

Leading article

Thrombophilia testing for whom, why and when – a review of current practices

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Abstract

Thromboembolic conditions have accounted for 1 in 4 deaths worldwide in 2010. Thrombosis, which is the common pathogenesis of myocardial infarction, ischaemic stroke, and venous thromboembolism (VTE), is the leading cause of death (1/5 deaths in 1990). Hospital-acquired VTE accounts for approximately 60 percent of all VTEs annually. Others include cancer-associated thrombosis and gender associated risks. In addition to the disease burden, it also causes a significant economic burden world-wide. Multiple diagnostic tests and treatment, prolonged hospital stay and follow-up care, including management of recurrent VTE can be extremely costly. Therefore, it is important to focus on VTE prevention so that health care systems can save money, improve outcomes and save lives. In this background, it is important to have a clear direction as to when, how and whom to test for thrombophilia. Testing for thrombophilias is often performed without a clear understanding of the clinical implications of such results. Guidelines vary in the appropriate use of thrombophilia testing. In this review, these variations in thrombophilia testing and the implications mentioned in the recent guidelines and other articles are discussed. The review of such guidelines, guidance statements, review articles conclude that screening for hereditary thrombophilia has very

limited advantage in the management of patients with thrombosis. Screening for antiphospholipid syndrome (APLS) is more important given its high rate of recurrence in both venous and arterial thrombosis. Screening for underlying haematological conditions such as myeloproliferative neoplasms (MPN) and paroxysmal nocturnal haemoglobinuria (PNH) is also considered important.

Introduction

The global incidence of venous thromboembolism is significantly high which leads to frequent testing for hereditary and acquired thrombophilia. With high costs associated with testing, careful selection of patients, appropriate timing of testing and application to patient management are important in providing high quality clinical care. This article reviews rational, effective use of thrombophilia testing and its impact on patient management.

1. Thrombophilia testing and clinical use

Testing for thrombophilias should be performed when such results would assist in improving the management. Testing has been recommended to assist in secondary prevention (to determine the duration of anticoagulation following a thrombotic event); in respect of hereditary disorders, to aid in primary prevention in relatives of affected patients¹. Guidance statements are based on analysis of the appropriate application of positive or negative test results and thereby inform these decisions¹. Inappropriate application of testing include cost, inappropriate timing of testing² misinterpretation of results^{3,4}, stress/anxiety^{4,5} and the possibility of genetic discrimination⁶. The appropriate application of testing is, patient satisfaction from having identified a risk factor underlying a thrombotic event and an increased probability of using prophylaxis in high risk situations by affected relatives⁷. The knowledge

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of the presence of hereditary thrombophilia is unlikely to affect the survival or the risk of post thrombotic syndrome in patients⁸. Studies have demonstrated that not all thrombophilias indicate a higher risk of recurrence of VTE. This raises questions regarding the need for thrombophilia testing⁹. Population screening is currently not recommended⁹.

1.1 Clinical use of testing for activated protein C resistance (APCR)/factor V Leiden (FVL) and prothrombin G20210A

APCR is the most frequent hereditary defect associated with VTE. The FVL seems to be responsible for more than 90% of APCR cases among the Caucasian white population⁹. However, African and Asian populations have a low incidence of FVL. The ethnicity of the prothrombin gene mutation (PT G20210A) is similar to that of FVL⁹. In contrast to a heterozygote with APCR/FVL with a 3-4 fold risk, a homozygote has a 11 fold increase. In PT G20210A it is 4 fold and 7 fold respectively⁹. A compound heterozygote has a 20 fold increase in developing a primary VTE¹⁰. In countries with high prevalence, the relative risk of oral contraceptive pill (OCP) associated VTE in APCR/FVL heterozygotes is increased by 5-30 fold, but the actual difference with those developing VTE on OCP compared to those not on OCP was 0.3% per year. In respect of the pregnant women the risk of VTE among carriers and non-carriers are 1.97% and 0.73% per 100 pregnancy-years respectively⁹. With this low absolute risk of VTE, routine APCR/FVL screening prior to commencement of OCP or prior to pregnancy was found to be not cost effective^{9,11}. No such data available in respect of PT G20210A⁹, but the relative risk for the primary VTE in the general population was less than for APCR/FVL. **Both APCR/FVL and PT G20210A do not have an association with arterial thrombosis and their association with recurrent VTE is minimal⁹**. Surgery is a known risk factor for DVT, but routine screening for these mutations to initiate VTE prophylaxis was not proven cost effective^{9,11}.

