



**College of American Pathologists
Consensus Conference XXXVI:
Diagnostic Issues in Thrombophilia**

November 9-11, 2001
Atlanta, Georgia

Conference Synopsis

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**Dr. Kujovich participated via teleconference in the discussion of Factor V Leiden on the first day of the deliberations.*

Introduction and General Considerations

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Abstract

Objective

To review the state of the art relating to laboratory testing for thrombophilia, as reflected by the medical literature and the consensus opinion of recognized experts in the field, and to make recommendations regarding laboratory testing (whom to test, when to test, what tests to perform, rationale for testing, and other issues) in the assessment of thrombotic risk in individual patients and their family members.

Data Sources

Review of the medical literature, primarily from the last 10 years, and the experience and opinions of experts in the field were used as sources.

Data Extraction and Synthesis

Participant authors evaluated the medical literature and prepared manuscripts with specific proposed recommendations. Drafts of all of the manuscripts were prepared and circulated to every participant in the College of American Pathologists Conference XXXVI on Diagnostic Issues in Thrombophilia prior to the conference. Each of the conclusions and associated recommendations was then presented for discussion. Recommendations were accepted if a consensus of 70 percent or more of the 27 experts attending the conference was reached. The results of the discussion were then used to revise the manuscripts and recommendations into final form.

Conclusions

Consensus was reached on 178 recommendations, all of which are presented in the manuscripts that follow. Detailed discussion of the rationale for each of these recommendations is found in the text of this article along with citations to justify the level of evidence for the recommendations. This is an evolving area of research, and it is certain that further clinical studies will change many of the recommendations, cause some to be deleted and others to be added in the future.

Introduction

At the meetings of the International Society on Hemostasis and Thrombosis (ISTH) held more than quarter century ago, the majority of the abstracts and educational programs presented were devoted to the bleeding diatheses. Studies of thrombotic disorders were still awaiting key discoveries that have led to the recent explosion of interest in vascular biology and thrombophilia. In contrast, the most recent meeting of the ISTH showed an order of magnitude increase in attendance and abstracts when compared to the initial meetings. More than 80 percent of the abstracts at the 2001 meeting were devoted to some aspect of the study of thrombosis (Report of the President to the XVIII congress of the ISTH, 10 July 2001). It is likely that this distribution did not reflect a reduction in the interest in the bleeding diatheses, but rather a profound growth in the interest in thrombosis.

The outcome of this growth in the study of thrombosis and vascular biology has been the discovery of a number of pathways involved in the regulation of hemostasis. Abnormalities in the components of these pathways have been elucidated and, following evaluation of patients, families, and populations with thrombophilia, associated with an increased risk of thrombosis. An outcome of this increase in information has been a concomitant growth in the number of plasma or molecular components that can be measured in the clinical laboratory. With tests being readily available, both laboratorians and clinicians have been struggling with all of the issues surrounding this testing, such as whom to test, when to test, what tests should be performed, and what are the best tests.

This problem is exemplified by a recent study examining test utilization to evaluate thrombophilia. In the population of patients studied, more than half were tested within the first week of the thrombotic event; in one third of the patients antithrombin (AT) was measured while the patient was taking heparin; and in about one fifth of the patients protein C and/or S was measured while the patient was orally anticoagulated. There were also many documented errors in the interpretation of the results.

The College of American Pathologists (CAP) XXXVI Consensus Conference: Diagnostic Issues in Thrombophilia, held November 9-11, 2001, in Atlanta, Georgia, was convened to provide recommendations concerning these important clinical issues. A total of 31 participants, 27 of whom were in attendance at the conference, contributed to this project. Members of the CAP Biochemical and Molecular Genetics Resource Committee (Ronald McGlennen and Richard Press) and Coagulation Resource Committee (John Brandt, Wayne Chandler, Timothy Hayes, Kandice Kottke-Marchant, Michael Laposata, Douglas Triplett, and John Olson) invited other pathologists and clinicians to participate in the conference. As a result, the participants prepared 16 manuscripts dealing with specific thrombophilic risk factors and two manuscripts dealing with the application of the factors in thrombotic conditions. The draft manuscripts were available in advance of the meeting and circulated to all participants.

Based on their review of the literature and their experience, the authors suggested specific recommendations. At the conference, the conclusions of the manuscripts were presented and the

recommendations discussed and voted on by the participants in attendance. Both the inherited and the acquired thrombophilias are discussed. Recommendations regarding patient management were not addressed at the conference; however, some of the authors have included in their manuscripts their own observations regarding the impact of testing on clinical decisions.

Recommendations were approved and included in the final publication only if more than 70 percent of those present agreed with the content and wording of the recommendation. Each recommendation has a “level of evidence” that the authors have assigned. The definitions of the levels of evidence are presented in Table 1, and the participants agreed upon the levels. Following the conference, the manuscripts were finalized, taking into account the discussion, suggestions, and finalized recommendations made at the conference. All manuscripts were then submitted to the *Archives of Pathology & Laboratory Medicine* and subjected to the journal’s peer review process.

The impetus for gathering this group of recognized experts for a consensus conference was the lack of a clear direction from the literature regarding many of the issues. As might be expected, many of the recommendations generated lively discussion during the conference and will be controversial in the opinion of the reader. Other experts will find some items with which they can agree and others with which they strongly disagree. The same was true of the conference participants. Nevertheless, the recommendations, as presented, were agreed upon by a significant majority and should provide useful guidance for clinicians and laboratorians, as well as indicate possible directions for further studies to help clarify the many outstanding issues.

The purpose of publishing the synopsis is to present the conclusions and recommendations that were discussed and approved at the consensus conference. It will serve as a quick reference for the clinician and laboratorian dealing with patients who suffer from thrombophilia.

Table 1. Criteria used for the Level of Evidence Assigned to Recommendations

Level 1	One or more well-designed prospective study(ies) or two or more well-designed retrospective studies
Level 2	Retrospective studies or multiple anecdotal studies that reach consensus
Level 3	Isolated anecdotal studies and/or consensus of experts

General Recommendations

Patients and especially asymptomatic family members should provide informed consent before testing for thrombophilia is performed. Written consent is required for molecular testing (e.g., factor V Leiden or prothrombin G20210A) in many jurisdictions, but, in general, oral consent for testing with appropriate notation in the medical record should be sufficient.	Level 3
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Individuals testing positive for a thrombophilia require counseling as to:	Level 3
<ul style="list-style-type: none">• the risks of thrombosis to them and their family members,• the importance of early recognition of the signs and symptoms of venous thromboembolism (VTE) that would require immediate medical attention,• the risks and benefits of antithrombotic prophylaxis in situations when their risk of thrombosis is increased, such as perioperative or peripartum.	
Laboratory testing for other inherited and acquired thrombophilias should be considered even after the identification of a known thrombophilia, since more than one thrombophilia could coexist, compounding the risk for thrombosis in many cases.	Level 1
When available, World Health Organization (WHO) standards, or standards that can be linked to the WHO standard, should be used to calibrate functional and antigenic assays.	Level 1
The effects of age and gender should always be taken into consideration when interpreting the results of antigenic and functional assays, and, whenever possible, reference ranges that are age and gender specific should be developed.	Level 1
Before concluding that a patient has an inherited thrombophilia, diagnostic assays for function or antigen should be repeated after excluding acquired etiologies of the defect.	Level 3

Laboratory Evaluation of Hypercoagulability with Venous or Arterial Thrombosis: Venous Thromboembolism, Myocardial Infarction, Stroke, and Other Conditions

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Venous Thromboembolism

Conclusion

The identification of protein C, protein S, or antithrombin deficiency or combined thrombophilias may be used to influence decisions on treatment duration or prophylaxis.

