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Roshani, S.

Citation

Roshani, S. (2012, January 12). *Venous and arterial thrombosis : associations and risk factors*. Retrieved from https://hdl.handle.net/1887/18334

Version:	Corrected Publisher's Version
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Downloaded from:	https://hdl.handle.net/1887/18334

Note: To cite this publication please use the final published version (if applicable).

Thrombophilia and Venous Thromboembolism: Implications for Testing

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Sara Roshani, Danny M. Cohn and Saskia Middeldorp

Semin Thromb Hemost. 2007

Abstract

In the last decades, the knowledge on the etiology of venous thromboembolism (VTE) has increased tremendously. In approximately half of patients presenting with VTE, one or more thrombophilic defects can be identified. This has led to widespread testing for thrombophilia, despite the fact that, at present, it is unclear whether this should have therapeutic consequences. Here we review the currently established hereditary and acquired thrombophilic defects, and focus on the pros and cons of testing in the setting of VTE. Thrombophilia is defined as a disorder associated with an increased tendency to venous thromboembolism (VTE). Thrombophilia can be acquired, such as in patients with cancer, or congenital, in which case a defect in the coagulation system is hereditary. Egeberg was the first to use the term thrombophilia in 1965, when he described a Norwegian family that had a remarkable tendency to VTE, based on a deficiency of antithrombin.[1] Since then, various laboratory abnormalities, both hereditary and acquired, have been discovered that increase the risk of VTE. This article reviews the currently established thrombophilic abnormalities and discusses the potential usefulness and implications of testing for thrombophilia.

Coagulation cascade and regulatory mechanisms

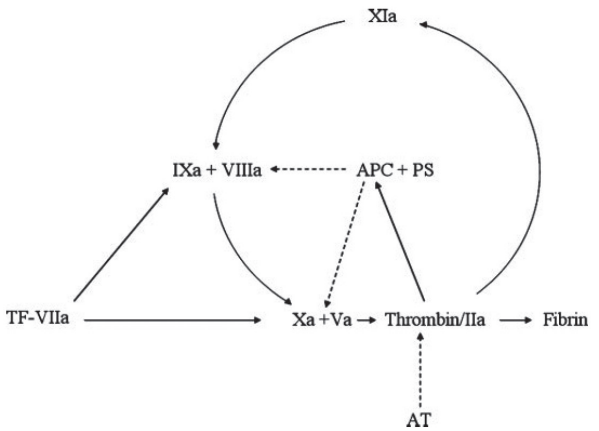
Well-established hereditary thrombophilia can be categorized into abnormalities of the natural anticoagulant system, elevation of plasma levels of coagulation factors, abnormalities in the fibrinolytic system, and miscellaneous hereditary conditions.

Fig. 1 depicts the current, simplified insight into the regulation of the coagulation system. Coagulation is initiated by a tissue factor (TF)/factor (F) VIIa complex that can activate FIX or FX. At high TF concentrations, FX is activated primarily by the TF/FVIIa complex, whereas at low TF concentrations, the contribution of the FIXa/FVIIIa complex to the activation of FX becomes more pronounced. Coagulation is maintained through the activation of FXI by thrombin. The coagulation system is regulated by the protein C pathway. Thrombin activates protein C. With protein S as a cofactor, activated protein C (APC) inactivates FVa and FVIIIa, which results in a downregulation of thrombin generation and consequently in an upregulation of the fibrinolytic system. Antithrombin is the other important natural anticoagulant that inhibits not only thrombin but also FXa and other coagulation factors by forming irreversible complexes. Furthermore, tissue factor pathway inhibitor (TFPI) inhibits the initiation of coagulation. Thrombin-activatable fibrinolysis inhibitor (TAFI) inhibits fibrinolysis, thereby protecting a formed thrombus from lysis in the presence of large amounts of thrombin.

