Comparative incidence of a first thrombotic event in purely obstetric antiphospholipid syndrome with pregnancy loss: the NOH-APS observational study

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The incidence of thrombosis in the purely obstetric form of antiphospholipid syndrome is uncertain. We performed a 10-year observational study of 1592 nonthrombotic women who had experienced 3 consecutive spontaneous abortions before the 10th week of gestation or 1 fetal death at or beyond the 10th week of gestation. We compared the frequencies of thrombotic events among women positive for antiphospholipid Abs (n = 517), women carrying the F5 6025 or F2 rs1799963 polymorphism (n = 279), and women with negative thrombophilia screening results (n = 796). The annual rates of deep vein thrombosis (1.46%; range, 1.15%-1.82%), pulmonary embolism (0.43%; range, 0.26%-0.65%), superficial vein thrombosis (0.44%; range, 0.28%-0.68%), and cerebrovascular events (0.32%; range, 0.18%-0.53%) were significantly higher in aPLAbs women than in the other groups despite low-dose aspirin primary prophylaxis. Women carrying 1 of the 2 polymorphisms did not experience more thrombotic events than women who screened negative for thrombophilia. Lupus anticoagulant was a risk factor for unprovoked proximal and distal deep and superficial vein thrombosis and women in the upper quartile of lupus anticoagulant activity had the highest risk. Despite data suggesting that aPLAbs may induce pregnancy loss through nonthrombotic mechanisms, women with purely obstetric antiphospholipid syndrome are at risk for thrombotic complications. (Blood. 2012;119(11):2624-2632)

Introduction

Antiphospholipid syndrome (APS) is characterized clinically by the occurrence of vascular events (arterial, venous, or small vessel thrombosis) and/or pregnancy morbidity (repeated unexplained abortions before the 10th week, unexplained fetal death at or beyond the 10th week, or premature birth before the 34th week because of preeclampsia).1-3 The mandatory laboratory criteria for APS are positive tests for antiphospholipid Abs (aPLAbs) on 2 or more occasions at least 12 weeks apart.2 The 3 tests exploring aPLAbs are lupus anticoagulant (LA) detected by coagulation assays, anticardiolipin Ab (aCL), and anti-β2 glycoprotein I Ab (aβ2GP1) detected by standardized ELISA.2 ELISA-detected positive Abs must be of IgG and or IgM isotype present in medium or high titer.

When identified in nonthrombotic women, pregnancy morbidity defines purely obstetric APS clinically. The risk of thromboembolism in women with purely obstetric APS remains unclear, although annual prevalence values between 7% and less than 1% have been described previously.4,6 Discrepancies between the estimated risk of thrombosis in purely obstetric APS patients has led to disagreement regarding the benefit of prescribing prophylactic anti-thrombotic treatment for such women.

The Nimes Obstetricians and Hematologists Antiphospholipid Syndrome (NOH-APS) study compared the frequencies of thrombotic events prospectively among groups of nonthrombotic women who had experienced a pregnancy loss fulfilling the APS clinical criteria and who were positive for aPLAbs, bore a constitutional thrombophilia (a F5 6025 or F2 rs1799963 polymorphism), or were negative for thrombophilia.

Methods

Patients

Between January 1, 1995 and January 1, 2005, 6318 patients with pregnancy loss during spontaneous pregnancies were referred for investigation to the Outpatient Department of Hematology, University Hospital of Nimes (Nîmes, France), a tertiary referral center. Exclusion criteria were any past thrombotic clinical antecedent or any treatment given during previous pregnancy attempts that may have modified the natural evolution of their condition, such as antithrombotics, immunosuppressives, or immunity-modulating drugs (eg, corticosteroids, hydroxychloroquine, or intravenous gammaglobulins). Women with pregnancy losses that could be explained by infectious, metabolic (including a fasting blood glucose...
concentration higher than 7mM), anatomic, or hormonal factors were also excluded. HIV-, hepatitis B–, or hepatitis C–seropositive women were excluded because of their particular vascular risk. A total of 4801 women fulfilled 1 of the 2 following inclusion criteria: (1) 3 unexplained consecutive spontaneous abortions before the 10th week of gestation that were not because of maternal anatomic or hormonal abnormalities or to paternal and maternal chromosomal causes (recurrent embryo loss subgroup); or (2) 1 unexplained death of a morphologically normal fetus at or beyond the 10th week of gestation with the normal fetal morphology documented by ultrasounds or by direct examination of the fetus (fetal loss subgroup). Patients were also categorized as primary aborters (no previous successful pregnancy) or secondary aborters (a previous successful pregnancy).

