THROMBOSIS AND HEMOSTASIS

Comparative incidence of pregnancy outcomes in thrombophilia-positive women from the NOH-APS observational study

Sylvie Bouvier,[1-3] Éva Cochery-Nouvellon,[1,2] Géraldine Lavigne-Lissalde,[1,2] Érick Mercier,[1-3] Pascale Fabbro-Peray,[4] Jean-Pierre Balducchi,[5] Pierre Marès,[6] and Jean-Christophe Gris[1-3]

[1]Department of Hematology, University Hospital, Nîmes, France; [2]Research team EA2992 "Dysfonction des Interfaces Vasculaires," University of Montpellier 1, Nîmes, France; [3]Laboratory of Hematology, Faculty of Pharmacy and Biological Sciences, Montpellier 1 University, Montpellier, France; and [4]Department of Biostatistics, Epidemiology and Medical Information, [5]Department of Internal Medicine, and [6]Department of Gynecology and Obstetrics, University Hospital, Nîmes, France

Key Points

- Fetal death is more frequent in women with prior abortions carrying *F5* rs6025 or *F2* rs1799963 polymorphisms vs nonthrombophilic women.
- Pregnancy complications are less frequent in LMWH-treated thrombophilic women with fetal loss vs untreated nonthrombophilic women.

The incidence of pregnancy outcomes in women with constitutive thrombophilia is uncertain. We observed women with no history of thrombotic events (nonthrombotic), 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 complications during a new pregnancy attempt among women carrying the *F5* rs6025 or *F2* rs1799963 polymorphism (n = 279; low-molecular-weight heparin [LMWH] treatment during pregnancy only in case of prior fetal death), and women with negative thrombophilia screening results as control women (n = 796; no treatment). Among women with prior recurrent abortions, thrombophilic women were at increased risk for fetal death. Among women with prior fetal death, thrombophilic women experienced less fetal death recurrences, less preterm births and preeclampsia, and more live births as they were treated with LMWH. In nonthrombotic *F5* rs6025 or *F2* rs1799963 heterozygous women with prior pregnancy loss, fetal loss may indicate a clinical subgroup in which future therapeutic randomized controlled trials testing the effect of LMWH prophylaxis are required in priority. (*Blood*. 2014;123(3):414-421)

Introduction

Pregnancy outcomes in symptomatic women carrying the *F5* rs6025 (factor V Leiden) or *F2* rs1799963 (prothrombin gene G20210A) polymorphism are still debated.

The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study systematically reviewed selected case-control studies to determine the clinical risks associated with thrombophilia in pregnancy.[1] Heterozygous *F5* rs6025 and *F2* rs1799963 polymorphisms were associated with early pregnancy loss in the first and second trimester (factor V Leiden: 172/243 [70.8%] vs 1632/2689 [60.7%], mean odds ratio [OR], 1.68; prothrombin gene polymorphism: 53/75 [60.3%] vs 657/1356 [48.5%], mean OR, 2.49), with late loss in the third trimester (factor V Leiden: 27/382 [7.1%] vs 124/1121 [11.1%], mean OR, 2.06; prothrombin gene polymorphism: 5/7 [71.4%] vs 35/113 [30.9%], mean OR, 2.66) and with preeclampsia (PE; factor V Leiden: 161/249 [64.7%] vs 1790/3673 [48.7%], mean OR, 2.19; prothrombin gene polymorphism: 42/71 [59.1%] vs 937/2028 [46.2%], mean OR, 2.54). Despite the increase in relative risk (RR), the absolute risk for adverse outcomes in pregnancy remained low. The subsequent meta-analysis of 10 prospective cohort studies only showed that women bearing the *F5* rs6025 polymorphism were at a small absolute increased risk for late pregnancy loss (from 511/16 158 [3.2%] in women testing negative for the polymorphism to 34/801 [4.2%] in women testing positive for the polymorphism, mean OR, 1.52). These results could not be further restricted to women with previous pregnancy complications and according to the clinical subtype of previous pregnancy losses.

The Nîmes Obstetricians and Hematologists Antiphospholipid Syndrome (NOH-APS) study observed a cohort of nonthrombotic women with previous pregnancy loss, recurrent unexplained spontaneous abortions, or a single fetal death, focusing on patients with APS.[3] The 2 included control groups, the first group consisting of women with a negative thrombophilia screening result and the other group consisting of those with a positive *F5* rs6025 or positive *F2* rs1799963 polymorphism, gave us the opportunity to evaluate the heterogeneity of new pregnancy outcomes in thrombophilic women with prior abnormal pregnancies, according to the heterogeneity of their clinical history.

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There is an Inside *Blood* commentary on this article in this issue.

