EXPERT REVIEWS

The real value of thrombophilia markers in identifying patients at high risk of venous thromboembolism

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Thrombophilia is defined as a condition predisposing to the development of venous thromboembolic complications. Over the past decades, there have been great advances in the understanding of the pathogenesis of venous thromboembolism (VTE) through the identification of several inherited and acquired risk factors. However, in spite of such progress, a number of questions remain unanswered. In particular, it is well known that some subjects carrying several risk factors for VTE will never experience a thrombotic episode while other individuals developed recurrent thromboembolic events with no known risk factor. In this review, we summarize the current knowledge on the various thrombophilia markers, and also discuss their role in the development of thrombotic complications.

KEYWORDS: acquired • arterial thrombosis • inherited • thrombophilia • venous thrombosis

Thrombophilia is defined as a hypercoagulable state leading to a thrombotic tendency [1–3]. In 1856, the German physician Rudolf Virchow conceived the theory of the triad, that is, endothelial injury, stasis of blood flow and hypercoagulability, to explain the etiology of thrombosis [4]. This concept was prophetic in that it has now been shown that all three components of the triad play active roles in the development of thrombotic events. During the past two decades, progress has been made in the identification and characterization of the cellular and molecular mechanisms that interdependently influence the Virchow's triad. It is now accepted that the combination of stasis and hypercoagulability, more than endothelial damage, is crucial for the occurrence of venous thromboembolism (VTE), venous thrombi being mainly constituted by fibrin and red blood cells and less by platelets. In contrast, platelets are essential for primary hemostasis, repair of the damaged endothelium and play a pivotal role in the development of arterial thrombosis [5].

Thrombophilic abnormalities can be inherited, acquired or mixed (both congenital and acquired) and the risk of VTE is different according to each abnormality (Table 1). Inherited thrombophilias include deficiencies of the natural anticoagulant proteins antithrombin, protein C and protein S, as well as the gain-of-function mutations in the factor V gene (FV G1691A Leiden) and prothrombin gene (prothrombin G20210A). Acquired thrombophilia is mainly represented by the presence of antiphospholipid antibodies, while the most frequently investigated mixed abnormality is mild-to-moderate hyperhomocysteinemia. In this review, we discuss the role of the main thrombophilia markers in the development of thrombotic events.

Antithrombin deficiency

Antithrombin is a single chain glycoprotein synthesized in the liver, belonging to the serine protease inhibitor (serpin) superfamily [6], which functions as a natural anticoagulant by binding to, and inactivating, thrombin and other serine proteases, such as activated factor X [7]. The result of this activity is a reduction in both the generation and the half-life of thrombin. In addition to the active site responsible for coagulation factor inactivation, the antithrombin molecule contains a heparin-binding site [8]. When exogenous heparin or endogenous heparan sulfate bind to this site, the ability of antithrombin to inactivate the

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Table 1. Inherited, acquired and mixed coagulation-related risk factors for thrombosis.

Inherited	Acquired	Mixed
Antithrombin deficiency	Antiphospholipid antibodies	Hyperhomocysteinemia
Protein C deficiency		Increased fibrinogen
Protein S deficiency		Increased factor VIII
Factor V Leiden		Increased factor IX
Prothrombin G20210A		Increased factor XI
		Increased lipoprotein (a)

above-mentioned activated factors is greatly enhanced. As expected, any type of mutation that leads to a reduction of plasma antithrombin levels or to a decreased ability to interact with either the activated factors or heparin will result in an increased risk of thrombosis [9].

Antithrombin deficiency is mainly transmitted as an autosomal dominant trait and patients usually present with recurrent VTE episodes during the second to third decade of life. The penetrance of this disease is very high, since most affected family members experience a thrombotic event by the age of 45 [10,11]. Antithrombin deficiency is probably the most severe of the inherited thrombophilias, causing more than 50-fold increased risk for VTE compared with that of individuals not carrying this defect [11]. In the general population, the estimated prevalence of antithrombin deficiency ranges from 2 to 17 per 10,000 individuals, and in patients with VTE is around 1% [12–14]. More than 250 gene variations have been identified in antithrombin deficiency, including missense and nonsense point mutations, insertions and deletions [15].

Two types of antithrombin deficiency can be distinguished. In type I, antithrombin activity and antigen level are both reduced in plasma owing to a lack of protein production or secretion by the mutant allele. In type II, low antithrombin activity contrasts with normal antigen levels, indicating functional defects in the molecule. Type II can be further subdivided into three subtypes characterized by impairment of the enzyme reactive site, of the heparin-binding site or by pleiotropic defects affecting antigen concentration and heparin-binding or enzyme activity [16]. The only individuals homozygous for antithrombin deficiency described so far carry heparin-binding site defects, suggesting that the other subtypes are associated with embryonic lethality [16].

Table 2 reports epidemiological data and association with VTE risk of the different thrombophilic risk factors. It should be outlined that risk estimates are generally based on selected family studies, so that the risk of venous thrombosis may not necessarily reflect the risk of thrombophilia in the general population.

