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MODERN TRENDS

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Thrombophilia-associated pregnancy wastage

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Objective: To critically review the literature regarding inherited thrombophilia and recurrent fetal loss. **Design:** English-language literature review.

Patient(s): Women who experienced repeated pregnancy wastage.

Intervention(s): Aspirin, glucocorticoids, heparin, and IV immunoglobulin for the prevention of miscarriage. **Main**

Outcome Measure(s): Live birth, miscarriage, preeclampsia, and pregnancy loss.
Result(s): Recurrent fetal loss and other placental vascular pathologies of pregnancy have long been associated with antiphospholipid syndrome, an acquired autoimmune thrombophilic state. The number of known heritable thrombophilic disorders has grown rapidly in recent years with the identification of activated protein C resistance, factor V Leiden mutation, and hyperhomocysteinemia as major causes of thrombosis. Data accumulated over the past 2 years suggest that heritable thrombophilia is associated with an increased risk of fetal loss and preeclampsia. The present review discusses potential pathogenetic mechanisms for this association and evaluates reported therapeutic regimens for the prevention of fetal loss in women with thrombophilia.
Conclusion(s): Placental thrombosis may be the final common pathophysiologic pathway in most women with habitual abortions and repeated pregnancy wastage. Prophylactic antithrombotic therapy is indicated in women with heritable thrombophilia and antiphospholipid syndrome and probably is more effective than the previously used modalities of prednisone, aspirin, and IV immunoglobulin. (Fertil Steril® 1999;72:765-74. ©1999 by American Society for Reproductive Medicine.)
Key Words: Thrombophilia, pregnancy wastage, factor V Leiden, hyperhomocysteinemia, anticardiolipin, protein S, protein C, antithrombin III, MTHFR, factor II

A full understanding *of* the inherited thrombophilias is becoming increasingly important in the management *of* high-risk gestations. These disorders are not only associated with an increased risk *of* thromboembolic disease during gestation and the puerperium but also with an increased incidence of preeclamptic toxemia, placental abruption, and poor obstetric outcome.

The successful outcome of pregnancy is dependent on the development of adequate placental circulation. Abnormalities of the placental vasculature may result in various gestational pathologies, including first- and second-trimester abortions, intrauterine growth retardation, intrauterine fetal death, and preeclampsia (1).

Habitual abortions (defined as ?3 spontaneous consecutive pregnancy losses) affect 1%-2% of women of reproductive age, and up to 5% have ?2 recurrent abortions. Interest in an acquired thrombotic autoimmune cause of recurrent pregnancy wastage has increased greatly with the discovery *of* the association *of* antiphospholipid (APL) antibodies, lupus anticoagulant (LAC), and anticardiolipin (ACL) antibodies with recurrent pregnancy loss (2-6).

Heritable thrombophilias are a group *of* genetic disorders *of* blood coagulation that result in an increased risk *of* thrombosis. Although these disorders have been clearly associated with venous thromboembolism, the role *of* most thrombophilic states in arterial thrombosis is less well established (7-12).

Several reports over the last 2 years have suggested a potential association *of* heritable thrombophilia with certain gestational pathologies (Table 1). The aim *of* the present report was to review recent data concerning thrombophilia and vascular placental pathology, to discuss potential pathophysiologic mechanisms

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TABLE 1

Placental vascular complications associated with thrombophilia.

Thrombophilia	Miscarriage	IUFD	Preeclampsia	HELLP
Antithrombin III deficiency	++	++		
Protein C deficiency	+	++		
Protein S deficiency	+	++		
Dysfibrinogenemia	+	+		
APC resistance	+	++	++	
Factor V Leiden mutation	++	++	++	+
Hyperhomocysteinemia	+	+	+	+
Factor II mutation		+		
Antiphospholipid syndrome	++	++	++	+
Combined defects	++	++	+	+

Note: Degree of association: + = possible association; ++ = definite association. APC = activated protein C; HELLP = hemolysis, elevated liver enzyme levels, low platelet count; IUFD = intrauterine fetal death.

Blumenfeld. Thrombophilia-associated pregnancy wastage. Fertil Steril 1999.

for this association, and to assess available therapeutic modalities for the prevention of placental vascular thrombosis to maximize successful gestational outcome.

ANTIPHOSPHOLIPID SYNDROME

In the last decade, autoimmune factors have been recognized as having a pathophysiologic role in recurrent pregnancy loss, even in women without clinically diagnosed autoimmune disease (1-6). Interest in an autoimmune cause of recurrent pregnancy wastage has increased greatly with the discovery of the association of APL antibodies with recurrent pregnancy loss (1-6). Approximately 10% of all patients who experience recurrent pregnancy loss have LAC, whereas ACL antibodies are found in 10%-13% of these patients (1-6, 12). Most of these patients are asymptomatic, without characteristics of an autoimmune disease (1-6, 12).

