Inflammatory markers in pediatric stroke: an attempt to better understanding the pathophysiology

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Abbreviations:

AIS — arterial ischemic stroke
BBB — blood-brain barrier
CRP — C-reactive protein
CI — confidence interval
ECM — extra cellular matrix
IL — interleukin
MCA — middle cerebral artery
MMP — metalloproteinase
rtPA — tissue plasminogen activator
sICAM — soluble intercellular adhesion molecule
sE-selectin — soluble E-selectin
sVCAM — soluble vascular cell adhesion molecule
TIMP — tissue inhibitors of metalloproteases
TNF — tumor necrotic factor
VEGF — vascular endothelial growth factor
VZV — varizella-zoster virus
vWF — von Willebrand factor

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Contributor's Statements:

Sarah E. Buerki: Dr. Buerki coordinated and supervised data collection, e.g. participant recruitment, drafted the initial manuscript, and approved the final manuscript as submitted.

Denis Grandgirard: Drs. Grandgirard and Leib designed the data collection instruments (laboratory analysis). Dr. Grandgirard was the main data analyst including statistics, data interpretation, critically reviewed the manuscript, and approved the final manuscript as submitted.

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Stephen L. Leib, Maja Steinlin: Drs. Leib and Steinlin conceptualized and designed the study, outlined the project data analysis and data interpretation.

Abstract

Background: The mechanisms of childhood and perinatal arterial ischemic stroke (AIS) are poorly understood. Multiple risk factors include cerebral arteriopathy, congenital cardiac disease, infection, sickle cell disease, and maternal-fetal conditions in neonates. For infections and parainfectious conditions being the most important a possible inflammatory pathophysiology has long been suspected. This pilot study aims to detect, whether there are any abnormalities of inflammatory markers associated with childhood and neonatal stroke.

Methods: The concentration of 23 different metalloproteinases (MMPs), tissue inhibitors of MMPs (TIMPs), endothelial factors, vascular cell adhesion proteins, and cytokines in plasma were measured in 12 children with AIS, 7 healthy age matched controls and 6 full term neonates with perinatal AIS.

Results: At the time of the acute event children with AIS had significantly elevated levels of MMP-9, TIMP4, IL-6, IL-8 and CRP compared to controls ($p < 0.05$). Except for lower IL-6 and CRP levels the pattern of children with a history of varicella-zoster virus (VZV) and other viral infections did not differ to the non-infectious group. Median levels of MMP-1, MMP-2, TIMP-1, TIMP-2, sE-selectin, sICAM-1, sVCAM-1, IL-8, IL-10, TNF-alpha, VEGF, Fetuin A were found to be higher in the neonatal group when compared with older children.

Conclusion: This pilot study supports the assumption of an inflammatory process and up-regulation of metalloproteinases and their inhibitors, and altered pattern of circulating pro-inflammatory cytokines, CRP and vWF levels in pediatric and neonatal AIS. It highlights the feasibility but also difficulties for similar larger future studies that should aim to clarify childhood stroke etiopathogenesis and consecutive further therapeutic options.

Introduction

Over the last two decades it has become evident that arterial ischemic stroke (AIS) in children can have many causes, inflammatory pathophysiology became more and more important within these multiple risk factors.¹ Several previous studies strongly suggest that inflammation and infectious mechanisms may be involved in the pathogenesis of the disease.^{2,3} Infections, especially upper respiratory infections, and parainfectious reactions appear among the most common risk factors for childhood stroke.^{4,5} Some studies demonstrate that up to one third of children with AIS had a history of varicella-zoster virus (VZV) infection in the preceding year, even more so in the preceding six months.⁶⁻⁸ In addition, other infections such as mycoplasma- and enterovirus-infections were shown to be related to vasculopathy in childhood stroke.^{9,10} Perinatal AIS is the most common cause of hemiplegic cerebral palsy, yet its etiology is poorly understood.¹¹ Among different independent risk factors, such as prolonged rupture of membranes and chorioamnionitis, underlying infectious / inflammatory mechanism play a role in a significant proportion of perinatal AIS.¹²

Studies evaluating biomarkers in pediatric stroke are scarce, although, AIS is known to be associated with a rise in systemic markers of endothelial activation, inflammation and oxidative stress.^{13,14} One study suggests that elevated D-dimers and C-reactive protein (CRP) contribute to childhood arterial ischemic stroke, whereas another study differentiates the role of more specific cytokines such as TNF- α , IL-2, IL-6, and IL-8.^{4,15} Recent research supports the concept of a reduced ADAMTS13, a Willebrand factor (vWF)-cleaving protease, corresponding to high levels of vWF, as a risk factor for pediatric stroke.¹³