Women had a standardized thrombophilia screening performed 3-6 months after the last pregnancy loss, which included a complete whole blood count and tests for fibrinogen, antithrombin, protein C, protein S, F5 6025 and F2 rs1799963 polymorphisms, JAK2 V617F mutation, LA, aCL, and a2GP1. Women with antithrombin, protein C, or protein S deficiency and women with an abnormal fibrinogen or the JAK2 V617F mutation were not included. Women with a completely negative thrombophilia screening and who agreed to participate in the observational study were temporarily assigned to the “negative” group and were numbered according to the order in which they entered the study. Women with an isolated F5 6025 or F2 rs1799963 polymorphism who agreed to participate in the observational study were assigned to the “constitutional thrombophilia” group. Women who were initially positive for aPLAbs without or with a positive F5 6025 or F2 rs1799963 polymorphism who agreed to participate in the observational study were temporarily included in the “aPLAb” group and had a second testing performed 6 months later. Those women persistently positive for aPLAbs at the 6-month test were definitively assigned to the aPLAb group. Finally, all women in the definitive constitutional thrombophilia and aPLAb groups who consented to participate were included in the study. As each of these women entered the study, the next woman from the ordered negative group candidates was definitively assigned to the negative group and included in the study (Figure 1).

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 Declaration of Helsinki of 1975 as revised in 1996. All the women gave their informed consent to participate.

Samples
Blood samples were obtained between 8:00 and 10:00 am after overnight fasting using a clean venipuncture procedure in which the first 2 mL of blood was systematically rejected. Clean samples were collected in tubes containing 1/10 volume of CTAD anticoagulant-antiplatelet mixture (0.109M trisodium citrate, pH 5.4; theophylline: 3.7mM adenosine; 0.198mM dipyridamole; Diatube-H; Becton Dickinson). After double centrifugation at 4000g for 20 minutes, aliquots of platelet-poor plasma were immediately stored at −80°C until tested and were subsequently used nonfiltered.

Two hundred healthy subjects (102 women and 98 men; mean age of 45 years; range, 18-82 years) were recruited in a local public health center during a systematic medical examination. Aliquots of plasma from each healthy individual were stored at −80°C and portions were also used to generate a pool of healthy plasma, which was also stored in aliquots at −80°C. Samples from subjects positive for aPLAbs (n = 2) were omitted from the pool of healthy plasma (n = 198). This pool was systematically generated from new samples every other year during follow-up, without significant variations of the computed normal threshold values.

Assays
All normal values were assessed from the analysis of the 200 healthy plasmas. The normal upper threshold values for these samples were assumed to be in the corresponding computed 99th percentile, the lowest to be in the corresponding 1st percentile.

F5 6023 and F2 rs1799963 polymorphisms and the JAK2 V617F mutation were demonstrated by PCR. Antithrombin was measured by chromogenic substrate assay (STA-Stachrom ATIII; Stago). Protein C and protein S activities were measured by chromometric assays (STA-Staclot Protein C and STA-Staclot protein S; Stago). Free protein S-antigen levels were measured by ELISA (Asserachrom Free protein S; Stago).

The methods used to test for aPLAbs have been described previously in detail. Briefly, LAs were detected using the following screening assays: activated partial thromboplastin time (aPTT; PTT-LA, Stago), dilute Russell viper venom time (Bioclot LA; Biopool), and tissue thromboplastin inhibition test using a 1:500 thromboplastin dilution (Neoplastin CI Plus; Stago). LAs were first identified in samples that were mixed 1:1 with healthy pooled plasma and were then confirmed by neutralization procedures according to the recommendations of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis. All coagulation assays were performed on the STA-R automatic coagulation analyzer (Stago). Plasma aCL IgG and IgM Abs were measured according to an adaptation of the in-house ELISA method that has been described previously by Reber et al. Plasma aβ2GP1 IgG and IgM Abs were measured according to an ELISA developed in-house using β2-glycoprotein I isolated from freshly frozen human citrated plasma as described by Horbach et al.

LAs were quantified using the results of the mixing test and are expressed as the ratio of the aPTT test result from the patient and healthy pooled plasma mixed 1:1, divided by the aPTT test result from the healthy pooled plasma (normal value < 1.22). All plasmas were secondarily quantified for the 4 solid-phase aPLAbs based on calibration curves performed using the Sapporo standards HICAL and EV2C9 kindly provided by The Binding Site staff (Saint Egrève, France). Normal values are: aCL-G < 0.85 μg/mL, aCL-M < 1.39 μg/mL, aβ2GP1-G < 0.89 μg/mL, and aβ2GP1-M < 0.99 μg/mL.

