WHAT IS THROMBOPHILIA?

Thrombophilia is a disorder of hemostasis in which there is a tendency for the occurrence of thrombosis. This tendency can be inherited or acquired. (Table 1) In general, heritable thrombophilia is associated with a predisposition to venous, not arterial, thromboembolism. 1

ACQUIRED THROMBOPHILIA

In many cases of venous thromboembolism (VTE), there is an obvious precipitating factor, such as the postoperative state. Acquired thrombophilic conditions should be considered in all cases of apparently spontaneous VTE. If the precipitating factor is not reversible then long-term anticoagulation may be indicated. Four important acquired conditions need special consideration. 1

a. Occult malignancy: Cancer is diagnosed in around 10% of patients within 6 months of presentation with apparently spontaneous deep vein thrombosis (DVT). It is generally recommended that a careful history and physical examination be carried out with further investigations ordered only as clinically indicated.

b. Myeloproliferative disease: The two most common myeloproliferative disorders, essential thrombocythemia and polycythemia vera, are both associated with thrombosis in the venous, arterial and microvascular compartments.

c. Paroxysmal nocturnal haemoglobinuria (PNH): This acquired clonal stem cell disorder results in a triad of thrombosis, hemolysis and marrow aplasia, but not all three features are necessarily present. Confirmation of PNH can be obtained by flow cytometric testing for CD55 and CD59, which are deficient in PNH red cells or leucocytes or by performing the older and less sensitive Ham’s test.

d. Antiphospholipid syndrome: This syndrome predisposes to both venous and arterial thrombosis. Clinical criteria include having one or more clinical episode of venous or arterial thrombosis, one or more unexplained fetal deaths (>10 weeks of gestation), or having three or more unexplained consecutive miscarriages (<10 weeks of gestation). Laboratory criteria include lupus anticoagulant present in plasma, or medium or high titers of antiphospholipid antibody of IgG or IgM isotype, or anti-beta2 glycoprotein-I antibody in serum or plasma. Antiphospholipid syndrome is diagnosed if at least one of the clinical criteria and one of the laboratory criteria is met. Laboratory tests should be performed twice, twelve weeks apart, and should be positive on both occasions. Long-term anticoagulation with warfarin is often recommended even after a single episode of VTE unless the antibodies or lupus inhibitor have disappeared. These antibodies are detected in 1% to 7% of healthy controls and do not require therapy in the absence of a history of venous or arterial embolism. 2, 3

INHERITED THROMBOPHILIA

Inherited thrombophilic disorders can be identified in up to 50% of patients presenting with VTE (Table 1). Some of these factors are much rarer in Asian and Indian population.

Table 1: Thrombophilic conditions and Associations

<table>
<thead>
<tr>
<th>Inherited</th>
<th>Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Prothrombin gene 20210 mutation</td>
<td>Immobility</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>Trauma</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>Postoperative state</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>Oral contraceptive pill</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>Increased plasma concentration</td>
<td>Antiphospholipid syndrome</td>
</tr>
</tbody>
</table>
| of factor VII, fibrinogen and other coagulation factors | Malignancy |}

*Partly determined by environment
a. Factor V Leiden (F5 R506Q – APC resistance)

The so-called APC resistance is mainly caused by a mutation in the Arg506 cleavage site of factor Va. The factor V Leiden, is inactivated more slowly by activated protein C than wildtype factor Va. It is the most prevalent thrombotic risk factor known in the Caucasian population, i.e. 3–7% carry the mutation, but it is very rare in native African and Asian populations. 4 Despite being a risk factor for VTE, the absolute risk of developing VTE is relatively low, with 80% of individuals never developing VTE by age 90. 5 Resistance to APC, can also occur in the absence of this mutation. These rare conditions are pregnancy, oral contraceptive use, hormone replacement therapy (HRT) or cancer. 2, 4

b. Prothrombin 20210A mutation

Poort et al (1996) described a mutation in the prothrombin gene which is associated with an increased level of prothrombin in the circulation. 4 The prevalence of this mutation ranges from 0.7% to 4% in Caucasian populations. 4

c. Antithrombin deficiency (also termed as Anti-Thrombin III or ATIII)

Antithrombin (AT) is an important natural anticoagulant of the coagulation system and inhibits the coagulation factors IIa, IXa, Xa, Xla, and Xlla. The prevalence of AT deficiency in the general population is very low and estimated to be approximately 0.02% in the general Caucasian population. 4 Low levels of AT may also be seen in acquired conditions such as liver disease, polytrauma, sepsis or consumptive coagulopathies. 7

d. Protein C deficiency

Protein C is a vitamin K-dependent glycoprotein that is synthesized in an inactive form in the liver. Upon activation, protein C is an important natural anticoagulant that, together with its co-factor protein S, suppresses thrombin generation by inhibition of coagulation factors Va and Vlla. Protein C deficiency is very rare with a prevalence of approximately 0.2% in the general population.