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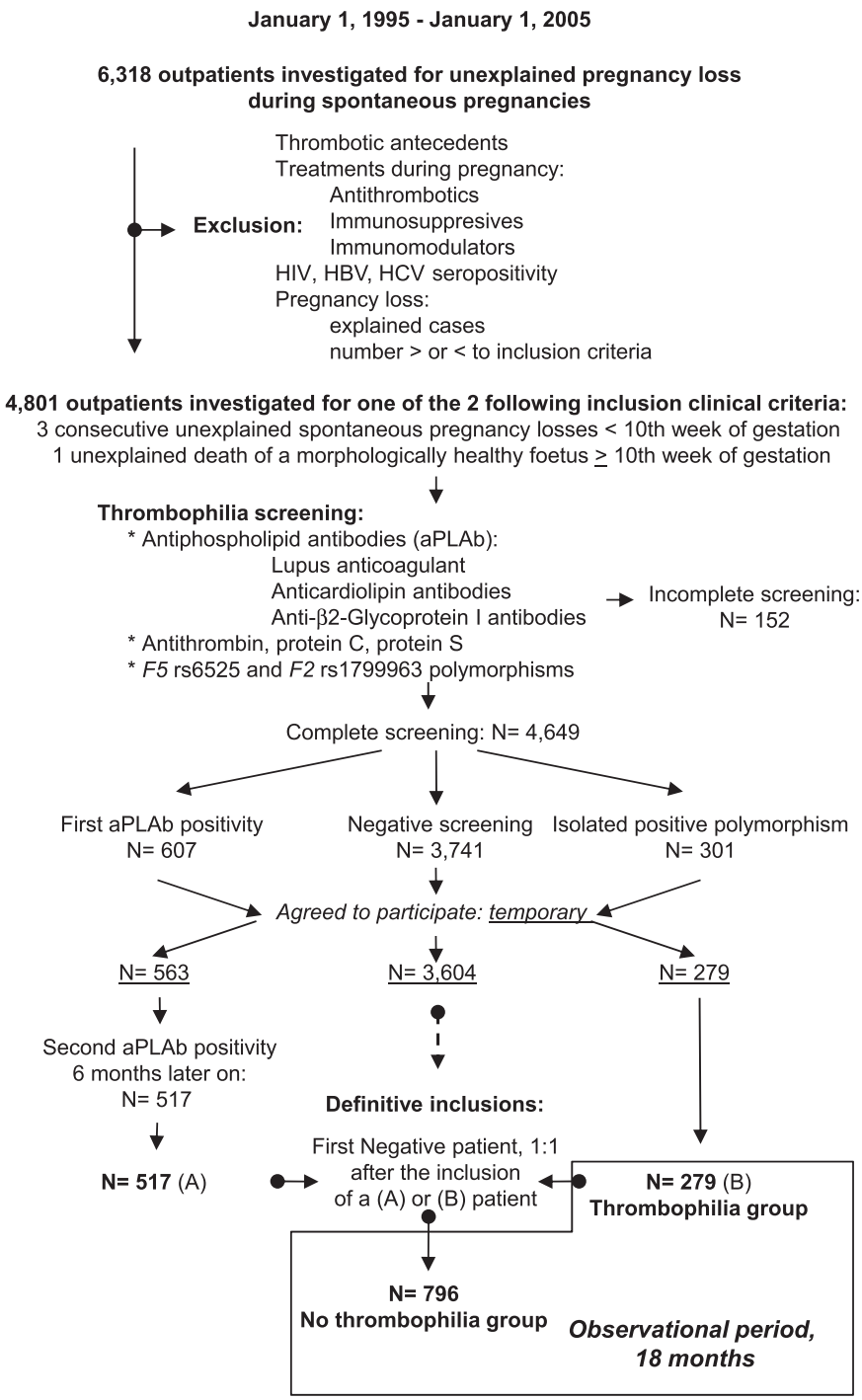

Figure 1. Patient recruitment diagram. The 2 groups of women with recurrent abortions or 1 fetal death: women with the *F5* rs6025 or *F2* rs1799963 polymorphism (Thrombophilia group), and women with negative antiphospholipid antibodies and a negative thrombophilia screening (No thrombophilia group). HBV, hepatitis B virus; HCV, hepatitis C virus.

Patients and methods

Patients

Recruitment of patients is presented in Figure 1 and has been described in detail elsewhere.[3] Briefly, 6318 women with pregnancy loss during spontaneous pregnancies were referred, during a 10-year period, for investigation to the Outpatient Department of Hematology, University Hospital of Nîmes (a tertiary referral center). Exclusion criteria were any history of thrombotic events (at least 1 clinical episode of venous, arterial, or small-vessel thrombosis, in any tissue or organ except the placenta, thrombosis being confirmed by objective validated criteria, ie, unambiguous findings by appropriate imaging studies or histopathology studies), or any treatment given during previous pregnancies that may have modified the natural evolution of their condition, such as antithrombotics, or immunosuppressive or immunity-modulating drugs. Women with pregnancy losses explained by infectious, metabolic, anatomic, or hormonal factors were also excluded. Women with seropositive results for HIV or hepatitis B or C were also excluded. A total of 4801 women fulfilled one of the 2 following inclusion criteria: (1) 3 unexplained consecutive spontaneous abortions before the 10th week of gestation, not caused by maternal anatomic or hormonal abnormalities or by paternal and maternal chromosomal causes (recurrent embryo loss subgroup); or (2) 1 unexplained death of a morphologically normal fetus (fetal loss) at or after the 10th week of gestation, with normal fetal morphology having been documented by ultrasound scan or direct examination of the fetus

(fetal loss subgroup). Patients were categorized as primary aborters (no previous successful pregnancy) or secondary aborters.

