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RESEARCH ARTICLE

Role of Endogenous Testosterone Concentration in Pediatric Stroke

Sandra Normann, MD,[1] Gabrielle de Veber, MD,[2] Manfred Fobker, MD,[3] Claus Langer, PhD,[3] Gili Kenet, MD,[4] Timothy J. Bernard, MD,[5,6] Barbara Fiedler, MD,[7] Ronald Sträter, MD,[1] Neil A. Goldenberg, MD, PhD,[5,6] and Ulrike Nowak-Göttl, MD[1]

AQ: 1

Previous studies have indicated a male predominance in pediatric stroke. To elucidate this gender disparity, total testosterone concentration was measured in children with arterial ischemic stroke (AIS; n = 72), children with cerebral sinovenous thrombosis (CSVT; n = 52), and 109 healthy controls. Testosterone levels above the 90th percentile for age and gender were documented in 10 children with AIS (13.9%) and 10 with CSVT (19.2%), totaling 16.7% of patients with cerebral thromboembolism overall, as compared with only 2 of 109 controls (1.8%; $p = 0.002$). In multivariate analysis with adjustment for total cholesterol level, hematocrit, and pubertal status, elevated testosterone was independently associated with increased disease risk (odds ratio [95% confidence interval]: overall = 3.98 [1.38–11.45]; AIS = 3.88 [1.13–13.35]; CSVT = 5.50 [1.65–18.32]). Further adjusted analyses revealed that, for each 1nmol/l increase in testosterone in boys, the odds of cerebral thromboembolism were increased 1.3-fold.

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The androgen testosterone is synthesized in the mitochondrion from its precursor steroid cholesterol by cleavage via 20,22-desmolase into pregnenolone, with subsequent conversion of pregnenolone into testosterone.[1] Testosterone determines the male phenotype during sexual differentiation, and promotes sexual maturation during puberty in boys.[1] The measurement of circulating testosterone is clinically relevant in 1) diagnosis and treatment of androgen disorders, 2) gender assignment in newborns with ambiguous genitalia, and 3) determination of pubertal stage in children with delayed or precocious puberty. Apart from circadian and seasonal variations, preanalytical factors influencing circulating testosterone concentration include diet (eg, fasting, intake of low or high glycemic meals, alcohol consumption), plasma volume (eg, hemoconcentration, hemodilution), illness, stress, and sexual activity.[2,3]

Apart from a variety of underlying medical conditions, several studies have suggested that pediatric arterial ischemic stroke (AIS)/cerebral sinovenous thrombosis (CSVT) is more common in boys than in girls.[4–7] The aim of the present study was to further elucidate this observed gender disparity by investigating a hypothesized relationship of elevated testosterone levels to increased risks of pediatric AIS/CSVT.

Materials and Methods

Ethics

The study was performed in accordance with the ethical standards laid down in the updated version of the 1964 Declaration of Helsinki and was approved by the medical ethics committee of the University of Münster, Germany. Written informed parental consent was uniformly obtained prior to study participation.

Subjects

A total of 124 unselected children with confirmed diagnosis of acute AIS[8] or CSVT ranging in age between neonate and 18 years (inclusive) were consecutively enrolled in the IPSS at the Münster, Germany participating center from January 2004 to January 2009. AIS and CSVT were confirmed by standard imaging methods, that is, duplex sonography (neonates only), magnetic resonance (MR) imaging employing a 1.5 or 3T magnet, or computed tomography (CT).[7] Neurovascular imaging was concomitantly performed in all cases, and was comprised in the latter instances of MR or CT venography/arteriography.[8–10] Plasma banking for research

AQ: 2

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Table 1. **Patient Demographics and Laboratory Parameters of Interest**

	AIS	CSVT	Control Group
Total number	72	52	109
Male (%)	36 (50)	31 (60)	53 (49)
Age, median y (range)	5.0 (0.5–18)	4.7 (0.5–18)	4.7 (0.5–18)
No. of children > Tanner 2 (%)	9 (12.5)	12 (23.0)	23 (21.1)
Male (%)	5 (14)	4 (13)	13 (25)
Cholesterol, median mg/dl (range)	155 (110–336)	149 (88–323)	157 (98–228)
Hematocrit, median % (range)	34.9 (25.7–45.6)	35.6 (25.3–46.0)	37 (28.0–47.0)
Female testosterone, median nmol/l (range)	0.1 (0.08–1.32)	0.26 (0.07–4.0)	0.1 (0.07–2.4)
Male testosterone, median nmol/l (range)	0.1 (0.1–21.7)	1.2 (0.1–23.3)	0.1 (0.07–29.6)
No. of subjects with testosterone >90th age- and gender-dependent percentiles (%)			
Total	10 (13.9)	10 (19.2)	2 (1.8)
Female	4 (11.1)	2 (9.6)	1 (1.8)
Male	6 (16.6)	8 (25.8)	1 (1.9)

