0.302] (0.426-1.182) vs. 0.916 [0.253] (0.50-1.437) respectively, p <.0001) and also lower ADAMTS13 Act:Ag ratio values (0.771 [0.188] (0.361-1.041) vs. 0.912 [0.183] (0.649-1.251) respectively, p<.0001). A significant negative statistical dependence was found between ADAMTS13-Act values and aADAMTS13-IgG concentration (ρ=-0.139, p=0.0017), and between the ADAMTS13 Act:Ag ratio and the levels of aADAMTS13-IgG (ρ=-0.381, p<.0001) (Figure 1).

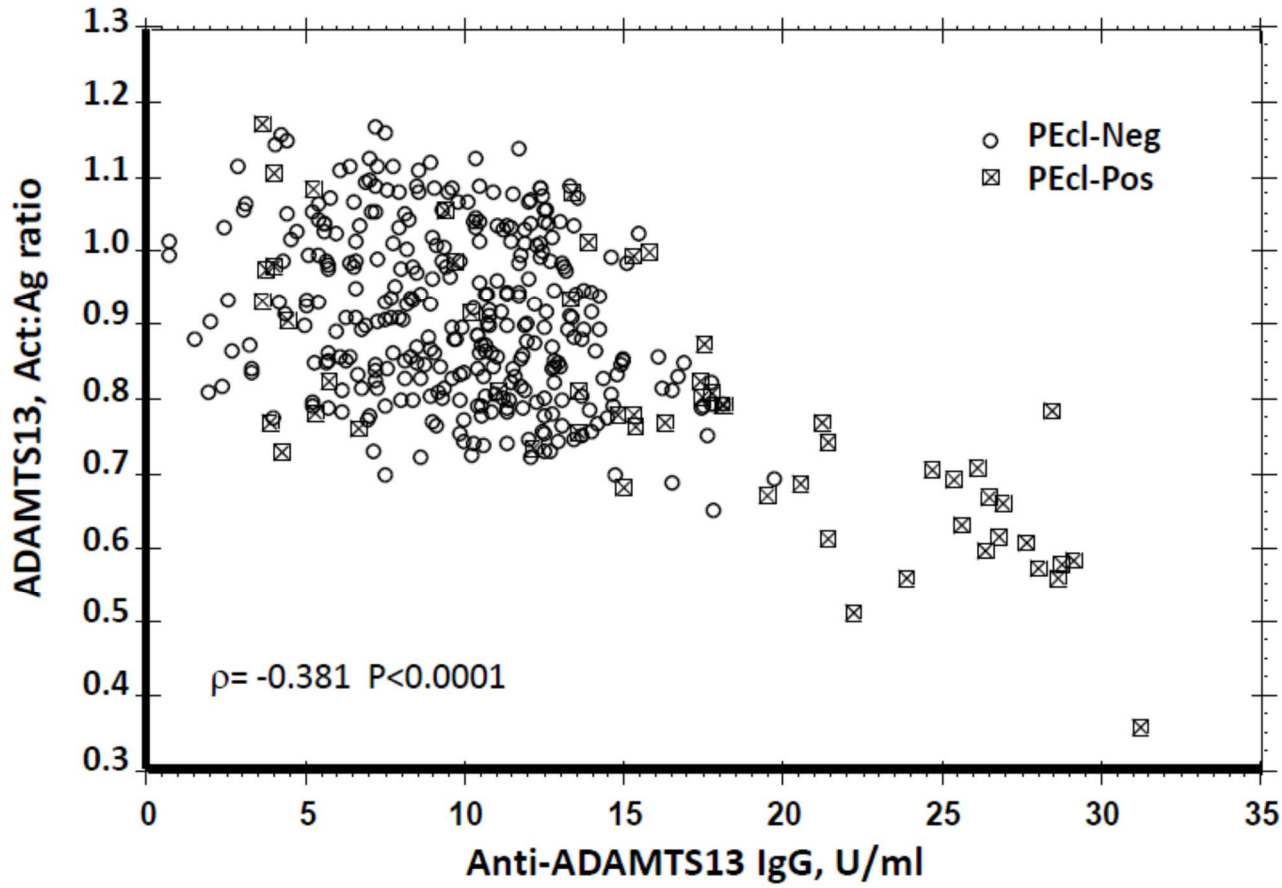

FIGURE 1. Relationship between values of the ADAMTS13 activity:antigen (Act:Ag) ratio and of the anti-ADAMTS13 IgG in pregnant oAPS women before the start of the low-dose aspirin-low molecular weight heparin prophylactic treatment, depending on the subsequent occurrence of pre-eclampsia. *PEcl: pre-eclampsia; Neg: negative; Pos: positive.*

3.3 Risk factors for PEcl (Table 3).

As placental diseases, including PEcl arise after the 19th completed WG, risk factors for PEcl were investigated on the subgroup of 383 women with an ongoing pregnancy after this limit.

