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Marianna Pina Rambaldi, Federico Mecacci, Secondo Guaschino and Michael J. Paidas

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Inherited and Acquired Thrombophilias

Marianna Pina Rambaldi, MD[1], Federico Mecacci, MD[1], Secondo Guaschino, MD[1,2], and Michael J. Paidas, MD[3]

Abstract

Thrombophilias represent an evolving story that continues to stir controversy for care providers and obstetrical patients. The predominant thrombophilic mutations include the factor V Leiden mutation, prothrombin gene mutation G20210A, methylene tetrahydrafolate reductase C667T, and deficiencies of the natural anticoagulants proteins C and S, and antithrombin. Prospective cohort studies have provided an accurate assessment of the risk of placenta-mediated complications posed by common inherited thrombophilic conditions. Acquired thrombophilic conditions consist of the antiphospholipid antibody syndrome (APAS) and hyperhomocysteinemia. Well-conducted, placebo-controlled, randomized trials have demonstrated no benefit of anticoagulation in women with recurrent pregnancy loss and inherited thrombophilia. The routine use of anticoagulation to prevent other placenta-mediated complications in the setting of inherited thrombophilia should be considered experimental until the results of adequate clinical trials are available. Heparin anticoagulation and antiplatelet therapies are the cornerstone of treatment of APAS in pregnancy.

Keywords

thrombophilia, pregnancy, preeclampsia, adverse pregnancy outcome, fetal demise, abortion, intrauterine growth restriction, acquired thrombophilia, inherited thrombophilia, abruption

Hemostatic Changes in Pregnancy

Pregnancy is associated with significant elevations in a number of clotting factors. Fibrinogen concentration is doubled, factors VII, VIII, IX, X, and XII increase 20% to 1000%, and von Willebrand factor increases 20% to 1000%, with maximum levels reached at term.[1] Prothrombin and factor V levels remain unchanged, while the levels of factors XIII and XI decline modestly. The overall effect of these changes is to increase the thrombin-generating potential. Coagulation activation markers are elevated in normal pregnancy , as evidenced by increased thrombin activity, increased soluble fibrin levels (9.2-13.4 nmol/L), increased thrombin–antithrombin (TAT) complexes (3.1-7.1 μg/L), and increased levels of fibrin D-dimer (91-198 μg/L).[2] In all, 50% (11 of 22) of the women had elevated TAT levels, and 36% (9 of 25) of the women had elevated levels of D-dimers in the first trimester.

During pregnancy, there are significant changes in the natural anticoagulant and fibrinolytic systems. Protein S (PS) levels significantly decrease in normal pregnancy. Mean PS-free antigen levels have been reported to be 38.9% ± 10.3% and 31.2% ± 7.4% in the second and third trimesters, respectively.[3] The PS carrier molecule, complement 4B-binding protein (C4BP), is increased in pregnancy and is one explanation for the diminished PS levels in pregnancy.[1] Levels of plasminogen activator inhibitor 1 (PAI-1) increase 3- to 4-fold during pregnancy; plasma PAI-2 values are low prior to pregnancy and reach concentrations of 160 μg/L at term. Table 1 summarizes the most relevant changes in the hemostatic milieu based on Paidas et al.[3] The prothrombotic hemostatic changes are exacerbated by pregnancy-associated venous stasis in the lower extremities resulting from compression of the inferior vena cava and pelvic veins by the enlarging uterus as well as a hormone-mediated increase in deep vein capacitance secondary to increased circulating levels of estrogen and local production of prostacyclin and nitric oxide.

Substantial changes must occur in local decidual and systemic coagulation, anticoagulant, and fibrinolytic systems to meet the hemostatic challenges of pregnancy, including avoidance of hemorrhage at implantation, placentation, and third stage of labor. In addition to the systemic prothrombotic, anticoagulant, and fibrinolytic changes, there are potent local hemostatic effects in the decidua that occur during pregnancy.[4,5] Progesterone augments perivascular decidual cell tissue factor and PAI-1 expression. Decidual tissue factor is critical in maintaining hemostasis as evidenced by experiments with

[1] High Risk Pregnancy Unit, University Hospital of Careggi, Florence, Italy
[2] Gynecology Unit, University Hospital Burlo Garofolo, Trieste, Italy
[3] Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA

Corresponding Author:
Marianna Pina Rambaldi, High Risk Pregnancy Unit, University Hospital of Careggi, Largo Brambilla 3, Florence 50124, Italy.
Email: mprambaldi@hotmail.it

Table 1. Hemostatic Changes in Pregnancy (after Bremme[1] and Paidas et al[3]).

Variables (Mean ± SD)	First Trimester[a]	Second Trimester[a]	Third Trimester[a]	Normal Range	Significance
Platelet, $\times 10^9$/L	275 ± 64	256 ± 49	244 ± 52	150-400	−
Fibrinogen, g/L	3.7 ± 0.6	4.4 ± 1.2	5.4 ± 0.8	2.1-4.2	+
Prothrombin complex, %	120 ± 27	140 ± 27	130 ± 27	70-30	+
Antithrombin, U/mL	1.02 ± 0.10	1.07 ± 0.14	1.07 ± 0.11	0.85-1.25	−
Protein C, U/mL	0.92 ± 0.13	1.06 ± 0.17	0.94 ± 0.2	0.68-1.25	−
Protein S, total, U/mL	0.83 ± 0.11	0.73 ± 0.11	0.77 ± 0.10	0.70-1.70	+
Protein S, free, U/mL	0.26 ± 0.07	0.17 ± 0.04	0.14 ± 0.04	0.20-0.50	+
Soluble fibrin, nmol/L	9.2 ± 8.6	11.8 ± 7.7	13.4 ± 5.2	<15	+
Thrombin-antithrombin, μg/L	3.1 ± 1.4	5.9 ± 2.6	7.1 ± 2.4	<2.7	+
D-Dimers, μg/L	91 ± 24	128 ± 49	198 ± 59	<80	−
Plasminogen activator inhibitor 1, AU/mL	7.4 ± 4.9	14.9 ± 5.2	37.8 ± 19.4	<15	+
Plasminogen activator inhibitor 2, μg/L	31 ± 14	84 ± 16	160 ± 31	<5	+
Cardiolipin antibodies positive	2/25	2/25	3/23	0	−
Protein Z, μg/mL[b]	2.34 ± 0.69	1.98 ± 0.54	1.93 ± 0.51		+
Protein S, %[b]		34.4 ± 11.8	27.5 ± 8.4		+

[a]First trimester, weeks 12 to 15; second trimester, week 24; and third trimester, week 35.
[b]First trimester, 0 to 14 weeks; second trimester, 14 to 27 weeks; and third trimester ≥27 weeks.

transgenic tissue factor knockout mice which have a significant risk of fatal postpartum hemorrhage.[6] It is worthwhile to note that obstetric conditions associated with impaired decidualization (eg, ectopic and cesarean scar pregnancy, placenta previa, and accreta) are associated with potential lethal hemorrhage.

Pharmacology of Anticoagulation in Pregnancy

The available anticoagulant drugs for the prevention and treatment of venous thromboembolism (VTE) include warfarin, unfractionated heparin (UFH), low-molecular-weight heparin (LMWH), factor Xa inhibitors, and direct thrombin inhibitors. However, heparins are the mainstay of therapy in pregnancy. The UFH enhances antithrombin (AT) activity, increases factor Xa inhibitor activity, and inhibits platelet aggregation. The LMWH is generated by chemical or enzymatic manipulation of UFH from a molecular weight of 15 ,000 to 4000 to 6500 Da. The smaller size impedes its AT but not antifactor Xa effects. Both LMWH and UFH cross the placenta, are considered safe for pregnancy, and are compatible with breastfeeding. Complications associated with heparins include hemorrhage, osteoporosis, and thrombocytopenia. Heparin-induced thrombocytopenia (HIT) occurs in 2 forms. Type I HIT typically occurs within days of heparin exposure, is self-limited, and is not associated with significant risk of hemorrhage or thrombosis. Type II HIT is an immunoglobulin-mediated syndrome and occurs in the setting of venous or arterial thrombosis, usually 5 to 14 days following initiation of heparin therapy. Type II HIT can be confirmed by serotonin release assays, heparin-induced platelet aggregation assays, flow cytometry, or solid phase immunoassay.