In one review, 65 LAC-positive, untreated women experienced spontaneous abortion or fetal death in 95% of their 242 gestations (11). In untreated LAC-positive women, a successful pregnancy with a healthy, live, full-term neonate is an unusual event, estimated to occur in <15% of cases (1, 3).

Lupus anticoagulant also has been associated with other obstetric problems, including fetal growth retardation, early onset severe preeclamptic toxemia, and chorea gravidarum (3, 9, 12). In addition, a severe postpartum syndrome has been described in LAC-positive patients that consists of fever, cardiac involvement, and pleural effusion (3, 13).

Closely related to LAC, ACL antibodies are APL antibodies detected by immunoassays that use cardiolipin (diphosphatidyl glycerol) as the solid phase (1, 3). The exact relation of ACL antibodies to LAC is still somewhat controversial (1, 3). In one report (14), it was found that 49% of patients with ACL antibodies had LAC, whereas in another report (15), all patients with LAC had ACL antibodies. However, not all women who had ACL antibodies also had LAC (3). Moreover, it has been suggested that LAC and ACL antibodies form two distinct but related subgroups of antibodies (16, 17). One interpretation of these conflicting data is that these two tests measure the same antibody with different degrees of sensitivity (1, 3).

In this context, LAC may be a subgroup of ACL antibodies (1, 3). Although ACL antibodies, like LAC, have been associated with recurrent fetal loss, thrombosis, thrombocytopenia, and neurologic disturbances (16-18), no distinct pathophysiologic role has been attributed to ACL antibodies, perhaps because they have not been studied as intensively as LAC (1, 3). Numerous investigators (1-6, 10-22) have noted the frequent association of fetal death with LAC, ACL antibodies, or both.

However, Simpson et al. (22) recently challenged the role of APL and ACL antibodies as a main causative pathophysiologic factor in first-trimester abortions. Their hypothesis was that a major pitfall in many previous studies was the fact that serum samples usually were obtained only from patients who already had experienced a pregnancy loss. Such an approach may not allow one to determine whether autoimmune antibodies were unrelated to, the result of, or the cause of pregnancy loss. These investigators obtained blood samples either before pregnancy or very early in pregnancy. They could not find an association between pregnancy loss and the presence of ACL or APL antibodies (22).

Nevertheless, to avoid confusion, it should be emphasized that in contrast to the controversy concerning the potential association between first-trimester pregnancy loss and ACL or APL antibodies, general consensus supports an association between midtrimester losses and adverse perinatal outcome (5, 11, 22, 23).

A difficulty in comparing studies is that the assays for ACL and APL antibodies are not unequivocally standardized (22, 24, 25). Rebar et al. (24) reported that qualitative positivity for immunoglobulin (Ig) G ACL antibodies ranged from 31%-60% among different tested kits, whereas the range was 6%-50% for IgM ACL antibodies. In addition, APL and ACL antibodies may not persist, especially IgM antibodies (22, 26).

Because both ACL antibodies and LAC react with phospholipids, terms for antibody to either have been used inappropriately interchangeably (3, 11). Lockshin et al. (18) compared the concordance and predictive ability for fetal death of the activated partial thromboplastin time and of IgG and IgM antibodies to cardiolipin in 50 pregnant women with systemic lupus erythematosus or LAC (3). Fetal death occurred in 77% of the patients with abnormal ACL antibody levels compared with only 5% of the patients with normal ACL antibody levels (18).

The calculated sensitivity for predicting fetal death was 0.55 for the activated partial thromboplastin time (LAC) versus 0.85 for the ACL antibodies; the specificity was 0.81 and 0.92, respectively (3, 18). These investigators (18) concluded that the measurement of ACL antibodies a better assay for the prediction of intrauterine fetal demise and was a more sensitive and specific test than the measurement of LAC.

The APL antibodies are a family of Igs that react with anionic phospholipids or anionic phospholipids-protein complex (16). Anticardiolipin antibody, the best known and most commonly examined antibody, is only one member of the APL antibody family, which also includes antiphosphatidylserine, antiphosphatidic acid, antiphosphatidylinositol, antiphosphatidylcholine, antiphosphatidylethanolamine, and reagin.

These Igs may be of IgG, IgM, or IgA isotypes. Patients often have a mixture of isotypes with differing reactivity toward negatively charged phospholipids (16).