Models	Variables	OR ($_{95\%}$CI)	P
Univariate			
	Fetal death	3.046 (1.554-5.970)	0.0012
	BMI	1.118 (1.028-1.216)	0.0090
	BMI > 30 kg.m^{-2}	2.384 (1.147-4.954)	0.0199
	Varicose veins	0.459 (0.209-1.009)	0.0527
	Tobacco	1.796 (0.805-4.007)	0.1524
	LA positivity	1.605 (0.8173-2.950)	0.1278
	LA activity	1.926 (1.028-3.608)	0.0406
	Triple positivity	1.637 (0.912-2.940)	0.0989
	ADAMTS13-Act * 100	0.950 (0.932-0.968)	< 0.0001
	ADAMTS13-Act, tertiles		
	1st tertile T1 (0.452; 0.831)	1	
	2nd tertile T2 (0.832-0.996)	0.318 (0.163-0.623)	0.0008
	3rd tertile T3 (0.997-1.437)	0.102 (0.039-0.268)	< 0.0001
	VWF :Ag	0.996 (0.991-1.002)	0.1991
	aADAMTS13-IgG	1.276 (1.193-1.365)	<0.0001
	aADAMTS13-IgG positivity	26.81 (12.96-55.49)	<0.0001
	aADAMTS13-IgG, tertiles		

	1st tertile T1 (0.66; 8.47) 2nd tertile T2 (8.48-11.96) 3rd tertile T3 (11.97-31.20)	1 0.333 (0.104-1.063) 4.382 (2.179-8.814)	 0.0634 < 0.0001
	ADAMTS13 Act/Ag * 100	0.917 (0.893-0.943)	<0.0001
	PlGF	0.823 (0.652-1.039)	0.1012
	sFlt1	1.018 (0.998-1.039)	0.0766
Multivariate 1*			
	aADAMTS13-IgG	1.263 (1.168-1.365)	< 0.0001
	ADAMTS13-Act	0.031 (0.003-0.284)	0.0021
	Fetal death	3.317 (1.408-7.816)	0.0061
	BMI	1.113(1.002-1.237)	0.0459
Multivariate 2*			
	aADAMTS13-IgG positivity	38.53 (14.87-99.84)	<0.0001
	ADAMTS13-Act, tertiles 1st tertile T1 (0.452; 0.831) 2nd tertile T2 (0.832-0.996) 3rd tertile T3 (0.997-1.437)	 1 0.297 (0.119-0.741) 0.109 (0.035-0.340)	 0.0093 0.0001
	BMI > 30 kg.m^{-2}	3.74 (1.263-11.07)	0.0172
	Fetal death	2.584 (1.056-6.324)	0.0376
	LA positivity	2.775 (1.010-7.825)	0.0499

Table 3. Risk factors of PEcl in oAPS women.
Results are restricted to putative predictors with p-value <0.25 in the univariate analysis and to those with a p-value <0.10 in the multivariate analysis, adjusted for predictors with univariate p<0.25.
LA: positive for lupus anticoagulant, aCL-M: positive for anticardiolipin IgM.

**: adjusted for the individual variables depicted in the univariate analysis. Multivariate models do not include variables describing the same marker.*

In univariate analyses, a number of risk factors were significant: fetal death and BMI among clinical variables; LA activity (strength) the sole retained aPLAb; and variables related to ADAMTS13-Act and aADAMTS13 IgG strongly associated with the risk of PEcl. Angiogenic factors (sFlt1 and PlGF) and VWF-Ag did not reach significance in univariate analysis. Multivariate models demonstrated that aADAMTS13-IgG and ADAMTS13-Act were strongly significant independent risk factors for PEcl, associated with, at a lower significance degree, fetal death, BMI and LA.