AIS = arterial ischemic stroke; CSVT = cerebral sinovenous thrombosis.

was performed via simultaneous enrollment in a local prospective cohort study of AIS and venous thromboembolism. As part of the latter study, 109 population-based healthy children served as controls. These pediatric controls had no history of chronic disease, thromboembolic events, or recent medication use, and were recruited in outpatient settings of preoperative clinics for minor/elective surgery or bone marrow donation; children with abnormal hematologic indices on complete blood count or elevated high-sensitivity C-reactive protein values were excluded from the control group. Pubertal status of cases and controls was classified according to Tanner (breast development for girls; pubic hair stage for boys).[11]

Blood Sample Collection and Laboratory Analysis

Blood samples in patients and controls were collected by peripheral venipuncture into plastic tubes (Sarstedt, Nümbrecht, Germany) in the morning after a 12-hour fasting period (4–6 hours for infants), and platelet-poor plasma was then isolated by centrifugation. In cases, blood samples were obtained 6–12 months postevent and at least 6 weeks following cessation of any anticoagulant therapy. Following isolation of plasma, plasma testosterone concentration (nmol/l) was immediately determined by immunoassay using the Modular Analytics E170 (Roche Diagnostics, Mannheim, Germany). Intra-assay coefficient of variation was 1.3% for high concentrations (23nmol/l) and 2.7% for low values (1.9nmol/l). The lower detection limit was 0.07nmol/l. Plasma total cholesterol concentrations were measured from the same plasma samples by photometric assay (CHOD-PAP method) on the Modular Analytics E170. Reference values for testosterone were derived from a pediatric healthy cohort (n = 156; Supplementary Data) that met the same criteria as, but was distinct from, the control group.

Statistical Analysis

Statistical analyses were performed with the StatView 5 software package (SAS Institute, Cary, NC) and the MedCalc software package (MedCalc, Mariakerke, Belgium). Apart from descriptive statistics, nonparametric statistics were employed. In addition, analysis of variance was performed to determine interactions between age, gender, and testosterone. Proportions were compared between groups using chi-square or Fisher exact testing, as appropriate. Logistic regression was used to evaluate for associations between analytic variables and incident AIS/CSVT, reported in each case as an odds ratio (OR) with corresponding 95% confidence interval (CI). Multiple logistic regression was performed to adjust for influences of hypothesized confounders and for covariates for $p < 0.2$ in univariate analyses of the relationship with incident AIS or CSVT. Patients and controls were frequency matched on age, gender, pubertal status (Tanner stage>2 vs ≤2), total cholesterol (the precursor of testosterone), and hematocrit (an indicator of hemoconcentration/hemodilution). Testosterone was evaluated as both a dichotomous variable (> vs ≤90th percentile values for age and gender) and a continuous variable. Correlations were determined by Spearman rank correlation test. For all hypotheses testing, alpha was set at 0.05.

Results

As shown in Table 1, the AIS/CSVT case group consisted of 57 females (46%) and 67 males (male/female ratio,1.28/1; $p = 0.63$), with a median age at diagnosis of 5 years (range, term neonate to 18 years). Testosterone showed a statistically significant but modest-strength correlation with hematocrit ($r = 0.441$; $p < 0.0001$); no correlation was evident between testoster-

Table 2. **Odds of Elevated Testosterone in Children With AIS (n = 72) and CSVT (n = 52) Versus 109 Healthy Controls (Univariate Logistic Regression)**

Analytic Variable	Univariate (Unadjusted) OR (95% CI)	*p*
Testosterone >90th percentile[a]	AIS: 3.46 (1.1–10.85)	0.032
	CSVT: 5.45 (1.71–17.35)	0.004
	AIS & CSVT: 4.2 (1.51–11.87)	0.006
Hematocrit per 1% increase	AIS: 0.86 (0.79–0.94)	0.0006
	CSVT: 0.92 (0.84–0.99)	0.046
	AIS & CSVT: 0.89 (0.84–0.95)	0.001
Cholesterol per 1mg/dl increase	AIS: 1.00 (0.83–1.01)	0.254
	CSVT: 1.0 (0.99–1.01)	0.918
	AIS & CSVT: 1.00 (0.99–1.01)	0.419
Puberty status > Tanner 2	AIS: 0.51 (0.23–1.15)	0.107
	CSVT: 1.1 (0.51–2.31)	0.827
	AIS & CSVT: 0.74 (0.40–1.36)	0.332

