Markers of Hereditary Thrombophilia with Unclear Significance

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Abstract

Thrombophilia leads to an increased risk of venous thromboembolism. Widely accepted risk factors for thrombophilia comprise deficiencies of protein C, protein S, and antithrombin, as well as the factor V "Leiden" mutation, the prothrombin G20210A mutation, dysfibrinogenemia, and, albeit less conclusive, increased levels of factor VIII. Besides these established markers of thrombophilia, risk factors of unclear significance have been described in the literature. These inherited risk factors include deficiencies or loss-of-activity of the activity of ADAMTS13, heparin cofactor II, plasminogen, tissue factor pathway inhibitor (TFPI), thrombomodulin, protein Z (PZ), as well as PZ-dependent protease inhibitor. On the other hand, thrombophilia has been linked to the gain-of-activity, or elevated levels, of α2-antiplasmin, angiotensin-converting enzyme, coagulation factors IX (FIX) and XI (FXI), fibrinogen, homocysteine, lipoprotein(a), plasminogen activator inhibitor-1 (PAI-1), and thrombin-activatable fibrinolysis inhibitor (TAFI). With respect to the molecular interactions that may influence the thrombotic risk, more complex mechanisms have been described for endothelial protein C receptor (EPCR) and factor XIII (FXIII) Val34Leu. With focus on the risk for venous thrombosis, the present review aims to give an overview on the current knowledge on the significance of the aforementioned markers for thrombophilia screening. According to the current knowledge, there appears to be weak evidence for a potential impact of EPCR, FIX, FXI, FXIII Val34Leu, fibrinogen, homocysteine, PAI-1, PZ, TAFI, and TFPI on the thrombotic risk.

Keywords

- thrombophilia
- risk factors
- unclear evidence

Zusammenfassung

Eine Thrombophilie führt zu einem erhöhten Risiko für venöse thromboembolische Ereignisse. Weithin anerkannte Risikofaktoren für eine Thrombophilie sind ein Mangel an Protein C, Protein S und Antithrombin, sowie die Faktor-V-"Leiden"-Mutation, die Prothrombin-G20210A-Mutation, eine Dysfibrinogenämie und, weniger eindeutig, erhöhte Faktor VIII-Aktivitäten. Neben diesen etablierten Markern sind in der Literatur auch Risikofaktoren von eher unklarem Stellenwert beschrieben. Zu diesen Risikofaktoren, bzw. Markern, gehören der Funktionsverlust (loss-of-activity), bzw. der Mangel, der Aktivität von ADAMTS13, Heparin-Cofaktor II (HCII), Plasminogen, Tissue Factor Pathway Inhibitor (TFPI), Thrombomodulin (TM), Protein Z (PZ) sowie des

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Protein Z-abhängigen Proteaseinhibitors (ZPI). Andererseits wurde eine Thrombophilie mit einem gain-of-activity, bzw. erhöhten Aktivitäten, von α2-Antiplasmin (a2-AP), Angiotensin-konvertierendem Enzym (ACE), den Gerinnungsfaktoren IX (FIX) und XI (FXI), Fibrinogen, Homocystein, Lipoprotein(a), Plasminogenaktivator-Inhibitor-1 (PAI-1) sowie dem Thrombin-aktivierbaren Fibrinolyse-Inhibitor (TAFI) in Verbindung gebracht. Im Hinblick auf die zugrundeliegenden molekularen Interaktionen, die einen Einfluss auf das Thromboserisiko haben könnten, stellen sich die Mechanismen für den endothelialen Protein-C-Rezeptor (EPCR) und den Faktor-XIII-(FXIII) Val34Leu komplexer dar. In der vorliegenden Arbeit soll ein Überblick über den aktuellen Wissensstand zur Bedeutung dieser Marker für das Thrombophilie-Screening bei Patienten mit venösen thromboembolischen Ereignissen gegeben werden. Hierbei zeigt sich nach derzeitigem Kenntnisstand eine schwache Evidenz für einen möglichen Einfluss von EPCR, F IX, F XI, F XIII Val34Leu, Fibrinogen, Homocystein, PAI-1, PZ, TAFI und TFPI auf das Thromboserisiko.