3.4 Risk factors for main PEcl subtypes (Table 4).

Outcomes, models	**Variables**	**OR ($_{95\%}$CI)**	**P**
A- early-onset PEcl			
Univariate			
	Fetal death	2.274 (1.070-4.834)	0.0327
	BMI	1.138 (1.030-1.258)	0.0113
	BMI > 30 kg.m-2	2.384 (1.010-5.628)	0.0474
	Varicose veins	0.426 (0.161-1.127)	0.0857
	Tobacco	1.796 (0.697-4.632)	0.2255
	ADAMTS13-Act * 100	0.933 (0.911-0.957)	< 0.0001
	ADAMTS13-Act, tertiles 1st tertile T1 (0.452; 0.831) 2nd tertile T2 (0.832-0.996)	 1 0.202 (0.085-0.480)	 0.0003

	3rd tertile T3 (0.997-1.437)	0.026 (0.003-0.192)	0.0004
	VWF-Ag	0.996 (0.989-1.002)	0.2067
	aADAMTS13-IgG	1.277 (1.188-1.373)	<0.0001
	aADAMTS13-IgG positivity	21.67 (9.627-48.76)	<0.0001
	aADAMTS13-IgG, tertiles 1st tertile T1 (0.66; 8.47) 2nd tertile T2 (8.48-11.96) 3rd tertile T3 (11.97-31.20)	 1 0.333 (0.088-1.262) 3.705 (1.656-8.293)	 0.1058 0.0014
	ADAMTS13-Ag	0.383 (0.079-1.872)	0.2360
	ADAMTS13 Act:Ag * 100	0.919 (0.891-0.948)	<0.0001
	PlGF	0.772 (0.584-1.019)	0.0678
	sFlt1	1.029 (1.005-1.054)	0.0196
Multivariate 1*			
	ADAMTS13-Act * 100	0.907 (0.864-0.952)	< 0.0001
	aADAMTS13-IgG	1.162 (1.055-1.280)	0.0023
	Fetal death	2.884 (1.111-7.487)	0.0295
	BMI	1.145 (1.009-1.300)	0.0355
	ADAMTS13-Ag	36.62 (1.107-1211)	0.0437
	sFlt1	1.042 (0.998-1.088)	0.0626
Multivariate 2*			
	aADAMTS13-IgG positivity	28.58 (9.906-82.47)	<0.0001
	ADAMTS13-Act, tertiles		

	1st tertile T1 (0.452; 0.831) 2nd tertile T2 (0.832-0.996) 3rd tertile T3 (0.997-1.437)	1 0.157 (0.054-0.462) 0.024 (0.003-0.190)	 0.0008 0.0004
	BMI > 30 kg.m-2	4.296 (1.312-14.07)	0.0160
	sFlt1	1.050 (1.004-1.099)	0.0327
B- Severe PEcl			
Univariate			
	Fetal death	2.843 (1.362-5.933)	0.0054
	BMI	1.121 (1.022-1.230)	0.0157
	BMI > 30 kg.m-2	2.235 (0.990-5.045)	0.529
	Varicose veins	0.608 (0.273-1.358)	0.2251
	LA	1.679 (0.849-3.318)	0.1361
	LA activity	1.801 (0.900-3.604)	0.0964
	Triple positivity	1.581 (0.826-3.027)	0.1666
	ADAMTS13-Act * 100	0.945 (0.925-0.965)	<0.0001
	ADAMTS13-Act, tertiles 1st tertile T1 (0.452; 0.831) 2nd tertile T2 (0.832-0.996) 3rd tertile T3 (0.997-1.437)	 1 0.270 (0.126-0.578) 0.072 (0.022-0.244)	 0.0007 <0.0001
	aADAMTS13-IgG	1.280 (1.193-1.373)	<0.0001
	aADAMTS13-IgG positivity	24.38 (11.25-52.83	<0.0001
	aADAMTS13-IgG, tertiles		