Abnormalities of the anticoagulant system

Most defects in the natural anticoagulant systems increase the tendency toward thrombosis. In the following paragraphs, deficiencies of the well-established thrombophilic abnormalities (i.e., deficiencies of protein C, protein S, and antithrombin) are discussed, as well as the evidence on less well-known candidates.

Figure 1. Regulation of blood coagulation. Coagulation is initiated by a tissue factor (TF)/factor (F) VIIa complex that can activate FIX or FX. At high TF concentrations, FX is activated primarily by the TF/FVIIa complex, whereas at low tissue factor concentrations the contribution of the FIXa/FVIIIa complex to the activation of FX becomes more pronounced. Coagulation is maintained



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Protein C Deficiency

Protein C deficiency appears to be quite rare, with a prevalence of 0.2% in the general population and 2.5 to 6% in patients with first VTE.[2,3] Numerous mutations in the gene coding for protein C lead to several types of protein C deficiency and are summarized in a published database.[4] In type I deficiency, levels of both antigen and activity are reduced, whereas in type II deficiency, antigen levels are normal but one or more functional defects lead to a decreased activity. Abnormalities in type II protein C deficiency can occur on sites of substrate binding, thrombomodulin interaction, or calcium binding. Most patients have heterozygous protein C deficiency with a protein C level 50% of normal. Homozygous protein C–deficient patients who completely lack the natural anticoagulant protein are extremely rare. These patients will develop a syndrome characterized by diffuse VTE with accompanying skin necrosis shortly after birth, known as neonatal purpura fulminans, which is fatal unless protein C is given in the form of protein C concentrate, fresh frozen plasma,[5] or FIX concentrate (which contains a large amount of protein C and S) together with heparin.[6] Homozygous or compound heterozygous patients with mutations leading to severely reduced but not absent levels of protein C do not have this clinical syndrome but may present with VTE in adulthood or will develop skin necrosis when exposed to vitamin K antagonists (VKA). These patients have protein C levels less than 20% of normal.[7]

Protein S Deficiency

The prevalence of protein S deficiency in the general population is estimated between 0.026% and 0.13% in a Scottish study among healthy blood donors, and at 1 to 2% patients with first VTE episode[8,9] . Protein S, in addition to being a cofactor to protein C, independently inhibits the prothrombinase and tenase complexes.[10] The pattern of inheritance is autosomal dominant. One third of patients with protein S deficiencies will have VTE by the age of 60 years. Homozygosity is associated with neonatal purpura fulminans.[5] Warfarin skin necrosis has been reported in protein S–deficient patients.[11]

Antithrombin Deficiency

Antithrombin directly inhibits thrombin and activated FIX, FX, and FXI by forming a covalent complex, a process that is accelerated 1000-fold by heparin and endogenous heparin-like substances. Antithrombin deficiency occurs in 0.02% of the general population and in 0.5 to 7.5% of patients presenting with a first VTE.[12] Traditionally, antithrombin-deficient patients are considered at higher risk for thrombosis than patients with other congenital thrombophilic states, although this observation may be caused by selection of severely thrombophilic families in the early days of thrombophilia testing, when only antithrombin deficiency was known. However, the rarity of the deficiency may point to a more severe clinical expression. The inheritance pattern is autosomal dominant, and almost all patients are heterozygous.

Activated Protein C Resistance and FV Leiden

FV Leiden (FVL) is responsible for at least 90% of APCresistant conditions. It is the most prevalent thrombophilic defect that occurs in 5 to 12% of the general population, with variations throughout the world.[13] APC, together with its cofactor protein S, regulates the coagulation cascade by inactivating FVa and FVIIIa. APC cleaves FVa in three sites, but cleaving the first site at amino acid position 506 is necessary for better access to the other two sites. The mutation in the first site is known as FVL, and in the other site is described as FV Cambridge (at position 306)[14] and FV Hong Kong.[15] In carriers of FVL, FVa is inactivated approximately 10 times more slowly than normal,[16] whereas the implications of the other mutations on APC resistance are not as strong.[17] No mutations at cleavage sites in FVIII were found that cause APC resistance.[18] Recently, through genome-wide scans in family studies, a locus on chromosome 18 was described that appeared to influence normal variation in APC resistance and FVIII levels, as well as susceptibility to thrombosis.[19] However, to date, this has not been confirmed by other groups. Other mechanisms for APC resistance are antiphospholipid antibody syndrome (APS),[16] high concentration of FVIII,[20] and reduced levels of protein S.[21]

