bjh review

Does thrombophilia testing help in the clinical management of patients?

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Summary

Thrombophilia can be identified in about half of all patients presenting with venous thrombosis. 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. Here, we review the most commonly tested thrombophilic abnormalities, i.e. protein C, protein S, and antithrombin deficiencies, the *F5* R506Q (factor V Leiden) and *F2* G20210A (prothrombin G20210A) mutations, and elevated levels of coagulation factor VIII, and their association with venous and arterial thrombosis as well as pregnancy complications. We conclude that testing for hereditary thrombophilia generally does not alter the clinical management of patients with venous or arterial thrombosis or pregnancy complications. Because testing for thrombophilia only serves limited purpose this should not be performed on a routine basis.

Keywords: thrombophilia, venous thrombosis, pulmonary embolism, cardiovascular diseases, pregnancy complications.

Thrombophilia can be identified in about half of all patients presenting with venous thrombosis, and appears to provide at least a partial explanation for a previously poorly explained disease (Weitz *et al*, 2004). Over the past decades, testing has increased tremendously for various indications (Coppens *et al*, 2007), but whether the results of such tests aid the clinical management of patients has not been settled. Here, we review the most commonly tested thrombophilic abnormalities, i.e. protein C, protein S, and antithrombin deficiencies, *F5* R506Q (factor V Leiden) and *F2* G20210A (prothrombin 20210A), and elevated levels of coagulation factor VIII, and their associations with venous and arterial thrombosis. Furthermore, we discuss whether and how this might help in the clinical management of patients.

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Definition of thrombophilia

Virchow (1856) proposed changes in the blood coagulability as one of the mechanisms that predispose to thrombosis. These changes in the blood coagulability, i.e. thrombophilia, indicate the presence of a hypercoagulable state leading to a thrombotic tendency. Important risk factors for thrombotic disease were mainly identified in studies comprising of families with a high incidence of thrombotic disease. However, acquired factors may also lead to a thrombotic tendency. Thrombophilia may therefore be defined as both an acquired or congenital abnormality of haemostasis predisposing to thrombosis (Table I). Several well-known factors predisposing to thrombophilia are described in more detail below.

Antithrombin deficiency

Antithrombin is an important natural anticoagulant of the coagulation system and inhibits the coagulation factors IIa, IXa, Xa, XIa, and XIIa. The inhibition, which occurs by the formation of covalent complexes, is accelerated 1000-fold by heparin. Homozygosity for antithrombin deficiency is very rare. Two types of antithrombin deficiency are defined (Lane *et al*, 1993, 1997). Type I, i.e. the classical and most common deficiency, is a quantitative deficiency with antithrombin plasma levels below 50% of normal. In type II antithrombin deficiency, the plasma levels are within the normal range but functional activity is impaired due to the production of a variant protein. Currently more than 80 different mutations are known in the antithrombin gene (*SERPINC1*, previously known as *AT3*) (Lane *et al*, 1997). The prevalence of antithrombin deficiency in the general population is very low and estimated to be approximately 0·02% in the general Caucasian population (Tait *et al*, 1994).

Protein C deficiency

Protein C is a vitamin K-dependent glycoprotein that is synthesized in an inactive form in the liver. Upon activation, activated protein C (APC) is an important natural anticoagulant that, together with its co-factor protein S, suppresses

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Table I. Prevalence of thrombophilia and relative risk estimates for various clinical manifestations.

	Antithrombin deficiency	Protein C deficiency	Protein S deficiency	*F5* R506Q mutation	*F2* G20210A mutation	Elevated levels of FVIII*	Lupus anticoagulant*	Anticardiolipin antibodies*	Anti-β2 GPI antibodies	Anti-prothrombin antibodies
Prevalence in the general population	0·02%	0·2%	0·03–0·13%	3–7%	0·7–4%	By definition chosen cut-off level	1–8%	5	3·4	14·6
Relative risk for a first venous thrombosis	5–10	4–6·5	1–10	3–5	2–3	5	3–10	0·7	2·4	1·4
Relative risk for recurrent venous thrombosis	1·9–2·6	1·4–1·8	1·0–1·4	1·4	1·4	1·3–6·7	2–6	1–6	–	–
Relative risk for arterial thrombosis	No association	No consistent association	No consistent association	1·3	0·9	3·1	10	1·5–10	–	–
Relative risk for pregnancy complications	1·3–3·6	1·3–3·6	1·3–3·6	1·0–2·6	0·9–1·3	1·1–1·2	No consistent data	No consistent data		

*In most studies, presence of these thrombophilic risk factors were only assessed once.
References are listed in the main text.
GPI, glycoprotein.

thrombin generation by inhibition of coagulation factors Va and VIIIa (Esmon, 2000).

Protein C is activated by thrombin bound to thrombomodulin on cell surfaces. Binding to the endothelial cell protein C receptor (EPCR), a type I transmembrane protein that is highly expressed on the endothelium of large blood vessels, enhances protein C activation more than 10-fold (Stearns-Kurosawa *et al*, 1996; Taylor *et al*, 2001). After being released from EPCR, APC is fully activated.

A deficiency in protein C is determined by measurement of plasma levels of protein C activity and antigen. There are two types of protein C deficiency. Type I deficiency is associated with a reduction in protein C activity as well as antigen levels (quantitative protein C deficiency). This is the most common type of protein C deficiency and is most likely to be the result of a reduced synthesis or stability of protein C. In type II deficiency the protein C activity is more reduced than the antigen levels due to the synthesis of abnormal protein C molecules (qualitative protein C deficiency).

