



# CLINICAL HEMOSTASIS REVIEW

*An Update on Advances and Issues in Hemostasis*

## Pediatric Thrombosis

Dorothy M. Adcock, MD

Thromboembolism is a disorder that largely affects adults. The incidence of childhood venous and arterial thrombosis is very low and occurs in only 0.07 per 10,000 children in the general population. The risk for developing thrombosis increases with age, increasing predominantly after the age of 40 years. In the adult population, the incidence of venous thrombosis is 1 in 1000 at 40 years of age and 1 in 100 at age 75 years.

The pediatric population is relatively protected from developing pathological thrombus until after puberty. In childhood, the vast majority of thrombotic events (TE) occur as a complication of therapy for serious underlying disease. Pathological thrombosis is essentially unheard of in the well child. Idiopathic TE occurs in less than 1% of newborns and in fewer than 5% of children. Approximately 40% of TE's in adults are idiopathic. The basis of this lower risk for developing TE in childhood is multifactorial. Children are relatively protected due to immaturity of the hemostatic system and lack of age-related acquired risk factors such as atherosclerotic vascular disease.

Development of the hemostatic system is a dynamic process. Levels of various hemostatic proteins vary with both age and hormonal influence. The hemostatic system is immature at birth, most proteins do not achieve adult levels until at least three to six months of age. Some proteins such as protein C, in fact, do not achieve adult levels until about 16 years of age. In the laboratory, immaturity of the hemostatic system can be demonstrated by both decreased rate and capacity for thrombin generation, as compared to adults. The rate and amount of thrombin generated is dependent on the concentration of procoagulant proteins as well as the levels of functional naturally occurring inhibitors of thrombin. Plasma pooled from a population aged 1-16 years showed 27% less thrombin generation in APTT-based assays, in comparison to adult plasma. Thrombin generation is lowest in pre-term and term infants, when procoagulant proteins tend to be at their lowest levels. Levels of  $\alpha$ -2 macroglobulin are increased during childhood and do not decrease to reach adult levels until the third decade of life.  $\alpha$ -2 macroglobulin binds to the active site of thrombin and serves as a direct thrombin inhibitor. Elevated levels of  $\alpha$ -2 macroglobulin are believed to contribute to the low incidence of TE during this time period.

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Objective: The reader will be able to identify the assays that should be performed in the evaluation of pediatric thrombosis.

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3176 S. Peoria Ct  
Aurora, CO 80014  
520.722.0797  
chr@coagulation.com

### CLINICAL ADVISORS

Dorothy M. Adcock, MD

### CONTRIBUTOR

Monica Thibault

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The incidence of pediatric thromboembolic disease is increasing. The most important contributing factor is improved tertiary care resulting in the survival of children that, 10 years ago, would have succumbed to their disease. Enhanced survival is now seen with such diseases as, acute lymphoblastic leukemia, a variety of solid tumors and congenital heart disease. Children with these disorders often require the placement of intravascular catheters which serve as an important risk factor for both venous and arterial thrombosis. Increased incidence of pediatric thrombosis is also a reflection of increasing recognition of this entity in this young population along with the application of improved diagnostic techniques.

In the age group from birth to 18 years, thrombosis occurs most commonly in two distinct periods - in those children less than 1 year of age and in the teen years.

### Venous Thromboembolic Disease

In the neonatal population, symptomatic venous thrombosis is reported in approximately 0.25/10,000 births while deep venous thrombosis (DVT) and pulmonary embolus (PE) occur at a rate of 0.07 and 5.3/10,000 hospital admissions respectively. Spontaneous venous thrombosis is very unusual in the pediatric population, accounting for only 5% of all TE's. The vast majority of pediatric thromboses occur as a secondary complication of underlying serious illness.

Over 80% of VTE in newborns and over 50% in children, occur as a result of placement of central venous lines (CVL). Of all pediatric patients with central venous lines, thrombosis can be detected in up to 50% if sensitive radiographic techniques are used. Many of these blood clots, however, are asymptomatic. There are multiple reasons that intravascular catheters promote the development

of blood clots including; catheter-induced endothelial damage, disruption of blood flow, the thrombogenic nature of the catheter surface and thrombogenicity of certain solutions infused.