Protein C deficiency

Protein C, described for the first time in 1979 [17], is a vitamin-K-dependent glycoprotein synthesized in the liver in an inactive form. Under physiological conditions, once activated by the thrombin–thrombomodulin complex, protein C acts as an anticoagulant by means of the proteolytic degradation of activated coagulant factors Va and VIIIa. As for other physiological inhibitors of coagulation, any mutation leading to a reduction of protein C activity increases the risk of VTE [18].

Inherited protein C deficiency is transmitted as a dominant autosomal trait [19] and more than 200 mutations in the protein C gene (*PROC*), which lies on 2q13-q14 chromosome, have been reported so far [20]. Heterozygous deficient people experience recurrent episodes of VTE, often before the age of 45. Homozygous individuals have a more severe clinical picture, not infrequently leading to neonatal purpura fulminans, a potentially fatal condition characterized by multiple thrombosis in small vessels leading to skin necrosis [14]. The penetrance of the disease is less than that of antithrombin deficiency, thus heterozygotes have a 15-fold increased risk for thrombosis compared with the general population [15]. However, the disorder is quite rare with prevalence in the population of only 0.2–0.4 and of 3% in patients selected for VTE. Similar to antithrombin deficiency, protein C deficiency can be divided in two subtypes. Type I, which is the most common, is characterized by a parallel reduction in plasma antigen level and activity, reflecting a reduced synthesis of a functional protein. The rarer type II is characterized by normal antigen level with reduced functional activity, reflecting normal synthesis of a dysfunctional protein. In type I, the majority of mutations are of the missense variety, leading to premature termination of synthesis or disruption of protein folding, whereas deletions and insertions occur with a much lower frequency (~10%). In type II, missense mutations are located mainly in the γ-carboxyglutamic acid and protease domains [21].

Protein S deficiency

Protein S is a vitamin-K-dependent protein of liver synthesis, which circulates in plasma in equilibrium between an inactive form, bound to a carrier called C4b-binding protein, and a free, functionally active form, which accounts for approximately 40% of the total plasma protein S [18]. Protein S functions as a cofactor of activated protein C (APC) for the degradation of activated factors Va and VIIIa. In addition, protein S acts as a cofactor of tissue factor pathway inhibitor in the inhibition of factor Xa. The bioavailability of protein S is closely linked to the concentration of the acute phase reactant C4b-binding protein, which acts as an important regulatory protein. Thus, all conditions (i.e., pregnancy, oral contraceptive use, acute thrombosis, inflammatory states and cancers) associated with increased C4b-binding protein levels also cause an increase in bound protein S and hence a reduction of the unbound free form [22].

Transmitted as an autosomal dominant trait, familial protein S deficiency has a clinical presentation very similar to that observed for protein C deficiency [23]. Thus, heterozygotes experience early recurrent VTE episodes and sometimes warfarin-induced skin necrosis, while rare homozygotes exhibit a very severe clinical picture with neonatal purpura fulminans. The penetrance of the disease is also similar to that of protein

Table 2. Prevalence of thrombophilia abnormalities and relative risk of thrombosis.

Thrombophilia abnormality	Prevalence (%)		Relative risk	
	General population	*Patients with VTE*	*First VTE*	*Recurrent VTE*
Antithrombin deficiency	0.02–0.17	1.1	50	2.5
Protein C deficiency	0.2–0.4	3.2	15	2.5
Protein S deficiency	0.03–0.1	2.2	6–10	2.5
Heterozygous factor V Leiden	2–10	20–50	7	1.4
Heterozygous prothrombin G20210A	1–4	5–10	3–4	1.4
Antiphospholipid antibodies	1–2	5–15	1–10	2–6
Hyperhomocysteinemia	5	10–15	1.5	0.9–2.7

VTE: Venous thromboembolism.

C deficiency, causing a nearly 10-fold increased VTE risk in affected individuals compared with the normal population. The prevalence of protein S deficiency in the general population is estimated at 0.03–0.1%, while it occurs in 2% of patients with VTE [14]. The risk of recurrence is similar to that of the other natural anticoagulant deficiencies (2.5-fold).

Three types of protein S defects have been described: type I is a quantitative deficiency, with decreased plasma levels of functional and immunoreactive total and free protein S; type II is a qualitative deficiency, with decreased cofactor activity but normal total and free protein S levels and type III is a quantitative deficiency, with reduced functional activity and free protein S antigen levels and normal total protein S levels [14]. The PROS1 gene database lists almost 200 different mutations associated with protein S deficiency, the majority being missense mutations or short deletions or insertions [24].

Factor V Leiden

In 1993, a poor anticoagulant response to APC was associated with an increased risk of VTE [25,26]. The so-called APC resistance is mainly caused by the G1691A mutation (chromosome 1q23) in the Arg506 cleavage site of FVa, described first in the city of Leiden in 1994 [27,28]. Factor V Leiden is inactivated by APC more slowly than wild-type FVa, thus promoting a hypercoagulable state and an increased susceptibility to VTE. The FV Leiden gain-of-function mutation has a dominant autosomal transmission and, in its heterozygous form, is the most common prothrombotic gene mutation in the Caucasian population, with a prevalence of about 3%, ranging from 2 to 10% with a gradient from Southern to Northern Europe [29]. This prevalence rises to 20–50% in patients presenting with a first episode of VTE. VTE risk is increased approximately sevenfold in heterozygous, while homozygotes exhibit a very high risk for VTE, up to 80-times the normal risk [30,31].

