Uncertain thrombophilia markers

Massimo Franchini1; Ida Martinelli2; Pier Mannuccio Mannucci3

1Department of Transfusion Medicine and Hematology, Carlo Poma Hospital, Mantova, Italy; 2Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy; 3Scientific Direction, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy

Summary

The development of venous thromboembolism (VTE), which includes deep-vein thrombosis and pulmonary embolism, may be associated with inherited or acquired risk factors that can be measured in plasma or DNA testing. The main inherited thrombophilias include the plasma deficiencies of the natural anticoagulants antithrombin, protein C and S; the gain-of-function mutations factor V Leiden and prothrombin G20210A; some dysfibrinogeniemias and high plasma levels of coagulation factor VIII. Besides these established biomarkers, which usually represent the first-level laboratory tests for thrombophilia screening, a number of additional abnormalities have been less consistently associated with an increased VTE risk. These uncertain causes of thrombophilias will be discussed in this narrative review, focusing on their clinical impact and the underlying pathogenetic mechanisms. Currently, there is insufficient ground to recommend their inclusion within the framework of conventional thrombophilia testing.

Keywords

Thrombophilia, venous thromboembolism, thrombophilia markers

Introduction

The term thrombophilia, which refers to an abnormality of blood coagulation leading to an increased risk of venous thromboembolism (VTE) (1–4), was first introduced in the mid-19th century by the German physician Rudolf Virchow, who formulated the theory of the triad, i.e. vessel wall damage, stasis and hypercoagulability in order to explain the pathogenesis of thrombosis (5). This concept was prophetic, because several studies have subsequently confirmed that all components of the triad play active roles in the development of VTE. In particular, great progress was made in the last two decades pertaining to the elucidation of the mechanisms underlying VTE and it is nowadays clear that the pathogenesis of VTE is multifactorial, including acquired and genetic risk factors (2). Among the latter, a number of abnormalities have been discovered, explaining together approximately 40 percent of all previously unexplained episodes of VTE, including deep-vein thrombosis [DVT] and pulmonary embolism [PE]) (3). Established thrombophilia includes the inherited deficiencies of the natural anticoagulant proteins antithrombin, protein C and protein S; the gain-of-function mutations in the factor V (factor V Leiden) and prothrombin genes (prothrombin G20210A); some dysfibrinogenemia and increased plasma levels of coagulation factor VIII (6). Robust evidence also exists on the association between non-O blood groups (i.e. A, B and AB) and a higher risk of VTE (7, 8), so that ABO blood groups are frequently included in the panel of first-level laboratory tests for thrombophilia screening. In addition to these validated biomarkers, others have been associated with an increased risk of VTE, as summarised in Table 1. Classic thrombophilia markers (left column of Table 1) are not discussed herewith, because they were the subject of a previous review article (9). Because the term thrombophilia usually refers to a tendency to develop venous thrombosis (not arterial), only abnormalities associated with an increased risk of VTE will be discussed.

Search methods

We reviewed the medical literature for published studies on risk factors for VTE. The MEDLINE electronic database was searched without temporal limits using English language restriction. The Medical Subject Heading and keywords used were: inherited thrombophilia, genetic thrombophilic risk factors, venous thromboembolism, deep-vein thrombosis, polymorphism, pulmonary embolism, hyperhomocysteinemia, fibrinogen, coagulation factors, procoagulants, hypofibrinolysis, factor VII, factor IX, factor X, factor XI, factor XIII, thrombin activatable fibrinolysis inhibitor, TAFI, plasminogen activator inhibitor-1, PAI-1, tissue factor pathway inhibitor, TFPI, methylenetetrahydrofolate reductase, MTHFR, mutation, thrombomodulin, endothelial protein C receptor, EPCR, lipoprotein(a), Lp(a), angiotensin-converting enzyme, ACE, protein Z, ADAMTS13. We also screened the reference lists of most relevant review articles on thrombophilia for further studies not captured in our initial literature search. Search

Correspondence to:
Pier Mannuccio Mannucci, MD
Via Pace 9, 20122 Milano, Italy
Tel.: +39 02 55038377, Fax: +39 02 50320723
E-mail: piermannuccio.mannucci@policlinico.mi.it