Level 2

Recommendations

Tests for factor V Leiden (FVL) (activated protein C resistance assays with factor V deficient plasma can be used as an initial test), functional protein C, functional protein S, functional antithrombin, and prothrombin G20210A mutation are appropriate in patients with venous thromboembolism (VTE), particularly for idiopathic venous thromboembolism, younger patients, and/or those with a family history of thrombosis.

Level 2,
with a
small
number of
level 1
studies

Anticardiolipin antibody and lupus anticoagulant assays are appropriate for patients with VTE, particularly if it is idiopathic or associated with autoimmune disease, or if it appears in the absence of a family history of venous thrombosis.

Level 2,
with some
level 1
studies

Testing for thrombophilia (tests noted in recommendations 1 and 2) is appropriate for patients with: Level 2

- a history of recurrent VTE
- VTE under the age of 50 years
- unprovoked VTE at any age; however, testing for protein C, protein S, and antithrombin deficiency may be of lower diagnostic yield in patients with a first lifetime VTE after age 50
- VTE at unusual sites (e.g., cerebral, mesenteric, portal, hepatic)
- VTE patients with a positive family history of VTE
- VTE secondary to pregnancy, oral contraceptive use, or hormone replacement therapy (HRT)

Although there is an association between homocysteine and venous thrombosis, the implications for testing are controversial. However, since elevated homocysteine can be lowered by treatment with vitamins B₁₂, folate and B₆, homocysteine testing may be considered for patients with VTE . Level 2

Heparin-induced thrombocytopenia should be considered for any patient who experiences venous thrombosis while exposed to heparin or within 30 days of heparin exposure, with a decrease in platelet count to less than 50 percent of baseline. Level 3

Prior to pregnancy or oral contraceptive use, it may be worthwhile to test asymptomatic female first-degree relatives of a proband with a defined inherited thrombophilia (for that identified defect). This is especially important for families with known antithrombin deficiency. Level 2

Controversial Recommendations

Testing for thrombophilia is controversial in patients with: Level 2

- a first, provoked VTE in older patients (age >50). In general, testing for thrombophilia is not recommended for VTE associated with active cancer or an intravascular device in adults.
- a first VTE related to selective estrogen receptor modulators (SERMs) or tamoxifen

After counseling, testing for thrombophilia is appropriate in asymptomatic first degree relatives of a proband with a known inherited thrombophilia. Such testing may be particularly useful in families with deficiencies of protein C, protein S, or antithrombin. Level 2

Arterial Thrombosis

Conclusions

C-reactive protein appears to predict future risk for coronary artery disease in healthy individuals as well as various patient populations, such as myocardial infarction or unstable angina patients. However, there is no consensus on who should be tested or how the test result should affect patient management (e.g. statin, aspirin therapies).	Level 1
Most, but not all, prospective studies find an association between lipoprotein (a) (Lp[a]) and myocardial infarction or related atherosclerotic disease. However, there is no clear consensus on who should be tested. Lp(a) can be reduced by estrogen (in women) or niacin therapy, but confirmation that such treatments will reduce future risk is awaiting further study.	Level 1 (with some conflicting results)

Recommendations

Antiphospholipid antibody (lupus anticoagulant and anticardiolipin antibody) assays can be considered for patients with arterial thrombosis, particularly in a young person or a person with no documented atherosclerosis.	Level 1 (with relatively few prospective studies to date)
Consider measuring homocysteine for patients with documented atherosclerotic arterial occlusive disease. Homocysteine concentration can be reduced by therapy with vitamins B ₁₂ , B ₆ , and folate, but confirmation that such treatments will reduce the risk of future cardiovascular events is awaiting further study.	Level 1
Routine testing for factor V Leiden and prothrombin G20210A is not recommended in patients with arterial thrombotic disease that is associated with atherosclerosis. However, these tests can be considered in certain unusual situations, such as patients with unexplained arterial thrombosis without atherosclerosis or young patients who smoke.	Level 2
Routine testing for protein C, protein S, and antithrombin is not recommended for patients with arterial thrombotic disease that is associated with atherosclerosis. However, these assays can be considered in certain unusual situations, such as young patients with unexplained arterial thrombosis without atherosclerosis.	Level 3
Heparin-induced thrombocytopenia should be considered in any patient who experiences arterial thrombosis while exposed to heparin or within 30 days of heparin exposure, with a decrease in platelet count to less than 50 percent of baseline.	Level 3
Although the following recommendation does not relate specifically to thrombophilia, it is included because of its well documented relationship to arterial vascular disease: A fasting lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) should be measured every five years in all adults aged 20 or older. More frequent testing may be indicated in certain individuals.	Level 1

Neurovascular Thrombosis

Conclusion

It is difficult to make a recommendation regarding lipoprotein (a) and stroke because the few level 1 studies that have been completed are conflicting.

Recommendations

Consider testing for antiphospholipid antibodies (anticardiolipin antibodies and lupus anticoagulants) in patients with unexplained stroke, particularly in a young person or a patient with autoimmune disease.	Level 1, with few prospective studies to date and some conflicting results
Consider testing for antiphospholipid antibodies in patients with cerebral venous thrombosis.	Level 3 (one level 2 study) and extrapolation from other venous thrombosis studies
Consider measuring homocysteine in patients with documented stroke or existing cerebrovascular disease. Homocysteine concentration can be reduced by therapy with vitamins B ₁₂ , B ₆ , and folate, but confirmation that such treatments will reduce the risk of future cardiovascular events is awaiting further study.	Level 1
Routine testing for factor V Leiden and prothrombin G20210A is not recommended in adult patients with arterial stroke; however, these tests can be considered in certain unusual situations, such as pediatric patients with stroke. These assays may also be useful for patients with cerebral venous thrombosis.	Level 2
Routine testing for protein C, protein S, and antithrombin deficiency is not recommended for adult patients with stroke. These assays can be considered in certain unusual situations, such as young patients with stroke (Level 2), patients who also have a personal or family history of venous thrombosis, or paradoxical emboli (Level 3). These assays are appropriate for patients with cerebral venous thrombosis (Level 3, and extrapolation from other venous thrombosis studies).	
Heparin-induced thrombocytopenia should be considered in any patient who experiences ischemic stroke or cerebral venous sinus thrombosis while exposed to heparin, or within 30 days of heparin exposure, with a decrease in platelet count to less than 50 percent of baseline.	Level 3

Diagnostic Studies for Thrombophilia in Women on Hormonal Therapy, in Women During Pregnancy, and in Children

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Pregnancy

Conclusions

The risk of venous thromboembolism (VTE) during gestation increases three- to fourfold.	Level 1
Thrombophilia can be identified in the majority of women with gestational VTE.	Level 1
Thrombophilia is associated with unexplained pregnancy loss (especially in the second and third trimesters).	Level 2
Other gestational vascular complications (preeclampsia, intrauterine growth retardation, placental abruption) are associated with thrombophilia.	Level 3
Combined thrombophilic conditions increase the risk for gestational complications.	Level 2

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Diagnostic Studies for Thrombophilia in Women on Hormonal Therapy, in Women During Pregnancy,
and in Children

Patients with prior VTE during pregnancy who have a thrombophilic state and who are at high risk for recurrence during subsequent pregnancy may receive antithrombotic prophylaxis during gestation and should receive antithrombotic prophylaxis in the postpartum period. Level 2

Prevention of pregnancy loss in women with thrombophilia by antithrombotic therapy is currently being evaluated in prospective randomized trials. Level 3

Recommendations

Women with VTE during pregnancy or in the postpartum period should be evaluated for thrombophilia. Level 1

Women with pregnancy loss that is either recurrent or late in the pregnancy (in the second or third trimester) should be evaluated for thrombophilia. Level 1

Whether women with other gestational vascular complications should be evaluated for thrombophilia is controversial. Level 3

Testing results for activated protein C (APC)-resistance and protein S obtained during pregnancy or the postpartum period should be interpreted with caution in view of physiologic changes. Level 3