Prothrombin G20210A

This polymorphism was first described in 1996 by Poort *et al.* [32], who showed that a G to A transition at nucleotide 20210 of the prothrombin gene within the 3′ untranslated region caused increased basal levels of functionally normal prothrombin, thereby conferring to heterozygotes an increased risk (~three- to fourfold) of developing VTE. Thus, these individuals exhibit a relatively low thrombotic risk, and most of them will not develop a thrombotic episode by age 50 years. In contrast, homozygosity for prothrombin gene mutation is much rarer and causes a higher thrombotic risk. The risk of VTE recurrence is similar to that of FV Leiden (1.4-fold). Like FV Leiden, the prothrombin G20210A mutation has a dominant autosomal transmission and is the second most common coagulation abnormality, with a prevalence of heterozygotes in populations of Caucasian descent ranging from 1 to 4%, with a gradient from Northern to Southern Europe. Prothrombin G20210A is found in 5–10% of VTE patients [33].

Antiphospholipid antibodies

Antiphospholipid antibodies are one of the most important acquired risk factors for thrombosis [34,35]. The corresponding syndrome is characterized by the presence of circulating antiphospholipid antibodies in plasma and either arterial and/or venous thrombosis, or pregnancy complications, particularly fetal loss [36]. The clinically relevant antiphospholipid antibodies include lupus anticoagulant, anticardiolipin and anti-β_2-glycoprotein I antibodies. These autoantibodies are directed against a wide variety of protein co-factors localized upon phospholipid membrane surfaces (β_2-glycoprotein I, prothrombin, protein C, protein S, annexin V, coagulation factor XII, etc.). The resulting complexes interact with several cell types, including endothelial cells, monocytes and platelets, all of which play important roles in hemostasis and thrombogenesis [37]. The relative risk of thrombosis varies depending on the type of antibody and is higher in the presence of lupus anticoagulant with or without anticardiolipin antibodies (odds ratio [OR]: 11.0; 95% CI: 3.81–32.3) and lower if only anticardiolipin antibodies are present at a clinically significant titer (>40 IU) (OR: 3.21; 95% CI: 1.11–9.28), or at a low titer <40 IU (OR: 1.56;

95% CI: 1.10–2.24) [38]. Antiphospholipid antibodies can be idiopathic, drug-related or associated with autoimmune (e.g., systemic lupus erythematosus or rheumatoid arthritis), lymphoproliferative or inflammatory diseases [39]. Incidental detection of antiphospholipid antibodies is not uncommon. In the case–control Leiden thrombophilia study [34], lupus anticoagulant was present in 0.9% of unaffected controls (and in 3.1% of the VTE cases) and β_2-glycoprotein I antibodies in 3.4% of controls (and in 7.5% of the patients). However, incidental antiphospholipid antibodies are associated with a low risk of thrombosis: in a large study including 178 asymptomatic carriers of antiphospholipid antibodies followed up for 26 months, no episode of thrombosis was detected [40].

Hyperhomocysteinemia

The amino acid homocysteine is formed from the demethylation of dietary methionine and its plasma levels are controlled by two metabolic pathways. The first involves the enzyme cystathionine β-synthase and requires vitamin B_6, the second involves the enzyme methionine synthase and requires both vitamin B_{12} and N^5-methyltetrahydrofolate reductase. Both genetic (e.g., mutations in N^5-methyltetrahydrofolate reductase and cystathionine β-synthase genes) and acquired factors (e.g., deficiencies of folate, vitamin B_{12} or vitamin B_6, advanced age, chronic renal failure and the use of antifolic drugs) interact to determine plasma homocysteine concentrations. As a consequence, hyperhomocysteinemia is considered a mixed factor (i.e., genetic and/or acquired) for thrombosis. Moderately increased plasma levels of homocysteine have been associated with a modest (1.5-times) increased thrombotic risk and approximately 5% of the general population has higher than normal levels of homocysteine, while the prevalence among VTE patients is about 10–15% [21,41].

High levels of coagulation factors

An association between elevated levels of factor VIII, but also of factors IX, XI and fibrinogen, and an increased risk of VTE has been demonstrated in several studies [21,42,43]. The plasma levels of these factors are not only influenced by age and inflammation, but are also under genetic control [11]. However, although a heritable component has been described for these clotting factors [44–46], to date no gene variations have been discovered accounting for such elevated levels. Hence most laboratories include only factor VIII plasma levels in the thrombophilia test panel.

