Prothrombotic Disorders in Infants and Children With Cerebral Thromboembolism

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Background: To our knowledge, the contribution of prothrombotic conditions to cerebral thromboembolism has never been prospectively studied in a large series of pediatric patients.

Methods: The Hospital for Sick Children, Toronto, Ontario, established a program in January 1992 to diagnose and treat children (term newborn to 18 years old) with arterial ischemic stroke or sinovenous thrombosis. The routine evaluation for prothrombotic conditions included plasminogen, antithrombin, protein C, free protein S, activated protein C resistance, IgG and IgM anticardiolipin antibody, and lupus anticoagulant. We analyzed samples taken within 2 years of the event. We report results on patients seen from January 1, 1992, to January 1, 1997.

Results: Ninety-two patients (47 males and 45 females) entered the program during the study interval. Patients ranged from newborn to 18 years in age. Arterial ischemic stroke occurred in 78% of patients while sinovenous thrombosis occurred in 22%. All were tested for prothrombotic disorders. One or more abnormal results were present in 35 (38%) of the 92 patients. The majority (21/35) had multiple abnormal test results. The abnormal test results were anticardiolipin antibody (33%), plasminogen (9.5%), activated protein C resistance (9%), protein C (7%), antithrombin (12.5%), lupus anticoagulant (8%), and free protein S (11.5%). Male sex predicted the presence of prothrombotic abnormalities (relative risk, 1.7; 95% confidence interval, 1.2-2.5), but stroke type (relative risk, 0.8; 95% confidence interval, 0.7-1.1), age group, and presence of other risk factors did not predict abnormal testing.

Conclusions: A significant proportion (38%) of children with cerebral thromboembolism had evidence of prothrombotic conditions. In particular, there was a predominance of children with anticardiolipin antibody (33%). These data support a recommendation that children with cerebral thromboembolism be evaluated for prothrombotic disorders.

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Thrombosis in the central nervous system occurs in both the sinovenous system (sinovenous thrombosis) and in the arterial system (arterial ischemic stroke). The diagnosis of cerebral thrombosis in children has been facilitated by the development and availability of sensitive and safe radiographic tests such as computed tomographic scans and magnetic resonance imaging with magnetic resonance angiography. The widespread use of these radiographic tests in children’s hospitals has contributed to an increasing awareness of thrombotic disease in the central nervous system.

At the same time that our ability to diagnose central nervous system thrombosis has increased, our understanding of blood coagulation and both congenital and acquired defects that present risk factors for thrombotic disease has significantly improved. The contribution of both quantitative and qualitative abnormalities in coagulation and fibrinolysis to extracerebral thrombosis has been delineated in adult patients and is being assessed in pediatric patients. However, little is known about the association between coagulation abnormalities and cerebral thrombotic events in children. Detecting acquired and congenital prothrombotic disorders is important because this information can influence both initial and long-term therapies. In addition, family studies are indicated if a child has a congenital prothrombotic disorder. Affected patients and family members require counseling regarding risk factors for thrombosis, the need for intermittent prophylaxis in the presence of acquired risk factors, and medical care by physicians knowledgeable about their disease.

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PATIENTS, MATERIALS, AND METHODS

PATIENT POPULATION

Consecutive children of all ages (full-term newborn to 18 years of age), with either sinovenous thrombosis or arterial ischemic stroke, admitted to the Hospital for Sick Children or referred to the follow-up Stroke Clinic at the Hospital for Sick Children between January 1, 1992, and January 1, 1997, were included in this study. No patients were excluded. All clinical, radiological, and general laboratory data were obtained as part of routine medical management. The diagnosis of sinovenous thrombosis and arterial ischemic stroke was based on clinical criteria and confirmatory computed tomographic scan, magnetic resonance imaging angiography, or venography, and/or by conventional angiography.

CLINICAL INFORMATION

Comprehensive clinical data forms were completed for each child and included the following information: age, weight, and sex, determined at the time of diagnosis; the presence of underlying or associated conditions including drug therapy; a family history of stroke or thromboembolic disease; and the type and duration of anticoagulant or antiplatelet therapy for each patient. Full clinical and radiographic details on this cohort will be published separately as part of the multicenter Canadian Pediatric Ischemic Stroke Registry analyses on arterial ischemic stroke and sinovenous thrombosis.

