A practical approach to thrombophilia testing

Introduction

Thrombophilia testing is increasingly requested by physicians to 'identify' a reason for thrombosis in young individuals. However, the timing, appropriate tests and, most importantly, the right interpretation of the results are often confusing for the general physician. This can have significant psychological and clinical implications for patients. This article discusses the suitability of thrombophilia testing in relation to the time of the test, preparation before the testing, interpretation of the results and further management based on these results.

What is thrombophilia?

'Inherited' thrombophilia is a genetically determined tendency to thrombosis. About 50% of patients presenting with an unprovoked venous thromboembolism have an identifiable inherited thrombophilic disorder (Middeldorp, 2011). The fact that only half of the cases who have clear-cut thrombophilic tendency can be identified as having an underlying predisposing condition is most relevant. This is because individuals and families with negative thrombophilia screening, but with definite thrombophilic tendency should be considered as having an as yet undiscovered thrombophilic defect. Such persons should be treated similarly to those who have been identified to have a thrombophilic diagnosis.

Inherited thrombophilias can be divided into 'loss-of-coagulation function' disorders (loss of natural anticoagulants, antithrombin, protein C and protein S) and 'gain-of-coagulation function' disorders (factor V Leiden and the prothrombin gene mutations) (Dahlback, 2008). The former conditions are less common but are potent risk factors for thrombosis, while the latter are more common but weaker risk factors for venous thromboembolism (Coppola et al, 2009).

In addition to inherited thrombophilia, individuals can develop spontaneous venous thromboembolism in the presence of lupus anticoagulant or antiphospholipid syndrome. This is an acquired condition where antibodies develop against several aspects of the coagulation pathway, interfering with the clotting mechanism and predisposing to thrombosis (Willis et al, 2012). Lupus anticoagulant is a much more common and stronger risk factor for venous thromboembolism than inherited thrombophilia and is also linked to arterial thrombosis and early pregnancy loss (Ruiz-Irastorza et al, 2010). One of the commonest mistakes with thrombophilia testing is that antiphospholipid screening is not done as part of the screening process.

Several other tests have been suggested to be indicative of thrombophilic tendency (*Table 1*). Controversy exists regarding the definite link between these different conditions and thrombophilia, and in most laboratories the testing is limited to the ones described before.

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In which patients should it be requested?

The value of obtaining laboratory evidence of thrombophilia is two-fold; to assess the risk of recurrence for the individual and the risk of thrombosis in their relatives. In this regard, the National Institute for Health and Clinical Excellence (2012) has recommended considerating testing in those who may be discontinuing anticoagulation following an unprovoked venous thromboembolism. They also recommend 'patients with a history of a first-degree relative with venous thromboembolism also should undergo testing for hereditary thrombophilia, but testing should not be extended to relatives' (National Institute for Health and Clinical Excellence, 2012). These messages are also stressed in the British Committee of Standards in Haematology guidelines on heritable thrombophilia screening (Baglin et al, 2010). The following clinical situations are also generally considered appropriate for thrombophilia testing:

- Unprovoked venous thromboembolism in patients less than 40 years of age
- Venous thromboembolism in those with a positive family history (two symptomatic family members)
- Venous thromboembolism secondary to pregnancy, oral contraceptive use or hormone replacement therapy
- Recurrent miscarriage (for lupus anticoagulant).

Before testing for thrombophilia, patients and especially asymptomatic family members should provide informed consent (Varga, 2008). Written consent should be obtained for molecular testing (e.g. factor V Leiden or prothrombin G20210A mutations) with appropriate recording in the medical records.

When should it be requested?

Thrombophilia testing should not be done in the acute setting of thrombosis, as the information does not change initial management (Baglin et al, 2010). In addition, acute illness or thrombosis can cause transiently reduced levels of several coagulation factors, including proteins C and S, antithrombin and procoagulant factors, as a result of their consumption into the blood clot (Johnson et al, 2012). Identifying low levels of these anticoagulant factors at this

Table 1. Tests included in thrombophilia screening

Tests done in most laboratories	Factor V Leiden mutation*
	Prothrombin G20210A mutation
	Antithrombin deficiency
	Protein C deficiency
	Protein S deficiency
	Antiphospholipid antibodies and lupus anticoagulant
Tests done in some special situations in specialized laboratories	Hyperhomocysteinaemia
	Raised factor VIII, IX and XI concentrations
	Raised von Willebrand factor
	Dysfibrinogenaemia
	Raised fibrinogen concentration
	Non-blood group O

*Activated protein C resistance is a screening test performed in some laboratories to identify those who may have factor V Leiden mutation. A negative result rules out this mutation. However, a positive result can also be caused by the patient taking the oral contraceptive pill or if the patient has high factor VIII levels

time would mean erroneous attribution of a thrombophilic state to these patients, which can have employment and insurance implications (Lochhead and Miedzybrodzka, 2007).

