

Laboratory testing for Thrombophilia in Pediatric Patients

On Behalf of the Subcommittee for Perinatal and Pediatric Haemostasis of the Scientific Standardization Committee of the International Society for Thrombosis and Haemostasis

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Introduction: Interest in genetic traits predisposing to thrombosis has blossomed as evolving research findings explain many familial syndromes of premature vascular disease. Plasma and DNA-based tests have been established to screen individuals for deficiencies or dysfunctions of these newly described proteins. Thrombophilia, or the genetic predisposition to thrombosis, has been determined in pediatric patients with thrombosis, from the newborn period through adolescence (1-9). In addition, acquired abnormalities in coagulation tests have been demonstrated to increase the risk of thrombosis in affected individuals (10-14). The Subcommittee on Perinatal and Pediatric Haemostasis of the International Committee of Thrombosis and Haemostasis formed a working group to consider recommendations for a standardized approach to laboratory testing of children with thrombosis.

Background: Certain principles have evolved in our early studies of infants and children with thrombosis.

First, while episodes of thrombosis in persons affected with thrombophilia most frequently present during adolescence and young adulthood, presentation during infancy and childhood is well-established. The manifestation of clinical thrombotic disease in infants and children with thrombophilia is skewed toward those children with more severe deficiencies or the concurrence of multiple thrombophilic traits. This has been most evident in neonates presenting with purpura fulminans and/or spontaneous large vessel thrombosis (15, 16). Therefore, a thorough laboratory evaluation should be done on every child with thrombosis, not limiting screening to a single trait already identified in the family. Children with recurrent unexplained thrombosis are likely to have a constitutional cause, especially if the family history is positive. In these cases, further evaluation for very rare, or even previously unrecognized thrombotic disorders is warranted.

Second, thrombosis in pediatric patients is multifactorial. The pediatric patient with thrombosis has an average of two and as many as four or more predisposing and triggering prothrombotic factors (1,6,7,15). Most pediatric patients with thrombosis have significant underlying medical diseases as shown on Table I. Catheters are present in approximately 25% of thrombosis cases. Genetic and acquired abnormalities of coagulation have been reported in the majority of children with thrombosis (1, 3, 17). Even children with two or more thrombophilic traits usually present clinically in the setting of trauma or inflammatory medical conditions. A recent study of children undergoing treatment for acute lymphoblastic leukemia determined that the risk of therapy-related thrombosis was increased 25 fold in children with underlying thrombophilia (2). This study suggested that laboratory screening of leukemic children prior to the institution of specific therapy offers the possibility of targeted interventions for prevention of thrombosis in high-risk children. The presence of prothrombotic conditions does not diminish the odds of detecting thrombophilic traits and should not be a deterrent to complete coagulation testing.

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Third, children are more likely than adults to have one or more significant genetic or acquired coagulation deficiencies that may require specific therapy for management of the acute thrombosis (15,16,18). Some examples include severe genetic deficiencies of protein C or protein S, as well as acquired deficiencies for which rapid replacement therapy may be necessary in order to interrupt consumptive coagulopathy or achieve successfully anticoagulation. It is often clinically useful to determine the level of coagulation regulatory proteins acutely rather than waiting until after long-term anticoagulant therapy may be discontinued. This may involve repeat testing for one or two proteins but will not substantially increase costs. In addition, antiphospholipid antibodies and their effects on coagulation proteins may be transient and may be missed if testing is delayed three to six months. Finally, genetic testing may be very important in managing future pregnancies. Evidence for the association of thrombophilic factors with thromboses in children is as follows:

Venous thrombosis: Genetic abnormalities of antithrombin III, protein C, protein S, factor V G1691A, Prothrombin G20210A, fasting homocysteine and elevated lipoprotein (Lp) (a) concentration have been reported in children with venous thrombosis (1-9). Antiphospholipid antibodies including the lupus anticoagulant and anticardiolipin antibody have been associated with venous and pulmonary thromboembolism in children (10-12). Dural sinus thrombosis is a form of venous thromboembolism (14).

Arterial thrombosis: Vascular injury, indwelling catheters, congenital malformations, cardiac disease or vasculitis are present in most children with arterial thromboses (19). However, thrombophilia should be excluded in affected children. High titer lupus anticoagulants are associated with arterial and venous thrombosis in children.