Protein C deficiency can be caused by numerous loss-of-function mutations in the protein C gene (*PROC*). More than 160 mutations in *PROC* are currently known to cause protein C deficiency, indicating that it is a very heterogeneous disorder (Reitsma *et al*, 1995). Protein C deficiency is very rare with a prevalence of approximately 0·2% in the general population (Miletich *et al*, 1987; Koster *et al*, 1995a).

Protein S deficiency

Protein S is the co-factor of protein C in the inactivation of factors Va and VIIIa. It circulates in a free (∼40%) form or bound to the acute phase C4b-binding protein (∼60%). Bound protein S has no protein C co-factor activity (Dahlback, 1986). Additional to the co-factor activity of protein S, it can inhibit the prothrombinase and tenase complexes independently (Hackeng *et al*, 1994). Three subtypes of protein S deficiency have been described. In type I deficiency, both the levels of total and free protein S are decreased. In type II, the co-factor activity of protein S is decreased but with normal levels of total and free protein S antigen levels. In type III deficiency (free protein S deficiency), free protein S levels are decreased but with normal levels of total protein S. Thus, type I and III are quantitative protein S deficiencies, whereas type II is a qualitative deficiency. Type II deficiency is very rare and difficult to diagnose. Also for protein S deficiency over 130 genetic mutations in the gene encoding protein S (*PROS1*) have been described (Gandrille *et al*, 1997, 2000). The prevalence of protein S deficiency is very low, ranging from 0·03% to 0·13% in the general Caucasian population (Dykes *et al*, 2001).

For all three natural anticoagulant factors environmental factors may lead to an acquired deficiency. Severe liver disease compromises its capacity to synthesize proteins, and will subsequently reduce the levels of many coagulation factors including antithrombin, protein C, and protein S. Severe

vitamin K deficiency, most often intentionally induced by the use of vitamin K antagonists, leads to acquired deficiencies of protein C and protein S (and other vitamin K-dependent coagulation factors, i.e. II, VII, IX and X), a combination that is not expected to be of genetic origin given the rarity of both hereditary deficiencies. A decrease in protein S activity is caused by estrogen excess, such as during pregnancy or use of oral contraceptives (Tans *et al*, 2000).

F5 *R506Q – APC resistance*

In contrast with the loss-of-function mutations in *PROC*, *PROS1* and *SERPINC1*, the gain-of-function mutations in the procoagulant factors of the coagulation system are more homogeneous and more prevalent in the general population.

In 1993, Dahlbäck *et al* reported that a poor anticoagulant response to activated protein C (APC) was associated with the risk of thrombosis (Dahlbäck *et al*, 1993). The so-called APC resistance is mainly caused by a mutation in the Arg506 cleavage site of factor Va (Bertina *et al*, 1994; Voorberg *et al*, 1994). The activated mutant factor V (*F5* R506Q), commonly called factor V Leiden, is inactivated more slowly by activated protein C than wildtype factor Va (Heeb *et al*, 1995; Kalafatis *et al*, 1995; Nicolaes *et al*, 1995; Aparicio & Dahlbäck, 1996). The *F5* R506Q mutation is the most prevalent thrombotic risk factor known in the Caucasian population, i.e. 3–7% carry the mutation, but it is very rare in native African and Asian populations (Rees, 1996).

Resistance to APC, although in the majority of cases caused by the *F5* R506Q mutation, can also occur in the absence of this mutation. This APC resistance may be caused by currently unidentified factors or by environmental factors. Acquired APC resistance is caused by changes in hormonal status occurring during pregnancy (Cumming *et al*, 1995), or the use of female hormones, i.e. oral contraceptives (Henkens *et al*, 1995; Olivieri *et al*, 1995; Meinardi *et al*, 1997; Rosing *et al*, 1997) or hormone replacement therapy (Curvers *et al*, 2002).

Prothrombin 20210A mutation

Poort *et al* (1996) described a mutation in the prothrombin gene (*F2*), i.e. the *F2* G20210A mutation, which is associated with an increased level of prothrombin in the circulation. Prothrombin is the precursor of the serine protease thrombin, which is a key enzyme in the blood coagulation. Although not as common as the *F5* R506Q mutation, the prevalence of the *F2* G20210A mutation is high in the general population with estimates ranging from 0·7% to 4% in Caucasian populations (Rosendaal *et al*, 1998).

