

# Thromboembolic Diseases in Neonates and Children

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## Key Words

Thrombosis · Thrombophilia · Children

## Abstract

Acquired and inherited prothrombotic risk factors increase the risk of thrombosis in neonates, infants and children. After suffering thrombosis white paediatric patients should be screened for common gene mutations, i.e. the factor V G1691A, factor II G20210A and MTHFR C677T genotypes, rare inherited prothrombotic risk factors, i.e. deficiencies of protein C, protein S, and antithrombin, plasminogen, probably inherited risk factors, i.e. fibrinogen, factor VIIIc, factor XII, new candidates, i.e. elevation of lipoprotein (a), and fasting homocysteine concentrations (3 - 6 months after thrombotic onset). Data interpretation is based on age-dependent reference ranges or the identification of causative gene mutations/polymorphisms with respect to individual ethnic backgrounds.

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## Introduction

Venous and arterial thrombosis are rare diseases being increasingly diagnosed and recognised also in infancy and childhood. Due to the special properties of the haemostatic system during infancy and childhood, symptomatic thrombotic manifestation occurs in 0.07/10,000 children, 5.3/10,000 admissions of children, and 2.4/1000 admissions of newborns to intensive care units. Within the entire childhood population, possibly due to the lower concentrations of antithrombin, heparin cofactor II and protein C along with a reduced fibrinolytic capacity neonates are at a 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 a reduced fibrinolytic activity [1].

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. Numerous clinical and environmental conditions (Table 1), such as periparturient asphyxia, neonatal infections, foetal diabetes, the use of central lines, trauma or surgery, dehydration, malignant diseases, renal diseases, autoimmune diseases, or the intake of oral contraceptives in adolescent girls resulted in elevated thrombin generation with subsequent thrombus formation in infancy and childhood [2-31].

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**Table 1.** Acquired risk factors for paediatric thromboembolism [2-31]

<b>Perinatal Diseases</b>	Birth asphyxia Respiratory distress syndrome Infants of diabetic mothers Neonatal Infections Necrotizing enterocolitis Dehydration Congenital nephrotic syndrome Polycythaemia
<b>Medical Interventions</b>	Central Lines Operations Renal transplantation Immobilisation Plaster Casts Extracorporeal membrane oxygenation
<b>Acute Diseases</b>	Trauma Sepsis Dehydration Acute Rheumatic Diseases Nephrotic syndrome Acute Lymphoblastic Leukemia
<b>Chronic Diseases</b>	Malignancies Renal Diseases Cardiac Malformations Chronic Rheumatic Diseases
<b>Drugs</b>	E. coli asparaginase Prednisone Coagulation factor concentrates Heparins Antifibrinolytic agents Oral contraceptives

Various genetic prothrombotic defects (Table 2), particularly those affecting the physiological anticoagulant systems, i.e. antithrombin-, protein C-, and protein S-deficiency, the mutation of coagulation factor V (G1691A), and the factor II variant (G20210A) have been well established as risk factors of thrombotic events [32,33]. In addition, metabolic diseases such as homozygous homocystinuria, and moderate hyperhomocysteinemia due to the homozygous methylenetetrahydrofolate reductase (MTHFR) polymorphism C677T have been described, as well as increased concentrations of lipoprotein (a), which have been recently shown to significantly enhance the risk for thromboembolic arterial and venous thrombosis in paediatric and adult patients [32-37]. Since the discovery of activated protein C resistance as a highly prevalent hereditary risk factor of thromboembolism, evidence has been accumulating that thrombophilia is a multifactorial disorder [38]. The association of multiple haemostatic prothrombotic defects or the combination of established prothrombotic risk factors with acquired environmental or clinical conditions greatly increases the risk of thrombosis not only in adults but also in infants and children (Level II) [36-41].

**Table 2.** Inherited prothrombotic risk factors.

<b>Common,</b>	Factor V G1691A gene mutation Factor II G20210A gene mutation Increased concentrations of Apolipoprotein (a) Homozygous C677T polymorphism in the methylenetetrahydrofolate reductase gene
<b>Rare</b>	Protein C-deficiency Protein S-deficiency Antithrombin-deficiency Heparin cofactor II-deficiency Increased homocysteine concentrations > 10 mol/l [79]
<b>Very rare</b>	Dysfibrinogenemia Plasminogenemia Homozygous homocystinuria
<b>Probably inherited</b>	Increased levels of factor VIIIc, IX, or fibrinogen Decreased levels of factor XII