A perplexing observation has been the marked intra-individual and interindividual heterogeneity of these antibodies (16). The laboratory heterogeneity can be explained in part by the varying specificity and sensitivity of laboratory tests (15, 16).

It was initially believed that thrombosis played a critical role in recurrent spontaneous abortions and intrauterine fetal death (16). Early reports stressed the placental insufficiency associated with extensive placental infarction (16). However, more recent studies have not unanimously confirmed the high frequency of placental infarction (1, 16, 18).

The placenta often is small for the gestational age (16). Retarded placental growth most likely is secondary to impaired blood flow, even though there is no evidence of infarction (16). Indeed, we and others have found impaired umbilical and uterine artery flow in pregnant women with systemic lupus erythematosus and the antiphospholipid syndrome (APLS) (3, 10, 21). The lack of appropriate trophoblastic invasion of the spiral arteries may explain the placental insufficiency (16). Placental bed biopsies have demonstrated marked atherosclerosis in the spiral arteries of women with APL antibodies (16, 27, 28). This accelerated atherosclerosis in some respects is similar to the accelerated atherosclerosis that develops after cardiac transplantation, coronary artery bypass surgery, and angioplasty (16).

It has been speculated (16) that a common link exists between these seemingly disparate states that may be explained by antibody-mediated vascular damage.

An attractive recent hypothesis is that APL antibodies decrease annexin V and thus may lead to coagulation. A study by Rand et al. (28) offers evidence that endogenous annexin V, whose physiologic function previously was unknown, has an antithrombotic role at the interface of trophoblasts and endothelial cells with circulating blood. The APL antibody-induced reduction in the level of annexin V at the apical surface of trophoblasts and endothelial cells may account for the thrombosis associated with the APLS (28).

Nevertheless, until 1996, approximately 30%-50% of all cases of spontaneous abortion had no identified cause. It has been suggested that these patients can be divided into two groups: those who exhibit hypofibrinolysis related to abnormal plasma levels of activators and inhibitors of fibrinolysis (10, 29, 30) and those who exhibit no apparent anomalies of hemostasis.

It appears that these patients may have a functional anomaly of the vascular endothelium characterized by high plasma levels of von Willebrand factor, tissue plasminogen activator, and plasminogen activator inhibitor-i, which may be associated with an increase in thrombin formation (10, 29, 30). This anomaly may lead to poor placental implantation or a very early insufficiency of the fetal-maternal circulation, because the placenta itself is the cause of increased thrombin formation. Thus, the reduction in thrombin formation could intuitively allow the reestablishment of a favorable hemostatic balance and encourage not only early placentation but potentially also carriage of the gestation to term.

INHERITED THROMBOPHILIA

The number of heritable thrombophilic disorders has grown rapidly since the original description of familial antithrombin III deficiency in 1965 (31). The first description of deficiency in the vitamin K- dependent factor protein C in 1981 (32) was followed shortly by reports on deficiencies of protein S, another vitamin K-dependent protein. The combined activity of activated protein C (APC) and protein S localized on platelet phospholipid surfaces results in the degradation of factor V to Vi and of factor VIII to VIIIi, significantly attenuating coagulation and fibrin formation (33).

In 1993, Dahlback et al. (34) suggested that the APC resistance observed with the plasma-based clotting assay is a common finding in patients with heritable thrombophilia. Shortly afterward, it was found that in most cases, APC resistance is associated with a point mutation R506Q at the cleavage site of factor V that results in factor V Leiden (35).

This mutation is the most common heritable thrombophilia in whites; it affects 1%-10% of the members of different subpopulations (36) but is not found in Native American or Mongoloid subpopulations (37). The mutation recently was ascribed to a single common origin in the white population (38).

ACTIVATED PROTEIN C RESISTANCE AND PREGNANCY WASTAGE

It is of interest that although factor V Leiden can be found in 20%-40% of patients with venous thrombosis (39, 40),

this mutation is found in 60% of patients with gestational thrombosis (41) and is a major cause of the thrombosis associated with the use of oral contraceptives (42). Moreover, it is now well established that acquired APC resistance without factor V Leiden is a common finding in patients with LAC or the APLS (43, 44), disorders that have long been associated with placental thrombosis and fetal loss. The high prevalence of the mutation and its major role in gestational thrombosis set the stage for studies examining placental thrombosis and fetal loss in patients with APC resistance and factor V Leiden.

Several recent reports have dealt with this association (Table 1). First, a preliminary report demonstrated an increase in second-trimester but not first-trimester fetal losses in women who had APC resistance diagnosed by a low APC sensitivity ratio with a plasma-based clotting assay compared with controls who had a normal APC sensitivity ratio (45). The EPCOT study, a multicenter collaborative evaluation of patients with heritable thrombophilia for the presence of fetal loss, recently revealed that although first- and second-trimester miscarriages were not more common in an unselected population of thrombophilic patients with factor V Leiden, the odds ratio for stillbirth was increased by twofold in patients with this mutation compared with controls (46).