	1st tertile T1 (0.66; 8.47) 2nd tertile T2 (8.48-11.96) 3rd tertile T3 (11.97-31.20)	1 0.444 (0.133-1.483) 4.56 (2.073-10.03)	 0.1875 0.0002
	ADAMTS13 Act/Ag * 100	0.917 (0.891-0.945)	<0.0001
	sFlt1	1.024 (1.001-1.047)	0.0386
Multivariate 1*			
	aADAMTS13-IgG	1.253 (1.159-1.356)	<0.0001
	ADAMTS13-Act *100	0.959 (0.936-0.983)	0.0009
	Fetal death	2.833 (1.132-7.090)	0.0261
	sFlt1	1.032 (1.003-1.061)	0.0293
	BMI	1.102 (0.985-1.234)	0.0907
Multivariate 2*			
	aADAMTS13-IgG positivity	29.29 (11.54-74.35)	<0.0001
	ADAMTS13-Act, tertiles 1st tertile T1 (0.452; 0.831) 2nd tertile T2 (0.832-0.996) 3rd tertile T3 (0.997-1.437)	 1 0.273 (0.108-0.691 0.074 (0.019-0.282)	 0.0061 0.0001
	LA positivity	3.007 (1.177-7.681)	0.0214
	BMI > 30 kg.m-2	3.245 (1.108-9.501)	0.0317
	Fetal death	2.483 (0.990-6.231)	0.0526
C- PEcl associated with foetal death *or* stillbirth			

***or* neonatal death.**			
Univariate			
	Fetal death	2.233 (0.697-7.160)	0.1764
	Age	1.108 (0.955-1.285)	0.1778
	BMI	1.121 (1.022-1.230)	0.0157
	Varicose veins	0.201 (0.026-1.552)	0.1239
	Tobacco	4.790 (1.539-14.92)	0.0068
	LA activity	2.840 (0.888-9.081)	0.0784
		1.581 (0.826-3.027)	0.1666
	Positivity for the *F5* rs6025 or *F2* rs1799963 polymorphism	4.892 (0.972-24.64)	0.0542
	ADAMTS13-Act * 100	0.942 (0.910-0.975)	0.0007
	ADAMTS13-Act, tertiles 1st tertile T1 (0.452; 0.831) 2nd tertile T2 (0.832-0.996) 3rd tertile T3 (0.997-1.437)	 1 0.157 (0.034-0.726) 0.140 (0.030-0.649)	 0.0178 0.0119
	aADAMTS13-IgG	1.352 (1.230-1.487)	<0.0001
	aADAMTS13-IgG positivity	29.25 (9.273-92.27)	<0.0001
	aADAMTS13-IgG, tertiles 1st tertile T1 (0.66; 8.47) 2nd tertile T2 (8.48-11.96) 3rd tertile T3 (11.97-31.20)	 1 0.444 (0.133-1.483) 5.13 (1.406-18.72)	 0.1872 0.0133

	ADAMTS13-Ag	7.474 (0.747-74.75)	0.0869
	ADAMTS13-Ag, tertiles 1st tertile T1 (0.532-0.920) 2nd tertile T2 (0.921-1.105) 3rd tertile T3 (1.106-1.680)	 1 0.912 (0.199-4.176) 2.088 (0.612-7.129)	 0.91 0.2397
	ADAMTS13 Act:Ag * 100	0.869 (0.826-0.915)	<0.0001
	sFlt1	1.033 (0.996-1.072)	0.0797
Multivariate 1*			
	Positivity for the *F5* rs6025 or *F2* rs1799963 polymorphism	15.93 (2.280-111.3)	0.0052
	LA activity	8.758 (1.820-42.14)	0.0068
	ADAMTS13 Act:Ag	0.898 (0.825-0.976)	0.0117
	aADAMTS13-IgG	1.174 (1.018-1.354)	0.0274
	sFlt1	1.044 (0.992-1.098)	0.0982
Multivariate 2*			
	ADAMTS13 Act:Ag	0.888 (0.821-0.961)	0.0030
	Positivity for the F5 rs6025 or F2 rs1799963 polymorphism	15.48 (1.932-108.6)	0.0093
	LA positivity	8.196 (1.403-47.87)	0.0195
	aADAMTS13-IgG positivity	7.148 (1.270-40.23)	0.0257

Table 4. Risk factors of PEcl subtypes in APS women.
Results are restricted to putative predictors with p-value <0.25 in the univariate analysis and to those with a p-value <0.10 in the multivariate analysis, adjusted for predictors with univariate p<0.25.

