Laboratory Investigation of Thrombophilia

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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.

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

<table>
<thead>
<tr>
<th>Congenital conditions</th>
<th>Acquired conditions</th>
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<tr>
<td>AT deficiency</td>
<td>Anti-phospholipid antibodies</td>
</tr>
<tr>
<td>PC deficiency</td>
<td>Hyperhomocysteinemia attributable to vitamin deficiency (or other non-genetic causes)</td>
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<tr>
<td>PS deficiency</td>
<td>APC resistance, not attributable to gene mutations</td>
</tr>
<tr>
<td>Dysfibrinogenemia</td>
<td>Increased factor VIII</td>
</tr>
<tr>
<td>FV Leiden mutation</td>
<td>Hyperhomocysteinemia attributable to congenital deficiency of CBS, MS, and MTHFR</td>
</tr>
<tr>
<td>Prothrombin G20210A mutation</td>
<td></td>
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<tr>
<td>Hyperhomocysteinemia attributable to congenital deficiency</td>
<td></td>
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<tr>
<td>of CBS, MS, and MTHFR</td>
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| 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* (>1220 units/L)             | 2.0 (22)                        |

* TAFI, thrombin-activatable fibrinolysis inhibitor.

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 B12), 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
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). How-
ever, 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 condi-
tion 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 de-
fects 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 thrombo-
embolism associated with these two conditions (10,15), it must be concluded that a general screening is not war-
ranted. Therefore, testing should be performed only in patients who have a history of unexplained thromboem-
bolism. 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* and types of assays to be included in the laboratory investigation of thrombophilia.

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

* Markers and types of assay listed here represent the authors’ choice. The reasons for choice are motivated and discussed in the text.
* 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 condition 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-β2-glycoprotein I and anti-prothrombin) is still to be defined, and no precise recommendations have been issued on their use to investigate 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 debrinated 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
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