Laboratory Investigation of Thrombophilia

Armando Tripodi[*] and Pier Mannuccio Mannucci

Until recently, laboratory diagnosis of thrombophilia was based on investigation of the plasmatic anticoagulant pathways to detect antithrombin, protein C, and protein S deficiencies and on the search for dysfibrinogenemia and anti-phospholipid antibodies/lupus anticoagulants. More recently, laboratory investigations have been expanded to include activated protein C (APC) resistance, attributable or not to the presence of the factor V Leiden mutation; hyperprothrombinemia attributable to the presence of the prothrombin gene mutation G20210A; and hyperhomocysteinemia attributable to impairment of the relevant metabolic pathway because of enzymatic and/or vitamin deficiencies. All of the above are established congenital or acquired conditions associated with an increased risk of venous and, more rarely, arterial thrombosis.

Testing is recommended for patients who have a history of venous thrombosis and should be extended to their first-degree family members. Because most of the tests are not reliable during anticoagulation, it is preferable to postpone laboratory testing until after discontinuation of treatment.

Whenever possible, testing should be performed by means of functional assays. DNA analysis is required for the prothrombin gene mutation G20210A. Laboratory diagnosis for anti-phospholipid antibodies/lupus anticoagulant should be performed by a combination of tests, including phospholipid-dependent clotting assays and solid-phase anti-cardiolipin antibodies. Hyperhomocysteinemia can be diagnosed by HPLC methods or by fluorescence polarization immunoassays.
© 2001 American Association for Clinical Chemistry

There is no a widely accepted definition for thrombophilia. Over the years, this term has been used to identify those disorders of hemostasis that are likely to predispose to thrombosis *(1)*. More recently, it has been defined as a tendency to develop thrombosis *(2)* as a consequence of predisposing factors that may be genetically determined, acquired, or both. The latter definition may be more useful because it includes situations that are apparently not directly linked to the hemostatic system (e.g., hyperhomocysteinemia). Whatever the definition, it is of relevance for this review that thrombophilia may be secondary to many conditions that can be investigated by laboratory testing. During the last few years, thrombophilia has contributed substantially to increased pressure on clinical laboratories, and demands for testing are increasing dramatically. However, they are not always justified because the patients to be investigated are not well defined. Furthermore, the appropriate timing for testing and the type of testing to be carried out are also somewhat confusing, so that clinical laboratories face the prospect of spending considerable amounts of time performing laboratory investigations that are not completely justified. Our aim is to review and update the situation with respect to the conditions associated with thrombophilia. We will also discuss (*a*) the reasons that testing should be performed, (*b*) who should be tested, (*c*) when and (*d*) where testing should be performed, (*e*) which kind of test is to be performed, and finally (*f*) the strategy (if any) to make testing cost-effective.

Conditions Associated with Venous Thromboembolism
The rapid expansion of knowledge during the last decade has made it possible to elucidate new mechanisms that regulate thrombogenesis. As a consequence, many conditions associated with venous thromboembolism have been unraveled. These constitute the starting point to establish laboratory investigations aimed at identifying the cause(s) of thromboembolism in patients with a past history of the disorder or to identify those who are at increasing risk to develop a thromboembolism.

Among the established conditions associated with an increased risk of venous thromboembolism that are of interest for this review, some are congenital and some are acquired (Table 1).

CONGENITAL CONDITIONS
Some of the inherited abnormalities of the anticoagulant mechanisms that operate in plasma are established risk

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, University and IRCCS Maggiore Hospital, 20122 Milan, Italy.

*Address correspondence to this author at: Hemophilia and Thrombosis Centre, Via Pace 9, 20122 Milan, Italy. Fax 39-02-5516093; e-mail armando.tripodi@unimi.it.

Table 1. Congenital and acquired conditions associated with venous thromboembolism.

Congenital conditions
    AT deficiency
    PC deficiency
    PS deficiency
    Dysfibrinogenemia
    FV Leiden
    Prothrombin G20210A mutation
    Hyperhomocysteinemia attributable to congenital deficiency of
      CBS,[a] MS, and MTHFR
Acquired conditions
    Anti-phospholipid antibodies
    Hyperhomocysteinemia attributable to vitamin deficiency or other
      nongenetic causes
    APC resistance, not attributable to gene mutations
    Increased factor VIII[b]

[a] CBS, cystathionine-β-synthase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase.
[b] May be at least in part congenital [see Ref. (21)].

factors for venous thromboembolism. They include anti-thrombin (AT),[1] protein C (PC), and protein S (PS) deficiencies (3) and the activated PC (APC) resistance phenomenon attributable (or not) to the presence of the factor V (FV) Leiden mutation (4, 5), which may be defined as a poor response of plasma to the anticoagulant action of APC. The relative risks associated with these abnormalities are not well established, but it is generally accepted that they range from AT deficiency (the most severe), to PC/PS deficiencies (intermediate severity), to APC resistance (the least severe). Congenital abnormalities of other plasmatic anticoagulant mechanisms, such as heparin cofactor II, are apparently not associated with thromboembolism (6) or require further evidence, such as the tissue factor pathway inhibitor (7). Apart from congenital abnormalities of the plasmatic anticoagulant mechanisms, other conditions that at least in principle might be associated with an increased risk of thromboembolism are congenital dysfibrinogenemia, which is characterized by the presence of abnormal fibrinogen in plasma, and congenital abnormalities of the fibrinolytic system. Although very rare, congenital dysfibrinogenemia is a risk factor for venous and arterial thrombosis (8), whereas abnormalities of the fibrinolytic system, once regarded as a risk factor for thrombosis, have not been confirmed in subsequent studies and are therefore not included in the laboratory investigation for thrombophilia (9). The last entry in the series of conditions firmly associated with an increased tendency to develop thromboembolism is the

[1] Nonstandard abbreviations: AT, antithrombin; PC, protein C; PS, protein S; APC, activated protein C; FV, factor V; LA, lupus anticoagulant; C4bBP, C4b binding protein; APTT, activated partial thromboplastin time; aCL, anti-cardiolipin; KCT, kaolin clotting time; and dRVVT, diluted Russell viper venom test.

presence of the mutation G20210A in the prothrombin gene, which may produce hyperprothrombinemia (10).