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases targeting extracellular proteins have an important role in the pathogenesis of neurological inflammatory processes. Elevation occurs in cerebrospinal fluid and plasma during ischemic stroke by BBB destruction, oedema formation, activation of pro-inflammatory cytokines (TNF- α , IL-1 β) and destruction of myelin protein.¹⁶

The overall hypothesis of this study is that children and neonates show an increased inflammatory reaction after arterial ischemic stroke that can be quantified by different biomarkers, including MMPs. The pilot study aims to identify circulating inflammatory biomarkers associated with childhood and

neonatal stroke at the time of the acute event and its evolution over time after 3 and 6 months, in order to gain new insight for future more expanded studies concerning etiopathogenesis of paediatric stroke.

Methods

Since January 2000, a population based registry referred to as the Swiss Neuropediatric Stroke Registry (SNPSR) for children living in Switzerland having suffered from an arterial ischemic stroke prospectively collects data on incidence, manifestation, risk factors and neurological outcome of these children by an active reporting system.¹⁷ For the study presented, parents of a child or a neonate, that was announced to the registry between February 2012 and May 2013 were asked for additional participation in the present study. A case-control approach was chosen for children with stroke. Age matched controls were recruited from children in a pre-surgical blood test for planned surgical intervention (orthopedic, inguinal herniotomy), not related to any inflammatory condition or brain disorder.

In all recruited patients, a series of inflammatory markers (details see below) were measured in plasma samples at t0 (within 3 days of acute event), and if possible at t1 (after 3 months) and t2 (after 6 months). In controls, the same inflammatory markers were measured at a single time point.

Measurement of inflammatory parameter concentration

The blood samples were collected at participating local health centers using heparin as an anticoagulant. Blood samples were centrifuged for 15 minutes at 1000 x g, within 30 minutes of collection, and resulting plasma was stored at – 80°C until analysis. Analysis was performed on a Bio-Plex 200 suspension array system (Bio-Rad, Hercules, CA, USA) using microsphere-based multiplex assays. All test were performed at the Institute for Infectious Diseases in Bern and analysed blinded (i.e. cases vs controls) within the same analytical batch. The concentrations of the following inflammatory parameters were assessed using the respective kits: MMP-1, MMP-2, MMP-9 (Fluorokine MAP human MMP kit, RnD Systems Inc. Minneapolis, MN, USA), TIMP-1, TIMP-2, TIMP-4 (Fluorokine MAP human TIMP kit, RnD Systems), sP-selectin, sE-selectin, sICAM-1, sVCAM-1 (Luminex Performance human adhesion

molecule kit, RnD Systems), IL-1beta, IL-6, IL-8, IL-10, TNF- α (Luminex Performance high sensitivity cytokines kit, RnD Systems), and VEGF, A2-M, CRP, Fetuin A, Fibrinogen, SAP, Haptoglobin, vWF (Milliplex MAP human cardiovascular disease panel 3, Merck Millipore, Darmstadt, Germany)

The assays were performed according to the manufacturer's instructions. Samples were diluted to fit in the dynamic range of the assay, when appropriate. Concentrations were calculated by Bio-Plex Manager software using a 5-parametric logistic standard curve derived from the recombinant cytokine standards provided in the kit.

Statistical analysis

Analysis included comparison of biomarker levels between children with stroke and controls, different age groups with stroke (children versus neonates), history of VZV during the past 6 months and recent viral infection (defined as parental recall of any infection within 4 weeks preceding the stroke) (children with or without VZV and infection), and evolution of the pattern of inflammatory markers over time. For statistical analysis, samples with analytical levels below the detection limits were arbitrarily assigned to values corresponding to the minimal detectable concentration provided by the kit's manufacturer's instructions.

Data were analyzed using Prism 5.0 (GraphPad, San Diego, CA, USA) and tested for normality using D'Agostino & Pearson omnibus normality test. Data with a normal distribution were compared using student t test, and data which did not follow normal distribution were compared using Mann Whitney test. For all statistical tests, values of $P < 0.05$ were considered as significant. Multiple testing correction was not done, because at least some of the tested hypotheses are likely to have some aspect in common (i.e. not statistically independent), for example common mechanisms of upregulation.