The UFH has a short half-life and is administered subcutaneously or via continuous infusion. Usually, patients receiving UFH require frequent laboratory monitoring and dosage adjustment. The LMWH is administered subcutaneously either once or twice daily. It has advantages over UFH including better bioavailability, longer plasma half-life, and more predictable pharmacokinetics and pharmacodynamics, but LMWH is much more expensive than UFH. The LMWH has a reassuring risk profile, including antenatal bleeding 0.43 (0.22-0.75), postpartum hemorrhage >500 mL 0.94 (0.61-1.37), wound hematoma 0.61 (0.36-0.98), thrombocytopenia 0.11 (0.02-0.32), HIT 0.00 (0.00-0.11), and osteoporosis 0.04 (<0.01-0.20).[7] Coumarins are vitamin K antagonists that block the generation of vitamin KH2. The latter serves as a cofactor for the posttranslational carboxylation of glutamate residues to γ-carboxyglutamates on the N terminal regions of prothrombin and factors VII, IX, and X as well as the anticlotting agents, protein C (PC) and PS.

Several other anticoagulants are now available which may have a role in limited circumstances in pregnancy. Danaparoid is another low-molecular-weight heparinoid and is especially useful in the cases of HIT and heparin allergy. Fondaparinux is a synthetic heparin pentasaccharide that complexes with the AT-binding site for heparin to permit the selective inactivation of factor Xa but not thrombin. Direct thrombin inhibitors represent another class of anticoagulants. Hirudin is a 65-amino acid protein derived from the medicinal leech (*Hirudo medicinalis*). It can be used in patients with HIT-2 and is readily available in a recombinant form, lepirudin. Argatroban is a synthetic direct thrombin inhibitor that competitively binds to thrombin's active site, has a short half-life (45 minutes), and is cleared by the liver, making it the direct thrombin inhibitor of choice for patients with renal failure. Bivalirudin is a 20-amino acid synthetic polypeptide analog of hirudin.

For therapeutic dosing of LMWH, the antifactor Xa level should be maintained at 0.6 to 1.0 U/mL, 4 to 6 hours after injection (eg, starting with 1 mg/kg enoxaparin subcutaneously every 12 hours). Again, treatment should continue for 20 weeks and then prophylactic doses given (eg, 40 mg enoxaparin subcutaneously every 12 or 24 hours, adjusted to maintain antifactor Xa levels at 0.1-0.2 U/mL 4 hours after an injection). Because regional anesthesia is contraindicated within 18 to 24 hours of LMWH

Table 2. Inherited Thrombophilias and Their Association With VTE.[11]

Ratio Thrombophilia Lifetime	Inheritance	Prevalence in European Pop. (From Large Cohort Studies), %	Prevalence in Patients With VTE (Range), %	Relative Risk or Odds VTE (95% CI)
FVL (homozy)	AD	0.07[a]	<1[a]	80 (22-289)
FVL (heterozy)	AD	5.3	6.6-50	2.7 (1.3-5.6)
PGM (homozy)	AD	0.02[a]	<1	>80-fold[a]
PGM (heterozy)	AD	2.9	7.5	3.8 (3.0-4.9)
FVL/PGM (compound heterozy)	AD	0.17[a]	2.0	20.0 (11.1-36.1)
Hyperhomocysteinemia	AR	5	<5	3.3 (1.1-0.0)[b]
Antithrombin def (<60% activity)	AD	0.2	1-8	17.5 (9.1-33.8)
Protein S def Heerlen S460P mutation or free S antigen <55%	AD	0.2	3.1	2.4 (0.8-7.9)
Protein C (<60% activity)	AD	0.2	3-5	11.3 (5.7-22.3)

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; FVL, factor V Leiden; PGM, prothrombin gene mutation G20210A; VTE, venous thromboembolism; heterozy, heterozygous; homozy, homozygous; Pop., population.
[a]Calculated based on the Hardy-Weinberg equilibrium.
[b]Odds ratio (OR) adjusted for renal disease, folate, and vitamin B_{12} deficiency, while OR are adjusted for these confounders.

administration, we recommend switching to UFH at 36 weeks or earlier if preterm delivery is expected. If vaginal or cesarean delivery occurs more than 12 hours from prophylactic or 24 hours from therapeutic doses of LMWH, anticoagulation-related problems with delivery are not anticipated. Protamine can partially reverse the anticoagulant effects of LMWH. Patients with AT deficiency represent the highest thrombogenic risk. Patients with AT deficiency, especially those with a history of past or current thromboembolism, should receive either plasma-derived or recombinant AT in the peripartum period.

Inherited Thrombophilias

In 1965, Egeberg, a Norwegian physician, reported a family with a partial AT deficiency, and in his classic article, he suggested the term thrombophilia, referring to hereditary or acquired conditions that predispose individuals to thromboembolic events.[8] After the description of AT deficiency, deficiencies of PC and PS were described in the 1980s.[9,10] Table 2 describes the association between inherited thrombophilias and VTE.[11]

Factor V Leiden

Interest in the thrombophilias significantly grew following the discovery of a relatively common genetic predisposition to clotting. In 1994, Dahlback and Hildebrand[12] reported an association between a mutation in the factor V gene and increased thrombotic risk, termed the factor V Leiden (FVL) mutation. The FVL mutation results from a substitution of adenine for guanine at the 1691 position of the 10th exon of the factor V gene, causing an amino acid substitution, namely, glutamine for arginine at position 506 in the factor V polypeptide (FV Q506). Then, factor V is rendered resistant to cleavage by activated PC (APC). The frequency of the FVL mutation varies among different ethnic groups. The mutation is present in 5.2% of caucasians, 1.2% of African Americans,[13] and 5% to 9% of Europeans, while it is rare in Asian and African populations.[14] The FVL mutation is primarily inherited in an autosomal dominant fashion.[15]

Heterozygosity of FVL confers a 5- to 10-fold increased risk of VTE, while homozygosity confers a greater than 25-fold increased risk of VTE.[16] Although FVL is present in 40% of pregnant patients with VTE, given the low incidence of thrombosis in pregnancy (1 in 1400) and the high incidence of the mutation in the European-derived population, the estimated risk of VTE among heterozygous pregnant patients without personal or family history of thrombosis is only 1.5%. However, the risk may be up to 17% among pregnant women with a personal or strong family VTE history.[17] In the multicenter prospective observational National Institute of Child Health and Development (NICHD) study, 134 FVL carriers were identified among 4885 (2.7%) gravidas.[18] No thromboembolic events occurred among the FVL carriers (0%, 95% confidence interval [CI]: 0-2.7), while 3 pulmonary emboli (PE) and 1 deep venous thrombosis (DVT) occurred in FVL noncarriers (0.08%, 95% CI: 0.02-0.21). Maternal FVL mutation carriage was not associated with increased pregnancy loss, preeclampsia, placental abruption, or small for gestational age (SGA) births.

Prothrombin Gene Mutation G20210A

A mutation in the prothrombin gene, prothrombin gene mutation (PGM) G20210A, was discovered in 1996, following the identification of the FVL mutation and was associated with a significantly increased risk of thrombosis.[19] The G20210 A polymorphism for prothrombin is a point mutation causing a guanine to adenine switch at nucleotide position 20210 in the 3′-untranslated region of the gene.[20] The prothrombin gene is located on chromosome 11p11-q12. The nucleotide switch results in increased translation and increased circulating levels of prothrombin. The presence of heterozygosity of the PGM mutation range from 2% to 3% of Europeans and leads to increased (150%-200%) circulating levels of prothrombin.[14] It accounted for 17% of thromboembolism in

pregnancy in 1 large case–control study.[21] The actual risk of clotting in an asymptomatic pregnant carrier is approximately 1 in 200 or 0.5%. The rarer condition, homozygosity for PGM, likewise confers a high risk of thrombosis, equal to that of homozygosity for FVL.[14]

Combined FVL and PGM Mutation

The prevalence of combined FVL and PGM heterozygous mutation is low in the general population, and it is present in 1 of 10 000 patients.[22] It has been suggested that the combination of these thrombophilic mutation has synergistic hypercoagulable effects during pregnancy and the puerperium.