ACQUIRED CONDITIONS
Among the acquired conditions associated with venous (and arterial) thromboembolism, a major role is played by the anti-phospholipid antibody syndrome (11) and moderate hyperhomocysteinemia (Table 1) (12). The anti-phospholipid antibody syndrome is characterized by repeated positive tests for lupus anticoagulant (LA) and/or solid-phase anti-phospholipid antibodies and by thrombocytopenia and fetal loss. A comprehensive discussion of all of the issues related to the syndrome is beyond the scope of this review; for more information, the reader may refer to a review by Triplett (11). Hyperhomocysteinemia may be caused by a congenital deficiency of the enzymes involved in its metabolism (12), but it may also be attributable to a poor dietary intake of vitamins that act as cofactors (folic acid and B_{12}), and therefore, it may be easily and effectively treated by dietary supplementation (13). Hyperhomocysteinemia has been shown to be a graded risk factor, with the risk increasing by 40% for every 5 μmol/L increase in homocysteine (14). These characteristics make homocysteine an appealing laboratory marker, and it is now increasingly included by many laboratories in the investigation for thrombophilia.

OTHER CONDITIONS ASSOCIATED WITH VENOUS THROMBOEMBOLISM
Numerous studies have been carried out to investigate whether other conditions are associated with an increased risk of thrombosis. Among these studies, the most comprehensive has been undertaken in The Netherlands (the Leiden Thrombophilia Study) (15). The authors enrolled consecutive patients with at least one episode of documented venous thromboembolism and a population of controls matched for sex, age, and living conditions to the patient population. The measurement of several plasmatic markers chosen among those that were most plausible revealed that high concentrations of procoagulant factors such as XI (16), VIII (17), IX (18), and fibrinogen (19) were indeed associated with an increased risk of venous thromboembolism (Table 2). Among them high factor VIII has

Table 2. Other conditions associated with venous thromboembolism.

Condition	Relative risk	Reference
Factor XI		
(>1200 units/L)	2.2	(16)
Factor IX		
(>1280 units/L)	2.5	(18)
Fibrinogen		
(>5 g/L)	4.0	(19)
TAFI[a]		
(>1220 units/L)	2.0	(22)

[a] TAFI, thrombin-activatable fibrinolysis inhibitor.

subsequently been confirmed by other investigators (20). Furthermore, high factor VIII was found to be at least in part genetically determined, as shown by familial clustering (21). According to the Leiden Thrombophilia Study, high plasma concentrations of thrombin activatable fibrinolysis inhibitor are an independent risk factor for venous thrombosis (22), but not plasma FV (23). Apparently all of the above are weak risk factors (Table 2), and the value of their measurement in the investigation of patients with thrombophilia (except for factor VIII) is to be established more conclusively.

Which Analytes Are to Be Included in the Investigation?

On the basis of the evidence provided by the available studies, it is in principle plausible to include all of the markers that may help to identify those conditions that are firmly associated with an increased risk of thrombosis. These include AT, PC, and PS deficiencies; dysfibrinogenemia; the syndrome of anti-phospholipid antibodies; APC resistance; hyperprothrombinemia; hyperhomocysteinemia; and high factor VIII concentrations (Table 3). However, this does not necessarily mean that they are useful and effective, and a more comprehensive discussion on this issue follows.

Why Should We Test?

In general, laboratory testing should be performed whenever the results may influence decisions on therapy or prevention. In the field of thrombophilia, results of testing are unlikely to influence the handling of acute events because the management of thrombosis is not dependent on its cause. In principle, results of testing might influence decision on prevention of (re)thrombosis (secondary prophylaxis). They may help clinicians decide how long and how intensively to treat patients. Even if no conclusive evidence has to date come from clinical studies to suggest that patients with genetic abnormalities (24–27) or anti-phospholipid antibodies (28) should be treated for a longer time or more intensively than patients with idiopathic venous thromboembolism, information on the causes of thrombosis may help clinicians to make decisions involving individual patients. Paradoxically, this might be even more relevant at a time when there are no accepted guidelines and decisions are made on a case-by-case basis. Testing may also be beneficial for those family members of the proband who are carriers of the defect but are still asymptomatic. These individuals may be offered primary prophylaxis that is usually not offered to their noncarrier counterparts when they are exposed to risk situations. Finally, testing may also help to identify those individuals with combined defects. Although not frequent, the combination of any given thrombophilic condition is not a very rare event if one considers that in some ethnic groups the FV and prothrombin mutations occur frequently in the general population (29, 30). There is evidence that individuals bearing combined genetic defects of PC (31) or PS (32) plus FV Leiden are at a higher risk of thromboembolism than those individuals with either defect alone. Furthermore, individuals with combined AT deficiency and FV Leiden are likely to develop thromboembolisms earlier in life than individuals with AT deficiency alone (33). Finally, an acquired risk factor, such as moderate hyperhomocysteinemia, may considerably increase the risk of thromboembolism in individuals who carry the FV Leiden mutation (34). The lesson that can be learned from the above observations is that for effectiveness to be maximized, laboratory screening should be comprehensive and include measurement of all of the above markers.

Who Should Be Tested?

In general, the prevalence of any of the above clinical conditions is not sufficient to justify screening of the general population. Possible exceptions may be FV Leiden and the prothrombin gene mutations, whose prevalences are relatively high, at least in Caucasians (29, 30). However, if one considers the relatively low risk of thromboembolism associated with these two conditions (10, 15), it must be concluded that a general screening is not warranted. Therefore, testing should be performed only in patients who have a history of unexplained thromboembolism. Neither age nor the presence of predisposing factors at the time of thromboembolism should be taken as strict criteria to decide on testing, because thromboembolism secondary to some of the above conditions may develop later in life and/or after exposure to circumstantial risk situations. Because of the beneficial effect that screening may have on asymptomatic individuals (see above), the laboratory investigation should be extended to all available first-degree family members of the proband. Affected members who have been identified should be

Table 3. Markers[a] and types of assays to be included in the laboratory investigation of thrombophilia.