LABORATORY EVALUATION

General

Throughout the study period, children diagnosed as having either sinovenous thrombosis or arterial ischemic stroke were evaluated for an acquired or congenital prothrombotic condition during their in-patient stay and/or routine clinic visits, if the relevant investigations had not been performed before the clinic visit. All assay results were compared with age-appropriate normal ranges previously published by us, which used the same laboratory techniques and were based on patients from the same geographic area.9 Abnormal results from patients receiving anticoagulants that would alter test results for prothrombotic conditions were labeled indeterminate. These included protein C, protein S, and activated protein C resistance during warfarin therapy, and antithrombin during standard heparin or low-molecular-weight heparin therapy. Abnormal results of assays that may be affected by the presence of thrombus that had been taken within 1 week of a thrombotic event were also called indeterminate (proteins C and S, antithrombin, plasminogen, or activated protein C resistance). Children with indeterminate results had repeated testing performed and were reclassified as having normal or abnormal prothrombotic testing, depending on the result of the subsequent test.

Sample Processing

Venous blood (either 3 or 5 mL) was placed in a tube containing 1.05-mmol/L buffered citrate solution for a final ratio of 1 part anticoagulant to 9 parts of serum. Plasma was immediately separated from cellular elements by double centrifugation at 3000 revolutions per minute for 15 minutes at 4°C. In addition, samples to be tested for lupus anticoagulant assays were filtered through a sterile 0.020-μm screen (Acrodisk, Gelman Sciences, Ann Arbor, Mich) to achieve complete platelet removal. Plasma was subsequently aliquotted, frozen at –70°C, and assays performed in batches every 2 to 4 weeks. All laboratory results were interpreted in the context of published age-matched normal values.9

Laboratory Assays

Protein C, antithrombin, and plasminogen were measured by chromogenic assay (Coamatic Protein C Assay and Coamatic Antithrombin Assay, Ortho Diagnostic System, Raritan, NJ; Spectrolyse Plasminogen SK, Biopool, Burlington, Ontario), free protein S by enzyme-linked immunosorbent assay (Biopool Protein S EID, Biopool, Burlingame, California), and activated protein C resistance using a commercial kit (Bioclot ACP, Biopool). Factor V Leiden was performed in a subset of patients by direct DNA analysis. Assays for lupus anticoagulant consisted of a dilute prothrombin time (Ortho Brain Thromboplastin, Ortho Diagnostic System), Russel Viper Venom test (Bioclot LA, Biopool), and activated protein C resistance using a commercial kit (Bioclot ACP, Biopool). Factor V Leiden was performed in a subset of patients by direct DNA analysis. Assays for lupus anticoagulant consisted of a dilute prothrombin time (Ortho Brain Thromboplastin, Ortho Diagnostic System), Russel Viper Venom test (Bioclot LA, Biopool), and activated protein C resistance using a commercial kit (Bioclot ACP, Biopool). Factor V Leiden was performed in a subset of patients by direct DNA analysis. Assays for lupus anticoagulant consisted of a dilute prothrombin time (Ortho Brain Thromboplastin, Ortho Diagnostic System), Russel Viper Venom test (Bioclot LA, Biopool), and Platelet Neutrophilic Assay (Advanced Biological Products Inc, Mississauga, Ontario).

STATISTICAL ANALYSIS

A priori hypotheses were formulated regarding possible predictors of positive prothrombotic testing that included stroke type (arterial ischemic stroke vs sinovenous thrombosis), age at event (older infants and children aged 1 month to 18 years vs newborns [<1 month]), sex, the presence of additional underlying causes, and the presence of a positive family history for thrombotic disorders. These hypotheses were tested in bivariate analysis using 2-group comparisons. Patients with or without a prothrombotic disorder were compared using 2-tailed Fisher exact test. Results are reported as relative risk (RR), with 95% confidence intervals (CIs).

At the Hospital for Sick Children, Toronto, Ontario, specialized coagulation and stroke clinics were established in 1992 in which hematologists and neurologists collaborated in the diagnosis and management of children with thromboembolic stroke. The purpose of this article is to describe the presence of both acquired and congenital prothrombotic disorders in a consecutive cohort of pediatric patients with sinovenous thrombosis and arterial ischemic stroke seen in these specialized follow-up clinics over a 5-year period.