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Abnormal fibrinolysis

Venous thrombosis may result from a hypofibrinolytic state (3). Defects of the fibrinolytic system are uncommon and may involve different components, including the proenzyme plasminogen and such regulators of fibrinolysis as plasminogen activator inhibitor 1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) (10). Congenital qualitative or quantitative plasminogen defects have not been causally linked to venous thrombosis (11). PAI-1, a serine protease inhibitor (serpin), is an important regulator of the fibrinolytic system through its inhibition of tissue andurokinase plasminogen activators (tPA and uPA) (12). A deletion/insertion (4G/5G) polymorphism in the promoter region of the PAI-1 gene (SERPNI) affects circulating levels of PAI-1, a three- to five-fold increased plasma levels of PAI-1 is measured in homozygotes for the 4G/4G mutation (13). The contribution of this mutation to thromboembolic events is, however, inconclusive (14). In a meta-analysis, a slightly increased risk of unexplained VTE was associated with the 4G allele compared with the 5G allele (odds ratio [OR] 1.153, 95% confidence interval [CI] 1.068–1.246) (15), but this finding was not replicated in prospective studies (16, 17). TAFI, also known as procarboxypeptidase B, is a plasma zymogen that inhibits fibrinolysis when converted to an active carboxypeptidase by such enzymes as trypsin, thrombin and plasmin. Its activation is increased more than 1,000-fold in the presence of the cofactor thrombomodulin (18). After activation, TAFI inhibits fibrinolysis through the removal of carboxy-terminal lysine residues from partially degraded fibrin (19), so that plasminogen binding sites are eliminated and plasminogen activation is blunted. Interindividual variations of TAFI plasma levels are genetically determined and several polymorphisms in the promoter region of the TAFI gene (CPB2) are associated with an increased level of this enzyme, which was found to be a mild risk factor for DVT (OR 1.7, 95% CI 1.1–2.5) in the Leiden Thrombophilia Study (20). In addition, higher TAFI levels were found in carriers of the factor V Leiden mutation who had developed VTE than in their asymptomatic relatives (21). Other genetic variants leading to abnormal fibrinolysis are those involving the genes encoding factor XIII and lipoprotein(a). The Val34Leu polymorphism of the catalytic A subunit of factor XIII (which stabilises fibrin by cross-linking α- and γ-chains and protects it from fibrinolysis) is associated with increased factor XIII levels in plasma but paradoxically provides a moderate protection from VTE (22–24). Lipoprotein(a) is a complex serum lipoprotein composed of a low-density lipoprotein particle linked to the apolipoprotein (a). Lipoprotein(a) has a structural homology with plasminogen and competes with it for binding to fibrin, inhibits tPA and impairs fibrinolysis (25). To date, several polymorphisms in the apolipoprotein (a) gene (LPA) were suggested to be major determinants of the plasma concentration of lipoprotein(a) (26). A meta-analysis found a statistically significant but modest association between high lipoprotein(a) levels (>300 mg/l) and VTE (OR 1.87, 95% CI 1.51–2.30) (30), and a recent study found an association between two LPA gene variants (rs10455872 and rs3798220) with systemic and coronary atherosclerosis but not with VTE (31). All in all, despite the increasing evidence on the role of high plasma lipoprotein(a) in the development of arterial thrombosis (25), data supporting its association with VTE are inconclusive (27–29).

Hyperhomocysteinaemia

High homocysteine levels in plasma are a mild risk factor for both venous and arterial thromboembolism, as demonstrated by multiple retrospective and prospective clinical studies, with a relative risk for VTE spanning between 2 and 3 (32, 33). Possible mechanisms include a toxic effect on endothelial cells, heightened smooth muscle cell proliferation and intimal thickening, impaired generation of vasodilators such as nitric oxide and prostacyclin, increased platelet adhesion, activation of factor V, induction of tissue factor activity and inhibition of tPA (34). The enzyme

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Abbreviations: MTHFR, methylenetetrahydrofolate reductase; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin-activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; Lp(a), lipoprotein a; EPCR, endothelial protein C receptor; ACE, angiotensin-converting enzyme; ADAMTS, A Disintegrin And Metalloprotease with ThromboSpondin-1-like domains; PZ, protein Z; ZPI, protein Z-dependent protease inhibitor.

