

Guidelines for the Laboratory Investigation of Inherited Thrombophilias. Recommendations for the First Level Clinical Laboratories

European Communities Confederation of Clinical Chemistry and Laboratory Medicine (EC4) Working Group on Guidelines for Investigation of Disease

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Recent advances in the laboratory diagnostic approach to inherited thrombophilia call for an update on laboratory strategies and organization. The present paper therefore deals in particular with: the panel test choice, timing and test appropriateness, and analytical methods in several clinical conditions. Specific recommendations are supported by the state-of-the-art in this branch. Clin Chem Lab Med 2003; 41(3):382–391

Key words: Clinical laboratory methods; Coagulation disorders; Coagulation inhibitors; Guidelines; Thrombophilia.

Abbreviations: APC, activated protein C; APCR, activated protein C resistance; MTHFR, methylenetetrahydrofolate reductase; SR, sensitivity ratio; TAFI, thrombin activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; VTE, venous thrombo-embolism.

Introduction

The aim of this document is to provide clinical laboratories with standard, evidence-based guidelines for the diagnosis of inherited thrombophilias, while focusing on the organizational and methodological aspects of laboratories, particularly clinical non-specialist laboratories involved in the first approach for thrombophilic subjects, here defined as "first level clinical laboratories". This aim is considered particularly relevant in view of: 1) the annual incidence of venous thrombo-embolism (VTE), which ranges from 20.8 to 145 cases per 100000 members of the general population in western countries (1–3); 2) and the progress recently made, with a wider availability of new laboratory techniques (4–6).

The present guidelines are limited to laboratory procedures for the clinical identification of genetically transmitted thrombophilia. Acquired conditions or other risk factors for venous and/or arterial thrombosis are not considered.

Until now, the genetic causes of thrombosis have been investigated in the setting of familial studies.

Findings in the larger studies have yielded limited information on the prevalence of abnormalities, and few studies therefore contain enough evidence for reliable conclusions to be drawn (7). As some genetically transmitted abnormalities are known to be risk factors for venous thrombosis, having a causal association with the disease (8), the laboratory identification of these factors is of particular importance.

Table 1 shows the evidence levels and recommendation grades used in this paper, as suggested by several societies, with some modifications (9, 10). In order to assign a more appropriate recommendation for the assay, we here include the principle that if a specific conclusion is reached in clinical trials using a specific laboratory method, the application of another method that is well correlated with the former method decreases the evidence level and, consequently, the related recommendation grade.

Table 1 Evidence-based grading system for a laboratory test.

Evidence levels	
Level I	Results from randomized controlled trials, confirmed in separate studies. Conclusions are applicable if the same test method is used.
Level IIa	Results from prospective cohort and case-control studies (applicable only to the same method).
Level IIb	Results from well-designed studies or case-control studies without randomization (applicable only to the same method).
Level III	Retrospective cohort studies. Study of sensitivity and specificity of a diagnostic test. Population-based descriptive studies. Opinions of experts. Level I or II evidence of study design but with the use of a different laboratory method well correlated to the first clinically investigated assay.
Recommendation grades	
Grade A	Based on level I of evidence
Grade B	Based on level II (a or b) of evidence
Grade C	Based on level III of evidence

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Defects

Antithrombin heterozygous deficiency occurs in about 1 in 2500 (0.04%) members of the general population (11). It has been estimated that the risk of developing a first episode of VTE is increased by 5- to 20-fold in heterozygous carriers (evidence level IIa) (12, 13). In type I deficiency, both the antigen and its activity are reduced; in type II deficiency, antithrombin activity is reduced, but the antigen is normal; type III, which is extremely rare, is due to a homozygous deficiency. A variant, IIc, with a defective heparin-binding site has also been identified; this is more frequent, but is associated with a lower risk, or has no effect on risk in its heterozygous form (8). The laboratory diagnosis is based on the finding of an antithrombin activity level of about 50% of the normal plasma value. As over 50 mutations have been identified (13, 14), any genetic approach to the diagnosis is difficult. The possibility of an acquired reduction in the antithrombin level must therefore be carefully considered when interpreting results in a clinical setting.