Marker	Type of assay
AT	Heparin cofactor activity against factor Xa
PC	Amidolytic assay with snake venom as activator
PS	Free antigen
APC resistance	APTT-based method without and with FV-deficient plasma
	Confirmation of positive and borderline results by FV genotyping
Dysfibrinogenemia	Thrombin and reptilase times
	Parallel analysis of immunologic and functional fibrinogen
Anti-phospholipid antibodies	Phospholipid-dependent tests (i.e., KCT, dRVVT) and aCL antibodies
Hyperprothrombinemia	Prothrombin genotyping
Hyperhomocysteinemia	HPLC, FPIA[b]
Factor VIII	Activity (clotting or amidolytic) or antigen measurement

[a] Markers and types of assay listed here represent the authors' choice. The reasons for choice are motivated and discussed in the text.

[b] FPIA, fluorescence polarization immunoassay.

kept informed about their future risk and counseled about appropriate prophylaxis at the time of exposure to challenging events (e.g., surgery, immobilization, and pregnancy).

When Is It Appropriate to Test?

Acute thromboembolic events with or without concomitant therapy may influence laboratory investigations (except for DNA analysis) or make difficult the interpretation of results. Hence, tests on plasma should be performed at least 6 months after the acute thrombotic episode. Furthermore, oral anticoagulants, given for prevention of thromboembolism after the acute event, affect the results of testing for PC, PS, and APC resistance. Therefore, it is preferable to defer the laboratory investigation until after discontinuation of oral anticoagulant treatment (at least 2 weeks). Circumstantial risk factors, such as oral contraceptives intake, surgery, immobilization, pregnancy, and others, are associated with an increased risk of thromboembolism. The occurrence of any such conditions in combination with any of the above genetic or acquired risk factors for venous thromboembolism (see above) may further increase the risk of thrombosis. Quantification of the relative increase in risk associated with the above combinations has been possible only for intake of oral contraceptives combined with FV Leiden (odds ratio = 34.7) (35) and the prothrombin gene mutation (odds ratio = 16.3) (36), which are the most prevalent genetic defects in the general population. The magnitude of this risk would suggest screening women, at least for these two genetic defects, before they are given oral contraceptives. Indeed, a cost/benefit analysis performed on the basis of the prevalence of the FV Leiden mutation and the risk of venous thrombosis in women taking oral contraceptives suggests that general screening even restricted to this setting is not cost-effective because the numbers of laboratory tests to be carried out to avoid one thrombotic event would be extremely high (37). On the other hand, in addition to a cost/benefit issue, this may also represent a philosophic and moral issue confronting humanity for which different societies may reach their own conclusions after full evaluation of the available information.

Where Should Tests Be Performed?

Most of the tests used to investigate thrombophilia (see below) require considerable experience and skill in the interpretation of their results. Hence, specialized coagulation laboratories are more suitable for this purpose. However, this does not necessarily mean that the same expertise cannot be found in laboratories from large or even small general hospitals. Probably the choice should rest on considerations that involve the best allocation of the economic resources provided by health services. One may envision a situation where a few coagulation laboratories act as specialized laboratories where patients from general hospitals are referred for investigation. However, a close connection between the clinic and the laboratory is an essential prerequisite for the effective management of thrombophilic patients, and in this respect, laboratories from general hospitals are more suited.

Which Tests?

Until recently, thrombophilia was investigated almost exclusively by means of plasma-based assays (phenotype). More recently, DNA analysis became available, and in some cases, genotype can now be used in combination with or instead of phenotype. Both types of analyses have advantages and disadvantages. Phenotype determination is much easier, even with simple instrumentation, but it is more difficult to standardize and the results may be variable. On the other hand, genotyping gives clear-cut results, but discrepancies between laboratories and methods cannot be ignored because they are not infrequent (38). Additional disadvantages of DNA analysis are that for some thrombophilic conditions (e.g., AT, PC, and PS deficiency), the underlying defect may be attributable to several different mutations. Therefore, it would be prohibitive to undertake comprehensive analysis of the whole gene on a routine basis. Furthermore, for some conditions, such as moderate hyperhomocysteinemia, the association of thromboembolism and the phenotype has clearly been established, whereas that for the genotype has not [see Ref. (12) for review]. Our choices for the markers involved in the investigation of thrombophilia are shown in Table 3, and our reasons for these choices will be discussed and supported in details.

AT

Measurement of the AT antigen is clearly not adequate to screen patients because it would leave undetected all cases of dysfunctional AT deficiency that typically have normal antigen concentrations and reduced functional activity. Functional assays for AT may be of two types: progressive inhibitory activity and heparin cofactor activity. The former is performed without heparin, the latter with heparin. Both activities may be assessed with thrombin or factor Xa as the target enzymes. Heparin cofactor activity is the test of choice to screen thrombophilic patients because it is able to detect all cases of AT deficiency of clinical relevance. Factor Xa seems more adequate than thrombin as the target enzyme because it allows better discrimination between carriers and noncarriers of the deficiency (39) and is not affected by the presence of the other main plasmatic inhibitor of thrombin, i.e., heparin cofactor II (39, 40). Subtle dysfunctions of the AT reactive site that affect thrombin more than factor Xa may go undetected with this assay. However, there is no conclusive evidence that the active site for thrombin is different from that for factor Xa, and few cases of discrepancies between anti-Xa and anti-thrombin activity have been reported (41).

PC

For PC, as for AT, antigen measurements should not be used to screen patients, but only for further characterization of defects identified by one or more functional assays. These may be based on measurement of the anticoagulant activity of APC exerted against the natural substrates factor VIIIa and Va, or of the amidolytic activity against small synthetic substrates. Both types of assays require preventive activation of plasmatic PC. This may in turn be achieved by thrombin, thrombin-thrombomodulin complex, or snake venom. Activation with thrombin-thrombomodulin and measurement of the anticoagulant activity should be the test of choice because it is likely to mimic in vivo conditions more closely than any other test. These tests are commercially available and may be easily adapted to automation in many coagulometers. However, they are potentially susceptible to artifacts because they may be affected by other conditions, such as APC resistance *(42)* or high concentrations of factor VIII *(43)*, and require considerable experience to interpret the results. All of these problems may be circumvented by use of amidolytic assays with snake venom as the activator. These assays may leave undetected cases of subtle PC dysfunction where the defect is restricted to the active site responsible for inactivation of the natural substrates, but not to the site responsible for splitting synthetic substrates. However, only a few such cases have been reported in the literature *(44, 45)*, so that one would assume that they are very rare and that only a limited number of affected patients would be missed by a diagnostic strategy using only amidolytic assays for PC measurement.

