

An approach to the investigation of thrombophilia

Beng H. CHONG FRCPA

Centre for Thrombosis and Vascular Research, University of New South Wales and Department of Haematology, Prince of Wales Hospital, Sydney, Australia

PATHOGENESIS OF THROMBOPHILIA

Thrombophilia is an inherited or acquired condition in which there is an increased tendency to develop thrombosis.

There are two systems of natural anticoagulant proteins which inhibit circulating activated coagulation factors and prevent these factors from causing pathological thrombosis.

Protein C, a vitamin K-dependent protein produced by the liver, is activated by **thrombomodulin** on the endothelial cell surface to its active form, activated protein C (MC). APC inactivates Factor Va and Factor VIIIa by proteolytic cleavage of its substrate proteins. The reactions require protein S, calcium and phospholipid as cofactors. Protein S is another vitamin K-dependent liver protein which exists either as a complex with C4 binding protein or as free protein S. It is the latter form of the protein that serves as a cofactor for APC. Hence deficiency of either protein C or protein S will result in a thrombophilic state. Recently a mutant form of Factor V (termed Factor Va_{Leiden}) was recognised; Factor Va_{Leiden} is resistant to the cleavage by APC.¹ The mutant Factor V is the result of a single amino acid substitution of arginine₅₀₆ by glutamine. Patients who inherit this genetic defect have an increased risk of venous thrombosis.

There is another system of anticoagulant proteins. **Antithrombin III (AT III)** neutralises predominantly thrombin, and **heparin** cofactor inhibits only thrombin. The reactions of these two anticoagulant proteins with activated factors

are enhanced several thousand fold by sulphated proteoglycans such as **heparin**. Like protein C and protein S deficiency, deficiency of AT III and **heparin** cofactor II may also lead to a **thrombophilic** state.

Similarly, unwanted fibrin clot formed in the vasculature is efficiently dissolved by the fibrinolytic system. This is mediated by a potent enzyme, **plasmin**. **Plasmin** circulates in the blood as an inactive precursor plasminogen, which is converted to its active form by endothelial cell-derived tissue plasminogen activator (**tPA**) or **urokinase**. The action of **tPA** and **plasmin** are neutralised by **tPA** inhibitor-1 (PAI-1) and **α2-anti-plasmin** respectively. An inherited or acquired decrease in **plasminogen** or **tPA** and conversely, an increase in PAI-1 or **α2-anti-plasmin** will lead to reduced fibrinolysis and consequently a thrombophilic state.² Furthermore, some patients with abnormal forms of fibrinogen (dysfibrinogenaemia) may have clots consisting of abnormal fibrin that is very resistant to lysis by **plasmin** and these individuals have an increased tendency to develop **thrombosis**.²

Among the aforementioned abnormalities, only inherited deficiency of protein C, protein S and AT III, Factor V_{Leiden} and dysfibrinogenaemia have been shown conclusively to be associated with an increased tendency to develop thrombosis (Table 1). Factor V_{Leiden} is most common; dysfibrinogenaemia as a cause of **thrombophilia** is very rare. The prevalence^{2,3} of the familial thrombophilias in patients with a history of thrombosis are given in Table 2.

TABLE 1: Familial thrombophilias

<u>Proven association</u>	<u>Unproven Association</u>
ATIII deficiency	Plasminogen deficiency
Protein C deficiency	Heparin cofactor II deficiency
Protein S deficiency	Increased histidine-rich glycoprotein
Activated Protein C resistance	Decreased tPA or increased PAI-1
Dysfibrinogenaemia	

Address for correspondence and reprint requests: Dr. Beng H. Chong, Department of Haematology, Prince of Wales Hospital, Cnr. High & Avoca Streets, Randwick, NSW 2031, Australia.