Protein C deficiency, studied in unselected patients, confers a 6.5- to 10-fold increase in the relative risk of VTE (12, 13, 15, 16) (evidence level IIa). The prevalence of the defect in healthy individuals is 0.2 to 0.3% (17, 18); type I and a type II deficiency have been identified and both forms are associated in most cases with a 50% reduction in activity. Several clinical conditions can reduce the protein C level and in some cases the laboratory diagnosis takes into account the demonstration of familial transmission. Numerous mutations have been found (19).

The prevalence of *protein S* deficiency, reflected in a low free protein S plasma concentration, was found to be 2.1% (16) in a Dutch population, 1.3% in an Italian population (20) (both with evidence level IIb), and 5.7% in a Spanish population (14) (level III). In a large Scottish study in which this type of deficiency was defined as a persistent value under the 1st percentile of a normal population, the prevalence of protein S free antigen deficiency was estimated to range from 0.03% to 0.13% (21). Numerous conditions, including inflammatory diseases, can decrease its concentration. It is difficult to interpret results in the different studies undertaken because the protein S level is reduced by several exogenous factors: age, sex, oral contraceptive use, and hormonal status (21). The true frequency of the inherited deficiency has not been investigated in the general population, but it is probably similar to that of protein C (0.3%). The relative risk due to reduced protein S levels is not clear. Some studies suggest a value of about 2 (22) (evidence level IIb). In other studies, however, the estimated odds ratio is almost 10 (12), especially if only family members with hereditary defects are assessed (13, 15). There is a type I and II defect, and also a type III defect, which is characterized by normal total protein S antigen but presents a reduction in the free protein S antigen and in the functional activity.

The prevalence of *factor V Leiden, activated protein C resistance (APCR)*, the most common known defect

in European countries, ranges from 2 to 15% in Caucasian populations (23), and is greater in northern European than in southern European populations, with an ethnic distribution (24). In the heterozygous form, there is a 3- to 8-fold increase in the risk of VTE (25, 26). In the homozygous form, the increase is 80-fold (27) (evidence level IIb). In a population with thrombosis, a prevalence of 20% has been found (25).

In the Caucasian population, the *G20210A prothrombin gene mutation* is detected in about 2% of subjects, with wide geographic differences (28). The odds ratio for VTE, estimated to be 2.5, is higher in relatives of thrombophilic patients (evidence level IIb) (6, 29, 30).

The prevalence of *moderate hyperhomocysteinemia*, a genetic risk factor acting in association with environmental factors (31, 32), is estimated at 5 to 25% in European population studies (33, 34). A common genetic cause of this condition is a variant of the methylenetetrahydrofolate reductase (*MTHFR*) gene (677C to T) that corresponds to a thermolabile variant of the enzyme, expressed only if folate intake is inadequate (35). It is not yet completely clear whether the genetic or the acquired condition leads to the risk of thrombosis; this issue is therefore included in the guidelines.

The combination of two defects or more increases the risk of thrombosis, and this is a relevant clinical condition. Other biochemical abnormalities associated with thrombophilia are either very rare (e.g., dysfibrinogenemia) or their role in the pathogenesis of VTE is not known as they have not been well investigated as risk factors. This category includes the plasminogen defect (36, 37), heparin cofactor II deficiency (38), fibrinolytic imbalance, increased histidine-rich glycoprotein and thrombin activatable fibrinolysis inhibitor (TAFI) (39), thrombomodulin abnormalities, tissue factor pathway inhibitor (TFPI) and factor XII deficiencies (40). Other conditions are affected by pre-analytical variables (7), thus their use is ineffective in the individual patient; these include the increased factor VIII (41, 42), IX (43), XI (44). All these conditions can be assessed in a second level laboratory in selected cases.