PS

Again functional assays should be the choice because it is logical to assume that dysfunctional PS deficiency may (although rarely) occur in thrombophilic patients. However, it must be considered that the available functional assays are based on the APC cofactor activity of PS *(46, 47)* and that these assays are not very specific. They are affected (probably to a greater extent than PC anticoagulant assays) by APC resistance *(48, 49)*. Hence, diagnoses based on these tests must be considered with caution. Until new and more specific assays become available, a reasonable alternative to screen thrombophilic patients for PS deficiency is antigen measurement. PS has a peculiar distribution in plasma: 60% of the whole protein is in a complex with the C4b binding protein (C4bBP), whereas 40% is free and active as APC cofactor *(50)*. Bound and free PS react differently with anti-PS antibodies in ELISA systems. In principle, the total or free antigen is measured, depending on the assay design. The measurement of the total antigen requires an ELISA system with incubation times long enough to allow complexed PS to be released from C4bBP and to react quantitatively with anti-PS antibodies bound to the plate *(51)*. The measurement of the free antigen requires either pretreatment of test samples to separate the free from the bound antigen before reaction with the antibody in the ELISA system or the use of a monoclonal antibody that recognizes only the free form of PS. Both alternatives are suitable for an accurate measurement of free PS antigen. The former is based on pretreatment of test plasma with polyethylene glycol, which precipitates only the PS-C4bBP complex. After centrifugation, supernatant plasma can be tested in the ELISA system to quantify the free antigen. The method is relatively simple *(52)*, but pretreatment of plasma must be standardized very carefully. New methods have recently become available that circumvent most of these problems. In addition to commercial methods based on monoclonal capture antibodies specific for the free PS antigen *(53)*, another method is becoming available in which the free PS antigen is captured in the ELISA system directly by the natural ligand C4bBP *(54)*. Another debated issue is whether it is necessary to assay for both the free and the total antigen (or either) in the investigation of thrombophilic patients. A recent study measured the antigen in a large kindred affected by PS deficiency documented by DNA analysis. The free antigen distinguished carriers from noncarriers of the PS deficiency much better than the total antigen in this family *(55)*. Although generalization of these results to other kindred groups is not possible, perhaps the free antigen, being closely related to the functional form of PS, is more useful than the total antigen to detect patients with PS deficiency.

APC RESISTANCE

APC resistance can be assessed in plasma with activated partial thromboplastin time (APTT)-based methods with and without APC, as originally described by Dahlback et al. *(4)*. These methods are simple and inexpensive. Furthermore, they are sensitive to the "APC resistance syndrome" as well as the FV Leiden mutation. It should be emphasized that FV Leiden accounts for most, but not all cases of APC resistance *(56)*. Another possibility is offered by APTT-based methods in which test plasma is prediluted with FV-deficient plasma *(57)*. This modification is highly (close to 100%) sensitive and specific for FV Leiden *(58, 59)* in both healthy controls and patients with suspected acute venous thromboembolism. Finally, a third possibility is DNA analysis to detect FV Leiden *(5)*. As mentioned, this does not cover all cases of APC resistance *(56)*. Recently, APC resistance has been reevaluated and was found to be an independent (from FV Leiden) risk factor for venous thrombosis *(60, 61)*. This suggests that the search for FV Leiden by DNA analysis, if used alone, would not identify all patients at risk. Therefore, we recommend measuring APC resistance by APTT-based methods with and without FV-deficient plasma and to confirm positive and borderline cases by DNA analysis for FV Leiden.

HYPERPROTHROMBINEMIA

Hyperprothrombinemia has been associated with the recently described mutation G20210A in the prothrombin gene (10). Here there are two options: DNA analysis to detect the mutation, and plasma analysis for prothrombinemia (10). Although hyperprothrombinemia is a risk factor for thrombosis independent from the presence of the gene mutation (10), it is unable to clearly distinguish carriers from noncarriers of the mutation (10). Therefore, if used alone it is not adequate to screen thrombophilic patients (62).

DYSFIBRINOGENEMIA

Thrombin and reptilase times should be used to screen the very rare cases of dysfibrinogenemia associated with an increased risk of thrombosis. Positive cases identified with these two simple tests should then be confirmed by parallel analysis of functional and immunoreactive fibrinogen. Typically, dysfibrinogenemia presents with normal or even higher than normal antigen and low functional activity.

ANTI-PHOSPHOLIPID ANTIBODY SYNDROME

The laboratory diagnosis of the anti-phospholipid syndrome is complicated by the lack of specific tests and the heterogeneity of the antibodies (11). Furthermore, problems with the tests available are compounded by the lack of standardization. To overcome these difficulties, the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis has issued consensus criteria that may be used to help laboratory diagnosis (63). Accordingly, thrombophilic patients should be screened both by phospholipid-dependent tests to detect LA and by assaying for anti-phospholipid antibodies with solid-phase ELISA tests to detect anti-cardiolipin (aCL) antibodies. According to the Scientific and Standardization Committee guidelines, LA may be considered if the following diagnostic criteria occur simultaneously (63): (a) one (or more) phospholipid-dependent test is prolonged; (b) the above prolongation is not corrected when plasmas from the patient and a healthy control are mixed; and (c) the prolongation recorded for patient plasmas is corrected by increasing the concentration of phospholipids in the test system. Among the phospholipid-dependent tests, APTT, kaolin clotting time (KCT), diluted Russell viper venom test (dRVVT), and diluted prothrombin time are the tests used most frequently to detect LA (11). Their sensitivities vary, and if used alone, none of them can ensure detection of all patients with LA. Specificity is also variable and limited. Recent evidence would suggest that the dRVVT is more specific than the KCT (or its modification) for those LAs associated with thrombosis (64). However, this awaits confirmation. Except for aCL antibodies (65), the value of the other ELISA tests (i.e., anti-β_2-glycoprotein I and anti-prothrombin) is still to be defined, and no precise recommendations have been issued on their use to inves-

tigate thrombophilic patients (66). In view of the above considerations, the present state of the art suggests relying on either KCT or its modifications (67) and dRVVT tests to detect LA, in addition to searching for aCL (IgG and IgM) antibodies. Positive results should be confirmed over time to ensure that the condition is not transient.