e. Protein S deficiency

Protein S is the co-factor of protein C in the inactivation of factors Va and Vlla. The prevalence of protein S deficiency is very low, ranging from 0.03% to 0.13% in the general Caucasian population. 4

f. Acquired deficiencies of AT, Protein C and S

Although mainly inherited, some of the thrombophilias can be acquired. Severe liver disease compromises its capacity to synthesize proteins, and will subsequently reduce the levels of many coagulation factors including anticoagulant factors AT, protein C, and protein S. Severe vitamin K deficiency, or oral anticoagulants, leads to acquired deficiencies of protein C and protein S. A decrease in protein S activity is caused by estrogen excess, such as during pregnancy or use of oral contraceptives. 4

WHEN TO TEST?

Testing should not be done immediately after a VTE. It is important to know that presence or absence of these thrombophilia does not affect the initial management in any patient and has a limited role in long term management of most patients. As acute phase reactants, plasma levels of AT and occasionally proteins C and S may transiently decrease, and fibrinogen and factor VIII levels may increase, with acute thrombosis. In general, thrombophilia testing should be delayed for at least 6 weeks to allow acute-phase
reactant proteins to return to baseline. Heparin therapy can lower AT levels and impair interpretation of clot-based assays for a lupus anticoagulant. Warfarin therapy reduces vitamin K–dependent factors, including proteins C and S. In those for whom temporary discontinuation of anticoagulation is not practical, heparin can be substituted for warfarin when testing protein C and S levels. Any abnormal result should be confirmed with repeat testing. DNA testing for the factor V Leiden and prothrombin G20210A mutations is unaffected by anticoagulation therapy.

WHY TO TEST?

The rationale to test is to prevent further thrombosis and determine duration of anticoagulation. There are no absolute indications for clinical diagnostic thrombophilia testing. Potential relative indications could include selected screening of asymptomatic or symptomatic family members of patients with a known familial thrombophilia, and testing symptomatic patients with incident or recurrent thrombosis. All of these potential indications are controversial and must be considered in the context of the clinical presentation.

ASSOCIATION BETWEEN THROMBOPHILIA AND A FIRST DEEP VENOUS THROMBOSIS

Although the relative risk of a first episode of venous thrombosis in patients with inherited thrombophilia is high, the absolute risk is low (Table 2). Thus if an asymptomatic person with thrombophilia is detected, there is no recommendation to start prophylactic anticoagulation.

ASSOCIATION BETWEEN THROMBOPHILIA AND RECURRENT DEEP VENOUS THROMBOSIS

Venous thrombosis has a tendency to recur. The cumulative incidence of a second episode is approximately 30% in 8 years. Two large follow-up studies assessed the risk of recurrent venous thrombosis associated with thrombophilic defects. Baglin et al (2003) showed that carriers of a thrombophilic defect did not have a highly increased risk of developing a recurrent venous thrombotic event. This was also observed in another large follow-up study by Christiansen et al (2005), which found no clear increased risk of recurrent venous thrombosis between the prothrombotic risk factors FVL, prothrombin gene mutation G20210A, and elevated levels of factor VIII. The outcomes regarding relative risk for recurrence ranged between two- and six-fold (Table 3 and 4).

ASSOCIATION OF THROMBOPHILIA WITH ARTERIAL THROMBOSIS

Although there is no established association between thrombophilia and arterial thrombosis, clinicians continue to order these tests in patients with arterial thrombosis. A survey in The Netherlands found that this was the indication for testing in almost a quarter of the ordered tests. Given that no differential treatment or secondary prevention will follow from the presence of thrombophilia there appears no benefit from thrombophilia testing in arterial thrombosis. The only condition where management might change is the antiphospholipid syndrome, where some experts recommend addition of oral anticoagulants to an arterial stroke. Even in this condition, there is no evidence from well-designed trials that supports this expert recommendation.