RESULTS

There were 92 children evaluated during the 5-year study period. Seventy-three had arterial ischemic stroke and 19 had sinovenous thrombosis. All patients underwent labo-
Thirty-five (38%) of the 92 patients had 1 or more abnor-
mal test results for a prothrombotic condition (ie, posi-
tive prothrombotic testing) compared with 57 patients with
normal test results (ie, negative prothrombotic testing).

The Table provides the results for the 35 patients
with positive testing for a prothrombotic disorder. The
presence of anticardiolipin antibody in 23 patients (33%)
was the most frequent abnormality. Twelve children had
anticardiolipin antibody as a single abnormality. The re-
mainder of the children with anticardiolipin antibody also
had other abnormalities, including protein S deficiency
(n = 3), plasminogen deficiency (n = 2), lupus antico-
agulants (n = 2), activated protein C resistance (n = 2),
antithrombin deficiency (n = 1), and plasminogen and
antithrombin deficiencies (n = 1). Of the remaining pa-
tients, many had combined deficiencies of coagulation
proteins, including antithrombin and protein S (n = 2),
antithrombin and protein C (n = 2), antithrombin and
plasminogen (n = 1), antithrombin, protein C, protein
S, and plasminogen (n = 2), and antithrombin, protein
C, and protein S (n = 2). Two children had lupus antico-
agulant and activated protein C resistance. The aver-
age interval from event to sample was 8 months (range,
1 day to 4.5 years). Of the 44 abnormal samples, 6 were
taken within 1 week of the clinical event. The remain-
der were obtained between 7 days and 1 month (n = 10),
1 to 3 months (n = 5), 3 to 6 months (n = 4), and 6 months
to 4.5 years (n = 19) after the clinical event. In patients
with anticardiolipin antibody in whom samples were avail-
able from multiple time points, the results went from ini-
tially normal to abnormal in 1 patient and initially
abnormal to normal in 4 patients.

Male sex was associated with positive prothrom-
botic testing (P = .01; RR, 1.7; 95% CI, 1.2-2.5). Of the
35 patients with positive prothrombotic testing, 25 (71%)
had arterial ischemic stroke and 10 (29%) had sinove-
nous thrombosis (Figure). The distribution was not sig-
ificantly different to that in the 57 patients with nega-
tive prothrombotic testing (48 [84%] with arterial
ischemic stroke and 9 [16%] with sinus venous thrombo-
sis) (P = .19; RR, 0.8; 95% CI, 0.7-1.1).

The median age of children with arterial ischemic
stroke was 4.9 years (age range, newborn to 15 years). The
median age of the children with sinovenous thrombosis
was 10 years (range, newborn to 18 years). The Figure
shows the distribution of the entire cohort by age and stroke
type. There was no significant difference in the ratio of nor-
mal to abnormal coagulation tests when analyzed by dif-
ferent age groups. In particular, prothrombotic abnor-
malities were not more likely in older infants and children
(1 month to 18 years) compared with newborns (<1 month)
(P = .42; RR, 1.45; 95% CI, 0.6-3.4).

The associated conditions in patients with arterial
ischemic stroke and sinovenous thrombosis varied. In chil-
dren with arterial ischemic stroke, cardiac disease was
the most common identified underlying disease. In chil-
dren with sinovenous thrombosis, dehydration was the
most common precipitating factor. There was insuffi-
cient power in our study to determine whether there were
any differences in underlying causes for patients with or
without a prothrombotic disorder.

A positive family history for thrombotic disorders
was present in only 1 of the 35 children with a prothrom-
botic abnormality, a child with a transient anticardio-
lipin antibody positivity. Four of the 57 patients with nega-
tive prothrombotic testing had family histories suggestive
of a congenital prothrombotic disorder.

**COMMENT**

The contribution of acquired and congenital prothrom-
botic hemostatic disorders to thrombotic stroke in chil-
dren is unknown but important to determine because of
the potential risk of recurrent events, availability of spe-
cific therapies and need to screen family members. This
analysis of the first 92 consecutively evaluated patients
from a single institutional program showed that ac-
quired prothrombotic hemostatic conditions exist in a
large proportion of children with thrombotic stroke while
congenital prothrombotic disorders are rare. Prothrom-
botic abnormalities were found in patients in all age
groups, with both stroke types, and whether there were
additional risk factors or a family history suggesting a co-
agulation abnormality.

