

Leader

Thrombophilia testing: what do we think the tests mean and what should we do with the results?

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Inherited thrombophilia is a genetically determined tendency to thrombosis.¹ In 1965 the first family with antithrombin deficiency was described, and for many years this was the only identifiable cause of thrombophilia.² More recently, pedigree and case-control studies have confirmed that the risk of venous thrombosis is increased by deficiencies of antithrombin, protein C, and protein S, and by resistance to activated protein C.^{3–5} Other candidate genetic factors are included in table 1.

The value of obtaining laboratory evidence of thrombophilia is the ability to predict the likelihood of recurrence in symptomatic patients and the risk of thrombosis in their relatives. Thus thrombophilia testing would be used to optimise the benefit/risk ratio of anticoagulant treatment. Therapeutic recommendations would have to be based on a risk-benefit analysis that considers the risk of the disease, the effectiveness of treatment, the risk of treatment, and the predictive value of the laboratory tests used to establish the diagnosis of thrombophilia. Venous thromboembolism is a common disease with a significant risk. The risk of death from recurrence in the first three months after a pulmonary embolus is 1–2%.⁶ The risk of recurrence of deep vein thrombosis is 17.5% after two years and 24.6% after five years.⁷ Treatment with oral anticoagulation is extremely effective as long as the international normalised ratio (INR) is maintained above 2.0.^{8,9} However, this treatment is potentially dangerous—in any one year there is a 1% chance of a major haemorrhage, and one quarter of these are fatal.¹⁰ Therefore the disease carries a high risk, and treatment, while effective, is also associated with significant danger. The ability to distinguish patients at high and low risk of thrombosis would help to optimise therapeutic decisions. The identification of laboratory evi-

dence of thrombophilia would seem a rational way to achieve this. Evidence based guidelines are currently limited to grade C recommendations.^{9,11,12} However, level I evidence is becoming available which will form the basis of grade A recommendations.^{13,14}

How might thrombophilia testing be used to help decide when and how to treat patients? Before beginning to answer this question, consideration must be given to the accuracy of the laboratory tests used to “diagnose” thrombophilia. Potential diagnostic inaccuracy was clearly illustrated by the Leiden group in 1993, when they published a report of a series of protein C deficient symptomatic families.¹⁵ There was a significant overlap of protein C levels in affected and unaffected members of families with mutations affecting the protein C gene. Distribution curves of protein C activity indicated that unaffected family members could have levels as low as 40% while affected members could have levels as high as 100% of normal. It is also clear that some laboratory methods produce discordance between phenotype and genotype.^{16,17} Therefore phenotypic testing will not identify or exclude a thrombophilic genetic defect with complete accuracy. While genetic testing for the factor V Leiden or F2G20210A¹⁸ mutations is relatively simple, the risk of venous thrombosis associated with these defects is only modest. The highest relative risk is associated with mutations affecting the functional plasma levels of the natural anticoagulants antithrombin, protein C, and protein S. In these cases the extent of possible mutations excludes routine genetic testing.¹ Therefore the finding of a low level of a natural anticoagulant on a single occasion is not diagnostic of primary deficiency of a natural

Table 1 Examples of genetic mutations associated with inherited thrombophilia

Established	Probable	Possible
Antithrombin (1%) Protein C (3%) Protein S (3%) Factor V Leiden (20%) F2G20210A (6%) Mutations causing high plasma homocysteine (unknown) Mutations causing dysfibrinogenaemia (very rare)	Mutations causing raised factor VIII (25%)	Other mutations causing APC resistance Mutations causing raised fibrinogen levels Mutations causing reduced thrombomodulin activity Mutations causing reduced TFPI activity Deletion/insertion polymorphism of the ACE gene

Approximate prevalence in unselected patients with deep vein thrombosis is shown in brackets where this is known. ACE, angiotensin converting enzyme; TFPI, tissue factor pathway inhibitor.

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anticoagulant. Conversely, a single normal result does not exclude a mutation affecting the antithrombin, protein C, or protein S genes. There are currently no guidelines on the number of times that phenotypic measurements should be repeated or the extent of family testing required to establish or exclude inherited antithrombin, protein C, or protein S deficiency.

