J Thromb Thrombolysis (2011) 31:275–281
DOI 10.1007/s11239-011-0572-y

Evidence-based approach to thrombophilia testing

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Published online: 23 February 2011

Abstract Thrombophilia can be identified in about half of all patients presenting with VTE. Testing has increased tremendously for various indications, but whether the results of such tests help in the clinical management of patients has not been settled. I use evidence from observational studies to conclude that testing for hereditary thrombophilia generally does not alter the clinical management of patients with VTE, with occasional exceptions for women at fertile age. Because testing for thrombophilia only serves limited purpose this should not be performed on a routine basis.

Keywords Thrombophilia · Venous thrombosis · Pulmonary embolism

Over seventy years ago, Nygaard and Brown [1] used the term thrombophilia to describe patients with an unexplained tendency for, mainly arterial, thrombotic events. Egeberg [2] used the term in 1965, when he described a Norwegian family that had a remarkable tendency to venous thromboembolism (VTE), and discovered that this was based on an inherited deficiency of antithrombin. In the 80s of the last century, inherited deficiencies of protein C and protein S were described [3, 4]. Since then various laboratory abnormalities, both hereditary and acquired, have been discovered that increase the risk of VTE. The most prevalent and relatively strong genetic risk factors are gain-of-function mutations, i.e. factor V Leiden that causes resistance to protein C (activated protein C, APC resistance) [5–7], and the prothrombin 20210A mutation that leads to higher levels of prothrombin [8]. Nowadays, inherited thrombophilia can be identified in about half of all patients presenting with VTE, and appears to provide at least a partial explanation for a previously poorly explained disease [9]. Over the past decades, testing has increased tremendously for various indications [10], but whether the results of such tests help in the clinical management of patients has not been settled [11, 12]. Here, I present an evidence-based approach for the most commonly tested inherited thrombophilias, i.e., deficiencies of antithrombin, protein C, or protein S, and the factor V Leiden and prothrombin G20210A mutations. In such an approach, more important than the strengths of their associations with VTE and its inherent absolute risks, is how testing for thrombophilia is able to reduce these risks.

Mechanisms of thrombophilia

The currently established inherited thrombophilias impact either the procoagulant or anticoagulant pathways. A simplified scheme of coagulation and fibrinolysis is depicted in the Fig. 1. Antithrombin, protein C and protein S are key players in the regulation of coagulation, hence they are referred to as natural anticoagulants. Antithrombin downregulates thrombin, and activated protein C, together with its cofactor protein S, inactivates factors Va and VIIIa. Factor V Leiden refers to a point mutation in the gene at position 1691, leading to a replacement of arginine (Arg) by glutamine (Gln) at amino acid position 506 of the factor Va protein, making it less susceptible for cleavage by activated protein C at this important site. The prothrombin 20210A mutation leads to a normal protein, but at higher levels.

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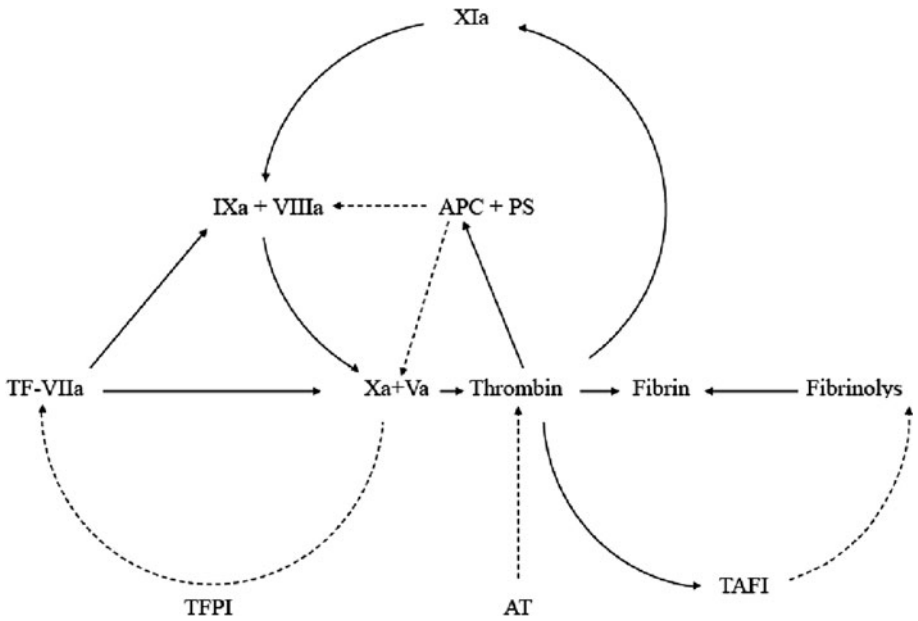

