Recurrent stroke: the role of thrombophilia in a large international pediatric stroke population

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Methods

Stroke Subtypes: Stroke subtypes in the enrolled children were reclassified according to explicit predefined criteria based on the TOAST criteria modified for children in which “vasculopathy” is substituted for “large vessel atherosclerosis”. Based on this classification in association with underlying diseases/co-morbidities, clinical data and the results of diagnostic studies including MR angiography, conventional angiography and Doppler ultrasonography, transthoracic and transesophageal echocardiography (ECHO) with saline contrast, and electrocardiography, the study patients were classified into four subgroups: (i) cardiac disease including persistent foramen ovale and mitral valve prolapse detected on ECHO as well as pre-diagnosed congenital heart disease (CHD) (ii) vasculopathy including dissection and other stenosis, e.g. focal cerebral and post-Varicella arteriopathy, post radiation and Down syndrome (iii) cryptogenic stroke, with no underlying disease/co-morbidities and no vasculopathy, and (iv) non-cardiac, non-vasculopathy and non-cryptogenic AIS, including stroke associated with other underlying diseases/co-morbidities such as systemic viral or bacterial infections including meningitis, post-vaccination, haemolytic-uraemic syndrome, malignancy or autoimmune disorders and others. Patients with both cardiac disease and vasculopathy were classified into the vasculopathy group. Ethnicity, age, gender and the proportion of stroke subtypes were collected.

Classification of vascular stroke type are as follows: vasculitis including post-Varicella vasculitis (n=72), dissection (n=46), transient cerebral arteriopathy (n=7), other specified vasculopathy (n=9: brain tumour n=1; post radiation n=2; Down syndrome n=3; catheter occlusion n=1; vasospasm n=2) and other unspecified vasculopathy including intracranial large vessel stenosis (n=76), short vessel stenosis with persistent transcranial Doppler-documented turbulence (n=24), large vessel occlusion (n=37) and hypoplastic arteries of unknown origin (n=17).

Antithrombotic treatment following first symptomatic stroke: At the discretion of the participating study centres, paediatric patients with a first stroke onset received antithrombotic therapy unless contraindicated by initial haemorrhagic infarction or other risk factors for haemorrhage.\textsuperscript{21-25}
Laboratory analyses: With written or oral parental consent, the mutations in factor 5 at rs6025 and factor 2 at rs1799963 (retrospective genotyping in children with stroke diagnosed prior 1995 [factor 5] - & 1997 [factor 2]), as well as circulating levels of lipoprotein (Lp) (a), fibrinogen, factor VIIIC, homocysteine, protein C, protein S, and antithrombin were investigated with standard laboratory techniques at stroke onset and/or repeated during routine follow up visits. To rule out possible laboratory pitfalls induced by the acute stroke onset, for example consumption of antithrombin, protein C or protein S or acute phase-induced elevation of fibrinogen, factor VIII or homocysteine, repeated laboratory thrombophilia work-up was performed at least three months after the acute stroke event and was repeated if abnormal. A non-homozygous type I deficiency (antithrombin, protein C) state was diagnosed when functional plasma activity and immunological antigen concentration of a protein (analysis of protein C and protein S at least three months after the index event and/or withdrawal of vitamin-K-antagonists) were confirmed to be below the age-related reference ranges. A non-homozygous type II deficiency (antithrombin, protein C) was diagnosed when low functional activity levels were found along with normal antigen concentrations at least 3 months after the index event, repeated if abnormal. The diagnosis of protein S deficiency was based on reduced free protein S antigen levels combined with decreased or normal total protein S antigen concentrations respectively at least 3 months after the index event, repeated if abnormal. Serum levels of Lp(a) > 30 mg/dl were considered elevated. Since data on normal values for fibrinogen and factor VIIIC in children are sparse, fibrinogen and factor VIIIC levels > age-dependent 90th percentiles, derived from the healthy control children were used as cut-off values. Criteria for the hereditary nature of a haemostatic defect were its presence in at least one further first or second-degree family member and/or the identification of a causative gene mutation.

*Reference values 1a and 1b

1a. Nowak-Göttl U, Limperger V, Kenet G, Degenhardt F, Arlt R, Domschikowski J, Clausnizer H, Liebsch J, Junker R, Steppat D. Developmental hemostasis: A lifespan from neonates and pregnancy to the young and elderly adult in a European white population. Blood Cells Mol Dis. 2017 Sep;67:2-13. doi:10.1016/j.bcmd.2016.11.012.

1b. Nowak-Göttl U, Junker R, Hartmeier M, Koch HG, Münchow N, Assmann G, von Eckardstein A. Increased lipoprotein(a) is an important risk factor for venous thromboembolism in childhood. Circulation. 1999 Aug 17;100(7):743-748.

Statistics: Patients with recurrent stroke were compared with patients without stroke recurrence. For the primary study objective, we calculated the symptomatic recurrence rate per 100 person-years and yearly incidence rates, based on the recruitment period and a median follow-up period of 35 months. Predictors possibly influencing symptomatic recurrence were defined a priori based on literature data and included stroke subtypes and prothrombotic risk factors (PR) significantly associated with a first AIS onset. Using a rule of
thumb for proportional hazards analysis including approximately 10 outcomes for each independent predictor, the final statistical model aimed to include seven predictors: calculated were the possible role of stroke subtypes on the recurrence rate per 100 person-years and the possible influence of thrombophilic risk factors on recurrent AIS. In order to evaluate an independent contribution to the risk of recurrent AIS and to adjust for further potential confounders (age at onset, gender, study centre) the hazard ratio (HR) together with 95% confidence intervals (CI) were estimated from Cox’s proportional hazards model. Variables were removed from the backward model if p was > 0.16. Stroke groups (cardiac, vascular, non-cryptogenic/-cardiac,-vascular) were compared to cryptogenic stroke. Patients with no thrombophilia were compared with those with thrombophilia. Statistical analyses were performed using the MedCalc software bvba (version 16.4.3, Ostend, Belgium) and StatView 5 software packages (SAS Institute Inc.). The recurrence rates were calculated as the number of recurrent events per 100 person-years.

For the secondary study objective, i.e. the time to recurrence, we calculated the probability of AIS-free survival (AFS) as a function of time utilizing the method of Kaplan and Meier (univariate analysis). The log rank test was used to test for differences in recurrence-free survival between groups. Patients were withdrawn from the survival analysis (censored cases) either at death unrelated to AIS recurrence or at loss to follow-up using data of the last clinical follow-up visit. To further test the relationship between independent and dependent variables, the likelihood ratio test was performed. Because of their apparently non-Gaussian distribution, continuous data are presented as median and minimum-maximum (min.-max.) values and were evaluated by non-parametric statistics including the Wilcoxon-Mann-Whitney U test. To compare frequency distributions of fatal outcome, χ² test and, if necessary, Fisher’s exact test was performed. In addition, numbers needed to screen (NNS) were calculated as previously described. ³⁰
Results

Figure 1 (supplement): Stroke–free survival with respect to thrombophilia status (none versus combined) is depicted (p=0.039).

Number at risk
no thrombophilia
786 171 81 29 6 0
multiple thrombophilia
54 13 3 1 0 0