3 Systemic Thromboembolism in Pregnancy: Heritable and Acquired Thrombophilias

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Abstract

Normal pregnancy is a hypercoagulable state. The predisposition to thrombosis may be exacerbated in women with heritable or acquired predisposition to thrombosis, known as thrombophilia. For a variety of reasons, the precise contribution of these thrombophilias to pregnancy morbidity is uncertain. However, there is evidence of an association between heritable thrombophilia and pregnancy morbidity including early and late pregnancy loss, preeclampsia, and intrauterine growth restriction. There also appears to be a weak association with placental abruption. Management of pregnant women with a thrombophilia relies on an accurate assessment of individual risk based on her personal and family history.

Keywords

Thrombophilia • Thromboembolism • Pregnancy • Hypercoagulable • Pregnancy morbidity • Pregnancy loss • Preeclampsia • Intra-uterine growth restriction

3.1 Introduction

Pregnancy results in an acquired hypercoagulable state due to pregnancy-associated changes in the hemostatic system (see Chap. 1). At delivery the placental bed spiral arteries, which lack a muscular layer, must quickly thrombose to limit and stop maternal hemorrhage. While contraction of the uterus is essential for prevention of major blood loss, it is likely that the evolutionary development of the hemostatic response to pregnancy (reviewed in Chap. 1) has provided a material survival advantage to both mother and fetus. However, the progressive hypercoagulability increases the risk of venous thrombosis during pregnancy (and the postpartum period) and in some women may contribute to pregnancy complications.

Venous thrombosis (deep vein thrombosis and pulmonary embolus, also referred to collectively as venous thromboembolism), pregnancy loss,

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preeclampsia, and intrauterine growth restriction are common pregnancy complications. These risks may be amplified in women with a heritable or acquired predisposition to thrombosis, so-called thrombophilia. Testing for heritable thrombophilias in women with previous pregnancy morbidity is now common in clinical practice. Consequently, hematologists and obstetricians are frequently asked for advice on intervention with antithrombotic therapy for subsequent pregnancies in women found to have laboratory evidence of heritable thrombophilia. However, the material contribution of heritable thrombophilia to pregnancy morbidity and hence the value of testing and using the results to inform clinical management decisions are still uncertain.

- The association between a diagnosis of heritable thrombophilia and pregnancy morbidity is weak as (1) the laboratory tests are imprecise, (2) the tests performed do not comprehensively assess the genetic framework of thrombophilia, and (3) both laboratory abnormalities and pregnancy morbidity are common and so it is inevitable that abnormalities are frequently found in women who are investigated.
- If there is a true association, then causation might be expected to be related to a common underlying pathology in which the likelihood of venous thrombosis and pregnancy morbidity is increased. However, while limited, studies reported so far do not support a common underlying pathology, at least for venous thromboembolism and pregnancy loss. Furthermore, based on biological plausibility, there is reason to believe that there may be different mechanistic pathology.
- Finally, if there is a causative link between an underlying predisposition to thrombosis and pregnancy morbidity, then testing for a limited number of thrombophilias using imprecise laboratory methodology may have little theoretical or practical clinical utility as the test results do not discriminate between women with and without an underlying predisposition to pregnancy morbidity.

The management of pregnancy-associated venous thromboembolism is detailed in Chap. 5 with specific treatment in relation to heritable thrombophilia addressed in Sect. 3.4.1 below. The association of pregnancy morbidity and late pregnancy complications with hereditary and acquired thrombophilias is reviewed in detail in Chap. 4. In this chapter, generic aspects of thrombophilia are considered, and a summary is presented of:

- The spectrum of established heritable thrombophilias associated with an increased risk of venous thrombosis
- The limitations of laboratory measurement and the implications for establishing a causal relationship and developing testing strategies that might have clinical utility
- An overview of heritable thrombophilia as it relates to pregnancy-associated venous thrombosis
- An overview of heritable thrombophilia as it relates to pregnancy morbidity

Acquired conditions that predispose to venous thrombosis, which therefore increase the risk of pregnancy-associated venous thrombosis, are included for the sake of completeness alongside the definite and possible heritable thrombophilias listed in Table 3.1.

3.2 Heritable Thrombophilias Associated with an Increased Risk of Venous Thrombosis

The heritable thrombophilias shown to be associated with at least a twofold increased risk of venous thrombosis are deficiencies of the natural anticoagulants antithrombin, protein C, and protein S, due to mutations in the corresponding genes *SERPIN1*, *PROC*, and *PROS*, and the two common mutations in genes encoding pro-coagulant factor: *F5*G1691A (FVR506Q, factor V Leiden) and *F2*G20210A (commonly referred to as the prothrombin gene mutation) (Table 3.1). The causal association between these heritable thrombophilias and venous thrombosis has been confirmed by comparing the prevalence of defects in patients with venous thrombosis and controls. The expression of heritable thrombophilia as a disease (venous thrombosis) is dependent on a strong gene-environ-

Table 3.1 Heritable and acquired conditions that predispose to venous thrombosis and hence which increase the risk of pregnancy-associated venous thrombosis

Heritable thrombophilia	Possible heritable component	Acquired risk factors for venous thrombosis
Antithrombin deficiency	High factor VIII/VWF	Increasing age (over 35 in pregnancy)
Protein C deficiency	High factor IX	Pregnancy
Protein S deficiency	High factor XI	COC/HRT
*F5*G1691A (factor V Leiden)	High fibrinogen	Obesity
*F2*G20210A (Sickle and thalassaemia disorders)	Factor XIII (qualitative) High homocysteine Hypofibrinolysis	Smoking Immobility Dehydration (hyperemesis) Hospitalization Antiphospholipid syndrome (APS) Heart failure Inflammatory disease Chronic respiratory disease Nephrotic syndrome Cancer Myeloproliferative disorders (PV, ET, PMF) Paroxysmal nocturnal hemoglobinuria (PNH)

VWF Von Willebrand Factor, *COC* combined oral contraceptive, estrogen containing, *HRT* hormone replacement therapy, *PV* polycythemia vera, *ET* essential thrombocythemia, *PMF* primary myelofibrosis

ment interaction and, in this respect, there is a strong interaction with pregnancy [1].