Antiphospholipid syndrome

Antiphospholipid syndrome is a non-inflammatory autoimmune disease characterised by thrombosis or pregnancy complications in the presence of antiphospholipid antibodies (Urbanus *et al*, 2008). Preliminary criteria for the diagnosis of definite antiphospholipid syndrome were formulated at an international consensus meeting in 1999 and updated in 2004 (Wilson *et al*, 1999; Miyakis *et al*, 2006). Clinical criteria include having one or more clinical episode of thrombosis, one or more unexplained fetal deaths (>10 weeks of gestation), or having three or more unexplained consecutive miscarriages (<10 weeks of gestation). Laboratory criteria include lupus anticoagulant present in plasma, or medium or high titers of anticardiolipin antibody of IgG or IgM isotype in serum or plasma, or anti-β2 glycoprotein-I antibody of IgG or IgM in serum or plasma. Antiphospholipid syndrome is diagnosed if at least one of the clinical criteria and one of the laboratory criteria is met. To prevent the detection of transiently present antiphospholipid antibodies, laboratory tests should be performed twice, twelve weeks apart, and should be positive on both occasions. Since the clinical criteria as described above are prevalent in the general population, the diagnosis of antiphospholipid syndrome is largely based on laboratory tests. Since data are limited, the prevalence of persistent lupus anticoagulant or antibodies against phospholipid in the general population is not well known. Although some population-based studies have estimated the prevalence of one or more positive tests, in most studies these were only assessed once (Ginsburg *et al*, 1992; Runchey *et al*, 2002; de Groot *et al*, 2005; Naess *et al*, 2006).

High levels of factors VIII

Elevated levels of coagulation factor VIII, but also of factors IX and XI lead to a hypercoagulable state (Koster *et al*, 1995b; van Hylckama Vlieg *et al*, 2000; Meijers *et al*, 2000). Although a heritable component has been described for these clotting factors, currently no polymorphisms have been discovered that can account for such elevated levels (Kamphuisen *et al*, 2000; Vossen *et al*, 2004; Bank *et al*, 2005). In most laboratories, only factor VIII levels are included in the thrombophilia test panel.

Association between thrombophilia and a first deep venous thrombosis

Initially, interest was mainly focused on the natural anticoagulants of the coagulation system. A deficiency in one of the natural anticoagulants that lead to an increased risk of venous thrombosis, i.e. antithrombin deficiency, was initially described by Egeberg (1965). It was reported that an inheritable deficiency in antithrombin led to lowered blood levels of antithrombin, which could cause a severe tendency to thrombosis in a family with a high incidence of thrombotic diseases. Individuals with antithrombin deficiency have historically been regarded to be at a very high risk of thrombosis, particularly in females during pregnancy. However, this perception is mainly based on small studies in which highly selected thrombophilic individuals were described (Hirsh *et al*, 1989; Conard *et al*, 1990). Several studies in families with at least one proband

with venous thrombosis and antithrombin deficiency have assessed the risk of a first episode of venous thrombosis in adult antithrombin deficient relatives. The incidence of first venous thrombosis in retrospective and prospective studies ranged between 0·4% and 1·7% per year (Sanson *et al*, 1999; Simioni *et al*, 1999; Vossen *et al*, 2005). In contrast to the relative risk for thrombosis of approximately 8–10 in deficient relatives as compared to those with normal antithrombin levels, a large population based case–control study found that antithrombin deficiency (measured as plasma levels <80 U/dl on two occasions) was associated with a five-fold (95% CI 0·7–34) increased risk of a first deep venous thrombotic event (Koster *et al*, 1995a).

The first reports of an increased risk of venous thrombosis associated with protein C deficiency appeared in 1981 (Griffin *et al*, 1981; Bertina *et al*, 1982; Comp *et al*, 1984). The prevalence of protein C deficiency is 2·5–6% in patients with a first episode of deep venous thrombosis (Heijboer *et al*, 1990; Pabinger *et al*, 1992). Heterozygous protein C deficiency is associated with a 4- to 6·5-fold increased risk of venous thrombosis [odds ratio (OR) 3·8, 95% confidence interval (CI) 1·3–10 if based on repeated measurements of protein C; OR 6·5, 95% CI 1·8–24 if based on DNA diagnosis] (Koster *et al*, 1995a). Homozygous protein C deficiency results in severe thrombotic complications of the foetus or at very early age, i.e. purpura fulminans (Branson *et al*, 1983; Marlar *et al*, 1989). The prevalence of homozygous protein C deficiency is estimated to be one in every 160 000–360 000 births (Miletich *et al*, 1987).

The risk of venous thrombosis associated with protein S deficiency was first described by Comp *et al* (1984). Conflicting results have been published on whether protein S deficiency is associated with an increased risk of venous thrombosis. In the Leiden Thrombophilia study, a large population-based case–control study on risk factors for venous thrombosis, no increased risk could be demonstrated (Koster *et al*, 1995a). However, family studies did show a strongly increased thrombotic risk associated with protein S deficiency [relative risk (RR) 17, 95% CI 7–45] (Simioni *et al*, 1999). Similarly to protein C deficiency, a homozygous state of protein S deficiency is associated with severe purpura fulminans in neonates (Mahasandana *et al*, 1990).

The *F5* R506Q mutation is the most common hereditary risk factor for venous thrombosis. Heterozygous carriers of the *F5* R506Q mutation have an approximately three- to five-fold increased risk of venous thrombosis whereas the risk in homozygous carriers is estimated to be increased 80 times (95% CI 22–289) (Koster *et al*, 1993; Bertina *et al*, 1994; Ridker *et al*, 1995a; Rosendaal *et al*, 1995; Pomp *et al*, 2007). APC resistance in the absence of the *F5* R506Q mutation is associated with an increased risk of venous thrombosis (Rodeghiero & Tosetto, 1999; de Visser *et al*, 1999; Tans *et al*, 2003).