Table 1: Haemostatic abnormalities associated with thrombophilia.
methylene-tetrahydrofolate reductase (MTHFR) plays an important role in the metabolism of homocysteine by converting 5,10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate. Hence MTHFR regulates the remethylation of homocysteine into methionine by 5-methyltetrahydrofolate (4). The substitution of cytosine by thymine at position 677 of the MTHFR gene (C677T polymorphism) renders the enzyme thermo-labile and less active, causing a mild to moderate hyperhomocysteinemia in homozygotes (4). The relationship between C677T variant and thrombosis is controversial. Indeed, while some investigators have reported an increased thrombotic risk in patients who were TT homozygotes (35, 36), other studies found no association between the polymorphism and cardiovascular risk (37, 38). For instance, the results of the Copenhagen Heart Study found no association between C677T homozygosity and ischaemic cardiovascular disease nor with VTE (39). No association with VTE was also observed in the large MEGA study (40). Similarly, in a recent meta-analysis involving more than 11,000 VTE cases and 21,000 controls no effect was found for C677T MTHFR (OR 1.38, 95% CI 0.98–1.93) (41). With this background, this thermolabile variant of MTHFR is no longer included in the diagnostic thrombophilia workup and the intermediate phenotype (plasma homocysteine levels) should be preferred. Similarly, although some recent but small studies have suggested a link between the A1298C MTHFR polymorphism (which has also been shown to decrease MTHFR activity) and retinal and cerebral venous thrombosis (42, 43), no clear association between this genetic variant and VTE emerges from the literature (44).

**Tissue factor pathway inhibitor**

Tissue factor pathway inhibitor (TFPI) inhibits coagulation by forming a quaternary complex with activated factor X, activated factor VII and tissue factor (45). Although low plasma levels of TFPI were reported to be a weak risk factor for first or recurrent VTE (46–48), this association was not confirmed by multivariable analysis in the frame of a population-based cohort study (49). The genetic basis for low TFPI levels is uncertain, because the results of studies investigating the relationship between gene polymorphisms, plasma levels and risk of VTE were inconclusive (4). For instance, the polymorphism at locus 536 of exon 7 of the TFPI gene (C356T) was associated with an increased VTE risk in some studies (50, 51), but not in others (52, 53).

**Elevated coagulation factor levels**

Elevated plasma levels of coagulation factors have been associated with an increased VTE risk (3, 4). Plasma factor levels are influenced by age and inflammation and a shift of the haemostatic balance toward a procoagulant state seems to be the most plausible mechanism for the increased thrombotic risk (3). While several studies have consistently demonstrated that high levels of factor VIII are a strong and independent risk factor for VTE (54–57), evidence for other factors (i.e. fibrinogen and factors V, VII, IX, X and XI) are more limited, being confirmed only for some of them (i.e. fibrinogen, factors IX and XI) (58–60) but not for others (i.e. factors V, VII and X) (58, 61–63). Fibrinogen levels higher than 5.0 g/l were associated with an almost four-fold increase of VTE risk (58), and such elevations were not explained by acute-phase reactions as measured by serum levels of C-reactive protein (64). In the Leiden Thrombophilia Study, individuals with factor IX levels higher than the 90th percentile (>129 U/dl) had a two-fold increased risk of VTE (OR 2.0, 95% CI 1.3–3.2) (59) after adjusting for age, sex, oral contraceptive use and high factor VIII and XI levels. For high factor XI levels a mildly increased risk of recurrent VTE (relative risk [RR] 1.6, 95% CI 1.0–2.8) was also subsequently observed (65). The Leiden Thrombophilia Study also showed that individuals with levels of factor XI higher than the 90th percentile (>120.8%) had an OR for VTE of 2.2 (95% CI 1.5–3.2), even after exclusion of known inherited and acquired risk factors (60).

The genetic basis of high levels of these procoagulants is only partially elucidated. A genome wide association study found that the single nucleotide polymorphisms rs2289252 and rs2036914 in the F11 gene were independently associated with plasma factor levels and DVT risk (66, 67). The association between the Malmö sequence variant in the F9 gene (rs6048) and VTE was suggested (68, 69). Finally, an extremely rare gain-of-function in the F9 gene (R338L; factor IX Padua) was detected in members of a single family who had several VTE episodes and very high plasma levels (eight-fold the normal level) of factor IX coagulant activity (70).

**Thrombomodulin deficiency**

Thrombomodulin is an endothelial cell transmembrane protein that plays a role of anticoagulant cofactor for thrombin-mediated activation of protein C (71). A number of genetic polymorphisms (1418 C/T, 1748 G/C, -133C/A, -33G/A, 3545 G/A) in the thrombomodulin gene that influence protein expression have been identified (72). Some of them seem to be associated with an increased risk of arterial thrombosis (73–75), but their link with VTE is less established (76–79). For instance, some studies failed to find an association between the 1418 C/T polymorphism and VTE risk (78, 80), while a more recent study found an even protective role against VTE for the 1418T allele associated with lower soluble thrombomodulin levels (81).