Central lines are most commonly placed into either the umbilical vein of a newborn or into the upper venous system of an infant or older child. The upper venous system is typically accessed through a peripheral vein or through a larger central conduit such as the jugular vein. Most DVT in the pediatric population therefore, involve the upper extremity. This is unlike DVT in adults that involve predominantly the deep venous system of the lower extremity. The typical presentation of catheter related acute DVT includes swelling, pain, and discoloration of the affected limb or with blockage of the jugular vein, swelling of the face and head. Long-term sequelae of venous thrombosis are significant and include most importantly, post-thrombotic syndrome (PTS). The potential for the development of PTS is enhanced in the very young patient due to immaturity of the fibrinolytic system. This leads to the potential for prolonged venous obstruction. Physiologically, PTS is due to damage to venous valvular system resulting in increased redistribution of blood flow from the deep to the superficial venous system. Clinically PTS leads to edema, impaired tissue viability and pain. These sequelae can last the patient's lifetime and result in significant morbidity.

The most common site of non-catheter related venous thromboses in the neonatal population are the renal veins. Renal vein thrombosis is typically idiopathic and generally occurs in the first few days of life. DVT of the lower extremities is the most common site for non-catheter related thrombosis in children.



## Arterial Thromboembolic Disease

In both children and adults, arterial thrombosis does not typically occur spontaneously, but rather, occurs in a setting of underlying vascular injury or disease. Atherosclerotic vascular disease is the most common predisposing factor for arterial thrombosis in the adult population. In the pediatric population, arterial thrombosis is almost always iatrogenic, secondary to arterial catheterization. Without the use of prophylactic heparin, thrombosis occurs in 40% of patients who undergo cardiac catheterization while it is seen in only 8% that receive anticoagulation. Non-catheter related arterial clots are rare in childhood and tend to occur in patients with arteritis, such as Takayasu's arteritis or Kawasaki's disease. Arterial blood clots may also originate in transplanted organs.

## Laboratory Evaluation

The role that congenital prothrombotic states play in the development of pediatric thrombosis is controversial. In children with TE, the reported incidence of prothrombotic disorders varies from 10% to 60%. Studies of families with PC, PS, AT deficiency or with either factor V Leiden or prothrombin G20210A mutations report an extremely low incidence of thrombosis in children less than 15 years of age. There is some doubt, therefore, regarding the usefulness of screening for congenital prothrombotic risk markers. This is especially true in children who have multiple acquired risk factors, such as a young cancer patient with a central venous catheter.

Despite such controversy, the British Committee for Standards in Haematology, Haemostasis and Thrombosis Task Force recommends laboratory evaluation for prothrombotic markers in any child with a clinically significant thrombosis, ischemic skin lesion

or purpura fulminans and in any child with a family history of purpura fulminans. The Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH) Subcommittee for Perinatal and Pediatric Thrombosis also suggests that every child with thrombosis undergo laboratory evaluation. It is furthermore, recommended that asymptomatic children should not be evaluated. Evaluation of the pediatric population with thromboembolic disease differs from that of adults, in a number of important ways, including assays performed, ranges used for interpretation and timing of the evaluation.

The ISTH subcommittee specifically recommends that when a prothrombotic risk evaluation is pursued, that a thorough evaluation be performed including markers for both congenital and acquired prothrombotic states. This is because it has been demonstrated that the pediatric patient with thrombosis has on average, 2 to 4 predisposing prothrombotic risk factors. Furthermore, in this age group the distinction between the risk factors for arterial and venous

thrombosis is blurred. In adults, there is a relatively clear association of risk factors with either arterial or venous thrombosis, however in young patients, risk factors do not seem to distinguish risk for venous or arterial disease.