Hormonal Therapy

Recommendations

Testing for thrombophilia is recommended in women who experience VTE as cerebral venous thrombosis during oral contraceptive or HRT. Level 1

Pediatrics

Conclusions

Thrombophilia is commonly found in children (particularly in infants) with VTE or stroke. Level 1

The association of multiple thrombophilias greatly increases the risk of thrombosis and/or recurrence of thrombosis in infants and children as it does in adults. Level 2

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Diagnostic Studies for Thrombophilia in Women on Hormonal Therapy, in Women During Pregnancy,
and in Children

The distribution of prothrombotic risk factors varies with respect to the ethnic background and the number of patients and controls investigated. Level 3

Recommendations

Testing for thrombophilia in children with venous or arterial thrombosis is recommended. The etiology and prevalence of thrombophilia differ when comparing children and adults. Level 1

Age-specific reference ranges should be used to interpret the results of thrombophilia testing in the pediatric and neonatal age groups. Level 3

Routine evaluation for thrombophilia for asymptomatic children of probands with inherited thrombophilia may be delayed until puberty. Level 3

Evaluation for thrombophilia for the siblings of probands with early symptomatic thromboembolism is recommended. Level 3

Clinical Utility of Factor V Leiden (R506Q) Testing for the Diagnosis and Management of Thromboembolic Disorders

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**Consensus Recommendations for Factor V Leiden Testing:
Who Should Be Tested?**

Conclusion

There is currently no evidence that the acute therapeutic management of venous thromboembolic events (duration and intensity of anticoagulation) should be different in patients with factor V Leiden (FVL).

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Hypothesis

The primary advantages of FVL testing would be the identification of high-risk patients who could benefit from either long term anticoagulant therapy or aggressive prophylaxis in temporary periods of high thrombotic risk.

Other direct clinical benefits of FVL testing would include the opportunity to detect:

- female probands for whom future decisions as to oral contraceptive use, hormone replacement therapy, or management of pregnancy complications could depend on FVL carrier status
- at-risk family members for whom future decisions as to antithrombotic prophylaxis, oral contraceptive use, hormone replacement therapy, or pregnancy complications could depend on FVL carrier status

Recommendations for FVL Testing

Because the discovery of an FVL mutation (by itself or in combination with other thrombophilias) would, in some situations, directly alter clinical management of the proband or lead to testing of family members, FVL testing is recommended in patient populations with a mutation prevalence above that of the normal population, such as individuals with venous thromboembolism (VTE) and a clinical suspicion of thrombophilia based upon any of the following criteria.

FVL testing is recommended in patients with:

A history of recurrent VTE	Level 2
A first VTE at less than 50 years of age	Level 1
A first unprovoked VTE at any age	Level 1
A first VTE at an unusual anatomic site such as the cerebral, mesenteric, portal, or hepatic veins	Level 2
A first VTE, at any age, in a subject with a first degree family member with a VTE before age 50	Level 1
A first VTE related to pregnancy, the puerperium, or oral contraceptive use	Level 1

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A first VTE related to hormone replacement therapy Level 3

An unexplained pregnancy loss during the second or third trimester Level 2

Testing for FVL is controversial in:

Young women smokers (age younger than 50) with a myocardial infarction Level 2

Older patients (age older than 50) with a first provoked VTE event in the absence of cancer or an intravascular device Level 3

A first VTE related to selective estrogen receptor modulators (SERMs) or tamoxifen Level 3

Selected cases of women with unexplained severe preeclampsia, placental abruption, or intrauterine growth retardation (IUGR) Level 3

After appropriate counseling, testing for FVL also may be indicated in:

Asymptomatic adult family members of probands with known prothrombin G20210A mutations, especially those with a strong family history of thrombosis at a young age Level 2

Asymptomatic female family members who are pregnant or are considering oral contraceptive use or pregnancy Level 2

Factor V Leiden testing is NOT recommended:

As a general population screen Level 1

As a routine initial test during pregnancy Level 2

As a routine initial test prior to or during oral contraceptive use (Level 2), hormone replacement therapy, or SERM therapy (Level 3)

As a prenatal test, newborn initial test, or as a routine test in asymptomatic prepubescent children Level 2

As a routine initial test in patients with arterial thrombotic events Level 1

FVL testing can be considered in certain unusual situations, such as patients with unexplained arterial thrombosis without atherosclerosis or young patients who smoke.

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Consensus Recommendations for Factor V Leiden Testing:
FVL Testing Method

Conclusions

The variable sensitivity and specificity of the first generation activated protein C (APC) resistance assay precludes its routine clinical utility in the evaluation of thrombophilia. However, the second generation APC resistance assay (with dilution of test plasma into Factor V deficient plasma) has, in some laboratories, a diagnostic specificity approximately equivalent to direct DNA-based mutation tests and is currently less costly. Definitive direct DNA-based methods to detect the FVL mutation are available in clinical diagnostic laboratories by any of several different in-house developed (not approved by the Food and Drug Administration [FDA]) methods. These direct mutation assays, when appropriately validated in a licensed clinical laboratory, are extremely accurate and precise for the detection of FVL.

Recommendations for FVL Testing Method

For patients, initial FVL testing may include either the second generation APC resistance functional assay or a direct DNA-based mutation method. Exceptions are noted below.	Level 2
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An initial DNA based testing method is recommended in:

Patients with a lupus anticoagulant and a markedly prolonged baseline activated partial thromboplastin time (aPTT) (which may interfere with the functional APC resistance assay)	Level 2
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Family members of subjects with known FVL mutations in order to avoid the need for follow-up confirmatory direct mutation testing	Level 3
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A negative second generation functional assay excludes the diagnosis of FVL. Confirmatory direct DNA-based testing is, however, recommended for patients with:

Borderline APC resistance (APCR) values	Level 1
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"Positive" APCR initial tests to definitively confirm both the diagnosis and the number of mutant alleles, and, for patients with very low APCR values, to distinguish heterozygotes, homozygotes, and those who are heterozygous for both FVL and a second mutation causing factor V deficiency	Level 3
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Laboratory testing for other inherited and acquired thrombophilic defects should be considered even after the identification of FVL, since the FVL allele often coexists with other disorders, and, when present, synergistically increases the thrombotic risk

Level 1

Management of Factor V Leiden Carriers with a History of Venous Thromboembolism

Conclusions

There is currently no evidence that the acute management of venous thromboembolic events (length or strength of anticoagulation) should be different in patients with inherited thrombophilia.

For FVL carriers, the duration of oral anticoagulation therapy must be tailored to the individual patient based on the risk of venous thromboembolism (VTE) recurrence and the risk of anticoagulant-related bleeding. Those patients with a higher risk of VTE recurrence could then benefit from a longer duration of anticoagulant therapy.

The risk of recurrent VTE associated with a heterozygous FVL mutation (without additional thrombophilic defects) is not firmly established.

Recommendations

Heterozygous or homozygous FVL carriers with a first lifetime deep vein thrombosis or pulmonary embolism should be treated in standard fashion, initially with heparin (either unfractionated or low molecular weight), followed by warfarin (target International Normalized Ratio [INR] 2.5; therapeutic range 2.0 - 3.0).

Level 1

In general, three to six months of oral anticoagulation therapy is recommended after a first lifetime VTE in FVL carriers, especially if the event was associated with a transient clinical risk factor (e.g., surgery, oral contraceptive use, pregnancy, or the puerperium).

