



## Hypercoagulable States and Thrombotic Risk Assessment

A delicate balance exists between clot formation and clot dissolution according to the concept of thrombo-hemorrhagic balance (hemostasis); any condition which shifts the balance in the direction of clot formation may lead to thrombosis. Since this theory was proposed, a number of genetic and acquired conditions have been associated with hyperactivity of the coagulation system which shift the balance toward clot formation, frequently resulting in the development of thrombotic disease. Inherited risk factors are usually implicated in early onset thrombotic disease (<15 years of age). Venous thrombosis is possibly the most common manifestation, occurring in approximately 1 in 1000 individuals per year. The accurate recognition of these hypercoagulable conditions is essential in the medical management of these patients.

The association of genetic or acquired deficiencies of specific coagulation factors including Protein C, Protein S, or antithrombin with thrombosis has been well documented since the 1980's. More recent studies have identified several other risk factors for the development of thrombosis, including activated protein C (APC) resistance, the prothrombin 20210 A allele, and elevated blood levels of homocysteine. Dysfibrinogenemia and high factor VIII levels, although less common, have also been linked with thrombophilia.

Heterozygous Protein C (PC) deficiency is relatively common, with a prevalence of 1 in 300 persons. Approximately 2 - 4% of patients with venous thrombosis have been shown to have congenital or acquired PC deficiency. PC functions as a potent natural anticoagulant when activated by the binding of thrombin to thrombomodulin on endothelial cell surfaces. Activated PC inhibits clot formation by proteolytically cleaving circulating factors Va and VIIIa. In addition, activated protein C promotes fibrinolysis by inhibiting the function of tissue plasminogen activator (TPA). In the laboratory, both functional and antigenic assays are used to detect PC deficiency, however, immunoenzymatic (antigenic)

assays are required for confirmation and typing of deficient patients (type 1 = quantitative deficiency; type 2 = qualitative deficiency).

Protein S (PS) deficiency occurs in approximately 2 - 5% of patients with venous thrombosis. In normal plasma, approximately 40% of PS circulates as a free, functionally active molecule, while 60% is complexed with C4b-binding protein (C4BP), a plasma protein of the classical complement pathway. The cofactor activity of free PS enhances the proteolysis of factors Va and VIIIa by activated PC. Free PS levels are usually reduced in cases where C4BP is elevated. C4BP is an acute phase protein which increases in concentration during inflammation, causing an increased risk of thrombosis in these conditions. The laboratory evaluation of PS should include the antigenic measurement of both total and free protein along with a measurement of functional activity. Like PC, PS levels are decreased by oral anticoagulant therapy; in anticoagulated patients, another vitamin K dependent protein (such as factor VII) can be measured and compared to determine if reduced levels are a result of therapy or represent an actual deficient state.

Antithrombin deficiency is seen in 2 - 4% of patients with venous thrombosis, and is demonstrated in nearly all cases of disseminated intravascular coagulation. In individuals with genetic deficiency, thrombosis may occur at a young age; the first thrombotic event in about half the patients occurs before age 25. Antithrombin regulates coagulation by inhibiting activated serine proteases (factors II, IX, X, XI, and XII), in the presence of heparin. Diagnosis requires measurement of functional activity, as well as antigen levels, to determine deficiency type.

In 1993, an hereditary abnormality, activated protein C (APC) resistance, was discovered and identified as the most common genetic risk factor for venous thrombosis, occurring in about 20% of patients with venous thrombosis. APC inhibits clotting by cleavage of factors Va and VIIIa. APC-resistance is most

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frequently caused by a genetic mutation in the factor V gene (factor V Leiden) at the primary cleavage site of factor Va by APC, rendering the cleavage site inaccessible. Functional assays utilizing the aPTT-based method are widely used to screen for APC-resistance. In normal plasma, the aPTT is prolonged with the addition of APC. A ratio of the aPTT with and without added APC is used to determine resistance. A PCR-based DNA assay for factor V Leiden is required for diagnosis.

New research techniques including gene sequencing have identified another genetic risk factor for thrombosis, the prothrombin 20210 A allele. This variant, which is associated with increased plasma prothrombin levels, was shown to occur in 1 - 2% of healthy subjects. In one study, the prevalence of this variant was 6.2% in a consecutive population of patients with thrombosis. While this variant is fairly common, this data implies that the relative risk associated with the prothrombin 20210 A allele is less than with deficiencies of other coagulation proteins.

Recent studies have clearly established a link between elevated blood levels of homocysteine and increased risk of recurrent thrombotic events. In one study, hyperhomocysteinemia was detected in 19% of patients under the age of 40 presenting with their first episode of venous thrombosis. Genetic and acquired abnormalities may be responsible for elevated blood levels of homocysteine. A mutation in the methylenetetrahydrofolate reductase (MTHFR) enzyme is the most common genetic factor leading to hyperhomocysteinemia. Acquired conditions include dietary deficiencies of vitamin B<sub>12</sub>, vitamin B<sub>6</sub> or folate, and renal failure. Hyperhomocysteinemia is now recognized to be an independent risk factor for arterial and venous thrombosis, although the mechanism of action has yet to be elucidated.

Acquired thrombosis risk factors are responsible for most thrombotic episodes that occur after age 45. These include various clinical disorders and conditions which may predispose to thrombosis. Among the most common is anti-phospholipid syndrome, in which the risk of thrombosis correlates with elevated levels of antiphospholipid antibodies. Laboratory assessment includes assays for the detection of antibodies to cardiolipin, phosphatidylserine, or B2GPI, and lupus anticoagulant panels. Elevated levels of factor VII, fibrinogen, and factor VIII / von Willebrand factor (vWF), acute phase reactants which are increased in various conditions, may also be associated with thrombophilia.

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~~Other diseases with an increased risk of~~  
thrombosis include: malignancy, myeloproliferative disorders,

polycythemia vera, congestive heart failure, atrial fibrillation, nephrotic syndrome, hyperlipidemia, and severe liver disease. Factors which may predispose to thrombosis include: surgery, immobilization, fractures, peurperium, paralysis, oral contraceptives, and hormone replacement therapy.

The incidence of thrombosis in individuals under 45 is surprisingly low when compared with the cumulative prevalence of genetic risk factors in the general population. This leads to the theory that thrombosis usually results from the simultaneous presence of multiple factors, including genetic defects and external influences. While some factors may predispose an individual for hypercoagulability, thrombosis may not be inevitable. The synergy created by the convergence of more than one risk factor may be required for thrombosis to occur. The diagnosis of these individuals should include a personal clinical history, physical examination, and possible family studies in addition to extensive laboratory testing.

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