Introduction

Thrombophilia refers to abnormal blood coagulation, leading to an increased risk of thromboembolism. Originally restricted to risk factors associated with venous thromboembolism (VTE), the concept of thrombophilia is now increasingly extended to thrombotic and embolic arterial events such as peripheral artery disease[1] or stroke.[2] In this narrative review, we will focus on thrombophilia leading to an increased risk of VTE. VTE is a common and serious blood clotting condition that includes both deep-vein thrombosis (DVT) and pulmonary embolism. DVT forms in deep veins, usually those of the legs. If the thrombus breaks free and becomes lodged to the lung arteries, DVT develops into pulmonary embolism. Thrombi in other parts of the body, such as in abdominal veins, are often excluded from clinical studies, and data on thrombophilia markers may not be directly related to these "atypical" events.

Thrombophilia can be either inherited or acquired. The two central mechanisms in inherited thrombophilia are the *loss of activity* of physiological anticoagulants and the *gain of activity* of physiological procoagulants. Widely accepted loss-of-activity thrombophilia markers include the deficiencies of protein C, protein S, and antithrombin while accepted gain-of-activity markers include the factor V "Leiden" (FVL) mutation R506Q, the prothrombin G20210A mutation, certain types of dysfibrinogenemia, and, to some extent, increased levels of factor VIII (FVIII; ►Table 1).[3–5]

Besides these classical and well-established markers of thrombophilia, a huge number of additional risk factors of unclear significance have been proposed and discussed in the literature. In the present review, based on the functions of the molecules, we aim to give an overview on the current knowledge of the significance of these markers for thrombophilia screening. We assume that a thrombophilia marker is unlikely to have clinical relevance if related to an odds ratio (OR) of less than 2.0 and that such a marker has no additional value in clinical decision making.[6]

The Fibrinolytic System

The fibrinolytic system plays a pivotal role in the regulation of hemostasis; because many other functions are associated with its active components, the fibrinolytic system is also referred to as the "plasminogen-plasmin system."[7] Plasminogen is activated to plasmin by urokinase-type plasminogen activator or tissue-type plasminogen activator in humans.[8] Plasmin is a serine protease that cleaves fibrin to break down blood clots but also cleaves fibronectin, thrombospondin, laminin, and von Willebrand factor (vWF).[9,10] The plasminogen activators are modulated by their inhibitor, called plasminogen activator inhibitor (PAI), of which three forms have been described: PAI-1, PAI-2, and PAI-3, as well as by nexin.[11] The active enzyme plasmin is modulated by its inhibitors α2-antiplasmin and α2-macroglobulin.[12] A third modulator is thrombin-activatable fibrinolysis inhibitor (TAFI), which can be activated by thrombin and inhibits clot lysis by removing carboxy-terminal lysine residues from fibrin, which subsequently prevents plasminogen from binding to fibrin.[13] A decreased fibrinolytic potential has been demonstrated to be a risk factor for venous and arterial thrombosis.[14] Low levels of plasminogen and high levels of inhibitors (PAI-1, TAFI, and a2-antiplasmin) are likely to contribute to this association. In fact, variation on clot lysis time could be explained by variations of these four molecules in more than 75% of patients investigated for clot lysis.[15] In the same study, elevated levels of PAI-1 and TAFI were associated with an increased risk of thrombosis, whereas the association for plasminogen and t-PA was lost after adjusting for markers of inflammation.

Plasminogen

Both quantitative and qualitative defects of plasminogen have been described. Although homozygous deficiency of plasminogen results in failure of the body to remove fibrin deposits in various organs, leading to pseudomembranous conjunctivitis, obstructive airway disorders, and other sequelae, there is no evidence for an increased risk of

Table 1 Current evidence for an association between markers of hereditary thrombophilia with unclear significance and VTE

Thrombophilia	Accepted markers (approx. relative risk)	Markers of unclear significance	
		Name	Current evidence for association with VTE
Loss-of-activity	AT deficiency (40–50) Protein C deficiency (15) Protein S deficiency (10)	ADAMTS13 α2-Antiplasmin EPCR mutations Heparin cofactor II MTHFR A1298C MTHFR C677T Plasminogen Protein Z TFPI TM mutations ZPI	No No Rather yes No No No No Rather yes Rather yes No No
Gain-of-activity	Factor V Leiden (heterozygous, 7; homozygous, 11–80) Prothrombin G20210A (heterozygous, 3–4; homozygous, 7–30) High FVIII (4)	ACE Clotting factor IX Clotting factor XI Clotting factor XIII Val34Leu Fibrinogen Homocysteine Lp(a) PAI-1 4G/5G Polymorphism TAFI	No Rather yes Rather yes Rather yes[a] Rather yes Yes No[b] Rather yes Rather yes