Fig. 1 Blood Coagulation and Fibrinolysis Simplified scheme of coagulation and fibrinolysis. Coagulation is initiated by a tissue factor (*TF*)—factor VIIa complex that can activate factor IX or factor X, leading to formation of the key enzyme thrombin (*factor IIa*). Tissue factor–dependent coagulation is rapidly inhibited by tissue factor–pathway inhibitor (*TFPI*). Coagulation is maintained through the activation by thrombin of factor XI. Through the intrinsic tenase complex (*factors IXa* and *VIIIa*) and the prothrombinase complex (*factors Xa* and *Va*), the additional thrombin required to down-regulate fibrinolysis is generated by the activation of thrombin-activatable fibrinolysis inhibitor (*TAFI*). The coagulation system is regulated by the protein C pathway. Thrombin activates protein C in the presence of thrombomodulin. Together with protein S (*PS*), activated protein C (*APC*) is capable of inactivating factors Va and VIIIa, which results in a down-regulation of thrombin generation and consequently in an up-regulation of the fibrinolytic system. The activity of thrombin is controlled by the inhibitor antithrombin (*AT*). The solid arrows indicate activation and the broken arrows inhibition

Thrombophilia testing for patients with venous thromboembolism

Thrombophilia testing is most often considered in patients with VTE, particularly if they are young, have recurrent episodes, have thrombosis at unusual sites, or have a positive family history for the disease. However, although such a strategy may lead to an increased yield of testing, the main question is whether a positive test result alters management. In general, VTE has a tendency to recur, with a cumulative incidence of a second episode of approximately 30% in 8 years [13]. Patients with a well recognized and transient clinical risk factor such as surgery, eliciting a first VTE have a very low risk of recurrence [13–15]. However, whether the presence of thrombophilia is able to predict recurrence is much less clear, with conflicting results in various studies that compared the prevalences of thrombophilia in patients with recurrent VTE with those in patients without recurrence [12]. A randomized controlled trial in which testing for thrombophilia in patients with a first episode of VTE is the intervention, and recurrent VTE is the outcome, would be ideal. Testing should then lead to a predefined strategy to prevent recurrence, with for instance a longer or indefinite duration of anticoagulant therapy. To my knowledge, no such trials have been successfully performed [11, 16].

Several follow-up studies have assessed the risk of recurrent VTE associated with thrombophilic defects. In a cohort of patients from Cambridge, those with a thrombophilic defect, including antithrombin, protein C, or protein S deficiency (HRs ranging from 1.0 to 2.9 for individual deficiencies, which were almost all combined with other thrombophilic defects), or the factor V Leiden mutation (HR 1.4, 95% CI 0.7–2.8), or the prothrombin 20210A mutation (HR 1.7, 95% CI 0.5–5.6), were not at highly increased risk of developing a recurrent venous thrombotic event [14]. In the Leiden Thrombophilia (LETS) study, no clearly increased risk of recurrent VTE was found for the prothrombotic mutations: factor V Leiden (HR 1.3, 95% CI 0.8–2.1), or prothrombin 20210A mutation (HR 0.7, 95% CI 0.3–2.0) [15]. A mildly increased risk of recurrent VTE was found in patients with a deficiency in one of the anticoagulants protein C, protein S, or antithrombin (HR 1.8; 95% CI 0.9–3.7). An Italian cohort study reported a similar risk increase of recurrence associated with deficiencies of the anticoagulants (antithrombin deficiency: HR 1.9, 95% CI 1.0–3.9, protein C or S deficiency: HR 1.4, 95% CI 0.9–2.2) [17]. In a large pooled study of

thrombophilic families, we observed a cumulative incidence of VTE recurrences after 10 years of 55% in relatives with a deficiency of antithrombin, protein C or protein S deficiency, as compared to 25% in those with the factor V Leiden mutation, the prothrombin mutation or high levels of FVIIIa [18].