Numerous acquired medical conditions and environmental factors increase the risk of venous thrombosis (Table 3.1). Risk factors for venous thrombosis generally interact synergistically. This means that risk factors are not additive, rather that they multiply. For example, if two risk factors A and B each increase the risk of venous thrombosis threefold, then the combination of factors increases the risk nine times (3 × 3), not six times (3 + 3). Pregnancy is an independent risk factor for venous thrombosis, so the presence of additional heritable and acquired thrombophilias and environmental factors act synergistically to increase the risk of venous thrombosis in pregnancy. The baseline risk of venous thrombosis in women of reproductive age is low at approximately 1 per 10,000. Consequently, the relative increased risk of venous thrombosis associated with pregnancy translates into an absolute risk of only 1 per 1,000 live births overall. However, in an individual woman, a relative increase in risk due to multiple interacting factors may translate into a high absolute risk. This is the basis for assessing the risk of venous thrombosis and offering thromboprophylaxis in high-risk pregnancies (see Chap. 5).

3.2.1 Antithrombin Deficiency

Antithrombin is a protease inhibitor. Based on kinetic rates of inhibition, its primary targets are thrombin and factor Xa, and hence antithrombin both regulates generation of thrombin and inhibits thrombin that has been generated. Inhibition of target proteases is increased approximately 1,000-fold by glycosaminoglycan activation of antithrombin, which is the mechanism by which heparin acts as a pharmacological anticoagulant. The activation process involves an induced conformational change in the structure of antithrombin which enables formation of an irreversible covalent complex with the target protease [2]. The complex undergoes a further dramatic conformational change involving both the inhibitor and the inhibited protein which alters the properties of each, resulting in rapid clearance from the circulation.

Two laboratory (intermediate) phenotypes of heritable antithrombin deficiency are recognized. Type I is characterized by a quantitative reduction of antithrombin with a parallel reduction in function (measured as inhibitory activity against factor Xa or thrombin) and the level of protein in the plasma (measured immunologically as the antigenic level). Type 2 deficiency is due to the

production of a qualitatively abnormal antithrombin protein characterized by disturbance of the complex inhibitory mechanism of protease inhibition as a result of a mutation in the *SERPINC1* gene. The functional activity is discrepantly low compared to the antigenic level. Type 2 deficiency is subclassified according to the nature of the functional deficit:

- Type 2 reactive site (RS) in which mutations alter the sequence of the mobile reactive center loop, thus reducing the ability to inhibit thrombin or factor Xa either in the presence or absence of heparin in a laboratory assay
- Type 2 heparin binding site (HBS) in which mutations affect the ability of antithrombin to bind and be activated by glycosaminoglycans, resulting in reduced ability to inhibit thrombin or factor Xa only in the presence of heparin in a laboratory assay
- Type 2 pleiotropic (PE) in which a single mutation produces multiple effects on the structure-function relationship of the molecule which is often associated with low plasma levels due to effects on either secretion or stability

Approximately 100 point mutations (missense, nonsense, or insertions or deletions causing frameshifts) and several whole or partial gene deletions have been identified as causes of type 1 deficiency. Numerous point mutations causing qualitative type 2 deficiency have been identified. Homozygous type 1 deficiency and type 2RS mutations are incompatible with life. Type 2 HBS and some PE mutations are associated with a lower risk of thrombosis; homozygosity, and compound heterozygosity, involving these mutations is compatible with life.

Functional activity assays typically use a chromogenic substrate and factor Xa as the target protease. The total amount of antithrombin protein can be measured immunologically with antibodies, for example, by enzyme-linked immunosorbent assay (ELISA). As antithrombin antigen levels may be normal or near normal in type 2 deficiency, immunological assays may fail to identify patients with these variants and so a functional assay should be used as the initial assay.

Although there is little reported variation in the plasma concentration of antithrombin both during healthy pregnancy and following delivery (as stated in Chap. 1), antithrombin levels may be slightly reduced in pregnancy and are reduced in women taking estrogen preparations, as well as in other situations. Consequently, the clinical significance of a low antithrombin level must be interpreted by an experienced clinician who is aware of all the relevant factors that may have influenced the test result in a specific patient.

3.2.2 Protein C Deficiency

Protein C is the zymogen precursor of activated protein C (APC). Protein C is activated to APC by thrombin bound to thrombomodulin on the endothelial surface. APC inactivates the activated cofactors (VIIIa and Va) and so inhibits thrombin generation. Factors VIII and V are activated by small amounts of thrombin during initiation of coagulation to nonenzymatic cofactors required for assembly of macromolecular complexes that are required for the full thrombin explosion. The enzymatic components of these complexes are factors IXa and Xa, and so inactivation of VIIIa and Va by APC leads to disassembly of the enzymatic complexes, thus attenuating thrombin generation.

Protein C deficiency is classified into type 1 and 2 defects on the basis of functional and antigenic assays. The relative risk of thrombosis in relation to type 1 and the various type 2 defects has not been characterized. Most heritable protein C deficiency is due to type 1 abnormalities. The majority of type 1 defects are due to point mutations. Multiple type 2 defects due to mutations in the *PROC* gene have been reported affecting the catalytic active site, the phospholipid-binding Gla domain, the propeptide cleavage activation site, and the sites of interaction with substrates or cofactors. In this case, there is discordance between the functional and antigenic levels.