The women underwent standardized thrombophilia screening at 3 to 6 months after the last pregnancy loss; screening included a complete whole blood count, and tests for fibrinogen, antithrombin, protein C, protein S, polymorphisms *F5* rs6025 (factor V Leiden polymorphism) and *F2* rs1799963 (prothrombin gene G20210A polymorphism), the *JAK2* V617F mutation, and antiphospholipid antibodies (aPLAbs).

The 152 women with an incomplete thrombophilia screening were not included. 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 completely negative thrombophilia screening results (N = 3741) and who agreed to participate in the observational study (N = 3604) were provisionally assigned to the "negative" group and were numbered according to the order in which they entered the study. Women with an isolated *F5* rs6025 polymorphism or isolated *F2* rs1799963 polymorphism (N = 301) and who agreed to participate in the observational study (N = 279) constituted the "thrombophilia" group.

Women who initially tested positive for aPLAbs, with or without the *F5* rs6025 or *F2* rs1799963 polymorphism, who agreed to participate in the observational study and who persistently tested positive for aPLAbs at the 6-month test (N = 517) were definitively assigned to the "APS" group. As each APS or thrombophilia woman entered the study, the next woman on the list of the negative group was definitively assigned to the "no-thrombophilia" group and was included in the study.

We do not discuss APS women in our present work. In total, 796 women in the no-thrombophilia group and 279 women in the thrombophilia group were studied (Table 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 in accordance with the Helsinki declaration of 1975 as revised in 1996. All of the women gave their informed consent to participate in this study.

Table 1. Clinical characteristics of the women at baseline and the characteristics at follow-up

	No-thrombophilia	Thrombophilia	*P*
N	796	279	
Age, y	30 (5) [17-44]	29 (4) [18-44]	.19
>35 y, n (%)	43 (5.4)	12 (4.3)	.47
Body mass index, kg/m^2 (%)	25.6 (4.5) [15.3-36.1]	25.9 (4.2) [13.5-34.1]	.19
>30 kg/m^2 (%)	78 (9.8)	29 (10.4)	.78
<18.5 kg/m^2 (%)	12 (1.5)	3 (1.1)	.82
Ethnicity (%)			.89
Whites	753 (94.6)	264 (94.6)	
Europeans	647 (81.3)	227 (81.4)	
North Africans	106 (13.3)	37 (13.2)	
Black Africans	36 (4.5)	12 (4.3)	
Asians	7 (0.9)	3 (1.1)	
PL subtypes (%)			
Embryonic PL <10 WG	483 (60.7)	93 (33.3)	<.0001
Fetal PL >10 WG	313 (39.3)	186 (66.6)	
Primary PL	549 (68.9)	185 (66.3)	.41
Secondary PL	247 (31.1)	94 (33.7)	
Inflammatory disease (%)	7 (0.9)	4 (1.4)	.66
Current tobacco users (%)	83 (10.4)	30 (10.8)	.88
Hypertension (%)	19 (2.4)	8 (2.9)	.83
Positive history in a first-degree relative (%)			
Venous thromboembolism	15 (1.9)	29 (10.4)	<.0001
Atherothrombosis	96 (12.1)	46 (16.5)	.062
Recurrent abortions	49 (6.2)	18 (6.5)	.86
Fetal death	33 (4.1)	26 (9.3)	.0011
Hyperlipidemia (%)			
hypercholesterolemia	42 (5.3)	13 (4.7)	.69
hypertriglyceridemia	34 (4.3)	11 (3.9)	.81
Interval between the inclusion and the new pregnancy attempt			
Duration, days (%)	156 (29) [95-386]	148 (31) [98-369]	.43

No-thrombophilia indicates women with a negative thrombophilia screening result. Thrombophilia indicates women testing positive for a *F5* rs6025 or *F2* rs1799963 polymorphism. Quantitative data are given as median (interquartile range) [range] values, qualitative data as number (percentage) values.

PL, pregnancy loss.

Methods

Follow-up. All patients were informed at the start of the study about the advisability of early blood testing in case of suspicion of a new pregnancy; they were then observed until 18 months after the final recruitments and any new pregnancy noted. Women with any positive pregnancy test result were instructed to contact their general practitioner for evaluation and, after discussion with a study physician, the possible initiation of prophylaxis.

Patients were clinically reevaluated each year in our outpatient department. Patient loss to follow-up was minimized by directly contacting the general practitioners and the patients themselves. A complete clinical check-up was performed. Any suspicion of developing systemic disease led to further adapted investigations for diagnosis. Symptoms were evaluated, and treatments received during the year were recorded. There was no systematic aPLAbs assessment if a new pregnancy occurred.