1.2 Testing for anti-thrombin III deficiency (AT III), protein C (PC) and protein S (PS) deficiency and their clinical impact

The prevalence of the above hereditary thrombophilic conditions in the Caucasian white and

non-Caucasian white population is <1%⁶. The first lifetime VTE in the western population for the above, accounts for <10%, but noted to be around 30% in the Asian population⁹. The above deficiencies are considered more serious as the lifetime risk for VTE is high⁶ with approximately 50% of patients developing VTE by 50 years of age. The risk of recurrence after a symptomatic VTE is noted to be 10% per year if the patient is not on anti-coagulation⁶. Therefore, it is believed that unlike in APCR/FVL, testing asymptomatic family members of affected patients for the above deficiencies is important⁹. *However, the guidance statements suggest, not screening family members of patients with VTE, as family history of VTE confers an excess risk of thrombosis. Therefore, it is suggested that such first-degree relatives should be advised to use thromboprophylaxis in a situation of high risk¹. Genetic testing to identify variants of the above deficiencies should be performed only if the results will influence the management¹².*

1.3 Testing for methyl tetrahydrofolate reductase (MTHFR) polymorphism and its clinical impact

Hyperhomocysteinemia is a weak risk factor for VTE if mild. However, if the homocysteine levels are >22μmol/L, the risk increases up to 4 fold and at lower levels the risk is minimal⁹. Markedly elevated levels of homocysteine are known to be associated with arterial thromboembolism. Although the risk of thrombosis may be elevated in patients with MTHFR polymorphisms, which are associated with elevated homocysteine levels, there is a high prevalence of these polymorphisms in the general population. This has not shown to increase the risk of either the first or recurrent VTE^{10,13}. The mutations associated are MTHFR C677T and A1298C, and testing for these especially in the presence of normal plasma homocysteine levels is not indicated. *Both American College of Medical Genetics (ACMG) and College of American Pathologists do not recommend testing for methyl tetrahydrofolate reductase (MTHFR) polymorphism as a component of thrombophilia screening in patients with recurrent pregnancy losses or for at-risk family members^{5,7}. No treatment designed to lower homocysteine levels has proven to change the thromboembolic risk¹⁴.*

With the knowledge of the clinical utility/disutility of screening for the above hereditary thrombophilic conditions, we will proceed to evaluate the situations of performing hereditary thrombophilia screening.

2. Hereditary thrombophilia screening for primary prevention in first degree relatives of VTE patients

2.1 Should asymptomatic first degree relatives of patients with VTE and hereditary thrombophilia undergo screening?

A study done in a cohort of 382 first degree family members of patients with VTE and hereditary thrombophilia who were tested and followed up for 9 years, revealed that twice as many thrombophilia carriers used prophylaxis in risk situations and the rate of provoked VTE was higher in this group with thrombophilia (0.58%/year in those with hereditary thrombophilia as opposed to 0.24%/year in those without)¹⁴. The difference was not significant. The study indicated a potential harm from screening, as family members who tested negative for a thrombophilic defect were less likely to use prophylaxis during high risk situations which could be detrimental¹.

Another large screening study done on family members of VTE patients revealed that asymptomatic carriers of a hereditary thrombophilic defect were at excess risk of thrombosis, with the risk varying according to the genetic defect¹⁵. Studies have further revealed that a family history of thrombosis alone carries an increased risk, even in the absence of any identifiable thrombophilia^{15,16}, indicating that a negative thrombophilia screening does not equate to normal VTE risk. Published guidelines vary substantially with regard to utility of family screening^{17,3,4,12}.

The consensus reached by a study of these published guidelines is that testing for thrombophilia in asymptomatic family members of patients with VTE and hereditary thrombophilia is not indicated as a family history of VTE by itself confers an excess risk of thrombosis and relatives should be counseled regarding use of prophylaxis in high risk situations¹. Primary prevention of thrombosis is not advocated regardless of the genetic defect because

the risk of bleeding may be higher than the actual risk of the first thrombotic event^{1,11,12}.

Hence screening for asymptomatic family members is not recommended⁹.

2.2 Is hereditary thrombophilia screening indicated in asymptomatic first-degree female relatives prior to using oestrogen containing medication?

NO – *The consensus reached along with the recommendations by the National Institute of Clinical Excellence (NICE) 2012⁷ was not to test for hereditary thrombophilia in these individuals as oestrogen is a known risk factor for thrombosis² and a family history of VTE predicts an excess risk of thrombosis even if thrombophilia testing was negative.*

2.3 Is hereditary thrombophilia screening necessary in asymptomatic first-degree female relatives contemplating pregnancy?