In another report from our center, 39 consecutively seen patients referred for evaluation of recurrent fetal loss of unknown cause were studied for APC resistance and factor V Leiden mutation (47). Nineteen (48%) of the 39 patients had factor V Leiden mutation (16 heterozygous and 3 homozygous) (47). Five of the 19 patients had a history of venous thrombosis, which in all cases was temporally related to pregnancy or the postpartum period (4 cases) or to lowdose estrogen therapy (1 patient). Of the 128 pregnancies experienced by these patients, 25 (19%) ended in live births; 9 (7%) of the 25 infants were premature and 16 (12%) were delivered at term (47). More than half the 128 gestations ended in early spontaneous abortions and 15% in secondtrimester abortions. Nine (47%) of 19 patients had at least 1 pregnancy with an intrauterine fetal death, which was associated in 2 patients with the syndrome of hemolysis, elevated liver enzyme levels, and a low platelet count (HELLP) (48).

Nine (23%) of the 39 women had a low APC sensitivity ratio (range, 1.76-2.05) without factor V Leiden mutation. These 9 patients with a low APC sensitivity ratio without factor V Leiden mutation also had significantly worse pregnancy outcomes compared with women who had a normal APC sensitivity ratio. These results suggest that APC resistance and factor V Leiden mutation are not uncommon in women with recurrent fetal loss and should be searched for particularly in patients who experience intrauterine fetal death (10).

The difference between the EPCOT study (46) and our results (47) stems from the definition of the study popula

tions. Unselected patients with factor V Leiden were included in the EPCOT study, whereas only patients with recurrent fetal loss of unknown cause were evaluated in our study (10, 47). Conceivably, only a few patients with factor V Leiden may experience fetal loss. Similar findings can be found in patients with APL antibodies. Although the APLS, a common autoimmune thrombophilic state, is well known to be associated with vascular placental abnormalities (49), it should be noted that most patients with APL antibodies do not have gestational abnormalities (5, 22).

Two case-control studies from Italy (50) and the United States (51) recently confirmed that factor V Leiden mutation is associated with an increased risk of fetal loss. The study by Grandone et al. (50) demonstrated a prevalence of 16% of factor V Leiden in women with ?2 unexplained fetal losses compared with 4% in controls, and the study by Ridker et al. (51) showed a 2.3-fold increase in the relative risk of fetal loss in women with factor V Leiden mutation.

Heritable deficiencies of the vitamin K-dependent natural anticoagulant, protein C, and of protein S, recently have been associated with an increased risk of gestational abnormalities. Two studies (46, 52) suggested that the increased risk was particularly significant for intrauterine fetal death.

ANTITHROMBIN III DEFICIENCY

Antithrombin III deficiency has long been associated with a significant thrombotic tendency throughout gestation and the puerperium; up to 70% of women with antithrombin III deficiency experience thrombosis during this period (53). In addition, an acquired decrease of antithrombin III plasma levels is a common finding in patients with preeclampsia (10). However, it was demonstrated only recently that hereditary antithrombin III deficiency is associated with miscarriages and intrauterine fetal death (46, 52).

Antithrombin III is a naturally occurring anticoagulant. It is a serine protease inhibitor that inactivates thrombin and factors IXa, Xa, XIa, and XIIa, and thereby limits the coagulation cascade. In conjunction with naturally occurring heparins, this inactivation is enhanced by a factor of 40,000 (therefore, postthrombosis heparin anticoagulation of a patient with antithrombin III deficiency has only a limited effect). Antithrombin III deficiency is a heterogeneous disorder, caused by >80 different mutations, most inherited in an autosomal dominant mode. Type I is the most common and results in both quantitative and qualitative reductions; type II results in a functional change only (53).

All subtypes have the same risk of thrombosis, except for type IIC, an abnormality of the heparin binding site, in which the risk is only 6% in the heterozygous state (53). In the rare type IIC homozygote individual, however, there is a much greater risk of thrombosis, which may involve the arterial circulation (53, 54). Antithrombin III deficiency is the most thrombogenic of all the inherited isolated heterozygous

thrombophilias, with at least a 50% chance of thrombosis over a lifetime (53). The risk of thrombosis during pregnancy has been estimated to be 51% (53), although this may be an overestimate in light of the difficulty in quantifying the number of undiagnosed parturients with uncomplicated pregnancies. The prevalence of antithrombin III deficiency is between 1:600 and 1:5,000 (53); it accounts for up to 5% of thromboses in selected groups of patients (53, 55).