Abbreviations: ACE, angiotensin-converting enzyme; EPCR, endothelial protein C receptor; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin-activatable fibrinolysis inhibitor; TM, thrombomodulin; VTE, venous thromboembolism; ZPI, protein Z–dependent protease inhibitor.
Notes: Risk markers are divided according to loss- or gain-of-activity of underlying proteins/enzymes and presented in alphabetical order. For widely accepted markers, the approximate relative risk for a first VTE is provided (see text for references). "No" indicates that available meta-analyses or the majority of published studies found no significant association. "Yes" indicates that available meta-analyses or the majority of published studies demonstrated an association with a statistically significant OR $\geq$2.0, that is, an at least twofold increased chance for VTE. "Rather yes" indicates that meta-analyses or the majority of studies demonstrated a statistically significant OR $\geq$1.0, but $<$2, that is, a chance that is only discreetly increased.
[a]This is a protective polymorphism.
[b]Retinal vein occlusions are an exception and an association has been established for this specific patient group.

thrombosis, neither for individuals with homozygous nor for individuals with heterozygous plasminogen deficiency.[16,17] In addition, various qualitative defects of plasminogen were identified in patients with thromboembolic events, but the majority of family members, though affected, were not symptomatic.[18] In summary, there is no evidence that plasminogen deficiency contributes to thrombophilia.

Plasminogen Activator Inhibitor 1

PAIs interfere with plasminogen activation, and elevated levels of PAIs should predispose to thromboembolism. At least three polymorphic variations in the human PAI-1 gene *SERPIN1* have been reported. Specific alleles were associated with altered levels of PAI-1 in plasma. The biological consequences of elevated PAI-1 levels are not fully clear. The deletion/insertion (4G/5G) polymorphism in the promoter region leading to elevated levels of circulating PAI-1 has been studied extensively.[19] In a meta-analysis, the 4G allele was associated with unexplained VTE with an OR of 1.153 (95% confidence interval [CI], 1.068–1.246).[20] A slightly increased arterial thrombotic risk (OR, 1.088; 95% CI, 1.007–1.175) was also reported,[21] but an association of this polymorphism with stroke could not be established.[22] In the end, there is preliminary evidence that the PAI-1 4G/5G polymorphism may be associated with a slightly increased risk of thromboembolism under yet-to-be-defined circumstances.

α2-Antiplasmin

There is no evidence for an association between elevated α2-antiplasmin levels and the risk of thrombosis. Two smaller studies on thrombotic events postsurgery did not show an association,[23,24] and elevated α2-antiplasmin levels were also not associated with increased risk of thrombosis in a study of fibrinolytic markers.[15] These findings are in accordance with the assumption that α2-antiplasmin, because it is present in abundance, cannot represent a limiting factor of fibrinolysis, while rather regulating steps *before* the final activation of plasminogen are more likely to be critical.[25]

Thrombin-Activatable Fibrinolysis Inhibitor

TAFI inhibits fibrinolysis when converted to its active form by thrombin (or plasmin).[26] Elevated levels of TAFI should therefore reduce clot lysis and lead to an elevated risk of thromboembolism. In the Leiden Thrombophilia Study (LETS), TAFI levels above the 90th percentile of the controls were associated with a slightly increased risk for thrombosis (OR, 1.7; 95% CI, 1.1–2.5), and adjustments for various possible confounders did not substantially affect this estimate.[27] Elevated levels of TAFI were also reported as a mild risk factor for recurrent VTE, even after adjustment for potentially confounding variables (risk ratio, 1.06; 95% CI, 1.0–1.16).[28]

A comparable association between elevated TAFI levels was also reported for patients from the MEGA study in multiple assays of fibrinolytic markers (OR, 1.6; 95% CI, 1.2–2.1 for the highest quartile).[15] In contrast, a retrospective family study on 1,940 patient relatives identified 187 individuals with high TAFI levels, but the adjusted relative risk did not show an association with thromboembolic events (relative risk, 0.8; 95% CI, 0.5–1.3).[29] In summary, a minor effect of elevated TAFI levels on the risk of thrombosis cannot be excluded.