Inherited thrombophilia & thrombotic risk

A number of family studies have been conducted to investigate the risk and incidence of VTE in carriers of thrombophilia versus non-carriers. These studies consistently indicate that a thrombotic risk gradient does exist, being higher in individuals with antithrombin, protein C, protein S deficiencies, homozygous FV Leiden and prothrombin G20210A or multiple abnormalities of the latter (severe thrombophilia) than in heterozygotes for FV Leiden or prothrombin G20210A (mild thrombophilia) [47–50]. Thus, carriers of antithrombin, protein C or protein S deficiencies have a 4- to 30-fold increased risk of VTE compared with non-carriers, being the highest incidence of VTE (0.9–4.0 per 100 person-years) observed in carriers of antithrombin deficiency [47,51]. Conversely, carriers of mild thrombophilic traits have a two- to sevenfold increased risk of VTE, with a much lower incidence of events than those with severe thrombophilia (0.14–0.67 per 100 person-years for FV Leiden; 0.05–0.42 per 100 person-years for prothrombin 20210A) [47–50]. Even low borderline plasma levels of antithrombin, protein C and protein S are associated with a twofold increased risk of VTE, to the same degree as that of mild thrombophilia [52]. The degree of severity of the thrombophilic defect has been found to influence the age of occurrence of the first thrombotic episode. Indeed, in a large family cohort, relatives of probands with thrombosis and a thrombophilic defect, who themselves had an antithrombin, protein C, or protein S deficiency, had VTE at a younger age (median 29 years) than those with FV Leiden, prothrombin G20210A or elevated FVIII levels (median 40 years) [47]. The definition of different classes of thrombotic risk for thrombophilic defects is also of great clinical relevance, as indefinite duration of anticoagulation is actually recommended after an unprovoked VTE event in carriers of severe thrombophilia [3]. A particular mention deserves the occurrence of VTE in pediatric population, whose incidence is increasing due to the improved survival of children with previously fatal disease and advances in pediatric care [53]. VTE in children is rare, being in the great majority of cases associated with primary underlying diseases, such as cancers, sepsis, congenital cardiovascular disorders or with therapeutic interventions, such as central venous line placement [53]. In this context, inherited thrombophilia has been described as an additional risk factor rather than a primary triggering factor, although a recent cohort study found that 9 of 21 (42.8%) antithrombin-deficient pediatric patients developed VTE spontaneously [54]. A meta-analysis of observational studies published from 1970 to 2007 on the impact of thrombophilia traits on VTE development or recurrence in children reported that all the investigated traits showed a significant association with first-ever VTE with ORs ranging from 2.63 (95% CI: 1.61–4.29) for the prothrombin mutation to 9.44 (95% CI: 3.34–26.66) for antithrombin deficiency [55]. A similar association between inherited thrombophilia and cerebrovascular occlusion (cerebral venous thrombosis and stroke) was found in another systematic review and meta-analysis [56]. Only few published data are available on the risk of recurrence of VTE in pediatric patients, suggesting a recurrence rate between 3% in neonates and 21% in children with idiopathic VTE [53,55]. However, it remains a controversial issue whether or not children from thrombophilia families should be screened for inherited thrombophilia.

Thrombophilia testing in high-risk situations

Clinical and epidemiological studies on the prevalence of the forementioned thrombophilic traits, as well as on their

association with the development of VTE (Table 2) have led to confirm the multifactorial nature of VTE, in which the thrombotic event is the result of multiple gene–gene and/or gene–environment interactions. In keeping with this model, inherited thrombophilia does interact with several other well-established acquired predisposing factors for VTE such as malignancy, inflammatory states, antiphospholipid antibodies, surgery, trauma, immobility, pregnancy-puerperium, use of oral contraceptives or hormone replacement therapy, elevated body mass index, severe infections and venous abnormalities [57]. Moreover, another important genetic factor, age, plays a pivotal role in modulating the thrombotic risk. Overall, this model provides a dynamic concept of the thromboembolic risk, encompassing a genetic predisposition (one or more co-inherited thrombophilic abnormalities) plus a variable contribution of environmental factors (potentially modifiable or preventable) during different ages of life, which may lead to overcome a threshold and hence trigger the VTE episode [57]. Therefore, thrombophilia markers cannot be interpreted in isolation, because interactions with other genetic and acquired risk factors are important determinants of the overall risk of VTE. Thus, it appears evident that an indiscriminate search for thrombophilia carriers is of no use and that a targeted screening strategy is potentially more useful [58].

In general, screening for thrombophilia is meaningful only when it does influence the management of affected patients. Our suggested indications for thrombophilia screening include (Box 1): unprovoked thrombosis and/or thrombosis at an age less than 50 years, VTE at unusual sites (such as hepatic, mesenteric and cerebral veins), recurrent VTE, asymptomatic relatives of patients with severe thrombophilia, VTE during pregnancy or in women taking oral contraceptives or under hormone replacement therapy. Notably, testing for thrombophilia in patients during the acute phase of a thromboembolic episode is not indicated, because many of those laboratory tests are functional assays (e.g., clot-base or chromogenic) that can be abnormal as a consequence of the inflammatory state rather than for the inherited defect [59].