Recommended timing of the pediatric evaluation differs from that of adults. The work-up of the pediatric patient should be undertaken in the acute phase of the event as results of this evaluation may impact therapy. This is particularly true in those with protein C, protein S or antithrombin deficiency. Results of the evaluation may also be useful in planning future pregnancies in the patient's family. In the adult population, it is common for the evaluation to take place around the time that warfarin therapy will be or has been discontinued.

Interpretation of prothrombotic markers must be made with the patient's age in mind. The hemostatic system of the neonate is significantly different from that of an adult. Most procoagulant and anticoagulant factors are reduced at birth and do not come in to the normal range until three to 6 months of age. As levels of protein C, protein S and antithrombin may be low physiologically in the neonate, there is often considerable overlap between the normal reference range for age and range seen with a heterozygous deficiency.

There is little consensus between experts regarding which assays should be performed in the evaluation of pediatric thrombosis. Most experienced pediatric hematologists agree that an evaluation should include at a minimum, assays for antithrombin, protein C, protein S, factor V Leiden, prothrombin G20210A and homocysteine. Other assays that may be considered include evaluation for lupus anticoagulant, anticardiolipin antibodies, lipoprotein (a), dysfibrinogenemia, and assays to determine the presence of abnormal fibrinolysis.

**Table 1. Recommended Laboratory Assays in the Pediatric Population to Evaluate Venous Thrombosis**

- Antithrombin Activity
- Protein C Activity
- Free Protein S Antigen and Total Protein S Antigen
- Factor V Leiden
- Prothrombin G20210A Mutation
- Homocysteine
- Lipoprotein (a)
- Lupus Anticoagulant Evaluation
- Anticardiolipin Antibodies

**Antithrombin:** Functional antithrombin levels are reduced at birth and reach adult levels by about 3 months of age. Antithrombin levels are reduced in children receiving L-asparaginase therapy and in those receiving unfractionated heparin therapy. It may be difficult to distinguish heterozygous AT deficiency from expected values in these situations. Repeat analysis 3 to 6 months after the acute thrombotic event or following cessation of therapeutic intervention is generally recommended. Family studies may also be of value. Heterozygous AT deficiency is rare and homozygous AT deficiency has rarely been reported.

**Protein C:** Functional PC levels are reduced at birth and reach adult levels by about 16 years of age. Approximately 40% of otherwise healthy children have functional PC levels in the range seen with heterozygous PC deficiency in adults. Functional levels may also be reduced in those with vitamin K deficiency, those on warfarin therapy, following an acute thrombotic event and as a result of serious illness. It may be difficult to distinguish heterozygous PC deficiency from expected values in these situations. Repeat analysis 3 to 6 months after the acute thrombotic event or following cessation of therapeutic intervention may be useful. Family studies may also be of value. Heterozygous PC deficiency occurs with an incidence in the general population of 0.2%. Homozygous PC deficiency is rare and usually presents as purpura fulminans within the first hours to first few days of life. Purpura fulminans is an acute, life-threatening, rapidly progressive, disorder of skin necrosis due to thrombosis of the microvasculature. Individuals with purpura fulminans have laboratory features of disseminated intravascular coagulation. Rather than skin necrosis, cerebral and retinal vein thrombosis may also be presenting features and

frequently occur *in utero*. With homozygous PC deficiency, laboratory evaluation reveals undetectable (<0.01 U/ml) PC levels. As PC levels can be significantly reduced in an ill neonate, the diagnosis of homozygous PC deficiency should be made only when there is an appropriate clinical picture and when both parents are confirmed heterozygous for PC. Homozygous PC deficiency is fatal unless immediately treated.

**Protein S:** In the evaluation of pediatric PS deficiency, free and total PS antigen assays should be performed. Free and total PS levels are physiologically low in the neonate but reach adult levels by about 4 and 10 months respectively. C4bBP is low in the neonate. Functional PS levels are further reduced in those with vitamin K deficiency or on warfarin therapy. It may be difficult to distinguish heterozygous PS deficiency from "expected" levels in these situations. Repeat analysis 3 to 6 months after the acute event may be useful. Family studies may also be of value. Heterozygous PS deficiency occurs with about the same frequency in the general population as PC deficiency. Homozygous PS deficiency is rare and usually presents as purpura fulminans within the first hours to first few days of life or with cerebral or retinal vein thrombosis. Laboratory evaluation reveals undetectable (<0.01 U/ml) PS levels. Homozygous PS is fatal unless immediately treated.