HYPERHOMOCYSTEINEMIA

Until recently, homocysteine has been measured mainly by HPLC-based methods with electrochemical or fluorometric detection (68). More recently, enzyme immunoassays (69) and fluorescence polarization immunoassays (70) have become commercially available. These methods are as reliable as the HPLC-based methods (71). However, they are more suited for use in general clinical laboratories because they require simpler instrumentation and less expertise than those required by HPLC.

FACTOR VIII

Either the antigen or the activity measurements have been used in the studies evaluating high factor VIII as a risk factor for venous thrombosis (17, 20). Therefore, assays for both the antigen and the activity are suitable for screening thrombophilic patients. The activity can be measured by coagulometers and APTT-based methods with factor VIII-deficient plasma, or by amidolytic methods (72). The antigen can be measured by ELISA-based methods. Whatever the choice, the cost per test is relatively high because of the requirement for factor VIII-deficient plasma or chromogenic substrate on one hand and commercial ELISA systems on the other.

Strategy to Make Screening Cost-Effective

The strategy used at present in thrombophilia testing is to investigate individually each of the hemostatic components known to be associated with increased risk of thromboembolism. This strategy is considerably more expensive and time-consuming than the strategy typically used to deal with bleeding in which global screening tests are first carried out and then single factor measurements are performed only on positive cases. This different approach is justified by the lack of screening tests able to globally investigate thrombophilia. Designing such tests is complicated and far-reaching because individual risk factors for thrombosis belong to regulatory systems that are apparently not linked or are only partially linked. These difficulties notwithstanding, attempts in this direction have been made, including development of a global test for the PC anticoagulant pathway (73) and measurement of the endogenous thrombin potential (74). In principle, the former could be taken as an index of the function of one of the anticoagulant pathways operating in plasma (including PC, PS, and APC resistance). The latter could be taken as an index of thrombin generation reflecting the overall balance of procoagulants and anticoagulants of plasma ex vivo. To be of value as screening tests, they must be sensitive enough to identify the vast

majority of patients who present with any of the defects of the regulatory systems associated with an increased risk of thromboembolism. The next section will be devoted to discussing the current situation with respect to the potential application of these two different approaches to investigate thrombophilia.

GLOBAL TESTS FOR THE PC ANTICOAGULANT PATHWAY
These tests have been investigated extensively because there are many commercial methods available that are based on the activation of endogenous PC by snake venom (e.g., Protac) and on the performance of paired clotting tests (PT, APTT, or dRVVT). The baseline clotting time (without snake venom) depends on the procoagulant strength of the plasma. The clotting time recorded when snake venom is added depends on the functionality of the PC anticoagulant pathway. It is prolonged over the baseline clotting time in healthy individuals, but not in patients with congenital deficiencies of PC or PS or with APC resistance. Numerous studies have been published over the years on the clinical evaluation of these tests. Almost invariably, their diagnostic efficacy has been found acceptable for PC deficiency and APC resistance, but not for PS deficiency, where variable proportions of patients with this deficiency were not identified by the global test *(75)*. Therefore, improvement of the sensitivity toward PS is required before these global tests can be proposed as suitable candidates to screen the PC anticoagulant pathway. Interestingly, a common feature of all of the studies is that the global test was abnormal in a considerable proportion of patients who presented with a history of thromboembolism with no identifiable specific defect *(75)*. The possibility that these tests are sensitive to as yet unidentified hemostatic defect(s) that impair the PC anticoagulant pathway is a reasonable explanation. Alternatively, it is possible that these tests are sensitive to subtle imbalances of the procoagulant/anticoagulant systems that occur without an apparent defect. A recent study published in abstract form has shown that an abnormality of the global test is indeed a risk factor for thrombosis independent from any of the specific abnormalities of the PC anticoagulant pathway *(76)*. Prospective studies are needed to evaluate the real value of these tests in the management of patients with thrombophilia.

THROMBIN POTENTIAL
Because thrombin generation is the ultimate event in the coagulation cascade that occurs shortly after activation of coagulation and before conversion of fibrinogen to fibrin, it is not surprising that the concept of a simple test able to record thrombin generation caused considerable interest at a time when the mechanisms of regulation of thrombogenesis were only partially understood *(77)*. However, it was not until recently that this concept has been developed by Hemker et al. *(78)* and adapted to the new technology (i.e., synthetic chromogenic substrate and automated analyzers). In a series of reports published over the last decade, these authors reported on how to record thrombin generation in vitro after activation of defibrinated plasma by means of intrinsic (cephalin) or extrinsic (tissue factor) activators. The area under the curve of thrombin generation recorded over time (also called thrombin potential) ultimately depends on the balance between procoagulants and anticoagulants *(78)*. Accordingly, the thrombin potential decreases in patients treated with antithrombotic drugs and increases in those at increased risk of thrombosis. To date, this concept has been tested in some groups known to have increased risk of thrombosis, such as women on oral contraceptives *(79)*, patients with the prothrombin G20210A mutation *(80)*, and patients treated with oral anticoagulants or heparin *(81)*. Modified tests in which APC was added to the test system have also been used to assess APC resistance in women taking oral contraceptives *(82)*. However, the complexity of the instrumentation needed to perform these tests *(83)* makes them relatively difficult to perform on a larger scale, and their value in the management of thrombophilic patients, albeit promising, is still to be evaluated.