The laboratory diagnosis of protein C deficiency is based on a functional assay. As protein C antigen levels may be normal or near normal in type 2 deficiency, immunological assays may fail

to identify patients with these variants, so a functional assay should be used as the initial assay. Most commercially available functional assays use a snake venom to activate protein C and a chromogenic substrate to quantify APC activity. A chromogenic assay will detect type 1 and most type 2 defects. The diagnosis of type 1 protein C deficiency is problematic because of the wide overlap in protein C activity between heterozygous carriers and unaffected individuals. The diagnosis of type 2 defects is problematic because a chromogenic assay will only detect defects affecting the enzymatic site.

Protein C levels are not affected by pregnancy or estrogen exposure. Acquired low levels of protein C occur during anticoagulant therapy with oral vitamin K antagonists, vitamin K deficiency, disseminated intravascular coagulation (DIC), and liver disease. Consequently, the clinical significance of a low protein C level must be interpreted by an experienced clinician who is aware of all the relevant factors that may have influenced the test result in a specific patient.

3.2.3 Protein S Deficiency

Protein S is a vitamin K-dependent glycoprotein produced in the liver, endothelial cells, and megakaryocytes. Protein S is a nonenzymatic cofactor for APC-mediated inactivation of factors VIIIa and Va and additionally is involved with tissue factor pathway inhibitor-dependent natural anticoagulation. Approximately 60 % of protein S circulates bound to C4b-binding protein and is inactive. The remaining 40 %, designated free protein S, is uncomplexed and is the active form. Free protein S increases the affinity of activated protein C for negatively charged phospholipid surfaces on platelets or the endothelium and increases complex formation of APC with the activated forms of factors VIII and V (VIIIa & Va). However, the degree of C4b binding has not yet been shown to be a determinant of thrombosis risk. In addition to APC cofactor activity, protein S has an independent anticoagulant activity as a cofactor for TFPI (tissue factor pathway inhibitor).

Protein S is usually quantified immunologically rather than measured functionally. Nowadays, monoclonal antibodies that detect only free protein are used to quantify free protein S. Functional protein S assays are imprecise and are not used in the majority of coagulation laboratories.

Protein S levels are significantly lower in females, so much so that different normal reference ranges are required for males and females. There is a significant risk of a false-positive diagnosis of protein S deficiency in women. Protein S levels are reduced by estrogens and fall progressively during normal pregnancy. Acquired low levels of protein S occur during anticoagulant therapy with oral vitamin K antagonists, vitamin K deficiency, DIC, and liver disease.

Protein S defects are divided into three types:

- In type I deficiency, both total and free protein S levels are low (and functional activity, if measured, is found to be low).
- Type II defects are characterized by reduced activity in the presence of normal total and free levels of protein S. Type II deficiency is difficult to diagnose because functional protein S assays are imprecise.
- In type III deficiency, the total protein S level is normal but the free protein S level is low. Some type III deficiency is thought to be a phenotypic variation of type 1 resulting from the same genetic mutations. However, it is now apparent that many patients with an apparent type III phenotype do not have heritable protein S deficiency. This may be related to an increase in C4b levels.

This complicated classification reflects the complexity of the biology of protein S but has no mechanistic reference to disturbance of natural anticoagulant activity. Given these limitations and the imprecision of laboratory methodology, the diagnosis of heritable protein S deficiency is less precise and the clinical implication of a low protein S level in an individual is more uncertain than it is for antithrombin or protein C.

3.2.4 *F5*G1691A (FVR506Q, Factor V Leiden)

Factor V is a cofactor required for thrombin generation. Factor V has no cofactor activity until cleaved by thrombin or factor Xa. Activated factor V (Va) is inactivated by APC (see Sect. 3.2.2 above). Resistance to activated protein C (APC resistance) is a laboratory phenomenon in which there is a suboptimal anticoagulant response to addition of APC to a patient's plasma. In 95 % of cases of familial APC resistance, this is due to the same point mutation in the gene for FV, a guanine to adenine transition at nucleotide position 1691 in exon 10 (*F5*G1691A), resulting in a mutant protein FVR506Q. The mutation is known as the factor V Leiden mutation and the mutant factor Va has normal procoagulant activity, but substitution of glutamine for arginine at position 506 (which is an APC cleavage site) results in slower inactivation by APC. Nowadays, the mutation is frequently detected by direct DNA analysis (rather than by a clotting assay) to detect the presence of the mutant protein.

The mutation is present in around 4 % of the Caucasian population and around 15 % of unselected consecutive Caucasian patients with a first venous thrombosis. The prevalence is highest in Northern Europeans. The mutation is infrequent in other populations. The high prevalence and founder effect suggest positive selection, and this may relate to a favorable effect on embryo implantation and hence reproduction [3] rather than a lower risk of fatal hemorrhage in females during childbirth, as originally thought.

Acquired APC resistance is common, in part often due to increased FVIII levels, and is observed in pregnancy and in association with estrogen exposure.

3.2.5 *F2*G20210A

A single nucleotide change of guanine to adenine at position 20210 in the 3′ untranslated region of the prothrombin gene is a mild risk factor for venous thrombosis. The prevalence of the *F2*G20210A mutation is around 2 % in Caucasians with a higher prevalence in Southern compared to Northern Europeans. The mutation increases the plasma level of prothrombin by around 30 %, but the mechanism responsible for this has not been identified. No specific clotting test for the presence of the mutation has been described, and diagnosis depends on detection of the genetic mutation by DNA analysis.

