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

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• ***Objective.*—To review the role of acquired and inherited prothrombotic risk factors that increase the risk of thrombosis in oral contraceptive users, during pregnancy, and in neonates, infants, and children; and to determine by the consensus opinion of recognized experts in the field which risk factors should be determined in which individuals at which time.**

***Data Sources.*—Review of the medical literature and current clinical practice by a panel of experts in the field of thrombophilia.**

***Data Extraction and Synthesis.*—The experts made an extensive review of the published literature and prepared a draft manuscript, which included preliminary recommendations. The draft manuscript was circulated to participants in the College of American Pathologists Conference XXXVI: Diagnostic Issues in Thrombophilia prior to the conference. The manuscript and recommendations were then presented at the conference for discussion. Recommendations were accepted if a consensus of the 26 experts attending the conference was reached. The results of the discussion were used to revise the manuscript into its final form.**

***Conclusions.*—This report reviews the options for testing for thrombophilic states in women using oral contraceptives, during pregnancy, and in neonates and children. General guidelines for testing in these clinical situations are provided, along with citation of the appropriate supporting literature.**

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This report focuses on diagnostic studies in thrombophilia related to pregnancy and its prevention by oral contraceptives, and in children. The discussion begins with the controversial issues related to thrombophilia testing of women using oral contraceptives. The subject of testing for thrombophilia in patients with pregnancy-associated venous thrombosis and fetal loss is then considered. The article concludes with a discussion of issues related to thrombosis testing in neonates and children.

THROMBOPHILIA IN WOMEN ON HORMONAL THERAPY

Oral Contraceptives and Hormone Replacement Therapy

The risk of venous thromboembolism (VTE) associated with the use of oral contraceptives containing ≥50 μg of ethinylestradiol has been estimated to be increased 4-fold.[1,2] Reduction of the ethinylestradiol content to 30 to 40 μg did not result in a decrease of VTE risk.[3] Two case-control and 2 cohort studies documented a 3- to 6-fold increase in the risk of VTE in healthy young women.[4–7] Women using third-generation oral contraceptives containing the progestins levonorgestrel and gestodene are associated with a 6- to 9-fold increased risk for VTE compared to nonusers. Of 16 studies comparing third-generation oral contraceptives to second-generation contraceptives containing the progestins levonorgestrel and norgestrel, 13 have demonstrated a 1.4- to 4.0-fold increased risk for VTE in users of third-generation oral contraceptives and 3 showed no difference.[8] The absolute risk for VTE in the first year of third-generation oral contraceptive use is 1 in 1000, which is 10-fold higher than in nonusers.

Proposed pathogenic mechanisms conferring thrombotic risk include an increase in levels of factors VII, VIII, and X; fibrinogen; and prothrombin.[9] Oral contraceptive use has also resulted in acquired activated protein C (APC) resistance,[10,11] which is more pronounced in users of third-generation compared to second-generation preparations.[12,13] The observed decrease in protein S only partially explains the acquired APC resistance in oral contraceptive users. A decrease of factor V[9] may also result in a reduced anticoagulant effect.[14]

The third-generation oral contraceptives (those containing desogestrel) produce greater increases in prothrombin, factor V, and thrombin-activatable fibrinolysis inhibitor, and greater decreases in protein S and factor V than do second-generation preparations containing levonorgestrel.[9] These changes may underlie the consistent epide-

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miologic findings of increased thrombotic risk in users of third-generation oral contraceptives over second-generation oral contraceptive users. These observations raise the likelihood that the relative risk for thrombosis for those with the factor V Leiden mutation using third-generation oral contraceptives exceeds that in second-generation oral contraceptive users with the factor V Leiden mutation.