Positive results for aPLAbs were categorized as follows according to the recommendations of the International Society on Thrombosis and Hemostasis subcommittee: type I, more than 1 laboratory criteria present (any combination); type IIa, LA present alone; type IIb, aCL-Ab present alone; and type IIc, aβ2GP1-Ab present alone. Triple positivity was defined by the association of a positive LA test, a positive aCL-Ab test (IgG and/or IgM), and a positive aβ2GP1-Ab test (IgG and/or IgM).

Follow-up
Patients were initially informed about symptoms of superficial vein thrombosis (SVT), deep vein thrombosis (DVT), pulmonary embolism (PE), transient ischemic attack (TIA) or stroke and were told to contact a study physician immediately if these symptoms occurred. Transient risk factors for venous thrombosis were detailed (surgery, trauma, significant immobilization or bed rest > 3 days, infectious disease, acute inflammatory reaction, airplane travel lasting more than 4 hours, and pregnancy) and if any of these occurred, the women were instructed to contact their general practitioner for evaluation and prophylaxis initiation after a discussion with a study physician.

Patients were systematically followed and clinically reevaluated each year in the outpatient department of our institution. Patient loss because of lack of follow-up was minimized by contacting the general practitioners and the patients themselves directly. A complete systemic and vascular clinical examination was performed. Any suspicion of developing systemic disease led to adapted investigations for diagnosis. Symptoms were evaluated and the treatments taken during the year were screened. The information about vascular symptoms and transient risk factors for venous thromboembolism (VTE) was repeated. No systematic venous ultrasonographic evaluations of the lower limbs were performed. Women from the aPLAb group had a systematic aPLAb assessment. No systematic aPLAb assessment was performed at the time any thrombotic events occurred.

Primary prophylaxis of thromboses
All of the patients with purely obstetric APS underwent primary prophylaxis treatment for chronic thrombosis consisting of low-dose aspirin (LDA; 100 mg/d). Compliance was monitored only by self-declaration of the patient-companion couples. No biologic assay was performed during follow-up to evaluate LDA compliance and effect. Women in the constitutional thrombophilia and negative groups did not receive prophylaxis for chronic thrombosis.

Contraception
Initial birth control, if any, was accomplished by mechanical methods, because most of the included women desired a new pregnancy. In the aPLAb and constitutional thrombophilia groups, the contraindications for estrogen-progestational oral contraceptives (OCs) was systematically discussed, and women seeking OCs during their follow-up period were given progesterin-only OCs: initially, 17-OH-progestrone or 19-norprogestrone derivatives or a low-dose second-generation progesterone (levonorgestrel) followed by a low-dose third-generation progesterone (desogestrel). In the negative group, women seeking an OCs were given levonorgestrel. Current OC use was defined as OC use during the month before the event.

Antithrombotics during new pregnancies
According to published guidelines, low-molecular-weight heparin (LMWH; enoxaparin, 4000 IU/d) plus LDA (100 mg/d) were prescribed for all pregnant aPLAb women from after the positive pregnancy test until delivery. These treatments continued during the 6-week postpartum period, with systematic monitoring and investigation for heparin-induced thrombocytopenia according to published recommendations. Patients from the constitutional thrombophilia group systematically received the enoxaparin prophylaxis (4000 IU/d) during the 6-week postpartum period. In patients with any previous fetal loss, enoxaparin was also prescribed during pregnancy. Patients from the negative group had no drug-mediated prophylaxis during pregnancy or postpartum. In untreated patients, a transient enoxaparin-mediated prophylaxis (4000 IU daily) was given systematically if a transient clinical risk factor existed for venous thrombosis occurring during pregnancy no matter if it was classic or pregnancy related (prolonged bed rest for premature delivery symptoms, fetal death or stillbirth, preeclampsia, abruptio placentae, cesarean section, and primary postpartum hemorrhage). Elastic compression stockings were prescribed systematically for all participants.
Table 1. Clinical characteristics of subjects at baseline and at follow-up