In order to investigate whether testing for thrombophilia reduces the risk of recurrent VTE in patients with a first episode of thrombosis, for instance by management alterations (such as prolonged use of anticoagulations, avoidance of or intensified prophylaxis in high risk situations) we selected 197 patients from the MEGA case control study who had had a recurrent event during follow-up [19]. We compared the proportion of these patients who had been tested with the proportion of 324 patients who did not have a recurrence during follow-up, matched for age, sex, year of event and geographical region. Thrombophilia tests were performed in 35% of cases and in 30% of controls. The OR for recurrence was 1.2 (95% CI 0.9–1.8) for tested versus non-tested patients, indicating that testing does not reduce the risk of recurrent VTE in patients who have experienced a first episode.

In conclusion, observational studies show that patients who have had VTE and have hereditary thrombophilia, are at most at a slightly increased risk for recurrence. Furthermore, in real life, no beneficial effect on the risk of recurrent VTE was observed in patients who had been tested for thrombophilia. In general, after a first episode of venous thrombosis, 3–6 months of anticoagulant therapy is considered to have the optimal balance between the risk of treatment (bleeding) and the benefit (prevention of an extension or recurrence of venous thrombosis) [20]. In the absence of trials that compared routine and prolonged anticoagulant treatment in patients testing positive for thrombophilia, prolonged anticoagulant therapy cannot be justified as it may cause more harm than benefit.

Testing for thrombophilia to modify the risk of a first deep venous thrombosis

The risk of VTE in individuals with thrombophilia is, by definition, increased as compared to the general population. In clinical practice, requests to estimate the risk of a first episode of VTE are most often done by asymptomatic individuals with a family history of VTE, with or without a known specific thrombophilic defect. It is important to realize that having a family history of VTE increases the risk of VTE in a first degree relative by approximately two-fold, regardless of the presence of a known hereditary thrombophilic defect [21]. Still, a potential advantage of testing patients with VTE for thrombophilia may be the identification of asymptomatic family members of thrombophilic patients in order to take preventive measures if tested positive.The risks of a first episode of VTE have been assessed in several family studies with similar design and are summarized in Table 1.

Although antithrombin deficiency has historically been regarded to be the most severe thrombophilic defect leading to a very high risk of thrombosis, in a large population based case–control study antithrombin deficiency (measured as plasma levels <80 U/dl on two occasions) was associated with a fivefold (95% CI 0.7–34) increased risk of a first deep venous thrombotic event.(22) Retrospective and prospective studies in families with at least one proband with VTE and antithrombin deficiency found an incidence of first VTE between 0.4% and 1.7% per year in deficient relatives [23–27]. Also for the other hereditary thrombophilias, the overall absolute risk of VTE in asymptomatic relatives has been assessed in numerous studies and is generally low, even during high risk situations such as pregnancy, the postpartum period, surgery, immobilisation, trauma and during the use of oral contraceptives [23, 27–37] (Table 1). Noteworthy, the risk estimates related to surgery, trauma and immobilisation as

Table 1 Estimated incidence of a first episode of venous thrombosis in carriers of various thrombophilic defects

	Antithrombin, protein C, or protein S deficiency [18, 23, 24, 26, 27]	Factor V Leiden, heterozygous [23, 27–29]	Prothrombin 20210A mutation [31, 32]	Factor V Leiden, homozygous [30, 35–37]
Overall (%/year, 95% CI)	1.5 (0.7–2.8)	0.5 (0.1–1.3)	0.4 (0.1–1.1)	1.8 (0.1–4.0)[a]
Surgery, trauma, or immobilization (%/episode, 95% CI)	8.1 (4.5–13.2)	1.8 (0.7–4.0)	1.6 (0.5–3.8)	–
Pregnancy (%/pregnancy, 95% CI)	4.1 (1.7–8.3)	2.1 (0.7–4.9)	2.3 (0.8–5.3)	16.3[b]
During pregnancy, %, 95% CI	1.2 (0.3–4.2)	0.4 (0.1–2.4)	0.5 (0.1–2.6)	7.0[b]
Postpartum period, %, 95% CI	3.0 (1.3–6.7)	1.7 (0.7–4.3)	1.9 (0.7–4.7)	9.3[b]
Oral contraceptive use (%/year of use, 95% CI)	4.3 (1.4–9.7)	0.5 (0.1–1.4)	0.2 (0.0–0.9)	–

Data apply to individuals with at least one symptomatic first-degree relative

[a] Based on pooled OR of 18 [8–40] and an incidence of 0.1% in non-carriers

[b] Data from family studies, risk estimates lower in other settings

shown in this table for a large part reflect the situation before standard prophylaxis was routine patient care.