Heterozygotes for factor V Leiden using oral contraceptives have a 35-fold increase in the risk for VTE compared to nonusers with normal factor V genotype[15–17] with a further increase in the risk in factor V Leiden homozygotes.[18] Likewise, the prothrombin G20210A mutation confers an increased risk for VTE, including cerebral vein thrombosis.[19] The uncommon deficiencies of antithrombin, protein C, and protein S are also associated with increased thrombotic risk in oral contraceptive users.[20,21]

As a corollary to the data on oral contraceptives, hormone replacement therapy is associated with a threefold increased risk of VTE. The combination of hormone replacement therapy and APC resistance increases the risk for VTE (odds ratio [OR] 13.3; 95% CI 4.3–41). The prothrombotic effects of the factor V Leiden mutation and hormone replacement therapy are additive rather than multiplicative.[22]

The incidence of fatal VTE in oral contraceptive users is 0.7 in 100 000. In heterozygotes for the factor V Leiden gene, it is in the range of 5 in 100 000. Since the absolute risk of VTE is estimated to be 1 in 200 to 1 in 500 in heterozygotes for factor V Leiden, the cost-benefit obtained from wide screening of the general population is questionable.[23] Currently, the cost of testing for factor V Leiden depends on the assays selected and the methods used to calculate the costs. Cost-benefit analysis suggests that screening for factor V Leiden would be cost-effective if the screening costs less than $9.[24] Theoretically, screening tests such as APC-resistance assays, and even possibly a protein C global assay, might be suitable for testing selected populations, such as oral contraceptive users, especially in areas where the prevalence of factor V Leiden is particularly high.

THROMBOPHILIA DURING PREGNANCY

Gestational Hypercoagulability and VTE

Pregnancy is an acquired hypercoagulable state with an increased thrombotic risk, which is also high during the first few months postpartum. The risk for VTE is increased 3-fold to 4-fold during gestation. This situation may result from an increase in procoagulants, such as factor VIII, and a decrease in physiological anticoagulants, such as protein S. Moreover, acquired APC resistance increases and fibrinolytic activity is reduced as the pregnancy progresses and the mother proceeds into the postpartum period. Inherited thrombophilia further increases the risk of VTE in pregnancy.[25,26] A thrombophilic risk factor can be found in the majority of women who present with gestational VTE. Factor V Leiden is found in 30% to 60%, the prothrombin G20210A mutation in 10% to 20%, and antiphospholipid antibodies in 10% to 20% of women with gestational VTE. In addition, antithrombin III, protein C, and protein S deficiencies are found in another 10% to 20%. The absolute risk of gestational VTE in carriers of factor V Leiden is 1 in 500, in carriers of the prothrombin mutation it is 1 in 200, and in carriers of both mutations it is 1 in 20.[25]

Gestational Hypercoagulability and Pregnancy Loss

Recurrent fetal loss (RFL) is a common problem. Of women in the reproductive age group, 1% to 2% experience 3 or more losses and 5% experience 2 or more losses. Recurrent fetal loss has a well-established association with certain acquired thrombophilic disorders, such as the antiphospholipid syndrome.[27] Several different antiphospholipid antibodies have recently been associated with RFL. Anti-phosphatidylethanolamine immunoglobulin (Ig) M (OR = 6.0; 95% confidence interval [CI] = 2.3–15.7; $P < .001$), anti-β_2-glycoprotein I IgG (OR = 4.4; 95% CI = 1.6–11.7; $P = .004$), anti-annexin V IgG antibodies (OR = 3.2; 95% CI = 1.2–8.1; $P = .02$), and the lupus anticoagulant (OR = 3.0; 95% CI 1.3–6.8; $P = .009$) have been found to be independent retrospective risk factors for unexplained early fetal loss.[28] These 4 markers were found in subsequent pregnancies to be associated with a significant risk of fetal loss, despite low-dose aspirin treatment.

A number of recent observations suggest an association between RFL and inherited thrombophilia. Forty-two (22%) of 188 pregnancies in women with protein C, protein S, or antithrombin III deficiency resulted in pregnancy loss, compared to 23 (11%) of 202 pregnancies in control subjects (OR = 2.0; 95% CI = 1.2–3.3).[29] In 15 women and 64 pregnancies with dysfibrinogenemia associated with thrombosis, 39% ended by miscarriage and 9% by intrauterine fetal death.[30]

Three case-control studies have evaluated the prevalence of the factor V Leiden mutation in women with RFL. Despite differences in ethnic white subpopulations and selection criteria for RFL, all 3 studies documented a significantly increased prevalence of the factor V Leiden mutation in women with RFL. Ridker et al[31] studied women with RFL without an extensive etiological workup, except for ruling out chromosomal abnormalities, and found a 2.3-fold increase in the prevalence of factor V Leiden in women with RFL.