LA: positive for lupus anticoagulant, aCL-G: positive for anticardiolipin IgG; aCL-M: positive for anticardiolipin IgM; aβ2GP1-G: positive for anti-β2-glycoprotein I IgG; aβ2GP1-M: positive anti-β2-glycoprotein I IgM.
**: adjusted for the individual variables depicted in the univariate analysis. Multivariate models do not include variables describing the same marker.*

Similar findings were obtained depending on PEcl subtype. Variables related with aADAMTS13-IgG and ADAMTS13-Act generally emerged as the strongest independent risk factors in the computed multivariate models depicting the risk of early-onset PEcl and severe PEcl. Yet different results were seen for PEcl cases associated with fetal death *or* stillbirths *or* neonatal death (*i.e.* PEcl cases with no living child after 28 days post-delivery): aADAMTS13-IgG remained an independent risk factor, as did ADMATS13 Act:Ag ratio, but LA and positivity for the *F5* rs6025 or *F2* rs1799963 thrombogenic polymorphisms were also strongly and independently associated with the risk.

3.5 Discrimination power of risk factors to predict PEcl (Table 5).

	PEcl	**Early-onset PEcl**	**Severe PEcl**	**PEcl, no living child**
ADAMTS13-Act	0.734 (0.660-0.808) P<0.001	0.788 (0.715-0.862) p<0.0001	0.749 (0.670-0.827) P<0.0001	0.740 (0.607-0.873) P<0.0001
ADAMTS13-Ag	0.499 (0.406-0.593) P=0.99	0.563 (0.458-0.667) P=0.241	0.517 (0.415-0.619) P=0.74	0.382 (0.210-0.553) P=0.177
ADAMTS13 Act:Ag	0.750 (0.656-0.843) P<0.0001	0.772 (0.601-0.843) P=0.0001	0.745 (0.638-0.852) P<0.0001	0.844 (0.712-0.974) P<0.0001
aADAMTS13-IgG	0.748 (0.642-0.855) P<0.0001	0.706 (0.576-0.836) P=0.002	0.739 (0.624-0.854) P<0.0001	0.706 (0.576-0.836) P=0.004

VWF-Ag	0.447 (0.357-0.538) P=0.025	0.436 (0.336-0.537) P=0.22	0.466 (0.368-0.563) P=0.49	0.456 (0.269-0.643) P=0.64
sFlt1	0.586 (0.495-0.677) P=0.064	0,630 (0,527-0,733) P=0.013	0.609 (0.511-0.707) P=0.029	0.646 (0.518-0.773) P=0.025
PlGF	0.568 (0.486-0.651) P=0.106	0.585 (0.489-0.681) P=0.084	0.574 (0.486-0.662) P=0.099	0.574 (0.441-0.706) P=0.277
PlGF:sFlt1	0.587 (0.497-0.677) P=0.059	0.628 (0.526-0.729) P=0.014	0.607 (0.511-0.704) P=0.030	0.637 (0.514-0.760) P=0.030

Table 5. Values (95% confidence intervals) of the area under (AUC) the receiver operating characteristic (ROC) curves describing the association of biological markers with pre-eclampsia (PEcl) and pre-eclampsia subtypes.

PEcl, no living child: pre-eclampsia associated with foetal death or stillbirths or neonatal death

The highest values of the AUCs under the ROC curves were globally obtained for the ADAMTS13-Act and ADAMTS13 Act:Ag variables, with mean values fluctuating around 0.75 (thus there is 75% chance that the variable is discriminatory between a positive and a negative case for the particular PEcl type), generally considered as an acceptable value. The best result was obtained for the ADAMTS13 Act:Ag ratio to predict PEcl cases with no living child after 28 days post-delivery (Figure 2). The 0.789 value maximized Youden index; its negative predictive value was excellent: 0.990 (negative likelihood ratio LR-: 0.239), but with a poor positive predictive value (0.164; LR+: 4.852).