The demonstration of a single defect appears inadequate for defining a thrombophilic status. More recently, a preference has been shown for the model of venous thrombosis with a multi-factorial basis, which is also influenced by other genetic polymorphisms and environmental factors (7). It is therefore important to identify the association between factors in the same subject, and it must be borne in mind that several familial defects with a potential role have not yet been identified.

Subjects who require screening

Screening for the general population is not justified (recommendation Grade C). All subjects with a confirmed episode of VTE must be considered for thrombophilia tests if acquired causes, such as cancer, have been ruled out, and at least one of the following is present: family history of VTE, recurrent idiopathic thrombosis, early age at onset, thrombosis after trivial provo-

cation or in unusual sites (recommendation Grade B) (45). It is also important to screen other first degree family members of a positive symptomatic patient, as the decision on treatment can be influenced by the following situations (recommendation Grade B): a combined oral contraceptive or hormonal replacement therapy is prescribed (46); surgery is to be performed (recommendation Grade C) (7). Screening is not cost-effective in asymptomatic subjects, particularly in women on oral contraceptives or hormone-replacement therapy (47).

When screening is required

In general, the clinical management of an acute thrombotic event is not influenced by the immediate demonstration of a specific defect, so the biochemical study can be postponed until the initial treatment period is over. The acute phase can produce misleadingly low functional protein C and protein S results with some commercial assays; also the use of heparin or oral anticoagulants can modify the results of these tests. Only genotyping tests are reliable at this stage.

Antithrombin and protein C determination are also useful during the acute phase in order to decide whether replacement therapy with antithrombin and protein C concentrates is to be given.

Laboratory Organization

The role of the clinical laboratory in the approach to these situations can be divided into two parts: screening capability and defect characterization. In particular, clinical laboratories must offer diagnostic tools for the former (recommendation Grade C) in view of the relatively high incidence of VTE, the frequency of inherited disorders, and their association with the onset of VTE. The main role of the laboratory in this first approach is to allow the selection of patients for referral to a second level laboratory.

In all cases, specific anamnestic information must be collected and reported by the prescriber or by the laboratory staff in order to facilitate a comprehensive interpretation of results: for familial thrombotic diseases, previous and current VTE, fetal loss, respiratory and cardiovascular diseases, acute ophthalmic defects, post-phlebotic syndrome, anticoagulant and hormonal therapy, and also, at the pediatric age, neurological symptoms.

Blood sampling

It is preferable to use a system involving direct collection of blood into a tube containing the anticoagulant; if a syringe is used, a small volume (≤ 20 ml) is recommended in order to prevent blood coagulation or platelet activation, without the application of excessive suction strength, as this would cause sample hemolysis (48).

As the blood container should not have an activating surface, plastic tubes are recommended; siliconed

glass tubes are also allowed, although care should be taken on filling to avoid the activation of platelets. If multiple specimens are collected, the coagulation specimen should be collected into the second or third tube.

In some cases, the blood specimen is drawn from an indwelling catheter: this can produce an incorrect volume (air leakage), sample dilution by infusate solution, or contamination with anticoagulants: at least the first 5 ml of blood, or six dead space volumes, should be discarded (49).

Determinations for coagulative or chromogenic tests call for the use of sodium citrate as the anticoagulant with a 1:10 blood dilution. A 0.109 or 0.105 M concentration is recommended (48) (Grade C). The citrate concentration of 0.129 M must be discharged because it is known to interfere with the correct standardization of one-stage coagulation tests, and it is reasonable to assume that it also influences coagulative methods for thrombophilia screening. However, the lower the citrate concentration, the greater the tolerance to tube filling errors (50).

If plasma is required, the routine coagulation testing procedure can be used in centrifugation, aliquoting, and freezing: when storing plasma for coagulation tests particular care must be taken to avoid platelet contamination with double centrifugation or with plasma filtration.