YES – Pregnancy and puerperium are considered as major transient risk factors for thrombosis resulting in a 5-10 times the baseline risk^{18,19}. These patients do not appear to have an excess risk of thrombosis in the absence of thrombophilia. (The risk would be the same as mentioned in 3.1) *BUT, testing is recommended as it would distinguish women at low or higher thrombotic risk which would assist in deciding the duration for prophylaxis^{20,21,12}.*

3.1 Secondary prevention following VTE in females in the reproductive age group

3.1.1 Is hereditary thrombophilia screening indicated in those contemplating pregnancy with a history of a provoked VTE by a minor transient risk factor which is non-oestrogen related?

NO – As per the RCOG guidelines, history of any DVT requires prophylaxis throughout the antenatal period²².

3.1.2 Is hereditary thrombophilia screening necessary in a woman in the reproductive age group, with a history of previous VTE either unprovoked, provoked by pregnancy or oral contraceptive pill (OCP)?

NO – She would require prophylaxis throughout the antenatal period irrespective of the thrombophilia status^{3,8,22}.

3.1.3 Is hereditary thrombophilia screening indicated in a woman in the reproductive age group with a history of a prior VTE provoked by a major provoking risk factor (surgery or major trauma)?

NO – As she does not have additional risk factors²².

3.2 Secondary prevention in patients other than females in the reproductive age group

3.2.1 Is hereditary thrombophilia screening indicated in patients after a PROVOKED VTE?

NO – The consensus is to NOT perform thrombophilia testing, as a positive result is not a sufficient reason to provide extended anticoagulation following an episode of provoked VTE¹.

3.2.2 Is hereditary thrombophilia screening indicated in patients following an UNPROVOKED VTE?

NO – These patients require being on long term anticoagulation,

- As the absolute risk for recurrent VTE is high, with a 5-year risk of 30% unless the patient is on extended duration anticoagulant therapy^{1,23}. Therefore, if the patient remains on indefinite anticoagulation, thrombophilia screening will not add additional benefit¹.
- However, after initial first 3 months, if it is necessary to stop anticoagulation in a patient due to:
 - high risk of bleeding
 - continuing anticoagulation is contrary to patients' values and preferences. Thrombophilia screening is recommended^{1,23}. BUT ONLY screening for antiphospholipid antibodies is recommended due to the high rate of recurrence in APLS.
- Following an unprovoked VTE, if thrombophilia screening is negative, it can falsely reassure clinicians that the risk of recurrent VTE is low resulting in a decision to stop anticoagulation

in patients at high risk of recurrence. The NICE guidelines recommend not screening for FVL/PT G20210A, protein C and S deficiency and AT III deficiency¹⁷.

3.2.3 Is hereditary thrombophilia screening indicated in patients following VTE at unusual sites?

NO – These sites include cerebral, retinal, hepatic, splanchnic, renal and upper limb^{24,25,12}.

- The above conditions can be associated with acquired causes such as PNH, MPNs and APLS.
- Retinal vein thrombosis has no association with thrombophilia.
- Upper limb thrombosis is most often associated with an acquired obstructive pathology.
- Renal vein thrombosis too has no association with thrombophilia and is most often associated with nephrotic syndrome or malignancy²⁶.

3.2.4 For secondary prevention in antiphospholipid syndrome (APLS)

APLS results in a higher risk of recurrence than hereditary thrombophilias which could promote the decision for prolonged anticoagulation in a patient.

- APLS has a moderate to high risk for recurrent VTE. Lupus anticoagulant alone confers a 11 fold increase in the risk, and those with anti-cardiolipin antibodies posing a milder risk of 1.6 fold¹⁵. Risk is found to be higher in triple positive APLS^{9,27}.
- Risk of recurrent thrombosis is high in APLS with rates of 12%, 17-26% and 30-44% at 1 year, 5 years and 10 years of diagnosis respectively^{10,27}.

In summary

- I. Screening for hereditary thrombophilia is not indicated for prevention of recurrence in patients with unprovoked VTE as they need to be on long term anticoagulation therapy due to such increased risk regardless of test status.
- II. Even in the case of thrombosis at unusual sites hereditary thrombophilia screening is not recommended.

4. Need for thrombophilia screening after arterial thrombosis

The pathogenesis of arterial thrombosis and venous thrombosis differ with arterial thrombosis resulting from platelet-rich thrombi caused by underlying atherosclerosis, whereas venous thrombosis occurs due to hypercoagulable states resulting in the formation of fibrin-rich thrombi.

