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Glial Fibrillary Acid Protein and Cerebral Oxygenation in Neonates Undergoing Cardiac Surgery

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Abstract

Background Neonates undergoing surgery for complex congenital heart disease are at risk of developmental impairment. Hypoxic–ischemic brain injury might be a contributing factor. We aimed to investigate the perioperative release of the astrocyte cytoskeleton component glial fibrillary acid protein and its relation to cerebral oxygenation.

Methods Serum glial fibrillary acid protein levels were measured before and 0, 12, 24, and 48 hours after surgery. Reference values were based on preoperative samples; concentrations above the 95th percentile were defined as elevated. Cerebral oxygenation was derived by near-infrared spectroscopy.

Results Thirty-six neonates undergoing 38 surgeries utilizing cardiopulmonary bypass were enrolled (complete data available for 35 procedures). Glial fibrillary acid protein was elevated after 18 surgeries (arterial switch: 7/12; Norwood: 5/15; others: 6/8; $p = 0.144$). Age at surgery was higher in cases with elevated serum levels (6 [4–7] vs. 4 [2–5] days, $p = 0.009$) and intraoperative cerebral oxygen saturation was lower ($70 \pm 10\%$ vs. $77 \pm 7\%$, $p = 0.029$). In cases with elevated postoperative glial fibrillary acid protein, preoperative cerebral oxygen saturation was lower for neonates undergoing the arterial switch operation ($55 \pm 9\%$ vs. $64 \pm 4\%$, $p = 0.048$) and age at surgery was higher for neonates with a Norwood procedure (7 [6–8] vs. 5 [4–6] days, $p = 0.028$).

Conclusions Glial fibrillary acid protein was elevated after ~50% of neonatal cardiac surgeries and was related to cerebral oxygenation and older age at surgery. The potential value as a biomarker for cerebral injury after neonatal cardiac surgery warrants further investigation; in particular, the association with neurodevelopmental outcome needs to be determined.

Keywords
- cardiopulmonary bypass
- congenital heart disease
- neonates
- neurology/neurologic (deficits
- disease
- injury)

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Introduction

Advances in surgical technique and perioperative care have led to a substantial increase of survival for neonates with complex congenital heart disease (CHD). Despite these improvements, neonates requiring corrective or palliative surgery are still at higher risk of later neurodevelopmental impairment.[1–3] The etiology is multifactorial and related to nonmodifiable patient-specific factors and potentially modifiable factors including surgical technique and perioperative care. Perioperative cerebral hypoxemia might be a relevant modifiable cause.[4] Particularly white matter injury has been documented by cerebral magnetic resonance imaging (MRI) before and after surgery in several previous studies.[5–11] Brain injury is usually subtle and clinical evident neurologic injury is fortunately relatively rare. As developmental assessment can only be performed with a relatively long period of latency, surrogate markers for clinically silent hypoxic-ischemic brain injuries in neonates with CHD are needed. Near-infrared spectroscopy allows real-time noninvasive measurement of cerebral tissue oxygen saturation and is frequently used for perioperative monitoring. Previous studies have reported relationships between perioperative cerebral oxygenation and abnormal findings on MRI or neurodevelopmental outcomes in neonates with complex CHD.[9,12–14] A brain-specific and sensitive biomarker released into the bloodstream after cellular damage, which permits the identification of patients at risk or even predicts long-term developmental outcomes, would be of great value for clinical practice and to develop strategies to improve the long-term neurologic outcome.