[a]Using age- and gender-specific cutoffs (Supplementary Table).
AIS = arterial ischemic stroke; CSVT = cerebral sinovenous thrombosis; OR = odds ratio; CI = confidence interval.

one and total cholesterol ($r = 0.049$; $p = 0.47$). Age exhibited significant interactions with testosterone levels in patients and controls ($p < 0.001$); therefore, testosterone data were analyzed as age- and gender-dependent percentiles. As shown in the Figure, testosterone levels above the 90th percentile for age and gender were documented in 10 children with AIS (13.9%) and 10 with CSVT (19.2%), totaling 16.7% of patients with cerebral thromboembolism overall, as compared with only 2 of 109 controls (1.8%; $p = 0.002$). In univariate logistic regression, testosterone levels above the 90th age- and gender-dependent percentiles were associated with a 4- to 5-fold increase in odds of overall cerebral thromboembolism (Table 2). In gender-specific subgroup analysis, the odds of cerebral thromboembolism associated with elevated testosterone levels remained significantly increased in boys (OR [95% CI]: overall = 7.93 [1.70–36.82]; AIS = 5.66 [1.07–30.01]; CSVT = 11.33 [2.19–58.43]). In girls, these odds were not increased significantly (OR [95% CI]: overall = 4.88 [0.52–45.31]; AIS = 5.89 [0.58–59.28]; CSVT = 3.23 [0.19–54.54]). In multivariate analysis (Table 3) with adjustment for pubertal status, hematocrit, and total cholesterol level, elevated testosterone above the 90th percentiles remained significantly associated with both pediatric AIS and CSVT.

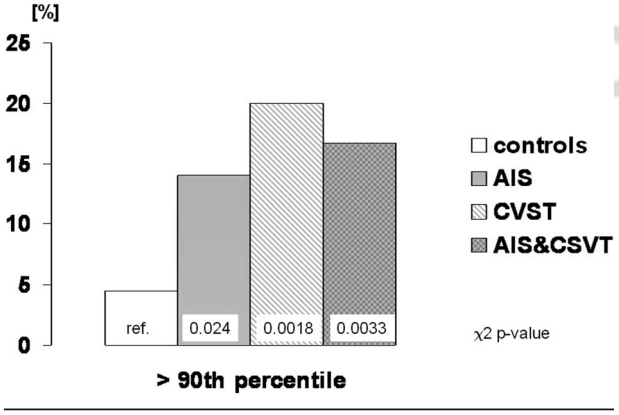

Fig. The proportions (%) of children above the 90th age- and gender-dependent testosterone percentiles with respect to disease groups are depicted. In addition, p *values (chi-square analysis) are given for the comparison of proportions elevated between the control group and each disease group. AIS = pediatric arterial ischemic stroke; CSVT = cerebral sinovenous thrombosis.*

Further dose-response analyses in boys, with adjustment for age, pubertal status, cholesterol, and hematocrit, revealed that the odds of cerebral thromboembolism increased by an average of 32% overall (OR = 1.32; 95% CI, 1.01–1.72), and 48% in CSVT in particular (OR = 1.48; 95% CI, 1.07–2.05), for each 1nmol/l increase in testosterone. By contrast, no significant change in odds of AIS was determined for increasing testosterone levels (OR = 1.19; 95% CI, 0.88–1.62).