The plasma homocysteine concentration in whole blood samples continues to rise very early. For this, two strategies are available: 1) cooling the blood immediately after venipuncture, placing the sample tube with EDTA on ice, and separating the plasma within 2 hours, or 2) drawing the blood into an acidified citrate tube that inhibits the red blood cell production of homocysteine (51).

Sample preservation

The cool preservation of plasma and blood samples is widely used, but this practice has not been well documented. Some studies show that antithrombin and protein C can be stored for 7 days at 6 °C. Protein S activity decreases by 12% at 8 hours' storage at 6 °C (52, 53). If the tests are not performed immediately, a rapid -20 °C or -80 °C cooling of plasma samples (and blood if required) is preferable (Grade C). No evidence is available on plasma storage for up to 3 months.

The assays

For the first level laboratory organization, at least one test should be available for each defect (Table 2). In all cases, repeat tests must be performed to investigate any abnormal phenotypic findings, so as to reach a reliable conclusion (recommendation Grade C) (7).

A correct interpretation of results also calls for overall screening for coagulative pathways, which should be performed with specific assays: prothrombin time test results can show the degree of hepatic protein synthesis and indicate the effects of any anticoagulant treatment; an APTT can reveal heparin therapy or con-

Table 2 Conditions involved in inherited thrombophilia.

Defects of proven value	Still not completely defined	Poorly defined defects
Antithrombin	Increased factor VIII	Plasminogen
Factor V Leiden	Increased factor VII	Heparin co-factor II
Protein C	Increased factor IX	Fibrinolytic imbalance
Protein S	Increased factor XI	Histidin-rich
Mutant prothrombin		Glycoprotein
Hyperhomocysteinemia		TAFI
Dysfibrinogenemia		TFPI
		Factor XII deficiency
		HR2 haplotype
		Thrombomodulin gene polymorphism

tamination that could interfere with some functional assays (54). A complete blood count with hematocrit and platelet count is also recommended. In some cases it is important to know the functional status of the liver, using appropriate laboratory tests. In clinical practice, a laboratory assessment for lupus-like anticoagulant is often associated with thrombophilia tests: this provides important information also for the interpretation of the level of inhibitors, but this aspect is not dealt with in the present document.

The simplest possible organization of the analytical test panel is achieved using a single global test for the protein C pathway and an antithrombin functional assay, a configuration that can be sufficient in a laboratory with a limited workload. In fact, these tests include the most serious (antithrombin) and the most frequent (APCR) defects, but they are quite insensitive for protein S activity < 60 u/dl (55) (evidence level III); nor can they detect mutant prothrombin or moderate hyperhomocysteinemia. Patients whose samples are negative must therefore be referred to another laboratory for complete screening. This procedure cannot be recommended because of lack of evidence.

The recommended specific tests

Antithrombin

All defects can be approached with a chromogenic heparin cofactor activity assay (recommendation Grade B) that measures the capacity of the plasma to inhibit an amount of factor enzyme added to the system. For this, two detection systems are available: anti-IIa and anti-Xa. The anti-Xa tests can also reveal any defective heparin-binding site. As this is a minor defect (19), an anti-IIa assay is sufficient for a screening procedure. Alternatively, the heparin-binding site variants can be detected by modifying the test protocol, choosing a short incubation phase with a low concentration of heparin. This methodology is reserved for widening analysis.

The measurement of antigenic protein can be useful in selected or positive cases in order to differentiate between type I and type II defects. Further second-level characterization of the antithrombin defect calls for bidimensional electrophoresis, with and without heparin added, to define the defective phenotype.