<table>
<thead>
<tr>
<th></th>
<th>Negative group</th>
<th>Constitutional thrombophilia group</th>
<th>aPLAb group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>796</td>
<td>279</td>
<td>517</td>
<td></td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 (5 [17-44])</td>
<td>29 (4 [18-44])</td>
<td>29 (4 [16-41])</td>
<td>.21</td>
<td></td>
</tr>
<tr>
<td><strong>Age &gt; 35 y</strong></td>
<td>43 (5.4%)</td>
<td>12 (4.3%)</td>
<td>15 (2.9%)</td>
<td>.086</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 30</td>
<td>78 (9.8%)</td>
<td>29 (10.4%)</td>
<td>60 (11.6%)</td>
<td>.58</td>
</tr>
<tr>
<td>≥ 18.5</td>
<td>12 (1.5%)</td>
<td>3 (1.1%)</td>
<td>5 (1%)</td>
<td>.66</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>753 (94.6%)</td>
<td>264 (94.6%)</td>
<td>489 (94.6%)</td>
<td>.87</td>
</tr>
<tr>
<td>European</td>
<td>647 (81.3%)</td>
<td>227 (81.4%)</td>
<td>420 (81.2%)</td>
<td>.49</td>
</tr>
<tr>
<td>North African</td>
<td>106 (13.3%)</td>
<td>37 (13.2%)</td>
<td>69 (13.4%)</td>
<td>.65</td>
</tr>
<tr>
<td>Black African</td>
<td>36 (4.5%)</td>
<td>12 (4.3%)</td>
<td>22 (4.2%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>7 (0.9%)</td>
<td>3 (1.1%)</td>
<td>6 (1.2%)</td>
<td>.16</td>
</tr>
<tr>
<td><strong>Pregnancy loss subtype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryonic at &lt; 10 WG</td>
<td>483 (60.7%)</td>
<td>93 (33.3%)</td>
<td>206 (39.8%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Fetal at ≥ 10 WG</td>
<td>313 (39.3%)</td>
<td>186 (66.6%)</td>
<td>311 (60.2%)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>549 (68.9%)</td>
<td>185 (66.3%)</td>
<td>342 (66.1%)</td>
<td>.49</td>
</tr>
<tr>
<td>Secondary</td>
<td>247 (31.1%)</td>
<td>94 (33.7%)</td>
<td>173 (33.9%)</td>
<td>.0001</td>
</tr>
<tr>
<td>Inflammatory disease</td>
<td>7 (0.9%)</td>
<td>4 (1.4%)</td>
<td>32 (6.2%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Current smokers</td>
<td>63 (10.4%)</td>
<td>30 (10.8%)</td>
<td>50 (9.7%)</td>
<td>.89</td>
</tr>
<tr>
<td>Hypertension</td>
<td>19 (2.4%)</td>
<td>8 (2.9%)</td>
<td>17 (3.3%)</td>
<td>.02</td>
</tr>
<tr>
<td><strong>Positive history in a first-degree relative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td>15 (1.9%)</td>
<td>29 (10.4%)</td>
<td>12 (2.3%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Atherothrombosis</td>
<td>96 (12.1%)</td>
<td>46 (16.5%)</td>
<td>53 (10.3%)</td>
<td>.043</td>
</tr>
<tr>
<td><strong>Hyperlipidemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>42 (5.3%)</td>
<td>13 (4.7%)</td>
<td>31 (6.0%)</td>
<td>.31</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>34 (4.3%)</td>
<td>11 (3.9%)</td>
<td>27 (5.2%)</td>
<td>.64</td>
</tr>
<tr>
<td>Varicose veins</td>
<td>187 (23.5%)</td>
<td>58 (20.8%)</td>
<td>117 (22.6%)</td>
<td>.65</td>
</tr>
<tr>
<td><strong>Hormonal contraception, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral, estropgestative</td>
<td>38 (4.8%)</td>
<td>2 (0.7%)</td>
<td>5 (0.9%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Oral, progestin only</td>
<td>5 (0.6%)</td>
<td>14 (5.0%)</td>
<td>27 (5.2%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Lipid-lowering drugs</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Follow-up duration, d</strong></td>
<td>3572 (1860)</td>
<td>3457 (1781)</td>
<td>3392 (1893)</td>
<td>.023</td>
</tr>
</tbody>
</table>

Quantitative data are given as median (interquartile range) and qualitative data as number (percentage) values. WG indicates weeks of gestation.

Outcome assessment

All suspected thrombotic events were confirmed objectively using clinically adapted objective methods as follows: whole-leg compression ultrasonography for SVT and DVT,14 perfusion lung scan or helical CT scan for PE, and neuroimaging using CT scans and MRI for TIA and stroke. TIA was considered for adjudication in cases of ischemic cerebral imaging. VTE was defined by the occurrence of DVT, PE, or both. Cerebrovascular events were defined by the occurrence of TIA or stroke.