Neonatal thrombosis: Neonates presenting with arterial and venous thrombosis have an increased risk of thrombophilia, particularly in settings with no obvious triggering event (20, 21). Multiple thrombophilic traits are often determined in term neonates with severe or multifocal thrombosis. Catheter-related thrombosis in preterm infants are usually caused by vascular damage and decreased blood flow. The benefit of screening for thrombophilia in this population has not been established (22).

Recommendations for laboratory testing in children with thrombosis:

Pediatric patients should be tested for a full panel of genetic and acquired prothrombotic traits. Detection of one thrombophilic factor does not exclude the existence of a second or third. The evaluation may be performed in stages, as shown on Table II. All tests in level I should be performed initially. Any abnormalities may be repeated in three to twelve months, off anticoagulation therapy. Age-dependent reference ranges have to be taken into account (23, 24). If all tests in Level I are negative and the affected child has a strong positive family history for thrombosis, recurrent thrombosis or life-threatening thrombosis, then Level II tests should be performed to determine a marker thrombosis risk and help decide long-term therapy. Tests in Level III are currently under investigation and have not yet been linked to thrombosis in children.

Recommendations for future studies:

Future studies are required for definitive recommendations regarding laboratory testing of children with thrombosis. To date, most studies of genetic risk factors for thrombosis have been conducted in individuals of northern European extraction (2,-4, 6-9,18), while thrombosis is known to affect children of all races and ethnicities. Studies are needed in diverse racial ethnic groups to determine genetic risk factors that can be applied to children of other race and ethnicity. Little data is currently available regarding the influence of maternal or fetal genes on thrombotic risk in the fetus and neonate. Cost and medical efficacy of testing preterm infants with catheter-related thromboses remains to be determined. Currently, no vascular or coagulation abnormality can be detected in one half of children with stroke. Further studies of risk factors for stroke in children, including platelet and endothelial cells factors, should be pursued. Finally, the role of thrombophilia in recurrence of childhood thrombosis, including implications for long-term anticoagulant therapy and recommendations for contact sports should be addressed (18).

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Table I: Underlying prothrombotic predisposing and provoking factors in children.

Indwelling vascular catheters
Infection
Trauma
Surgery
Vascular malformation or damage
Malignancy
Chemotherapy with L-asparaginase
Cardiac disease
Prosthetic cardiac valves
Systemic lupus erythematosus
Rheumatoid Arthritis
Crohn's disease
Ulcerative colitis
Primary antiphospholipid antibody syndrome
Polycythemia
Sickle Cell Anemia and other hemoglobinopathies
Renal Disease
Diabetes Mellitus
Appendicitis
Inflammation

Table II. Recommendations for Laboratory Testing in Children with Thrombosis

Level I: Basic evaluation for all children

Complete blood count with hematocrit, white blood count and platelet count
Antithrombin III
Protein C activity
Free and total protein S antigen
Factor V G1691A and/or functional activated protein C resistance assay
Prothrombin G20210A
MTHFR T677T and/or fasting homocysteine level
Lipoprotein (a)
Lupus anticoagulant
Anticardiolipin antibodies
Sickle Cell: screen or hemoglobin electrophoresis

Level II: Extended evaluation for children with normal Level I values and recurrent thrombosis, a positive family history for thrombosis or severe thrombosis:

ELT: euglobulin clot lysis time
Plasminogen
Dysfibrinogenemia evaluation: fibrinogen activity, antigen, thrombin time, reptilase time, fibrin degradation products, consider crossed immunoelectrophoresis
PAI: plasminogen activator inhibitor
Heparin cofactor II
PNH: paroxysmal nocturnal hemoglobinuria (Sucrose hemolysis)
If not previously performed:
Functional activated protein C resistance (modified assay)
Fasting homocysteine level
Hemoglobin electrophoresis
ESR: erythrocyte sedimentation rate, CRP: C-reactive protein

Level III (Currently under investigation)

Factor VIII
Factor XII
Factor XI
Von Willebrand factor level and Multimers
Spontaneous platelet aggregation
Platelet receptor polymorphisms
TPA (tissue plasminogen activator)
TFPI (Tissue factor pathway inhibitor)