The formation of a clot (thrombus) depends on a
complex interaction between the coagulation and fibri-
nolytic systems. Congenital deficiencies of antithrom-

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* Twenty-one of 35 children had multiple abnormalities as described in the
"Results" section of the text.
These deficiencies have all been described in adults with sinovenous thrombosis,11-13 and deficiencies of plasminogen, protein C, and protein S have been reported in adults with arterial ischemic stroke.15-18 Activated protein C resistance was found at only a slightly increased incidence with childhood stroke. In our study, activated protein C resistance occurs in 5% of the white population,19 which increases the risk of thrombosis whether it is attributable to the factor V Leiden mutation or not.20 Deficiencies in the fibrinolytic system, for example congenital deficiency of plasminogen, also result in thrombotic complications.21

In children with stroke, deficiencies of protein C, protein S, and antithrombin,22-29 and activated protein C resistance30 have been reported, although one retrospective case series30 reported no association of these abnormalities with childhood stroke. In our study, activated protein C resistance was found at only a slightly increased incidence to that reported in the general population.19 The frequency of other congenital prothrombotic conditions is difficult to determine from our study because not all patients were investigated for all abnormalities, follow-up testing was incomplete, and family studies were not performed in most cases. Further studies will be required to confirm the relative frequency of congenital prothrombotic disorders in children with stroke.

Acquired deficiencies of protein C and protein S associated with clinical thrombosis occur in patients with sepsis and viral infections such as varicella.31-33 Our patients frequently had combinations of deficiencies in protein C, protein S, antithrombin, and plasminogen. The presence of multiple coagulation protein deficiencies is more consistent with an acquired than a congenital prothrombotic disorder. Because cerebral thromboses, especially arterial ischemic strokes, are usually associated with smaller thrombi than noncerebral thromboses, plasma concentrations of coagulation proteins are less likely to be significantly decreased. The multiple abnormalities in our patients were unlikely to be attributable to the sinovenous thrombosis or arterial ischemic stroke per se since the mean interval from event to testing was 8 months and abnormal hemostatic results within 1 week of the stroke event were considered to be indeterminate and subsequently repeated. Our results suggest that acquired deficiencies of coagulation proteins are present in children following thromboembolic stroke, but their causative role remains to be determined.

Antiphospholipid antibodies consist of both lupus anticoagulant and anticardiolipin antibody, and are a heterogeneous group of antibodies that react with proteins bound to phospholipids and interfere with functional coagulation tests.34 The presence of antiphospholipid antibodies is the most common acquired prothrombotic state in cerebral35 and noncerebral34,36 thrombosis in adults. Children with antiphospholipid antibodies and systemic lupus erythematosus have a significant risk of arterial or venous thrombosis, of which 50% occur in the central nervous system,37,38 and antiphospholipid antibodies have been increasingly reported in children with cerebral ischemia.39 The increased prevalence of antiphospholipid antibodies in our large cohort confirms the association between antiphospholipid antibodies and cerebrovascular disease in children. In our study antiphospholipid antibodies were found in children of all ages who did not have systemic lupus erythematosus as a primary diagnosis. Further studies are needed to confirm the importance of transient and persistent antiphospholipid antibodies and the prognostic implications of each. Whether antiphospholipid antibodies are involved in the pathogenesis of stroke remains to be proven.

In conclusion, to our knowledge, this is the first large prospective cohort study of prothrombotic abnormalities in children with arterial ischemic stroke or sinovenous thrombosis. The presence of prothrombotic abnormalities in infants and children at all ages, in both arterial ischemic stroke and sinovenous thrombosis, with and without additional risk factors or a family history suggesting a coagulation disorder suggests that all children with these forms of stroke should be evaluated for prothrombotic abnormalities. Further studies are required to define the pathogenic significance of these prothrombotic abnormalities. Finally, large prospective follow-up studies will be needed to determine if the recurrence risk of sinovenous thrombosis or arterial ischemic stroke is increased in children with prothrombotic abnormalities.

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REFERENCES