Protein C

At the first level, a functional assay using a protein C activator derived from snake venom is recommended (recommendation grade B) (54). Clotting and/or chromogenic detection are useful (56) but have some differences: the chromogenic test is simpler and more stable, and is suitable for type I and type II defects, but in some cases it is of limited sensitivity. With the clotting methods, a misleadingly low protein C level may be obtained in the presence of factor V Leiden (57). Moreover, protein C may be underestimated in patients with elevated factor VIII levels (58) or anti-phospholipid antibodies (59). In the first screening, the chromogenic test is preferable (55). About 10% of type II defects (defined type IIb) may be lost; the choice of the functional test to be used depends on the clinical context of each different laboratory. A complementary additional test is an antigenic assay for protein C, which is useful in differentiating between the different types of deficiency.

Protein S

The first test recommended for deficiency screening is the free protein S antigenic assay (recommendation Grade B) (60). Several techniques are available for this purpose: the most commonly used test is the measurement of protein S after separation of the C4bBP-bound protein S by precipitation with polyethylene glycol. The method, employed for antigen detection in large patient series, is an enzyme-linked immuno-adsorbent assay (ELISA); the "Laurell" test, suitable for both total and free protein S, is affected by imprecision problems. Rapid latex particle turbidimetric assays, using monoclonal antibodies for distinct epitopes of free protein S, have been validated only in analytical correlation studies. Further studies should therefore be conducted to test their validity in a clinical setting.

Functional protein S assays (clotting), which are sensitive to the three types of defects, are widely employed, being a valid alternative to antigenic free protein assay. However, their use has not yet been well documented.

Some factors affect the specificity of functional tests: reduced levels are observed in subjects on hormonal

therapy (61), in carriers of activated protein C (APC) resistance (62), and in some patients with anti-phospholipid antibodies. Low values should therefore be further investigated by means of the immuno-reactive assay of free protein S (54) (Grade C) if a functional protein S assay has been used in the initial screening test.

Further complementary tests are immunoassay for total protein S and C4b-binding protein measurement.

Factor V Leiden

The screening test employed is APC resistance. The most commonly used test is based on the prolongation of an APTT if activated protein C is added. The resultant expression is the sensitivity ratio (SR) (APC-SR). It is not advisable to normalize the data by dividing the ratio by the APC-SR of a pooled normal plasma, because this carries a risk of including a single factor V Leiden carrier in the pool, thus markedly decreasing sensitivity (63). Some variants of the original test, with different sensitivities, are available on the market. There is evidence that a correction with factor V-deficient plasma improves tests up to a 100% sensitivity for the Leiden mutation (64). This approach is therefore recommended (Grade C).

Another test used is based on Xa clotting time with and without APC. This test, also expressed as a sensitivity ratio, can be improved by diluting the unknown sample with factor V-deficient plasma. It appears to have a high sensitivity to the genetic defect (64).

A genetic assay for factor V Leiden mutation is also available in diagnostic kits and can be used instead of the APC resistance test if cost considerations suggest that it is advisable. Examples of methodologies are: restriction endonuclease digestion of PCR amplicons, allele-specific PCR, and allele-specific oligonucleotide probe hybridization (66) (Grade C).

The clinical relevance of a non-Leiden APC resistance is currently under discussion, and in such cases a thrombophilic condition is suspected, but has not yet been demonstrated.

G20210A prothrombin gene mutation

This defect can be detected only through DNA analysis, usually undertaken using a PCR-based method; pro-

thrombin levels, which are increased in the carriers of this condition, are not suitable for screening (evidence level III) (67).

Hyperhomocysteinemia

The HPLC method has been used in most clinical studies demonstrating an increased risk of VTE associated with fasting hyperhomocysteinemia (evidence level IIa). Enzyme immunoassay and fluorescence polarization immunoassay show a good correlation with HPLC (68), but there is a lack of documentation justifying their use in clinical practice. Baseline fasting measurement is recommended for the detection of hyperhomocysteinemia. It is also important to check that the patient has not modified his or her diet in the weeks preceding blood collection, in particular with vitamin supplementation. Post-methionine oral load homocysteine determination should also be performed in order to identify any defect in the metabolic pathway, but the value of this test in the assessment of thrombophilic risk has not yet been established. Mutations have been identified in some of the methionine pathway enzymes, in particular the common thermolabile MTHFR variant, but these tests are not indicated for thrombophilia screening because they are not associated with VTE.