Lipoprotein(a)

Lipoprotein(a) (Lp(a)) is not part of the fibrinolytic system, but this cholesterol-rich lipoprotein consists of a low-density lipoprotein domain attached to apolipoprotein(a), which has partial homology to plasminogen. Lp(a) and plasminogen compete for fibrin binding, which in theory should impair fibrinolysis whenever Lp(a) is available in higher-than-normal concentration. Recent in vitro evidence, however, questions the relevance of this mechanism.[30] Without doubt, Lp (a) is an independent risk factor for atherosclerotic cardiovascular diseases through mechanisms associated with increased atherogenesis and thrombosis, but its contribution to VTE is less clear.[31] In a systematic review and meta-analysis based on case–control studies, a significant, but modest association between Lp(a) and VTE has been reported (OR, 1.56; 95% CI, 1.36–1.79).[32] In contrast, in a pooled analysis of population-based cohort studies, the fully adjusted hazard ratio for VTE was nonsignificant (hazard ratio, 1.00; 95% CI, 0.94–1.07).[33] In addition, genetic association studies were also unable to demonstrate an association between defined genetic variants which lead to elevated Lp (a) and the risk VTE.[34,35] Despite this, a recent meta-analysis of studies that aimed to lower Lp(a) concentrations using evolocumab, a monoclonal antibody blocking the PCSK9 enzyme, demonstrated a significant VTE risk reduction in patients whose baseline Lp(a) was above the median (hazard ratio, 0.52; 95% CI, 0.30–0.89), whereas evolocumab had no such effect in the low Lp(a) group.[36] This does not prove an effect of Lp(a) itself; additional metabolic changes following PCSK9-blockage may be responsible for the observed effects.

Unlike in other VTEs, thrombophilia does not seem to play a major role in retinal vein occlusion (RVO),[37] while common cardiovascular risk factors, such as hypertension, diabetes, and hyperlipemia, were reported to be predisposing factors.[38] RVO is therefore often considered a complication of atherosclerosis rather than a form of venous thrombosis. According to a recent meta-analysis, elevated Lp(a) also seems to have effects on an unusual thrombotic event (OR, 2.38; 95% CI, 1.7–3.34).[39] Although we do not think that Lp(a) is a relevant thrombophilia marker for VTE in general, we recommend including Lp (a) in the thrombophilia workup for RVOs.

Elevated Levels of Coagulation Factors

An elevated activity level of FVIII is a widely accepted thrombophilia marker. It is well documented through several studies that the thrombotic risk is approximately three- to fivefold increased for FVIII levels $\geq$150 IU/dL.[40] In addition, the impact of elevated FVIII levels on the risk of recurrent thrombosis is also well documented.[41] Only recently, in a large population-based case–control study, elevated FVIII levels were associated with a high risk of VTE after orthopaedic surgery (OR, 18.6; 95% CI, 7.4–46.9), comparable to the risk associated with the presence of FVL (OR, 17.5, 95% CI, 4.1–73.6).[42] Despite solid evidence for an association between elevated FVIII levels and VTE, it is still a matter of debate whether or not FVIII should be included into thrombophilia screening. The widespread use of one-stage clotting assays with their analytical shortcomings (mainly overestimating FVIII levels), the large intra-individual variation, the potential influence of a previous thrombotic event, any acute phase reaction, any underlying disease such as cancer, and the lack of a defined cut-off value make the use of FVIII as a thrombophilia factor problematic. It has been demonstrated that a shortened activated partial thromboplastin time (aPTT) is a risk factor for thrombosis and that this association between a short aPTT and VTE is only partially mediated by high FVIII levels.[43] Accordingly, it could be worth to look at other factors that affect aPTT, especially FXI, FIX, and fibrinogen.

Factor XI

The LETS demonstrated an OR of 2.2 (95% CI, 1.5–3.2) for VTE for individuals with a FXI level above the 90th percentile.[44] In a subsequent nested case–control study where the authors looked at levels of factors IX through XIII in 462 cases and 1,047 controls, only elevated levels of factors IX and XI were associated with an increased risk of VTE after adjustment for age, sex, ethnicity, and study.[45] With further adjustment for body mass index and diabetes, only elevated FXI levels remained associated with an elevated VTE risk: OR, 1.8 (95% CI, 1.3–2.7). Finally, a prospective study where 16,299 participants, initially free of VTE, were followed up over time, 606 VTEs occurred; the age, ethnicity, sex, and study-adjusted hazard ratio was 1.51 (95% CI, 1.16–1.97) for the highest versus lowest quintile of FXI, indicating a mild association.[46] Only few studies on plasmatic FXI levels and the risk of VTE did not find an association.[47] In contrast, studies that focused on polymorphisms in the *F11* gene gave contradictory results. Regarding *F11* rs2289252, a Swedish case–control study found an association (hazard ratio, 1.8; 95% CI, 1.1–3.0), whereas, using the same SNP, no association was identified in a Portuguese study (OR, 1.09; 95% CI, 0.75–1.59).[48,49] We conclude that elevated FXI levels are most likely a mild risk factor for VTE.