Level 1

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Clinical Utility of Factor V Leiden (R506Q) Testing for the Diagnosis and Management of
Thromboembolic Disorders

The need for lifelong anticoagulation after a first episode of VTE in FVL carriers has not been established by appropriate clinical trials. Therefore, indefinite anticoagulation should be recommended only after careful consideration of the risks and benefits. Indefinite anticoagulation may be recommended for:

FVL carriers with an idiopathic or life-threatening VTE event (especially with reduced cardiopulmonary functional reserve)	Level 2
FVL carriers with more than one hereditary thrombophilia (or homozygous carriers of one hereditary thrombophilia)	Level 2
FVL carriers with additional persistent clinical risk factors (e.g., malignant neoplasm or antiphospholipid antibodies)	Level 2
Hereditary thrombophilia patients (or any patient) with recurrent unprovoked VTE should receive indefinite anticoagulation therapy.	Level 1
Women with FVL and a history of unprovoked VTE should receive prophylactic anticoagulation with heparin or low molecular weight heparin during pregnancy and for at least six weeks postpartum.	Level 2
After orthopedic surgery, because of a possible increase in the risk of VTE recurrence, FVL carriers with a history of VTE may require a higher intensity and/or more prolonged VTE prophylaxis, especially if the prior VTE was idiopathic or the patient has other persistent VTE risk factors (e.g., obesity, malignant neoplasm, or chronic immobility).	Level 2
Routine anticoagulation therapy is not recommended for FVL carriers with atherosclerotic arterial occlusive disease; however, among carriers with myocardial infarction or stroke, anticoagulation therapy for secondary prevention may be appropriate.	Level 3

Management of Factor V Leiden Carriers with No Thrombotic History

Long term primary antithrombotic therapy is not recommended for asymptomatic FVL carriers.	Level 3
FVL carriers (with or without previous VTEs) should receive appropriate prophylaxis when exposed to risk factors for VTE.	Level 1

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Standard prophylaxis recommendations are sufficient for most types of surgery. A possible exception is an asymptomatic FVL carrier undergoing hip replacement surgery, who might be at increased risk of symptomatic deep venous thrombosis (DVT) for several months thereafter. These hip surgery patients might then be considered for extended out-of-hospital prophylaxis, especially in association with obesity or prolonged immobilization.

Level 2

Prophylactic anticoagulation is not routinely recommended in pregnant FVL carriers with no history of thrombosis. Decisions about anticoagulation should be individualized based on the underlying defect (heterozygous versus homozygous) and coexisting risk factors. Asymptomatic women who do not receive anticoagulation should be followed closely throughout pregnancy and offered a six-week postpartum course of warfarin.

Level 2

Clinical and Laboratory Management of the Prothrombin G20210A Mutation

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Conclusions

There is currently no evidence that the acute therapeutic management of venous thromboembolic events (length or strength of anticoagulation) should be different in patients with the prothrombin G20210A mutation.

Hypothesis: The primary advantages of prothrombin G20210A testing would be the identification of high-risk patients who could benefit from either long-term anticoagulant therapy or aggressive prophylaxis in temporary periods of high thrombotic risk.

Other direct clinical benefits of testing for the prothrombin G20210A mutation would include the opportunity to detect:

- Female probands for whom future decisions about oral contraceptive use, hormone replacement therapy, or management of pregnancy complications could depend on prothrombin G20210A carrier status.
- At-risk family members for whom future decisions about antithrombotic prophylaxis, oral contraceptive use, hormone replacement therapy, or pregnancy complications could depend on prothrombin G20210A carrier status.

Definitive direct DNA-based methods to detect the prothrombin G20210A mutation are available in clinical diagnostic laboratories, by any of several different in-house developed (not approved by the Food and Drug Administration [FDA]) methods. These direct mutation assays, when appropriately validated in a licensed clinical laboratory, are extremely accurate and precise for the detection of prothrombin G20210A.

Specific Recommendations

Prothrombin G20210A testing is recommended in patient populations with a mutation prevalence above that of the normal population, such as those with venous thromboembolic events and a clinical suspicion of thrombophilia based upon any of the following criteria.

Prothrombin G20210A testing is recommended in patients with:

A history of recurrent venous thromboembolism (VTE)	Level 2
A first VTE at less than 50 years of age	Level 1
A first unprovoked VTE at any age	Level 1
A first VTE at an unusual anatomic site such as the cerebral, mesenteric, portal, or hepatic veins	Level 2
A first VTE at any age in a subject with a first-degree family member with a VTE before age 50	Level 1
A first VTE related to pregnancy, the puerperium, or oral contraceptive use	Level 1
A first VTE related to hormone replacement therapy	Level 3
Unexplained pregnancy loss during the second or third trimester	Level 2

Testing for prothrombin G20210A is controversial in:

Young women smokers (age younger than 50) with a myocardial infarction	Level 2
Older patients (age older than 50) with a first provoked VTE event in the absence of cancer or an intravascular device	Level 3
A first VTE related to selective estrogen receptor modulators (SERMs) or tamoxifen	Level 3

Selected cases of women with unexplained severe preeclampsia, placental abruption, or intrauterine growth retardation (IUGR)	Level 3
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After appropriate counseling, testing for prothrombin G20210A also may be indicated in:

Asymptomatic adult family members of probands with known prothrombin G20210A mutations, especially those with a strong family history of thrombosis at a young age	Level 2
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Asymptomatic female family members who are pregnant or are considering oral contraceptive use or pregnancy	Level 2
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Prothrombin G20210A testing is NOT recommended:

As a general population screen	Level 1
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As a routine initial test during pregnancy	Level 2
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As a routine initial test prior to or during oral contraceptive use (Level 2), hormone replacement therapy, or SERM therapy (Level 3)

As a prenatal test, newborn initial test, or as a routine test in asymptomatic prepubescent children	Level 2
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As a routine initial test in patients with arterial thrombotic events	Level 1
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Antithrombin Deficiency: Issues in Laboratory Diagnosis

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Conclusions

Antithrombin is an important heparin-dependent regulator of hemostasis by inhibition of thrombin, factor Xa, factor XIa, and factor XIIa.

Reference Ranges

Antithrombin levels are low in neonates and infants and increase to adult levels by approximately one year of age; levels are then slightly higher than those of adults up to age 16.

Antithrombin reference ranges are similar in men and women.

Antithrombin levels increase in post-menopausal women and in women using high-dose oral contraceptives, but the reference ranges are not affected.

There is a wide range of functional antithrombin values in normal adult individuals (83-128%).

Prevalence of deficiency

Antithrombin deficiency is uncommon in the general population, approximately 0.07-0.16%.

The prevalence of antithrombin deficiency in patients with venous thrombosis is approximately 2% (range 1-8%).

Association with thrombosis

Heterozygous antithrombin deficiency is associated with venous thrombosis.

Most (greater than 50%) heterozygous antithrombin deficient individuals have a thrombotic event by age 30.

Homozygous type IIb antithrombin deficiency may be associated with venous thrombosis early in life; other types of homozygous antithrombin deficiency are incompatible with survival.

Heterozygous antithrombin deficiency is associated with an increased (5 to 50 times greater) risk of venous thrombosis.

Co-inheritance of another thrombophilic risk factor in addition to antithrombin deficiency results in a further increased risk for thrombosis.

The association of antithrombin deficiency with arterial thrombosis is uncertain.

Acquired deficiency

Impaired synthesis: liver disease, malnutrition, premature infancy, inflammatory bowel disease, burns

Increased consumption: disseminated intravascular coagulation (DIC), hemolytic transfusion reaction, malignancy, L-asparaginase therapy, acute thrombosis, heparin therapy, urinary protein loss (nephrotic syndrome).