Primary prophylaxis of thrombosis. Women in the thrombophilia and no-thrombophilia groups were not taking any chronic prophylaxis for thrombosis.

Antithrombotics during new pregnancies. Low-molecular-weight heparin (LMWH; enoxaparin, 40 mg/day ie, 4000 U/day) was given to all patients from the thrombophilia group with any fetal loss antecedent,[4] from the positive pregnancy test to delivery, whereas thrombophilic women with previous recurrent abortions received no antithrombotic treatments. Compliance with the LMWH treatment was monitored only by self-declaration by the patient-companion couples and systematic examination of subcutaneous injection sites at each medical examination. Monitoring and investigation for heparin-induced thrombocytopenia was performed according to published recommendations.[5] Enoxaparin injections were stopped at the onset of uterine contractions indicating imminent delivery. Patients of the no-thrombophilia group had no drug-mediated prophylaxis during pregnancy.

Outcome assessment. Events occurring during the first pregnancy during the observational period were systematically reviewed.

Thrombotic events were objectively confirmed as described elsewhere.[3]

Fetal death was defined as the intrauterine demise of a conceptus known to be alive at or beyond 10 completed weeks of gestation (WG) documented by (1) detection of fetal heart tones, (2) ultrasonic detection of a conceptus with biometric measurements indicating a gestational age at or beyond 10 WG, or (3) delivery of a dead conceptus whose size indicated a gestational age at or beyond 10 WG. All pregnancy losses before 10 WG were considered spontaneous abortions of either an anembryonic or embryonic nature.

Women were classified as having hypertension if they were using hypertensive medication or had either a systolic blood pressure of ≥140 mm Hg or a diastolic blood pressure of ≥90 mm Hg, both on 2 readings taken with the patient in a supine position 5 minutes apart, and on 2 separate occasions. PE was defined as diastolic blood pressure increasing to >90 mm Hg, or systolic blood pressure increasing to >140 mm Hg on 2 occasions at least 4 hours (h) apart after 20 WG, accompanied by a significant proteinuria (>0.3 g in a 24-h urine sample). Women with preexisting hypertension were scored as having PE if there was a new onset of proteinuria. PE was classified as severe if 1 or more of the following was observed: severe hypertension (diastolic >110 mm Hg or systolic >160 mm Hg), eclampsia (seizures), pulmonary edema, proteinuria >5g per 24 h, renal insufficiency as revealed by abnormal

creatininemia levels, abnormally high liver enzyme levels (aspartate aminotransferase or alanine aminotransferase levels >70 IU/L) with abdominal pain, or low platelet counts (<100 G/L). HELLP syndrome was defined according to the criteria established at the University of Tennessee: hemolysis on peripheral blood smear (schistocytes) with serum lactate dehydrogenase levels >600 IU/L, serum aspartate aminotransferase levels >70 IU/L, and platelet count <100 000/μL. PE with onset before 34 WG was defined as early-onset PE.

Biometry-based gestational age was assessed according to charts derived from published data,[6,7] and newborn weight percentiles were taken from standard French birth weight charts. Babies with birth weights lower than the corresponding gestational age- and sex-adjusted, customized 10th percentile were scored as small-for-gestational age (SGA). Severe SGA was defined as weight below the fifth percentile, very severe SGA as below the third percentile.

Placental abruption (PA) was defined according to classic clinical prenatal signs and symptoms: vaginal bleeding accompanied by nonreassuring fetal status or uterine hypertonicity, or visualization of abruption by ultrasound scanning, and evidence of retroplacental clots during examination of the delivered placenta. Cases were confirmed by histopathological diagnosis.

Neonatal mortality (NM) was defined as death before age 28 days; death before age 8 days was recorded as early neonatal mortality and death after age 8 days as late neonatal mortality.

Major bleeding events were classified according to the definitions of the Subcommittee on Control of Anticoagulation of the International Society on Thrombosis and Haemostasis[8]: fatal bleeding; and/or symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular, or pericardial bleeding; intramuscular bleeding with compartment syndrome; and/or bleeding that led to the hemoglobin concentration decreasing to 20 g/L or lower, or that led to transfusion of 2 or more units of red blood cells.

All events were adjudicated by a committee of independent experts blinded to the thrombophilia screening results.

Statistical analysis. Quantitative data were described by median, interquartile range, and range values. Qualitative data were described by values and percentages. Comparisons between baseline characteristics and risk factors were performed using the Student *t* test, the Mann-Whitney test, the Kruskal-Wallis test, the χ^2 test, and the Fisher exact test when appropriate.

Definition of groups and analysis of the impact of biological covariates on pregnancy outcome incidences were based on values obtained at the initial inclusion.

All analyses were based on pregnancy outcomes that occurred during the first pregnancy attempt, treated as described above, after initial investigations and recruitment.

We compared the pregnancy outcomes between groups estimating a RR using the Mantel-Haenszel method, with a 95% confidence interval (CI), providing a *P* value from the associated Mantel-Haenszel χ^2 test.