Major bleeding events were defined according to the Control of Anticoagulation Subcommittee of the International Society on Thrombosis and Hemostasis15: fatal bleeding; symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intrarticular, or pericardial bleeding; intramuscular bleeding with compartment syndrome; and/or bleeding that led to a decrease of hemoglobin level of 20 g/L or more or that led to transfusion of 2 or more units of RBCs. All events were adjudicated by a committee of independent experts blinded to the thrombophilia screening results.

Statistical analysis

Quantitative data are described by median, interquartile range, and range values. Qualitative data are described by values and percentages. Comparisons between baseline characteristics and risk factors were performed using the Student, Mann-Whitney, Kruskall-Wallis, χ², and Fisher exact tests as appropriate.

Definition of groups and analysis of the impact of biologic covariates on thrombotic incidences were based on values obtained at the initial inclusion.

All analyses were based on the first thrombotic event that occurred during follow-up, which was determined by central adjudication. For women who did not have a thrombotic event, censoring time was defined as the time from randomization to the earliest of the following: death, nonavailability for follow-up, or January 1, 2010. For women in the aPLAb group whose aPLAbs became negative during follow-up, censoring time was defined as the time from randomization to the last positive assessment.

Time-to-event methods (eg, log-rank tests and Cox regression) were used to compare thrombotic incidences between groups. The outcomes analyzed were VTE, PE, DVT (either proximal or distal), SVT, TIA, stroke, or stroke/TIA. Cox-proportional hazards models were stratified according to clinical variables that might have a putative effect on the occurrence of the outcomes (adjusted hazard ratio: age, body mass index, thrombotic familial antecedents, ethnicity, smoking history, hypertension, hypercholesterolemia, hypertriglyceridemia, current OC use, current progestin-only OC use, varicose veins, embryonic/fetal pregnancy loss, primary/secondary pregnancy loss, and any associated systemic disease). Smoking was defined as smoking at least one cigarette a day. Women were classified as hypertensive if they were using hypertensive medication or had either a systolic blood pressure of 140 mmHg or higher or a diastolic blood pressure of 90 mmHg or higher on 2 readings taken in a supine position 5 minutes apart on 2 separate occasions. Hypercholesterolemia was defined as a fasting cholesterol concentration above 5.2mM, and hypertriglyceridemia as a fasting triglyceride concentration above 1.7mM. The outcome comparisons are presented as annualized rates and adjusted hazard ratios with 95% nominal confidence intervals.

Putative predictors of the various outcomes among the clinical predictors (age, body mass index, thrombotic familial antecedents, ethnicity, smoking history, varicose veins, embryonic/fetal pregnancy loss, primary/secondary pregnancy loss, and initial inflammatory disease) and the biologic predictors at randomization (positive LA, positive aCL-G, positive aCL-M, positive aβ2GP1-G, positive aβ2GP1-M, triple positivity and the
Constitutional thrombophilias

Categories of positive aPLAb

<table>
<thead>
<tr>
<th></th>
<th>Negative group</th>
<th>Constitutional thrombophilia group</th>
<th>aPLAb group</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>0</td>
<td>0</td>
<td>319 (61.7%)</td>
</tr>
<tr>
<td>LA: activity</td>
<td>0.99 (0.09) [0.82-1.20]</td>
<td>1.02 (0.09) [0.79-1.21]</td>
<td>1.42 (0.78) [0.79-2.68]</td>
</tr>
<tr>
<td>Anti-cardiolipin IgG</td>
<td>0</td>
<td>0</td>
<td>244 (47.2%)</td>
</tr>
<tr>
<td>aCL-G titer, μg/ml</td>
<td>0.7 (0.21) [0.05-0.84]</td>
<td>0.41 (0.22) [0.07-0.84]</td>
<td>0.70 (1.37) [0.05-7.1]</td>
</tr>
<tr>
<td>Anti-cardiolipin IgM</td>
<td>0</td>
<td>0</td>
<td>372 (71.9%)</td>
</tr>
<tr>
<td>αCL-M titer, μg/ml</td>
<td>0.54 (0.32) [0.08-1.35]</td>
<td>0.51 (0.29) [0.05-1.34]</td>
<td>1.60 (1.55) [0.05-18.1]</td>
</tr>
<tr>
<td>Anti-β2-glycoprotein I IgG</td>
<td>0</td>
<td>0</td>
<td>114 (22.1%)</td>
</tr>
<tr>
<td>a2GP1-G titer, μg/ml</td>
<td>0.23 (0.14) [0.05-0.86]</td>
<td>0.26 (0.18) [0.02-0.84]</td>
<td>0.57 (0.55) [0.03-6.9]</td>
</tr>
<tr>
<td>Anti-β2-glycoprotein I IgM</td>
<td>0</td>
<td>0</td>
<td>210 (40.6%)</td>
</tr>
<tr>
<td>a2GP1-M titer, μg/ml</td>
<td>0.41 (0.24) [0.04-0.98]</td>
<td>0.47 (0.34) [0.02-0.98]</td>
<td>0.72 (0.85) [0.05-19.4]</td>
</tr>
<tr>
<td>Categories of positive aPLAb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>383 (74.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>31 (6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIb</td>
<td>103 (19.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIc</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA + aCL + a2GP1</td>
<td>149 (28.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constitutional thrombophilias</td>
<td></td>
<td></td>
<td>17 (3.3%)</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of thrombophilias at baseline