Recommendations for Testing Antithrombin

Whom to test

Inclusion of antithrombin testing in general thrombophilia evaluation is included in "[Laboratory Evaluation of Hypercoagulability with Venous or Arterial Thrombosis: Venous Thromboembolism, Myocardial Infarction, Stroke, and Other Conditions](#)"

Isolated testing for antithrombin is recommended when an individual from a family with known antithrombin deficiency requires testing. Level 1

Isolated testing for antithrombin is recommended as a confirmatory test when an abnormal antithrombin was found in the initial test, either in the same or a different laboratory. Level 1

Routine measurement of antithrombin is not recommended prior to starting oral contraceptive or hormone replacement therapy unless there is a family history of antithrombin deficiency. Level 2

How to test

Antithrombin amidolytic assays are recommended for initial testing for antithrombin deficiency. Level 1

There is no need to routinely perform antithrombin antigen assays. Level 1

Antithrombin antigen assays may be useful for distinguishing type I from type II antithrombin deficiency. Level 1

Pharmacologic agents (especially heparin) and other causes of acquired antithrombin deficiency should be taken into consideration in interpretation of antithrombin results. Level 1

Before a diagnosis of hereditary antithrombin deficiency is rendered, patients with low antithrombin values should have this finding confirmed on a subsequent sample after exclusion of acquired etiologies. Family studies may be of additional help. Level 1

When to test

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Antithrombin Deficiency: Issues in Laboratory Diagnosis

It is preferable not to test for antithrombin deficiency during an acute event (thrombotic, surgical, etc.); however, a normal antithrombin value in the setting of an acute event excludes antithrombin deficiency.

Level 2

Testing for antithrombin deficiency is best done at least five days after cessation of heparin therapy.

Level 2

Laboratory Issues in Diagnosing Abnormalities of Protein C, Thrombomodulin, and Endothelial Cell Protein C Receptor

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Conclusions

Protein C is an important physiologic regulator of hemostasis in the degradation of factors Va and VIIIa.

Protein C levels are low in neonates and infants and increase to adult levels during adolescence.

Adult protein C levels are independent of age and sex, but they may be higher in post-menopausal women.

There is a wide range of antigenic and functional protein C values in normal adult individuals (approximately 70-140%) with a log-normal distribution. There may be an overlap in values between heterozygous and normal individuals at the low end of the reference range. Laboratory and assay-specific reference ranges should be established.

Higher protein C levels can be observed in patients with nephrotic syndrome or ischemic heart disease, in pregnant patients, and in patients using oral contraceptives or hormone replacement therapy.

Decreased protein C levels due to decreased synthesis/post-translational modification can be observed in patients with vitamin K deficiency or hepatic disease and in patients using warfarin therapy. Decreased protein C levels due to increased turnover may be observed in patients with consumptive coagulopathy, renal insufficiency, acute thrombosis, acute respiratory distress syndrome (ARDS), plasma exchange, breast cancer, or massive hemorrhage, as well as in the postoperative state.

Protein C deficiency is uncommon in the general population, approximately 0.2-0.4%.

Heterozygous protein C deficiency is usually associated with protein C levels more than three standard deviations below the mean of a laboratory's reference range, while the classification is less clear if the protein C level is between three and two standard deviations below the mean. The diagnosis of protein C deficiency should be made with caution when the protein C functional level is between three and two standard deviations below the mean of the laboratory's reference range.

The prevalence of heterozygous protein C deficiency in patients with venous thrombosis is approximately 4% (range 1.5-11.5%).

Heterozygous protein C deficiency is associated with an increased risk of venous thrombosis.

Co-inheritance of another thrombophilic risk factor, such as factor V Leiden, in addition to protein C deficiency, generally results in a further increased risk for thrombosis. Co-inheritance of the prothrombin G20210A mutation may not increase thrombotic risk with protein C deficiency.

Homozygous protein C deficiency is rare (1:500,000 to 1:750,000), but it is a significant cause of neonatal venous thrombosis. It usually presents as purpura fulminans neonatorum.

Protein C deficiency is not associated with a risk for arterial thrombosis.

Recommendations

Whom to test

Inclusion of protein C testing in general thrombophilia evaluation is included in "Laboratory Evaluation of Hypercoagulability with Venous or Arterial Thrombosis: Venous Thromboembolism, Myocardial Infarction, Stroke, and Other Conditions."

Isolated testing for protein C is recommended when an individual from a family with known protein C deficiency requires testing.	Level 1
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In addition, testing would be limited to protein C for a confirmatory test when an abnormal protein C was found in the initial test, either in the same or different laboratory.	Level 1
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Assay for protein C is not recommended prior to starting oral contraceptive use or hormone replacement therapy unless there is a family history of protein C deficiency.	Level 2
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College of American Pathologists

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and Endothelial Cell Protein C Receptor

Infants with purpura fulminans neonatorum should have protein C levels measured to evaluate for the presence of homozygous protein C deficiency. Level 1

How to test

Protein C amidolytic assays are recommended for initial testing for protein C deficiency. Level 1

Protein C clottable assays may detect some forms of protein C deficiency missed by amidolytic assays, but they are subject to multiple interferences and should be used for initial testing with caution. Level 1

In the presence of a lupus anticoagulant, the amidolytic assay is recommended for initial testing. Level 2

There is no need to routinely perform protein C antigen assays. Level 2

Protein C antigen assays may be useful for distinguishing type I from type II protein C deficiency. Level 1

A calibrated plasma should be used to construct assay standard curves. These plasmas should be calibrated against the current World Health Organization (WHO) International Standard Protein C Plasma. Level 2

Before a diagnosis of hereditary protein C deficiency is rendered, patients with low protein C values should have this finding confirmed on a subsequent sample after exclusion of acquired etiologies. Family studies may be of additional help. Level 1

Oral vitamin K inhibitors, such as warfarin, and other causes of acquired protein C deficiency should be taken into consideration in interpretation of protein C results. Level 1

When to test

It is preferable not to test for protein C deficiency during an acute event (thrombotic, inflammatory, surgical, etc.); however, a normal protein C value in the setting of an acute event excludes protein C deficiency. Level 1

Elective testing for protein C deficiency is best done 30 days after cessation of warfarin therapy due to the interindividual variation in metabolism of warfarin. Other vitamin K antagonists, such as phenprocoumon, with a 150 hour half-life, require a longer delay before testing. Level 1

A Review of the Technical, Diagnostic, and Epidemiological Considerations for Protein S Assays

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Conclusions

General considerations

Protein S (PS) assays present a diagnostic challenge because the ultimate reference (gold standard) for the determination of free and functional PS assays remains the PEG precipitation procedure, which is difficult to reproduce. In addition, free PS levels, especially in PS-deficient individuals, appear to be disproportionately sensitive to the time, temperature and dilutional conditions of the assays in comparison to the levels of non PS-deficient individuals.

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A Review of the Technical, Diagnostic, and Epidemiological Considerations for Protein S Assays

Reference range

Protein S levels are lower (measuring approximately 35% of adult normal) in neonates and infants, but they increase to adult levels by 1 year of age.

Adult protein S levels vary with age, sex, and hormonal status.

Protein S levels are higher in men than in women.

Protein S levels increase with age in women.

Protein S levels do not increase with age in men.

Protein S levels are lower in women prior to menopause and during pregnancy, and they are lower in women taking oral contraceptives or on hormone replacement therapy.

Prevalence

Protein S deficiency is very uncommon in the general population (0.2-0.5%).

Prevalence of heterozygous Protein S deficiency in patients with venous thrombosis ranges from 1-3%.

There is no well-defined association of PS deficiency with arterial disease.

The prevalence of PS deficiency should be determined in very large studies and, in particular, in Asians and Africans, where no such studies have been performed.

Association with thrombosis

In thrombophilic families the cumulative venous thrombosis risk is 50% by age 45 for heterozygotes.

Rare cases of homozygous PS deficiency have been reported in association with neonatal purpura fulminans.

Additional co-existent risk factors may increase the thrombosis risk in thrombophilic families.

Currently, when a patient with thrombosis and a negative family history is identified with PS deficiency, it is completely unknown what the risk is of recurrent thrombosis for this patient or of first thrombosis for affected family members.

A reliable diagnostic standard should be developed to evaluate new tests regarding sensitivity and specificity. There are almost no data of this sort available to support rational choice of assay methodology for the clinical laboratory.