In a cohort study of 270 pregnancies in patients carrying FVL mutation and 215 controls, the frequency of VTE was 6.4% for FVL heterozygous, 16.7% for homozygous, 20% for double heterozygous, and 1.2% for noncarriers. The relative risks (RRs) of developing pregnancy-related VTE in women who were carriers of a combined heterozygous when compared to noncarriers were 15.4 (95% CI: 1.4-164).[23] In a study of 119 consecutive women with a history of VTE during pregnancy and the puerperium and 233 controls, it was seen that a combined polymorphism of PGM and FVL is associated with an increased risk of thromboembolic events compared with the risk of either mutation alone.[17] Patients carrying the combination of FVL and PGM heterozygous mutation have a RR of 84 (95% CI: 19-369), with a probability of 4.7% having a thrombosis during pregnancy even without a personal or positive family history.[21] In the same study, no association between genetic risk factors and pregnancy-associated complications was reported. In contrast, Martinelli and collaborators did not find an increased risk of thrombosis, comparing 52 double heterozygous carriers to 104 FVL heterozygous, 104 PGM heterozygous, and 104 nontrombophilic women. Thrombotic events occurred in 1.8% of double carriers (95% CI: 0.5-6.3), 1.5% of FVL carriers (95% CI: 0.5-4.3), 1% of PGM carriers (95% CI: 0.2-3.6), and 0.4% of noncarriers (95% CI: 0-2.5). All the events happened in the postpartum period.[24]

Protein S Deficiency

The PS is a vitamin K-dependent 69 000 molecular weight glycoprotein, which has several anticoagulant functions including its activity as a nonenzymatic cofactor to the anticoagulant serine protease APC.[25] The PS has a plasma half-life of 42 hours, longer than PC whose half-life is approximately 6 to 8 hours. Circulating PS exists in both free (40%) and bound (60%) forms. Plasma PS is reversibly bound (60%) to C4BP, which serves as a carrier protein for PS. The PS also has an APC-independent anticoagulant function in the direct inhibition of the prothrombinase complex. The PS also inhibits thrombin activatable fibrinolysis inhibitor.[26] The PS deficiency occurs in 0.03% to 1.3% of the population, and inheritance is autosomal dominant.[27] The PS deficiency presents with 1 of 3 phenotypes, type I, marked by reduced total and free forms; type II, characterized by normal free PS levels but reduced APC cofactor activity; and type III, in which there are normal total but reduced free PS levels. Of note, different mutations have highly variable procoagulant sequelae, making it extremely difficult to predict which patients with PS deficiencies will develop thrombotic sequelae.

Pregnancy is associated with decreased levels of PS activity and free PS antigen in most patients.[28] Most normal pregnancies acquire some degree of resistance to APC when measured by the first-generation global assays and tests that measure endogenous thrombin potential.[29,30] Factor X's activation to factor Xa and its involvement in the activation of prothrombin is a central element in the generation of thrombin. It is possible that derangements in the control of factor Xa contribute to adverse prothrombotic sequelae in pregnancy.

Until recently, the significance and degree of the decrease in PS levels commonly seen in pregnancy had not been adequately evaluated. Paidas et al[3] compared second and third trimesters PS levels in 51 healthy women with a normal pregnancy outcome with 51 healthy women with a poor pregnancy outcome. The PS levels were significantly lower in the second and third trimesters among patients with APO. A small case–control study performed in patients from larger, multicenter, and prospective study also found lower levels of PS activity and free antigen in the second and third trimesters.[18]

Protein C Deficiency

The PC is a vitamin K-dependent 62 000 molecular weight glycoprotein substrate that is a precursor to a serine protease, APC.[31] The PC is activated to APC by thrombin in the presence of thrombomodulin (TM) on the surface of endothelial cells. The APC with PS and factor V as cofactors inactivate factors Va and VIIIa. The inactivation of factors Va and VIIIa decreases the generation of thrombin. Deficiencies of PC result from numerous mutations, although 2 primary types are recognized, type I in which both immunoreactive and functionally active PC levels are reduced and type II where immunoreactive levels are normal but activity is reduced.[32] The prevalence of PC deficiency is 0.2% to 0.5%, and its inheritance is autosomal dominant.

The reported pregnancy and puerperal risk of thromboembolism with PC and PS deficiencies appears modest, ranging from 5% to 20%, and may be overstated because of ascertainment biases.[32] Preston et al[33] have reported that the risk of stillbirth is modestly increased with an adjusted odds ratio (OR) of 2.3 (95% CI: 0.6-8.3). The risk of miscarriage appears to be minimal with PC deficiencies (OR 1.4; 95% CI: 0.9-2.2) or not significant.[32,34]

Antithrombin Deficiency

The AT, a vitamin K-independent glycoprotein, is a pivotal component of the natural anticoagulant system, acting as a major inhibitor of thrombin and other serine proteases. The anticoagulant effect of heparin occurs via increase in AT's inhibitory activity of thrombin. Deficiency of AT is the most thrombogenic of the inherited thrombophilias, with a 70% to

90% lifetime risk of thromboembolism.[32] In addition to its thrombin inhibitory properties, AT can also inactivate factors Xa, IXa, VIIa, and plasmin. The anticoagulant activity of AT is increased 5000- to 40 000-fold by heparin. Deficiencies in AT result from numerous point mutations, deletions, and insertions and are usually inherited in an autosomal dominant fashion.[30] The 2 classes of AT deficiency are

1. Type I, the most common deficiency, is characterized by concomitant reductions in both antigenic protein levels and activity.
2. Type II deficiency, which is characterized by normal antigenic AT levels but decreased activity.

Type II deficiency is further classified by the site of the mutation (eg, reactive site, heparin-binding site [HBS], and pleiotropic functional defects). The type II HBS variant appears to have the least clinical significance. Because the prevalence of AT deficiency is low, 1 in 1000 to 1 in 5000, it is only present in 1% of the patients with thromboembolism. The risk of thrombosis among affected patients is as high as 60% during pregnancy and 33% during the puerperium.[32] With no previous history of VTE, 31% of patients with hereditary AT deficiency will develop a VTE during pregnancy, and if there is a history of previous thromboembolism, the recurrence rate is 49%.[35] Preston et al[33] reported adjusted OR of 1.7 (95% CI: 1.0-2.8) and 5.2 (95% CI: 1.5-18.1) for miscarriage and stillbirth, respectively. However, because of its low prevalence compared to that of fetal loss, preeclampsia, intrauterine growth restriction (IUGR), and abruption, AT deficiency is rarely the cause of these disorders.[36]

Protein Z Deficiency

Protein Z is a 62-kDa vitamin K-dependent plasma protein which serves as a cofactor for a protein Z-dependent protease inhibitor (ZPI) of factor Xa.[37,38] Protein Z is critical for regulation of factor Xa activity in addition to tissue factor pathway inhibitor.[39-41] Protein Z increases rapidly during the first months of life followed by slow increases during childhood, with adult levels reached during puberty.[42,43] Protein Z deficiency influences the prothrombotic phenotype in patients with FVL, and low plasma protein Z levels have been reported in patients with antiphospholipid antibodies (APAs).[44,45] There is a high prevalence of protein Z deficiency in patients with unexplained early fetal loss (10-19th weeks).[46] Gris et al[47] found an increased risk of fetal loss associated with protein Z deficiency (OR 6.7; 95% CI: 3.1-14.8; $P < .001$) and noted that the patients with late fetal loss and recurrent miscarriages had lower protein Z levels.