TABLE 2: Prevalence of familial thrombophilia in patients with thrombosis

AT. III deficiency	1.1	—	7.5%
Protein C deficiency	1.0	—	7.8%
Protein S deficiency	0.5	—	5.0%
Activated Protein C resistance	21	—	40%
Total	24	—	56%

Besides familial thrombophilias, there are a number of well-recognised acquired thrombophilic states. An association between cancer and thrombosis has been known for many years. Patients with malignancy such as lung, ovarian, pancreatic and mucosa-secreting gastrointestinal tract carcinoma: may have unusual forms of thrombosis including migratory superficial thrombophlebitis, renal vein and portal vein thrombosis. The thrombosis of these patients may progress despite what is usually considered adequate anticoagulant therapy. Budd-Chiari syndrome has been reported in patients with hepatoma, renal cell and adrenal carcinoma⁵ as well as polycythaemia rubra vera (PRV).⁶ Patients with myeloproliferative disorders, particularly essential thrombocythaemia and PRV have increased risk of cerebral thrombosis and digital ischaemia. Other acquired conditions known to have an association with thrombosis are antiphospholipid syndrome, cancer chemotherapy, post-operative state, nephrotic syndrome, estrogen therapy, paroxysmal nocturnal haemoglobinuria (PNH) and heparin-induced thrombocytopenia (Table 3).²

Who should be investigated for possible underlying thrombophilia?

The incidence of venous thrombosis increases with age and is common in individuals over the age of 60 years. It frequently occurs after major surgery. Young patients, particularly <40 years of age, who present with thrombosis for the first time especially without an obvious precipitating cause or with only minor provocation, should be investigated for an underlying thrombophilic state. Other groups of patients who should also be investigated include those with a strong family history of thrombosis and those with recurrent thrombosis or thrombosis at an unusual site. Patients with coumarin-induced necrosis or neonatal purpura fulminans (which suggests the presence of protein C or S deficiency) and heparin resistance (which occurs frequently in

AT III deficiency) should also be investigated (Table 4). In addition, those with positive history or physical signs suggestive of the presence of an acquired thrombophilic state such as thrombophlebitis migrans, generalised oedema (a suggestive feature of nephrotic syndrome) and haemolysis or bone marrow hypoplasia (suggestive of PNH) should have the appropriate investigations to look for the specific underlying prethrombotic conditions (Table 5²).

Tests for familial thrombophilias

Patients suspected to have an inherited thrombophilic state, particularly young patients with a strong family history of thrombosis should have laboratory tests for AT III, protein C and protein S deficiency and APC resistance in the first instance as these are the common familial thrombophilias. If the tests are negative and a familial thrombophilia is still strongly suspected clinically, it would be appropriate to look for rarer causes like dysfibrinogenaemia or conditions in which the association with thrombosis is weak, such as heparin cofactor II deficiency (Table 1).

Tests for AT III, protein C and protein S

Tests for AT III, protein C and protein S deficiency include both antigenic or functional assays. The former tests are usually enzyme-linked immunosorbant assays (ELISA), immuno-electrodiffusion assays and nephelometry. The

TABLE 3: Acquired thrombophilias

Malignancy
Cancer chemotherapy
Antiphospholipid antibody
Myeloproliferative disorder
Heparin-induced thrombocytopenia
Post-operative state
Oestrogen therapy
Nephrotic syndrome
Paroxysmal nocturnal haemoglobinuria

TABLE 4: Clinical manifestations of thrombophilia

Family history of thrombosis Thrombosis at a young age or in infancy Recurrent or idiopathic thrombosis or thrombosis following trivial provocation Thrombosis at an unusual site Coumarin necrosis or neonatal purpura fulminans Heparin resistance Recurrent thrombosis despite adequate therapy
--

functional tests are either clotting tests or chromogenic assays. It is preferable that both types of assays be performed. However, if only one **type** of test is **being** done because of financial or other reasons, one should choose the functional assays as they will detect both quantitative and qualitative defects. For protein S assays, it is important to use assays that measure free and not total protein S as it is the levels of **free** protein S that correlate with the occurrence of thrombosis.