In women with RFL of unknown cause, studies by Grandone et al[32] and by Brenner et al[33] have suggested that evaluation for factor V Leiden mutation is highly warranted, as a significant percentage of women with RFL are found to be carriers of the mutation. It should be emphasized, however, that other reports failed to document an association between factor V Leiden mutation and RFL.[34]

In populations in which homozygosity for factor V Leiden is highly prevalent, a significant association of this state with RFL can also be demonstrated.[33] The risk for RFL is greater in homozygotes than in heterozygotes with the factor V Leiden mutation.[35] Female siblings of thrombophilic women with the factor V Leiden mutation are also at an increased risk for RFL.[36] Women with thrombophilia have an increased percentage of losses at later stages of gestation.[33] However, APC resistance and the factor V Leiden mutation can also be associated with recurrent first trimester pregnancy loss.[37]

A potential explanation for the association between RFL and APC resistance is that APC resistance increases progressively throughout normal pregnancy, to some extent in correlation with changes in factor VIII, factor V, and protein S levels.[38] Transient elevations in APC resistance can be documented during normal gestations even in women with a normal factor V genotype. Naturally, APC resistance is greater during gestation in women with factor V Leiden mutation. Activated protein C resistance in

Table 1. Acquired Risk Factors for Pediatric Thromboembolism

Perinatal diseases
Birth asphyxia
Respiratory distress syndrome
Infants of diabetic mothers
Neonatal infections
Necrotizing enterocolitis
Dehydration
Congenital nephrotic syndrome
Polycythemia
Medical interventions
Central lines
Surgery
Renal transplantation
Immobilization
Plaster casts
Extracorporeal membrane oxygenation
Acute diseases
Trauma
Sepsis
Dehydration
Acute rheumatic diseases
Nephrotic syndrome
Acute lymphoblastic leukemia
Chronic diseases
Malignancies
Renal diseases
Cardiac malformations
Chronic rheumatic diseases
Drugs
Asparaginase
Prednisone
Coagulation factor concentrates
Heparins
Antifibrinolytic agents
Oral contraceptives

the absence of the factor V Leiden mutation has also been associated with pregnancy loss,[39] possibly as a result of other mutations that confer APC resistance.

A meta-analysis of 10 case control studies evaluated the role of the MTHFR T/T genotype and hyperhomocysteinemia in women with pregnancy loss. In 5 of the 6 case-control studies, the MTHFR T/T genotype was not found to be a significant risk factor for recurrent early pregnancy loss.[40] However, elevated fasting and post–methionine-loading homocysteine levels were found to be associated with recurrent early pregnancy loss (pooled OR = 2.7; 95% CI = 1.4–5.2 and OR = 4.2; 95% CI = 2.0–8.8, respectively).[40]

A recent study by Martinelli et al[41] demonstrated that the factor V Leiden and the prothrombin G20210A mutations are associated with an approximate 3-fold increase in the risk of late fetal loss. Eleven (16%) of 67 women with late fetal loss had either the factor V or the prothrombin mutation versus 13 (6%) of the 232 control subjects. The relative risk of late fetal loss in carriers of the factor V Leiden and prothrombin mutations was 3.2 (95% CI = 1.0–1.9) and 3.3 (95% CI = 1.1–10.3), respectively.

Combinations of thrombophilic states may further increase the risk for RFL. The European Prospective Cohort on Thrombophilia (EPCOT) study documented the highest OR for stillbirth (OR = 14.3; 95% CI = 2.4–86) in women with combined thrombophilic defects.[42]

Late Gestational Vascular Complications

Activation of blood coagulation and endothelial cell stimulation are recognized events in preeclampsia[43]; these events are clinically characterized by gestational hypertension, edema, and proteinuria.