Among others, the glial fibrillary acid protein might be a suitable biomarker. This protein is part of the astrocyte cytoskeleton and is thought to be specific to the central nervous system. In previous studies, serum levels were associated with neurologic outcome after traumatic brain injury and cardiac arrest in adults and with acute brain injury and death in children with extracorporeal life support.[15–17] In addition, glial fibrillary acid protein concentrations were predictive for neurodevelopmental outcome in neonates with perinatal asphyxia and hypoxic ischemic encephalopathy and for the occurrence of periventricular white matter injury in premature infants.[18,19] Up to now there is limited data regarding glial fibrillary acid protein levels in children with CHD.[20–25] Especially the value of glial fibrillary acid protein as a predictor of developmental outcome after cardiac surgery is relatively unknown.[25,26]

This study aimed to determine perioperative glial fibrillary acid protein levels in neonates undergoing cardiac surgery utilizing cardiopulmonary bypass and to evaluate the association between serum levels and cerebral tissue oxygenation. We hypothesized that higher glial fibrillary acid protein levels are related to impaired perioperative cerebral oxygenation.

Methods

The study was designed as a prospective observational cohort study. Neonates with CHD up to 28 days of age undergoing cardiac surgery utilizing cardiopulmonary bypass were eligible for enrollment. Exclusion criteria included proven or clinically suspected genetic syndrome, weight at surgery of less than 2500 g, history of birth asphyxia, or preexisting brain injury.

The study protocol has been approved by the institutional research ethics committee. Written informed consent was provided for all subjects.

All patients received standard care during the perioperative period. In terms of bypass management, the pH-stat method was used for cooling to the desired temperature. Patients undergoing the Norwood procedure were operated on in deep hypothermia with selective cerebral perfusion during aortic arch reconstruction. Hemofiltration was routinely used before weaning from bypass.

Glial Fibrillary Acidic Protein

Glial fibrillary acid protein levels were obtained before surgery as well as 0, 12, 24, and 48 hours after surgery. Blood was drawn from indwelling arterial or central venous lines. Samples were centrifuged and aliquots stored at –20°C until analysis. Protein concentrations were determined by enzyme-linked immunosorbent assay (ELISA) with a commercially available ELISA-platform (Abbexa, Cambridge, United Kingdom). Samples were analyzed according to the manufacturer's instructions; all samples were assayed in duplicates.

Due to the lack of validated reference values, these were defined based on preoperative samples after exclusion of outliers and extreme values. Concentrations >95th percentile were defined as elevated. For statistical analysis, cases with glial fibrillary acid protein values in the normal range were compared with patients who had elevated concentrations at any time after surgery.

Routine Monitoring and Near-Infrared Spectroscopy

Routine perioperative monitoring included continuous measurement of arterial oxygen saturation and invasive arterial and central venous blood pressure (IntelliVue, Philips Healthcare, Best, the Netherlands). Arterial blood gases, including lactate levels, were obtained at 1 to 2-hour intervals; central venous blood gases were sampled at 4-hour intervals.

Near-infrared spectroscopy probes were placed on the patient's midline forehead and slightly to the right of midline on the T10-L2 posterior flank. Cerebral and somatic tissue oxygen saturations were monitored continuously (INVOS 5100, Medtronic, Minneapolis, Minnesota, United States). Regional oxygen saturation values determined by near-infrared spectroscopy were matched to the hemodynamic and respiratory data for 12 hours before and 48 hours after surgery. Mean values were calculated for the 12 preoperative hours (baseline), for the first 4 postoperative hours (early postoperative course), and for the entire 48-hour postoperative period. The intraoperative course was divided into five periods (pre-bypass, cooling, low-flow, rewarming, off-pump) and mean values were calculated for each period and for the entire intraoperative course. To estimate cerebral oxygen extraction, the difference between the arterial and the corresponding cerebral oxygen saturation measurement was calculated. Comparisons were made

between neonates with elevated postoperative glial fibrillary acid protein and cases with glial fibrillary acid protein in the normal range. Subgroup analyses were performed for neonates undergoing the Norwood procedure or the arterial switch operation.

Statistics

Continuous variables are expressed as mean and standard deviation or median and interquartile range as appropriate and categorical data as count and percentages. We employed Fisher's exact test for analysis of categorical data. Continuous variables were compared with the Student's *t*-test for two independent samples or—in case of non-normally distributed data—with the Mann–Whitney *U*-Test or Kruskal–Wallis test. Correlations were calculated using the Pearson correlation coefficient. All statistical analyses were performed with the statistical software package SPSS (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, New York, United States). A value of $p < 0.05$ was considered statistically significant.