Factor IX

The LETS identified elevated levels of FIX as a risk factor for VTE.[50] Using the 90th percentile measured in control subjects (P(90) = 129 U/dL) as the cut-off, a two- to threefold increased risk for individuals with elevated FIX levels was identified. Adjustment for age, sex, oral contraceptive use, and high levels of factors VIII and XI did not affect this association. Even after exclusion of individuals with known genetic disorders, the OR for elevated FIX levels was still 2.5 (95% CI, 1.6–3.9). Assessing the risk of future VTE, Cushman's

longitudinal, nested case–control study demonstrated only a very mild association of high FIX levels with VTE after adjustment for age, sex, ethnicity, and study (OR, 1.4; 95% CI, 1.0–2.0).[45] This association was lost after additional adjustment for diabetes mellitus and body mass index. A mild effect of elevated FIX levels on the risk of venous thrombosis cannot be excluded.

Fibrinogen

Fibrinogen is the major procoagulatory protein in plasma by mass; its levels are between 1.5 and 3.5 g/L. An association between increasing levels of fibrinogen (for each g/L) and VTE risk was reported in a small study group of 199 patients with a first episode of thrombosis and 199 controls (OR, 1.4; 95% CI, 1.02–1.95).[51] Notably, when the study group was divided in strata according to their fibrinogen levels, the 95% CIs indicated insufficient evidence to conclude that patients and controls differed significantly. Fibrinogen is an acute-phase reactant and its levels increase in inflammation, but also under environmental stimuli (e.g., smoking). Therefore, a subsequent study included 474 thrombosis patients and 474 matched controls, which were part of the LETS.[52] High-sensitive C-reactive protein (CRP) was included in the laboratory panel. The CRP-adjusted OR was 4.3 (95% CI, 1.7–10.5) for a fibrinogen level greater than 5 g/L and 1.6 (95% CI, 1.0–2.6) for a fibrinogen level greater than 4 g/L and was basically comparable to the unadjusted OR, indicating that the mild risk associated with elevated fibrinogen levels could not be attributed to inflammatory stimuli. Using the same set of data, stratification by age showed that the VTE risk associated with high levels of fibrinogen was mainly increased in older patients.[53] Even the highest fibrinogen levels ($>$5 g/L) were not associated with VTE risk in patients younger than 45 years (OR, 1.5; 95% CI, 0.7–3.3), whereas in patients older than 45 years, even fibrinogen levels greater than 3 g/L were associated with VTE risk (OR, 1.8; 95% CI, 1.3–2.6).

Factor XIII

FXIII becomes activated through thrombin and crosslinks fibrin γ- and α-chains, stabilizing the blood clot. The FXIII B subunit is highly polymorphic. Of three common single nucleotide polymorphisms (A8259G, C29470T, and A30899G), one (A8259G) results in an amino acid substitution, His95Arg.[54] The relationship between His95Arg and VTE was investigated in two independent study cohorts, including 685 patients and 763 controls, revealing a pooled OR of 1.5 (95% CI, 1.1–2.0). The FXIII A subunit carries a polymorphism, Val34Leu, which was associated with elevated FXIII levels and moderate protection from VTE in some,[55] but not all, studies.[56] A meta-analysis calculated a combined ORs for VTE of 0.63 (95% CI, 0.46–0.86) for homozygotes and 0.89 (95% CI, 0.80–0.99) for heterozygotes.[57]