It is clear that the 2% annual major bleeding risk associated with continuous anticoagulant treatment outweighs the risk of venous thrombosis [38, 39].

For women at fertile age who wish to use oral contraceptives, or have an increased risk of VTE during and after pregnancy, Tables 2, 3 indicate the effect of either avoidance of these risk factors (for oral contraceptives), or taking preventing measures during and/or after pregnancy. The risk of additional venous thrombotic events induced by the use of oral contraceptive is outlined in Table 2, and can be applied to women who have a positive first degree relative with VTE who has a known thrombophilic defect. The values clearly indicate that women with antithrombin, protein C or protein S deficiency have a high risk of VTE provoked by use of oral contraceptives, and that it may be worthwhile to test women from these families to advise those with a deficiency to avoid use of the contraceptive pill. However, it should be noted that women without a deficiency still have a markedly increased risk of oral contraceptive related VTE compared to the general population (0.7 vs. 0.03% per year of use), probably because of co-segregation of yet unknown thrombophilias. Thus, a negative thrombophilia test may lead to false reassurance. These risk estimates are very different for the more common thrombophilias such as factor V Leiden, with a large number of women needing to avoid use of oral contraceptives to avoid 1 VTE, and a number needed to test of 666. Table 3 indicates the number needed to test to install prophylactic measurements around pregnancy, again applicable to women from thrombophilic families. Only for women with antithrombin, protein C or protein S deficiency, or those who are homozygous for factor V Leiden, the risks during pregnancy may outweigh the nuisance of daily subcutaneous low-molecular-weight heparin injections and skin reactions, and the very small risk for severe complications of anticoagulant therapy during pregnancy [40–42]. Whether the 80% of pregnancy-related episodes occurring in the 6–12 weeks postpartum justifies prophylaxis during this period is a matter of the physicians' and patients' preference in which the values from Table 3 can be useful. It is noteworthy that in these family studies the risk of pregnancy-related VTE in women who do not have the thrombophilic defect is approximately 0.5%, a value in the same range as in the general population [43]. Hence, withholding prophylaxis to women from thrombophilic families who do not have the defect is supported by evidence.

In conclusion, only in limited situations thrombophilia testing in asymptomatic relatives may be useful. This seems particularly the case in families with antithrombin, protein C or protein S deficiency, or homozygosity for factor V Leiden, and appears limited to women who intend to become pregnant and perhaps, in individual cases of women who would like to use oral contraceptives. In general, appropriate counselling with knowledge of absolute risks should help in making an informed decision in which patient's preferences can be taken into account.

Thrombophilia testing for other clinical indications

Although numerous studies have investigated the association between thrombophilia and arterial cardiovascular diseases, positive and negative studies are equally available [44]. There is no evidence that the presence of inherited thrombophilia should lead to different secondary prevention, and testing in this clinical setting is not justified.

Table 2 Estimated number of asymptomatic thrombophilic women who should avoid using oral contraceptives to prevent one VTE, and estimated number needed to test

Thrombophilia	Risk on OC per year (%)	Risk difference per 100 women	N not taking OC to prevent 1 VT	N of female relatives to be tested
Antithrombin, protein C, or protein S deficiency				
Deficient relatives	4.3[a]	3.6	28	56
Non-deficient relatives	0.7[a]			
Factor V Leiden or prothrombin mutation				
Relatives with the mutation	0.5[a]	0.3	333	666
Relatives without the mutation	0.2[a]			
Family history of VTE				
General population, no family history	0.03[b]	0.02	5000	None
General population, positive family history	0.06[b]	0.04	2500	None

[a] Based on family studies as outlined in Table 1

[b] Based on a population baseline risk of VTE in young women of 0.01% per year [64], a relative risk of VTE by use oral contraceptives of three [65], and a relative risk of two of VTE by having a positive family history [21]