3.2.6 Other Candidate Heritable Thrombophilias

A number of other anticoagulant proteins have been investigated as potential factors causing thrombophilia, but a relationship between venous thrombosis and low protein levels or associated gene mutations has not been established.

Increased levels of factors VIII, IX, and XI are associated with an increased risk of venous thrombosis, but a heritable basis for high levels associated with venous thrombosis is not established. There is equivocal evidence for a causal relationship between fibrinogen levels and venous thrombosis. Polymorphisms in the prothrombin gene have been described that may further increase the risk of venous thrombosis associated with the *F2*G20210A mutation, but the effect is mild. It was previously thought that deficiency of factor XII was a risk factor for venous thromboembolism, but subsequent investigation strongly indicates that this is unlikely. A protective effect against venous thrombosis has been reported for a polymorphism in the factor XIII gene (FXIIIV341L).

A causal relationship between levels of specific individual proteins involved in regulating fibrinolysis and venous thrombosis has not been established. However, in a case–control study using a global measure of fibrinolytic potential, there was an approximately doubled risk of venous thrombosis in patients with clot lysis times above the 90th percentile of controls [4]. Further analysis of a larger study confirmed this finding and demonstrated that hypofibrinolysis in combination with established acquired and genetic risk factors, such as *F5*G1691A, had a synergistic effect on venous thrombosis risk [5].

The genetic basis for hypofibrinolysis in these patients was not investigated.

Hyperhomocysteinemia may be caused by genetic abnormalities but only the severe inherited abnormalities of homocysteine metabolism (homozygous cystathionine beta-synthase deficiency and homozygous deficiency of methylenetetrahydrofolate reductase) result in congenital homocystinuria associated with an increased risk of both arterial and venous thrombosis, as well as premature atherosclerosis and mental retardation, epilepsy, and skeletal and eye problems. Fifty percent of patients present with venous or arterial thrombosis before the age of 30 years. The thermolabile variant of methylenetetrahydrofolate reductase (MTHFR), due to a common genetic polymorphism (C677T), is not a risk factor for venous thrombosis [6, 7].

3.2.7 Antiphospholipid Syndrome (APS)

The antiphospholipid syndrome (APS) is the most common acquired form of thrombophilia. APS is diagnosed when a patient with arterial or venous thrombosis (or pregnancy morbidity in women) is found to have antiphospholipid antibodies (anticardiolipin, aCL; and/or lupus anticoagulant, LA; and/or anti-beta-2-glycoprotein I, aß$_2$-GPI). The updated international consensus (revised Sapporo) classification criteria for definite antiphospholipid syndrome [8] require the presence of a LA and/or IgG or IgM aCL present in medium or high titer (i.e., >40 GPL or MPL or > the 99th percentile) and/or aß$_2$GPI (IgG and/or IgM) >99th percentile. These aPL should be persistent, defined as being present on two or more consecutive occasions at least 12 weeks apart. The international consensus criteria were originally designed for scientific clinical studies, and there remains a need for firm diagnostic criteria for routine clinical use which may differ from these. APS has conventionally been divided into primary and secondary forms, the latter being associated with systemic lupus erythematosus (SLE) or a related rheumatological condition. However, this distinction was abandoned in the revised Sapporo classification [8] on the basis that it is unknown whether APS and SLE are two diseases coinciding in an individual, underlying SLE offers a setting for the development of APS, or APS and SLE represent two elements of the same process.

Laboratory test results are subject to considerable pre-analytical variation. In addition, transiently abnormal results may be found in normal healthy individuals. For these reasons, for a patient to be considered to have antiphospholipid activity (aCL, LA, or aß2-GPI), test results must be positive on two separate occasions. The probability of misdiagnosing APS has been reduced by stricter criteria for antibody titers (>40 GPL or MPL for aCL or >90th percentile for aCL or aß2-GPI) and demonstration of persistence of antibodies (present on at least 2 consecutive occasions at least 12 weeks apart) [9]. Positivity in all 3 assays (aCL, LA, aß2-GPI) is associated most strongly with thrombosis and pregnancy complications. Recent evidence suggests that the antibodies most strongly associated with thrombosis and pregnancy morbidity are against domain I of β2-GPI; these antibodies are responsible for lupus anticoagulant activity specifically associated with clinical events and are responsible for positive aCL results. While the criteria for diagnosis of APS are unlikely to change again soon, it is possible that the laboratory identification of clinically relevant antibodies to domain I β2-GPI will eventually simplify the diagnosis and improve the clinical utility of laboratory tests.

3.3 Limitations of Laboratory Measurement and the Implications for Establishing a Causal Relationship and Developing Testing Strategies with Clinical Utility

The laboratory diagnosis of heritable thrombophilias is difficult as the tests are subject to considerable pre-analytical variables. Low levels of antithrombin, protein C, and protein S occur in a variety of circumstances and test results, and the

clinical implications of both positive and negative results, are frequently misinterpreted. If testing is performed during pregnancy, results must be interpreted with reference to the effect of the pregnancy.

Functional assays should be used for which accuracy and imprecision are acceptable. However, no single method will detect all defects. Even in families with characterized defects, a phenotypic assay may fail to accurately discriminate affected and nonaffected individuals. True heritable deficiencies may not be detected and false positive diagnoses are common.

Low levels of antithrombin, protein C, or protein S may relate to age, sex, acquired illness, or drug therapy, so interpretation requires knowledge of the patient's condition at the time of blood sampling. Low levels of antithrombin, protein C, or protein S suspected to be the result of heritable mutations should be confirmed on one or more separate samples. Demonstrating a low level in other family members supports a diagnosis of heritable deficiency, and characterization of the genetic mutation can be confirmatory.