Whether or not the presence of a thrombophilic abnormality is able to predict VTE recurrence is still a matter of debate [60]. The estimated risk of recurrence for VTE is approximately 5% per year, although idiopathic cases tend to recur more frequently (~20% in the first 2 years) than unprovoked cases [57]. Several prospective studies have investigated the risk of VTE recurrence in carriers of either mutations, with conflicting results [60–63]. The risk of recurrence among heterozygotes for FV Leiden or prothrombin G20210A has been recently revised by at least three meta-analyses [64–66], and it was found to be 1.4- to 1.6-fold higher in patients with FV Leiden and 1.3- to 1.4-fold higher in those with the prothrombin mutation. Data on natural anticoagulant deficiencies are less extensive, because only a few studies with a limited number of patients assessed the risk of recurrence. However, in probands and their deficient relatives belonging to the EPCOT prospective cohort, the incidence of recurrent VTE was 10.5% per patient-year in patients with antithrombin deficiency and 3.5% per patient-year in carriers of FV Leiden [67]. In a retrospective investigation on probands with a deficiency of natural anticoagulants and their deficient relatives, the incidence of recurrent VTE was confirmed to be high, being 7.7% per patient-year (10% for antithrombin deficiency, 6% for protein C deficiency and 8.4% for protein S deficiency) [68].

Box 1. Authors' suggested indications for thrombophilia testing.

Clinical condition

- Idiopathic thrombosis and/or age <50 years at the time of first venous thrombosis
- Unusual sites of venous thrombosis (hepatic, mesenteric, splenic, portal, cerebral)
- History of recurrent venous thrombosis
- Asymptomatic relatives of patients with severe thrombophilia
- Venous thrombosis during pregnancy or in women taking oral contraceptives or under hormone replacement therapy

Another important issue regards the screening of asymptomatic relatives of patients with inherited thrombophilia. The rationale for this approach consists in the possibility of identifying those individuals carrying of a thrombophilic trait who may benefit from targeted thromboprophylaxis in high-risk situations (pregnancy, puerperium, surgery, immobilization and trauma). A number of prospective and retrospective studies have specifically investigated the VTE risk among relatives of individuals with inherited thrombophilia [69,70]. Collectively, these studies reported that the VTE incidence among relatives was higher in carriers of an antithrombin, protein C or protein S defect (with a range of 0.36–4.0% per individual-year) than in carriers of FV Leiden (0.19–0.58% per individual-year) and prothrombin G20210A (0.11–0.37% per individual-year) [69]. Considering these data globally, while a screening in asymptomatic relatives of patients with severe thrombophilia is warranted, more uncertainty exists regarding the usefulness of a familial screening among the relatives of probands with mild thrombophilia. However, as the risk of VTE has been consistently reported to be higher in asymptomatic carriers of mild thrombophilia who have a family history of VTE than in those with no family history [69–72], this approach to testing seems to be reasonable.

As regards the gender-related risk factors for VTE, a universal screening for thrombophilia before exposure to environmental risk circumstances such as oral contraceptives or pregnancy, has been demonstrated not to be cost-effective, but it should be considered in well-selected cases [73–75]. For instance, the use of third-generation combined oral contraceptives is associated with a twofold increased risk of VTE [76] and a supra-additive effect is observed (up to fivefold) when an inherited thrombophilic risk factor is also present [77]. However, because the expected absolute incidence of VTE among unselected carriers is relatively low, not exceeding 6.2 events per 1000 women-years, screening has a limited value in women with no personal

or family history of VTE [73]. By contrast, testing for inherited thrombophilia before the prescription of oral contraceptives might be beneficial for women who are relatives of symptomatic carriers of severe thrombophilia, because in this category the absolute incidence of VTE is much higher (4–10 events per 100 women-years for antithrombin, protein C and protein S deficiencies) [78]. However, it should be outlined that in these families, women without such deficiencies also have a markedly increased risk of oral contraceptive-related VTE compared with the general population (0.7 vs 0.04% per year of use) [79]. This phenomenon, which identifies families with a strong thrombotic tendency probably due to co-segregation of still unknown thrombophilia defects, should be carefully taken into account when oral contraceptives are prescribed.

The absolute incidence of VTE among post-menopausal women on hormone replacement therapy is higher than among oral contraceptive users, due to their older age. Accordingly, unrestricted or family-driven screening before prescribing hormone replacement therapy has been proposed by a number of investigators, because this therapeutic procedure is associated in thrombophilic carriers with a relatively high VTE risk [73]. Finally, pregnant women with asymptomatic inherited thrombophilia are considered at high VTE risk, and thus eligible for prophylaxis with low molecular weight heparin during antenatal and the 6-week puerperium periods, but only if they have a family history of VTE, severe thrombophilia or a mild thrombophilia with additional risk factors (family history of VTE, immobility, obesity, age >35 years, gross varicose veins) [80,81].

Expert commentary & five-year view

During the past 30–40 years, our knowledge on the genetic abnormalities predisposing to VTE has dramatically increased. Thus, from the analysis of the available data it appears that the thrombotic risk is higher in carriers of natural anticoagulant deficiencies and in the presence of homozygous defects or multiple abnormalities (severe thrombophilia) than in heterozygotes for FV Leiden and prothrombin G20210A (mild thrombophilia). This thrombotic risk gradient should be evaluated in the framework of other (genetic and/or acquired) coexisting risk factors for first or recurrent VTE when assessing the need and duration of primary or secondary antithrombotic prophylaxis. Future research should be focused on the identification of novel thrombophilic factors in order to explain (and prevent) more cases of unprovoked thrombosis, and to clarify the molecular mechanisms underlying the complex interaction between genetic and environmental risk factors for thrombosis.