The study has been approved by the research ethics board of the University Hospital of Berne, Switzerland (Study Nr. 2149).

Results

During a period of 15 months, inflammatory biomarkers were measured in 12 affected children (median age 7.6 years, range 1.8-15.6) and 6 term neonates (median age 3 days, range 1-7) with AIS. All of the study participants had confirmed AIS.

Detailed clinical information and the timing of the blood tests (T0) is summarised in **Table 1**. Median T0 in the children's group was 3.5 days (range 1-14), and 5.3 days (range 1-9) in the neonatal group. There was no seasonal trend of stroke dates. All children and neonates underwent MRI as well as MR angiography, which detected in all but 2/12 children cerebral arteriopathy, whereas only 2/6 neonates had a detectable vascular anomaly.

Five of the 12 children had a recent (defined as parental recall of any infection within 4 weeks preceding the stroke) viral infection; all of them also have had VZV infection during the past 6 months. A 15.6 years old female, denying a recent infection was later tested positive for mycoplasma pneumonia: rise of IgM within 4 weeks, but no immunoglobulin class switch from IgM to IgG, which is generally accepted as evidence of a recent *M. pneumoniae* infection. Three of the 12 children had an underlying cardiac pathology, but only one underwent a recent cardiac surgery previous to their stroke. One 11.9 years girl with a chronic ischemic cardiopathy had heart transplantation 7 days prior to stroke. One of the other cardiac patients had a Down's syndrome, malignant infarction of middle cerebral artery (MCA) led to death 3 days after stroke. A 14.6 years old female with known Beckwith-Wiedemann Syndrome had severe MCA infarction as well, but received bridging thrombolysis with mechanical thrombectomy resulting in recanalisation.

Figure 1 shows the significant up-regulation of MMP-9, TIMP4, IL-6, IL-8, and CRP in serum specimens from children with AIS compared to 7 healthy age matched controls (Mann Whitney test: $p=0.58$). Levels of TIMP-1 and vWF were also increased in children with stroke, but not significantly compared to controls (**Table 2**).

In 6/12 children a second blood withdrawal after 3 months at t1, and in 4/12 a third control at t2 after 6 months could be performed (**Figure 2**). Within this small group, no specific trend was apparent over time,

therefore no specific conclusion can be made, but some parameters such as vWF seem to remain elevated over time in 2/4 children.

Except for CRP and IL-6 levels patterns of the 5/12 children with VZV and recent infection did not differ significantly from patterns of children without VZV and infection in their history (**Figure 3**). Both, CRP and IL-6, were lower in the group of children with VZV and infection, however, still elevated compared to the controls. By calculating the normalized Euclidean distance including all parameters, we could conclude that the pattern of the patient with the positive *M. pneumoniae* serologies was more closely related to that of the stroke patients with VZV and infection.

Neonates and children differed significantly in their pattern of inflammatory markers. The group of neonates was relative homogeneous, since all were born at full term and none had perinatal complications reported. All became symptomatic by acute neonatal seizures within 7 days after birth, subacute ischemia was confirmed by MRI in each case. When comparing children and neonates with stroke, median levels of circulating MMP-1, MMP-2, TIMP-1, TIMP2, sE-selectin, sICAM-1, sVCAM-1, IL-8, IL-10, TNF-alpha, VEGF, Fetuin A, were found to be higher in neonates than in children, whereas there was no significant difference in MMP-9, IL-6, CRP and vWF levels (**Table 2**). Unfortunately, it was not possible to recruit healthy age matched neonate controls, and no standardized values are available for that age group. Measurements over time in the neonatal group were achieved in 3 patients, showing down regulation of sE-selectin, sICAM-1, sVCAM-1, and IL-10 at t1 (after 3 months) and t2 (after 6months) (**Figure 2**).

Discussion

The findings, of elevated levels of circulating inflammatory biomarkers, support an underlying inflammatory pathophysiology in AIS in children, independent of history of previous VZV- or other infection. Even though the number of study participants of this pilot study is very small, the important differences in levels of certain biomarkers indicate an inflammatory reaction in these stroke patients. For these reasons a future more expanded study would be indicated.

In all of the 12 children with AIS the MMP-9, TIMP-4, IL-6, IL-8, and CRP levels were elevated compared to the controls. The fact that unrelated to - underlying other risk factors - the biomarker profile of children with stroke was different compared to controls, suggests that cerebral ischemia triggers an inflammatory response in childhood AIS, and most likely in perinatal AIS as well.