### Thrombotic Locations in Paediatric Patients

The most common sites of thrombus formation in neonates are the renal veins, vena caval occlusion, and periparturient thromboembolic stroke [2-7,23,24,42,43]. In addition, high rates of catheter-related thrombosis in neonates, infants and children have been reported [2-10,13,19]. Central venous lines lead to thrombus formation and thrombus growth near the catheter implantation site, especially when prothrombotic risk factors are involved. Further locations of childhood thromboembolism reported are cerebral venous thrombosis, portal and mesenteric vein thrombosis [46,47], while arterial vascular occlusions have been reported mainly as ischaemic stroke [27,35,42,43], and, catheter-related thrombosis in the aorta, the femoral artery and the subclavian artery respectively.

Purpura fulminans is a life-threatening event characterised histologically by microvascular thrombosis in the dermis followed by perivascular haemorrhage. Haemorrhagic necrosis of the adrenal glands (Waterhouse-Friderichsen syndrome), renal cortical necrosis may also occur. Clinically, progressive purpuric skin lesions and diffuse oozing from skin puncture sites are observed, often within hours after birth. The lesions are initially red and flat, quickly become indurated and necrotic, and may result in gangrene. The known underlying causes of purpura fulminans are disseminated intravascular coagulation (DIC), for example in response to bacterial septicaemia, i.e. for example *B β*-haemolytic streptococcal disease, *Neisseria meningitidis* or *streptococcus pneumoniae*. In addition, in

neonates congenital absence of protein C or protein S, or the presence of homozygous or heterozygous factor V G1691A mutation have been reported [41,48-53].

### **Prothrombotic Risk Factors in Paediatric Thromboembolism**

Underlying triggering factors and genetic prothrombotic conditions mentioned above, as well as acquired antiphospholipid antibodies play a potential role in the paediatric population with symptomatic venous thrombosis [53, 54], or ischaemic cerebrovascular accidents [27,44]. Suitable data are available from case series [55-65], and case-control studies in Caucasian paediatric patients suffering from venous thrombosis [43,56] or ischaemic stroke [27,35,61,64] with respect to prothrombotic risk factors (Level II).

### **Screening Tests, Time Point of Testing, and Interpretation of Laboratory Results**

Besides a step-wise protein-based diagnostic procedure - the corresponding antigen concentration is not reduced when the activity of a protein is within its normal range - DNA based assays are recommended. Suitable protein-based assays, i.e. APC-resistance [66,67], protein C activity, free and total protein S antigen, antithrombin activity, fibrinogen concentration, plasminogen activity, coagulation factors VIIIc and XII, lipoprotein (a) [68,69], and fasting homocysteine concentrations should be investigated along with DNA-based assays, i.e. factor V G1691A mutation, prothrombin G20210A variant and MTHFR C677T genotype.

Commercially available lipoprotein (a) assays are not yet standardised. However, a working group supported by the National Institutes of Health/National Heart, Lung and Blood Institute has previously evaluated [22] lipoprotein (a) assays using reference material developed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and a well-defined reference assay which is based on the immunodetection of non-repetitive epitopes within Lp(a) [68]. Overall in this trial reported, the results of various Lp(a) assays correlated well. However, individual assays for the measurement of apolipoprotein (a) were biased by 6% to 31% towards higher or lower lipoprotein (a) values so that at a given lipoprotein (a) risk threshold some assays will overestimate and some will underestimate the thrombotic risk. Therefore, and because lipoprotein (a) serum levels are mainly determined by a genetic size polymorphism of its main protein component it is useful to include also the analysis of apo(a) phe-

notypes [69].

In addition, rare prothrombotic defects, e.g. dysfibrinogenemia, hypo-/or dysplasminogenemia, heparin cofactor II deficiency, increased levels of histidine-rich glycoprotein, 2 macroglobulin, 1-antitrypsin, protein z, low tissue factor pathway inhibitor concentrations or further genetic polymorphisms, should be kept in mind. Besides testing for prothrombotic defects as stated above, all symptomatic children with thrombosis should be screened for antiphospholipid or anticardiolipin antibodies and the presence of lupus anticoagulants [53,54].