Carriers of the *F2* G20210A mutation have a two- to three-fold increased risk of thrombosis (Poort *et al*, 1996; Brown *et al*, 1997). This mutation is associated with elevated levels of prothrombin, which is suggested to be the mechanism by which the mutation increases the risk of venous thrombosis. Elevated levels of prothrombin in the absence of the *F2* G20210A mutation are associated with a two-fold increased risk of venous thrombosis (95% CI 1·5–3·1 for the highest *versus* the lowers quartile) (Poort *et al*, 1996).

High levels of other procoagulant factors have also been associated with an increased risk of venous thrombosis. A high level of factor VIII, i.e. above 150%, is associated with a five-fold increased risk of thrombosis, which was shown to be independent of acute-phase reactions (Koster *et al*, 1995b; Kraaijenhagen *et al*, 2000; Kamphuisen *et al*, 2001; O'Donnell & Laffan, 2001; Bobrow, 2005). Also, high levels of fibrinogen and factors IX and XI have been associated with an approximately two-fold increased risk of thrombosis (Koster *et al*, 1994; van Hylckama Vlieg *et al*, 2000; Meijers *et al*, 2000).

A cause of acquired thrombophilia is the antiphospholipid syndrome. The presence of lupus anticoagulants is most strongly associated with the risk of thrombosis. The presence of antibodies to anticardiolipin, β_2-Glycoprotein I, and prothrombin have been associated with thrombosis in some, but not all, studies (Galli *et al*, 2003; Galli & Barbui, 2005). More recently, it was shown that the presence of anti-β_2-Glycoprotein I antibodies was associated with an increased risk of a first episode of deep venous thrombosis (de Groot *et al*, 2005). This test seems less affected by problems of standardisation (Urbanus *et al*, 2008). A large prospective follow-up study found an increased risk of venous thrombosis associated with the presence of anti-prothrombin antibodies (Bizzaro *et al*, 2007).

Deep venous thrombosis is a multicausal disease. Numerous studies indicated that the risk of venous thrombosis is highly increased when more than one risk factor is present within one individual. This became first apparent in families with thrombophilia, in which two or more genetic defects were often found (Zoller *et al*, 1995; van Boven *et al*, 1996; Koeleman *et al*, 1997; Makris *et al*, 1997; Meinardi *et al*, 2001, 2002). The finding that the combination of *F5* R506Q with oral contraceptive use considerably increases the risk was the first example of a clear gene–environment interaction in the aetiology of venous thrombosis (Vandenbroucke *et al*, 1994).

Association between thrombophilia and recurrent deep venous thrombosis

Venous thrombosis has a tendency to recur. The cumulative incidence of a second episode is approximately 30% in 8 years (Prandoni *et al*, 1996a). It has been consistently shown that patients with a clear clinical risk factor eliciting a first deep venous thrombotic event have a very low risk of recurrence (Prandoni *et al*, 1996a; Baglin *et al*, 2003; Christiansen *et al*, 2005). However, the evidence on laboratory testing for thrombophilia to predict the risk of a recurrent thrombotic

event is much more challenging than its association with a first thrombotic event.

Conflicting results have been published on the risk of recurrent thrombosis associated with *F5* R506Q and *F2* G20210A. While some studies reported an increased risk of recurrent thrombotic events in all carriers of *F5* R506Q (Eichinger *et al*, 1997, 2002; De Stefano *et al*, 1999; Kearon *et al*, 1999; Lindmarker *et al*, 1999; Rintelen *et al*, 1996), others found no increased risk or only in individuals homozygous for the mutation (Ridker *et al*, 1995b; Marchetti *et al*, 2000; Simioni *et al*, 2000; Miles *et al*, 2001; Vink *et al*, 2003). Also, double heterozygosity for *F5* R506Q and *F2* G20210A, or concomitance of other thrombophilic defects appear to increase the risk of recurrent venous thrombosis (De Stefano *et al*, 1999; Meinardi *et al*, 2002).

Elevated levels of factor VIII and IX have been associated with an increased risk of recurrence in single studies (Kyrle *et al*, 2000; Weltermann *et al*, 2003).

More recently, two large follow-up studies assessed the risk of recurrent venous thrombosis associated with thrombophilic defects. Baglin *et al* (2003) showed that carriers of a thrombophilic defect, i.e. antithrombin, protein C, or protein S deficiency [hazard ratios (HRs) ranging from 1·0 to 2·9 for individual deficiencies, which were almost all combined with other thrombophilic defects], or *F5* R506Q (HR 1·4; 95% CI 0·7–2·8), or *F2* G20210A (HR 1·7; 95% CI 0·5–5·6) did not have a highly increased risk of developing a recurrent venous thrombotic event. This was also observed in the large follow-up study by Christiansen *et al* (2005), which found no clear increased risk of recurrent venous thrombosis the prothrombotic risk factors: *F5* R506Q (HR 1·3; 95% CI 0·8–2·1), *F2* G20210A (HR 0·7; 95% CI 0·3–2·0), and elevated levels of factor VIII (HR 1·3; 95% CI 0·8–2·1). Only a deficiency in one of the anticoagulants protein C, protein S, or antithrombin, showed a mildly increased risk of recurrent venous thrombosis (HR: 1·8; 95% CI 0·9–3·7). De Stefano *et al* (2006) reported a similar risk of recurrence associated with deficiencies of the anticoagulants (AT deficiency: HR: 1·9; 95% CI 1·0–3·9, protein C and S deficiency: HR: 1·4; 95% CI 0·9–2·2).