Table 3 Estimated number of asymptomatic thrombophilic women who should use LMWH prophylaxis during pregnancy and/or the postpartum period to prevent pregnancy-related VTE, and estimated number needed to test

Thrombophilia	N of female relatives to be tested to prevent VTE during pregnancy[a]	N of female relatives to be tested to prevent VTE postpartum[a]
Antithrombin, protein C, or protein S deficiency	83	33
Factor V Leiden or prothrombin mutation, heterozygous	250	60
Factor V Leiden, homozygous	14	10

[a] Based on family studies as outlined in Table 1

These estimates apply to women with a positive family history of VTE and assume a 100% efficacy of prophylaxis with LMWH

Inherited thrombophilia has also been implicated in pregnancy complications, such as recurrent miscarriage and fetal death, analogous to the clinical manifestations that are part of the antiphospholipid syndrome [45]. The association between hereditary thrombophilia varies depending on type of thrombophilia and timing of pregnancy failure [46], and there is a modest association also between thrombophilia and other adverse pregnancy outcomes, most notably preeclampsia and intra-uterine growth retardation [47]. However, whether this association can be considered causal remains controversial, as many other factors play a role in the risk of pregnancy complications [48–51]. Therapeutic options to prevent pregnancy complications in women with thrombophilia comprise aspirin as well as (low-molecular-weight) heparin. For women with recurrent pregnancy loss, there is no evidence supporting treatment [50, 52]. Observational research is hampered by severe methodological flaws or inconsistent results. Two randomized trials have not used an adequate comparator, i.e. no treatment or placebo [53, 54]. Two recent randomized controlled trials in women with unexplained recurrent miscarriage, the ALIFE study from The Netherlands and the SPIN study from Scotland, were unable to demonstrate a beneficial effect of anticoagulant therapy, compared to no pharmacological treatment or placebo [55, 56]. Although the ALIFE study was underpowered for subgroup analyses, an a priori planned analysis in women with inherited thrombophilia showed a relative risk for live birth of 1.31 (95% CI 0.74–2.33) for the combined intervention compared to placebo, and 1.22 (95% CI 0.69–2.16) for aspirin, with corresponding absolute difference in live birth rates of 16.3% (95% CI −18.2 to 50.8) and 11.8% (95%CI −21.1 to 44.6) respectively [55]. The possibility that one or both of these interventions might be beneficial in such women warrants further study in adequately powered, controlled trials. With the current evidence, using anticoagulant therapy to improve the prognosis of a pregnancy in women with pregnancy complications should be considered experimental.

Drawbacks of thrombophilia testing

Disadvantages of testing patients with a venous thrombosis for thrombophilia might be the high costs of testing [57]. Although some cost-effectiveness studies have been published on the topic of testing for thrombophilia, which concluded that in some scenarios testing could indeed be cost-effective, the number of assumptions from inconsistent observational studies seriously hamper their interpretation [58, 59]. The psychological impact and consequences of knowing to be a carrier of a (genetic) thrombophilic defect is a potential drawback of testing [60]. Most studies that focused on impact of testing for thrombophilia showed that patients had experienced low psychological distress following thrombophilia testing [61, 62]. Nevertheless, a qualitative study described several negative effects of both psychological and social origin [63]. Finally, difficulties in obtaining life or disability insurances are frequently encountered by individuals who are known carriers of thrombophilia, regardless whether they are symptomatic or asymptomatic [63].

Conclusions

Despite the increasing knowledge about the etiology of venous thrombosis, testing for hereditary thrombophilia generally does not alter the clinical management of patients with VTE. There are a few exceptions. For some asymptomatic women at fertile age who come from families with a tendency for VTE and a known thrombophilic defect, a positive test may lead to the decision to install prophylaxis during or postpartum in case of pregnancy, or the individual decision to not use oral contraceptives.

In conclusion, because testing for hereditary thrombophilia does not affect clinical management of most patients with VTE, it only serves limited purpose and should not be performed on a routine basis.