Putative predictors of various outcomes among the clinical predictors (age, body mass index, thrombotic familial antecedents, ethnicity, smoking history, varicose veins, preexisting hypertension, embryonic/fetal pregnancy loss, primary/secondary pregnancy loss, and initial inflammatory disease), the metabolic markers at inclusion (hypercholesterolemia defined as a fasting cholesterol concentration >5.2 mmol/L and hypertriglyceridemia as a fasting triglyceride concentration >1.7 mmol/L) and the biological predictors (positive *F5* rs6025 or *F2* rs1799963 polymorphism) were evaluated in women from the Constitutional Thrombophilia group, first by univariate analysis then by multivariate analysis. For multivariate models, a stepwise variable selection was performed, starting with all of the variables from the univariate models having $P < .20$ as potential predictors, with adjustment being finally performed for all variables with $P < .20$ in the univariate models. The final model included only main effects with $P < .10$.

All tests were 2 sided and were assessed at the 5% significance level.

Statistical analyses were performed using SAS-Windows software (version 9.1).

Results

Baseline patient characteristics

This study included 1075 nonthrombotic women with unexplained pregnancy loss (Table 1), the dominant inclusion criterion being recurrent unexplained embryonic pregnancy loss in the no-thrombophilia group but fetal loss in the thrombophilia group. The heterozygous *F5* rs6025 polymorphism was the main thrombophilia (n = 176, 63%), and the heterozygous *F2* rs1799963 polymorphism was evident in 103 women (37%). There were no homozygote women and 11 double-heterozygote women (3.9%). Fetal death or venous thrombotic histories were also more frequent in the first-degree relatives of women included in the Constitutional Thrombophilia group. The interval between the inclusion and the observed new pregnancy attempt was not different between the groups. A globally normal fecundity rate was evidenced, nearly all included women (1069/1075) initiating a new pregnancy attempt during the observational period.

New pregnancy outcomes in women with prior recurrent abortions

Almost all women had a new positive pregnancy test result after inclusion, with no difference between thrombophilic and nonthrombophilic women (Table 2). None of the women in this subgroup received any antithrombotics during pregnancy.

Thrombophilic women demonstrated similar spontaneous abortion recurrence rates as nonthrombophilic women (13/93 [14%] vs 92/480 [19.2%], respectively), but as pregnancies evolved, higher rates of fetal death were observed (7/80 [8.8%] vs 9/388 [2.3%], respectively; $P = .0063$). A nonsignificant trend for higher PE rates (5/75 [6.6%] vs 11/384 [2.9%], respectively) and early-onset PE rates (3/75 [4%] vs 4/384 [1%], respectively), higher preterm live birth rates (12/73 [16.4%] vs 35/379 [9.2%], respectively), and higher early neonatal mortality rates (2/73 [2.7%] vs 2/379 [0.5%], respectively) was also evidenced in thrombophilic women.

New pregnancy outcomes in women with prior fetal death

A new pregnancy could be diagnosed after inclusion in almost all women (Table 3).

Treated thrombophilic women in this subgroup experienced fewer fetal deaths than untreated nonthrombophilic women (19/185 [10.3%] vs 72/311 [23.2%], respectively; $P = .0007$). Consequently, they more frequently gave birth to living neonates (130/185 [70.3%] vs 156/311 [50.2%], respectively; $P < .0001$), with fewer cases of preterm living births before 37 weeks (16/130 [12.3%] vs 39/156 [25%], respectively; $P = .0106$).

Whereas the comparison of the rates of all PE cases between thrombophilic and nonthrombophilic women did not reach statistical significance, treated thrombophilic women experienced less cases of severe PE (3/139 [2.2%] vs 15/180 [8.3%], respectively; $P = .0242$) and less cases of early-onset PE before 34 weeks (1/139 [0.7%] vs 11/180 [6.1%], respectively; $P = .0394$) than untreated nonthrombophilic women. Finally, we observed a nonsignificant trend toward lower rates of SGA babies (19/139 [13.7%] vs 37/180 [20.6%], respectively; $P = .115$) and placenta-mediated complications as a whole (26/139 [18.7%] vs 50/189 [27.8%], respectively; $P = .064$) in these LMWH-treated thrombophilic women compared with the non-LMWH–treated, nonthrombophilic women.

Table 2. Outcomes of new pregnancies in women from the thrombophilia group and no-thrombophilia group with prior recurrent abortions