Evidence of an association between deficiencies of antithrombin, protein C, or protein S and arterial thrombosis is limited to case reports and small studies that are generally hampered by the low prevalence of these thrombophilias. No cases of antithrombin deficiency were found in two studies of young patients with myocardial infarction. Although case reports suggest that antithrombin deficiency may be associated with stroke, studies in neonates, children and young adults with ischemic stroke revealed no association with AT deficiency. Three case–control studies did not demonstrate an increased prevalence of protein C deficiency in young patients with myocardial infarction as compared to controls. Similarly, there is no convincing evidence of an association with stroke. Larger studies have not found an association between protein S deficiency and myocardial infarction or ischemic stroke. Numerous studies have investigated whether FVL is a risk factor for myocardial infarction. Several large cohort studies including the Physicians’ Health Study, Cardiovascular Health Study, and the Copenhagen Heart Study did not find an association between FVL and myocardial infarction. There is no evidence from randomized trials that supports the use of thrombophilia testing in arterial thrombosis.
no established relationship between prothrombin G 20210A mutation and myocardial infarction \textsuperscript{14} or stroke. \textsuperscript{17,18} Testing for thrombophilia in arterial thrombosis is not recommended, except for anti-phospholipid syndrome, if clinically indicated.

**CLINICAL MANAGEMENT OF PATIENTS WITH VENOUS THROMBOSIS**

After a first episode of venous thrombosis, 3–6 months of anticoagulant therapy is considered to have the optimal balance between the risk of treatment, i.e. bleeding, and the benefit, i.e. the prevention of an extension or recurrence of venous thrombosis. As outlined above, the presence of hereditary thrombophilia in patients with venous thrombosis does not strongly increase the risk of recurrence after discontinuation of anticoagulant therapy. For patients with antiphospholipid antibody syndrome, there is a significant risk of recurrence.\textsuperscript{19} This observation has led to the recommendation to treat patients with known antiphospholipid antibodies for at least 12 months. With rare exceptions, the therapy for acute thrombosis is no different for those with than for those without a recognized thrombophilia.\textsuperscript{9}

**PREVENTION OF PRIMARY VENOUS THROMBOEMBOLISM THROUGH THROMBOPHILIA SCREENING**

Universal population screening: Consensus exists that screening of the general population to assess venous thrombosis risk is not cost effective.

Selective population screening: Before oral contraceptive pill use: Thrombophilia screening to facilitate primary prevention of VTE is a reasonable consideration only for women who are about to begin using oral contraceptives. The annual risk of venous thrombosis in an otherwise healthy woman of reproductive age is around 0.01%. (Table 2) The substantially increased risk of primary thromboembolism in women with deficiencies in protein C, protein S, or AT who take oral contraceptive agents is well documented, leading van Vlijmen et al\textsuperscript{20} to recommend that women with these deficiencies should not take oral contraceptive agents.\textsuperscript{20} Even this is controversial. A 10-fold increase in the rate of VTE amounts to only 3 cases per 1000 people per year.\textsuperscript{2} A more practical recommendation is that women should be informed that the risk of thrombosis during oral contraceptive therapy is increased significantly in those who carry thrombophilic conditions. Given this information, women can decide if they want to be screened before receiving oral contraceptives.\textsuperscript{9} As the OCP is the most acceptable and effective form of contraception for many women, a clear understanding of the absolute risk of VTE is required:\textsuperscript{1}

a. Firstly, most women with FVL will not have VTE even after using OCP for many years.

b. Secondly, 10,000 women would have to be screened and the OCP withheld in 500 otherwise healthy FVL heterozygotes to prevent one episode of VTE.

c. It is noteworthy that OCP-related venous thrombosis is most likely to occur in the first 6–12 months of use. Thus, if a woman has been using the OCP for several years without complications, discontinuation on the basis of the family history or test results may not be justified in every case.

Selective population screening: Before HRT use: As with OCP use, the greatest risk is in the first 6–12 months of use.

Selective population screening: Pregnancy: In women who have had a previous VTE there is no clear benefit to thrombophilia testing before pregnancy. The recommendations are that if there is any previous VTE, irrespective of cause or risk, post partum prophylaxis with oral anticoagulation is recommended as about 50% of pregnancy-related VTE occur in the first 6 weeks after delivery.\textsuperscript{21} If VTE was idiopathic or related to previous pregnancy or estrogen use, there is a sufficiently high risk of VTE during pregnancy, and heparin throughout pregnancy is recommended. In asymptomatic women detected to have thrombophilia, either observation alone or heparin throughout pregnancy are valid options.\textsuperscript{21}