Quantitative data are given as median (interquartile range) [range] values and qualitative data as number (percentage) values.

F5 6025 or F2 rs1799963 polymorphisms were evaluated in women from the aPLAb group, first by univariate analysis and then by multivariate analysis. For multivariate models, a stepwise variable selection was performed, starting with all of the variables from the univariate models having P < .25 as potential predictors, with adjustment being finally performed for all variables with P < .25 in the univariate models. If triple positivity was an eligible potential predictor, multivariate analysis only considered clinical predictors and Leiden polymorphisms. The final model included only main effects with P < .10. For cases in which a significant biologic predictor emerged from the multivariate models, a new analysis was performed categorizing the biologic covariate into 4 quartiles according to the activity levels. P values were considered statistically significant at a level of .05 or less. Statistical analyses were performed using SAS- Windows Version 9.1 software.

Results

Baseline patient characteristics

The NOH-APS study included 1592 women (Table 1). Recurrent unexplained embryonic loss was the most frequent inclusion criterion in the negative group and fetal loss was the most frequent inclusion criterion in the other groups. A VTE history or an atherothrombotic history was more frequent in the aPLAb group than in the negative group and the 2 other groups. Few women were initially taking OCs. Second-generation estro-progestative compound use was less frequent in the negative group than in the 2 other groups.

In the aPLAb group, aCL-M Abs or LA were the 2 features present most frequently (Table 2). Three-quarters of the women were positive for more than one marker, 19.9% of the women were positive only for aCL Abs (8.5% had only aCL-M Abs). None had only a2GP1 Abs. Nearly one-third of the patients exhibited triple positivity defined without any isotype restriction, which was due in part to the high frequency of aCL-M–positive patients. Systematic aPLAb assessment during follow-up revealed that 26 initially positive women became negative for all aPLAbs: 6 of 31, 6 of 59, and 14 of 44 among the patients with an initially isolated positive LA, aCL-G, and aCL-M, respectively.

Follow-up vascular data

The median follow-up duration was close to 9.5 years and was slightly shorter for the aPLAb group (Table 1). Thirty-seven patients (2.3%) were lost to follow-up. No patient died during follow-up.

Nineteen patients (1.2%) were diagnosed with cancer (in the negative group, n = 9; in the constitutional thrombophilia group, n = 2; in the aPLAb group, n = 8) and 11 (2.1%) aPLAb patients developed systemic lupus erythematosus. None of them developed any symptomatic thrombosis.

The number of DVT (total, proximal, and distal subtypes), PE, and SVT events was higher in aPLAb patients than in the other groups (Table 3). There were no cases of isolated PE, the 31 adjudicated events being associated with DVT. Women from the aPLAb group experienced more strokes and more TIA or stroke than did the women in the negative or constitutional thrombophilia groups. Among the 12 cases of stroke, all were ischemic and 8 had at least 1 probable cause: large artery atherosclerosis (n = 3); lacune (calling for small artery disease; n = 2); or cardioembolism (n = 3).