Most often arterial thromboses occur secondary to underlying systemic disorders such as dyslipidaemia, hypertension, diabetes mellitus and atherosclerosis¹⁷. Therefore, thrombophilia screening is not indicated. However, young patients or those lacking above mentioned risk factors for arterial thrombosis should be investigated for antiphospholipid syndrome⁷.

Of the hereditary thrombophilias, FVL/PT G20210A and AT III deficiency have no associated risk for arterial thrombosis⁹. Protein S has shown to be associated with coronary artery disease in middle aged men with no other comorbidities⁹. No association has been found with protein C and S deficiencies and stroke.

In contrast to hereditary thrombophilias, antiphospholipid antibodies are associated with arterial thrombosis. This can manifest as pregnancy loss in 9% of women, stroke in 14% and myocardial infarction (MI) in 11%¹⁰.

Also, in young patients with strokes there is evidence that lipoprotein (a) (LPa) can be associated with arterial strokes even though they are mainly associated with cardiovascular ischaemic attacks¹⁰.

In summary

In arterial thrombosis hereditary thrombophilia screening is NOT recommended. Screening is indicated only for APLS, MPN, PNH, sickle carriers and LPa.

5. Paediatric thrombosis, neonatal thrombosis, purpura fulminans and stroke in children

- Neonates and children presenting with purpura fulminans need to be tested for protein C and S deficiency¹².

- In neonates with stroke thrombophilia screening is not routinely recommended¹².
- Neonates presenting with multiple unexplained thrombosis and suggestive of catastrophic antiphospholipid syndrome (CAPS), testing for antiphospholipid antibodies and heritable thrombophilia should be considered¹².

6. Timing for thrombophilia assessment

- i. Thrombophilia testing is affected when done at the stage of an acute thrombosis or anticoagulant therapy^{1,28}. Natural anticoagulants protein C, S and AT III levels are low, soon after a thrombotic event and best screened 3 months after a thrombotic event.
- ii. Anticoagulants such as heparin and direct oral anticoagulants (DOACs) can give a false positive result in the assays for lupus anticoagulant and makes interpretation difficult in the presence of warfarin.
- iii. The tests for thrombophilia that can be reliably done while on anticoagulation are the genotype-based tests such as FVL and PT G20210A and antibody titers for cardiolipin and beta-2 Glycoprotein-1. They can also be performed accurately at any point during the care of the patient. The ideal timing for testing of the remaining, (lupus anticoagulant, PC, PS and AT) is after a minimum of 3 months of anticoagulation treatment. Anticoagulants should be withheld for a minimum of 1 week before testing^{1,12}.
- iv. Antenatal and the postpartum period are acquired hypercoagulable states. Coagulation factors such as fibrinogen and factor VIII show an increase but protein S shows a significant decrease^{7,29}.
- v. Pregnancy and oestrogen use reduce the levels of protein S. Therefore, it is highly recommended to test when not pregnant or not using oestrogen preparations¹.
- vi. It has been noted that lupus anticoagulant and anticardiolipin IgG antibody levels will be falsely high especially during the first trimester³⁰.

In summary

Testing for thrombophilia should not be performed soon after a thrombotic event, while on anti-coagulation, on OCP or during pregnancy.

Conclusion

After an intensive study of many research articles, guidelines and reviews on the topic of thrombophilia screening it would be justifiable to conclude the following:

- Hereditary thrombophilia screening is not indicated as population screening, prior to commencement of oestrogen therapy, pregnancy, provoked DVT, unprovoked DVT or arterial thrombosis (strokes).
- Hereditary thrombophilia screening is indicated only as primary prevention in asymptomatic first-degree female relatives in the reproductive age group of a patient with VTE to decide on duration of antenatal thrombo-prophylaxis. For secondary prevention in patients of this age group if pregnant require antenatal and postnatal thromboprophylaxis. Therefore, thrombophilia screening is NOT indicated.
- The presence of antiphospholipid antibodies which has a high primary and recurrent risk for VTE and arterial thrombosis needs to be screened in patients after an unprovoked VTE not willing to continue with extended duration anticoagulation, due to risk of bleeding, personal preference or converting to DOACs after 3 months of warfarin.
- Young stroke patients without any comorbidity for arterial thrombosis need to be screened for APLS and other haematological disorders such as MPNs, PNH and sickle carrier states.
- MTHFR polymorphism is no longer considered as an indication requiring screening due to its high prevalence in the general population and relative low risk of thrombosis.
- Testing for thrombophilia is ideally done 3 months after an acute event and after having stopped anticoagulation and while on bridging therapy (LMWH) for a minimum of 1 week. LMWH should be stopped 24 hours prior to testing.

- In neonatal strokes hereditary thrombophilia screening is not recommended routinely.

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