Perhaps methods such as next-generation sequencing may help to detect new very rare traits associated with a high risk of VTE.

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Key issues

- Thrombophilia is defined a hypercoagulable state leading to a thrombotic tendency.
- Thrombophilic abnormalities can be inherited, acquired or mixed (both congenital and acquired).
- Thrombophilic abnormalities cause blood hypercoagulability through the impairment of the anticoagulant or the potentiation of procoagulant pathways.
- During the past 30–40 years, our knowledge on genetic abnormalities causing thrombophilia has greatly expanded.
- Both inherited and acquired risk factors should be taken into account when assessing the individual risk of thrombosis.
- Universal screening for inherited thrombophilia is unjustified.
- Screening asymptomatic relatives of carriers of severe thrombophilia is recommended.

References

Papers of special note have been highlighted as:
•• of considerable interest

1. Martinelli I, Bucciarelli P, Mannucci PM. Thrombotic risk factors: basic pathophysiology. Crit Care Med 2010; 38(Suppl 2):S3-9
2. Previtali E, Bucciarelli P, Passamonti SM, Martinelli I. Risk factors for venous and arterial thrombosis. Blood Transfus 2011; 9(2):120-38
3. Martinelli I, De Stefano V, Mannucci PM. Inherited risk factors for venous thromboembolism. Nat Rev Cardiol 2014; 11(3):140-56

•• **A recent extensive review of the literature on genetic risk factors for venous thromboembolism.**

4. Virchow R. Phlogose und Thrombose im Gefäßsystem; Gesammelte Abhandlungen zur Wissenschaftlichen Medizin. Staatsdruckerei; Frankfurt: 1856
5. Lippi G, Franchini M, Targher G. Arterial thrombus formation in cardiovascular disease. Nat Rev Cardiol 2011;8(9):502-12

•• **An updated review of the mechanisms leading to arterial thrombosis.**

6. Spencer F, Becker RC. Diagnosis and management of inherited and acquired thrombophilias. J Thromb Thrombolysis 1999;7(2):91-104
7. Mammen EF. Antithrombin: its physiological importance and role in DIC. Semin Thromb Hemost 1998;24(1):19-25
8. Roemisch J, Gray E, Hoffmann JN, Wiedermann CJ. Antithrombin: a new look at the actions of a serine protease inhibitor. Blood Coagul Fibrinolysis 2002;13(8): 657-70

9. Mammen EF. Clinical relevance of antithrombin deficiencies. Semin Hematol 1995;32(4 Suppl 2):2-6
10. Buchanan GS, Rodgers GM, Branch DW. The inherited thrombophilias: genetics, epidemiology, and laboratory evaluation. Best Pract Res Clin Obst Gynecol 2003; 17(3):397-411
11. Franchini M, Veneri D, Salvagno GL, et al. Inherited thrombophilia. Crit Rev Clin Lab Sci 2006;43(3):249-90
12. Wells PS, Blajchman MA, Henderson P, et al. Prevalence of antithrombin deficiency in healthy blood donors: a cross-sectional study. Am J Hematol 1994;45(4):321-4
13. Tait RC, Walker ID, Perry DJ, et al. Prevalence of antithrombin deficiency in the healthy population. Br J Haematol 1994; 87(1):106-12
14. De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes and management. Blood 1996;87(9):3531-44
15. Lane DA, Kunz G, Olds RJ, Thein SL. Molecular genetics of antithrombin deficiency. Blood Rev 1996;10(2):59-74
16. Finazzi G, Caccia R, Barbui T. Different prevalence of thromboembolism in the subtypes of congenital antithrombin III deficiency: review of 404 cases. Thromb Haemost 1987;58(4):1094
17. Kisiel W. Human plasma protein C. Isolation, characterization and mechanism of activation by alpha-thrombin. J Clin Invest 1979;64(3):761-9
18. Simioni P. The molecular genetics of familial venous thrombosis. Bailliere's Clin Haematol 1999;12(3):479-503
19. Griffin JH, Evatt B, Zimmerman TS, et al. Deficiency of protein C in congenital thrombotic disease. J Clin Invest 1981; 68(5):1370-3
20. Reitsma PH, Bernardi F, Doig RG, et al. Protein C deficiency: a database of mutations, 1995 update. On behalf of the Subcommittee on Plasma Coagulation Inhibitors of the Scientific and Standardization Committee of the ISTH. Thromb Haemost 1995 73(5):876-89
21. Franco RF, Reitsma PH. Genetic risk factors of venous thrombosis. Hum Gen 2001;109(4):369-84
22. Borgel D, Gandrille S, Aiach M. Protein S deficiency. Thromb Haemost 1997;78(1): 351-6
23. Comp PC, Esmon CT. Recurrent venous thromboembolism in patients with a partial deficiency of protein S. N Engl J Med 1984;311(24):1525-8
24. García de Frutos P, Fuentes-Prior P, Hurtado B, Sala N. Molecular basis of protein S deficiency. Thromb Haemost 2007;98(3):543-56
25. Dahlback B, Carlsson M, Svesson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C. Proc Natl Acad Sci USA 1993; 90(3):1004-8
26. Koster T, Rosendaal FR, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet 1993;342(8886-8887):1503-6
27. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369(6475):64-7
28. Voorberg J, Roelse J, Koopman R, et al. Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V. Lancet 1994;343(8912):1535-6
29. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet 1995;346(8983):1133-4
30. Rosendaal FR, Koster T, Vandenbroucke JP , Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden. Blood 1995;85(6):1504-8
31. Cushman M, Tsai AW, White RH, et al. Deep vein thrombosis and pulmonary embolism in two cohorts: the longitudinal investigation of thromboembolism etiology. Am J Med 2004;117(1):19-25
32. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88(10): 3698-703
33. Rosendaal FR, Doggen CJ, Zivelin A, et al. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998;79(4):706-8
34. De Groot PG, Lutters B, Derksen RH, et al. Lupus anticoagulants and the risk of a first episode deep vein thrombosis. J Thromb Haemost 2005;3(9):1993-7
35. Naess IA, Christiansen SC, Cannegieter SC, et al. A prospective study of anticardiolipin antibodies as a risk factor for venous thrombosis in a general population (the HUNT study). J Thromb Haemost 2006; 4(1):44-9
36. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4(2):295-306
37. Galli M, Barbui T. Antiphospholipid antibodies and thrombosis: strength of association. Hematol J 2003;4(3):180-6