Thrombophilia and Arterial Thrombosis
There is no solid evidence that congenital deficiencies of the main anticoagulant pathways of blood coagulation (including APC resistance) may increase the risk of arterial thrombosis. Although studies have shown that FV Leiden *(84)*, APC resistance *(85)*, or the prothrombin G20210A gene mutation *(86)* may be contributory risk factors for myocardial infarction or cerebrovascular disease in selected groups of patients, the most comprehensive prospective study, carried out on American physicians, showed that FV Leiden does not increase the risk of myocardial infarction or stroke *(87)*. Hence, laboratory screening for the above conditions in patients who present with arterial thrombosis should be considered of little value. In contrast, these patients should be investigated to detect anti-phospholipid antibodies *(11)*, hyperhomocysteinemia *(12)*, and dysfibrinogenemia *(8)*, which are frequently associated with arterial thrombosis. The value of investigation of fibrinogen (polymorphisms), factor VII (both polymorphisms and plasma concentrations), factor XIII, thrombomodulin, fibrinolysis, and platelet-membrane glycoprotein gene polymorphisms is still controversial [see Ref. *(88)* for review].

Conclusions
Thanks to the recent progress made in the understanding of regulatory mechanisms for thromboembolism, it is now possible to assess the risk of thrombosis associated with many thrombophilic conditions. Most of these conditions can be investigated by relatively simple laboratory methods. Although the cost of such investigations does not warrant screening of the general population, the careful selection of the patients to be investigated, of the timing of testing, and of the tests to be carried out may be of value

to open new perspectives for the management of thrombophilic patients and to prevent future events in patients who are affected by genetic defects but are still asymptomatic. Future efforts should be aimed at developing a strategy that makes testing more cost-effective. This might be achieved by developing screening tests able to assess globally the increased tendency toward thromboembolism seen in thrombophilic patients.

References

1. British Committee for Standards in Haematology. Guidelines on investigation and management of thrombophilia [Review]. J Clin Pathol 1990;43:703–10.

2. Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia. Part I [Review]. Thromb Haemost 1996;76:651–62.

3. De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes and management [Review]. Blood 1996;87:3531–44.

4. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C. Prediction of a cofactor for activated protein C. Proc Natl Acad Sci U S A 1993;90:1004–8.

5. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C [Letter]. Nature 1994;369:64–7.

6. Bertina RM, van der Linden IK, Engesser L, Muller HP, Brommer EJ. Hereditary heparin cofactor II deficiency and the risk of development of thrombosis. Thromb Haemost 1987;57:196–200.

7. Ariens RA, Alberio G, Moia M, Mannucci PM. Low levels of heparin-releasable tissue factor pathway inhibitor in young patients with thrombosis. Thromb Haemost 1999;81:203–7.

8. Haverkate F, Samama M. Familial dysfibrinogenemia and thrombophilia. Report on a study of the SSC subcommittee on fibrinogen [Review]. Thromb Haemost 1995;73:151–61.

9. Bauer KA. Conventional fibrinolytic assays for the evaluation of patients with venous thrombosis: don't bother [Editorial]. Thromb Haemost 2001;85:377–8.

10. 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 increase in venous thrombosis. Blood 1996;88:3698–703.

11. Triplett DA. Antiphospholipid-protein antibodies: laboratory detection and clinical relevance [Review]. Thromb Res 1995;78:1–31.

12. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis [Review]. Thromb Haemost 1999;81:165–76.

13. Malinow MR, Duell PB, Hess DL, Anderson PH, Kruger WD, Phillipson BE, et al. Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. New Engl J Med 1998;338:1009–15.

14. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease, probable benefits of increasing folic acid intakes. JAMA 1995;274:1049–57.

15. Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C. Leiden Thrombophilia Study. Lancet 1993;342:1503–6.

16. Meijers JCM, Tekelenburg WLH, Bouma BN, Bertina RM, Rosendaal FR. High levels of factor FXI as a risk factor for venous thrombosis. N Engl J Med 2000;342:696–701.

17. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet 1995;345:152–5.

18. Van Hylckama Vlieg A, van der Linden IK, Bertina RM, Rosendaal FR. High levels of factor FIX increase the risk of venous thrombosis. Blood 2000;95:3678–82.

19. Koster T, Rosendaal FR, Reitsma PH, van der Velden PA, Briet E, Vandenbroucke JP. Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case control study of plasma levels and DNA polymorphism. The Leiden Thrombophilia Study (LETS). Thromb Haemost 1994;71:719–22.

20. O'Donnell J, Tuddenham EG, Manning R, Kemball-Cook G, Johnson D, Laffan M. High prevalence of elevated factor VIII levels in patients referred for thrombophilia screening: role of increased synthesis and relationship to the acute phase reaction. Thromb Haemost 1997;77:825–8.

21. Kamphuisen PW, Lensen R, Houwing-Duistermaat JJ, Eikenboom JCJ, Harvey M, Bertina RM, Rosendaal FR. Heritability of elevated factor VIII antigen levels in factor V Leiden families with thrombophilia. Br J Haematol 2000;109:519–22.

22. Van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk of deep vein thrombosis. Blood 2000;95:2855–9.

23. Kamphuisen PW, Rosendaal FR, Eikenboom JCJ, Bos R, Bertina RM. Factor V antigen levels and venous thrombosis. Risk profile, interaction with factor V Leiden, and relation with factor VIII antigen levels. Arterioscler Thromb Vasc Biol 2000;20:1382–6.

24. Sarasin FP, Bounameaux H. Decision analysis model of prolonged oral anticoagulant treatment in factor V Leiden carriers with first episode of deep vein thrombosis. BMJ 1998;316:95–9.

25. Sanson BJ, Simioni P, Tormene D, Moia M, Friederich PW, Huisman MV, 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;94:3702–6.

26. Marchetti M, Pistoro A, Barosi G. Extended anticoagulation for prevention of recurrent venous thromboembolism in carriers of factor V Leiden: cost effectiveness analysis. Thromb Haemost 2000;84:752–7.

27. van den Belt AGM, Hutten BA, Prins MH, Bossuy PMM. Duration of oral anticoagulant treatment in patients with venous thromboembolism and a deficiency of antithrombin, protein C or protein S. A decision analysis. Thromb Haemost 2000;84:758–63.

28. Khamashta MA, Cuadrado MJ, Mujic F, Taub NA, Hunt BJ, Hughes GRV. The management of thrombosis in the antiphospholipid antibody syndrome. New Engl J Med 1995;332:993–7.

29. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet 1995;346:1133–4.

30. Rosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, et al. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost 1998;79:706–8.

31. Koeleman BPC, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C deficient families. Blood 1994;84:1031–5.

32. Zoller B, Berntsdotter A, Garcia de Frutos P, Dahlback B. Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. Blood 1995;85:3518–23.

33. Van Boven HH, Reitsma PH, Rosendaal FR, Bayston TA, Chowdhury V, Bauer KA, et al. Factor V Leiden (FVR506Q) in families with inherited antithrombin deficiency. Thromb Haemost 1996;75:417–21.

34. Ridker PM, Hennekens CH, Selhub J, Miletich JP, Malinow MR, Stampfer MJ. Interrelation of hyperhomocyst(e)inemia, factor V

Leiden, and the risk of future venous thromboembolism. Circulation 1997;95:1777–82.

35. Vandenbroucke JP, Koster T, Briet E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral contraceptive users who are carriers of factor V Leiden. Lancet 1994;344:1453–7.

36. Martinelli I, Taioli E, Bucciarelli P, Akhavan S, Mannucci PM. Interaction between the G20210A mutation of the prothrombin gene and oral contraceptive use in deep vein thrombosis. Arterioscler Thromb Vasc Biol 1999;19:700–3.

37. Vandenbroucke JP, van der Meer FJM, Helmerhorst FM, Rosendaal FR. Factor V Leiden: should we screen oral contraceptive users and pregnant women? BMJ 1996;313:1127–30.

38. Preston FE, Kitchen S, Jennings I, Woods TAL. A UK National External Quality Assessment Scheme (UK NEQAS) for molecular genetic testing for the diagnosis of familial thrombophilia [Letter]. Thromb Haemost 1999;82:1556–7.

39. Demers C, Henderson P, Blajchman MA, Wells MJ, Mitchell L, Johnston M, et al. An antithrombin III assay based on factor Xa inhibition provides a more reliable test to identify congenital antithrombin III deficiency than an assay based on thrombin inhibition. Thromb Haemost 1993;69:231–5.

40. Conard J, Bara L, Horellou M, Samama MM. Bovine or human thrombin in amidolytic at III assays. Influence of heparin cofactor II. Thromb Res 1986;41:873–8.

41. Tripodi A, Krachmalnicoff A, Mannucci PM. Characterization of an abnormal antithrombin (Milano 2) with defective thrombin binding. Thromb Haemost 1986;56:349–52.

42. Faioni EM, Franchi F, Asti D, Mannucci PM. Resistance to activated protein C mimicking dysfunctional protein C: diagnostic approach. Blood Coagul Fibrinolysis 1996;7:349–52.

43. De Moerloose P, Reber G, Bouviar CA. Spuriously low levels of protein C with Protac activation clotting assay [Letter]. Thromb Haemost 1988;59:543.

44. Faioni EM, Hermida J, Rovida E, Razzari C, Asti D, Zenali S, et al. Type II protein C deficiency: identification and molecular modeling of two natural mutants with low anticoagulant and normal amidolytic activity. Br J Haematol 2000;108:265–71.

45. Bovill EG, Tomczak JA, Grant B, Bhushan F, Pillemer E, Rainville IR, et al. Protein C$_{vermont}$: symptomatic type II protein C deficiency associated with two GLA domain mutations. Blood 1992;79:1456–65.

46. Preda L, Tripodi A, Valsecchi C, Lombardi A, Finotto E, Mannucci PM. A prothrombin time-based functional assay of protein S. Thromb Res 1990;60:19–32.

47. Wolf M, Boyer-Neumann C, Martinoli JL, Leroy-Matheron C, Amiral J, Meyer D, et al. A new functional assay for human protein S activity using activated factor V as substrate [Letter]. Thromb Haemost 1989;62:1144–5.

48. Faioni EM, Franchi F, Asti D, Sacchi E, Bernardi F, Mannucci PM. Resistance to activated protein C in nine thrombophilic families: interference in a protein S functional assay. Thromb Haemost 1993;70:1067–71.

49. Faioni EM, Boyer-Neumann C, Franchi F, Wolf M, Meyer D, Mannucci PM. Another protein S functional assay is sensitive to resistance to activated protein C [Letter]. Thromb Haemost 1994;72:648.

50. Dahlback B. The protein C anticoagulant system: inherited defects as basis for venous thrombosis. Thromb Res 1995;77:1–43.

51. Tripodi A, Bertina RM, Conard J, Pabinger I, Sala N, Mannucci PM. Multicenter evaluation of three commercial methods for measuring protein S antigen. Thromb Haemost 1992;68:149–54.

52. Malm J, Laurell M, Dahlback B. Changes in the plasma levels of vitamin-K dependent proteins C and S and of C4b-binding protein during pregnancy and oral contraception. Br J Haematol 1988;68:437–43.

53. Amiral J, Grosley B, Boyer-Neuman C, Marfaing-Koka A, Peynaud-Debayle E, Wolf M, et al. New direct assay of free protein S antigen using two distinct monoclonal antibodies specific for the free form. Blood Coagul Fibrinolysis 1994;5:179–86.

54. Giri TK, Hillarp A, Hardig Y, Zoller B, Dahlback B. A new direct, fast and quantitative enzyme-linked ligandsorbent assay for measurement of free protein S antigen. Thromb Haemost 1998;79:767–72.

55. Simmonds RE, Ireland H, Lane DA, Zoller B, Garcia de Frutos P, Dahlback B. Clarification of the risk for venous thrombosis associated with hereditary protein S deficiency by investigation of a large kindred with a characterized gene defect. Ann Intern Med 1998;128:8–14.

56. Zoller B, Svensson PJ, He X, Dahlback B. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. J Clin Invest 1994;94:2521–4.

57. Jorquera JI, Montoro JM, Fernandez MA, Aznar J. Modified test for activated protein C resistance [Letter]. Lancet 1994;344:1162–3.

58. Tripodi A, Negri B, Bertina RM, Mannucci PM. Screening for the FV:Q506 mutation. Evaluation of thirteen plasma-based methods for their diagnostic efficacy in comparison with DNA analysis. Thromb Haemost 1997;77:436–9.