As well as specific limitations relating to individual factors, there are a number of common generic issues which limit accuracy and precision of laboratory diagnosis and consequently contribute to limiting the clinical utility of thrombophilia testing. These can be summarized as follows:

- The laboratory diagnosis of heritable thrombophilias is difficult as the tests are subject to numerous biological and pre-analytical variables.
- The fact that venous thrombosis has a multiple genetic basis with incomplete penetrance and a strong gene-environment interaction makes counseling in relation to thrombophilia testing uncertain.
- In families with known heritable thrombophilias, the risk of venous thrombosis can be increased in unaffected members as well as affected, so a negative thrombophilia result does not exclude an increased risk of venous thrombosis.
- Even in families with characterized defects, a phenotypic assay may fail to accurately discriminate affected and nonaffected individuals.
- True heritable deficiencies may not be detected and false positive diagnoses are common.
- Low levels of antithrombin, protein C, and protein S occur in a variety of circumstances, and test results and the clinical implications of both positive and negative results are frequently misinterpreted.
- Testing for heritable thrombophilias in selected patients, such as those with a strong family history of unprovoked recurrent thrombosis, may influence decisions regarding duration of anticoagulation. Unfortunately, in this regard, identifying patients for testing is not straightforward as criteria for defining thrombosis-prone families have not been validated and the association between family history of thrombosis and detection of inherited thrombophilia is weak.

In order to limit inaccuracy and imprecision, the British Society for Haematology has published clinical guidelines for testing for heritable thrombophilia [10] which include the following generic recommendations:

- Testing at the time of acute venous thrombosis is not indicated as the utility and implications of testing need to be considered and the patient needs to be counseled before testing. As treatment of acute venous thrombosis is not influenced by test results, testing can be performed later.
- The prothrombin time (PT) should be measured to detect the effect of oral vitamin K antagonists which will cause a reduction in protein C and S levels.
- Functional assays should be used to determine antithrombin and protein C levels.
- Chromogenic assays of protein C activity are less subject to interference than clotting assays and are therefore preferable.
- Immunoreactive assays of free protein S antigen are preferable to functional assays. If a protein S activity assay is used in the initial screen, low results should be further investigated with an immunoreactive assay of free protein S.

- Repeat testing for identification of deficiency of antithrombin, protein C, and protein S is indicated, and a low level should be confirmed on one or more separate samples. Deficiency should not be diagnosed on the basis of a single abnormal result.

In addition to factors that limit the accuracy and precision of laboratory testing, there is potentially a fundamental flaw in attempting to quantify the degree of thrombophilia in an individual patient by using a dichotomous testing strategy in which a limited number of factors are designated normal or abnormal. The "thrombophilic condition" is dependent on a large complex genetic framework subject to strong environmental influence [1, 11, 12].

3.4 Overview of Heritable Thrombophilia as It Relates to Pregnancy-Associated Venous Thrombosis

3.4.1 Treatment of Pregnancy-Associated Venous Thrombosis

There are limited data in relation to treatment specifically of pregnancy-associated venous thrombosis in women with heritable thrombophilia. However, there is no evidence that issues that have been clarified in nonpregnant patients are different in pregnant women:

- There is no evidence that heritable thrombophilia should influence the initial intensity of anticoagulation with heparin.
- When warfarin is introduced following delivery, there is no evidence that heritable thrombophilia should influence the intensity of anticoagulation.
- Warfarin-induced skin necrosis is extremely rare, even in patients with protein C or S deficiency, such that most individuals with protein C or S deficiency do not develop skin necrosis.
- There is no evidence that recurrent venous thrombosis while on anticoagulant treatment is more likely in patients with heritable thrombophilia.

In nonpregnant patients with antithrombin deficiency, heparin resistance is infrequent and recurrence or extension of thrombosis while on treatment is no more frequent than that observed in individuals without antithrombin deficiency. However, there are anecdotes of pregnant women with heritable antithrombin deficiency who have low anti-Xa levels despite therapeutic doses of low-molecular-weight heparin. It is advisable for pregnant women with venous thrombosis and antithrombin deficiency to be referred urgently to a hematologist with appropriate expertise for supervision of treatment.

The most important clinical factor predicting likelihood of recurrent venous thrombosis is whether or not a first episode of venous thrombosis was unprovoked or provoked. Pregnancy is a relatively strong provocation for venous thrombosis and the risk of spontaneous recurrent venous thrombosis after pregnancy-associated venous thrombosis in women with heritable thrombophilia is low, and long-term anticoagulation is not indicated. Long-term prospective cohort outcome studies have shown that finding a heritable thrombophilia does not reliably predict recurrence in unselected patients even after an episode of unprovoked venous thrombosis. However, studies were not powered to exclude an increased risk of recurrence specifically in relation to rare thrombophilias, such as antithrombin or protein C deficiency. Therefore, it remains uncertain if mutations affecting the *SERPINC1*, *PROC*, and *PROS* genes causing deficiency of the corresponding protein might predict a sufficiently high risk of thrombosis to justify long-term (life-long) anticoagulation after a single episode of venous thrombosis. Following an episode of pregnancy-associated venous thrombosis, women with heritable thrombophilia should be referred to a thrombophilia specialist for consideration of future management, including duration of anticoagulation and need for thromboprophylaxis in subsequent pregnancies.

