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Common Sense Pathology

A REGULAR CASE-BASED SERIES ON PRACTICAL PATHOLOGY FOR GPs

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Thrombophilia

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Thrombophilia

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Introduction

The term thrombophilia refers to a disorder of haemostasis in which there is a tendency for the occurrence of thrombosis. Environmental or acquired risk factors for thrombosis have been known for centuries. However, in the past few decades, an increasing number of hereditary risk factors have also been identified (see Table 1, opposite page). Clinical thrombosis usually results from the interaction between multiple risk factors, and even in patients with inherited thrombophilia, is often associated with an acquired risk factor such as surgery, pregnancy or use of oral contraceptives.

Not all patients with venous thromboembolism (VTE) will require laboratory testing for thrombophilia, and even today there is still much controversy about which patients should be tested. Some have argued that widespread testing of thrombosis patients for prothrombotic abnormalities has been prematurely adopted into clinical practice, because there is little evidence that their identification leads to improved clinical outcomes. Testing is expensive and does not necessarily alter patient management. Therefore a careful selection of patients for thrombophilia testing is advised to

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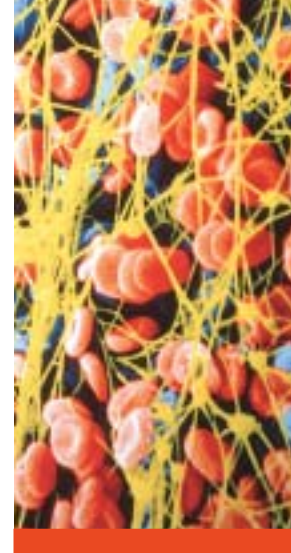
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improve reliability of results and to have an acceptable cost/benefit ratio. This article will give an overview of the thrombophilia testing available and attempt to provide guidance as to when it should be performed, based on our current knowledge. In general, patients identified as having laboratory evidence of thrombophilia should be referred to a haematologist or vascular specialist for further assessment and management.

What tests are available?

The initial laboratory evaluation in patients with venous thrombosis should include a complete blood count, coagulation studies, liver and renal function tests, and urinalysis. These tests may indicate a possible myeloproliferative disorder (including polycythemia rubra vera and essential thrombocythemia), paroxysmal nocturnal haemoglobinuria or nephrotic syndrome. An otherwise unexplained prolongation of the activated partial thromboplastin time (APTT) that does not correct on 1:1 dilution with normal plasma may indicate a lupus anticoagulant (see next page).

Specific thrombophilia tests include genetic, coagulation-based and antibody-based tests (see Table 2).

They include:

1. Factor V Leiden (genetic test)

This genetic mutation prevents the normal inactivation of the factor V molecule by activated protein C (APC), and results in increased levels of procoagulant factor Va. It was originally described as APC resistance and can also be detected using a coagulation test. Factor V Leiden occurs in about 5% of Caucasians and the risk of venous thrombosis is increased three- to sevenfold in heterozygotes, and 80-fold in homozygotes. It is important to note that certain acquired conditions (including pregnancy, oral contraceptive use and elevated factor VIII levels) may also result in APC-resistance on coagulation testing. Therefore Factor V Leiden should always be confirmed by specific genetic analysis.

2. Prothrombin G20210A mutation (genetic test)

This variation of the prothrombin gene is associated with elevated plasma levels of the procoagulant molecule prothrombin, and an increased risk of venous thrombosis. It increases the risk of venous thrombosis almost threefold.

Table 1: Causes of thrombophilia

Acquired

- Advanced age
- Previous thrombosis
- Immobilisation
- Major surgery
- Orthopaedic surgery
- Malignancy
- Oral contraceptives
- Hormone replacement therapy
- Pregnancy
- Antiphospholipid syndrome
- Essential thrombocythemia
- Polycythemia rubra vera
- Paroxysmal nocturnal haemoglobinuria
- Hyperhomocysteinaemia (also has hereditary component)

Inherited

Deficiency of circulating natural anticoagulant factors

- Protein C deficiency
- Protein S deficiency
- Antithrombin deficiency

Increased levels of procoagulant factors

- Factor V Leiden
- Prothrombin gene mutation

Table 2: Specific thrombophilia tests

- Factor V Leiden gene mutation (activated protein C resistance)
- Prothrombin G20210A mutation
- Antithrombin level
- Protein C level
- Protein S level
- Lupus anticoagulant
- Anticardiolipin antibody
- Homocysteine level
- MTHFR 677C>T gene mutation



3. Antithrombin, protein C and protein S deficiencies (coagulation tests)

Antithrombin, protein C and protein S are circulating natural anticoagulants. Both quantitative and qualitative inherited deficiencies exist and the association with venous thromboembolism is well documented.