The only common exception to avoiding testing in the initial stages of a venous thromboembolism episode is if a patient has a high likelihood of having antiphospholipid syndrome and requires treatment with unfractionated heparin. This is because lupus anticoagulant can affect the clotting screen results (especially the activated partial thromboplastin time), making the monitoring of heparin with activated partial thromboplastin time unreliable (anti-factor Xa assay is preferred in such cases) (Ballard and Marques, 2012).

A reasonable testing policy in most circumstances is to continue warfarin for 3–6 months, and then interrupt treatment for thrombophilia testing. Ideally at least 2 weeks should elapse after warfarin has been discontinued before testing for thrombophilia is considered to avoid the effects of warfarin on vitamin K-dependent anticoagulation factors including protein C and S and also lupus anticoagulant (Favaloro et al, 2009). In the case of low molecular weight heparin, at least 48 hours should have elapsed to avoid the effect this drug may have on the anticoagulant antithrombin in some cases (Favaloro et al, 2009).

What do the results mean?

In the case of factor V Leiden and prothrombin gene mutations, the result is given as normal, heterozygous or homozygous. Homozygosity for these mutations is uncommon and usually presents at a younger age. There is approximately an 80-fold increase in lifetime risk of venous thromboembolism in patients with homozygous factor V Leiden mutation (Ballard and Marques, 2012).

Heterozygosity is the commonest positive result: 8% of Caucasians in the United States are heterozygous for factor V Leiden mutation while only 1 in 5000 are homozygous (Ballard and Marques, 2012). In rare instances, compound heterozygosity for both mutations can exist in the same person which further increases the risk of thrombosis. It is best to explain to patients who are heterozygous for these mutations that they are at a slightly increased risk of thrombosis than those who do not carry these mutations. It is also important to stress that in the presence of additional risk factors for thrombosis (*Table 2*), they should be receiving thromboprophylaxis, if there are no contraindications.

With respect to natural anticoagulants, low levels usually mean an inherited thrombophilic state. It is unusual to get low levels of multiple anticoagulant factors at the same time. Simultaneous low levels of protein C and S usually indicate warfarin contamination of the test sample (Favaloro et al, 2009; Ballard and Marques, 2012). However, low levels of individual anticoagulants can co-exist with the mutations described above, which means a higher risk than a single abnormality. It is important in this context to bear in mind that the natural anticoagulants can decrease in several other disease states (*Table 3*) (Favaloro et al, 2009; Ballard and Marques, 2012). If one or more of these conditions co-exists, it may be difficult to interpret these results. It may, however, be considered that lower levels noted in these situations would put such individuals at an increased risk of thrombosis. In the case of natural anticoagulant deficiency, it is good practice to repeat and confirm the results since laboratory assays are variable. and age- and sex-specific ranges exist.

Lupus anticoagulant positivity is an important acquired cause of thrombosis. This test is usually done in combination with anticardiolipin antibodies (and anti-beta2-glycoprotein I antibodies). If both tests are positive, it suggests the diagnosis of antiphospholipid syndrome in a patient with arterial or venous thrombosis or pregnancy morbidity (Miyakis et al, 2006). The revised Sapporo criteria for antiphospholipid syndrome recommend repeating lupus anticoagulant and anticardiolipin antibody tests and anti-beta2-glycoprotein I antibodies, if possible, on two separate occasions at least 12 weeks apart before confirmation of the diagnosis. This is based on the fact that transient positivity can occur in those who had a recent infection. Strongly positive anticardiolipin antibody titres in combination with lupus anticoagulant suggest higher risk of thrombosis (Sangle and Smock, 2011).

Table 2. Acquired risk factors for venous thromboembolism

Increasing age
Obesity
Malignancies including solid tumours and haematological cancers
Postoperative state
Hormones (combined oral contraceptive pill and hormone replacement)
Pregnancy
Medications (chemotherapy, tamoxifen, thalidomide)
Indwelling venous catheters and devices
Medical illnesses (especially vasculitides, inflammatory bowel disease and nephrotic syndrome)

What should be done about the results?