Several recent reports suggest an association between APC resistance (with the factor V Leiden mutation) and early onset of severe preeclampsia. In one study, 14 (8.9%) of 158 women with severe preeclampsia were found to be heterozygous for the factor V Leiden mutation, compared with 17 (4.2%) of 403 normotensive gravida controls (P = .03).[44] Similarly, in another study, the factor V Leiden mutation was documented in 19% of women with preeclampsia, compared to 7% of control subjects.[45]

Hyperhomocysteinemia was documented in 26% of women with placental abruption, in 11% of the cases with intrauterine fetal death, and in 38% of women delivering babies whose birth weight was below the fifth percentile, compared with an estimated 2% to 3% in the general control population.[46] Likewise, hyperhomocysteinemia was documented in 26 (31%) of 84 women with previous placental infarcts or abruption, compared to 4 (9%) of 46 control subjects.[47] In the Hordaland Homocysteine Study, plasma homocysteine levels were evaluated in 5883 women with 14 492 gestations. The study, which is the largest performed to date, reported an increased risk in these subjects with elevated plasma homocysteine for preeclampsia (OR = 1.33), stillbirth (OR = 2.11), early labor (OR = 1.41), and placental abruption (OR = 3.03).[48]

Seventeen of 27 women with placental abruption had APC resistance, compared to 5 of 29 control subjects (OR = 8.2; 95% CI = 3.6–12.7).[37] Factor V Leiden was documented in 8 (30%) of 27 patients, compared to 1 (3%) of 29 control subjects.[49]

The high prevalence of genetic thrombophilias in women with pregnancy-related vascular thromboembolism[50,51] and the thrombotic changes in the placentae of the majority of women with complications, venous thrombophilia and stillbirth,[52] suggest that antithrombotic drugs may have a therapeutic benefit to preserve the pregnancy in women with gestational vascular complications.

Data on treatment of women with inherited thrombophilia and pregnancy loss are predominantly uncontrolled and include small series of patients treated mostly with low-molecular-weight heparin. A recent collaborative study demonstrated the safety of using low-molecular-weight heparin during 486 gestations.[53] A successful outcome was reported in 83 (89%) of 93 gestations in women with a history of recurrent pregnancy loss and in all 28 gestations in women who experienced preeclampsia during a previous pregnancy.[53] In women with thrombophilia, 46 (75%) of 61 pregnancies treated with low-molecular-weight heparin resulted in a live birth, compared to a success rate of only 20% in these same 50 women in prior gestations without antithrombotic therapy.[54] However, the optimal dosage of low-molecular-weight heparin is unknown and should be optimized by prospective randomized trials, which are currently underway.

THROMBOPHILIA IN NEONATES AND CHILDREN

Description of the Problem

Venous and arterial thrombosis are increasingly being recognized in infancy and childhood. Symptomatic thrombotic manifestations are recorded in 0.07 of 10 000

Table 2. Prevalence Rate of Prothrombotic Risk Factors in White Children*

	Controls	Patients	Odds Ratio (95% CI)
Risk factors in venous thrombosis[88,110]			
Factor V Leiden G1691A and A1691A	15/370	83/261	11.0 (6.2–19.7)
Factor V Leiden G1691A	14/370	77/261	10.6 (5.9–19.3)
Factor V Leiden A1691A	1/370	6/261	8.7 (1.0–72.6)
Prothrombin G20210A	4/370	11/261	4.1 (1.3–12.8)
Protein C deficiency	3/370	24/261	12.4 (3.7–41.6)
Protein S deficiency	3/370	15/261	7.5 (2.1–26.0)
Antithrombin deficiency	0/370	9/261	. . .
Lp(a) > 30 mg/dL	19/370	78/261	7.2 (3.7–14.5)
Risk factors in spontaneous stroke[89]			
Protein C deficiency	2/296	9/148	9.5 (2–44.6)
Factor V Leiden G1691A	12/296	30/148	6.0 (2.97–12.1)
Prothrombin G20210A	4/296	9/148	4.7 (1.4–15.6)
MTHFR 677TT	31/296	35/148	2.6 (1.5–4.5)
Lp(a) > 30 mg/dL	14/296	39/148	7.2 (3.8–13.8)
Risk factors in neonatal stroke[97]			
Protein C deficiency	. . .	6/91	. . .
Factor V Leiden G1691A	10/182	17/91	3.9 (1.7–9.0)
Prothrombin G20210A	4/182	4/91	2.0 (0.5–8.3)
MTHFR 677TT	20/182	15/91	1.6 (0.8–3.3)
Lp(a) > 30 mg/dL	10/182	20/91	4.8 (2.2–10.9)