References

1. Nygaard KK, Brown GE (1937) Essential thrombophilia: report of five cases. Arch Intern Med 59(1):82–106
2. Egeberg O (1965) Inherited antithrombin III deficiency causing thrombophilia. Thromb Diath Haemorrh 13:516–530
3. Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C (1981) Deficiency of protein C in congenital thrombotic disease. J Clin Invest 68(5):1370–1373
4. Comp PC, Esmon CT (1984) Recurrent venous thromboembolism in patients with a partial deficiency of protein S. N Eng J Med 311(24):1525–1528
5. Dahlback B, Hildebrand B (1994) Inherited resistance to activated protein C is corrected by anticoagulant cofactor activity found to be a property of factor V. Proc Natl Acad Sci USA 91(4):1396–1400
6. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H et al (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369(6475):64–67
7. Voorberg J, Roelse J, Koopman R, Buller HR, Berends F, ten Cate JW et al (1994) Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V. Lancet 343(8912):1535–1536
8. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) 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 88(10):3698–3703
9. Middeldorp S, Levi M (2007) Thrombophilia: an update. Semin Thromb Hemost 33(6):563–572
10. Coppens M, van Mourik JA, Eckmann CM, Buller HR, Middeldorp S (2007) Current practice of testing for hereditary thrombophilia in The Netherlands. J Thromb Haemost 5:1979–1981
11. Cohn DM, Vansenne F, de Borgie CA, Middeldorp S (2009) Thrombophilia testing for prevention of recurrent venous thromboembolism. Cochrane Database Syst Rev (1):CD007069
12. Middeldorp S, van Hylckama Vlieg A (2008) Does thrombophilia testing help in the clinical management of patients? Br J Haematol 143:321–335
13. Prandoni P, Lensing AW, Cogo A, Cuppini S, Villalta S, Carta M et al (1996) The long-term clinical course of acute deep venous thrombosis. Ann Int Med 125(1):1–7
14. Baglin T, Luddington R, Brown K, Baglin C (2003) Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet 362:523–526
15. Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR (2005) Thrombophilia, clinical factors, and recurrent venous thrombotic events. JAMA 293(19):2352–2361
16. Cohn DM, Middeldorp S (2008) Vroegtijdige beeindiging van het onderzoek naar het nut van trombofilietests bij een eerste veneuze trombo-embolie: het NOSTRADAMUS-onderzoek. Ned Tijdschr Geneeskd 152(38):2093
17. De Stefano V, Simioni P, Rossi E, Tormene D, Za T, Pagnan A et al (2006) The risk of recurrent venous thromboembolism in patients with inherited deficiency of natural anticoagulants antithrombin, protein C and protein S. Haematologica 91(5):695–698
18. Lijfering W, Brouwer JL, Veeger NJ, Bank I, Coppens M, Middeldorp S et al (2009) 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 113(21):5314–5322
19. Coppens M, Reijnders JH, Middeldorp S, Doggen CJ, Rosendaal FR (2008) Testing for inherited thrombophilia does not reduce recurrence of venous thrombosis. J Thromb Haemost 6:1474–1477
20. Buller HR, Agnelli G, Hull RD, Hyers TM, Prins MH, Raskob GE (2004) Antithrombotic therapy for venous thromboembolic disease. The seventh ACCP conference on antithrombotic and thrombolytic therapy. Chest 126:401S–428S
21. Bezemer ID, van der Meer FJ, Eikenboom JC, Rosendaal FR, Doggen CJ (2008) The value of family history as a risk indicator for venous thrombosis. Arch Intern Med 169(6):610–615
22. Koster T, Rosendaal FR, Briet E, van der Meer FJ, Colly LP, Trienekens PH et al (1995) Protein C deficiency in a controlled series of unselected outpatients: an infrequent but clear risk factor for venous thrombosis (Leiden Thrombophilia Study). Blood 85(10):2756–2761
23. Simioni P, Sanson BJ, Prandoni P, Tormene D, Friederich PW, Girolami B et al (1999) The incidence of venous thromboembolism in families with inherited thrombophilia. Thromb Haemost 81(2):198–202
24. Sanson BJ, Simioni P, Tormene D, Moia M, Friederich PW, Huisman MV et al (1999) The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. Blood 94:3702–3706
25. Vossen CY, Conard J, Fontcuberta J, Makris M, van der Meer FJ, Pabinger I et al (2005) Risk of a first venous thrombotic event in carriers of a familial thrombophilic defect. The European Prospective Cohort on Thrombophilia (EPCOT). J Thromb Haemost 3(3):459–464
26. Friederich PW, Sanson BJ, Simioni P, Zanardi S, Huisman MV, Kindt I et al (1996) Frequency of pregnancy-related venous thromboembolism in anticoagulant factor-deficient women: implications for prophylaxis. Ann Int Med 125:955–960
27. Bucciarelli P, Rosendaal FR, Tripodi A, Mannucci PM, De Stefano V, Palareti G et al (1999) 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 19(4):1026–1033
28. Middeldorp S, Henkens CMA, Koopman MMW, van Pampus ECM, van der Meer J, Hamulyak K et al (1998) The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. Ann Int Med 128(1):15–20
29. Middeldorp S, Meinardi JR, Koopman MMW, van Pampus ECM, Hamulyak K, van der Meer J et al (2001) A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. Ann Int Med 135(5):322–327
30. Segal JB, Brotman DJ, Necochea AJ, Emadi A, Samal L, Wilson LM et al (2009) 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. JAMA 301(23):2472–2485
31. Bank I, Libourel EJ, Middeldorp S, van Pampus ECM, Koopman MMW, Hamulyak K et al (2004) Prothrombin 20210A mutation: a mild risk factor for venous thromboembolism but not for arterial thrombotic disease and pregnancy-related complications in a family study. Arch Int Med 164(17):1932–1937
32. Coppens M, van der Poel MH, Bank I, Hamulyak K, van der Meer J, Veeger NJ et al (2006) A prospective cohort study on the absolute incidence of venous thromboembolism and arterial