Global assessments could be useful in the prediction of the risk of recurrent venous thrombosis. Elevated levels of D-dimer have been associated with an increased risk of thrombosis. However, with regard to recurrent thrombosis, mainly a high negative predictive value has been reported (Palareti *et al*, 2002; Eichinger *et al*, 2003). The usefulness of thrombin generation tests in predicting first or recurrent venous thrombosis remains to be established. Most individual single nucleotide polymorphisms (SNPs) associated with an increased risk of a first deep venous thrombosis, are not associated with the risk of a recurrent event. However, multiple SNP analysis may prove to be useful in the prediction of recurrent thrombosis. Recently, we have reported an increased risk of recurrent venous thrombosis associated with carriers of multiple SNPs that were individually associated with only a mild increased risk of a recurrent event. However, it was concluded that, although the risk of a recurrent event is increased when more than one SNP is present within one individual, the number of carriers is low, indicating that the clinical utility of multiple SNP analysis at present would be limited (van Hylckama Vlieg *et al*, 2008).

The risk of recurrence in antiphospholipid or anticardiolipin antibodies was investigated in various studies (Prandoni *et al*, 1996b; Rance *et al*, 1997; Schulman *et al*, 1998; Zanon *et al*, 1999; de Groot *et al*, 2005; Lim *et al*, 2006). The outcomes regarding relative risk for recurrence ranged between two- and six-fold. These results are hard to interpret, as these studies did not test the antiphospholipid antibodies or lupus anticoagulant repetitively (as suggested by international guidelines (Miyakis *et al*, 2006). Moreover, duration of anticoagulant treatment differed substantially.

Association of thrombophilia with arterial thrombosis

Arterial thrombosis is considered to occur when a (until then) subclinical atherosclerotic plaque ruptures. Atherosclerosis is a multi-factorial condition, of which most risk factors don't overlap with those for venous thrombosis. It has been hypothesised that thrombophilia particularly increases the risk of arterial events in the absence of overt atherosclerotic lesions in the vessel wall. However, whether thrombophilia plays a role in arterial thrombosis remains controversial. Moreover, whether the presence of thrombophilia affects the risk of recurrent arterial thrombosis is unknown.

Evidence of an association between deficiencies of antithrombin, protein C, or protein S and arterial thrombosis is limited to case reports and small studies that are generally hampered by the low prevalence of these thrombophilias. No cases of antithrombin deficiency were found in two studies of young patients with myocardial infarction; one study investigated its prevalence among 70 survivors of myocardial infarction before the age of 36 years (Rallidis *et al*, 2003), and another study in 75 patients with myocardial infarction before the age of 45 years who had no evidence of coronary atherosclerosis (Dacosta *et al*, 1998). Although case reports suggest that antithrombin deficiency may be associated with stroke (Arima *et al*, 1992; Martinez *et al*, 1993), studies in neonates, children and young adults with ischemic stroke revealed no association with antithrombin deficiency (Gunther *et al*, 2000; Hankey *et al*, 2001; Carod-Artal *et al*, 2005).

Also for hereditary protein C deficiency, there is no strong association with myocardial infarction. Although patients have been described with protein C deficiency and myocardial infarction (Peterman & Roberts, 2003; Tiong *et al*, 2003), three case–control studies did not demonstrate an increased prevalence of protein C deficiency in young patients with myocardial infarction as compared to controls (Hayashi *et al*, 1997; Rallidis *et al*, 2003; Segev *et al*, 2005). Studies on the relationship between protein C deficiency and ischemic stroke have focused on patient populations with a low atherosclerotic

burden, particularly young adults and children. Positive associations have been reported mainly in small studies and case reports (Grewal & Goldberg, 1990; Kohler *et al*, 1990; De Stefano *et al*, 1991; Martinez *et al*, 1993; deVeber *et al*, 1998). Larger studies, also among unselected patients with ischemic stroke, did not demonstrate a higher prevalence of protein C deficiency in stroke patients (Douay *et al*, 1998; Ganesan *et al*, 1998; Munts *et al*, 1998; Hankey *et al*, 2001).

Several case reports exist regarding young patients who develop myocardial infarction without evidence of significant coronary artery disease and are then found to have protein S deficiency (Beattie *et al*, 1997; Manzar *et al*, 1997). However, larger studies have not found an association between protein S deficiency and myocardial infarction (Rallidis *et al*, 2003; Segev *et al*, 2005). Likewise, although small studies and case reports have suggested that protein S deficiency increases the risk of developing ischemic stroke (Girolami *et al*, 1989; Green *et al*, 1992), larger studies, also focusing on patient populations with a low atherosclerotic burden including children and neonates, have not confirmed this relationship (Douay *et al*, 1998; Munts *et al*, 1998; deVeber *et al*, 1998; Hankey *et al*, 2001).