* CI indicates confidence interval; Lp(a), lipoprotein (a); and ellipses, insufficient data.

total children, 5.3 of 10 000 children admitted to hospitals, and 2.4 of 1000 newborns admitted to intensive care units. Possibly owing to the lower concentrations of antithrombin, heparin cofactor II, and protein C, along with a reduced fibrinolytic capacity, neonates are at greater risk of thromboembolic complications than older children. The incidence of vascular accidents decreases significantly after the first year of life, with a second peak during puberty and adolescence, again associated with reduced fibrinolytic activity.[55]

Thrombus formation and thrombus growth are the result of local coagulation activation, combined with a disturbance in the balance between coagulation and fibrinolysis, leading to a prothrombotic state. In infancy and childhood, numerous manipulations and conditions (Table 1), such as birth asphyxia, neonatal infections, fetal diabetes, the use of central lines, trauma or surgery, dehydration, malignant diseases, renal diseases, autoimmune diseases, and the use of oral contraceptives in adolescent girls, result in elevated thrombin generation with subsequent thrombus formation.[56–85]

Various genetic prothrombotic defects, such as the factor V Leiden mutation and the prothrombin G20210A mutation, have been well established as risk factors for thrombotic events,[86,87] as well as antithrombin, protein C, and protein S deficiency. In addition, metabolic diseases, such as hyperhomocysteinemia and increased concentrations of lipoprotein (a) (Lp[a]), have been recently shown to significantly enhance the risk of thromboembolic arterial and venous thrombosis in pediatric and adult patients.[86–91] Since the discovery of APC resistance as a highly prevalent hereditary risk factor of thromboembolism, evidence has been accumulating that thrombophilia not infrequently involves multiple risk factors in the same patient.[92] The combination of genetic prothrombotic risk factors with acquired environmental or clinical conditions greatly increases the risk of thrombosis in children as well as adults.[90–95]

The most common sites of thrombus formation in neonates are the renal veins, vena cava, and the vessels where occlusion produces a thromboembolic stroke.[56–61,71,78,96,97] High rates of catheter-related thrombosis in neonates, infants, and children have been reported.[56–64,67,73] Central venous lines lead to thrombus formation and thrombus growth near the catheter implantation site, especially when prothrombotic risk factors are involved. Other sites of childhood thromboembolism include cerebral vein thrombosis[73,98,99] and portal and mesenteric vein thrombosis.[100,101] Arterial vascular occlusions are mainly ischemic strokes (Table 2)[81,89,96,97] and catheter-related thrombosis in the aorta, the femoral artery, and the subclavian artery.

Purpura fulminans is a life-threatening event characterized by microvascular thrombosis in the dermis followed by perivascular hemorrhage. Hemorrhagic necrosis of the adrenal glands (Waterhouse-Friderichsen syndrome) and renal cortical necrosis may also occur. Clinically, progressive purpuric skin lesions and diffuse oozing from skin puncture sites are observed, often within hours of birth. The lesions are initially red and flat, but quickly become indurated and necrotic and may result in gangrene. The known underlying causes of purpura fulminans in neonates are disseminated intravascular coagulation, for example, in response to bacterial septicemia caused by β-hemolytic streptococcus, *Neisseria meningitidis*, or *Streptococcus pneumoniae* infection; a congenital deficiency of protein C or protein S; and the presence of a homozygous or a heterozygous factor V Leiden mutation.[95,102–107]

The genetic prothrombotic conditions described, as well as the acquired antiphospholipid antibodies, play a contributory role in the pediatric population with symptomatic venous thrombosis[107,108] or an ischemic cerebrovascular accident.[81,98] Table 2 summarizes results from case series and case-control studies[109–114] in white children with venous thrombosis[88,110] or ischemic stroke[81,89,115–119] with respect to individual thrombotic risk factors.