To identify prothrombotic risk factors and conditions responsible for vascular accidents in children a laboratory investigation is indicated. Based on the data obtained from case-control studies (Level I: [7,18,19,36]; Level II: [27,34,35,43,47,56,61]), at least the symptomatic propositus should be screened in a specialised coagulation unit for prothrombotic defects. In addition, since a recent prospective study on recurrent vascular occlusion after a first episode of spontaneous venous thromboembolism, i.e. thrombosis in the absence of further secondary causes, has indicated a subgroup of paediatric patients suffering from combined prothrombotic risk factors, to be at high risk of recurrent thrombosis a search for multiple risk factors is justified in selected patient groups [36].

With respect to the Mendelian theory of inheritance, approximately 50% of siblings of a symptomatic propositus suffering from a combined prothrombotic defect carry one single risk factor, while 25% carry two or more gene mutations/polymorphisms. Thus, based on the fact that an effective prophylactic anticoagulant therapy is available in risk situations, a screening for prothrombotic risk factors has to be discussed also in non-symptomatic siblings and further first degree family members [71].

To prevent results of protein-based assays from being affected by the acute thrombotic onset plasma samples should be obtained at least 3 to 6 months after the thrombotic episode. In addition, also the use of oral anticoagulant medication influence protein-based assays. Therefore it is recommended to draw fresh plasma samples for coagulation analyses at least 14-30 days after withdrawal of oral anticoagulation. In contrast, since DNA-based assays are influenced neither by the acute thrombotic onset nor anticoagulation and thrombolytic therapy, screening can be performed for genetic mutations/polymorphisms immediately at the onset of the vascular accident. Since Lp(a) levels are increasing during the first year of life [72], reaching twofold values compared with birth values at the age of approximately one year repeated testing after 8 to 12 months following the acute thrombotic onset is mandatory, when including Lp(a) in the screening programme in Caucasian neonates suffering from thromboembolism. A repeated test-

**Table 3.** Recommended screening and re-screening for prothrombotic risk factors in Caucasian neonates and children: Protein- and DNA-based methods (may vary within different ethnic backgrounds).

Protein based assays at onset	DNA based methods at onset	Protein based assays: repeated testing
APC-R (APC-resistance) Protein C-activity Free protein S-antigen Antithrombin-activity Fibrinogen Plasminogen Factor VIIIc, factor XII Lipoprotein (a) Fasting homocysteine concentration Antiphospholipid /anticardiolipin IgM/IgG	Factor V G1691A Factor II G20210A MTHFR C677T Further prothrombotic polymorphisms	Protein C-activity Free protein S-antigen Antithrombin-activity Fibrinogen Plasminogen Factor VIIIc, factor XII Lipoprotein (a) Fasting homocysteine concentration Antiphospholipid /anticardiolipin IgM/IgG

ing is also necessary in paediatric patients with increased anticardiolipin/ antiphospholipid IgM or IgG antibodies, or lupus anticoagulants.

For all plasma-based assays a clotting abnormality should be documented as a defect only if the plasma level of a protein is outside the limits of its normal range [55,73,74]. Besides classification based on age-dependent normal reference ranges and confirmation of a suspected protein-based prothrombotic defect in a second plasma sample (3 to 6 months later; without oral anticoagulation), criteria for the hereditary nature of a haemostatic risk factor is the identification of a causative gene mutation [32,33].

A type I deficiency state can be diagnosed when the functional plasma activity and immunological antigen concentration of a protein is below the age-related limit. A type II deficiency is present when repeatedly low functional activity levels are combined with normal antigen concentrations. As in adults, the diagnosis of protein S-deficiency is based on reduced free protein S antigen levels combined with decreased or normal total protein S antigen concentrations.

### Final Conclusions

The distribution of prothrombotic risk factors vary in different countries with respect to the ethnic population background and the number of patient/controls investigated

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[75-78]. Thus, to estimate the individual patient risk in paediatric patients suffering thromboembolism, it is recommended that symptomatic patient groups should be investigated in comparison with age- and gender-matched healthy controls from the same geographic catchment areas. Based on these data a step-wise screening of common and rare prothrombotic risk factors is recommended.

Since there are little data available so far with respect to the presence of inherited prothrombotic risk factors in paediatric populations others than Jews [39,64] or Caucasian, [7,19,34,36,43,47,56,61] the following recommendations (Level I: [7,18,19,36]; Level II: [27,34,35,43,47,56,61]) are restricted to Caucasian German and Austrian children with venous thrombosis or stroke.[7,19,34,36,43,47,56,61] In the latter cohort a screening of inherited prothrombotic risk factors listed in Table 3, including the measurement of Lp(a) concentrations is recommended along with the evaluation of underlying clinical conditions (Table 1).

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