Paidas et al[3] found that there was a significant decrease in the protein Z levels in patients ($n = 51$) with a variety of APO, including IUGR, preeclampsia, preterm delivery, and bleeding in pregnancy, compared with women ($n = 51$) with normal pregnancy outcomes (second trimester 1.5 ± 0.4 vs 2.0 ± 0.5 μg/mL, $P < .0001$ and third trimester 1.6 ± 0.5 vs 1.9 ± 0.5 μg/mL, $P < .0002$).

Protein Z levels at the 20th percentile (1.30 μg/mL) were associated with an increased risk of APO (OR 4.25; 95% CI: 1.536-11.759), with a sensitivity of 93% and specificity of 32%. Mean first trimester protein Z level was significantly lower among patients with APO compared with pregnant controls (1.81 ± 0.7 vs 2.21 ± 0.8 μg/mL, respectively; $P < 0.001$). Gris et al[47] carried out a prospective, randomized trial comparing the LMWH enoxaparin (40 mg/d) with low-dose aspirin (100 mg/d) in 160 women with 1 unexplained fetal loss (≥10th week of gestation) and FVL, prothrombin 20210, or PS deficiency. Treatments were started at 8 weeks' gestation. The live birth rate was 86% in the enoxaparin-treated women versus 29% in the aspirin-treated group (OR for live birth with LMWH 15.5; 95% CI: 7-34). Birth weights were higher, and there were fewer SGA infants in the enoxaparin group. Gris et al[47] found that the presence of protein Z deficiency or the presence of protein Z antibodies was more frequently present in cases of treatment failures ($P = .20$ and .019, respectively) as was the complex of protein Z deficiency positive antiprotein Z antibodies ($P = 0.004$); 15 of the 20 cases led to pregnancy failure, 9 being treated with aspirin and 6 with enoxaparin. Both the groups of patients received 5 mg/d folic acid, in addition to aspirin or heparin therapy.

Elevated Levels of Type I PAI

The PAIs are serine protease inhibitors, often referred to as serpins (serine protease inhibitors) with diverse functions, including blood coagulation, fibrinolysis, and cell migration.[48,49] The PAI-1 and PAI-2 regulate tissue- and urokinase-type plasminogen activators, respectively (tPA and uPA). The tPA and uPA regulate fibrin degradation via the conversion of plasminogen to plasmin and are also involved in the remodeling of extracellular matrix.[50] The PAI-1 and PAI-2 are found in the blood of women with normal pregnancies, and their levels tend to rise with advancing gestation.[51] In patients with preeclampsia, the vascular endothelium is responsible for the majority of the elevated PAI-1 plasma levels, with platelets accounting for a smaller proportion.[52] Unlike PAI-1, which is found in a variety of nonpregnant disease states, PAI-2 expression has been identified in a limited number of cells, principally placental trophoblasts, macrophages, and various malignant cell lines.[52,53]

It is well known that pregnancy is associated with elevated levels of PAI-1, and higher levels are noted in cases of preeclampsia or IUGR (either during manifestations of the disease process or shortly prior to their manifestation in the case of preeclampsia). Homozygosity for the 4G/4G mutation in the PAI-1 gene leads to a 3- to 5-fold increased level of circulating PAI-1. The significance of the 4G/4G PAI-1 mutation is uncertain. The contribution of this prothrombotic mutation to thromboembolic events has been called into question as evidenced by the recent review by Francis.[54] A large multicenter study did not find a relationship between any of the inherited thrombophilic conditions and fetal loss but achieved approximately 30% power to detect a difference.[55] Polymorphisms of the TM gene are associated with an increased risk of thrombosis, but the pregnancy implications are unclear at this time.[56] Interestingly, pregnant patients with

thrombophilia and subsequent APO have been demonstrated to exhibit a decreased first trimester response to TM in an activated partial thromboplastin time (APTT) system.[57]

Hyperhomocysteinemia and Methylene Tetrahydrofolate Reductase Thermolabile Mutant gene Mutation C677T

Homocysteine is generated from the metabolism of the amino acid methionine. It normally circulates in the plasma at concentrations of 5 to 16 μmol/L. Deficiencies in vitamins B6, B12, and folic acid can result in elevated levels of homocysteine in the setting of inherited hyperhomocysteinemia. Homocysteine levels can vary with diet; however, and normal levels in pregnancy are slightly lower than nonpregnant values.

Hyperhomocysteinemia can be diagnosed by measuring fasting homocysteine levels by gas chromatography mass spectrometry or other sensitive biochemical means. The disorder is classified into 3 categories according to the extent of the fasting homocysteine elevation, severe (>100 μmol/L), moderate (25-100 μmol/L), or mild (16-24 μmol/L). Methionine loading can improve diagnostic sensitivity. Severe hyperhomocysteinemia results from an autosomal recessive homozygous deficiency in either cystathionine β synthase (CBS; prevalence of 1 in 200 000) or methylene tetrahydrafolate reductase (MTHFR). Clinical manifestations of hyperhomocysteinemia include neurologic abnormalities, premature atherosclerosis, and recurrent thromboembolism.

The mild and moderate forms can result from autosomal dominant (heterozygote) deficiencies in CBS (0.3-1.4% of population) or from homozygosity for the 667C-T MTHFR thermolabile mutant present in 11% of white European populations.[32] Patients with mild or moderate hyperhomocysteinemia are at risk of atherosclerosis, thromboembolism, fetal neural tube defects, and possibly recurrent abortion. There are conflicting data on the link between hyperhomocysteinemia and recurrent spontaneous abortion.[58-60] An older meta-analysis of the association between hyperhomocysteinemia and pregnancy loss prior to 16 weeks suggested a weak association with an OR of 1.4 (95% CI: 1.0-2.0).[61] The natural history of the MTHFR mutation in pregnancy has not been well documented. The meta-analysis by Rey et al[34] concluded that MTHFR was not associated with an increased risk of fetal loss. A subsequent meta-analysis concluded that MTHFR C677T mutation was not associated with early pregnancy loss.[62]

Acquired Thrombophilia

The well-characterized antiphospholipid antibody syndrome (APAS) has been defined by the combination of VTE, obstetric complications, and APAs, commonly termed the Sapporo criteria for APAS.[63] Since 2005, the revised criteria for the classification of APAS have been in place and is summarized in Table 3.[64] Current accepted obstetric complications include at least 1 fetal death at or beyond the 10th week of gestation, at least 1 premature birth before the 34th week as a sequelae of preeclampsia or uteroplacental insufficiency, or at least 3 unexplained consecutive spontaneous abortions at or before the 10th week. The APA must be present on 2 occasions at least 12 weeks apart. The APAs are immunoglobulins directed against proteins bound to negatively charged surfaces, usually anionic phospholipids.[65] The APAs can be detected by screening for antibodies that:

- directly bind these protein epitopes (eg, anti-β2-glycoprotein-1, prothrombin, annexin V, APC, PS, protein Z, ZPI, high- and low-molecular-weight kininogens, tPA, factor VII(a), and XII, the complement cascade constituents, C4, and CH, and oxidized low-density lipoproteins antibodies);
- are bound to proteins present in an anionic phospholipid matrix (eg, anticardiolipin and phosphatidylserine antibodies); or
- exert downstream effects on prothrombin activation in a phospholipid milieu (ie, lupus anticoagulants).[66]