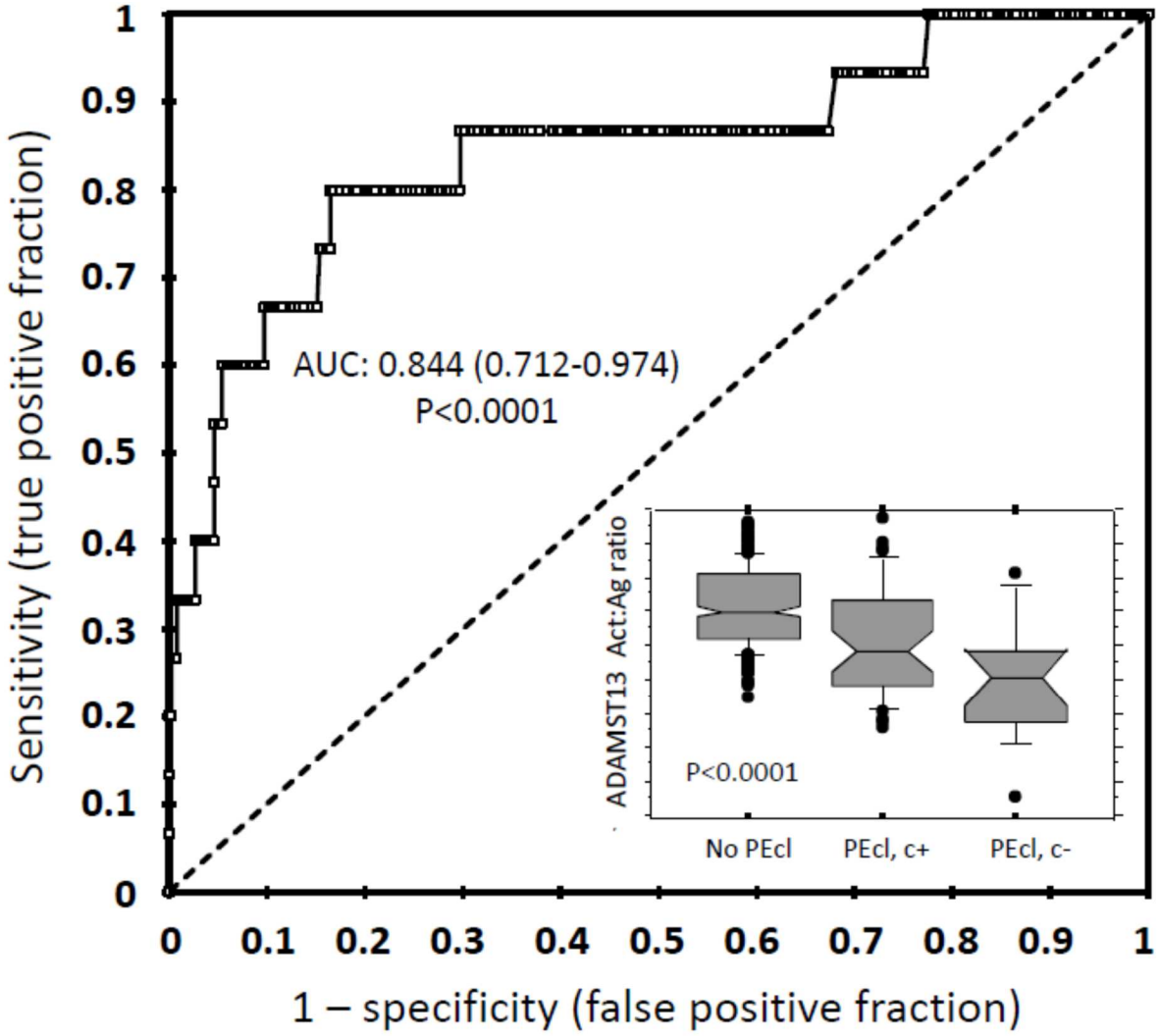

FIGURE 2. Receiver operating characteristic curve analyzing the discrimination power of the ADAMTS13 activity:antigen (Act:Ag) ratio in predicting pre-eclampsia with no child.
AUC: area under the curve; PEcl: pre-eclampsia; c+: with a living child after 28 days post-delivery; c-: with no living child after 28 days post-delivery.

4. Discussion

Women with a diagnosis of oAPS still develop an abnormal rate of placental diseases, mainly PEcl, during new pregnancies even when treated according to guidelines. PEcl is one of the numerous conditions that can present with microangiopathic hemolytic anemia and thrombocytopenia, and is associated with an early quantitative decrease of ADAMTS13 (10). As ADAMTS13 autoantibodies and ADAMTS13 dysfunction have been reported in APS [11], we looked for an association between

ADAMTS13-related markers and the subsequent occurrence of PEcl in our cohort of newly pregnant women with purely oAPS [7].