Numerous studies have investigated whether *F5* R506Q is a risk factor for myocardial infarction. Several large cohort studies including the Physicians' Health Study (Ridker *et al*, 1995b), the Cardiovascular Health Study (Cushman *et al*, 1998), and the Copenhagen Heart Study (Juul *et al*, 2002) did not find an association between *F5* R506Q and myocardial infarction. In a meta-analysis the pooled odds ratio for myocardial infarction was 1·3 (95% CI 0·9–1·7) (Boekholdt *et al*, 2001). Several studies have suggested that *F5* R506Q may be a risk factor in rare cases where myocardial infarction occurs without evidence of atherosclerosis. The presence of *F5* R506Q was significantly higher among people who developed myocardial infarction without evidence of coronary artery disease, than among those with coronary artery disease or healthy controls (Mansourati *et al*, 2000). Also, a similar phenomenon was observed among young women, who are at very low risk of having significant coronary artery disease (Rosendaal *et al*, 1997). An interaction between *F5* R506Q and other classical risk factors, most notably smoking, was observed. Whereas the presence of one risk factor led to a moderately elevated risk, *F5* R506Q carriers who smoked had a 32-fold increased risk of myocardial infarction compared with non-smoking, non-carriers of *F5* R506Q (95% CI 7·7–133). Despite these positive findings, other studies did not confirm the association between and myocardial infarction at a young age. For instance, the Italian Atherosclerosis Thrombosis and Vascular Biology Study compared 1210 survivors of myocardial infarction before the age of 45 years with 1210 controls and found no evidence for an association (Atherosclerosis Thrombosis and Vascular Biology Italian Study Group, 2003). The pooled odds ratio in patients developing myocardial infarction before the age of 55 years in the previously mentioned meta-analysis remained 1·3 but became borderline significant (95% CI 1·0–1·6) (Boekholdt *et al*, 2001). Similar findings exist for the relationship between *F5* R506Q and stroke. Large studies investigating unselected patients with ischemic stroke observed no significant association (Ridker *et al*, 1995b; Cushman *et al*, 1998; Hankey *et al*, 2001). Again, results in patients with a low atherosclerotic burden have been inconsistent. Whereas in one study of 106 women with an ischemic stroke before the age of 45 years, *F5* R506Q was not a risk factor (Longstreth *et al*, 1998), a larger study among 468 stroke and transient ischaemic attack patients before the age of 60 years found a 2·6 (95% CI 1·5–4·6) increased risk in *F5* R506Q carriers, and an 8·8-fold (95% CI 2·0–38·0) risk in smoking carriers of *F5* R506Q as compared to non-smoking non-carriers (Lalouschek *et al*, 2005). Some studies in children with stroke suggest that *F5* R506Q may be a risk factor in this highly selected group (Zenz *et al*, 1998). An Israeli study among 65 children with ischemic stroke estimated a five-fold higher risk in children with *F5* R506Q (OR 4·8, 95% CI 1·4–16·5) (Kenet *et al*, 2000).

The relationship between *F2* G20210A and the risk of myocardial infarction has been subject of a number of studies, but results have been inconsistent. In the Study of Myocardial Infarctions Leiden among 560 men who suffered a first myocardial infarction before the age of 70 years, *F2* G20210A was associated with a slightly increased risk of myocardial infarction (OR 1·5, 95% CI 0·6–3·8). However, the risk was mainly increased in the presence of classical cardiovascular risk factors, e.g., smoking, hypertension, diabetes mellitus, or obesity with risk increases between three- and six-fold (95% CIs 1·5–6·7 and 3·0–12·5) (Doggen *et al*, 1998). A family study revealed that *F2* G20210A carriers were at an elevated risk of myocardial infarction as compared with their family members without the mutation (OR 4·7, 95% CI 1·0–22·5) (Bank *et al*, 2004a). However, a subanalysis of the Physicians' Health Study observed no significant association between *F2* G20210A and risk of myocardial infarction (Ridker *et al*, 1999). Another study, of 539 patients with myocardial infarction, found an OR of 0·7 (95% CI 0·3–1·6) (Croft *et al*, 1999). This lack of association was confirmed in a meta-analysis that found an overall OR of 1·1 (95% CI 0·8–1·6) (Boekholdt *et al*, 2001). However, this meta-analysis estimated a borderline significantly elevated risk of developing myocardial infarction at young age in *F2* G20210A carriers (pooled odds ratio 1·9, 95% CI 1·0–3·5), but this was not confirmed by the large Atherosclerosis Thrombosis and Vascular Biology Study among survivors of myocardial infarction before the age of 45 years (Atherosclerosis Thrombosis and Vascular Biology Italian Study Group, 2003). Similar inconsistent observations apply to the relationship between *F2* G20210A and stroke. Several large studies among unselected patients with ischemic stroke showed no association with *F2* G20210A (Ridker *et al*, 1999; Hankey *et al*, 2001; Smiles *et al*, 2002). Findings in young stroke patients have shown inconsistent results. Patients with documented ischemic stroke before the age of 50 years and without cardiovascular risk factors were five times more likely to carry *2* G20210A than controls (De Stefano *et al*, 1998). A study of 468 patients with cerebrovascular disease before the age of 60 years found an elevated risk of stroke among male

carriers of the *F2* G20210A mutation (OR 6·1, 95% CI 1·3–28·3) but not among women (Lalouschek *et al*, 2005). In contrast, two other large studies observed no significant association between *F2* G20210A and risk of developing stroke at young age (Austin *et al*, 2002; Pezzini *et al*, 2005).

In conclusion, no firmly established increased risk for arterial cardiovascular diseases, e.g. myocardial infarction and ischemic stroke, and inherited thrombophilia, is present. Some studies suggest that thrombophilia may increase this risk of arterial cardiovascular disease in young patients, particularly when classical risk factors, such as smoking, are present. However, positive studies are equally counterbalanced by negative studies. A clear-cut explanation for the discrepant findings between studies, e.g., differences in patient selection, is not available.