There is currently no evidence that a laboratory abnormality should influence the intensity of heparin or warfarin treatment. In a review of 70 thrombotic events in 57 individuals with antithrombin deficiency, heparin resistance was infrequent and recurrence or extension of thrombosis while on treatment was no greater than that observed in individuals without antithrombin deficiency.¹⁹ Warfarin-induced skin necrosis is extremely rare, even in patients with protein C or S deficiency. Most individuals with protein C or S deficiency do not develop skin necrosis, and there is no indication that the first few days of oral anticoagulant treatment should be different. The intensity of maintenance therapy with warfarin should not be influenced by laboratory evidence of inherited thrombophilia. There is no evidence as yet that recurrence on treatment is more likely in patients with laboratory evidence of thrombophilia. A target INR of 2.5 is sufficient for patients with the FV Leiden mutation in the heterozygous or homozygous state, even in combination with deficiency of a natural anticoagulant.²⁰ Further studies are required to determine if deficiency of antithrombin, protein C, or protein S should be an indication for a higher target INR, although this seems unlikely from case reports and descriptive family studies.

Recurrence of venous thromboembolism after stopping oral anticoagulant treatment has not been shown to be predictable by thrombophilia testing. While there are many family studies and small series indicating a high frequency of recurrence in patients with thrombophilia, a direct comparison with patients and families without laboratory evidence of thrombophilia is required. This comparison has been made for symptomatic patients who are heterozygous for the factor V Leiden mutation. Two initial studies indicated a higher risk of recurrence after stopping oral anticoagulant treatment in patients with the factor V Leiden mutation.^{21 22} However, the risk did not appear sufficient to justify lifelong anticoagulant treatment in all patients.^{20 23 24} In two recent prospective studies, factor V Leiden and *F2G20210A* mutation detection did not predict a greater risk of recurrence after stopping oral anticoagulant treatment.^{13 14} The relative risk of recurrence after stopping oral anticoagulant treatment in patients with antithrombin, protein C, or protein S deficiency has not been determined. At present it is uncertain whether symptomatic patients should receive long term anticoagulation.^{11 12} Until evidence is available the duration of treatment should generally be the same for patients with and without laboratory evidence of thrombophilia.

In thrombosis-prone families, identifying asymptomatic individuals with the same laboratory abnormality as symptomatic members may help to reduce the likelihood of thrombosis, as high risk episodes can be avoided or prophylaxis given during risk periods. The risk of contraceptive pill related thrombosis in affected family members of thrombosis-prone families with thrombophilia has now been shown to be significantly increased.⁵ Similarly, the risk of thrombosis in association with surgery, trauma, or immobility is significantly increased. It is unlikely that studies will randomise affected family members to prophylaxis or not during high risk periods. Therefore, a historical comparison is the most likely type of study that will be performed. Given this, it would seem sensible at present to recommend that affected females from thrombosis-prone families should generally avoid the combined oral contraceptive pill. In addition affected family members should probably receive anti-thrombotic prophylaxis for surgical procedures at an earlier age, for example from the age of 15 years rather than after 40 years of age. A combination of graded pressure stockings and a low molecular weight heparin is now a reasonable recommendation. The risk of bleeding with a low molecular weight heparin is small and there is a very low risk of heparin induced thrombocytopenia and thrombosis (HITT).

In conclusion, thrombophilia testing does not predict likelihood of heparin resistance, heparin failure, or warfarin induced skin necrosis. At present there is no conclusive evidence that recurrence on treatment with warfarin with a target INR of 2.5 is greater in patients with thrombophilia than in those without. There is no conclusive evidence that patients with thrombophilia are more likely to suffer an earlier recurrence once treatment is stopped. There is no doubt that the relative risk of thrombosis is increased in affected family members of thrombosis-prone families, but this translates to a relatively low absolute risk per year, and long term primary prophylaxis is not warranted. Studies confirming a beneficial outcome from thrombophilia testing and risk related prophylaxis for members of thrombosis-prone families are required. However, it is unlikely that a strategy based on current thrombophilia testing will be completely effective. While venous thromboembolism is associated with single gene defects in some families, it is now apparent that the likelihood of a thrombotic event is increased considerably when the haemostatic balance is altered by more than one mutation.^{25 26} The polygenic basis of venous thrombosis and the influence of environmental factors make it difficult to predict the likelihood of thrombosis. Furthermore, clinical outcome studies which estimate the risk of one or more specified mutations may be flawed owing to the contribution of additional but unidentified genetic factors. It is possible that most patients who suffer a thrombotic event have an underlying hypercoagulable state.²⁷ As it is impossible at present to identify the multiple individual factors affecting thrombosis risk, it might be more beneficial

to use a “global” test to identify hypercoagulability. Unfortunately, this is also impossible at present.