The tests recommended are summarized in Table 3.

Calibration

A WHO calibrator is available for antithrombin (1st IRP 72/1). Internationally accepted standards have been proposed for protein C (1st IS 86/622) and protein S (1st IS 93/590). Commercial materials must be tested against these standards (54). It is preferable to avoid the use of a normal plasma pool as a calibrator (Grade C).

Analytical performance

For homocysteine, an inter-individual CV of 32.9% has been reported in an Australian population (69) and of 9.4% in a limited Caucasian Dutch population (evidence Grade C). Data for protein C and S variability have been studied in a large British (Scottish) population: inter-individual CVs were 17.5% for protein C (70)

Table 3 Methods recommended for first level diagnostic laboratory.

Defect	Screening assay	Alternative procedure	Complementary test
Antithrombin	Chromogenic anti IIa	Chromogenic anti Xa	Antigen assay Two-dimensional IEF
Factor V Leiden	APC-SR with factor V-deficient plasma dilution	Polymorphism 1691 of factor V gene	Polymorphism 1691 of factor V gene
Protein C	Coagulative assay	Chromogenic assay	Protein C antigen assay
Protein S	Antigen free assay	Functional coagulative	Total and free antigenic assay
Mutant prothrombin	Polymorphism of factor II gene		
Hyperhomocysteinemia	HPLC	Immunoassay	Polymorphism of <i>MTHFR</i> gene

and 22% and 24% for protein S total and free antigen, respectively (71). We therefore recommend a maximal CV of 10% or less for QC inter-assays for these tests (recommendation Grade C).

Internal quality assurance and participation in external quality assessment schemes, or proficiency test programs, are mandatory for all assays (54).

Results report

The ISTH-IUPAC-IFCC (72) has issued recommendations for reporting and naming components and units (Grade C).

Reference ranges for coagulation inhibitors must be calculated locally by age and sex (73). If a complete screening test is performed, we recommend that an annotation should be made on an appropriate form in order to facilitate the correct interpretation of results. In all cases in which screening is positive, a second analysis must be made in a newly collected blood sample in order to confirm the results (Grade C) (7). First-degree relatives must also be invited by the laboratory staff to undergo appropriate tests.

Results interpretation

As it is difficult in some cases to correctly interpret thrombophilia test results, any interpretation must be made in the light of individual clinical circumstances and under the supervision of an experienced clinician or clinical scientist (54) (Grade C). In particular, it is important to consider all the factors that may affect the tests, including age, sex-related variations, liver function, hormonal status, pregnancy, acute phase response to inflammatory diseases, and anticoagulant treatment.

The laboratory must establish reference ranges for all the assays and tests used, but it is of utmost importance to define the optimal cut-off value for discriminating between carriers and non-carriers of a defect (74). Although this interpretation is usually easy for antithrombin, protein C, and APC-SR modified with factor V-deficient plasma, this process can be very complicated in the presence of decreased functional protein S levels. It is therefore advisable to repeat blood sampling and testing in all cases of positive or doubtful results obtained with non-genotyping tests. Moreover, a deficiency can be definitively demonstrated through the transmission study of relatives. This applies in particular to any protein S deficiency, which is otherwise often overestimated.

It is important to consider all the known acquired causes of change to a thrombophilia marker in order to avoid any misinterpretation. Some specific variations in coagulation inhibitors are as follows.

Antithrombin values may be decreased in patients on heparin treatment and in those with thrombosis, and the nephrotic syndrome also causes a loss of antithrombin. More marked decreases are found in patients with disseminated intravascular coagulation and severe liver disease.