Among patients with a first idiopathic venous thromboembolism, there is little clinical trial evidence supporting a specific recommendation for longer-term treatment based on the results of thrombophilia testing (Middeldorp and van Hylckama Vlieg, 2008). Decisions regarding duration of anticoagulation (lifelong or not) in patients with venous thromboembolism should be made with reference to:

1. Whether the episode was provoked or not
2. The presence and absence of other risk factors
3. The risk of anticoagulant therapy-related bleeding (Baglin et al, 2010).

In other words, positivity or negativity of results from heritable thrombophilia is unlikely to impact on the risk of venous thromboembolism recurrence. Despite the fact that thrombophilia testing cannot predict recurrence or duration of anticoagula-

Table 3. Common causes of acquired deficiencies in antithrombin, protein C and protein S

Pregnancy (antithrombin and protein S)
Liver disease (all three)
Sepsis (all three)
Acute thrombosis (all three)
Nephrotic syndrome (antithrombin and protein S)
Disseminated intravascular coagulation (all three)
Drugs such as heparin and L-asparaginase (antithrombin)
Use of warfarin (protein C and S)

tion, many patients with recurrent venous thromboembolism are tested for these abnormalities with significant cost implications. The worrying aspect of this habit is the discontinuation of anticoagulation in those who may have negative results which puts them at risk of recurrence. A prior unprovoked venous thromboembolism is the biggest risk factor for a recurrence, not thrombophilia positivity. D-dimer testing at the end of a period of anticoagulation is useful in predicting recurrence in those who have had an unprovoked venous thromboembolism (Palareti et al, 2006).

Counselling those with positive thrombophilia results

Individuals testing positive for inherited thrombophilia tests require counselling as to:

- The slightly increased risk of thrombosis for them and their family members
- The importance of early recognition of the signs and symptoms of venous thromboembolism that would require immediate medical attention, especially if they are not receiving anticoagulation
- The risks and benefits of antithrombotic prophylaxis in situations when their risk of thrombosis is increased, such as perioperative or peripartum settings or with oestrogen-containing treatments (contraceptive pill or hormone replacement).

Controversies in thrombophilia testing

The association between inherited thrombophilia and arterial cardiovascular diseases like stroke or myocardial infarction in younger individuals is not well-established but those with antiphospholipid syndrome are at higher risk of these conditions (Middeldorp and van Hylckama Vlieg, 2008; Baglin et al, 2010). Similarly, controversy exists about the relationship between pregnancy complications and heritable thrombophilia although antiphospholipid syndrome is clearly linked to recurrent miscarriages (de Jong et al, 2011; Bennett et al, 2012). Unusual site thromboses like retinal vein, intra-abdominal and intracranial venous thromboses are not linked to thrombophilic states categorically and testing is not generally advised, although it is often performed (Tait et al, 2012). Lastly, the role of several other factors like elevated levels of coagulation factors and hyperhomocysteinaemia as a causative factor for venous thromboembolism remains under debate.

Conclusions

Several considerations have to be made before testing for thrombophilia in individuals who have unprovoked venous thromboembolism. Some common pitfalls are summarized in *Table 4*. BJHM

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Table 4. Common pitfalls in thrombophilia testing

Testing after an acute thrombosis
Not including tests for lupus anticoagulant in thrombophilia screening for a patient with venous thromboembolism
Interpreting results in the presence of anticoagulants or conditions which cause low levels of natural anticoagulants
Believing negative thrombophilia tests means that there is no thrombophilic tendency
Informing patients that a positive thrombophilia result means an increased risk of recurrence

TOP TIPS

- Thrombophilia testing can be normal despite a strong family history of thromboembolism.
- Adequate counselling is needed before performing thrombophilia testing, after discussion of the implications of positive and negative results.
- Lupus anticoagulant and anticardiolipin antibodies should be part of thrombophilia testing.

KEY POINTS

- Thrombophilia testing should only be done after careful consideration of the benefits it may have for future management.
- These tests are ideally not done at the initial presentation with thrombosis.
- Thrombophilia testing does not predict future recurrences in most cases.
- Consent should be obtained in all cases of thrombophilia testing.
- Several co-existing states can affect thrombophilia results.