Sanson et al. (52) reported an increase in fetal loss in women with antithrombin III deficiency. In the EPCOT study (46), antithrombin III deficiency was associated with the highest risk for miscarriage and with a 5.2-fold increase in stillbirth, the highest of all isolated thrombophilic states. These data are consistent with the severe gestation-associated thrombotic tendency observed in antithrombin III-deficient women and emphasize the importance of prophylactic anticoagulation in this setting (10, 53).

FIBRIN FORMATION AND STABILIZATION

The final steps of the coagulation pathway are the transformation of fibrinogen to fibrin, fibrin polymerization, and cross-linking of fibrin by activated factor XIII. Hereditary abnormalities of fibrinogen have been associated with fetal loss (56). Twenty percent of all reported dysfibrinogenemias are manifested by thrombosis (57).

In a recent survey of 15 women with hereditary dysfibrinogenemia associated with thrombosis, a high incidence of gestational abnormalities was noted (57). Of 64 pregnancies, only 34 (52%) ended in normal delivery; miscarriages were documented in 39% and stillbirths in 9% (57). Seven of the 15 women experienced postpartum thrombosis, which emphasizes again the link between thrombophilia, gestation-associated thrombosis, and fetal loss (10, 53, 57).

However, it should be noted that fetal loss has been reported in patients with dysfibrinogenemia even without thrombosis, as well as in patients with hypofibrinogenemia and factor XIII deficiency (56, 58), suggesting that additional mechanisms other than placental thrombosis may operate in these patients.

HYPERHOMOCYSTEINEMIA

Homocysteine is derived from dietary methionine and is present in plasma in low concentrations of 5-15 μmol/L. Within cells, homocysteine is transsulfurated to cystathionine by cystathionine (3-synthase or remethylated to methionine by a pathway involving methylene tetrahydrofolate reductase (MTHFR) and methylene synthase (53). Folic acid, vitamin B_6, and vitamin B_{12} are important cofactors that participate in these metabolic pathways (59). Inherited deficiencies of enzymes of these pathways lead to homozygous hyperhomocysteinemia, which is associated with
plasma homocysteine levels of >50 μmol/L and characterized by thromboembolism and atheroma progression in childhood (60). Nutritional deficiencies of folic acid, vitamin **B_6,** and vitamin B_{12} are the main causes of acquired mild to moderate (15-50 μmol/L) hyperhomocysteinemia (61).

The most accurate and predictive laboratory method for diagnosing hyperhomocysteinemia is a fasting homocysteine level or a methionine loading test. The latter is more cumbersome but may show less intra-individual variation (53); as yet, there is no consensus. The cutoff point in the latter test is >50 μmol/L for premenopausal women (53). Methionine loading should be performed with caution, particularly during pregnancy, because of evidence that the subsequent acute hyperhomocysteinemia may cause endothelial dysfunction (53, 62).

Initially, homocystinuria (resulting in severe hyperhomocysteinemia of >100 μmol/L) was recognized (53). This is an autosomal recessive condition that results from homozygous cystathionine synthase deficiency or homozygous MTHFR deficiency associated with a phenotype that includes premature atherosclerosis and early recurrent venous thrombosis (53).

Mild (16-24 μmoUL) and moderate (25-100 μmol/L) fasting hyperhomocysteinemia may be due to either heterozygous cystathionine synthase deficiency or homozygosity for the thermolabile mutant of MTHFR (53). The latter condition occurs in approximately l l% of Europeans (63). It is now apparent that although such individuals do not have the general phenotypic increased risk of atherosclerosis and venous thrombosis, for women, the risk of bearing an offspring with a neural tube defect or experiencing recurrent pregnancy wastage is increased (53).

Theoretically, common mutations or polymorphisms in one of the methionine pathway enzymes may lead to hyperhomocysteinemia. Such a mutation recently has been demonstrated in MTHFR. This C677T substitution leads to a thermolabile MTHFR variant that has 50% abnormal activity. Homozygosity for this mutation can be found in 5%15% of normal individuals across different ethnic populations (64). A large body of clinical and experimental evidence suggests an association between hyperhomocysteinemia and arterial thrombosis (65). Several studies have suggested a threefold increase in the risk of cardiovascular disease in subjects who are homozygous for thermolabile MTHFR, particularly in association with nutritional deficiency (66).