3.4.2 Prevention of Pregnancy-Associated Venous Thrombosis

Pregnancy is associated with a five to tenfold increased risk of venous thrombosis compared to nonpregnant women of comparable age and has an absolute risk of 1 per 1,000 deliveries. There is an increased relative risk of pregnancy-associated venous thrombosis in women with thrombophilia (Table 3.2), but this translates into a low absolute risk. For example, the relative risks of 34 and 8 associated with homozygosity and heterozygosity for the factor V Leiden mutation, respectively equate to absolute risks of 3.4 and 0.8 %, based on an overall absolute risk of 0.1 % (1 per 1,000 deliveries). Based on the calculated odds ratios in Table 3.2, absolute risks of pregnancy-associated venous thrombosis would only be expected to exceed 1 % for homozygosity for the factor V Leiden mutation. However, where a statistically significant increase in risk is demonstrated, the possibility of rates greater than 1 % cannot be excluded (based on the upper 95 % confidence intervals) for deficiencies of antithrombin, protein C, and protein S; heterozygosity for the *F5*G1691A (factor V Leiden); and homozygous and heterozygous *F2*G20210A mutations. Homozygosity for the thermolabile variant of MTHFR (C677T) is not associated with an increased risk of pregnancy-associated venous thrombosis (Table 3.2).

The risk of thrombosis, compared to the general age-matched female population, is increased 100-fold in pregnancy in women with a previous thrombosis. Thrombosis in pregnancy rarely occurs in women whose initial venous thrombosis was provoked, unless the provocation was use of an estrogen-containing contraceptive. In general, the absolute risk of pregnancy-associated venous thrombosis in women with heritable thrombophilia with no previous history is small, but the risk is considered greatest in women with antithrombin deficiency, those homozygous for the FVR506Q or the *F2*G20210A mutations or those who are double heterozygotes for FVR506Q and *F2*G20210A. The number of women with these defects is very small. The most appropriate management of these women is uncertain and recommendations are based on low-level evidence. Retrospective studies in women with laboratory evidence of thrombophilia and previous venous thrombosis for whom detailed information of the type of thrombophilia was available indicate that the rate of recurrence is similar in women with and without thrombophilia. However, a limitation of studies published to date is that women with high-risk thrombophilias were excluded (deficiency of antithrombin, protein C, and protein S, and combined defects).

In women with a previous history of venous thrombosis, the major factor in determining whether prophylaxis should be given is whether or not the prior venous thrombosis was provoked. If the episode was unprovoked, prophylaxis should be considered and thrombophilia testing is not required if prophylaxis is given. In women with a first provoked event, the decision to test or not should be influenced by the strength of the provocation, for example, thrombosis associated with major trauma and subsequent immobility would not be an indication for prophylaxis or testing. In women with a first-degree relative with thrombosis, the decision to test should be influenced by whether or not the event in the relative was unprovoked or provoked and the strength of the provocation. If the event in the first-degree relative was pregnancy or COC-associated, then testing and finding thrombophilia should prompt consideration of prophylaxis, particularly if the symptomatic relative was known to have the same defect, especially deficiency of antithrombin or protein C. When testing in pregnancy is performed, it is necessary to interpret the results with reference to the effect of pregnancy on the test results.

Table 3.2 Results of systematic review of thrombophilia in pregnancy

	Pregnancy-associated VTE	Recurrent pregnancy loss in first trimester	Non-recurrent second trimester loss	Late pregnancy loss	Preeclampsia	Intrauterine growth restriction
Antithrombin deficiency	(8/11)/(242/815)			(1/1)/(17/61)	(1/1)/(57/131)	
	4.7	–	–	7.6	3.9	–
	(1.3–17.0)			(0.3–196)	(0.2–97)	
Protein C deficiency	(23/32)/(232/715)			(3/234)/(18/524)	(3/3)/(60/104)	
	4.8	–	–	3.0	5.1	–
	(2.1–10.6)			(0.2–38.5)	(0.3–102)	
Protein S deficiency	(16/28)/(250/911)			(14/15)/(258/801)	(14/20)/(158/402)	
	3.2	–	–	20.1	2.8	–
	(1.5–6.9)			(3.7–109)	(0.8–10.6)	
Homozygous *F5*G1691A	(29/91)/(145/1,248)	Heterozygous and homozygous	–	(7/212)/(2/118)	(4/5)/(608/1,143)	(1/1)/(60/153)
	34.4			2.0	1.9	4.6
	(9.9–120)			(0.4–9.7)	(0.4–7.9)	(0.2–115)
Heterozygous *F5*G1691A	(96/226)/(263/1,595)	(173/287)/(1,390/2,285)	(34/58)/(98/432)	(27/382)/(124/1,121)	(161/249)/(1,790/3,673)	(25/49)/(512/1,147)
	8.3	**1.9**	**4.12**	**2.1**	**2.2**	2.7
	(5.4–12.7)	(1.01–3.6)	(1.9–8.8)	(1.1–3.9)	(1.5–3.3)	(0.6–12.1)
Homozygous *F2*G20210A	(2/2)/(40/253)	–	–	–	–	–
	26.4					
	(1.2–559)					
Heterozygous *F2*G20210A	(42/61)/(277/1,005)	(54/78)/(627/1,428)	(4/11)/(22/271)	(15/36)/(348/1,134)	(42/71)/(937/2,028)	(25/44)/(583/1,375)
	6.8	**2.7**	8.6	**2.7**	**2.5**	2.9
	(2.5–18.8)	(1.4–5.3)	(2.2–34)	(1.3–5.5)	(1.5–4.2)	(0.6–13.7)
Homozygous MTHFR (C677T)	(20/128)/(89/543)	(22/39)/(21/368)		(69/323)/(198/1,059)	(221/482)/(1,234/3,205)	(62/121)/(460/961)
	0.7	0.9	–	1.3	**1.4**	1.2
	(0.2–2.5)	(0.4–1.7)		(0.9–1.9)	(1.1–1.8)	(0.8–1.8)