•• **An extensive review of the literature describing the association between antiphospholipid antibodies and thrombosis.**

38. Wahl DG, Guillemin F, de Maistre E, et al. Meta-analysis of the risk of venous thrombosis in individuals with antiphospholipid antibodies without underlying autoimmune disease or previous thrombosis. Lupus 1998;7(1):15-22
39. Harris EN, Gharavi AE, Hughes GRV. Antiphospholipid antibodies. Clin Rheum Dis 1985;11(3):591-609
40. Girón-González JA, García del Río E, Rodríguez C, et al. Antiphospholipid syndrome and asymptomatic carriers of antiphospholipid antibody: prospective analysis of 404 individuals. J Rheumatol 2004;31(8):1560-7
41. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. Thromb Haemost 1999;81(2):165-76
42. Kyrle PA, Minar E, Hirschl M, et al. High plasma levels of factor VIII end the risk of recurrent venous thrombembolism. N Engl J Med 2000;343(7):457-62
43. Koster T, Blann AD, Briet E, et al. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet 1995; 345(8943):152-5
44. Kamphuisen PW, Houwing-Duistermaat JJ, van Houwelingen HC, et al. Familial clustering of factor VIII and von Willebrand factor levels. Thromb Haemost 1998;79(2): 323-7
45. Vossen CY, Hasstedt SJ, Rosendaal FR, et al. Heritability of plasma concentrations of clotting factors and measures of a prethrombotic state in a protein C-deficient family. J Thromb Haemost 2004;2(2):242-7
46. Bank I, Libourel EJ, Middeldorp S, et al. Elevated levels of FVIII:C within families are associated with an increased risk for venous and arterial thrombosis. J Thromb Haemost 2005;3(1):79-84
47. Lijfering W M, Brouwer JL, Veeger NJ, et al. Selective testing for thrombophilia in patients with first venous thrombosis: results from a retrospective family cohort study on

absolute thrombotic risk for currently known thrombophilic defects in 2479 relatives. Blood 2009;113(21):5314-22

48. Bucciarelli P, Rosendaal FR, Tripodi A, et al. Risk of venous thromboembolism and clinical manifestations in carriers of antithrombin, protein C, protein S deficiency, or activated protein C resistance: a multicenter collaborative family study. Arterioscler Thromb Vasc Biol 1999;19(4): 1026-33
49. Simioni P, Sanson BJ, Prandoni P, et al. Incidence of venous thromboembolism in families with inherited thrombophilia. Thromb Haemost 1999;81(2):198-202
50. Brouwer JL, Veeger NJ, Kluin-Nelemans HC, van der Meer J. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. Ann Intern Med 2006;145(1):807-15
51. Sanson BJ, Simioni P, Tormene D, et al. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. Blood 1999; 94(11):3702-6
52. Bucciarelli P, Passamonti SM, Biguzzi E, et al. Low borderline plasma levels of antithrombin, protein C and protein S are risk factors for venous thromboembolism. J Thromb Haemost 2012;10(9):1783-91
53. Kenet G, Nowak-Götl U. Venous thromboembolism in neonates and children. Best Pract Res Clin Haematol 2012;25(3): 333-44
54. Limperger V, Franke A, Kenet G, et al. Clinical and laboratory characteristics of paediatric and adolescent index cases with venous thromboembolism and antithrombin deficiency. An observational multicenter cohort study. Thromb Haemost 2014. [Epub ahead of print]
55. Young G, Albisetti M, Bonduel M, et al. Impact of inherited thrombophilia on venous thromboembolism in children: a systematic review and meta-analysis of observational studies. Circulation 2008; 118(13):1373-82
56. Kenet G, Lütkhoff LK, Albisetti M, et al. Impact of thrombophilia on risk of arterial ischemic stroke or cerebral sinovenous thrombosis in neonates and children: a systematic review and meta-analysis of observational studies. Circulation 2010; 121(16):1838-47
57. Coppola A, Tufano A, Cerbone AM, Di Minno G. Inherited thrombophilia: implications for prevention and treatment of venous thromboembolism. Semin Thromb Hemost 2009;35(7):683-94
58. Wu O, Robertson L, Twaddle S, et al. Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study. Screening for thrombophilia in high-risk situations: a meta-analysis and cost-effectiveness analysis. Br J Haematol 2005;131(1):80-90
59. Whitlatch NL, Ortel TL. Thrombophilias: when should we test and how does it help? Semin Respir Crit Care Med 2008;29(1): 25-39
60. Kyrle PA, Rosendaal FR, Eichinger S. Risk assessment for recurrent venous thrombosis. Lancet 2010;376(9757):2032-9