Prothrombin G20210A Mutation

The second most prevalent form of thrombophilia is prothrombin G20210A. This mutation is found in 1% of the population, in 4 to 8% of patients with a first VTE.[22] The proposed mechanism for thrombosis is elevation in the levels of normal prothrombin.[23] A prothrombin level higher than 115% (90th percentile) results in a 2.1-fold increased VTE risk also in the absence of the prothrombin 20210A mutation.[23,24] However, carriers and noncarriers cannot be distinguished using the prothrombin level because there is a high degree of overlap between the groups.[24]

Elevated Levels of Coagulation Factors

As is the case for prothrombin (FII), increased levels of coagulation FVIII, FIX, FXI, and fibrinogen increase the risk of VTE.[25–28] In several studies, elevated FVIII levels were shown to be quite prothrombotic, with a dose-dependent 5- to 10-fold relative risk increase.[25,29,30] For each 10% increase of FVIII, the VTE risk increases by 10% (95% confidence interval, 0.9 to 21.0),[30] and this is independent of an acute-phase role of FVIII.[29,31] Although the etiology of persistently elevated FVIII is not clear, it appears to be determined in part genetically in some patients,[30,32] but not all studies confirm this.[33] Elevated FIX was also shown to increase the risk of VTE 2.5-fold, independent of several potential confounders and other genetic defects.[26] Elevated FXI is associated with a 2-fold risk increase for VTE.[27] The likely mechanism for an elevation of FXI is excessive thrombin generation, which in turn leads to more thrombin deposition and downregulation of fibrinolysis by activation of TAFI.[34] Finally, fibrinogen levels >500 mg/dL are associated with approximately a 4-fold risk increase of VTE,[28] which was not explained by acute-phase reactions as measured by C-reactive protein.[31] Elevated levels of FV,[35] FVII,[28] and FX,[36] have not shown to increase the risk for thrombosis.

Miscellaneous (candidate) Risk Factors

Hyperhomocysteinemia

Severe hyperhomocysteinemia or homocystinuria is a rare condition that is associated with strongly elevated levels of homocysteine (>100 mmol/L), premature arterial and VTE, mental retardation, and a Marfan-like stature.[37] It is caused by homozygosity or compound heterozygosity of mutations in cystathionine-b-synthase, whereas heterozygosity results in mild to moderate homocysteinemia. More than 90 mutations in this gene are known to cause homocystinuria.[38] The second gene that may be involved is methylenetetrahydrofolate reductase (MTHFR). Several mutations are known to increase the levels of homocysteine; homozygosity leading to MTHFR deficiency, however, is rare. Contrary to homocystinuria, mild hyperhomocysteinemia is common and, depending on chosen cutoff values, occurs in 5 to 10% of the population. A common thermolabile C677T polymorphism results in mild hyperhomocysteinemia in homozygotes who are low in folate, vitamin B12, or vitamin B6.[39] It has remained a matter of debate whether mild homocysteinemia is a cause or consequence of VTE. The current opinion is that the association is mild; a 5 mmol/L increase in plasma level increases the risk for VTE by 1.27 in prospective studies, and by 1.60 in retrospective studies.[40] The presence of the homozygous TT677 MTHFR polymorphism increases the risk 1.20-fold, with no observed effect in studies in North America. It was hypothesized that this differential effect is explained by the higher folate intake in this part of the world.