Risk of Primary VTE in asymptomatic family members with hereditary thrombophilia: A potential advantage of testing patients with venous thrombosis for thrombophilia may be the identification of asymptomatic family members of thrombophilic patients in order to take preventive measures if tested positive. As detailed previously, the risk for a first episode of venous thrombosis in relatives with thrombophilia is increased two- to ten-fold. Nevertheless, the overall absolute risk in thrombophilic families is generally low, even during high-risk situations such as pregnancy, puerperium, surgery, immobilisation, trauma and during the use of oral contraceptives (Table 2). It is clear that the 2% annual major bleeding risk associated with continuous anticoagulant treatment outweighs the risk of venous thrombosis.\textsuperscript{22,23}

Risk of VTE Recurrence in Patients with Thrombophilia: There is convincing evidence that symptomatic people with positive thrombophilia tests do not have a higher rate of recurrence than those who test negative.\textsuperscript{9,10} The presence of a single thrombophilic factor confers only a statistically insignificant increase in risk (RR = 1.2 and 1.5 according to Christiansen et al\textsuperscript{9} and Baglin et al\textsuperscript{10}, respectively. In patients with 2 or more thrombophilias, risk increases modestly (RR = 1.2).\textsuperscript{10} On the other hand, antiphospholipid syndrome with circulating anticardiolipin antibodies definitely increases the risk of recurrence.\textsuperscript{2,24} Two recent studies highlighted the relevance of clinical factors, which are easily determined at the bedside (provoked versus idiopathic VTE), and the risk of thrombosis recurrence.\textsuperscript{9,10}

**ADVANTAGES AND DRAWBACKS OF THROMBOPHILIA TESTING**

The obvious advantage of thrombophilia testing is that it provides a possible cause for VTE. Often, the patient wants to know the reason why they developed a VTE and the chances of recurrence.
Table 5: Arguments For and Against Thrombophilia Testing in venous thromboembolism patients.

<table>
<thead>
<tr>
<th>In Favor of testing</th>
<th>Against Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing provides the opportunity to define the cause of thrombosis</td>
<td>Half of all VTE patients will not have a known thrombophilic disorder; but these patients probably do have a thrombophilic disorder that has not been discovered yet. Finding thrombophilia does not affect any treatment decision. Cost of testing is significant.</td>
</tr>
<tr>
<td>Results influence treatment decisions</td>
<td>Results of inherited thrombophilia testing do not affect initial or later treatment. Initial treatment is the same. For future prophylaxis, all patients with previous VTE are at increased risk of recurrence and need prophylaxis in high risk situations, irrespective of presence or absence of thrombophilia. Exception: Antiphospholipid syndrome patients should receive prolonged anticoagulation.</td>
</tr>
<tr>
<td>Testing is useful for screening asymptomatic family members of an affected proband</td>
<td>There is no evidence that screening family members affects subsequent risk of thrombosis in only the members who test positive. Testing may create anxiety in asymptomatic family members. Asymptomatic family members who test positive for inherited thrombophilia do not require routine prophylactic anticoagulation.</td>
</tr>
<tr>
<td>Testing is useful in managing patients with previous VTE who are considering pregnancy</td>
<td>Management should be the same regardless of results of thrombophilia workup.</td>
</tr>
</tbody>
</table>

Table 6: Investigations for Thrombophilia

<table>
<thead>
<tr>
<th>Test</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full blood counts and peripheral smear</td>
<td>For myeloproliferative disorders, PNH, sickle cells, spherocytosis</td>
</tr>
<tr>
<td>PT</td>
<td>As baseline and for monitoring</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (APTT)</td>
<td>As baseline and monitoring for heparin; using a thromboplastin that is relatively sensitive to the presence of a lupus anticoagulant would show a prolonged APTT if lulus anticoagulant is present.</td>
</tr>
<tr>
<td>Lupus anticoagulant panel</td>
<td></td>
</tr>
<tr>
<td>Antiphospholipid (anti-cardiolipin antibody) ; anti-b2 glycoprotein 1 antibodies</td>
<td>For anti-phospholipid syndrome</td>
</tr>
<tr>
<td>Activated Protein C (APC) – resistance ratio</td>
<td>Commonest cause is Factor V Leiden</td>
</tr>
<tr>
<td>Factor V Leiden mutation genotyping</td>
<td>Usually done if APC- resistance ratio is low, confirms mutation in factor V Leiden</td>
</tr>
<tr>
<td>Prothrombin G20210G mutation genotyping</td>
<td>If positive, suggests a predisposition to VTE</td>
</tr>
<tr>
<td>Protein C, S and AT (Antigen and Activity)</td>
<td>If low, suggests a possible cause of hereditary thrombophilia. Occasionally low levels are acquired.</td>
</tr>
<tr>
<td>Flow cytometry for PNH</td>
<td>If PNH is suspected</td>
</tr>
<tr>
<td>Chemistry, ESR</td>
<td>For organ dysfunction, inflammatory disease</td>
</tr>
<tr>
<td>X Ray chest</td>
<td>For possible malignancy</td>
</tr>
<tr>
<td>Selected Markers of malignancy: PSA, CA-125, beta-HCG, CEA, Colonoscopy, mammogram.</td>
<td>If clinically suspected. General screening for malignancy is not recommended.</td>
</tr>
</tbody>
</table>