Tests for APC resistance

APC resistance may be detected by a clotting test or a DNA assay. The current clotting tests are either **APTT-based** or dilute Russell viper venom (**dRVVT**) time-based. Using the **APTT**-based tests, there is considerable overlap between the results of individuals with and without the defect. The **dRVVT-based** test is much more specific in our hands. These tests should only be used as a screening test and if the test is positive, it should always be **confirmed** by DNA testing. There are a range of assays that can be used to detect Factor V and these are shown in Table 6. Among these tests the most commonly used is allele-specific restriction analysis (ASRA).

Helpful points for investigations of familial thrombophilias

Tests for **AT III**, protein C, protein S and clotting tests for APC resistance should not be performed **during** acute thrombosis or less than six weeks after resolution of thrombosis as anticoagulant proteins are frequently consumed during acute thrombosis and their levels may remain low for some time thereafter. Similarly, activated factors or their activation **peptide** fragments may interfere with the APC resistance tests. Oral anticoagulant therapy will reduce plasma levels of proteins C and S and tests performed in patients receiving oral anticoagulant will give falsely low results. Both oral anticoagulant and **heparin** will interfere with the APC resistance clotting tests. If any of the tests are positive, it is helpful to repeat it at least once before the patient is labelled for life as a familial thrombophilic. If the test is positive, a family study is required to detect other family members at risk of thrombosis. A second abnormality should also be looked for, as some families have two co-existing thrombophilic conditions e.g. APC resistance and protein S deficiency. These individuals with two abnormalities have a higher risk of thrombosis. Finally, it is also important to exclude acquired conditions that may give rise to low plasma levels of **AT III**, protein C or

TABLE 5: Tests for an underlying thrombophilia in patients with suggestive abnormalities

<u>Suggestive abnormalities</u>	<u>Tests</u>
Malignancy: Thrombophlebitis migrans , recurrent thrombosis despite adequate therapy	FBE, Stool for occult blood. Chest X-ray, Abdominal CT scan, endoscopy, colonoscopy
Nephrotic Syndrome: Generalised oedema	Urinary proteins, serum albumin, etc.
Paroxysmal nocturnal haemoglobinuria: Haemolysis, pancytopenia	Sugar water test, Ham test

TABLE 6: DNA tests for Factor V_{Leiden} (APC Resistance)

Allele-specific restriction analysis (ASRA)
Allele-specific oligonucleotide (ASO) hybridisation
Allele-specific PCR (ARMS)
Base sequencing

TABLE 7: Acquired conditions with decreased AT III, Protein C and/or Protein S

<u>Decreased AT III</u>	<u>Decreased Proteins C & S</u>
Liver disease	Liver disease
DIC	DIC
Nephrotic syndrome	Neonatal period
Major surgery	Vit K antagonist & L-asparaginase therapy
Pregnancy	Pregnancy, SLE, acute inflammation, oestrogen therapy (Protein S decreased)
Heparin, L-asparaginase, & oestrogen therapy	

TABLE 8: Markers of *in vivo* platelet activations

Platelet changes:	(a) Increased platelet surface expression of P-selectin
	(b) Presence of activated GPIIb-IIIa which binds PAC-1 monoclonal antibody
Urinary changes:	(a) Increased urinary excretion of prostaglandin metabolites
Plasma changes:	(a) Elevated plasma levels of PF4, β-thromboglobulin and P-selectin

protein S² before a diagnosis of familial thrombophilia is made (Table 7).

Investigations for acquired thrombophilia

A list of common acquired **thrombophilia** is given in Table 3. In some patients, the presence of these conditions is clinically obvious or there may be a suggestive history or physical sign (Table 5). If so, the appropriate tests should be performed to look for the underlying thrombophilic state. If **not**, it is appropriate to request lupus inhibitor and **anticardiolipin** assays to detect antiphospholipid antibody syndrome, full blood counts to look for a myeloproliferative syndrome, and chest x-ray and stool for occult blood to detect occult lung and **gastrointestinal** cancer. These investigations can be justified as these conditions may present with thrombosis without clinically overt symptoms and signs of the underlying conditions. In the elderly, more