Anticoagulant Mechanisms

Tissue Factor Pathway Inhibitor

TFPI is a protease inhibitor and exerts its inhibitory effect mainly by inhibiting the tissue factor–FVIIa complex in a FXa-dependent manner.[58] Accordingly, low plasma concentrations of TFPI might increase the risk for thrombosis. The OR for VTE in subjects who had free antigen levels of TFPI below the 10th percentile, as compared with those who had levels above this value, was 1.7 (95% CI, 1.1–2.6) in the Leiden Thrombophilia study.[59] A subsequently performed nested case–control study demonstrated relevant effects of various confounding factors: compared with those in the upper 95%, the bottom 5% of free TFPI levels had an age-, sex-, ethnicity-, and study-adjusted, nonsignificant OR for VTE of 1.35 (95% CI, 0.86–2.12).[60] Additional adjustment for coagulation factor activities retrieved a moderate risk in this analysis (OR, 1.93; 95% CI, 1.05–3.53). In addition, moderate risk for recurrent VTE was identified in a prospective trial and patients after VTE were followed up after withdrawal of anticoagulation.[61] Genetic markers for TFPI levels are more difficult to interpret. In a recent meta-analysis, the *TFPI* rs8176592 polymorphism was associated with an increased risk of VTE, while no association was found for rs10931292 or rs10153820 and venous thrombosis. However, associations varied between ethnical groups.[62]

Thrombomodulin

Thrombomodulin (TM) is an endothelial thrombin receptor that modulates the activity of thrombin toward activation of anticoagulant protein C as well as TAFI. Accordingly, lack of function of TM results in abnormalities of regulation of the coagulation–fibrinolysis system.[63] Single nucleotide polymorphisms or mutations of the TM gene that influence protein expression or function have been associated with complement-mediated thrombotic microangiopathy and are potentially linked to arterial thrombosis.[64–68] With respect to the risk of VTE, sequence variations in the promotor as well as in the coding regions of the TM gene (*THBD*) have been found to be rare and/or could not be identified as independent risk factors for VTE.[69,70] On the contrary, an even reduced risk of VTE has been described for the c.1418C $>$ T polymorphism, which leads to replacement of Ala455 by Val in the TM molecule. This finding was substantiated by lower levels of soluble thrombomodulin (sTM) as well as gain-of-activity of the membrane-bound molecule.[71] However, other studies failed to demonstrate an association of the c.1418C $>$ T polymorphism with the risk of VTE.[72–74] Overall, there is currently no evidence that sequence variations in *THBD* correlate with an increased risk of VTE.

Endothelial Protein C Receptor

Activation of protein C on endothelial cells is augmented by the endothelial cell protein C receptor (EPCR), which promotes binding and alignment of the molecule to the thrombin–TM complex.[75] A soluble form of EPCR (sEPCR), which lacks the transmembrane domain and cytoplasmic tail, is present in plasma.[76] In contrast to transmembrane EPCR, sEPCR acts as an inhibitor of the protein C pathway by binding to both, protein C and activated protein C (APC) with similar affinity, thereby interfering with activation of the zymogen as well as the anticoagulant activity of the enzyme.[77] Sequence variants in the EPCR gene (*PROCR*) have

been found to be associated with both, cellular EPCR expression and sEPCR plasma levels. For instance, a 23bp insertion in exon 3 of PROCR that results in a stop codon downstream the insertion site has been described.[78] The EPCR 23bp insertion is very rare in patients with VTE as well as in patients with myocardial infarction. Accordingly, no association between the EPCR 23bp insertion and an increased arterial or venous thrombotic risk could be established.[79–81] Furthermore, two *PROCR* haplotypes (H1 and H3) with opposed functional phenotypes have been identified. Haplotype H1, usually tagged by the 4678G > C sequence variation (3′ UTR), is associated with increased plasma levels of anticoagulant APC and reduced levels of sEPCR. Haplotype H3, usually tagged by 4600A > G (exon 4, resulting in Ser219Gly), is associated with increased shedding of EPCR from the endothelium and, thus, increased levels of procoagulant sEPCR.[82] However, data from the LETS did not reveal a strong association between EPCR haplotypes and VTE risk.[83] A comprehensive meta-analysis demonstrated a weak association between the H3 haplotype and the risk of VTE (OR, 1.22; 95% CI, 1.11–1.33).[84] More recently, it has been described that both haplotypes may play a role with respect to the thrombotic risk in the younger population,[85] while a protective role against arterial thrombosis was suggested in patients with antiphospholipid syndrome.[86] In conclusion, there is currently only weak evidence for an association between EPCR haplotypes and the risk for VTE.