(continued)

Table 3.2 (continued)

	Pregnancy-associated VTE	Recurrent pregnancy loss in first trimester	Non-recurrent second trimester loss	Late pregnancy loss	Preeclampsia	Intrauterine growth restriction
Hyperhomocysteinemia	(33/37)/(128/235)	(12/16)/(47/113)		(2/7)/(16/55)	(37/41)/(257/364)	
	6.2	4.3	–	1.0	**3.5**	–
	(1.4–28.4)	(1.3–13.9)		(0.2–5.6)	(1.2–10.1)	
Lupus anticoagulant	(59/107)/(581/1,728)		(9/17)/(13/178)	(15/242)/(124/730)	(63/89)/(426/981)	
	3.0	–	14.3	2.4	1.5	–
	(1.03–8.6)		(4.7–43)	(0.8–7.0)	(0.8–4.6)	
Anticardiolipin antibodies	(127/149)/(869/1,956)	(116/120)/(551/647)		(52/242)/(124/730)	(130/217)/(803/2,428)	(7/60)/(15/800)
	3.4	**5.0**	**–**	**3.3**	**2.7**	**6.9**
	(1.3–8.7)	(1.8–14.0)		(1.6–6.7)	(1.7–4.5)	(2.7–17.7)

Data from Robertson et al. [13]

Each box indicates in brackets number of women in total (women with thrombophilia/women with event)/(women with no thrombophilia/women with event), odds ratios calculated on the random effects model (not the fixed effect model) to provide a more conservative result, indicated to 1 decimal place with 95 % confidence intervals in brackets.

Statistically significant results are indicated in bold if n >20 for thrombophilia group

In summary:

- Women should be assessed for risk of pregnancy-associated venous thrombosis primarily in relation to clinical risk factors; this assessment should be performed when first seen in pregnancy and again if circumstances change during pregnancy, for example, the woman develops preeclampsia or is admitted to hospital.
- Most women with a previous unprovoked venous thrombosis or pregnancy or COC-related thrombosis will qualify for thromboprophylaxis on the basis of clinical risk alone, so testing for heritable thrombophilia may not be contributory.
- Women with a previous event due to a major provoking factor, for example, surgery or major trauma, would not usually require prophylaxis or testing.
- Women with a previous event due to a minor provoking factor, for example, travel, should be tested and considered for prophylaxis if a thrombophilia is found.
- In asymptomatic women with a family history of venous thrombosis, testing is not required if the clinical risks alone are sufficient to result in thromboprophylaxis during pregnancy.
- Asymptomatic women with a family history of venous thrombosis should be tested if an event in a first-degree relative was unprovoked or provoked by pregnancy, COC exposure, or a minor risk factor. The result will be more informative if the first-degree relative has a known thrombophilia, so the interpretation of the result in the asymptomatic woman is with reference to the defect in the symptomatic affected relative.

3.5 Overview of Heritable Thrombophilia as It Relates to Pregnancy Morbidity

There is evidence of an association between heritable thrombophilia and pregnancy morbidity including early and late pregnancy loss, preeclampsia, and intrauterine growth restriction. There also appears to be a weak association with placental abruption [13]. A simple hypothesis is that thrombophilia may increase the risk of placental insufficiency due to placental vascular thrombosis. If thrombophilia results in pathology which mechanistically results from thrombosis, it might be expected that women with a predisposition to venous thrombosis would have a higher incidence of pregnancy morbidity thought to be due to placental vascular thrombosis. However, in a case–control study, pregnancy loss was no more frequent in women with a history of venous thrombosis than in controls, although pregnancy-induced hypertension and preeclampsia were more common. The stillbirth rate was not significantly higher [14]. The placental vasculature is not formed until 10–12 weeks of gestation so a thrombotic pathology does not explain why the majority of women with thrombophilia have early pregnancy loss before 12 weeks. An alternative "non-thrombotic" hypothesis is that trophoblast apoptosis is the underlying mechanism. In vitro studies have demonstrated that antiphospholipid antibodies inhibit trophoblast differentiation and placentation [15, 16]. Experimental studies in mice with genetic disruption of the protein C pathway indicate that the fetal loss that occurs before 10 weeks is similarly due to inhibition of trophoblast growth by coagulation proteases [17].

Meta-analysis indicates an increased prevalence of thrombophilias in case–control studies comparing women with pregnancy complications to those without. However, the point estimates of odds ratios calculated from case–control studies are low, indicating that any causal association is weak. Importantly, the predictive value of a positive thrombophilia test result for recurrence of a particular pregnancy complication has not yet been determined. Randomized trials demonstrating that the presence of a thrombophilia should modify management of subsequent pregnancies have yet to be reported. Nevertheless, many clinicians have instituted a policy of offering anticoagulant drugs to women with a history of pregnancy morbidity, particularly recurrent miscarriage or stillbirth, on the basis of finding laboratory evidence of a thrombophilic defect. Thromboprophylactic dose of low-molecular-

weight heparin (LMWH) is usually the preferred option and, while the risk of treatment is low, it is not zero. Allergic skin reactions occur in 1–2 % [18] although heparin-induced thrombocytopenia with thrombosis that often manifests with skin necrosis at injection sites rarely if ever occurs in pregnancy. Regional analgesia is considered to be contraindicated if within 12 h of subcutaneous prophylactic dose heparin [19]. There seems to be little if any risk of osteoporosis with the use of low-dose LMWH for the duration of a pregnancy. A review of almost 3,000 women prescribed low-dose LMWH in pregnancy revealed a very low incidence of complications including bleeding [18].