During an inflammatory process, MMPs are involved in the degradation of extracellular matrix (ECM) components of the BBB. The MMP activity is mainly regulated by natural tissue inhibitors (TIMPs). Thus the elevation of TIMP-4 in our stroke patients is most likely related to high MMP-9. MMP 9 attacks major components of the basal lamina including type IV collagen, laminin, and fibronectin therefore playing a key role in delayed opening of the BBB after stroke, especially in states of systematic inflammation. It is also implicated in the development of hemorrhagic transformation, particularly with tissue plasminogen activator (tPA) treatment. Therefore, understanding the exact role of MMP-9 and other MMPs might have important diagnostic implications for stroke, and development of new therapeutic strategies aimed at modulating MMPs.¹⁸

MMPs can be divided in sub-groups, i.e. MMP-2 and MMP-9 belong to the sub-group of gelatinases. Besides their natural inhibitors (TIMPs), gelatinases can be inhibited by synthetic inhibitors, statins, and through tetracycline compounds.¹⁹ Doxycycline is a second-generation tetracycline, which has shown to have anti-inflammatory effects by reduction of cytokine release, and the inhibition of MMPs. Its protective mechanism and anti-inflammatory properties had broad beneficial effects on brain and cochlea improvement, as well as on survival in a model of pneumococcal meningitis in infant rats.²⁰ Due to protection of BBB against cleavage by gelatinases (MMP-2 and -9), tetracyclines seem to be also an

attractive agent applied for reduction of hemorrhage risk during thrombolytic therapy. A combination therapy with recombinant tPA (rtPA) and minocycline can extend the therapeutic window for thrombolysis; in adult animal studies minocycline seems to be a relatively selective inhibitor of MMP-9. It reduces infarct volume, MMP-9 plasma level and risk of bleeding during late, 6-h thrombolysis with rtPA after embolic focal ischemia.²¹ Efficacy and safety of adding minocycline to rtPA in patients is currently evaluated in Australian prospective multicenter study.²² The fact that we observe an activation of MMP-9 in childhood stroke supports the idea that children might also profit from this add-on therapy. This is especially important, as for children delay to diagnosis is still significantly longer than for adults.²³ Patients of the study with VZV and recent infection show a different pattern of inflammatory biomarkers than children without history of infection. Their IL-6 and CRP are lower than in the other children with stroke. Raised serum levels of IL-6 in the context with ischemic stroke were found previously and several studies suggest that IL-6 has a detrimental effect on infarct size and neurological outcome in adult patients with cerebral ischemia.²⁴ CRP has also a probable prognostic role and could therefore be a candidate biomarker. Thus, the lower but still increased CRP and IL-6 levels in our VZV/infection patients, compared to the children without history of infection, may indicate a probable better outcome. Whether these children have a lower inflammatory reaction due to smaller ischemic areas (and thus better outcome), or vice versa, has yet to be determined. However, this might explain why antiviral and immunosuppressive treatments in VZV associated stroke might have no benefit.²⁵ Elevated TIMP-1 ($p = 0.055$) and vWF ($p = 0.054$) levels could be seen in the stroke group but not in controls. Recent studies found raised vWF and therefore reduced ADAMTS13 activity as a risk factor for pediatric AIS. Our findings support this, as vWF levels are high, and even over time in some children with stroke. ADAMTS13 is thought to influence the flexibility of the BBB and is a possible protective factor in stroke.²⁶ Thus, elevated vWF and consequently reduced ADAMTS13 might not be a secondary effect of ischemia, but a pre-existing profile provoking stroke in childhood. Different levels of biomarkers in children with AIS compared to controls are most likely mainly a consequence of stroke, but could also reflect an underlying innate and adaptive alteration of immune response. Innate immunity

represents a fast but relatively blunt inflammatory and toxic response to invading microorganisms, but also interacts with several modified endogenous antigens.²⁵

We found that the pattern of the neonates with stroke differs significantly over the pattern of children with AIS for several inflammatory parameters. Median levels of the majority of measured markers were found to be higher in the neonatal group; interestingly, there was no significant difference in MMP-9, IL-6, CRP and vWF levels. Whether this has to do with the immaturity of the brain in general or is a consequence of stroke, needs further research. There is limited data, but a small pilot study found that in 50 preterm and term neonates MMP-2 and MMP-9 plasma activities were gestational age dependent, with highest levels observed in neonates at 33-36 week gestation.²⁷ Further studies hypothesize that intrinsic developmental differences in basement membrane and ECM formation may contribute to a somehow preserved BBB integrity after acute neonatal arterial stroke.²⁸