Venous thrombotic events associated with APA include DVT with or without acute pulmonary embolism (APE), while the most common arterial events include cerebral vascular accidents and transient ischemic attacks. At least half of the patients with APA have systemic lupus erythematosus (SLE). Anticardiolipin antibodies were associated with an OR of 2.17 (95% CI: 1.51-3.11; 14 studies) for any thrombosis, 2.50 (95% CI: 1.51-4.14) for DVT and APE, and 3.91 (95% CI: 1.14-13.38) for recurrent VTE.[67] Patients with SLE and lupus anticoagulants were at a 6-fold greater risk of VTE compared to patients with SLE without lupus anticoagulants, while patients with SLE with anticardiolipin antibodies had a 2-fold greater risk of VTE compared to patients with SLE without these antibodies. The lifetime prevalence of arterial or venous thrombosis in affected patients with APAs is approximately 30%, with an event rate of 1% per year.[66] These antibodies are present in up to 20% of the individuals with VTE.[68] A review of 25 prospective, cohort, and case–control studies involving more than 7000 patients observed an OR range for arterial and venous thrombosis in patients with lupus anticoagulants of 8.65 to 10.84 and 4.09 to 16.2, respectively, and 1 to 18 and 1 to 2.51 for anticardiolipin antibodies.[66] Antiphosphatidylserine-dependent antiprothrombin immunoglobulin (Ig) G (IgG) antibodies have been linked to second trimester fetal loss.[69] However, data on clinical association of antiprothrombin antibodies, which include antibodies against prothrombin alone and antibodies to the phosphatidylserine–prothrombin complex, are not yet included in the classification criteria for APAS.[64] In a large, prospective cohort of 1155 women in whom a panel of APAs was evaluated in the first trimester to determine their relationship to adverse pregnancy outcome, the combinations of antiphosphatidylethanolamine IgG antibody plus anticardiolipin antibody IgG (OR 17.5; 95% CI: 4.7-66.7) or antiphosphatidylethanolamine IgG antibody plus lupus anticoagulant (OR 22.2; 95% CI: 5.4-909) predicted severe pregnancy-induced hypertension with 30.8% sensitivity and 99.2% specificity.[70]

There is a 5% risk of VTE during pregnancy and the puerperium among patients with APA despite treatment.[71] Recurrence

Table 3. Diagnosis of Antiphospholipid Antibody Syndrome.[60]

Definite APS is considered if at least one of the following clinical criteria and at least one of the following laboratory criteria are satisfied:
Clinical criteria
Obstetrical
- History of 3 unexplained consecutive spontaneous abortions ≤10 weeks gestational age (GA)
- History of 1 unexplained fetal death ≥10 weeks
- GA (morphologically and karyotypically normal)
- History of preterm delivery <34 weeks GA, as a sequelae of preeclampsia or uteroplacental insufficiency, including the following:
  Nonreassuring fetal testing indicative of fetal hypoxemia (eg, abnormal Doppler flow velocimetry waveform)
  Oligohydramnios (amniotic fluid index less than or equal to 5 cm)
  Intrauterine growth restriction (IUGR) less than the 10th percentile
  Placental abruption

Nonobstetrical
- Arterial thrombosis, including: cerebrovascular accidents, transient ischemic attacks, myocardial infarction, amaurosis fugax
- Venous thromboembolism (VTE), including deep venous thrombosis (DVT), pulmonary emboli (PE), or small vessel thrombosis

Laboratory criteria
Should be present on 2 occasions, more than 12 weeks apart, and not more than 5 years prior to clinical manifestation:
- Anticardiolipin antibody
  IgG or IgM isotype, present in medium or high titers (ie, 40 GPL or MPL, or > 99th percentile)
- Anti-β2 GPI antibody
- IgG or IgM isotype (>99th percentile)
  Lupus anticoagulant in plasma, utilizing one of the following tests:
  Dilute Russell viper venom time (dRVVT)
  Lupus anticoagulant

Abbreviations: APS, antiphospholipid syndrome; GPI, glucose phosphate isomerase; GPL IgG phospholipid units; Ig, immunoglobulin; MPL, IgM phospholipid units.

risks of up to 30% have been reported in APA-positive patients with a prior VTE; thus, long-term prophylaxis is required in these patients. A severe form of APAS is termed catastrophic antiphospholipid syndrome, which is defined by potential life-threatening variant with multiple vessel thromboses leading to multiorgan failure.[72] In the Euro-Phospholipid Project Group (13 countries included), DVT, thrombocytopenia, stroke, PE, and transient ischemic attacks were found in 31.7%, 21.9%, 13.1%, 9.0%, and 7.0%, respectively.

The APAs are associated with obstetric complications in approximately 15% to 20% including fetal loss after 9 weeks' gestation, abruption, severe preeclampsia, and IUGR. For lupus anticoagulant-associated fetal loss, reported OR range from 3.0 to 4.8, while anticardiolipin antibodies display a wider range of reported OR of 0.86 to 20.0.[65] It is unclear whether APA is also associated with recurrent (3 or more) early spontaneous abortions in the absence of stillbirth. In patients with APA, 50% or more of pregnancy losses occur after the 10th week.[73] Patients with APA more often display initial fetal cardiac activity compared to patients with unexplained first trimester spontaneous abortions without APA (86% vs 43%; $P < .01$).[74] The APAs have been commonly found in the general obstetric population, with one survey demonstrating that 2.2% of such patients have either IgM or IgG anticardiolipin antibodies with most such women having relatively uncomplicated pregnancies.[75] Other factors may have a role in the pathogenesis of APA. Potential mechanisms by which APA induce arterial and venous thrombosis as well as adverse pregnancy outcomes include APA-mediated impairment of endothelial TM and APC-mediated anticoagulation, induction of endothelial tissue factor expression, impairment of fibrinolysis and AT activity, augmented platelet activation and/or adhesion, and impairment of the anticoagulant effects of the anionic phospholipid-binding proteins β2-glycoprotein-I and annexin V.[76,77] In the murine model, complement activation, namely, C5 complement split product C5a, has been required for tissue factor expression in APA-treated mice.[78] A specific anti-C5a monoclonal antibody was able to reverse the thrombogenicity of APAs.[79] Heparin's inhibition of aberrant complement activation has explained its protective effects on preventing fetal loss caused by APAs.[80,81] However, heparin does not completely prevent all of antiphospholipid-related obstetric complications as has been demonstrated in clinical studies. One explanation may be that APAs limit trophoblast migration by downregulating interleukin 6 secretion and signal transducer and activator of transcription 3, and heparin does not prevent these effects.[82]

Inherited Thrombophilia and Pregnancy Complications

Inherited thrombophilic conditions have been evaluated in a variety of obstetric complications, including preeclampsia and related conditions, early and late fetal loss, IUGR, and abruptio placentae.

Preeclampsia

Several studies (mostly case controlled) have evaluated the relationship between heterozygous FVL and severe preeclampsia. The FVL was identified in 4.5% to 26% of patients with severe preeclampsia, eclampsia, or hemolysis, elevated liver enzymes,

and low platelet count syndrome.[83-87] The systematic review by Alfirevic et al[36] suggested a positive association between FVL and preeclampsia and/or eclampsia (OR 1.6; 95% CI: 1.2-2.1). The PGM was identified in up to 9.1% of the cases, whereas PS deficiency was reported in 5% to 25% of cases.[83,85,87] Although the preponderance of the early studies evaluating severe preeclampsia demonstrated a positive association with inherited thrombophilias, there is also evidence demonstrating no association. In a study by Livingston et al,[87] maternal and fetal genetic thrombophilias (FVL, MTHFR, and PGM) were compared in 110 patients with severe preeclampsia and 97 normotensive patients with healthy outcomes. There were no differences in the rate of FVL (4.4% vs 4.3%), MTHFR (9.6% vs 6.3%), or PGM (0% vs 1.1%) between the 2 groups. Similar findings were noted in white (n = 47) and African American (n = 63) women and in those with early- or late-onset severe preeclampsia. In addition, there were no differences in the frequency of the studied genetic thrombophilias in cord blood in severe preeclampsia and normotensive groups. In 2009, the Montreal Preeclampsia Study reviewed 113 patients with preeclampsia from a cohort of 5162 patients, compared to 443 control subjects.[88] This case–control study did not find an increased risk of preeclampsia, including early-onset or severe preeclampsia, in association with maternal FVL mutation. The latest systematic review and meta-analysis, encompassing 10 prospective cohort studies and evaluating the association of FVL and PGM and placenta-mediated complications in prospective cohort studies, demonstrated no significant association between FVL and preeclampsia (OR = 1.23; 95% CI: 0.89-1.70) or between PGM and preeclampsia (OR = 1.25; 95% CI: 0.79-1.99).[89] In contrast, after that, a big nested case–cohort study of pregnant women in Denmark was published by Lykke and collaborators; in a cohort of more than 100 000 women, they genotyped for FVL, PGM, and MTHFR in 2032 cases and 1851 random controls. It was found that FVL increased the risk of severe preeclampsia by OR 1.6 (CI: 1.1-2.4) and MTHFR by OR 1.3 (CI: 1.1-1.6), while PTM did not increase such risk.[90]