Protein Z and Protein Z–Dependent Protease Inhibitor

Protein Z (PZ) acts as a cofactor during inhibition of FXa by the PZ–dependent protease inhibitor (ZPI).[87] Recent data suggest that ZPI/PZ also functions as a physiological inhibitor of prothrombinase-bound FXa, questioning the current idea that FXa within the prothrombinase complex is protected from inhibition by protease inhibitors.[88] Accordingly, low plasma levels of either PZ or ZPI may increase the thrombotic risk. Several PZ (*PROZ*) and ZPI gene (*SERPINA10*) polymorphisms that influence functional protein plasma levels have been described, while their impact on the risk of VTE remains debatable.[89,90] In 2001, Vasse et al demonstrated a link between PZ deficiency and ischemic stroke, while no such association was found in patients with VTE.[91] Regarding both PZ and ZPI plasma levels, a relationship with venous thrombosis was also not detected in the LETS.[92] In contrast to these findings, a meta-analysis comprising 28 case–control studies revealed a significant association between low PZ levels and venous thromboembolic diseases (OR, 2.18; 95% CI, 1.19–4.00).[93] Furthermore, it has been proposed that low plasma levels of PZ may increase the risk of VTE associated with established thrombophilic risk factors (e.g., the FV Leiden mutation).[94,95] Regarding ZPI, several sequence variations in *SERPINA10*, including two stop mutations (R67X and W303X) have been identified with higher frequency in patients with thrombosis.[90,96] However, a subsequently performed meta-analysis did not reveal an association between these mutations and an increased risk for VTE.[97] Also other identified *SERPINA10* sequence variations showed no association with the risk for VTE.[98,99] In conclusion, there is weak evidence for an association between low levels of PZ and VTE.

Heparin Cofactor II

Heparin cofactor II (HCII) is a member of the serine protease inhibitor (SERPIN) family that binds to dermatan sulfate on endothelial cells and inhibits thrombin.[100] However, although contradictory reports have been published,[101,102] even homozygous deficiency of HCII has not been associated with an increased risk of thrombosis.[103–106]

Homocysteine Metabolism

Homocysteine is an intermediate in the metabolism of the essential amino acid methionine, which, among other food, is present in meat and dairy products. Homocysteine is toxic in higher concentrations and needs to be metabolized in the human body by one of the two following pathways: remethylation or transsulfuration. Remethylation (from homocysteine back to methionine) requires folate which will be converted to 5-methyltetrahydrofolate by the activity of an enzyme, methylene tetrahydrofolate reductase (MTHFR). Vitamin B12 is a relevant cofactor for this reaction. Transsulfuration (from homocysteine to cysteine) uses a different set of enzymes and depends on the availability of vitamin B6. Finally, the kidneys remove remaining homocysteine from the plasma. According to this complex metabolism, the following factors can affect homocysteine levels in the patient's plasma: food or food supplementation as a source of methionine, homocysteine, folate, vitamin B12, and vitamin B6; genetic polymorphisms affecting MTHFR activity; and renal function.[107]

MTHFR C677T, MTHFR A1298C

Genetic polymorphisms of MTHFR can affect enzyme activity and may lead to higher levels of homocysteine, an effect which can be overcome by folate substitution.[108] Indeed, it has long been suspected that in populations with high oral intake of folate, reduced activity of MTHFR does not correlate with an increased risk of VTE.[109] A meta-analysis including 11,000 VTE cases did not find a correlation between the MTHFR C677T polymorphism and VTE.[5] A more recent meta-analysis had two interesting findings: the MTHFR C677T polymorphism was associated with the risk of VTE, but only in Asians, not in Europeans; and the MTHFR A1298C polymorphism was not associated with the risk of VTE.[110] It is very likely that the difference between Asian and European patients is an effect of nutrition rather than of ethnicity: many countries in the western world enrich industrially manufactured food with folate.[111] Because of the aforementioned complex metabolism of homocysteine, and because of the evidence from meta-analyses, MTHFR polymorphisms should not be included in the diagnostic workup of thrombophilia.

Homocysteine Plasma Level

The mechanisms by which homocysteine increases the risk of VTE are unclear but may include effects on endothelial

cells, platelets, and coagulation factors.[112] An association between hyperhomocysteinemia and the risk of VTE has been demonstrated in a meta-analysis (OR, 2.95; 95% CI, 2.08–4.17),[113] an association that was underlined in various smaller studies thereafter.[114–116] It should be noted that lowering the homocysteine level has no clear impact on patients with arterial thrombotic events,[117] and evidence for VTE patients is lacking.