	Thrombophilia, 93		No-thrombophilia, 483			
Group, N	**n/N**	**Incidence**	**n/N**	**Incidence**	**RR (95% CI)***	***P***
New pregnancies	93/93	1.000	480/483	0.994		
Prophylactic LMWH treatment during pregnancy	0/93	0	0/480	0		
Spontaneous abortions before 10 WG	13/93	0.140	92/480	0.192	0.734 (0.429-1.255)	.2584
Ongoing pregnancies at 10 WG	80/93	0.860	388/480	0.808	1.063 (0,969-1.1656)	.1988
Fetal deaths (% of new pregnancies)	7/93	0.075	9/480	0.019	4.039 (1.543-10.58)	.0045
Fetal deaths (% of ongoing pregnancies at 10 WG)	7/80	0.088	9/388	0.023	3.801 (1.458-9.908)	.0063
Ongoing pregnancy at 20 WG (% of new pregnancies)	75/93	0.806	384/480	0.800	1.007 (0.903-1.122)	.9073
Live births (% of new pregnancies)	73/93	0.774	379/480	0.790	1.027 (0.912-1.156)	.6639
Live births (% of ongoing pregnancies at 10 WG)	73/80	0.913	379/388	0.977	0.934 [0.871-1.001)	.0543
Preterm live births <37 WG (% of live births)	12/73	0.164	35/379	0.092	1.794 (0.979-3.289)	.0587
Preterm live births <34 WG (% of live births)	5/73	0.068	13/379	0.034	2.013 (0.740-5.474)	.1706
PE (% of ongoing pregnancies at 20 WG)	5/75	0.066	11/384	0.029	2.346 (0.839-6.555)	.1040
Severe PE	2/75	0.027	6/384	0.016	1.720 (0.354-8.360)	.5014
PE <34 WG	3/75	0.040	4/384	0.010	3.870 (0.884-16.94)	.0724
Eclampsia	1/75	0.013	3/384	0.008	1.720 (0.181-16.31)	.6366
HELLP syndrome	1/75	0.013	2/384	0.005	2.580 (0.237-28.09)	.4366
SGA (% of ongoing pregnancies at 20 WG)	11/75	0.147	43/384	0.112	1.320 (0.714-2.440)	.3758
Severe SGA <p5	6/75	0.080	21/384	0.055	1.474 (0.616-3.529)	.3835
Very severe SGA <p3	4/75	0.053	13/384	0.034	1.588 (0.532-4.737)	.4071
PE+SGA	3/75	0.040	7/384	0.018	2.211 (0.585-8.359)	.2421
PA (% of ongoing pregnancies at 20 WG)	2/75	0.027	5/384	0.013	2.064 (0.408-10.44)	.3810
Placenta-mediated complications: PE and/or SGA and/or PA (% of ongoing pregnancies at 20 WG)	14/75	0.187	49/384	0.128	1.474 (0.859-2.530)	.1589
NM (% of live births)	2/73	0.027	4/379	0.011	2.618 (0.488-14.02)	.2615
Early NM, day 1-d 7	2/73	0.027	2/379	0.005	5.233 (0.749-36.56)	.0952
Late NM, day 8-d 27	0/73	0	2/379	0.005	1.0351 (0.050-21.34)	.9822

*Thrombophilic vs nonthrombophilic women.

Safety outcomes in the antithrombotic-treated pregnant women

Prophylactic LMWH treatments were given to 185 thrombophilic pregnant women. No treatment had to be stopped for safety reasons; we observed no major bleeding events.[8] There were no cases of heparin-induced thrombocytopenia, symptomatic osteoporosis, or thrombosis. Patients who received epidural or spinal analgesia or anesthesia experienced no complications, with no case of epidural hematoma or hemorrhagic or neurologic complications.

Three patients (1.6%) experienced allergic skin reactions after enoxaparin injections, and these symptoms resolved after switching for another prophylactic LMWH. Easy bruising, at least at the injection site, was frequent (n = 97 [52.4%]), and sometimes also in cutaneous friction or contact zones (n = 39 [21.1%]).

Primary postpartum hemorrhage occurred in 15 (8.1%) of the 185 treated patients and in 51 (6.4%) of the 796 nonthrombophilic women ($P = .41$); severe primary postpartum hemorrhage with an estimated blood loss of >1000 mL occurred in 3 treated patients (1.6%) and in 10 nonthrombophilic women (1.3%, $P = .97$).

Predictors in the thrombophilia group

Heterogeneous therapeutic treatment of the thrombophilic patients during their new pregnancy led us to evaluate the putative predictors of various pregnancy outcomes: first, in cases of previous recurrent abortions; second, in cases of previous fetal loss. None of the predictors reached statistical significance for any outcome after univariate analyses was performed in each subgroup defined by its inclusion criterion. *P* values systematically exceeded .20 in the recurrent abortion group. *P* values between .10 and .20 were rarely observed in the fetal death group: they remained higher than .10 when a multivariate analysis was possible (data not shown).

Discussion

Our observational study of nonthrombotic *F5* rs6025 or *F2* rs1799963 heterozygous patients with pregnancy loss shows heterogeneous results.

Thrombophilic patients with prior recurrent abortions, who did not receive any antithrombotic treatment during their new pregnancy attempt, had an increased risk for fetal death comparatively to untreated thrombophilia-negative women sharing the same obstetric history.

Thrombophilic patients with prior fetal death, who received LMWH treatment (prophylactic dosage) during their new pregnancy attempt, were at lower risk for the development of fetal death recurrence, premature live birth, PE, and early-onset PE. They more frequently gave birth to a living neonate than untreated thrombophilia-negative women sharing the same obstetric history.