Table 3. Conclusions and Recommendations*

Pregnancy

Conclusions

- The risk of VTE during gestation increases 3- to 4-fold. *Level 1*
- Thrombophilia can be identified in the majority of women with gestational VTE.[25] *Level 1*
- Thrombophilia is associated with unexplained pregnancy loss (especially in second and third trimester).[29–37,39–42,52] *Level 2*
- Other gestational vascular complications (preeclampsia, intrauterine growth retardation, placental abruption) are associated with thrombophilia.[45–51] *Level 3*
- Combined thrombophilic conditions increase the risk for gestational complications.[33,42,52] *Level 2*
- Patients with prior VTE during pregnancy who have a thrombophilic state are at high risk for recurrence during subsequent pregnancy, may receive antithrombotic prophylaxis during gestation, and should receive antithrombotic prophylaxis in the postpartum period.[26] *Level 2*
- Prevention of pregnancy loss in women with thrombophilia by antithrombotic therapy is currently being evaluated in prospective randomized trials.

Recommendations

- Women with VTE during pregnancy or in the postpartum period should be evaluated for thrombophilia.[1] *Level 1*
- Women with pregnancy loss that is either recurrent or late in the pregnancy (second and third trimester) should be evaluated for thrombophilia.[29–37,39–42,52] *Level 1*
- Whether women with other gestational vascular complications should be evaluated for thrombophilia is controversial.[44–51] *Level 3*
- Testing results for APC resistance and protein S obtained during pregnancy or the postpartum period should be interpreted with caution in view of physiologic changes.

Hormonal therapy

Recommendation

- Testing for thrombophilia is recommended in women who experience VTE as cerebral venous thrombosis during oral contraceptive use or HRT.[15–22] *Level 1*

Pediatrics

Conclusions

- Thrombophilia is commonly found in children (particularly in infants) with VTE or stroke.[88,89,97,101,108,110,111,118] *Level 2*
- The presence of multiple thrombophilias greatly increases the risk of thrombosis and/or recurrence of thrombosis in infants and children, as it does in adults.[90] *Level 1*
- The distribution of prothrombotic risk factors varies with respect to the ethnic background and the number of patients/controls investigated.[129–132] *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.[61,72,73,90] *Level 1*; [81,88,89,97,101,110,115] *Level 2*
- Age-specific reference ranges should be used to interpret the results of thrombophilia testing in the pediatric and neonatal age groups.
- Routine evaluation for thrombophilia for asymptomatic children of probands with inherited thrombophilia may be delayed until puberty.[125] *Level 3*
- Evaluation for thrombophilia of the siblings of probands with early symptomatic thromboembolism is recommended.[125] *Level 3*

* VTE indicates venous thromboembolism; HRT, hormone replacement therapy; and APC, activated protein C. Definition of levels of evidence: Level 1, 1 or more well-designed prospective studies; Level 2, retrospective studies or multiple anecdotal studies that reach consensus; and Level 3, isolated anecdotal studies and/or the consensus of expert practitioners.

Diagnostic Testing

Suitable assays for APC resistance,[120,121] protein C activity, free and total protein S antigen, antithrombin activity, fibrinogen concentration, plasminogen activity, factor VIII, Lp(a),[122,123] and fasting homocysteine concentration should be investigated along with DNA-based assays, notably the factor V Leiden mutation when indicated by the APC-resistance assay, the prothrombin G20210A mutation, and possibly the MTHFR C677T genotype if the homocysteine concentration is elevated.