Association with pregnancy complications

Analogous to the clinical manifestations that are part of the antiphospholipid syndrome (Opatrny *et al*, 2006), family studies in the 1990s were the first to demonstrate that hereditary thrombophilia was also associated with an increased risk for miscarriage (Preston *et al*, 1996; Sanson *et al*, 1996; Meinardi *et al*, 1999). Since then, numerous studies have investigated this issue. From meta-analyses, it can be concluded that the association between hereditary thrombophilia varies depending on type of thrombophilia and timing of fetal loss (Rey *et al*, 2003) and that there is a modest association also between thrombophilia and other adverse pregnancy outcomes, most notably preeclampsia and intra-uterine growth retardation (Robertson *et al*, 2006). However, whether this association can be considered causal remains controversial, as many other factors, most notably structural or numeric chromosomal abnormalities, play a role in the risk of pregnancy complications (Rai & Regan, 2006; Middeldorp, 2007a,b; Rodger *et al*, 2008).

Implications of thrombophilia for clinical management

Many patients and clinicians are motivated to find an explanation for their disease. It should be realised however, that the existence of a thrombophilic defect does not exclude other risk factors, given the multi-causal aetiology of thrombosis. The relevant issue is under what circumstances a positive result on a thrombophilia test helps in the clinical management. Generally, this would consist of the need to adjust the therapeutic regime in thrombophilic patients, and the possibility of identifying asymptomatic family members (for subsequent preventive measures).

Clinical management of patients with venous thrombosis

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, i.e. bleeding, and the benefit, i.e. the prevention of an extension or recurrence of venous thrombosis (Buller *et al*, 2004). As outlined above, the presence of hereditary thrombophilia in patients with venous thrombosis does not strongly increase the risk of recurrence after discontinuation of anticoagulant therapy. In the absence of trials comparing routine and prolonged anticoagulant treatment in patients that tested positive for thrombophilia, with the current knowledge available prolonged anticoagulant therapy cannot be justified as it may cause more harm than benefit. An attempt to prevent recurrent thrombosis in the long-term without inducing an unacceptable number of major bleeding episodes was investigated in a trial comparing prolonged anticoagulant treatment with vitamin K antagonists with a low intensity (International Normalized Ratio below 2·0) and conventional intensity in patients after a first episode of venous thrombosis, with and without thrombophilia (Kearon *et al*, 2003). The lower intensity anticoagulation increased the incidence of recurrent venous thrombosis (1·9% vs. 0·6% in the conventional intensity group), without reducing major bleeding complications (0·96% vs. 0·93%).

For patients with antiphospholipid antibody syndrome the issue is more complicated. Patients with a first episode of venous thrombosis and antiphospholipid antibodies (tested once 6 months after the diagnosis of venous thrombosis) had an cumulative incidence of 29% recurrence during 4 years of follow-up, as compared to 14% in the patients without these antibodies (RR 2·1, 95% CI 1·3–3·3) (Schulman *et al*, 1998). This observation has led to the recommendation to treat patients with known antiphospholipid antibodies for at least 12 months (Buller *et al*, 2004; Ruiz-Irastorza *et al*, 2007). Nevertheless, guidelines refrain from giving recommendations regarding the routine testing of consecutive patients with venous thrombosis. Even if the prevalence of persistently positive tests was 10%, 10 patients would need to be tested in order to find one patient with venous thrombosis based on antiphospholipid syndrome in whom prolonged anticoagulant treatment would be installed. To our knowledge, no cost-effectiveness assessments have been performed. Based on retrospective studies with highly selected patients, it was assumed that patients with antiphospholipid antibody syndrome should be treated with a high intensity anticoagulant regime (Khamashta *et al*, 1995). However, two randomised trials showed that a higher intensity of anticoagulation with vitamin K antagonists in patients with antiphospholipid antibodies did not reduce the risk of recurrence, but led to an increase in the bleeding risk (Crowther *et al*, 2003; Finazzi *et al*, 2005).

Identification of asymptomatic family members with hereditary thrombophilia

A potential advantage of testing patients with venous thrombosis for thrombophilia may be the identification of asymptomatic family members of thrombophilic patients in

order to take preventive measures if tested positive. As detailed previously, the risk for a first episode of venous thrombosis in relatives with thrombophilia is increased two- to ten-fold. Nevertheless, the overall absolute risk in thrombophilic families has been assessed in many studies and is generally low, even during high-risk situations such as pregnancy, puerperium, surgery, immobilisation, trauma and during the use of oral contraceptives. Estimates are listed in Table II. It is clear that the 2% annual major bleeding risk associated with continuous anticoagulant treatment outweighs the risk of venous thrombosis (van der Meer *et al*, 1996; Palareti *et al*, 1996). It is of note that the risk estimates related to surgery, trauma and immobilisation, as shown in Table II, mainly reflect the situation before standard prophylaxis was routine patient care. Low risk during pregnancy in asymptomatic women with thrombophilia does not justify prophylaxis with low-molecular-weight heparin during the nine months of pregnancy. Although the risk of severe complications, such as bleeding, osteoporosis and heparin-induced thrombocytopenia is very small, the nuisance of daily subcutaneous injections and skin reactions in one-third of women is high (Bank *et al*, 2003; Deruelle *et al*, 2006). Whether the 80% of pregnancy-related episodes occurring 6–12 weeks postpartum justifies prophylaxis during this period is a matter of the physicians' and patients' preference. The number needed to treat to prevent one postpartum thrombosis is 25 in case of a deficiency in the natural anticoagulants and approximately 50 in patients with the common thrombophilias. Finally, it is clear that risk of a first venous thrombosis during use of oral contraceptives should be weighed against the disadvantage of other contraceptive methods.