4. Lupus anticoagulant (coagulation test) and anticardiolipin antibody (serum antibody test)

The lupus anticoagulant and anticardiolipin antibody are both acquired autoantibodies directed against phospholipid-protein complexes (antiphospholipid antibodies). They can be associated with venous and/or arterial thrombosis as well as recurrent miscarriage, as part of the antiphospholipid syndrome. Laboratory diagnosis relies on specialised coagulation assays for the lupus anticoagulant and immunoassays for anticardiolipin antibody. The lupus anticoagulant is one of the causes of a prolonged APTT. It is important to always request both lupus anticoagulant and anticardiolipin antibody in a patient with suspected antiphospholipid syndrome because many patients will have only one type of antiphospholipid antibody. Transiently positive antiphospholipid antibody tests may occur and are generally not considered to be associated with thrombosis.

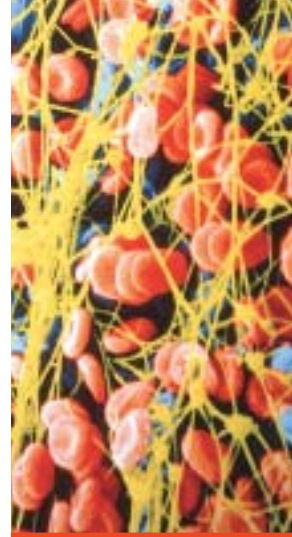
5. Hyperhomocysteinaemia (immunoassay) and methylene tetrahydrofolate reductase (MTHFR) 677C>T mutation (genetic test)

Homocysteine is a non-protein amino acid and hyperhomocysteinaemia has been found to promote atherosclerosis as well as venous and arterial thrombosis. Elevated levels can result from genetic- or nutrient-related disturbances in the pathways for homocysteine metabolism. Homocystinuria is a rare genetic disease characterised by very high levels of homocysteine, arterial vascular disease and venous thrombosis. More recently it has been suggested that even mildly elevated levels of homocysteine ($>18\mu\text{mol/L}$) are associated with an increased risk of thrombosis, and that this may be caused by a common variant in the MTHFR gene, known as 677C>T mutation. Whether this is a significant risk factor for VTE is still unclear, and results from future meta-analyses may prove more informative. Homocysteine levels can be measured in fasting blood specimens using immunoassay. Measurement of levels post-oral methionine loading (uncommonly used) may increase the sensitivity for detection of hyperhomocysteinaemia. Genetic testing can be used to detect the 677C>T MTHFR mutation.

Which patients should be tested?

Only a minority of patients suspected of having venous thromboembolism on clinical grounds actually have the disease, therefore it must first be established that the diagnosis of venous thromboembolism has clearly been made by objective means, such as ultrasound, radiology or nuclear scanning. After establishing the diagnosis, comprehensive clinical assessment is critical and should reveal most acquired causes of thrombophilia, and a detailed personal and family history should also be recorded. Women should be specifically questioned about oral contraceptive use, hormone replacement therapy, pregnancy and any history of miscarriage.

There is no clear consensus about which patients to test for thrombophilia, and there are generally two approaches to this issue — the conservative approach and the liberal approach. Clinicians who advocate a conservative approach argue that testing all or most patients with an initial episode of VTE is expensive and cost-ineffective. They say this is because there is a paucity of evidence about how, if at all, the clinical management of patients with thrombophilia and VTE differs from those patients who do not have a specific inherited thrombophilia. Testing of any patient or their relatives can generate needless anxiety if a genetic thrombophilic factor is identi-



fied, or alternatively promote false reassurance in those with a negative test. Advocates of the liberal approach suggest that although there is insufficient evidence, it is biologically plausible to consider longer or indefinite anticoagulant treatment in patients with 'severe' thrombophilia associated with a high risk of VTE, such as carriers of homozygous or double heterozygous gain-of-function mutations and those with antithrombin deficiency, particularly if VTE occurred in the absence of transient risk factors. Thrombophilia testing may also help to decide on the usefulness of primary prophylaxis in the presence of transient risk factors in asymptomatic relatives of thrombosis patients with thrombophilia. Knowledge of the existence of thrombophilia is also likely to benefit women in the post-partum period when the risk of VTE is high and antithrombotic prophylaxis should be considered.