Commercially available Lp(a) assays are not yet standardized. However, a working group supported by the National Institutes of Health/National Heart, Lung and Blood Institute has evaluated 22 Lp(a) assays using reference material developed by the International Federation of Clinical Chemistry and Laboratory Medicine in a well-defined reference assay based on the immunodetection of nonrepetitive epitopes within Lp(a).[122] Overall in the reported trial, the results of various Lp(a) assays correlated well. However, individual assays for the measurement of apolipoprotein (a) were biased by 6% to 31% toward higher or lower Lp(a) values, so that at a given Lp(a) threshold, some assays overestimate and some underestimate the thrombotic risk. For this reason and because Lp(a) serum levels are determined mainly by a genetically determined size polymorphism of its main protein component, it may be useful to include the analysis of apolipoprotein (a) phenotypes.[123]

In cases with a strong family history for thrombosis and an affected child, but no positive test results for the more common abnormalities, rare prothrombotic defects (eg, dysfibrinogenemia, hypoplasminogenemia, dysplasminogenemia, heparin cofactor II deficiency, increased levels of histidine-rich glycoprotein, and other genetic mutations and polymorphisms) should be kept in mind. Besides testing for the inherited prothrombotic defects listed, all symptomatic children with thrombosis should be screened for antiphospholipid or anticardiolipin antibodies and the presence of lupus anticoagulants.[107,108]

Purpura fulminans, a complication of disseminated intravascular coagulation, is unlikely if there is no biochemical evidence of accelerated fibrinolysis. Therefore, in addition to the measurement of a partial thromboplastin time, prothrombin time, and platelet count, screening tests for disseminated intravascular coagulation should include a functional fibrinogen assay, functional antithrombin as-

say, and a D-dimer assay. Protein C, protein S, and the factor V Leiden mutation should be investigated in neonates with disseminated intravascular coagulation complicated by purpura fulminans.[124]

A recent prospective study on recurrent vascular occlusion after a first episode of spontaneous VTE has identified a subgroup of pediatric patients suffering from combined prothrombotic risk factors who are at high risk of recurrent thrombosis. A search for multiple risk factors is justified in selected patient groups.[90] According to the mendelian theory of inheritance, approximately 50% of siblings of a symptomatic propositus suffering from 2 prothrombotic defects carry a single risk factor, while 25% carry 2 or more gene mutations/polymorphisms. Because an effective prophylactic anticoagulant therapy is available for use in high-risk situations, screening for prothrombotic risk factors should be considered in symptomatic siblings and first-degree family members.[125]

Since DNA-based assays are influenced neither by acute thrombotic onset nor by anticoagulation and thrombolytic therapy, screening can be performed for genetic mutations or polymorphisms immediately at the onset of a thrombotic event. Oral anticoagulant medication influences certain assays and, therefore, it is recommended that fresh plasma samples for coagulation analyses be drawn at least 14 to 30 days after withdrawal of oral anticoagulation therapy. To reduce the effect of an acute thrombotic event on protein C, protein S, and antithrombin, plasma samples should ideally be obtained 3 to 6 months or more after the thrombotic episode. Since Lp(a) levels increase during the first year of life,[126] attaining a 2-fold value over birth level by approximately 1 year, repeat testing 8 to 12 months after the acute thrombotic onset is mandatory when including Lp(a) in the screening program of white neonates suffering from thromboembolism. Repeat testing is also necessary in pediatric patients with increased anticardiolipin/antiphospholipid IgM or IgG antibodies or lupus anticoagulants to fully assess thrombotic risk.

For all plasma-based assays, a clotting abnormality should be documented as a defect only if the level is outside the limits of its normal range.[109,127,128] Besides classification based on age-dependent normal reference ranges and confirmation of a suspected prothrombotic defect in a second sample (3–6 months later without oral anticoagulation), the criterion for the diagnosis of a hereditary hemostatic risk factor is the identification of a causative gene mutation.[86,87] Also, the distribution of prothrombotic risk factors varies with respect to ethnic background.[129–132]

The conclusions and recommendations for laboratory testing for thrombophilia in women on hormonal therapy, during pregnancy, and in children are presented in Table 3.

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