This brings into focus the concept that multiple hereditary and acquired risk factors may act synergistically to increase the expression of thrombosis (53). Indeed, the Hordaland study (67) recently demonstrated that most subjects with homocysteine levels of >40 p.mol/L had the C677G mutation combined with low folate status. A number of studies

have clearly suggested that folic acid supplementation may decrease and often normalize homocysteine levels in hyperhomocysteinemic patients (53).

Folic acid deficiency is common in pregnancy and, like the MTHFR mutation, has been associated with an increased risk of neural tube defects (68). Mild hyperhomocysteinemia has been associated with spontaneous abortions, placental infarctions, and abruptio placentae (68). Prospective placebo-controlled studies are indicated to assess fetal outcome after folic acid supplementation in patients with hyperhomocysteinemia.

We recently studied (27) the prevalence of homozygous thermolabile MTHFR in 76 women with fetal loss and 106 controls and found a borderline increase in the relative risk of fetal loss (relative risk = 2.2; 95% confidence interval, 0.6-8.0). Women >30 years of age who present with a first episode of venous thrombosis are seven times more likely to have increased homocysteine levels than matched controls (53, 69).

In a group of patients with recurrent thrombosis, hyperhomocysteinemia was 2-3 times more common than in a control group (70). The specific association of hyperhomocysteinemia and thrombosis in pregnancy has not been settled unequivocally to date (53). Before diagnosis of the condition is attempted during pregnancy, normal levels of homocysteine need to be established, unless the polymerase chain reaction is used to detect the G667CT thermolabile mutation directly (53).

Women who have experienced ?2 consecutive pregnancy losses before 17 weeks of gestation are 2-3 times more likely to be homozygous for the thermolabile variant of MTHFR than are matched control women with successful pregnancy outcomes (71). This is associated with higher fasting and postloading homocysteine levels and lower folate concentrations (72). It is unknown whether folate supplementation reduces the risk of pregnancy loss (53).

Dekker et al. (73) found that 18% of parturients with severe early onset preeclamptic toxemia had a positive methionine loading test result. More recently, the same group found hyperhomocysteinemia in 26% of women who had placental abruption, 11% of those who experienced intrauterine fetal death after 16 weeks of gestation, and 38% of those who were delivered of an infant whose weight was below the fifth percentile, compared with an estimated 2%-3% of the general control population (74).

FACTOR II G20210 MUTATION

In 1996, Poort et al. (75) reported that polymorphisms in the prothrombin gene are associated with an increased risk of venous thrombosis. This finding has been verified by other investigators. Factor II mutation is found in 1%-3% of the normal population, with a higher incidence in Southern Europe and Israel (76). In a study of 76 women with fetal loss (27), factor II mutation was found in 7.8% compared with 3.8% of 106 controls (relative risk = 1.95; 95% confidence interval, 0.83-46).

COMBINED THROMBOPHILIC DEFECTS

Data that have emerged over the past 4 years suggest that manifested thrombophilia often is a multigenic disorder. Thus, coexistence of factor V Leiden with protein C mutation or with protein S mutation may increase the likelihood of expression of thrombosis (77-80). Likewise, co-inheritance of factor V Leiden and hyperhomocysteinemia may be manifested by severe thrombotic events (81). Of particular interest, the coexistence of factor V Leiden and homozygous hyperhomocysteinemia (81) and the combination of factor V Leiden and the familial APLS (82) may result in thrombosis and recurrent fetal loss. It is not surprising therefore that the EPCOT study revealed the highest odds ratio for stillbirth (14.3; 95% confidence interval, 2.4-86) in patients with combined thrombophilic defects (46).

Dekker et al. (73) found a variety of thrombophilic defects in 85 women with a history of severe early onset preeclampsia, including protein S deficiency (25%), ACL antibodies (29%), APC resistance (6%), and hyperhomocysteinemia (18%). It is of interest that 13 patients had combinations of thrombophilic defects that emphasized the role of combined thrombophilia in observed placental pathology (73). In our recent study of 76 women with fetal loss (27), 6 (7.9%) had combined thrombophilic polymorphism compared with 1 (0.9%) of 106 controls (P<.02).

ACTIVATED PROTEIN C RESISTANCE AND PREECLAMPSIA

There is ample evidence that thrombosis in placental vessels results in placental infarctions in patients with preeclampsia. Activation of blood coagulation and endothelial cell stimulation and injury are basic and fundamental findings in preeclampsia (83, 84).

Activated protein C resistance and factor V Leiden recently have been associated with early onset severe preeclampsia (73, 85, 86). Dekker et al. (73) reported that 16% of women with severe early onset preeclampsia were APC resistant. In another study, 14 (8.9%) of 158 women with severe preeclampsia were heterozygous for the factor V Leiden mutation compared with 17 (4.2%) of 403 normotensive controls (P=.03) (85).