These observational results must be interpreted with caution. The absence of academic control groups (untreated thrombophilic women with prior fetal loss and treated nonthrombophilic women with prior fetal loss) cannot lead to definitive conclusions. LMWH treatments may have had no effect or even some deleterious effects if thrombophilias are protective factors against bad pregnancy

Table 3. Outcomes of new pregnancy attempts in women from the thrombophilia group and no-thrombophilia group with prior fetal death

	Thrombophilia, 186		No-thrombophilia, 313			
Group, N	**n/N**	**Incidence**	**n/N**	**Incidence**	**RR (95% CI)***	***P***
New pregnancies	185/186	0.995	311/313	0.994		
Prophylactic LMWH treatment during pregnancy	185/185	1	0/311	0		
Spontaneous abortions before 10 WG	36/185	0.195	83/311	0.267	0.750 (0.533-1.056)	.0998
Ongoing pregnancies at 10 WG	149/185	0.805	228/311	0.740	1,090 (0.989-1.202)	.0835
Fetal deaths (% of new pregnancies)	19/185	0.103	72/311	0.232	0.444 (0.277-0.712)	.0007
Fetal deaths (% of ongoing pregnancies at 10 WG)	19/149	0.128	72/228	0.313	0.407 (0.257-0.646)	.0001
Ongoing pregnancy at 20 WG (% of new pregnancies)	139/185	0.751	180/311	0.579	1.299 (1.145-1.475)	.0001
Live births (% of new pregnancies	130/185	0.703	156/311	0.502	1.385 (1.198-1.600)	<.0001
Live births (% of ongoing pregnancies at 10 WG)	130/149	0.872	156/228	0.684	1.270 (1.142-1.413)	<.0001
Preterm live births <37 WG (% of live births)	16/130	0.123	39/156	0.250	0.499 (0.292-0.850)	.0106
Preterm live births <34 WG (% of live births)	7/130	0.054	19/156	0.122	0.448 (0.194-1.032)	.0593
PE (% of ongoing pregnancies at 20 WG)	12/139	0.086	21/180	0.117	0.740 (0.377-1.452)	.381
Severe PE	3/139	0.022	15/180	0.083	0.246 (0.073-0.833)	.0242
PE <34 WG	1/139	0.007	11/180	0.061	0.118 (0.015-0.901)	.0394
Eclampsia	1/139	0.007	5/180	0.028	0.259 (0.031-2.192)	.215
HELLP syndrome	1/139	0.007	4/180	0.022	0.324 (0.037-2.864)	.311
SGA (% of ongoing pregnancies at 20 WG)	19/139	0.137	37/180	0.206	0.665 (0.401-1.104)	.115
Severe SGA <p5	9/139	0.065	19/180	0.106	0.613 (0.286-1.314)	.209
Very severe SGA <p3	4/139	0.029	12/180	0.067	0.432 (0.142-1.309)	.138
PE+SGA	5/139	0.036	13/180	0.072	0.498 (0.182-1.364)	.175
PA (% of ongoing pregnancies at 20 WG)	2/139	0.014	6/180	0.033	0.432 (0.089-2.106)	.299
Placenta-mediated complications: PE and/or SGA and/or PA (% of ongoing pregnancies at 20 WG)	26/139	0.187	50/180	0.278	0.673 (0.443-1.024)	.064
NM (% of live births)	2/130	0.015	4/156	0.026	0.608 (0.113-3.265)	.562
Early NM, day 1-d 7	1/130	0.008	3/156	0.019	0.405 (0.043-3.849)	.432
Late NM, day 8-d 27	1/130	0.008	**1/156**	0.006	1.215 (0.077-19.24)	.889

*Thrombophilic vs nonthrombophilic women.

outcomes. However, this is not the described tendency,[1,2] and our thrombophilia-positive untreated patients with prior recurrent abortions experienced more fetal death cases than their thrombophilia-negative untreated control patients.

We studied previously symptomatic patients. We may have selected a group of women in whom thrombophilia is only one of the individual characteristics cooperating with a series of biological cofactors and mechanisms, yet unknown, altogether contributing to the risk for abnormal pregnancy outcomes. LMWH use in a secondary prophylaxis setting may be the main determinant of our results, which cannot apply to primary prophylaxis. The suspected interactions may per se explain most of the clinical effects that we observed. Experimental in vitro data show that LMWH may promote extravillous trophoblast development, being able to stimulate their invasive properties.[9,10] Series of placental bed biopsies from late, sporadic miscarriage with a normal karyotype showed reduced trophoblast invasion features,[11] which were negative in cases of early embryonic demise.[12] Heparin-binding epidermal growth factor–like growth factor triggers chemotaxis of endometrial stromal cells,[13] which may be modified by heparin. The classic complement pathway inhibition in pregnant women treated with heparin may also be relevant,[14] altered complement regulation being the rule in PE.[15] However, invasion of the decidua by extravillous trophoblasts is accompanied by thrombin generation from decidual cell–expressed tissue factor.[16] In thrombophilic women, excess thrombin formation may abnormally enhance expression of soluble fms-like tyrosine kinase-1,[16] a potent inhibitor of angiogenesis involved into PE, thus prone to be regulated by LMWH.