Comparisons between vascular outcomes are shown in Table 3. The upper limits of the annualized rates of venous events for women in the negative group were low: 0.22% for PE, 0.61% for DVT, 0.36% for proximal DVT, and 0.35% for distal DVT. Upper limits of the annualized rates of arterial events were 0.14% for TIA and 0.12% for stroke in the negative patients. The incidences of venous and arterial thrombotic events for the patients in the constitutional thrombophilia group were not...
The predictors of the various thrombotic events were evaluated in aPLAb patients (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). Proximal and distal DVTs were categorized into either provoked events (n = 9 and n = 16, respectively) or unprovoked events (n = 29 and n = 14, respectively). LA was a predictor of VTE and DVT events combined, unprovoked proximal DVT, unprovoked distal DVT, and SVT. After categorization into quartiles (Q1: 0.79-1.05; Q2: 1.051-1.42; Q3: 1.421-1.83; and Q4: > 1.83), the higher LA activity quartiles were correlated with higher risks of thrombotic events. Patients positive for aCL-M were significantly protected against PE, provoked proximal DVT, and provoked distal DVT. An F5 6025 polymorphism enhanced the risk of TIA. None of the putative predictors we studied appeared to be significant risk factors for stroke.

The mean incidence of triple positivity, which is a high-risk aPLAb profile in asymptomatic carriers, was 1.18% for VTE, 0.36% for PE, and 0.20% for stroke plus TIA. The computed risks were globally concordant with those of being LA positive only for most of the vascular events (supplemental Table 1). Although triple positivity was a predictor of PE, LA was not.

Follow-up safety data

Primary vascular prophylaxis with LDA in aPLAb patients did not induce any major bleeding events. Minor hemorrhages were limited to local minor bruising (mainly as a result of trauma), venipuncture-related bruising, and minor gum bleeding because of tooth brushing. Heavy menses, defined as a Janssen score17 higher than 185, was infrequent (n = 16). No major bleeding events occurred. Thrombophilia testing was discontinued during pregnancy.

Discussion

Our observational results in patients with pregnancy loss show that the risks of venous thromboembolism and of cerebrovascular
manifestations are higher in women with purely obstetric APS than in women without APS. These risks are not higher in women carrying the $F_5$ 6025 or $F_2$ rs1799963 polymorphism, 2 prevalent low-risk constitutive thrombophilias. These elevated risk levels were apparent despite the use of LDA primary vascular prophylaxis in APS women.

Purely obstetric APS patients who experienced a new pregnancy during follow-up were treated using the combination of LDA and LMWH for obstetrical purposes according to expert recommendations, even if the option of monotherapy with LDA has not been definitively ruled out by others. The addition of LMWH may have contributed to the limitation of vascular events during pregnancy. Patients also received a chronic LDA-mediated primary prophylaxis against thrombosis, which reflects to a great extent clinical practice, but which is not evidence-based medicine, pointing to crucial lacks in that clinical setting.

Before the present study, few data were available on the risk of VTE in women with purely obstetric APS. Management currently depends on expert opinion and on the perception of the physician. Limited retrospective studies have detected either a higher or a slightly elevated frequency of thrombotic events after pregnancy loss in APS. The adjusted rate of VTE events was almost twice as high in women with APS compared with patients without APS. Furthermore, this rate was close to the one observed in asymptomatic aPL-positive subjects receiving LDA primary prophylaxis (2.7%). Our data from patients receiving primary prophylaxis with LDA suggest that APS is an important predictor of subsequent VTE. Further evaluations are needed to determine an evidence-based mode of prevention.

We found the overall cerebrovascular risk to be nearly doubled in APS women compared with those without APS, but observed few events. A population-based study found relative odds of stroke that were similar to those found in our study, despite the absence of prophylactic antiplatelet treatments. The RATIO study found strikingly higher risks in aPL-positive women, particularly for LA and ischemic stroke (odds ratio = 43). In comparison, we observed very few stroke cases, but aPL-positive patients had a primary prophylaxis that might have modified the spontaneous arterial risks.

Among all aPLAbs, LA was the unique risk factor independent of other aPLAbs for various clinical subtypes of the venous thrombotic disease, including proximal unprovoked DVT, distal unprovoked DVT, and SVT. We also found a dose-effect relationship between LA intensity and clinical outcome. LA is considered to be the most powerful predictor of thrombosis. Strong risk factors for thrombosis are generally associated with spontaneous, rather than provoked, thrombotic events, which fits with the Rosendaal thrombosis potential model. Being aCL-M positive lowered the risk of provoked proximal and distal DVTs. ACL-M-positive status was associated with late pregnancy losses between 13 and 24 weeks in a meta-analysis study, but there are no strong data linking aCL-M to vascular morbidity. The discrepancy between aCL-M-positive status and the risk for the 2 APS main clinical areas may explain this protective effect of aCL-M: the
absence of a switch from IgM synthesis to IgG synthesis might prevent more pathogenic Abs from being produced.