Thrombophilia testing may be done after counseling that the decision regarding the duration of anticoagulation would be determined primarily by clinical factors. Testing for research or academic reasons is another indication, but patient should not bear the cost of these investigations.

Absence of thrombophilia does not mean that the patient is not at risk of recurrence. The highest risk of recurrence is in patients who developed VTE without any precipitating cause, irrespective of any detectable thrombophilia. Similarly, a provoked VTE has a low recurrence rate, even if there is presence of a thrombophilia. There may be a change in management when a diagnosis of antiphospholipid syndrome is made, or in relation to pregnancy and oral contraceptive use.

People are prone to overestimate the thrombosis risk if they test positive. This emphasises the importance of counselling people before embarking on thrombophilia testing. Good clinical practice demands that tests are carried out for carefully considered clinical reasons and that doctors must be in a position to give their patients an accurate and thorough understanding of the implications of results, be they normal or abnormal.

The disadvantages of testing patients with a venous thrombosis for thrombophilia include the cost of testing and the psychological impact and consequences of a person knowing that they are a carrier of a (genetic) thrombophilic defect. In most cases of asymptomatic carriers of thrombophilia, no change in management is recommended except for possible prophylaxis in high risk situations such as surgery (Table 5).

**HOW TO TEST?**

The tests should be guided by clinical evaluation. Performing a battery of tests, especially expensive “thrombophilia” screens is not recommended. The type of tests which can be performed is noted in Table 6.

**IMPORTANT PRACTICAL PITFALLS IN TESTING**

Uncritical interpretation of laboratory results leads to misdiagnosis and thrombophilia testing exemplifies this. If erroneous and overdiagnosis are to be avoided, the following points must be recognised:
a. Normal ranges for antithrombin and proteins C and S are wide and patients with deficiency may have levels that are only slightly below normal. Repeat testing is often required for diagnostic confidence.

b. Pregnancy induces a state of resistance to the anticoagulant effect of activated protein C, which mimics the presence of FVL.

c. Pregnancy and OCP use lead to a fall in plasma protein S concentration.

d. Antithrombin concentration is reduced in acute thrombosis by heparin treatment and in pre-eclampsia.

e. Proteins C and S are vitamin K dependent and their concentrations are reduced by warfarin treatment.

WHO SHOULD BE TESTED?

To Identify Predisposing Factors and For Research Purposes

To determine the cause of VTE, currently recommended indications for thrombophilia testing include idiopathic or recurrent venous thromboembolism; a first episode of venous thromboembolism at a “young” age (e.g., < 40 years); a family history of venous thromboembolism (in particular, a first-degree relative with thrombosis at a young age); venous thrombosis in an unusual vascular territory (e.g., cerebral, hepatic, mesenteric, or renal vein thrombosis); and neonatal purpura fulminans or warfarin-induced skin necrosis.

For Clinical Management

More recently, there is consensus of many experts that for clinical management, thrombophilia testing is hardly ever needed. Despite the increasing knowledge about the multifactorial etiology of venous thrombosis, testing for hereditary thrombophilia generally does not alter the clinical management of most patients with venous or arterial thrombosis. In arterial thrombosis, there is no role for testing for hereditary thrombophilia. Although, there is a higher relative risk of venous thromboembolism in families with a tendency for venous thrombosis and a known thrombophilic defect, a positive test may lead to postpartum anticoagulant prophylaxis in case of pregnancy, or the individual decision to not use oral contraceptives. According to current guidelines, testing for antiphospholipid antibody syndrome may be justified in patients with venous or arterial thrombosis or well-defined pregnancy complications. There is an intermediate strength recommendation to prolong anticoagulant treatment in case of venous thrombosis, and to use vitamin K antagonists instead of aspirin in case of arterial thrombosis in patients who fulfill the laboratory criteria for antiphospholipid antibody syndrome. With the current knowledge, the value of routine testing of patients with a venous or arterial thrombosis remains questionable, and may lead to overtreatment with hemorrhagic complications or unnecessary concern in those tested positive.

REFERENCES