Intrauterine Growth Restriction

Infante-Rivard et al[91] found rates of 4.5% and 2.5% for FVL and PGM, respectively, when IUGR was defined as less than 10th percentile. In a systematic review, FVL and PGM were associated with an increased risk of IUGR: OR 2.7 (95% CI: 1.3-5.5) and 2.5 (95% CI: 1.3-5.0), respectively, in 10 case–control studies.[92] However, in 5 cohort studies (3 prospective and 2 retrospective), the RR was 0.99 (95% CI: 0.5-1.9). The authors concluded that both FVL and PG confer an increased risk of giving birth to an IUGR infant, although this may be driven by small, poor quality studies that demonstrated extreme associations. In the 2008 meta-analysis by Dudding et al, FVL was not associated with IUGR, with a pooled OR of 1.15 (95% CI: 0.95-1.39).[93] Most recently, in the systematic review and meta-analysis of 10 prospective cohort studies, Rodger et al demonstrated no significant association between FVL and SGA (OR = 1.0; 95% CI: 0.80-1.25) or PGM and SGA (OR 1.25; 95% CI: 0.92-1.70).[89] In the recent study on the Danish cohort, it was found that FVL increased the risk of SGA defined as less than third percentile by OR 1.4 (CI: 1.1-1.8) while PTM and MTHFR did not.[90] Prevalence rates of 11% to 23% have been reported for PS deficiency.[94-96]

Alfirevic et al[36] found a significant association between PS deficiency and IUGR (OR 10.2; 95% CI: 1.1-91).

Abruptio Placentae and Thrombophilia

The determination of the relationship between thrombophilias and abruptio placentae (decidual hemorrhage) has been challenging because of the limited number of studies and confounding variables, including chronic hypertension and cigarette and cocaine use.[97,98] De Vries et al[95] found that 9 (29%) of 31 patients with abruption had a PS deficiency, compared with their general population prevalence of 0.2% to 2%. The prevalence of FVL, PGM, and PS deficiency was in the ranges of 22% to 30%, 18% to 20%, and 0% to 29%, respectively.[1,85]

The association between abruption and FVL is less clear compared to preeclampsia and IUGR, because there is much less data on patients with abruption. Kupferminc et al reported a modest association between FVL and abruption with an OR of 4.9 (95% CI: 1.4-17.4).[85] A small case–control study of 27 patients with abruption compared to 29 control participants revealed 29.6% carrier rate of FVL compared to 3.4% of controls.[99] Procházka and associates conducted a retrospective case–control study among 102 women with placental abruption and 2371 controls.[100] They reported a nonsignificant increase in FVL carriers among affected patients compared with controls, with OR of 1.5 (95% CI: 0.9-2.7). Furthermore, they noted that 20% of women with a prior abruption had a first-degree relative with a history of VTE compared to 6.7% of controls, suggesting a higher prevalence of inherited thrombophilia among women with abruption. The same group, in 2007, again retrospectively reviewed 180 women with placental abruption and 196 controls and found a significantly increased carrier rate for FVL compared with controls, with OR of 3.0 (95% CI: 1.4-6.7).[101] Alfirevic et al conducted a systematic review and reported a strong association between placental abruption and homozygosity for FVL, with OR 16.9 (95% CI: 2.0-141.9).[36] A milder, but statistically significant, association was also seen for FVL heterozygosity, with OR 6.7 (95% CI: 2.0-21.6). In the multicenter prospective observational NICHD report from 2005, nested carrier–control analysis revealed no difference between FVL carriers and noncarriers in the development of abruption.[18] In the recent systematic review and meta-analysis, Rodger et al demonstrated no significant association between FVL and abruption (OR = 1.85; 95% CI: 0.92-3.70) or PGM and abruption (OR 2.02; 95% CI: 0.81-5.02).[89] Finally, in the most recent nested case–cohort study of pregnant women in Denmark, it was found that FVL increased the risk of abruption by OR of 1.7 (CI: 1.2-2.4) while PTM and MTHFR did not.[90] Regarding MTHFR and hyperhomocysteinemia, in a very well done 2007 case–control study from the New Jersey-Placental Abruption group, homozygosity for neither the

C677CT (OR 0.60; 95% CI: 0.33-1.18) nor the A1298C (OR, 2.28; 95% CI: 0.82-6.35) variants in MTHFR was associated with placental abruption.[102] This contrasts with a previously published meta-analysis, which found that hyperhomocysteinemia yielding a stronger pooled OR of 5.3 (95% CI: 1.8-15.9) for abruption, than did homozygosity for the MTHFR mutation, with OR 2.3 (95% CI: 1.1-4.9).[103]

Fetal Loss

Several studies have found strong associations between FVL and second or third trimester fetal loss but not with early first trimester losses. The gaseous milieu of the uteroplacental circulation during early pregnancy is one containing low oxygen levels, with intervillous oxygen pressures at 8 to 10 weeks of 17 $\pm$ 6.9 mm Hg and rising to 60.7 $\pm$ 8.5 mm Hg by 13 weeks.[104] The hypoxic environment may result from trophoblast plugging of the spiral arteries and low Doppler flow in the uterine vasculature and allows for undetectable levels of the damaging superoxide dismutase in trophoblasts prior to 10 weeks.[105,106] This same effect, at a later gestational age, would have a contrasting effect to the larger embryo or fetus, providing biologic plausibility for the association with pregnancy loss at later gestations in the following studies. In a retrospective cohort study, Roque et al evaluated 491 patients with a history of various obstetric complications and reported that FVL carrier status was paradoxically protective against losses at <10 weeks with an OR 0.23 (95% CI: 0.07-0.77) but significantly associated with losses >14 weeks with an OR of 3.71 (95% CI: 1.68-8.23).[59] Moreover, women who experienced only euploid losses were not more likely to have an identified thrombophilia than women who experienced only aneuploid losses (OR 1.03; 95% CI: 0.38-2.75). This protective effect of FVL on early pregnancy is also seen within the in vitro fertilization population, where implantation rates were substantially higher among FVL carriers than among noncarriers (90% vs 49%; $P = .02$).[107] Supportive findings were reported in a large prospective study comparing 843 women with thrombophilias, of whom 571 had 1524 pregnancies, to 541 control women of whom 395 had 1019 pregnancies.[33] Of the thrombophilias studied, the authors noted a statistically significant association with stillbirth (OR 3.6; 95% CI: 1.4-9.4) but not spontaneous abortion (OR 1.27; 95% CI: 0.94-1.71). Although underpowered to evaluate the impact of individual thrombophilic conditions, the same trend could be discerned for FVL with higher ORs for stillbirth (2.0; 95% CI: 0.5-7.7) than for spontaneous abortion (0.9; 95% CI: 0.5-1.5). Dudding and Attia demonstrated that FVL is most strongly linked to later fetal loss, finding no association between first trimester losses and FVL but a consistent and graded increase in risk with each second/third trimester fetal loss (OR 2.4; 95% CI: 1.1-5.2 for isolated stillbirth and OR 10.7; 95% CI: 4.0-28.5 for 2 or more stillbirths).[108] In contrast, Rey et al found that FVL was significantly linked to both recurrent loss prior to 13 weeks (OR 2.01; 95% CI: 1.13-3.58) and nonrecurrent fetal loss (OR 1.73; 95% CI: 1.18-2.54).[34] Although the exact gestational age when FVL transitions from protective to detrimental is unknown, it is clear that by the second trimester it is a risk factor. Thus, it is not surprising that FVL is linked to stillbirth. One retrospective case–control study of over 2000 women with recurrent fetal losses showed a striking association between FVL and stillbirth >22 weeks with an OR of 4.51 (95% CI: 1.81-11.23).[109] This finding was confirmed in a more contemporary prospective case-controlled trial of 5000 women in which stillbirth was defined as intrauterine demise of fetuses >500 g.[110] This study showed a significant association with FVL (OR of 10.9; 95% CI: 2.07-56.94). In the recent systematic review and meta-analysis, Rodger et al demonstrated a significant association between FVL and fetal loss (OR = 1.52; 95% CI: 1.06-2.19) andbetween PGM and fetal loss (OR 1.13; 95% CI: 0.64-2.01).[89] Of note, the absolute risk of pregnancy loss in women with FVL was 4.2% when compared to 3.2% for FVL-negative women, representing a 52% higher risk of pregnancy loss associated with FVL. Prior meta-analysis and systematic reviews evaluating the PGM have found a statistically significant link with ORs of 2.49 (95% CI: 1.24-5)[111] and 2.3 (95% CI: 1.1-4.8).[34] Less data exist for the rarer inherited deficiencies of PS, PC, and AT. The meta-analysis by Rey et al reported an association between PS deficiency and recurrent late (>22 weeks or <25 weeks) fetal loss (OR: 14.7; 95% CI: 1.0-2181) as well as nonrecurrent fetal losses at >22 weeks (OR 7.4; 95% CI: 1.3-43).[30] This relationship was strengthened by the meta-analysis of Alfirevic et al, which found that PS deficiency was associated with an increased risk of stillbirth (OR 16.2; 95% CI: 5.0-52.3).[36] Saade and McLintock reported an association between PS deficiency and late fetal loss, with adjusted OR 41 (95% CI: 4.8-359).[112] In a systematic review, PS deficiency was associated with late pregnancy loss with OR of 20.1 (95% CI: 3.7-109).[111] Regarding PC deficiency, Preston et al have reported that the risk of stillbirth is modestly increased (adjusted OR 2.3; 95% CI: 0.6-8.3) but not miscarriage (OR 1.4; 95% CI: 0.9-2.2).[33] However, Alfirevic et al found no association between PC deficiency and stillbirth.[36] A relative paucity of data exists concerning obstetric complications and the rare condition of hereditary AT deficiency. In the largest retrospective cohort study, AT deficiency was associated with a significant increased risk of stillbirth at >28 weeks (OR 5.2; 95% CI: 1.5-18.1) but a more modest association with miscarriage <28 weeks (1.7; 95% CI: 1.0-2.8).[33]