Globally, 10% of the women had positive ADAMTS13 antibodies, some women had a low ADAMTS13 Act:Ag ratio suggestive of ADAMTS13 dysfunction [11], and a negative statistical dependence was noted between these two variables. ADAMTS13 antibodies could therefore possibly inhibit ADAMTS13 function, which deserves further investigation. No significant correlations were found according to aPLAb subtypes. However, a clearer understanding of the link between ADAMTS13 antibodies and aPLAbs is required, despite other autoantibodies coexisting with aPLAbs in patients with APS (20). Interestingly, aβ2GP1 antibodies from patients with APS have been shown to inhibit the function of ADAMTS13 in vitro [21].

When focusing on women with a viable pregnancy after 19 completed WG, a significant association was found between early levels of ADAMTS13 activity, antibody and Act:Ag ratio, and the global risk of PEcl occurrence, and also of the risk of occurrence of PEcl subtypes (early-onset PEcl, severe PEcl, PEcl with no child). The clinical risk increased as ADAMTS13 activity and Act:Ag ratio levels decreased, and as ADAMTS13 antibody levels increased. These risk factors demonstrated discriminatory power, with significant AUC values, but insufficient to allow a robust early diagnosis in practice as the positive predictive values were too low. Nevertheless, strong negative predictive values could help to exclude the patients without risk and to select those who should benefit from a second-line prognostic approach, if any. This is currently under investigation.

ADAMTS13 activity physiologically decreases progressively during pregnancy from 12-16 weeks until the end of early puerperium [22]. ADAMTS13 is decreased in PEcl [10, 23, 24] and HELLP syndrome [25]. The anti-angiogenic factor sFlt1, responsible for the pathophysiology of early-onset and severe PEcl [26, 27], can induce thrombotic microangiopathy in mice [28], an effect which is inhibited by recombinant ADAMTS13 therapy [28], suggesting that ADAMTS13 activity may be a critical determinant for the development of thrombotic microangiopathy secondary to the sFlt1-

mediated PlGF inhibition in PEcl pregnancies. The normal progressive decrease of ADAMTS13 during pregnancy might thus have potentiated the impact of initially low but not deficient ADMTS13 activities in some of our oAPS patients, thus facilitating the microangiopathic component participating in early-onset and severe PEcl cases. Recent results also demonstrate the expression of ADAMTS13 mRNA and protein in normal and abnormal placental tissues and its role in promoting angiogenesis and trophoblastic cell development, supporting the potential role of the ADAMTS13-VWF pathway in normal pregnancy and pathogenesis of PEcl [29].

A set of data also strongly suggests that complement may play a role in the development of placental disease with oAPS. Risk variants in complement regulatory proteins are described in patients with aPLAbs who develop PEcl [30], a significant complement activation is demonstrable in the plasma and placenta samples of APS women developing adverse pregnancy outcomes [31] and complement activation can predict adverse pregnancy outcome in women with aPLAbs [32]. In mice, a synergistic effect exists between severe ADAMTS13 deficiency and complement activation in pathogenesis of thrombotic thrombocytopenic purpura [33]. One may imagine that activation of the complement cascade *via* aPLAbs (may be also *via* ADAMTS13 antibodies), associated with initial low ADAMTS13 levels, enhance the VWF-dependent placental microthrombotic risk while pregnancy, especially during the third trimester, generates a further decrease in ADAMTS13, thus favoring PEcl. This hypothesis needs a careful validation by specific investigations. Confirmation would lead to testing, in pregnant oAPS women with low initial ADAMTS13 levels showing evidences of risk factors for placental diseases (high sFlt1/PlGF ratio, elevated uterine resistance index and uterine artery notch), complement inhibitors (eculizumab, ravulizumab) and recombinant ADAMTS13 substitution.

The limitations of our study include the single-center design, the overall low number of PEcl cases, the retrospective study of frozen samples, the absence of any control group allowing analysing the aPLAb-dependence of the ADAMTS13-related markers. Strength of this study include a homogeneous and well characterised group of women receiving a single type of treatment during

the index pregnancy, a careful phenotyping of the various placental diseases, and measurements performed in a single laboratory by personnel blinded to the outcomes.