59. Svensson PJ, Zoller B, Dahlback B. Evaluation of original and modified APC-resistance tests in unselected outpatients with clinically suspected thrombosis and in healthy controls. Thromb Haemost 1997;77:332–5.

60. de Visser MCH, Rosendaal FR, Bertina RM. A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis Blood 1999;93:1271–6.

61. Rodeghiero F, Tosetto A. Activated protein C resistance and factor V Leiden mutation are independent risk factors for venous thromboembolism. Ann Intern Med 1999;130:643–50.

62. Grunewald M, Germowitz A, Beneke H, Guethner C, Griesshammer M. Coagulation factor II activity determination is not useful as a screening tool for the G20210A prothrombin gene allele [Letter]. Thromb Haemost 2000;84:141–2.

63. Brandt JT, Triplett DA, Alving B, Scharrer IM. Criteria for the diagnosis of lupus anticoagulants: an update [Review]. Thromb Haemost 1995;74:1185–90.

64. Galli M, Finazzi G, Bevers EM, Barbui T. Kaolin clotting time and dilute Russell's viper venom time distinguish between prothrombin-dependent and $\beta2$-glycoprotein I-dependent antiphospholipid antibodies. Blood 1995;86:617–23.

65. Loizou S, McCrea JD, Rudge AC, Reynolds R, Boyle CC, Harris EN. Measurement of anticardiolipin antibodies by an enzyme-linked immunosorbent assay: standardization and quantitation of results. Clin Exp Immunol 1986;62:738–45.

66. Carreras LO, Forastiero RR, Martinuzzo ME. Which are the best biological markers of the antiphospholipid syndrome? [Review]. J Autommun 2000;15:163–72.

67. Chantarangkul V, Tripodi A, Arbini A, Mannucci PM. Silica clotting time (SCT) as a screening and confirmatory test for detection of the lupus anticoagulants. Thromb Res 1992;67:355–65.

68. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications [Review]. Clin Chem 1993;39:1764–79.

69. Frantzen F, Faaren AL, Alfheim I, Nordhei AK. Enzyme conversion immunoassay for determining homocysteine in plasma or serum. Clin Chem 1998;44:311–6.

70. Shipchandler MT, Moore EG. Rapid, fully automated measurement

of plasma homocyst(e)ine with Abbott IMx analyzer. Clin Chem 1995;41:991–4.

71. Tripodi A, Chantarangkul V, Lombardi R, Lecchi A, Mannucci PM, Cattaneo M. Multicenter study of homocysteine measurement. Performance characteristics of different methods, influence of standards on interlaboratory agreement of results. Thromb Haemost 2001;85:291–5.

72. Tripodi A, Mannucci PM. Factor VIII activity as measured by an amidolytic assay compared with one-stage clotting assay. Am J Clin Pathol 1986;86:341–4.

73. Robert A, Eschwege V, Hameg H, Drouet L, Aillaud MF. Anticoagulant response to *Agkistrodon contortrix* venom (AVC test): a new global test to screen for defects in the anticoagulant protein C pathway. Thromb Haemost 1996;75:562–6.

74. Hemker HC, Willems GM, Beguin S. A computer assisted method to obtain the prothrombin activation velocity in whole plasma independent of thrombin decay process. Thromb Haemost 1986; 56:9–17.

75. Tripodi A, Akhavan S, Asti D, Faioni EM, Mannucci PM. Laboratory screening of thrombophilia. Evaluation of the diagnostic efficacy of a global test to detect congenital deficiencies of the protein C anticoagulant pathway. Blood Coagul Fibrinolysis 1998;9:485–9.

76. Rosendaal FR, van der Meer FJM, Visser TH, Wagner C. ProcC global screening test and the risk of thrombosis [Abstract]. Thromb Haemost 1999;(Suppl):731.

77. Denson KWE, Biggs R. Laboratory diagnosis, tests of clotting function and their standardization. In: Biggs R, ed. Human blood coagulation haemostasis and thrombosis. Oxford: Blackwell Scientific Publication, 1972:278–332.

78. Hemker HC, Wielders S, Kessels H, Beguin S. Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. Thromb Haemost 1993;70:617–24.

79. Rotteveel RC, Roozendaal KJ, Eijsman L, Hemker HC. The influence of oral contraceptives on the time integral of thrombin generation (thrombin potential). Thromb Haemost 1993;70:959–62.

80. Kyrle PA, Mannhalter C, Beguin S, Stumpflen A, Hirschl M, Weltermann A, et al. Clinical studies and thrombin generation in patients homozygous or heterozygous for the G20210A mutation in the prothrombin gene. Arterioscler Thromb Vasc Biol 1998;18:1287–91.

81. Wielders S, Manjari M, Michiels J, Rijkers DTS, Cambus JP, Knebel RWC, et al. The routine determination of the endogenous thrombin potential, first results in different forms of hyper- and hypocoagulability. Thromb Haemost 1997;77:629–36.

82. Rosing J, Middeldorp S, Curvers J, Thomassen MCLGD, Nicolaes GAF, Meijers JCM, et al. Low dose oral contraceptives and acquired resistance to activated protein C: a randomized cross-over study. Lancet 1999;354:2036–40.

83. Hemker HC, Beguin S. Phenotyping the clotting system [Review]. Thromb Haemost 2000;84:747–51.

84. Rosendaal FR, Siscovick DS, Schwartz SM, Beverly RK, Psaty BM, Longstreth WT, et al. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. Blood 1997;89:2817–21.

85. Van der Bom JG, Bots ML, Haverkate F, Slagboom PE, Meijer P, de Jong PTVM, et al. Reduced response to activated protein C is associated with increased risk for cerebrovascular disease. Ann Intern Med 1996;125:265–9.

86. De Stefano V, Chiusolo P, Paciaroni K, Casorelli I, Rossi E, Molinari E, et al. Prothrombin G20210A mutant genotype is a risk factor for cerebrovascular ischemic disease in young patients. Blood 1998;91:3562–5.

87. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisemberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. New Engl J Med 1995;332:912–7.

88. Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombosis. Blood 2000;95:1517–32.