cardiovascular disease in asymptomatic carriers of the prothrombin 20210A mutation. Blood 108:2604–2607
33. Martinelli I, Bucciarelli P, Margaglione M, De Stefano V, Castaman G, Mannucci PM (2000) The risk of venous thromboembolism in family members with mutations in the genes of factor V or prothrombin or both. Br J Haematol 111(4):1223–1229
34. Martinelli I, Legnani C, Bucciarelli P, Grandone E, De Stefano V, Mannucci PM (2001) Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia. Thromb Haemost 86(3):800–803
35. Middeldorp S, Libourel EJ, Hamulyak K, van der MJ, Buller HR (2001) The risk of pregnancy-related venous thromboembolism in women who are homozygous for factor V Leiden. Br J Haematol 113(2):553–555
36. Middeldorp S, van der Meer J, Hamulyak K, Buller HR (2002) Counseling pregnant women with factor V Leiden homozygosity: use absolute instead of relative risks. Thromb Haemost 87(2):360–361
37. Martinelli I, De Stefano V, Taioli E, Paciaroni K, Rossi E, Mannucci PM (2002) Inherited thrombophilia and first venous thromboembolism during pregnancy and puerperium. Thromb Haemost 87(5):791–795
38. van der Meer FJ, Rosendaal FR, Vandenbroucke JP, Briet E (1996) Assessment of a bleeding risk index in two cohorts of patients treated with oral anticoagulants. Thromb Haemost 76(1):12–16
39. Palareti G, Leali N, Coccheri S, Poggi M, anotti C, D'Angelo A et al (1996) Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISCOAT) Italian Study on Complications of Oral Anticoagulant Therapy. Lancet 348:423–428
40. Bank I, Libourel EJ, Middeldorp S, van der Meer J, Buller HR (2003) High rate of skin complications due to low-molecular-weight heparins in pregnant women. J Thromb Haemost 1(4):859–861
41. Deruelle P, Denervaud M, Hachulla E, Ducloy-Bouthors AS, Valat AS, Puech F et al (2006) Use of low-molecular-weight heparin from the first trimester of pregnancy: a retrospective study of 111 consecutive pregnancies. Eur J Obstet Gynecol Reprod Biol 127(1):73–78
42. Bates SM, Greer IA, Pabinger I, Sofaer S, Hirsh J (2008) Venous thromboembolism, thrombophilia, antithrombotic therapy, and pregnancy: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 133(6 Suppl):844S–886S
43. Heit JA, Kobbervig CE, James AH, Petterson TM, Bailey KR, Melton LJ III (2005) Trends in the incidence of venous thromboembolism during pregnancy or postpartum: a 30-year population-based study. Ann Intern Med 143(10):697–706
44. Boekholdt SM, Kramer MH (2007) Arterial thrombosis and the role of thrombophilia. Semin Thromb Hemost 33(6):588–596
45. Opatrny L, David M, Kahn SR, Shrier I, Rey E (2006) Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a metaanalysis. J Rheumatol 33(11):2214–2221
46. Rey E, Kahn SR, David M, Shrier I (2003) Thrombophilic disorders and fetal loss: a meta-analysis. Lancet 361:901–908
47. Robertson L, Wu O, Langhorne P, Twaddle S, Clark P, Lowe GDO et al (2006) Thrombophilia in pregnancy: a systematic review. Br J Haematol 132(2):171–196
48. Middeldorp S (2007) Thrombophilia and pregnancy complications: cause or association? J Thromb Haemost 5(Suppl. 1): 276–282
49. Middeldorp S (2007) Pregnancy failure and heritable thrombophilia. Semin Hematol 44(2):93–97