At present, it would seem most appropriate to follow the recommendations of the College of American Pathologists and perform specific thrombophilia testing in the patient groups listed in Table 3.

Additionally, testing for antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibody) is considered appropriate for patients with VTE, particularly if the VTE is idiopathic, associated with autoimmune disease or in the absence of a family history of venous thrombosis.

The College of American Pathologists also recommend that before pregnancy or oral contraceptive use, it may be worthwhile to test asymptomatic, female, first-degree relatives of a proband with a defined inherited thrombophilia (for that identified defect). This testing is especially important for families with known antithrombin deficiency. Testing for thrombophilia is also considered appropriate in asymptomatic first-degree relatives of a proband with a known inherited thrombophilia. Such testing may be particularly useful in families with deficiencies of protein C, protein S, or antithrombin.

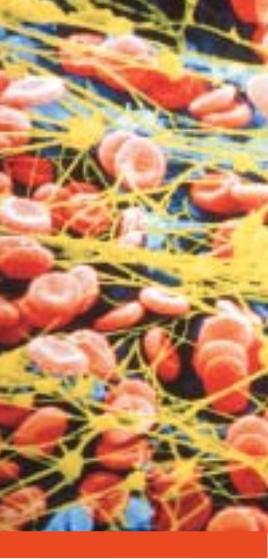
Complete thrombophilia testing is not justified in patients presenting with arterial thrombosis because hereditary thrombophilia is principally a risk factor for venous thromboembolism. However, testing for antiphospholipid antibodies and hyperhomocysteinaemia may be appropriate.

Thrombophilia testing and Medicare

When requesting protein C, protein S, antithrombin levels, or any genetic tests, specific clinical information is required for a Medicare rebate to be obtained. This includes a personal history of VTE; a previous abnormal or indeterminate test result; or a first-degree relative of a person with a proven thrombophilia defect (state which defect). This must be clearly stated on the request form.

Table 3: Who to consider for specific thrombophilia testing

- History of recurrent VTE
- VTE before age 50
- Unprovoked VTE at any age (however, testing for protein C, protein S, and antithrombin deficiency may be of lower diagnostic yield in patients with a first VTE after age 50)
- VTE at unusual sites (eg, cerebral, mesenteric, portal, hepatic veins)
- VTE patients with a positive family history of VTE
- VTE secondary to pregnancy, oral contraceptives, or hormone replacement therapy
- Combination of arterial and venous thrombosis (antiphospholipid antibodies)



What are the pitfalls in thrombophilia testing?

There are some important practical limitations of thrombophilia testing that deserve special mention. It may take up to several weeks to obtain results because such testing is not performed on a daily basis. Most tests (apart from DNA analysis) are affected by the post-thrombotic state, and should be performed a few months after an acute episode whenever possible. Testing should only be performed in specialised laboratories, and when an abnormality is detected, the test should generally be repeated on another occasion to confirm the diagnosis.

The existence of underlying conditions that influence normal haemostasis (such as pregnancy, liver disease, oral contraception, hormonal replacement therapy) should be considered when any laboratory diagnosis for thrombophilia is performed, because they can affect the result of thrombophilia coagulation assays.

The use of heparin or warfarin may affect thrombophilia testing, and if possible, it is best to wait until patients are no longer receiving anticoagulation. Specifically, antithrombin levels are reduced by heparin, and the diagnosis of protein C or protein S deficiency is difficult and not recommended in patients receiving warfarin therapy. Diagnosis of a weak lupus anticoagulant can also be difficult in warfarinised patients.

What to do with the results?