**Resistance to Activated PC:** Due to variability in FVIII levels and diminished thrombin generation in childhood, the activated PC resistance (APCR) assay is not recommended as a screening assay for the presence of the factor V Leiden mutation in the pediatric population. This assay rather, should be performed in addition to the DNA based assay for factor V Leiden. APCR, even in the absence of factor V Leiden is a prothrombotic risk factor. The risk

associated with APCR is graded, that is, the greater the resistance to APCR, the greater the risk for thrombosis.

**Factor V Leiden and Prothrombin G20210A:** These mutations represent single nucleotide polymorphisms and can be identified in the laboratory using a variety of molecular techniques. These assays therefore are not affected by age of the patient, underlying physiological condition or concurrent medications. In the Caucasian population, these mutations in the heterozygous state occur with a frequency of 5% and 2% respectively and increase risk of venous thrombosis in both children and adults. While the presence of a homozygous state for either or both of these mutations is less common, thrombotic risk is enhanced.

**Table 2. Less Common or Well-established Causes of Thrombosis in the Pediatric Population.**

- Elevated PAI-1
- Dysfibrinogenemia
- Plasminogen Deficiency
- Heparin Cofactor II Deficiency

**Homocysteine:** Elevated homocysteine levels are associated with an increased risk of both venous and arterial thrombosis. Plasma or serum homocysteine levels can be quantitated using immunological methods or gas chromatography. Thrombotic risk seen with elevated homocysteine levels is graded. Increased risk for thrombosis occurs in an elderly population when levels exceed 18 to 20  $\mu\text{mol/L}$ , but in childhood, levels are generally greater than 100  $\mu\text{mol/L}$  before risk is increased. There are a number of mutations in the homocysteine pathway that have the potential to result in

hyperhomocysteinemia. If homocysteine levels are significantly increased in the pediatric population, evaluation for mutations in cystathionine  $\beta$  synthetase or methylenetetrahydrofolate reductase should be investigated. Certain medications that inhibit the folate/B12 pathway such as methotrexate or renal failure may increase homocysteine levels.

Abnormalities of the fibrinolytic system: It is controversial as to whether moderate or even severe plasminogen deficiency is associated with an increased thrombotic risk. Patients with severe plasminogen deficiency suffer from ligneous conjunctivitis that appears to respond to plasminogen eye drops. Elevated levels of plasminogen activator inhibitor – 1 (PAI-1) may theoretically cause hypofibrinolysis and lead to increased thrombotic risk. The relationship between elevated PAI-1 levels and thrombotic risk however is not clear-cut.

Lupus anticoagulant and antiphospholipid antibodies: Antibodies to phospholipid antibodies and the presence of lupus anticoagulant are associated with both venous and arterial thrombotic disease in children and adults. Passive transfer of these antibodies to neonates from affected mothers is a rare occurrence but may be associated with severe perinatal thrombosis.

Lipoprotein (a): Levels of lipoprotein (a) are genetically determined and are influenced little if at all by diet or lipid lowering medications. Elevated concentrations of circulating lipoprotein (a) are associated with an increased risk of venous thrombosis in children. Lipoprotein (a) shares significant structural homology with plasminogen and it is believed that lipoprotein (a) competes with plasminogen for fibrin binding sites leading to impaired fibrinolysis.

## Conclusion

The incidence of pediatric thrombosis is increasing due to improved survival of children with serious pediatric illness, improved diagnostic modalities and increased recognition of thrombosis occurring in this population. National and International Registries for Pediatric Thrombosis are on-going with the hope that these will provide the data necessary to determine the optimum diagnostic and therapeutic modalities for those children suffering from thromboembolic disease. ▲

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