Early Pregnancy Loss and Thrombophilia

The association between early pregnancy loss and thrombophilia has also yielded conflicting results. In systematic reviews, the diversity among included studies implies that meta-analyses are performed including heterogeneous studies.[34,62,113] Factors influencing results include inclusion of isolated or recurrent fetal loss, presence or absence of successful live birth in obstetric history, gestational age cutoff for evaluation, and inclusion of proper control groups. There was no increased risk of loss with MTHFR C677T.[62] Roque et al[59] reported that the OR for having thrombophilia was actually significantly lower in women with

recurrent embryonic losses. The paternal or fetal genetic contribution has not been well studied to date. In a study of 357 couples with a history of 3 or more pregnancy losses under 12 weeks, the presence of multiple thrombophilic mutations in either partner was associated with a significantly increased risk of pregnancy loss (RR 1.9, range 1.2-2.8).[114]

Thrombophilia, Prior History of Poor Pregnancy Outcome, and Recurrence

The rate of recurrence is still being defined for patients who have had a prior adverse pregnancy outcome and harbor an inherited thrombophilic condition such as FVL, the PGM G20210A, or PS deficiency, . Martinelli et al[115] found that of 82 women with late fetal loss, 7 had a recurrence, and 2 (28.6%) of 7 had FVL. Kupferminc et al[85,86,116] have consistently reported very high rates of pregnancy complications in women with prior poor obstetric outcomes, 83% of the pregnancies in 18 women, 66% of the pregnancies in 9 multiparous women with PGM, and 77% occurrence of complications in 9 multiparous women. In a cohort of 28 patients with thrombophilia (heterozygous PGM and prior APO), Kupferminc et al[116] reported that 7 of 62 pregnancies were normal. According to the meta-analysis by Rey et al,[34] the presence of PGM was associated with recurrent fetal loss before 25 weeks (n = 690 women; OR 2.56; 95% CI: 1.04-6.29) and with nonrecurrent fetal loss after 20 weeks (5 studies, n = 1299; OR 2.3; 95% CI: 1.09-4.87). Rey et al also found that late fetal loss was associated with FVL mutation (n = 1888; OR 3.26; 95% CI: 1.82-5.83), PGM (n = 1299; OR 2.30; 95% CI: 1.09-4.87), and PS deficiency (n = 878; OR 7.39; 95% CI: 1.28-42.83) but not MTHFR, PC deficiency, or AT deficiency. Several factors impact studies concerning thrombophilia and pregnancy complications, including the heterogeneity of the populations studied, small sample size, rarity of the end point evaluated, number of thrombophilias assayed for, detection methods employed, lack of consistent assessment of fetal thrombophilia status, and potential ascertainment biases.[62,117] These limitations have been confirmed from 2 independent studies on fetal genotype.[118,119] Another confounding factor is pregnancy history and the severity of the pregnancy complication, which significantly impact the recurrence and occurrence of pregnancy complications in subsequent pregnancy, without considering thrombophilia.[120]

More recent larger prospective studies have suggested lower rates of recurrence. For example, women who had a first pregnancy loss and who were selected from 2 family cohorts of first-degree relatives of probands with FVL or PGM and a history of documented VTE or premature atherosclerosis were prospectively followed in a second pregnancy.[121] Their risk of loss of the subsequent pregnancy was higher than in women with a successful first pregnancy (25% vs 12%, RR 2.0; 95% CI: 1.4-3.0). The live birth rate of the second pregnancy after an early first loss (≤12 weeks of gestation) was 77% (95% CI: 62-87) for carriers and 76% (95% CI: 57-89) for noncarriers (RR 1.0; 95% CI: 0.8-1.3). After a late first loss (>12 weeks), the live birth rates were 68% (95% CI: 46-85) and 80% (95% CI: 49-94) for carriers and noncarriers, respectively (RR 0.9; 95% CI: 0.5-1.3). In another prospective cohort study of 2480 patients with recurrent pregnancy loss, patients with FVL who had fetal loss had a favorable 98% live birth rate in subsequent pregnancy.[122]

Regarding FVL and PGM and the risk of composite pregnancy complications in prospective studies, results have been mostly negative. For example, in the systematic review and meta-analysis comprising 4 prospective cohort studies, Rodger et al demonstrated no significant association between FVL (OR 1.08; CI: 0.87, 1.35) or PGM (OR 1.27; CI: 0.94, 1.71) and a composite of placenta-mediated complications.[89] These results are in contrast to 1 prospective cohort study of nulliparous women (n = 2034), which demonstrated that the PGM was associated with an increased risk of a composite of adverse pregnancy outcomes (OR 3.58; 95% CI: 1.20-10.61).[123]

Prevention of Adverse Pregnancy Outcomes in the Setting of Thrombophilia

Heparin and aspirin administration is the best strategy for the treatment of recurrent pregnancy loss associated with APAS, according to the Cochrane review of 2002.[124] This approach has been associated with a 54% reduction in pregnancy loss and is better than aspirin alone. Steroid administration is associated with an excessive risk of prematurity and therefore is not recommended as a first-line prevention strategy. However, the most recent evidence suggests that although UFH and aspirin confer a significant benefit in live births in APAS, the efficacy of LMWH plus aspirin remains unproven, highlighting the urgent need for large controlled trial.[125]