Systematic reviews of the results of studies reported up to 2005 investigating the association between heritable thrombophilia and pregnancy loss have been published [13, 20–22]. The summary of results of the most recent is shown in Table 3.2.

3.5.1 Pregnancy Loss

The largest study of pregnancy loss and thrombophilia investigated an association with the factor V Leiden mutation in over 1,000 consecutive Caucasian women [23]. No association was demonstrated with congenital APC resistance (due to the factor V Leiden mutation), but acquired APC resistance was more common in women with a history of miscarriage. Acquired APC resistance reflects the physiological hypercoagulable state of pregnancy, and there may be an association between pregnancy loss and other pregnancy morbidity; however, in this study, the women were tested in the non-pregnant state. A retrospective analysis of 64 women homozygous for the factor V Leiden mutation compared pregnancy outcomes to those in 54 age-matched control women [24]. The stillbirth rate in the affected women was 3.3 % compared to 1.7 % in the controls and rates of miscarriage were 12 and 10 % respectively, results that were not significantly different. While the statistically insignificant results may have resulted from the low power of the study, a major difference in outcome between heterozygous and homozygous women is unlikely, a finding suggested also by systematic review (Table 3.2). The only prospective controlled study investigating the association between heritable thrombophilia and pregnancy loss showed no increased risk in 48 affected women compared to 60 controls [25].

The systematic review published by Robertson and colleagues [13] showed that recurrent first trimester pregnancy loss in association with anticardiolipin antibodies was higher than for any heritable thrombophilia, and second trimester pregnancy loss was strongly associated with lupus anticoagulant activity (Table 3.2). Late pregnancy loss appeared to be most strongly associated with protein S deficiency, but the number of women with protein S deficiency was only 15 and, as indicated in Sect. 3.3, the diagnosis of heritable protein S deficiency is often inaccurate.

Individual studies have produced conflicting findings on the association between MTHFR homozygosity and recurrent pregnancy loss: some found an association [26, 27] but others did not [28–30], and a meta-analysis suggested that there is no association (Table 3.2). Adequate folic acid supplementation silences the phenotypic expression of this polymorphism.

3.5.2 Preeclampsia and Intrauterine Growth Restriction

The association between heritable thrombophilia and preeclampsia and intrauterine growth restriction appears to be similar to other pregnancy morbidity, but fewer data are available. Anticardiolipin antibodies appear to be relatively strongly associated with growth restriction (Table 3.2).

A meta-analysis suggested that MTHFR homozygosity was associated with an increased risk only for preeclampsia (Table 3.2). However, this polymorphism may be associated with an increased risk of other pregnancy complications, including placental abruption or infarction [31], preeclampsia [32, 33] and pregnancy-induced hypertension [34]. As mentioned above, adequate folic acid supplementation silences the phenotypic expression of this polymorphism.

3.5.3 Recommendations

Randomized controlled trials, with a no treatment or a placebo arm, in women with a history of pregnancy complications are in progress. The British Society for Haematology has recommended that results from these trials should be awaited before recommending that anticoagulant drugs are given to pregnant women based on testing for heritable thrombophilia, but the issue remains contentious [10]. Provisional studies suggest a benefit of intervention in women with thrombophilia but the benefit, if true, may not be restricted to women with thrombophilia. In small studies, live birth rates of around 70 % were reported compared to live birth rates of around 30 % in previous pregnancies. However, these studies involved small cohorts and there were no control groups. If hypercoagulability, and a related mechanism such as protease-induced trophoblastic apoptosis, is a material contributory factor in many cases of pregnancy morbidity, then administration of low-dose LMWH may be beneficial regardless of whether or not there is laboratory evidence of thrombophilia. If there is an increased relative risk in women with a laboratory "marker," then a beneficial effect will be more readily demonstrated in women with thrombophilia, even though the magnitude of benefit may be the same in women with the same underlying pathology but no identifiable thrombophilia "marker." Therefore, until the results of trials in women at high risk of pregnancy complications and the results of trials specifically in women with thrombophilia are known, many experts suggest that decisions to use low-dose heparin for prevention of pregnancy morbidity should not be made in relation to the results of thrombophilia tests.

In all future studies, criteria for both diagnosis of heritable thrombophilia and pregnancy morbidity must be clearly defined a priori [35]. Many studies to date have not defined criteria for the diagnosis of thrombophilia, they have often relied on results of single laboratory measurements in individuals, and they have not used strict criteria for pregnancy morbidity, for example, the use of ultrasound for accurate clinical assessment.

3.6 Purpura Fulminans in the Newborn

Purpura fulminans is a rare syndrome characterized by progressive hemorrhagic skin necrosis that occurs in neonates with congenital severe protein C or S deficiency at birth or in the first few days of life (an alternative form of the condition occurs in association with infection in children and adults, and this condition is typically associated with acquired severe protein S deficiency). If a neonate develops purpura fulminans, levels of protein C and S should be measured urgently. Levels are normally low at birth, but the condition is associated with undetectable levels. Measurement of levels in the parents may help to interpret the neonate's results. Neonatal purpura fulminans due to deficiency of protein C or S requires urgent replacement therapy with factor concentrate or fresh frozen plasma when concentrate is not immediately available.

In cases of pregnancy where one partner is known to have protein C deficiency, some experts would consider testing the other partner to determine if there is the possibility of the child having severe protein C deficiency at birth, with a view to antenatal detection of a homozygous infant. However, this approach is extremely problematic and counseling and interpretation of test results must be undertaken by an expert. Given the unreliability of phenotypic diagnosis, genetic analysis is mandatory if antenatal diagnosis is going to be considered with a view to termination. Some experts would consider this approach only if there was a previously affected infant.

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