Determinants of protein S levels

Co-morbid disease states can influence protein S levels

PS is a vitamin K dependent protein; thus, treatment with oral vitamin K antagonists and vitamin K deficiency are associated with decreased levels of PS.

Testing Recommendations

Whom to test

Isolated testing for PS is recommended when an individual from a family with known PS deficiency requires testing. Level 1

Isolated testing for PS is recommended as a confirmatory test when an abnormal PS was found in the initial test, either in the same or different laboratory. Level 1

It is not recommended to measure PS during pregnancy or the postpartum period for the purpose of diagnosing hereditary deficiency. Level 1

Test results for PS obtained during pregnancy should be interpreted with caution in view of physiologic changes that can influence PS levels. Level 1

How to test

Initial testing can be performed by either a functional or immunoassay for free PS. Level 1

Functional PS assays may detect some forms of PS deficiency missed by free PS immunoassays, but they are subject to multiple interferences and should be used for initial testing with caution. Level 1

If the functional PS result is abnormal, confirm it with a monoclonal free PS immunoassay. Level 1

If the initial PS result is low by any method, then this finding should be confirmed on a subsequent sample after exclusion of acquired etiologies. Family studies may be of additional help. Level 1

In the presence of a lupus anticoagulant, the monoclonal free PS immunoassay is recommended for initial testing. Level 1

A calibrated plasma should be used to construct assay standard curves. These plasmas should be calibrated against the current World Health Organization (WHO) International Standard PS Plasma. Level 1

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A Review of the Technical, Diagnostic, and Epidemiological Considerations for Protein S Assays

There is no need to routinely perform total PS antigen assays. Level 1

Total PS antigen may be useful for distinguishing subtypes of PS deficiencies. Level 1

In rare instances, immunoassays for C4b binding protein (BP) might be useful in distinguishing the subtypes of PS deficiencies. Level 1

Gender-specific, and for women age-specific, reference ranges should be considered for PS. One must also consider use of oral contraception and hormone replacement therapy, which can reduce levels of PS substantially. Level 1

When to test

It is preferable not to test for PS deficiency during an acute event (thrombotic, inflammatory, surgical, etc.); however, a normal PS value in the setting of an acute event excludes a PS deficiency. Level 1

Elective testing for PS deficiency is best done at least 30 days after cessation of warfarin therapy due to the half life of warfarin (47 hours) and the half life of PS (42.5 hours). Other oral vitamin K antagonists, such as Phenprocoumon, with a half life of 140 hours, require longer delays. Level 1

Hyperhomocyst(e)inemia and Thrombophilia

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Conclusions

Retrospective case control studies consistently demonstrate an association between hyperhomocyst(e)inemia and both venous thromboembolism (VTE) and arterial thrombosis.

Plasma total homocysteine (tHcy) measured 4-6 hours after methionine loading has been shown to be associated with both VTE and arterial thrombosis, independently of the baseline tHcy level. However, for practical reasons, relatively few centers routinely perform methionine loading in the evaluation of subjects with suspected hyperhomocyst(e)inemia.

Prospective studies investigating the relationship between hyperhomocyst(e)inemia and arterial thrombosis have demonstrated conflicting results, but overall there appears to be a weak positive association.

Fewer prospective data are available on the association between hyperhomocyst(e)inemia and VTE. The results of the existing studies are not uniform. Therefore, it remains controversial whether homocysteine is a risk factor for VTE.

There is no evidence that heterozygosity for the C677T mutation in 5,10 methylenetetrahydrofolate reductase (MTHFR) is either associated with hyperhomocyst(e)inemia or is a risk factor for venous or arterial thrombotic disease.

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Hyperhomocyst(e)inemia and Thrombophilia

Although homozygosity for the C677T mutation in MTHFR is associated with higher plasma tHcy levels, it is not itself an independent risk factor for arterial or venous thrombosis.

Although plasma tHcy can be reduced by therapy with vitamins B₆, B₁₂, and folic acid, only a single study has demonstrated that the lowering of tHcy reduces the rate of restenosis following percutaneous coronary angioplasty. It is as yet unclear whether therapeutic intervention, either primary or secondary, reduces the risk of other arterial thrombotic events or VTE.

Recommendations

Who should be tested for hyperhomocyst(e)inemia

Consider testing patients with documented atherosclerotic (coronary artery, cerebrovascular or peripheral vascular) disease for hyperhomocyst(e)inemia.	Level 1
Homocysteine concentration can be reduced by therapy with vitamins B ₆ , B ₁₂ , and folic acid; however, confirmation that such treatments will reduce the risk of future cardiovascular events awaits further investigation.	Level 1
Due to insufficient data, it is controversial whether testing for hyperhomocyst(e)inemia is indicated in VTE	Level 2

How to test for hyperhomocyst(e)inemia

Both high-pressure liquid chromatography (HPLC) and immunoassay are acceptable methods for measurement of plasma tHcy	Level 2
Gender and local population-specific reference ranges are strongly recommended.	Level 1
Samples drawn in ethylenediaminetetraacetic acid (EDTA) should be kept on ice if separation of plasma cannot be performed within about 30 minutes.	Level 1
Secondary causes of hyperhomocyst(e)inemia should be considered, and some (such as vitamin B12 deficiency) are important to exclude before initiating therapy with folic acid.	Level 3
Genotyping for either the 677 or 1298 mutations in MTHFR is not recommended in subjects with hyperhomocyst(e)inemia.	Level 2

When to test for hyperhomocyst(e)inemia

Although plasma tHcy is frequently measured in samples drawn after overnight fasting, it is unclear whether it is necessary to insist on fasting specimens.	Level 3
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Hyperhomocyst(e)inemia and Thrombophilia

In the interpretation of plasma tHcy levels, it should be noted that levels may be elevated for several months following myocardial infarction or stroke.

Level 1

Plasminogen and Tissue Plasminogen Activator Deficiency as Risk Factors for Thromboembolic Disease

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Conclusions

In some populations the incidence of heterozygous plasminogen deficiency appears to be slightly higher in patients with a history of thrombosis than it is in the general population.

Family studies do not support the hypothesis that heterozygous plasminogen deficiency is associated with an increased risk of thrombosis.

Homozygous plasminogen deficiency is associated with ligneous conjunctivitis.

There is no consistent clinical evidence of an association between homozygous plasminogen deficiency and risk of thrombosis.

There is no consistent evidence of a relationship between decreased tissue plasminogen activator (tPA) and risk of thrombosis.

Recommendations

Determination of plasminogen concentration (activity or antigen) should not be part of the routine evaluation of patients with thrombophilia.

Plasminogen activity should be determined in patients suspected of having ligneous conjunctivitis.

There is no indication for measuring tPA in patients with thrombophilia.

There is no indication for routine assessment of genetic abnormalities/ polymorphisms of plasminogen or tPA in the evaluation of the risk of venous thromboembolism (VTE) or arterial thrombosis.

The Contact System

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Conclusions

There is no evidence to support hypercoagulability in patients homozygously deficient in prekallikrein (PK), high-molecular weight kininogen (HK), or factor XI.

Apparent association with thrombosis and mid-range activity levels more likely than not due to antiphospholipid syndrome/lupus anticoagulant (APLS/LA).

Increases in factor XIIa in coronary artery disease are not more accurate than more conventional cardiac risk factor

Recommendations

Routine measurement of the activities of HK, PK, and factor XII or their activated products is not recommended as part of a hypercoagulable evaluation.	Level 2
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Routine measurement of the activities of HK, PK, and factor XII or their activated products is not recommended as part of acute coronary syndrome evaluation.	Level 2
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Dysfibrinogenemia and Thrombosis

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Conclusions

The term *dysfibrinogenemia* encompasses a variety of abnormalities of fibrinogen function and may be either congenital or acquired in origin.