Other natural anticoagulants

TFPI inhibits the coagulation process during its earliest phase. Levels below the 10th percentile of values in control subjects were associated with a 1.7-fold risk increase for VTE in one study.[41]

Fibrinolytic system

Generally, impaired overall fibrinolysis appears to be associated with an increased risk for VTE, although the role of the individual components is not very clear.[42] No relationship has been found between deficiencies of plasminogen, levels of polymorphisms in the gene, and levels of polymorphisms in the gene coding for

tissuetype plasminogen activator, whereas increased levels of tissue plasminogen activator inhibitor type 1 have shown conflicting results.[42] High levels of TAFI have been shown consistently to increase the risk for VTE.[43–45]

Miscellaneous

Although it has been hypothesized that deficiencies in the contact coagulation pathway, in particular FXII, may increase the risk of thrombosis because of a reduced fibrinolytic capacity, several studies have indicated that FXII deficiency does not increase the risk of VTE.[46,47]

Acquired Thrombophilia

Antiphospholipid antibody syndrome

APS is an autoimmune disorder defined as venous and/ or arterial thrombosis or recurrent pregnancy loss in the presence of persistent antibodies against phospholipids or phospholipid-binding proteins, and is categorized into primary and secondary status based on presence of autoimmune diseases such as systemic lupus erythematosus.[48] The syndrome is not common in consecutive patients with VTE, although the prevalence of isolated antiphospholipid antibodies measured on a single occasion in healthy subjects is not uncommon, and is only a weak risk factor for VTE.[46] The presence of lupus anticoagulant, however, is associated with a 5- to 16- fold increased risk for VTE.[49]

Implications of testing for thrombophilia

Given that, at present, a thrombophilic defect can be demonstrated in more than 50% of the patients who present with a VTE, the tendency to test patients for thrombophilia has increased tremendously. However, the usefulness and cost effectiveness of testing is a matter of debate.[51,52] The (dis)advantages and implications of thrombophilia testing are discussed in the following section. Reasons for testing for thrombophilia might be clarification of the cause, the possibility to adjust therapeutic regimes of VTE in thrombophilic patients for the optimal prevention of recurrence, and the possibility to track asymptomatic family members (and subsequently take preventive measures). Conversely, testing

for thrombophilia might lead to needless expenses, anxiety, and social problems.

Reasons to Test for Thrombophilia

It is often argued that patients and their doctors would like to have an explanation for the episode of VTE, although this has never been explicitly studied. It should be realized however, that the existence of a thrombophilic defect does not exclude other reasons for a prothrombotic state. For example, a 60-year-old male presenting with an idiopathic deep VTE of the leg might have an occult cancer as well as a thrombophilic defect. An important argument in favor of testing for thrombophilia would be the possibility to adjust therapeutic measures for treatment of a VTE (by means of intensity or duration of treatment). The optimal therapy for VTE depends on the risk of recurrence, the (dis)- comfort of the therapy and the risk of side effects, such as (major) bleeding. The estimated risk of recurrence for VTE, in general, is 5% per year,[52,53] although idiopathic episodes tend to recur more frequently (20% in the first 2 years) compared with provoked episodes.[54] Standard therapy for patients with a first VTE includes anticoagulant treatment with VKAs for 3 to 6 months, with international normalized ratios between 2 and 3.[55] This therapy ensues an annual bleeding risk of 0.25% for fatal bleeding and 1.0% for life-threatening bleeding.[56,57] A different approach to thrombophilic patients, compared with nonthrombophilic patients, is only justified if the former have a different risk of recurrence. Even though thrombophilia has shown to increase the risk of a first VTE, it is to date still controversial whether thrombophilia also increases the risk of recurrent VTE. The estimated relative risk of recurrence in patients with thrombophilia is small, compared with patients without thrombophilia (Table 1). The estimated odds ratios (ORs) for the natural anticoagulant deficiencies, as described mainly in retrospective analyses, were 2.5.[58–60] In one prospective study, the follow- up of the Leiden Thrombophilia Study (LETS), the risk of recurrence appeared even more moderate, with an OR of 1.8.[61] Two meta-analyses studied the risks of recurrence in patients with the common thrombophilias: FVL and the prothrombin G20210A mutation. The risk of recurrence was found consistently to be 1.3- to 1.4-fold higher in patients with FVL and 1.4- to 1.7-fold higher in patients with the prothrombin mutation.[62,63] For the other thrombophilic defects, less data are available. Three studies assessed the risk of recurrence in patients with high levels of FVIII coagulant