The identification of early markers for the risk of late-pregnancy, placenta-mediated complications in conventionally treated pregnant women with oAPS, with high negative predictive values, might potentially help select the women whose prophylactic treatment does not need escalating strategies adding adjunctive therapies [34, 35], which are controversial among experts [36]. A prospective multicentric study of an early evaluation of ADAMTS13 in newly pregnant women with oAPS is warranted. Understanding the association between APS, aPLAbs, ADAMTS13 antibodies and ADAMTS dysfunction might open new avenues for innovative tailored therapeutic developments.

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Authorship

Contributions: J.-C. G. conceived the study. J.-C. G., V.B. and J.K. designed the study, performed the statistical analyses and wrote the paper. S.B., J.K., É. C.-N. and É. M. performed the research, contributed to data analysis and to the writing of the paper. V.L., A. P.-M. and A.M. contributed to data analysis and to the writing of the paper.

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Conflict of interest

The authors have no competing financial interests to declare.

Ethics approval and consent to participate

The study was approved by Nîmes University Hospital' Institutional Review Board and ethics committee (IRB n° 20.03.06) and performed in accordance with the policy on bioethics and human biological samples of French laws on clinical research and in accordance with the 1996 revised version of the 1975 Helsinki declaration. All the women gave their informed consent to participate.

Data availability statement

The dataset supporting the conclusions of this article are available in the clinical data repository of the University Hospital of Nîmes, Place du Pr. Robert Debré, 30029 Nîmes cedex 9, France, and by directly contacting the corresponding author.

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Consent for publication

Participating authors grant permission for publication.

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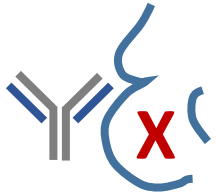
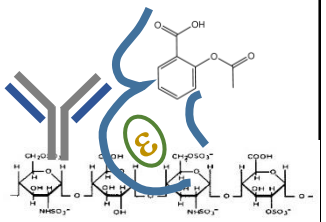
Early ADAMTS13 testing associates with pre-eclampsia in antiphospholipid syndrome

Cohort and methods

NOH-APS cohort
* Pregnancy losses
* Antiphospholipid antibodies aPL-Abs
* Obstetric antiphospholipid syndrome oAPS

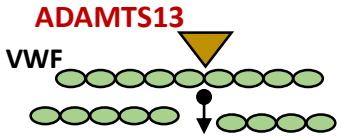


Observation of first pregnancy after diagnosis
* July 1st, 1995 - September 1st, 2007
* Prophylactic LMWH plus low-dose aspirin
* N=517



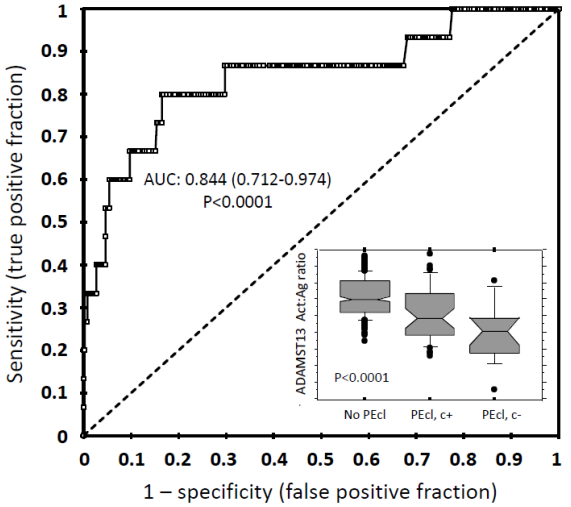
Primary endpoint: pre-eclampsia PEcl
* Secondary: pre-eclampsia subtypes

Retrospective study, stored plasma samples
* ADAMTS13-related laboratory markers
  - Activity, antigen, IgG autoantibodies



Results

Pregnant oAPS women with subsequent PEcl:
* higher ADAMTS antibodies
* lower ADAMTS13 activities
* lower ADAMTS13 activity:antigen ratios
* All are risk factors for PEcl and PEcl subtypes



Prediction
of PEcl with no living child (c-)
by
the ADMTS13 activity:antigen ratio.
Negative predictive value: 0.990

ADAMTS13 testing might help identify pregnant women with oAPS not requiring escalating treatment stategies to prevent PEcl