Triple aPLAb positivity in subjects positive for LA, aCL, and aβ2GP1 Abs has been described as a high-risk aPLAb profile in asymptomatic carriers.16 The mean incidence of venous and arterial (mainly arterial) events for these patients may be as high as 5.3% per year, and male sex is a strong indicator. Our female recruitment and LDA prophylaxis may explain the differences in these results. In some univariate analyses, triple positivity, like LA, was found to be a risk factor. However, high LA activities were associated with higher risks after multivariate analysis. Finally, a significant amount of triple positivities were associated with positive aCL-M, in which thrombotic impact is questionable.

Our study has several limitations. This is not a multicentric study. Our results apply only to patients referred by primary care physicians to the hematologists, gynecologists, and obstetricians of a tertiary referral center. A significant number of patients may have been available for investigation through a purely private medical sector. Because LA has been strongly associated with recurrent miscarriage before the 24th week of gestation in women without autoimmune disease,29 this might have also induced a recruitment bias if LA-positive patients were favored among our purely obstetric APS women. The absence of an association between early miscarriages and aβ2GP1 Abs may explain the low frequency of patients positive for aβ2GP1 Abs in our population of women and the absence of association between aβ2GP1 Abs and subsequent thrombotic events. Most of the lost embryos were not submitted to karyotype analysis; a significant number of recurrent early losses may have been because of embryonic chromosomal abnormalities. Nevertheless, embryonic karyotyping is not mandatory for APS clinical criteria.2 We also did not check any biologic parameter of poor responsiveness to LDA in APS women, and therapeutic compliance was assessed only through self-reporting. We did not look for asymptomatic venous thrombosis systematically and did not evaluate patients for the presence of atherosclerotic lesions using, for example, ultrasonographic methods.

Another limitation of our study is that only aPLAbs patterns at randomization were used for analysis. We observed some variations in the aPLAbs patterns during follow-up, but women whose aPLAbs all became negative were censored at the time of last positive assessment; therefore, a bias in the global evaluation of the thrombotic risks in case of aPLAb positivity is unlikely. We did not determine aPLAbs during follow-up in women with initially negative aPLAbs, and it may be that some of them subsequently revealed aPLAbs, which may limit our results. The variations of the aPLAb patterns may have some impact on the evaluation of the thrombotic risk predictors in aPLAb-positive women. However, there is currently no available integrated system allowing evaluating the risks according to the continuous variations of the 5 APS markers during follow-up. We have initiated new algorithm and statistical developments, hoping for adapted solutions, but these will have to be validated.

Our study also has several strengths. The annualized rates of VTE events in our negative/nonthrombophilic group were similar to those reported previously.6 The rate of stroke in this group also overlaps the overall incidence rate described for subjects under the age of 45.30,31 A cohort study showed that women who experience recurrent spontaneous pregnancy loss or stillbirth are not at higher risk of stroke later in life.32 We were able to rely heavily on the Nîmes Obstetricians and Haematologist (NOHA) administrative region hospital medical network.33 This network was able to recruit a substantial number of patients, particularly purely obstetric APS women. Follow-up lengths tended to be long for these patients and losses to follow-up were very limited because of the efficiency of the NOHA human network. We therefore believe that the number of symptomatic vascular events that occurred in patients and that were not registered into the database were minimal or absent.

The results of studies in mouse models suggest that obstetric APS may be a nonthrombogenic syndrome,34-37 but mechanisms of pregnancy loss involving thrombosis have been described recently36-38 and this agrees with our observational results, at least in a subset of patients. LDA-mediated primary prophylaxis was well tolerated in our purely obstetric APS patients, which might have limited the numbers of subsequent thrombotic events. Multicentric prospective, randomized, controlled studies are warranted to determine the efficacy of this prophylaxis in this patient population.

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Authorship

Contribution: J.-C.G, J.-P.B., P.M., J.-P.G., I.Q., and M.D. designed the research and wrote the manuscript; N.M. and P.-F.P. performed the statistical analysis and wrote the manuscript; J.-C.G., J.-P.G., J.-P.B., P.M., and I.Q. performed the research; and S.B., E.C.-N., and E.M. contributed analytical tools and wrote the manuscript.

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Comparative incidence of a first thrombotic event in purely obstetric antiphospholipid syndrome with pregnancy loss: the NOH-APS observational study

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