Limitations

Biomarker studies are challenging, especially when focusing on establishing etiologic associations. There are several limitations to this study.²⁹

The number of patients was too small to perform more detailed, especially multivariate statistical analyses. Number of patients was limited, as blood taking for the study was only possible during blood taking for diagnostic or therapeutic purposes. In addition, for the same reason range of time of blood taking was too expanded. For future studies, a study setting where blood taking in the acute event with delayed agreement of patient and parents would be of huge advantage. Follow up measurements were limited for similar reasons. In future studies, this could be overcome, if the present study would be embedded in a larger study, maybe asking for MR imaging at certain time points which would involve an additional blood puncture for contrast enhancement.

Higher numbers of cases would enable differentiation between pediatric stroke risk profiles, its primary mechanisms such as inflammation, and secondary responses to brain injury and illness.

Several possible confounders have to be considered. Although clinical stroke severity is difficult to define, as well as the actual stroke volume, both might influence the levels of inflammatory biomarkers. The same is probably true for subjects with underlying heart disease and cardiac surgical intervention. Also, reperfusion injury after intervention might influence the systemic inflammatory response. The cohort is too small to tell whether age could have an influence, but it might be considered as a possible confounder for future bigger studies as well. All these problems would have to be addressed by bigger numbers of patients, which would enable multivariate or regression analyses in statistics.

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Conclusion

Despite small number of participants and numerous technical challenges, the data of this descriptive pilot study clearly point towards an underlying inflammatory response during neonatal and childhood stroke and support a future larger study. The differences in endothelial dysfunction and inflammatory markers in children and neonates support both hypotheses: different inflammatory patterns in subgroup of strokes, but maybe also different vulnerabilities to stroke. The patterns in childhood stroke with beside temporary changes of marker also possible innate abnormalities support the theory of a certain “vulnerability” of endothelial cells for inflammation and therefore focal arteriopathy. The demonstrated possible role of inflammatory biomarkers in childhood and neonatal arterial ischemic stroke might be important. Improved understanding of inflammatory biomarkers and their signal cascades, after ischemic stroke, could have diagnostic and, by modulating MMPs, therapeutic implications.

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Table 1. Clinical details and timing of blood test (T0) on 12 affected children (1A) and 6 term neonates (1B) with AIS.

1A

Study ID	Sex	Age at stroke (years)	T0 (days)	Cerebral arteriopathy (yes/no)	VZV/recent infection (yes/no)	Seasonal timing of stroke (month)	Other anomalies
1	m	1.7	5	yes	yes	January	
2	f	2.0	1	no	no	June	Tricuspid atresia
3	f	4.0	1	yes	yes	August	
4	f	5.3	8	yes	yes	February	
5	f	6.0	1	no	yes	May	
6	f	6.3	1	yes	no	March	B cell prolymphocytic leukemia without CNS involvement
7	m	6.9	2	yes	yes	May	Polycystic kidney disease
8	f	7.8	6	yes	no	June	Trisomy 21, atrioventricular canal defect
9	f	8.8	1		no	April	
10	f	11.9	14	yes	no	March	Cardiac transplantation because of cardiac artery anomaly
11	f	14.6	1	yes	no	March	Beckwith Wiedemann syndrome
12	f	15.6	1	yes	yes	April	Mycoplasma PCR positive in Serum

1B

Study ID	Sex	Age at stroke (days)	T0 (days)	Cerebral arteriopathy (yes/no)	Gestational age (weeks)	Seasonal timing of stroke (month)	Birth weight (grams)	Adaption at birth (APGAR)
13	m	0	8	no	37 6/7	July	4040	9/10/9
14	m	1	1	no	40 6/7	March	3800	7/8/9
15	f	2	7	yes	40 2/7	November	3540	8/9/10
16	m	4	3	yes	40 5/7	November	3380	1/5/9
17	f	4	4	no	40 1/7	March	4180	6/8/8
18	m	7	9	no	40 6/7	February	3695	7/8/8

Table 2. Children with stroke (n=12), neonates with stroke (n=6) and control children (n=7)