Acquired forms of dysfibrinogenemia appear to be weakly associated, if at all, with thrombosis.

Using current laboratory methods, congenital dysfibrinogenemia is found rarely in patients with venous thrombosis.

The relative risk of thrombosis is unknown, although there is a significant association between some forms of congenital dysfibrinogenemia and venous thrombosis.

The thrombin time is the most frequently used screening assay to detect dysfibrinogens in general, but it suffers from a significant lack of sensitivity and specificity.

Specific Recommendations

Although there is an association between some rare dysfibrinogens and thrombosis, testing for the identification of dysfibrinogens in patients with thrombophilia is not recommended. Level 2

The diagnosis of inherited dysfibrinogenemia associated with thrombosis requires extensive analysis of the fibrinogen protein, the genetic defect, and family studies. Level 3

Factor XIII Polymorphisms and Venous Thromboembolism

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Conclusion

Evidence is conflicting regarding the association of factor XIII Val34 polymorphism with risk of venous thromboembolism, although most studies suggest a protective effect. However, no pathophysiologically relevant mechanism has been proposed. Further studies are needed before routine screening of this polymorphism can be recommended.

Recommendation

Measurement of plasma factor XIII levels or polymorphisms is not recommended in evaluating thrombophilia.

Level 2

Heparin Cofactor II Deficiency

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Recommendation

Routinely testing patients with thromboembolic disease for heparin cofactor II (HCII) deficiency is not recommended at the present time.

Level 1

Plasminogen Activator Inhibitor-1 and Venous Thromboembolism

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Conclusions

There is conflicting evidence regarding the relation between an elevated plasminogen activator inhibitor-1 (PAI-1) level or PAI-1 polymorphisms and risk of venous thromboembolism.

The predictive value of PAI-1 plasma concentrations is uncertain, and there are several important physiologic covariates in regulation of plasma levels.

Common polymorphisms in the PAI-1 gene affect plasma concentrations.

There is insufficient information to recommend use of PAI-1 plasma levels or genotype in evaluating thrombophilia.

Recommendations

The measurement of plasma PAI-1 levels or polymorphisms is not recommended in evaluating thrombophilia.

Level 1

Elevated Hemostatic Factor Levels as Potential Risk Factors for Thrombosis

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Fibrinogen

Conclusions

Elevated fibrinogen is associated with an increased risk of arterial thrombosis, but there is a lack of prospective treatment data showing that lowering fibrinogen reduces risk and there is a lack of universal assay standardization making comparison of fibrinogen levels between institutions difficult.

Level 1

CAP Consensus Conference XXXVI: Diagnostic Issues in Thrombophilia
Elevated Hemostatic Factor Levels as Potential Risk Factors for Thrombosis

Recommendations for clinical testing of elevated factor levels

Although fibrinogen is an independent predictor of arterial thrombosis, the lack of prospective treatment data and the lack of universal assay standardization, routine testing for elevated fibrinogen is not recommended. Level 1

Fibrinogen is not a well established risk factor of venous thrombosis. Therefore, routine measurement of fibrinogen concentration is not recommended. Level 1

Prothrombin (Factor II)

Conclusions

See accompanying article, "[Clinical and Laboratory Management of the Prothrombin G20210A Mutation](#)," on prothrombin levels and DNA mutations. Level 1

Recommendations for clinical testing of elevated factor levels

See accompanying article, "[Clinical and Laboratory Management of the Prothrombin G20210A Mutation](#)," on prothrombin levels and DNA mutations. Level 1

Factor V

Recommendations for clinical testing of elevated factor levels

Owing to the limited amount of data linking factor V concentration and myocardial infarction, and no evidence of association with venous thrombosis, routine testing for factor V is not recommended. Level 2

Factor VII

Recommendations for clinical testing of elevated factor levels

Because factor VII is not an independent risk factor for thrombosis, routine measurement of factor VII concentration is not recommended. Level 1

Factor VIII

Conclusions

Elevated factor VIII activity is associated with an increased risk of venous thrombosis. Level 1

The risk is familial and independent of increased von Willebrand factor or non-O blood group. Level 1

To be evaluated, the factor VIII activity must be the patient's baseline level, free of acquired variables that increase levels. Level 1

Recommendations for clinical testing of elevated factor levels

Owing to assay and sample variables, as well as the lack of established direct effects, causal relationships, or diagnostic cutoffs, it is controversial to measure factor VIII in patients with thrombophilia. However, measurement of factor VIII activity may be indicated when there is an additive venous thromboembolism (VTE) risk, as in known thrombophilic families where some affected members are more symptomatic than others. Level 1

Assay of factor VIII is not recommended for the evaluation of arterial thrombotic risk. Level 1

Factor IX

Conclusions

Elevated factor IX may be associated with an increased risk of venous thrombosis. Level 1

Recommendations for clinical testing of elevated factor levels

Owing to limited data, routine measurement of factor IX concentration is not recommended. Level 2

Factor X

Recommendations for clinical testing of elevated factor levels

Level 1

Because factor X is not an independent risk factor for arterial or venous thrombosis, routine measurement of factor X concentration is not recommended.

Factor XI

Conclusions

Elevated factor XI may be associated with an increased risk of venous thrombosis.

Level 2

Recommendations for clinical testing of elevated factor levels

At present, factor XI levels are not indicated to assess individual venous thrombotic risk

Level 2

von Willebrand Factor

Recommendations for clinical testing of elevated factor levels

Because von Willebrand factor is not an independent risk factor for venous or arterial thrombosis, routine measurement of von Willebrand factor antigen or activity is not recommended.

Level 1

Platelet Count Monitoring and Laboratory Testing for Heparin-Induced Thrombocytopenia: Recommendations of the College of American Pathologists

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Contribution of Dr. T. Warkentin to “Acquired Immune-Mediated Thrombophilia”

Douglas A. Triplett (Coordinator)
Theodore E. Warkentin (HIT)
Joel L. Moake (TTP)

Recommendations for platelet count monitoring for early detection of heparin-induced thrombocytopenia (HIT)

Patients at risk:

Highest risk for heparin-induced thrombocytopenia (HIT)—postoperative patients receiving prophylactic-dose or therapeutic-dose unfractionated heparin: minimum monitoring during heparin therapy, every second day from day 4 to day 10*	Level 1
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Intermediate risk for HIT—medical/obstetrical patients receiving prophylactic- or therapeutic-dose unfractionated heparin, postoperative patients receiving prophylactic-dose low-molecular-weight heparin, or patients receiving intravascular catheter “flushes” with unfractionated heparin: minimum monitoring during heparin therapy, two or three times from day 4 to day 10*, when practical**	Level 1
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CAP Consensus Conference XXXVI: Diagnostic Issues in Thrombophilia
Platelet Count Monitoring and Laboratory Testing for Heparin-Induced Thrombocytopenia: Recommendations of
the College of American Pathologists

Low risk for HIT—medical/obstetrical patients receiving prophylactic- or therapeutic-dose low-molecular-weight heparin, medical patients receiving only intravascular catheter “flushes” with unfractionated heparin: routine monitoring is not recommended***	Level 2
The crucial time period for monitoring “typical-onset” HIT is between days 4 to 10* after starting heparin, where the highest platelet count from day 4 (inclusive) onwards represents the “baseline.”	Level 1
For a patient recently exposed to heparin (within the past 100 days), a repeat platelet count obtained within 24 hours following re-initiation of heparin is recommended to identify patients with “rapid-onset” HIT due to already circulating HIT antibodies.	Level 1
A platelet count should be measured promptly and compared with recent values in a patient who develops thrombosis during or soon after heparin therapy or in a patient who develops an unusual clinical event in association with heparin therapy (e.g., heparin-induced skin lesions; acute systemic reaction post-intravenous heparin bolus).	Level 2
A platelet count fall of 50% or greater from baseline can indicate HIT, even if the platelet count nadir remains above $150 \times 10^9/L$; rarely, platelet count declines of even lesser magnitude attributable to HIT can be associated with thrombotic events.	Level 1
<i>* first day of heparin use = day zero; platelet count monitoring should be extended beyond day 10 if the platelet count begins to fall unexpectedly during the day 4 to 10 period, or if heparin therapy is interrupted and restarted because of an intervening surgical or procedural intervention.</i>	
<i>** platelet count monitoring may not be practical when low-molecular-weight heparin is given to outpatients</i>	
<i>*** monitoring as per the “intermediate” risk group is appropriate if one or more doses of unfractionated heparin were given prior to initiating therapy with low-molecular-weight heparin</i>	