50. Rodger MA, Paidas MJ, Mclintock C, Middeldorp S, Kahn SR, Martinelli I et al (2008) Inherited thrombophilia and pregnancy complications revisited: association not proven causal and antithrombotic prophylaxis is experimental. Obstet Gynecol 112(2):320–324
51. Branch DW, Gibson M, Silver RM (2010) Clinical practice recurrent miscarriage. N Engl J Med 363(18):1740–1747
52. Kaandorp SP, Di Nisio M, Goddijn M, Middeldorp S (2009) Aspirin or anticoagulants for treating recurrent miscarriage in women without antiphospholipid syndrome. Cochrane Database Syst Rev (1):CD004734
53. Gris JC, Mercier E, Quere I, Lavigne-Lissalde G, Cochery-Nouvellon E, Hoffet M et al (2004) Low-molecular-weight heparin versus low-dose aspirin in women with one fetal loss and a constitutional thrombophilic disorder. Blood 103:3695–3699
54. Brenner B, Hoffman R, Carp H, Dulitzky M, Younis J, for the LIVE-ENOX Investigators (2005) Efficacy and safety of two doses of enoxaparin in women with thrombophilia and recurrent pregnancy loss: the LIVE-ENOX study. J Thromb Haemost 3(2):227–229
55. Kaandorp SP, Goddijn M, van der Post JA, Hutten BA, Verhoeve HR, Hamulyak K et al (2010) Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med 362(17):1586–1596
56. Clark P, Walker ID, Langhorne P, Crichton L, Thomson A, Greaves M et al (2010) SPIN: the Scottish Pregnancy Intervention Study: a multicentre randomised controlled trial of low molecular weight heparin and low dose aspirin in women with recurrent miscarriage. Blood 115(21):4162–4167
57. Machin SJ (2003) Pros and cons of thrombophilia testing: cons. J Thromb Haemost 1(3):412–413
58. Marchetti M, Pistorio A, Barosi G (2000) Extended anticoagulation for prevention of recurrent venous thromboembolism in carriers of factor V Leiden: cost-effectiveness analysis. Thromb Haemost 84:752–757
59. Wu O, Robertson L, Twaddle S, Lowe GD, Clark P, Greaves M et al (2006) Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. Health Technol Assess 10(11): 1–110
60. Cohn DM, Vansenne F, Kaptein AA, de Borgie CA, Middeldorp S (2008) The psychological impact of testing for thrombophilia: a systematic review. J Thromb Haemost 6:1099–1104
61. van Korlaar I, Vossen CY, Rosendaal FR, Bovill EG, Naud S, Cameron LD et al (2005) Attitudes toward genetic testing for thrombophilia in asymptomatic members of a large family with heritable protein C deficiency. J Thromb Haemost 3(11):2437–2444
62. Legnani C, Razzaboni E, Gremigni P, Ricci Bitti PE, Favaretto E, Palareti G (2006) Psychological impact of testing for thrombophilic alterations. Thromb Haemost 96(3):348–355
63. Bank I, Scavenius MPRB, Buller HR, Middeldorp S (2004) Social aspects of genetic testing for factor V Leiden mutation in healthy individuals and their importance for daily practice. Thromb Res 113:7–12
64. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J (2007) Incidence and mortality of venous thrombosis: a population-based study. J Thromb Haemost 5(4):692–699
65. Vandenbroucke JP, Rosing J, Bloemenkamp KWM, Middeldorp S, Helmerhorst FM, Bouma BN et al (2001) Oral contraceptives and the risk of venous thrombosis. N Eng J Med 344(20): 1527–1535