Analytes median (min-max)	Children with stroke		Control children		Neonates with stroke		P1 [*]	P2 [†]
MMP-1, ng/ml	0.60	(0.06-11,4)	0.06	(0.06-1,03)	4.08	(2.65-6.69)	0.10	0.01
MMP-2, ng/ml	202.6	(94.2-389.8)	244.6	(94.2-297.3)	442.3	(364.4-529.3)	0.79	0.001
MMP-9, ng/ml	67.3	(31.3-253.7)	33.7	(17.2-65.7)	139.2	(39.9-212.2)	0.04	0.25
TIMP-1, ng/ml	56.5	(37.3-90.3)	41.3	(32.6-54.4)	135.8	(75.0-182.1)	0.06	0.001
TIMP-2, ng/ml	73.3	(47.2-153.1)	102.3	(61,1-113.3)	184.7	(131.1-280.8)	0.65	0.001
TIMP-4, ng/ml	0.91	(0.43-2.42)	0.39	(0.25-0.76)	0.49	(0.32-0.83)	0.002	0.04
sP-selectin, ng/ml	30,4	(13,5-111,2)	24,2	(8,05-37,7)	46,5	(28,5-57,9)	0.37	0.08
sE-selectin, ng/ml	36,1	(10,52-56,6)	37,0	(10,4-50,2)	59,1	(33,7-109,7)	0.74	0.03
s-ICAM-1, ng/ml	66,9	(42,4-167,9)	67,7	(27,1-84,7)	96,7	(73,90-125,8)	0.87	0.03
s-VCAM-1, µg/ml	0.86	(0.64-2.7)	0.78	(0.54-1.05)	2.4	(1.7-3.1)	0.37	0.002
IL-1beta, pg/ml	3.6	(0.36-11.72)	10.46	(0.36-26.68)	9.115	(2.9-26.29)	0.07	0.13
IL-6, pg/ml	4.3	(0.89-329.1)	1.21	(0.39-3.86)	3.78	(0.97-9.63)	0.003	0.65
IL-8, pg/ml	5.66	(1.75-24.95)	2.82	(1.93-6.65)	25.02	(9.82-50.84)	0.04	0.002
IL-10, pg/ml	1.24	(0.65-5.05)	1.15	(0.66-3.23)	3.675	(1.35-14.4)	0.44	0.035
TNF-alpha, pg/ml	8.555	(3.48-24.59)	4.49	(2.78-12.04)	14.62	(10.55-22.05)	0.42	0.02
VEGF, pg/ml	12.8	(2.92-88.11)	5.54	(2.08-26.84)	38.16	(10.26-116.3)	0.19	0.03
A2-M, mg/ml	1.75	(1.23 -2.2)	1.59	(0,80-2.05)	1.59	(1.52 -1.69)	0.57	0.52
CRP, µg/ml	5,91	(0,13-98,3)	0,12	(0,03-4,07)	2,94	(0,60-69,98)	0.007	0.92
Fetuin, µg/ml	223,6	(131,4-307,3)	235,2	(192,6-264,0)	337,7	(249,36-412,4)	0.59	0.001
Fibrinogen, mg/ml	0.27	(0.02-1.89)	0.20	(0.14-0.29)	0.35	(0.17-0.51)	0.19	0.82
SAP, µg/ml	3,79	(2,60-8,66)	3,11	(1,57-4,08)	2,12	(1,92-4,59)	0.10	0.07
Haptoglobin, mg/ml	0.54	(0.23-2.7)	0.35	(0.11-0.99)	0.02	(0,001-1.39)	0.29	0.04
vWF, µg/ml	8,87	(4,02-66,88)	3,96	(0,93-12,93)	10,68	(5,55-21,95)	0.06	0.98

* P value for the comparison “Children with stroke” vs “Control Children” (Mann Whitney test)

† P value for the comparison “Children with stroke” vs “Neonates with Stroke” (Mann Whitney test)

Figure 1. Analytes with statistical significance for the comparison control children versus children with stroke

Figure 2. 7 children were analyzed in a time-dependent fashion: 4 children with stroke, for which 2 children have t0 and t1, and 2 have t0, t1 and t2 (2A). 3 neonates, for which 2 have t0 and t1 and only 1 has t0, t1 and t2 (2B).

Figure 3. Analytes with statistical significance for the sub-group analysis of children with stroke with or without varicella-zoster virus (VZV) history

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