**Endothelial protein C receptor**

The endothelial protein C receptor (EPCR) plays a key role in the protein C anticoagulant pathway, being predominantly expressed on the surface of endothelial cells of large blood vessels and increasing up to 20-fold the rate of protein C activation by the thrombin-thrombomodulin complex (82). A soluble form of EPCR is also produced, probably generated through proteolytic cleavage of the receptor by a metalloprotease (83). Although a number of genetic variants in the EPCR gene (PROCR) influence EPCR expression and plasma levels of soluble EPCR, the interest of investigators was focused on the H1 and H3 haplotypes (84). The PROCR H1 haplotype (tagged by the rare allele of 4678G/C, rs9574) was associated with increased plasma levels of activated
protein C, reduced levels of sEPCR and lower VTE risk, whereas the PROCR H3 haplotype (tagged by the rare allele of 4600A/G, rs867186) was associated with reduced EPCR function (by promoting cellular shedding of EPCR), increased levels of soluble EPCR and a higher VTE risk (85–87). A recent meta-analysis confirmed a modest positive association between the PROCR single-nucleotide polymorphism rs867186 variant and VTE (OR 1.22, 95% CI 1.11–1.33) (88).

**Angiotensin-converting enzyme**

The angiotensin-converting enzyme (ACE), which is present at high concentrations on the surface of vascular endothelial cells, plays an important role in the modulation of vascular tone by converting angiotensin I to angiotensin II, a potent vasoconstrictor. In addition, it influences haemostasis by inhibiting fibrinolysis and inducing platelet activation and aggregation (89). Several studies have evaluated whether or not the insertion/deletion (I/D) polymorphism in the ACE gene, which accounts for approximately half of the inter-individual variability of circulating ACE serum levels (D/D subjects have the highest ACE activity) (90), is associated with an increased risk of VTE, but results are controversial (91–94). A meta-analysis made by pooling data from 14 studies failed to support an association between the D/D genotype and VTE (OR 1.206, 95% CI 0.951–1.531) (95). Interestingly, the pharmacological inhibition of the renin-angiotensin system did reduce the risk of VTE in patients with atherosclerotic disease (96).

**Protein Z / protein Z-dependent protease inhibitor**

Protein Z is a vitamin K-dependent glycoprotein which serves as a cofactor of the protein Z-dependent protease inhibitor (ZPI) of activated factor X (97). A number of genetic variants in the protein Z gene regulate plasma levels (98). The role of reduced plasma levels and related gene polymorphisms on the occurrence of VTE remains unclear (99). Although the results of a meta-analysis were consistent with a role for low protein Z in VTE (OR 2.18, 95% CI 1.19–4.00) (100), a number of case-control studies found no association between plasma protein Z, gene polymorphisms and VTE risk (101, 102). Thus, if an association exists, it is perhaps exerted only in the presence of other thrombophilia markers, such as factor V Leiden (103). Likewise, the available clinical data regarding the role and relevance of ZPI levels on the VTE risk are contradictory (104).

**ADAMTS13 polymorphisms**

ADAMTS13 (A Disintegrin And Metalloprotease with Thrombospondin-1-like domains) is a metalloproteinase responsible for the modulation of the molecular size of von Willebrand factor multimers. Although a dysregulation of this enzyme has been associated with various conditions characterised by a risk of micro-vascular thrombosis (105), only few and conflicting data are available on the role on VTE risk of ADAMTS13 gene polymorphisms associated with decreased activity/antigen levels of the plasma protease (106, 107).

**Conclusions**

In the last 20 years, in addition to established thrombophilia biomarkers, other markers were investigated for their association with VTE. Little evidence of their clinical usefulness is available, so that their laboratory testing is not recommended unless it is done for investigational purposes. There are commercial kits, often based upon DNA technology, that propose these markers in the frame of screening panels meant to diagnose thrombophilia. Beside being clinically useless, they carry the unwarranted risk to label as carrier of a genetic trait people who obtain no benefit from such knowledge, whereas genetic testing is always accompanied by fear and anxiety.

**Conflicts of interest**

None declared.

**References**