Acquired thrombophilia states should be mentioned. Of all the laboratory tests that are commonly performed, those that detect antiphospholipid activity appear to have the greatest potential for predicting clinical outcome. For example, patients with antiphospholipid activity may benefit from a higher intensity of oral anticoagulant treatment. A target INR of 3.5 should be considered for these patients.²⁸ Recurrence after stopping treatment is also more likely in these patients, and treatment for as long as activity is persistent should be considered.^{13 28}

In this short review I have attempted to indicate some of the uncertainties of thrombophilia testing when applied to symptomatic patients with venous thromboembolism and their asymptomatic relatives. Most recommendations are based on level II evidence, although level I evidence is now appearing. So what do I actually do in clinical practice? The first disease category in which I perform thrombophilia testing is venous thromboembolism. I screen all unselected patients with an episode of venous thromboembolism. I then treat with standard therapy with a target INR of 2.5 using the BCSH (British Committee for Standards in Haematology) guidelines as a reference for duration of treatment.⁹ On completion of the initial treatment period I perform thrombophilia tests. These consist of functional measurement of antithrombin and protein C, using chromogenic assays, and an antigenic measurement of protein S. Antiphospholipid activity is detected by quantitation of anticardiolipin antibody by enzyme linked immunosorbent assay (ELISA), and detection of lupus anticoagulant activity with a dilute Russel’s viper venom clot time and a silica clot time, with phospholipid corrections. A standard activated protein C (APC) sensitivity ratio is measured and genotyping is performed for detection of the factor V Leiden mutation and the *F2G20210A* mutations. At the moment I do not measure factor VIII or von Willebrand protein levels, as accuracy is significantly affected by preanalytical variables. I restart patients with antiphospholipid activity on warfarin and continue for as long as there is evidence of activity. I monitor activity while patients are on warfarin with anticardiolipin titres and by the ratio of factor V in one stage prothrombin and activated partial thromboplastin based assays.²⁹ I also continue warfarin treatment indefinitely in patients who are homozygous for the factor V Leiden mutation as there is a high risk of early recurrence in my experience.

If a patient suffers a recurrence after stopping treatment I treat for two years with a target INR of 2.5, without interruption.¹³ If a patient suffers a recurrence on treatment I treat indefinitely with a target INR of 3.5.⁹

Testing for hereditary thrombophilia also helps me to advise relatives and their doctors when I am questioned about thrombosis risk. For example if a thrombophilic defect is found

in an index patient I would test for this in other family members, if a therapeutic decision could be influenced by the result. One typical scenario would be a female first degree relative of the propositus who wishes to take the combined oral contraceptive pill. I would test for the specific defect previously confirmed in the index patient, as thrombosis risk is greater in affected than in non-affected family members.⁵ A second scenario might be a first degree relative who required surgery. If they had the specific defect that was present in the propositus, I would recommend the combined use of a low molecular weight heparin and graded pressure stockings even if they were less than 40 years old. Furthermore, I would recommend at least 10 days of heparin prophylaxis and encourage the use of pressure stockings for at least 14 days and then up to four weeks or until fully mobile. As these are only grade C recommendations I do not at present initiate a “comprehensive family study” but simply test for the specific familial defect if a clinical scenario develops. If in the future evidence becomes available that clinical decisions based on testing influence clinical endpoints, there will then be a case for offering proactive testing of asymptomatic family members. Finally, I would only accept at least two abnormal phenotypic results as indicative of a likely genetic defect. I consider repeat testing mandatory. In selected families, for example those with more than two members who have died of venous thromboembolism, I would consider lifelong anticoagulant treatment after a single event. I would also consider treatment of young asymptomatic members of such families if there is strong concordance between the defect and thrombotic events in older members.

The second disease category in which I perform thrombophilia testing is recurrent miscarriage and fetal loss. In this situation I would test specifically for antiphospholipid activity, as there is an evidence basis for treating affected women with heparin.³⁰ I would not test for hereditary thrombophilic defects as there is as yet no evidence basis for treating women with abnormalities.

There is no other disease category in which I recommend thrombophilia testing. While defective regulation of thrombin generation may have biological relevance to other thrombotic disorders, for example atherosclerosis and arterial thrombosis, there is no evidence that thrombophilia testing should influence clinical management decisions in either selected or unselected patients. “Thrombophilia screening” of the general population is not justified.

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