expensive and invasive investigations may be necessary if these preliminary tests are negative and occult cancer is still strongly suspected. In patients with negative preliminary tests, useful clues may appear on follow-up. Alternatively, tests for *in vivo* platelet activation and activation of coagulation and fibrinolysis may be performed. These are listed in Tables 8, 9 and 10. The presence of markers of *in vivo* activation of **platelets**,^{7,8} **blood** coagulation and **fibrinolysis**⁹ in the absence of clinical thrombosis would suggest that there may be an ongoing stimulus to thrombogenesis. This may prompt the clinicians to investigate the patients more extensively for an **underlying** thrombophilic condition or to observe the patients more closely on follow-up with benefit of earlier diagnosis of thrombosis when it occurs? These activation markers are useful to monitor response of thrombosis to treatment or response of the underlying condition (that induces the thrombosis to treatment) for

TABLE 9: Plasma markers of activation of coagulation

<u>Biochemical step</u>	<u>Marker</u>
Fibrinogen-Fibrin	Fibrinopeptides A & B
Prothrombin-Thrombin	Prothrombin Frag. (F ₂) Thrombin-ATIII complex
Protein C-APC	Protein C Act. Peptide APC-PCI Complex
Factor IX-Factor IXa	FIX Act. Peptide, FIXa-ATIII
Factor X-Factor Xa	FX Act. Peptide, FXa-ATIII

TABLE 10: Plasma markers of fibrinolysis

<u>Biochemical Step</u>	<u>Marker</u>
Plasminogen-Plasmin	Plasmin-Anti-Plasmin Complex
Plasmin action on fibrin/fibrinogen	B ₁₋₄₂ fragment B ₁₅₋₄₂ fragment, FDP, low fibrinogen
Plasmin action on cross-linked fibrin	D-dimer

example response of a cancer to chemotherapy or surgery.

REFERENCES

1. **Zoller B, Dahlback B.** Resistance to activated protein C caused by a Factor V gene mutation. *Curr Op Haematol* 1995; **2:358-64.**
2. Hirsh J, Prins MH, Samama M. Approach to the thrombophilic patient. In: **Colman R, Hirsh J, Mareder VJ, Salzman EW, eds:** Hemostasis and Thrombosis: Principles and Clinical Practice. 3rd Edition. Philadelphia: JB Lippincott, 1994: **1543-61.**
3. **Heijboer H, Brandjes DPM, Buller HR, Sturk A, ten Cate JW.** Deficiency of coagulation-inhibiting and **fibrinolytic** proteins in outpatients with **deep-vein** thrombosis. *N Engl J Med* 1990; **323: 1512.**
4. Sack GH, **Levin J, Bell WR.** Trousseau's syndrome and other manifestations of chronic disseminated coagulopathy in patients with neoplasms: Clinical, pathophysiologic, and therapeutic features. *Medicine (Baltimore)* 1977; **56: 1.**
5. Valla D, Casadevall N, **Lacombe C, et al.** Primary myeloproliferative disorder and hepatic vein thrombosis. A prospective study of erythroid colony formation **in vitro** in 20 patients with Budd-Chiari syndrome. *Am Intern Med* 1985; **103: 329.**
6. **Klein AS, Sitzmann JV, Colman J, Herlong FH, Camerom JL.** Current management of the **Budd-Chiari** syndrome. *Ann Surg* 1990; **212: 144.**
7. Chong BH, Murray B, Berndt MC, **Dunlop L, Brighton T, Chesterman CN.** Plasma P-selectin is increased in thrombotic consumptive platelet disorders. *Blood* 1994; **83: 1535-41.**
8. Kaplan KL, Laboratory markets of platelet activation. In: **Colman R, Hirsh J, Mareder VJ, Salzman EW, eds:** Hemostasis and Thrombosis: Principles and Clinical Practice. 3rd Edition. Philadelphia: JB Lippincott, 1994: **1180-96.**
9. Bauer KA, Weitz JL. Laboratory markets of coagulation and **fibrinolysis.** In: **Colman R, Hirsh J, Mareder VJ, Salzman EW, eds:** Hemostasis and Thrombosis: Principles and Clinical Practice. 3rd Edition. Philadelphia: JB Lippincott, 1994: **1197-1210.**