Clinical management of patients with arterial thrombosis

Despite the inconsistent but generally absent association of hereditary thrombophilia with arterial thrombosis, a survey in The Netherlands found that this was the indication for testing in almost a quarter of the ordered tests (Coppens *et al*, 2007). Given that no differential treatment or secondary prevention will follow from the presence of thrombophilia we strongly argue against testing. Vitamin K antagonists are generally recommended for patients with arterial thrombosis and a definite diagnosis of antiphospholipid antibody syndrome, but there is no evidence from well-designed trials that supports this expert recommendation (Ruiz-Irastorza *et al*, 2007).

Clinical management of patients with thrombophilia and pregnancy complications

Therapeutic options to prevent pregnancy complications in women with thrombophilia comprise aspirin as well as (low-molecular-weight) heparin. There is currently no evidence supporting treatment for women with recurrent pregnancy loss (Walker *et al*, 2003; Di Nisio *et al*, 2005; Middeldorp, 2007a; Rodger *et al*, 2008). Observational research is hampered by severe methodological flaws or inconsistent results. Two published randomised trials did not use an adequate comparator, i.e. no treatment or placebo (Gris *et al*, 2004; Brenner *et al*, 2005). Currently, randomised controlled trials with a no treatment or placebo arm are being carried out and results should be awaited before implementing a potentially harmful intervention in pregnant women with inherited thrombophilia and a history of pregnancy complications. For women with antiphospholipid antibody syndrome, aspirin and low-molecular-weight heparin treatment is often suggested although the evidence is limited (Empson *et al*, 2005; Jauniaux *et al*, 2006).

Drawbacks of thrombophilia testing

The disadvantages of testing patients with a venous thrombosis for thrombophilia include be the cost of testing, which is approximately €500, for a complete thrombophilia screen (Machin, 2003). Although some cost-effectiveness studies have been published regarding the testing for thrombophilia, which concluded that in some scenarios testing could indeed be cost-effective, the number of assumptions from inconsistent

Table II. Estimated incidence of a first episode of venous thrombosis in carriers of various thrombophilic defects (data apply to individuals with at least one symptomatic first-degree relative).

	Antithrombin, protein C, or protein S deficiency	*F5* R506Q mutation	*F2* G20210A mutation	Elevated levels of FVIII*
Overall (%/year, 95% CI)	1·5 (0·7–2·8)	0·5 (0·1–1·3)	0·4 (0·1–1·1)	0·3 (0·2–0·5)–1·3 (0·5–2·7)
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)	1·2 (0·4–1·8)
Pregnancy (%/pregnancy, 95% CI)	4·1 (1·7–8·3)	2·1 (0·7–4·9)	2·3 (0·8–5·3)	1·3 (0·4–3·4)
During pregnancy, %, 95% CI	1·2 (0·3–4·2)	0·4 (0·1–2·4)	0·5 (0·1–2·6)	1·0 (0·3–2·9)
Postpartum period, %, 95% CI	3·0 (1·3–6·7)	1·7 (0·7–4·3)	1·9 (0·7–4·7)	0·3 (0·1–1·8)
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)	0·6 (0·2–1·6)

*Classification of elevated levels of FVIII > 150% based on a single measurement.
References are listed in the main text.

observational studies seriously hamper their interpretation (Marchetti *et al*, 2000; Wu *et al*, 2006). The psychological impact and consequences of a person knowing that they are a carrier of a (genetic) thrombophilic defect is a potential drawback of testing (Cohn *et al*, 2008). Most studies that focused on impact of testing for thrombophilia showed that patients had experienced low psychological distress following thrombophilia testing (van Korlaar *et al*, 2005; Legnani *et al*, 2006). Nevertheless, a qualitative study described several negative effects of both psychological and social origin (Bank *et al*, 2004b).

Conclusions

Despite the increasing knowledge about the multifactorial etiology of venous thrombosis, testing for hereditary thrombophilia generally does not alter the clinical management of patients with venous or arterial thrombosis or pregnancy complications. There are a few exceptions. For some asymptomatic women at fertile age who come from families with a tendency for venous thrombosis and a known thrombophilic defect, a positive test may lead to the decision to install prophylaxis postpartum in case of pregnancy, or the individual decision to not use oral contraceptives. According to current guidelines, testing for antiphospholipid antibody syndrome may be justified in patients with venous or arterial thrombosis or well-defined pregnancy complications, although no formal studies about its cost-effectiveness have been carried out. There is an intermediate strength recommendation to prolong anticoagulant treatment to 12 months in case of venous thrombosis, and to use vitamin K antagonists instead of aspirin in case of arterial thrombosis in patients who fulfil the laboratory criteria for antiphospholipid antibody syndrome. Finally, women with pregnancy complications and a definite diagnosis of antiphospholipid antibody are usually treated with aspirin and low-molecular-weight heparin.

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

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