•• **An extensive review of the literature describing the various risk factors for venous thromboembolism recurrence.**

61. Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet 2003;362(9383):523-6
62. Christiansen SC, Cannegieter SC, Koster T, et al. Thrombophilia, clinical factors, and recurrent venous thrombotic events. J Am Med Assoc 2005;293(19):2352-61
63. Lijfering WM, Middeldorp S, Veeger NJ, et al. Risk of recurrent venous thrombosis in homozygous carriers, and double heterozygous carriers of factor V Leiden and prothrombin G20210A. Circulation 2010; 121(15):1706-12
64. Ho WK, Hankey GJ, Quinlan DJ, Eikelboom JW. Risk of recurrent venous thromboembolism in patients with common thrombophilia: a systematic review. Arch Intern Med 2006;166(7):729-36
65. Marchiori A, Mosena L, Prins MH, et al. The risk of recurrent venous thromboembolism among heterozygous carriers of factor V Leiden or prothrombin G20210A mutation. a systematic review of prospective studies. Haematologica 2007; 92(8(1107):1114
66. Segal JB, Brotman DJ, Necochea AJ, et al. Predictive value of factor V Leiden and prothrombin G20210A in adults with venous thromboembolism and in family members of those with a mutation: a systematic review. J Am Med Assoc 2009; 301(23):2472-85
67. Vossen CY, Walker ID, Svensson P, et al. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005;25(9):1992-7
68. Brouwer JL, Lijfering LM, Ten Kate MI, et al. High long term absolute risk of recurrent venous thromboembolism in patients with hereditary deficiencies of protein S, protein C, or antithrombin. Thromb Haemost 2009;101(1):93-9
69. Rossi E, Ciminello A, Za T, et al. In families with inherited thrombophilia the risk of venous thromboembolism is dependent on the clinical phenotype of the proband. Thromb Haemost 2011;106(4): 646-54
70. Holzhauer S, Goldenberg NA, Junker R, et al. Inherited thrombophilia in children with venous thromboembolism and the familial risk of thromboembolism: an observational study. Blood 2012;120(7): 1510-15
71. Bezemer ID, van der Meer FJ, Eikenboom JC, et al. The value of family history as a risk indicator for venous thrombosis. Arch Intern Med 2009;169(6): 610-15
72. Bucciarelli P, De Stefano V, Passamonti SM, et al. Influence of proband's characteristics on the risk of venous thromboembolism in relatives with factor V Leiden or prothrombin G20210A polymorphisms. Blood 2013;122(15):2555-61
73. Wu O, Robertson L, Twaddle S, et al. Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study. Screening for thrombophilia in high-risk situations: a meta-analysis and cost-effectiveness analysis. Br J Haematol 2005;131(1):80-90
74. Vandenbroucke JP, van der Meer FJM, Helmerhorst FM, et al. Factor V. Leiden: should we screen oral contraceptive users and pregnant women? Br Med J 1996; 313(7065):1127-30
75. Clark P, Twaddle S, Walker ID, et al. Cost-effectiveness of screening for the factor V Leiden mutation in pregnant women. Lancet 2002;359(9321):1919-20
76. Battaglioli T, Martinelli I. Hormone therapy and thromboembolic disease. Curr Opin Hematol 2007;14(5):488-93
77. Wu O, Robertson L, Langhorne P, et al. Oral contraceptives, hormone replacement therapy, thrombophilias and risk of venous thromboembolism: a systematic review: the Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. Thromb Haemost 2005;94(1):17-25
78. Mahmoodi BK, Brouwer JL, Ten Kate MK, et al. A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening

asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin. J Thromb Haemost 2010;8(6):1193-200

79. Middeldorp S. Evidence-based approach to thrombophilic testing. J Thromb Thrombolysis 2011;31(3):275-81

80. Lussana F, Dentali F, Abbate R, et al. Italian Society for Haemostasis and Thrombosis. Screening for thrombophilia and antithrombotic prophylaxis in pregnancy: guidelines of the Italian Society for Haemostasis and Thrombosis (SISET). Thromb Res 2009;124(5):e19-25

81. Lockwood C, Wendel G; for the Committee on Practice Bulletins–Obstetrics. Practice bulletin no. 124: inherited thrombophilias in pregnancy. Obstet Gynecol 2011;118(3):730-40