The HELLP syndrome is a severe presentation of preeclampsia manifested by hemolysis, elevated levels of liver enzymes, and a low platelet count. A potential association between factor V Leiden and HELLP syndrome in two women has been reported (48). Therapy with low-molecular-weight heparin throughout pregnancy in three successive

TABLE 2

Therapeutic modalities for the prevention of fetal loss in thrombophilic patients.

	Therapeutic modality				
Thrombophilia	Steroids	Aspiri	Heparin	LMWH	Factor concentrates
Antithrombin III deficiency			+++	+++	
Protein C/protein S deficiency			+++	+++	
Factor V Leiden mutation		+++		+++	
Antiphospholipid syndrome	+	++	+++	+++	
Combined defects		+	+++	+++	

Note: Therapeutic benefit: + = equivocal; + + = substantial; +++ = high. LMWH = low-molecular weight heparin.

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pregnancies resulted in normal deliveries. It is of interest that 53% of the women with severe early onset preeclampsia in the report by Dekker et al. (73) had HELLP syndrome. Grandone et al. (86) recently showed a predisposition for preeclampsia in women with either the factor V Leiden or the thermolabile MTHFR mutation.

The finding of APC resistance without the factor V Leiden mutation in association with fetal loss and preeclampsia deserves an explanation. Acquired APC resistance is common in the APLS and has been suggested to play a major role in the pathogenesis of thrombosis in patients with this syndrome (43, 44). Antiphospholipid antibodies have a broad spectrum, with specificity for several targets, including the prothrombinase complex (LAC), endothelial cells, platelets, phospholipids, and cardiolipin. Selective APL antibodies directed toward the protein C/thrombomodulin/protein S/platelet phospholipid system may lead to APC resistance. In vitro data suggest that this could be the case (87). This hypothesis is strengthened by evidence from an animal model that thrombomodulin is essential for normal embryonic development (88).

THERAPEUTIC REGIMENS

Whereas most investigators agree on the causal association between the APLS and adverse pregnancy outcome, the optimal therapeutic modality has not been well defined. Treatment regimens suggested thus far include immunosuppression with glucocorticosteroids or high-dose IV immunoglobulin and antithrombotic therapy with antiplatelet agents and anticoagulants (Table 2).

Lubbe et al. (89) were the first to report the results of treatment with aspirin and prednisone; since then, many groups have adopted this therapy and the gestational success rate has improved from < 15% without therapy to 30%-78% with therapy (53, 90). Although many reports have shown a significant improvement in pregnancy outcome, the available data are limited by the small number of patients in individual studies involving various treatment protocols, which had not been compared prospectively. Moreover, information does not always exist concerning the number of prior miscarriages in each patient, the level of APL antibodies, and the presence or absence of previous thromboembolic phenomena.

The prolonged use of corticosteroids during pregnancy may be associated with significant maternal and fetal morbidity. Encouraging preliminary results from several nonrandomized trials suggest that aspirin alone in dosages as low as 75 mg/d is an effective therapy (91, 92). The role of corticosteroid therapy has been challenged by two recent studies that suggest that the improvement in outcome is mainly due to concomitant antithrombotic therapy (93, 94).

Most of the treatment trials of the APLS during pregnancy have been case-control or cohort studies (90). In one of the few prospective randomized trials, Cowchock et al. (94) conducted a multicenter study of 20 patients who were randomly assigned to receive treatment with either prednisone or low-dose heparin. A higher incidence of preterm delivery, premature rupture of the membranes, and preeclamptic toxemia was demonstrated in the group that received prednisone (90, 94). Silver et al. (93) conducted a study of 39 patients who were randomly assigned to receive treatment with both prednisone and aspirin or aspirin alone. The group that received both prednisone and aspirin had a significantly higher incidence of preterm delivery (93). Although neither of these studies (93, 94) had a placebo group, they suggest a better prenatal outcome with the use of aspirin or heparin than with prednisone (90).

Intravenous immunoglobulin has been prescribed to women with the APLS and fetal loss on an individual basis and success rates have ranged widely. In an experimental model, it was concluded that the passive transfer of ACL antibodies might have a direct effect on fetal outcome (95).