Our results in nonthrombophilic women show 79.6% (95% CI, 76%-83%) of new pregnancy attempts ending in live births in women with prior recurrent abortions, but only 50% (95% CI, 45%-56%) of these attempts leading to a living neonate in women with prior fetal death. In this latter group, 26.7% (95% CI, 22%-32%) and 23.2% (95% CI, 18%-28%) of new pregnancy attempts ended in abortion or fetal death, respectively. Our observations are on line with available results.[17-19] In a retrospective Dutch study on pregnancy after a first loss in women with the *F5* rs6025 or *F2* rs1799963 polymorphism, the live birth rates were 80% (95% CI, 49%-94%) in noncarriers after a late loss after 12 WG and 76% (95% CI, 57%-89%) after an early loss, thus overlapping our CIs despite a nonsimilar time frame–related dichotomization of events.[20] Thus, prior unexplained fetal loss defines a population of women with a poor natural prognosis in the subsequent pregnancy. Dichotomization of pregnancy losses according to the 10 WG threshold, as in APS clinical criteria,[21] should also be regarded as more systematic outside of any aPLAb context.

Our results in untreated women with the *F5* rs6025 or *F2* rs1799963 polymorphism and prior recurrent early losses, which show 78.5% (95% CI, 76%-83%) of new pregnancy attempts ending in live births, are also in agreement with published data in women with a first pregnancy loss before 12 WG describing a 77% success rate (95% CI, 62%-87%).[19]

Our data are globally in line with a recent multicentric observational Italian study suggesting that LMWH prophylaxis may reduce the risk for spontaneous miscarriages in *F5* rs6025 or *F2* rs1799963-positive women, particularly in those with previous obstetric events.[22]

They also, at least partly, resemble the results of a multicentric randomized controlled trial performed in women with inheritable thrombophilia and prior PE, mainly *F5* rs6025 or *F2* rs1799963 heterozygosity, which showed a strong effect of LMWH on the occurrence of early-onset PEs.[23]

Our study had some limitations. First, we included heterogeneous patients; this study was not a randomized controlled trial but only an observational study. Genome-wide scan in affected sibling pairs with miscarriages has suggested genetic linkage,[24] and such a genetic background may act as a strong confounder. Second, our study was not an academic multicenter study, even if several primary and secondary centers from our administrative area participated in the initial screening of patients. Some patients may have been treated independently from our network. Third, most of the lost embryos were not submitted for karyotype analysis, which may have explained some losses. Our study could not evaluate preclinical, very early losses. We probably did not always study the first pregnancy occurring during follow-up. Fourth, in treated patients, therapeutic compliance was assessed only through self-reporting.

Our study also had several strengths. We could rely on the Nîmes Obstetricians and Haemotologists administrative region-hospital medical network,[4] which could recruit a substantial number of patients.[3] A new pregnancy was the rule after inclusion, without any losses to follow-up, because of limited time between inclusion and new attempts. Pregnancy outcomes could be objectively diagnosed using international criteria or definitions. Therefore, we believe that the number of events that was not registered into the database was minimal.

Because of the lack of definitive randomized controlled trials giving strong conclusions, we understand the latest American College of Chest Physicians grade 2C conservative recommendation suggesting that women with inherited thrombophilia and a history of pregnancy complications not use antithrombotic prophylaxis.[25] However, observational evidence obtained in our current study and in other studies[22,23] may give some valuable clues and keys for a precise successful design of future randomized controlled trials, if any. Thrombophilic women with a history of fetal death, not abortions (and probably also late pregnancy complications) should probably be the most promising clinical target. Waiting for any substantial progress of precision medicine in the obstetric field may allow classification of pregnancy complications on causal molecular determinants, thus defining accurate groups of patients more prone to targeted therapeutic randomized controlled trials.

An initial miscarriage has been associated with a higher risk for obstetric complications in the next continuing pregnancy.[26] The *F5* rs6025 or *F2* rs1799963 polymorphism affects pregnancy outcomes.[1] Thrombophilic women with prior recurrent abortions experienced more fetal death than nonthrombophilic women. Thrombophilic women with prior fetal loss, receiving LMWH treatment during a new pregnancy attempt, experienced less pregnancy complications than untreated nonthrombophilic women.

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Authorship

Contribution: J.-C.G., J.-P.B., and P.M. designed the research and wrote the paper; P.F.-P. performed the statistical analysis and wrote the paper; S.B., J.-C.G., G.L.-L., J.-P.B., and P.M. performed the research and contributed to the analysis of the data; and S.B., E.C.-N., E.M., and G.L.-L. contributed analytical tools and wrote the paper.

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Correspondence: Jean-Christophe Gris, Laboratory of Hematology, University Hospital Caremeau, Place du Pr. Robert Debré, 30029 Nîmes cedex 9, France; e-mail: jean.christophe.gris@chu-nimes.fr.

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