Discussion

The present cohort study provides evidence that gender differences in the occurrence of pediatric AIS or CSVT are associated with elevated endogenous testosterone concentrations and that risk of cerebral thromboembolism increases in a concentration-dependent fashion with testosterone levels among males. The latter was not found in female stroke children, likely due to the

Table 3. **Adjusted Odds of Having Elevated Testosterone in Children With AIS (n = 72) and CSVT (n = 52) Versus 109 Healthy Controls (Multivariate Logistic Regression)**

Analytic Variable	Univariate (Unadjusted) OR (95% CI)	*p*
Testosterone >90th percentile[a]	AIS: 3.88 (1.13–13.35)	0.031
	CSVT: 5.50 (1.65–18.32)	0.005
	AIS & CSVT: 3.98 (1.38–11.45)	0.010
Hematocrit per 1% increase	AIS: 0.85 (0.77–0.95)	0.002
	CSVT: 0.96 (0.83–1.09)	0.299
	AIS & CSVT: 0.89 (0.84–0.95)	0.012
Cholesterol per 1mg/dl increase	AIS: 1.01 (0.99–1.02)	0.194
	CSVT: 1.00 (0.99–1.01)	0.941
	AIS & CSVT: 1.00 (0.99–1.01)	0.336
Puberty status > Tanner 2	AIS: 0.97 (0.4–2.6)	0.959
	CSVT: 2.08 (0.8–5.4)	0.129
	AIS & CSVT: 1.42 (0.7–3.1)	0.366

[a]Using age- and gender-specific cutoffs (Supplementary Table).
AIS = arterial ischemic stroke; CSVT = cerebral sinovenous thrombosis; OR = odds ratio; CI = confidence interval.

fact that testosterone levels were less often elevated in girls compared with boys (10.5% vs 20.9%). Thus, the female cohort may still be underpowered to detect a statistically significant difference between girls compared with healthy controls. Our data are concordant with knowledge gained from previous studies demonstrating that pediatric stroke or venous thrombosis is more common in boys than in girls.[4–7,12] In our study, the odds ratio for elevated testosterone is higher for patients with CSVT than AIS, suggesting that other factors may also be important in AIS pathophysiology. However, the fact that the proportion of children with Tanner stage >2 was lower in AIS (12.5%) than CSVT (23%) may have contributed to the lower OR observed in the former group.

In adults, associations between anabolic/androgenic steroid abuse and acute arterial or venous thrombosis have been demonstrated.[13–17] Underlying mechanisms have been suggested to involve interactions of androgens with platelet aggregation,[18] coagulation and fibrinolytic proteins, and the vasculature.[19] In in vitro work and animal studies, testosterone supplementation has been shown to elicit increased platelet production of thromboxane A2 along with a downregulation of the prostacyclin Pg12 production in aortic smooth muscle cells, leading to increased platelet aggregation via interaction of platelets and vasculature.[18,20] Furthermore, an observation was made in humans that bleeding in men with hypogonadism, characterized by decreased testosterone levels, was linked to hypoactive platelets. The latter finding supports the influence of testosterone on platelet function.[21] The effect of exogenous supraphysiological doses of testosterone has been investigated as a hormonal contraceptive treatment for males.[22] Anderson and coworkers reported increases in hematocrit and prothrombin fragment F1+2 and decreases in protein C- and protein S levels with this testosterone therapy.[22] Thus, a testosterone-mediated hypercoagulable state may be caused via interaction between various components of the coagulation system, including platelet hyperaggregation, downregulation of coagulation inhibitors such as protein C or protein S, and increased blood viscosity. Such effects on platelet function and the intrinsic anticoagulant system have been well established for estrogen, which has received far greater emphasis than testosterone in the thromboembolism literature.

Limitations of the present study are principally related to the testosterone assay methodology. Whereas the assay employed is relatively insensitive to very low concentrations of testosterone (as may be found in prepubertal males and in females),[23] our principal focus in the analysis on dichotomous categorization of testosterone as elevated versus nonelevated based on 90th percentile values largely overcomes this limitation. In addition, we found a significant interaction between testosterone and age, which supports the hypothesis of effect modification by age. However, the numbers were too small to stratify for different age groups in our analysis. Notwithstanding these potential limitations, the use of robust reference ranges established in a cohort of healthy children distinct from the control group in the study, the restriction of testosterone determination to convalescent samples in the case group, and the analytic adjustment for key confounders all serve to enhance the validity of the present work.[1,2]

AQ: 3

Future research should seek to evaluate whether elevated testosterone levels serve as a risk factor for cerebral thromboembolism among neonates and older children alike, and the influence of testosterone on recurrence risk in pediatric AIS and CSVT. In addition, further investigation into the mechanisms of testosterone-induced hypercoagulability, platelet activation, and vascular response is warranted. It is hoped that such work will lead to the identification of future novel interventions in pediatric cerebral thromboembolism.

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES **1**

AQ1: PE: Unstructured abstract okay?

AQ2: AU: Please spell out IPSS.

AQ3: AU: Please check: " women may be difficult to detect)" deleted.