Therapy with IV immunoglobulin in pregnant women with the APLS results in transient suppression of LAC but not of ACL antibodies (96)c

The current focus of therapeutic modalities deals with the relative role of heparin compared with aspirin. A recent study demonstrated an increase in the rate of successful pregnancy from 19% without therapy to 70% with aspirin therapy in all women; heparin also was used in those with previous thrombosis (97)c

The reason that aspirin alone is effective in certain patients with the APLS but others respond only to anticoagulants is still unknown. In theory, an abnormally increased ratio of thromboxane A_2 to prostacyclin can predispose patients to thrombosis and abortions, and patients with this abnormality may respond to aspirin. Likewise, anticoagulants can be more useful in patients with the APLS who manifest APC resistance because of inhibition of the phospholipid-dependent activation of protein C by autoantibody (10)c However, these theoretic assumptions should be assessed by prospective randomized clinical trials.

The identification of several autoantibodies brings into focus the concept that the APLS and the habitual abortions associated with it may be caused by antibodies raised against one or more of the six different negatively charged phospholipids. Yetman and Kutteh (19) recently examined the prevalence of ACL antibodies compared with other APL antibodies in patients with recurrent pregnancy loss. Anticardiolipin antibodies were detected in 17c3% of patients with recurrent pregnancy loss compared with only 4% of the control population. Eighty-seven (10c1%) of the 866 women in this study (19) had no evidence of ACL antibodies but had elevated titers of another APL antibody. Isolated IgG-type antibodies were directed most frequently against phosphatidylinositol, cardiolipin, and phosphatidylethanolamine (19)c

From a practical point of view, when ACL antibodies are not identified in a patient with a clinical history of recurrent pregnancy loss, a search for LAC and other markers of thrombophilia (ice., APC resistance) should be performed before a therapeutic modality is selected.

Despite recent findings regarding the APLS and its treatment with aspirin alone or in combination with prednisone, a recent study (5) challenged the therapeutic efficacy of these agents. Laskin et al. (5) randomly assigned 202 women with at least one autoantibody (i.e., antinuclear antibody, DNA antibody, antilymphocyte antibody, ACL antibody, or LAC) and at least two previous unexplained pregnancy losses to receive either prednisone (0c5-0c8 mg/kg of body weight per day) and aspirin (100 mg/d) or placebo. Live infants were born to 66 women in the treatment group (56%, P=.19) (5)c More infants were born prematurely in the treatment group than in the placebo group (62% versus 12%, P<.001) (5)c These investigators concluded that therapy with prednisone and aspirin is not effective in promoting live birth and that it

increases the risk of premature birth in women with autoantibodies and recurrent fetal losses.

Two prospective randomized studies recently showed that therapy with heparin plus low-dose aspirin results in significantly better outcome than therapy with low-dose aspirin alone in patients with the APLS (98, 99)c In a study by Kutteh (98), viable infants were delivered in only 11 (44%) of 25 women who received aspirin and SC heparin (P<.05). In a study by Rai et al. (99), the rate of live birth in patients who were treated with low-dose aspirin and heparin was 71% (32 of 45 pregnancies) compared with only 42% (19 of 45 pregnancies) in women who were treated with aspirin alone (P<.01).

It should be emphasized that despite the progress obtained with current therapeutic regimens, complications still occur in a substantial number of pregnant women with the APLS. In the study by Rai et al. (99), one quarter of the successful pregnancies were delivered prematurely, suggesting that the optimal therapeutic regimen has yet to be established (10, 100)c

The role of low-molecular-weight heparin in this setting deserves study in prospective clinical trials. The advantages of low-molecular-weight heparin over unfractionated heparin are a higher antithrombotic ratio (which means less bleeding for a better antithrombotic effect), a longer half-life with the need for only one injection per day, a smaller injected volume, and less heparin-induced thrombocytopenia. A recent unpublished collaborative study demonstrated the safety of using low-molecular-weight heparin in >400 pregnant women. During the past 4 years, we treated 63 pregnancies in 42 women who presented with thromboembolism and/or recurrent fetal loss with the low-molecularweight heparin enoxaparin throughout gestation (Brenner B, Blumenfeld Z, unpublished data). The dosage used was 40 mg/d except in patients with combined thrombophilia or those with abnormal findings on Doppler velocimetry that suggested decreased placental performance, in whom the dosage was increased to 40 mg twice a day. In patients with a history of thrombosis, therapy was continued for 4-6 weeks after delivery. Forty-five (71%) of the 63 pregnancies resulted in live births. Larger prospective randomized trials are warranted to assess the potential advantages of lowmolecular-weight heparin compared with unfractionated heparin.

In conclusion, heritable thrombophilia and the APLS are major causes of fetal loss. Extensive laboratory evaluation of thrombophilia is indicated in women with recurrent fetal loss, particularly in cases of intrauterine fetal demise. Prophylactic antithrombotic therapy is indicated in women with the APLS. Our extrapolation of preliminary reports in women with heritable thrombophilia suggests that antithrombotic therapy should be administered.

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