activity (FVIII:c) compared with patients with normal levels. One case-control study showed that elevated levels of FVIII:c above 150% were associated with an approximate 2-fold increased risk of recurrent VTE, compared with patients with a single episode.[30] In two cohort studies, the estimated relative risk of recurrent VTE was 6-fold increased in those with FVIII:c levels above the 90th percentile, corresponding to 234% and 294%, respectively.[64,65] These results could not be reproduced in the LETS follow-up study, in which an OR of only 1.3 was found.[61] The data on estimated risk of recurrence for elevated levels of FIX and FXI are scarce, but their impact on recurrence seems negligible.[61] The attribution of mild hyperhomocysteinemia in terms of risk of recurrence appears low (1.8 to 2.7),[66–68] and treating hyperhomocysteinemia did not show a decrease in the number of recurrences. [67] The risk of recurrence in antiphospholipid or anticardiolipin antibodies was investigated in four studies. [69–72] The outcomes regarding relative risk for recurrence range between 2- and 6-fold. These results are difficult to interpret, given that in these studies the antiphospholipid and anticardiolipin antibodies or lupus anticoagulant were not tested repetitively (as suggested by international guidelines[48]). Moreover, duration of anticoagulant treatment differed substantially. Adjustment of anticoagulant treatment in thrombophilic patients after a first VTE has only been addressed for a difference in intensity. This has not shown to be beneficial in patients with VTE, regardless of thrombophilia. Reducing the intensity of VKAs below 2.0 led to an increase of recurrence risk (1.9 versus 0.6%),[73,74] whereas major bleeding complications did not differ between a low-intensity and regular-intensity treatment (0.96 versus 0.93%).[73] A higher intensity of VKA in patients with antiphospholipid antibodies showed no reduction in the risk of recurrence, but led to an increase in the bleeding risk.[75,76] Whether clinical outcome of patients with VTE and thrombophilia improves with prolongation of anticoagulant has never been investigated. Current trials focus on whether such an intervention outweighs the bleeding risk, given that it is known that oral anticoagulant medication prevents VTE by more than 90%, as long as it is used.[77] Finally, a potential advantage of testing patients with VTE for thrombophilia may be the identification of asymptomatic family members. These individuals have a 2- to 10-fold increased risk for VTE as compared with noncarriers.[78–81] Regardless of this increased relative risk, the overall absolute risk remains low (Table 2). It is often argued that asymptomatic family members with

thrombophilia may benefit from targeted prevention in high-risk situations (e.g., pregnancy, puerperium, surgery,immobilization, and trauma), and the avoidance of acquired risk factors, most notably oral contraceptives. It is clear from Table 2 that bleeding risk associated with continuous anticoagulant treatment outweighs the risk of VTE. It is notable that the figures considering surgery, trauma, and immobilization, as shown in Table 2, have been collected for the larger part in times before standard prophylaxis was routine patient care. For pregnancy, 80% of the episodes occur in the postpartum period. Whether this should lead to administration of prophylaxis in the postpartum period is a matter of physician and patient preference, given that the number needed to treat is 25 in case of a deficiency in the natural anticoagulants and approximately twice as high in patients with the common thrombophilias. Finally, it is clear from data in Table 2 that the use of oral contraceptives should be weighed against the disadvantages of other contraceptive methods.