Protein C levels can be related to blood lipid levels

(75), sex and age (70). Liver disease, DIC, lupus-like anticoagulants, factor V inhibitors, and coumarins reduce protein C activity.

Protein S is affected by several pre-analytical factors. Moreover, particular attention must be paid when interpreting low values in young females (71) and during pregnancy. Anti-phospholipid antibodies, liver disease, DIC, and coumarins also decrease the protein S level. Most of the above-mentioned situations produce a more marked finding of a reduction if a functional assay is used.

The *homocysteine* level, as mentioned above, is also affected by several factors.

Some Particular Clinical Situations

Patients with recent or acute phase VTE

As stated above, only genetic tests for factor V Leiden and mutant prothrombin are reliable at this stage. Measurement of inhibitors, although indicated in some cases, should be undertaken at a later stage, after anticoagulant treatment has finished. However, family members can also be studied at this time if an inherited defect is suspected. This measure can be effective if preventive precautions are taken for the relatives.

Oral contraceptives or hormone-replacement therapy

It is of utmost importance to achieve the correct laboratory management of female candidates for screening for inherited thrombophilia; this is usually done in the context of a familial study. No evidence is available for the definition of a screening test that could be conducted prior to all hormonal treatment (42). It is well known that hormones can modify several coagulation tests, thus carrying a risk of erroneous interpretation of results. Therefore, if coagulation inhibitors are to be measured for clinical purposes, it is safe to do so 2 months after interruption of any hormonal therapy (55). Some authors have found that the risk of venous thrombosis is at its greatest in female defect carriers in the first few months of treatment (76). Venous thrombosis in the initial stage of oral contraceptive treatment may indicate the presence of an inherited clotting defect.

Pregnancy

The relationship between recurrent fetal loss and inherited thrombophilia is a controversial issue: association studies demonstrate a significantly increased risk in the presence of a plasmatic thrombophilic defect (75–81). As yet no clinical studies have been conducted to establish an evidence-based use of anticoagulant therapy in pregnant women with a coagulation defect (82). Therefore, these diagnostic tests are not justified for this purpose (7) (Grade C).

Pregnancy, particularly in months 7 to 9, produces marked changes in the levels of coagulation inhibitors. Some markedly decreased levels of antithrombin and

protein C and factor V Leiden are associated with the HELLP syndrome and other complications (83–85). Although these observations are applicable to a population study, they are not necessarily applicable to individual cases, as this would require documentation from clinical studies. The analysis of inhibitors in this context is therefore not recommended for extensive use (Grade C).

Thrombosis in the newborn

It is difficult to interpret levels of inhibitors in the neonatal period for a diagnosis of heterozygous deficiency. The plasma measurement of antithrombin, protein C and protein S must be employed only in patients whose condition is severe, in order to rule out any homozygous defect. A familial study is mandatory in all such cases. Some reference levels have been proposed in limited series (86–88). The condition defined as “Purpura fulminans” represents the rare situation produced by the homozygous defect of antithrombin or protein C.

Cerebrovascular events

Venous intracranial thrombosis, a rare but well-documented condition, is similar to peripheral venous thrombosis, as far as its diagnostic management is concerned.

Some recent studies indicate the possibility of arterial ischemic stroke in the young, or children, who are carriers of the genetic polymorphism of factor V, prothrombin or MTHFR (89–91). The data from these studies, however, require confirmation from findings in larger series. We believe that an appropriate laboratory study should also be made in cases of arterial thrombosis, limited to symptomatic subjects at the pediatric age (7).

Children with thrombosis may require specific therapy for the management of acute episodes. For example, in children with severe genetic protein C or protein S deficiency, prompt replacement therapy may be required. It is therefore often of clinical value to determine the level of proteins regulating coagulation immediately, rather than waiting until long-term anticoagulant therapy has been discontinued (Grade C) (92, 93).

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