Initial published studies conducted on inherited thrombophilia showed benefit using LMWH and low-dose aspirin,[126-131] but the low number of patients and inadequate study design of such studies did not permit firm recommendations regarding the antenatal administration of any prophylaxis for the sole indication of the prevention of APO.[132] According to a cochrane review,[133] based upon an extensive literature search for 1966 to 2004, women with a history of 2 or more spontaneous losses or 1 fetal demise without apparent cause other than inherited thrombophilia, only 2 trials were available for review. The first study was conducted by Gris et al[47] and compared administration of low-dose aspirin (100 mg/d) with 40 mg/d enoxaparin from the 8th week of gestation in a cohort of patients with a prior loss after 10 weeks and the presence of heterozygous FVL, PGM, or PS deficiency. The authors found that 23 of 80 patients treated with aspirin and 69 of 80 patients treated with enoxaparin had a successful pregnancy (OR 15.5; 95% CI: 7-34; $P < .0001$). Birth weights were higher, and there were less SGA infants in the enoxaparin group. The study has been criticized for its randomization strategy.[130] The other study was the trial reported by Tulppala et al,[134] which involved 82 patients and compared 50 mg aspirin with placebo starting at the time of positive urine pregnancy test, in women with 3 or more unexplained consecutive losses. No differences

were noted in the aspirin compared with the placebo group (RR 1.00 [0.78-1.29]). Regarding prevention of recurrent fetal loss, 2 excellent randomized, placebo-controlled trials have recently been completed. Kaandorp and colleagues conducted a randomized trial, in which these investigators enrolled 364 women between the ages of 18 and 42 years who had a history of unexplained recurrent miscarriage and were attempting to conceive or were less than 6 weeks pregnant.[135] Patients were randomly assigned to receive daily 80 mg of aspirin plus open-label subcutaneous nadroparin (at a dose of 2850 IU, starting as soon as a viable pregnancy was demonstrated), 80 mg of aspirin alone, or placebo. Live birth rates did not differ significantly among the 3 study groups. The proportions of women who gave birth to a live infant were 54.5% in the group receiving aspirin plus nadroparin (combination-therapy group), 50.8% in the aspirin-only group, and 57.0% in the placebo group (absolute difference in live birth rate: combination therapy vs placebo, −2.6 percentage points; 95% CI: −15.0 to 9.9; aspirin only vs placebo, −6.2 percentage points; 95% CI: −18.8 to 6.4). Neither aspirin combined with nadroparin nor aspirin alone improved the live birth rate, as compared with placebo, among women with unexplained recurrent miscarriage, irrespective of thrombophilia status. Clark and colleagues conducted a multicenter, randomized controlled trial in the United Kingdom and New Zealand to determine whether treatment with enoxaparin and low-dose aspirin, along with intensive pregnancy surveillance, reduced rate of pregnancy loss compared with intensive pregnancy surveillance alone in women with history of 2 or more consecutive previous pregnancy losses.[136] Participants (n = 294) presenting for initial antenatal care at fewer than 7 weeks' gestation with history of 2 or more consecutive previous pregnancy losses at 24 or fewer weeks' gestation and no evidence of anatomic, endocrine, chromosomal, or immunologic abnormality were randomly assigned to receive either enoxaparin 40 mg subcutaneously and 75 mg of aspirin orally once daily along with intense pregnancy surveillance or intense pregnancy surveillance alone from random assignment until 36 weeks gestation. Of the 147 participants receiving pharmacologic intervention, 32 (22%) pregnancy losses occurred, compared with 29 (20%) losses in the 147 patients receiving intensive surveillance alone, giving an OR of 0.91 (95% CI: 0.52-1.59) of having a successful pregnancy with pharmacologic intervention. These investigators observed no reduction in pregnancy loss rate with antithrombotic intervention in pregnant women with 2 or more consecutive previous pregnancy losses. Collectively, the results of these 2 studies confirm the need for well-conducted randomized trials in determining the optimal approach to prevent recurrent miscarriage and clinicians should await results of such studies before routinely obtaining inherited thrombophilia evaluations and placing patients on anticoagulation prophylaxis regimens to solely to prevent placenta-mediated complications.

Finally, in 2011 De Vries and collaborators conclude de FRUIT randomized controlled trial in the Netherlands and published their results. They randomized 139 women with previous preterm delivery <34 weeks, early-onset hypertensive disorders of pregnancy or SGA and inheritable thrombophilia in 2 arms receiving either LMWH with low-dose aspirin or aspirin alone. All participants were less than 12 weeks of gestation at enrollment and were negative for APA. The investigators found a reduction in risk of recurrent hypertensive disease before 34 weeks of 8.7% (CI: 1.9-15.5) with a number needed to treat of 12. This reduction disappeared when considered recurrent hypertensive disease irrespective of gestational age.[137]

In conclusion, this study suggests that a well-selected population with history of previous severe complication early in pregnancy could benefit from a prophylactic treatment. Anyway, we still need large-scale prospective association studies and clinical trials to provide the most robust evidence to guide practice patterns,

Table 4. Screening for Inherited and Acquired Thrombophilia.

PC (activity)
PS (free antigen level)
ATIII (activity)
Factor V Leiden (PCR)
Prothrombin gene mutation 20210A (PCR)
Homocysteine, fasting
Platelet count
Lupus anticoagulant
Anticardiolipin antibody IgG, IgM, and IgA
beta II glycoprotein I IgG, IgM, and IgA

Abbreviations: ATIII, Antithrombin III; Ig, immunoglobulin; PC, protein C; PCR, polymerase chain reaction; PS, protein S.

Thrombophilia Screening: Testing and Candidates

Recognizing that there is controversy over several aspects of thrombophilia as detailed in this chapter, Table 4 shows which test to perform if an evaluation of inherited and acquired thrombophilia is indicated. The recommendation for thrombophilia continues to evolve. Routine screening is not advised, and a tailored approach is advised at this time.[138] Antenatal administration of heparin anticoagulation for the sole indication of preventing pregnancy complications, irrespective of thrombophilia, should still be considered experimental, until randomized trials are completed.[139]

Management of Acquired and Inherited Thrombophilias: Prevention and Treatment Strategies

Guidelines summarized by Bates et al provide 1 approach to anticoagulation in obstetrical patients.[140] For pregnant women with no history of VTE who are homozygous for FVL or PGM, postpartum prophylaxis for 6 weeks with prophylactic or intermediate dose LMWH or vitamin K antagonists is indicated. If these patients have a positive family history for VTE, antepartum prophylaxis with LMWH is suggested (level of evidence: grade 2B). For patients with all other thrombophilias and no prior VTE,

antepartum clinical vigilance is suggested. Postpartum prophylaxis is indicated only in case of a positive family history for VTE (level of evidence: grade 2C). For women with inherited thrombophilia and a history of pregnancy complications, they suggest not to use antithrombotic prophylaxis (level of evidence: grade 2C). For women at high risk of preeclampsia, antepartum low-dose aspirin is recommended. For patients meeting criteria for APAS with a history of recurrent early pregnancy loss or unexplained late pregnancy loss, and no history of VTE, antepartum administration of prophylactic or intermediate-dose UFH or prophylactic LMWH combined with aspirin is recommended. For women with 2 or more miscarriages but without APLA or thrombophilia, they recommend against antithrombotic prophylaxis (level of evidence: grade 1B). During pregnancy, LMWH is preferred over UFH.

Conclusions

Hereditary and acquired thrombophilic mutations comprise a heterogenous group of conditions. Potential complications include venous and, in some cases, arterial thromboembolism. Prospective cohort studies have demonstrated that common inherited thrombophilias conditions, such as FVL and PGM, are weakly, if at all, associated with a variety of placenta-mediated complications, such as early or late pregnancy loss, preeclampsia, abruption, and fetal growth restriction. Pregnancies complicated by thrombophilic conditions require an individualized approach to their antepartum and postpartum care, after a comprehensive assessment of all of their risk factors. At this time, recommendations for managing patients should be based upon the available data from adequately conducted, completed, randomized clinical trials. Other recommendations are available from consensus panel documents. Several randomized trials are underway to determine the optimal prevention and management strategies in patients at risk of placenta-associated complications, with and without thrombophilias.

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