Table 1. Estimated Relative Risk of VTE Recurrence in Patients with Thrombophilia

Type of Thrombophilia	Relative Risk
Natural anticoagulant deficiencies	1.8-2.5[58-61]
FVL	1.3-1.4[62,63]
Prothrombin 20210A	1.4-1.7[62,63]
Elevated FVIII:c	1.3-6[61,64,65]
Elevated levels of FIX	1.2[61]
Elevated levels of FXI	0.6[61]
Mild hyperhomocysteinemia	1.8-2.7[66-68]
Antiphospholipid antibodies	2-6[69-72]

VTE, venous thromboembolism; FVL, factor V Leiden; FVIII:c, factor VIII coagulation activity; F, factor.

Table 2. Absolute Risk of VTE in Asymptomatic Carriers of Thrombophilia

Type of Thrombophilia	Overall Risk (%/year)	Surgery, Trauma, or Immobilization (%/episode)	Pregnancy (%/pregnancy)	Oral Contraceptive Use (%/year of use)
Natural anticoagulant deficiencies	0.4-4.0[78,87-91]	8.1[78]	4.1[78]	4.3[78]
FVL	0.1-0.7[78,80,81,87,90,92,94]	1.8-2.4[81]	1.9-2.1[78,81]	0.5-2.0[78,81]
Prothrombin 20210A	0.1-0.4[94-97]	2.0[95]	2.8[95]	0.2[95]
Elevated FVIII:c	0.3[97]	1.2[97]	1.3[97]	0.6[97]
Mild hyperhomocysteinemia	0.2[98]	0.9[98]	0.5[98]	0.1[98]

VTE, venous thromboembolism; FVL, factor V Leiden; FVIII:c, factor VIII coagulant activity; F,factor.

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Reasons Not to Test for Thrombophilia

Disadvantages of testing patients with a VTE for thrombophilia might be the cost of testing, which is approximately *s*500.[51] Several sophisticated studies focused on the cost effectiveness of testing for thrombophilia. [82,83] These studies focused on selected patient groups, because universal testing was considered less cost effective. It is of note that the external validity of the results may be distorted by the fact that the findings were based on a range of various assumptions. One study, by Marchetti et al,[82] assessed the cost effectiveness of testing for double heterozygosity of FVL and the prothrombin mutation, and subsequently prolonging anticoagulant therapy in those tested positive for both common thrombophilias. This strategy was considered cost effective, given that testing all patients with VTE provided one additional day of life at the cost of $13.624/quality-adjusted life-years.[82] The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening study assumed that testing for thrombophilia might be efficacious in high-risk situations.[83] The cost effectiveness of four different testing scenarios was calculated: (1) testing all women prior to prescription of oral contraceptives and restricting prescription only to those tested negative for thrombophilia; (2) testing all women prior to prescribing hormone replacement therapy and restricting prescription only to those tested negative for thrombophilia; (3) testing women at the onset of pregnancy and prescribing prophylaxis to those tested

positive for thrombophilia; and (4) testing all patients prior to major elective orthopedic surgery and prescribing extended thromboprophylaxis to those tested positive for thrombophilia. It was concluded in this study that the second scenario would be most cost effective, compared with the other scenarios. Nevertheless, selective screening based on the presence of previous personal or family history of VTE was considered to be more cost effective than universal testing in the four different scenarios.

Furthermore, the psychological impact and consequences of knowing that one is a carrier of a (genetic) thrombophilic defect could be regarded as a drawback of testing. Most studies that focused on impact of testing for thrombophilia showed that patients had experienced low psychological distress following thrombophilia testing. [84,85] Nevertheless, qualitative studies described several negative effects. In the study by Bank et al,[86] it is mentioned that parents were worried that their children "would be negatively influenced by factor V Leiden" and that some carriers "felt stigmatized." Finally, a disadvantage of testing for thrombophilia could be its potential social consequences (for instance, problems with health insurance or life insurance), although little data are available on this issue.

Conclusions

The discovery of newer thrombophilic defects has led to a deeper insight in the development of VTE during the last decades. However, testing patients with VTE, with respect to its advantages and disadvantages, has so far no direct consequences in terms of different treatment strategies. Given the absence of clear benefits, thrombophilia testing should be performed with restraint.

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