Other Thrombophilic Markers

Angiotensin-Converting Enzyme

The angiotensin-converting enzyme (ACE) plays a major role in the regulation of blood pressure by converting angiotensin I to angiotensin II.[118] ACE may also exert prothrombotic effects by inhibition of fibrinolysis and activation of platelets.[119] High ACE activity could therefore be associated with an increased risk of VTE. Half the variance of ACE activity is related to an insertion/deletion polymorphism,[120] and this polymorphism has been analyzed in most association studies. A meta-analysis based on 14 studies including 3,448 cases did not show evidence for an association between the ACE insertion/deletion genotype and VTE (OR, 1.206; 95% CI, 0.951–1.531).[121] In contrast, two studies in patients who were taking ACE inhibitors or angiotensin II receptor blockers and suffered from atherosclerosis or atrial fibrillation showed a reduction in the risk of venous thrombus formation.[122,123] However, no ACE polymorphism and/or data on enzyme activity levels were provided. Thus, the described effects could be based on a mechanism that differs from processes directly mediated by ACE.

Apolipoprotein E

Apolipoprotein E (ApoE) is a polymorphic glycoprotein that plays a role in cholesterol transport as well as cell membrane maintenance and repair.[124] While polymorphisms/genotypes of the ApoE gene (APOE) are discussed to be associated with atherosclerosis, cardiovascular disease, or ischemic stroke,[125–127] data on a corresponding risk of VTE are limited and contradictory. For instance, the ApoE*2 allele was described to be linked to deep venous thrombotic events in women.[128] Furthermore, the ApoE*3/4 genotype was found to be associated with an increased risk of VTE in a small pilot study (OR, 1.31; 95% CI, 1.30–10.48).[129] In contrast, other studies did not find any significant associations.[130,131]

ADAMTS13

ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) is a plasma glycoprotein that regulates the size distribution of vWF.[132] Due to the presence of uncleaved, ultra-large vWF multimers, severe lack of ADAMTS13 activity leads to thrombocytopenic purpura, a specific type of thrombotic microangiopathy.[133] Potential association between decreased ADAMTS13 activity and an increased risk for VTE is thought to be due to correspondingly increased vWF plasma levels.[134] Indeed, it has been shown that patients with an ADAMTS13 activity less than 1st quartile of normal showed an increased risk of VTE (OR, 1.6; 95% CI, 1.05–2.55), while the combination of low ADAMTS13 activity and high vWF antigen levels was associated with a 15-fold increased risk (95% CI, 7.80–33.80).[135] Furthermore, analysis by next-generation sequencing in patients with VTE without identified established risk factors revealed the presence of rare coding single-nucleotide variants within the ADAMTS13 gene (*ADAMTS13*). However, the association of these sequence variations with the risk of VTE needs to be verified.[136,137]

Conclusion

When determining the causes of VTE in a patient, it can be useful to include thrombophilia screening, bearing in mind that VTE is a multifactorial disorder and numerous other factors may play a more prominent role in triggering VTE in an individual patient, such as cancer or major surgery. Evidence of thrombophilia may have impact on the duration of anticoagulation.[138] In addition, in families with a history of VTE and thrombophilia, screening of family members (and subsequent primary antithrombotic prophylaxis in situations of high thrombotic risk) may help reduce the incidence of VTE and VTE-related disease burden.[139] While established markers of thrombophilia represent the fundamentals of thrombophilia screening, they are absent in most VTE patients. In search of alternative explanations for VTE in these patients, numerous additional markers have been described. In an attempt to appraise these markers, we reviewed the corresponding literature as described in the present paper. At present, none of the markers does add significantly to clinical routine thrombophilia testing. As summarized in ►Table 1, current evidence suggests that, for some of these markers, an association with VTE has not been demonstrated: plasminogen, α2-antiplasmin, TM gene mutations, ZPI, MTHFR gene polymorphisms, ADAMTS13, HCII, Lp(a), and ACE. We believe that these markers are of no benefit in patient care outside studies. In cases of supposed evidence of a given association between the presence of the marker and thrombophilia, analysis may be considered, although most associations are weak or very weak and further evidence from well-designed studies is warranted: TFPI, EPCR mutations, PZ, PAI-1, TAFI, homocysteine, FIX, FXI, fibrinogen, FXIII Val34Leu.

Other perspectives are needed to better understand the potential impact of novel markers. For instance, with respect to the interpretation of genetic association studies, most approaches looked at polymorphisms as markers of plasma levels. As discussed elsewhere, it will be necessary to look more closely for effects certain mutations may have on function or activity.[140] In addition, well-designed future studies must include extensive multiparameter testing, since it is evident that several players in coagulation, but also inflammation and metabolism, have significant impact on the risk of a first or recurrent VTE, also through mutual influence, in both a positive and negative sense.

Conflict of Interest
None declared.

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