Recommendations for laboratory testing for HIT antibodies

HIT antibody testing is recommended for patients in whom there is clinical suspicion of HIT based upon the temporal features of the thrombocytopenia or based upon the occurrence of new thrombosis during, or soon after, heparin treatment.	Level 1
Acute serum or plasma should be used for testing whenever possible, since HIT antibodies are transient and may not be detectable even a few weeks after clinical HIT.	Level 1
An antigen assay is an appropriate screening test for most laboratories that can perform enzyme-immunoassays; however, confirmatory testing using a sensitive washed platelet activation assay (e.g., platelet serotonin release assay, heparin-induced platelet activation [HIPA] test) may be appropriate if antigen assay results are weakly positive (“indeterminate”) or positive testing occurs in a patient with low pre-test probability for HIT. In these situations, a negative activation assay suggests the patient likely did not have HIT.	Level 1
Washed platelet activation assays (e.g., platelet serotonin release assay, HIPA test) have high sensitivity and specificity for clinical HIT; however, these assays are technically demanding and are most appropriate for reference laboratories.	Level 1

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Platelet Count Monitoring and Laboratory Testing for Heparin-Induced Thrombocytopenia: Recommendations of
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If the platelet aggregation test (PAT) using citrated platelet rich plasma is used as an initial test for HIT, a positive test generally supports the diagnosis of HIT, and further testing is usually not required. However, given the lower sensitivity of the PAT, a negative test does not exclude HIT in a patient with a moderate or high pre-test probability for HIT. In these situations, further testing with the antigen assay or washed platelet activation assay (or both) should be performed.

Level 1

Antiphospholipid Antibodies

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Conclusions

Preanalytical variables, including preparation of platelet poor plasma (PPP) and freeze thawing effect (if plasma is frozen and tested at a later date), can influence the outcome of testing.

Establishing the diagnosis of lupus anticoagulants (LA) requires use of the guidelines as developed by the Scientific Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies.

"Integrated test systems" are currently preferable when testing for LA. Examples include commercially available dRVVT (screen)/dRVVT (confirm; e.g., increased phospholipid content). Other systems include Staclot LA®, dilute prothrombin time/screen (dPT), and confirmatory reagent (dPT high phospholipid). Venom-based assays include Textarin Time and Taipan Venom Time.

When performing enzyme-linked immunosorbent assays (ELISAs), systems that use high sensitivity "microtiter plates" are preferred. Alternatively, flow cytometry may offer a more sensitive and specific test system.

Recommendations: lupus anticoagulants and antiphospholipid antibodies

Anticardiolipin antibody (ACA) and lupus anticoagulant (LA) assays are appropriate for patients with venous thromboembolism (VTE), particularly if the VTE is idiopathic or associated with autoimmune disease or if there is no family history of venous thrombosis.	Level 1
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Anticardiolipin antibody and lupus anticoagulant testing may be considered for patients with arterial thrombosis, particularly in a young person or a person with no documented atherosclerosis.	Level 2
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Anticardiolipin antibody and lupus anticoagulant assays should be considered for patients with unexplained stroke, particularly in a young person or a patient with autoimmune disease. Level 1

Testing for lupus anticoagulant and anticardiolipin antibody should be considered for patients with cerebral venous thrombosis. Level 3

Women with pregnancy loss that is either recurrent or late in the pregnancy (second and third trimester) should be evaluated for antiphospholipid antibodies (LA and APA). Level 1

In order to demonstrate persistence, any positive test (APA or LA) must be confirmed by repeat testing after 6 weeks. Level 2

Recommendations: lupus anticoagulant

Platelet poor plasma used for lupus anticoagulant testing should have a platelet count <10,000/uL. Level 1

The use of commercially available, integrated test systems for measuring LA is recommended, for example, the StaClot LA or dRVVT. Level 1

Patients being treated with anticoagulants and specimens containing anticoagulants should not be tested for LA; however, if patients on oral anticoagulants or heparin must be tested, the results must be interpreted with caution. Level 2

Recommendations: antiphospholipid antibodies

IgG ACA testing is recommended for the evaluation of thrombophilia. Although frequently measured, the risk of incident or recurrent thrombosis associated with IgM ACA and IgA ACA is uncertain. Elevated titres (>40 GIU) are most closely associated with thrombophilia. Level 2

ELISAs for anti prothrombin and anti b2GP1 antibodies may be performed in addition to ACA testing in the evaluation of thrombophilia, however, prospective studies involving the use of assays for anti prothrombin and anti b2GP1 antibody for the evaluation for thrombophilia are limited. Level 3

Laboratory diagnosis of lupus anticoagulant

Demonstration of an abnormal phospholipid dependent screening test of hemostasis (e.g. APTT, dRVVT, KCT, dPT, Textarin Time, Taipan Time).

CAP Consensus Conference XXXVI: Diagnostic Issues in Thrombophilia
Antiphospholipid Antibodies

Failure to correct the prolonged screening coagulation tests on mixing with normal platelet poor plasma (criteria necessary to identify the presence of a circulating anticoagulant [synonym: inhibitor]).

Shortening or correction of the prolonged screening tests upon the addition of excess phospholipids or hexagonal phase phospholipids.

Rule out other coagulopathies (e.g., factor VIII inhibitors, presence of heparin).

Thrombotic Thrombocytopenic Purpura and the Hemolytic-Uremic Syndrome

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Conclusions

Von Willebrand factor (vWF)-cleaving metalloprotease: ADAMTS 13 (a disintegrin and metalloprotease with thrombospondin domains).

Thrombotic thrombocytopenic purpura (TTP): ADAMTS 13 activity is less than about 5-10% of the activity in normal pooled plasma

Hemolytic uremic syndrome (HUS) and other thrombotic microangiopathies: ADAMTS 13 activity varies over a broad range, but is not less than about 10% of normal.

Chronic relapsing TTP: ADAMTS 13 gene mutations (chromosome 9q34) causes activity levels that are chronically less than about 5-10% of normal.

Acquired TTP: ADAMTS 13 activity is transiently less than about 5-10% of normal as a result of transient antibody inhibition or defect in enzyme production/survival.

Familial or recurrent HUS may be caused by deficient plasma levels of factor H as a consequence of chromosome 1q32 mutations. Factor H normally suppresses the activity of the C3bBb C3 convertase in the alternative complement pathway.

The procedures for estimation of vWF-cleaving metalloprotease activity (ADAMTS 13) using citrate-plasma are either lengthy or developmental.

The presently available tests are not capable of rapidly confirming the clinical diagnosis of TTP and are only available in highly specialized reference laboratories.

Recommendations

The diagnosis of TTP remains clinical/pathologic. However, if the diagnosis is uncertain, it may be appropriate to collect (before plasma infusion/exchange) a citrate-plasma sample for the later determination of vWf-cleaving metalloprotease activity (ADAMTS 13) in a reference laboratory. The activity value may influence subsequent therapeutic decisions.

Level 1

HUS remains a clinical/pathologic diagnosis; however, factor H measurement in familial or recurrent HUS may be appropriate.

Level 1