Once a patient with a specific thrombophilic risk factor has been identified, they should be referred to a specialist in haematology or vascular medicine for further management. Treatment options vary, and the individual patient's clinical scenario is often more important in determining management than the result of specific thrombophilia testing. There are also a group of patients who, from a clinical point of view, appear to have thrombophilia, but have no identifiable thrombophilic risk factor on laboratory testing. One possibility is that these patients have a defect that has not yet been identified. Referral for specialist care should still be considered, because they may require long-term anticoagulation.

Case 1

A 68-year-old male has a right proximal DVT (confirmed on venous duplex ultrasound) after discharge from hospital for an elective total hip arthroplasty performed twelve days before. He has no previous history of VTE and received routine peri-operative prophylaxis with low molecular weight heparin. Routine pre-operative blood tests showed no significant abnormality.

What other aspects of the patient's history are important to consider?

This patient has several acquired risk factors for VTE that include advanced age, immobilisation, and orthopaedic surgery. If the patient is known to have an underlying active malignancy, then the use of long-term anticoagulation with low molecular weight heparin rather than warfarin should be considered since it has been shown to be more effective. The presence of a family history of VTE may be of interest, but is unlikely to be of clinical significance in this patient.

What blood tests would you order?

This patient has normal renal function and no evidence of haematological abnormality on routine pre-operative blood tests. Before starting therapeutic anti-coagulation it is also important to check baseline coagulation tests, because a prolonged APTT potentially caused by a lupus anticoagulant may be missed. There is no indication to perform specific thrombophilia testing in this patient because there are several acquired risk factors for VTE and the precipitating event (surgery) is temporary.



Case 2

A 27-year-old female presents with a painful and swollen left calf, which she first noticed two days ago while walking to work. She has been otherwise well and has no other symptoms. She has not sustained any recent trauma to her leg or been immobilised. A lower limb venous duplex ultrasound shows a DVT involving the left posterior tibial vein extending into the distal popliteal vein and she is commenced on low molecular weight heparin.

What other aspects of the patient's history are important to consider?

It is important to establish if there are any potential precipitating factors for VTE (as listed in Table 1), particularly whether the patient is pregnant or on oral contraceptive. It is also important to determine if this is the first episode of VTE and whether or not the patient has a family history. The patient should also be questioned about previous pregnancies and miscarriages.

What blood tests would you order?

This patient has a documented DVT occurring at a relatively young age. Questioning reveals that this is her first thrombotic episode and that she is not pregnant or on the oral contraceptive pill. There are no obvious precipitating factors. She states that both her mother and maternal aunt have had multiple episodes of VTE and have been on warfarin. It would be appropriate to perform specific thrombophilia testing in this patient. However, it is important to remember that coagulation-based tests can be affected by the acute post-thrombotic phase as well as anticoagulants, so it is preferable to perform these types of tests after the patient ceases their anticoagulation. DNA (genetic) tests can be performed at any time.

Case 3

A 34-year-old female presents with right-sided weakness and dizziness. You arrange for a cerebral CT scan, which shows a cerebral infarct. She is admitted to hospital for further management including anti-coagulation. She tells you she has never been unwell before and is on no regular medication.

What other aspects of the patient's history is important?

This patient has had a documented cerebral infarct at a relatively young age, therefore it is important to look for an underlying predisposing factor. There is no history of hypertension, cardiac anomaly or previous cerebrovascular disease. However, on further questioning she tells you that she has had four consecutive spontaneous miscarriages, all before the 10th week of gestation. Investigations for possible maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes have all been excluded. She has no previous or family history of VTE.

What blood tests would you order?

This history is highly suggestive of the antiphospholipid syndrome, which is defined as the presence of vascular thrombosis (venous and/or arterial) or specific pregnancy complications in association with persistently positive antiphospholipid antibody tests. This patient has two clinical complications, and should be tested for both lupus anticoagulant and anticardiolipin antibody. However, it is important to remember that a weakly positive lupus anticoagulant may be missed in a patient taking warfarin, and should therefore be re-tested for once the patient stops therapy. The diagnosis of antiphospholipid syndrome requires that either lupus anticoagulant or anticardiolipin antibody be positive on repeat testing performed at least six weeks after the initial test.

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