Results

Patients

A total of 36 neonates were enrolled in the study between May 2015 and September 2016. Underlying diagnosis is given in ►**Table 1**. The diagnosis was made prenatally in 20 (55.6%) patients. Two patients (5.6%) were born preterm, and extracardiac malformations were seen in four (11.1%) patients. Prior to enrollment, four neonates had undergone pulmonary artery banding and another four required a balloon atrial septostomy. With one exception, all neonates received prostaglandin-E1 infusion for maintaining ductal patency. Preoperative adverse events were noted in six cases including clinical deterioration with signs of multiorgan failure, the need for intubation or inotropic support, unplanned cardiac surgery, or intervention.

Surgeries and Postoperative Course

The 36 neonates underwent 38 surgical procedures (►**Table 2**). Postoperative complications were noted after 14 (36.8%) surgeries, mostly within the first 48 hours. Transient rhythm disturbances ($n = 5$) were most common, others included sepsis, low-cardiac output, and shunt thrombosis. Clinically overt neurological complications were not noted in any case. Two patients died within the study period. One patient deceased 91 days after the Norwood procedure due to shunt thrombosis, another with the borderline left heart structures 28 days after a biventricular repair approach.

Table 1 Cardiac diagnosis

Hypoplastic left heart syndrome and other single ventricle lesions	16 (44.4%)
Transposition of the great arteries	12 (33.3%)
Ventricular septal defect + aortic arch abnormality[a]	4 (11.1%)
Common arterial trunk	2 (5.6%)
Total anomalous pulmonary venous drainage	1 (2.8%)
Partial anomalous pulmonary venous drainage + VSD	1 (2.8%)

Abbreviation: VSD, ventricular septal defect.
[a]Interrupted aortic arch ($n = 1$) and coarctation ($n = 3$).

Table 2 Surgical data and postoperative course

Type of surgery		
Norwood procedure	15	(39.5%)
Arterial switch operation	12	(31.6%)
Aortic arch repair (+VSD closure)	6	(15.8%)
Others[a]	5	(13.2%)
Surgical data		
Age at surgery (d)	5	(3–7)
Weight at surgery (kg)	3.42	±0.44
Cardiopulmonary Bypass (min)	139	±37
Aortic cross-clamp (min)	68	±30
Selective cerebral perfusion[b] (min)	43	±12
Temperature nadir (°C)	22.0	±4.3
Primary chest closure (*n*)	32	(84.2%)
Postoperative course		
Mechanical ventilation (h)	87	(61–118)
Duration of inotropic support (h)	26	(18–73)
Intensive care unit stay (d)	11	(6–33)
Hospital stay (d)	25	(12–49)

Abbreviation: VSD, ventricular septal defect.
[a]Others: repair of anomalous pulmonary venous return ($n = 2$), repair of common arterial trunk ($n = 2$), atrioseptectomy and pulmonary artery banding ($n = 1$).
[b]Selective cerebral perfusion ($n = 17$).

Perioperative Glial Fibrillary Acid Protein Serum Concentrations

►**Fig. 1** shows pre- and postoperative glial fibrillary acid protein serum concentrations. No samples were available after three procedures. Compared with preoperative baseline values, median postoperative serum concentrations were not significantly different at any time point. ►**Table 3** compares values between cases undergoing the arterial switch operation, the Norwood procedure, or other surgeries, respectively.

Elevated Preoperative Glial Fibrillary Acid Protein Concentrations

Preoperative glial fibrillary acid protein levels were above the 95th percentile (> 4 µg/L) in seven cases. Six patients (transposition of the great arteries, $n = 4$; common arterial trunk with interrupted aortic arch, $n = 1$; ventricle septum defect with aortic arch hypoplasia and coarctation, $n = 1$) had markedly elevated glial fibrillary acid protein concentrations (►**Fig. 1**, extreme values). Preoperative cerebral oxygen saturation was not different between cases with elevated

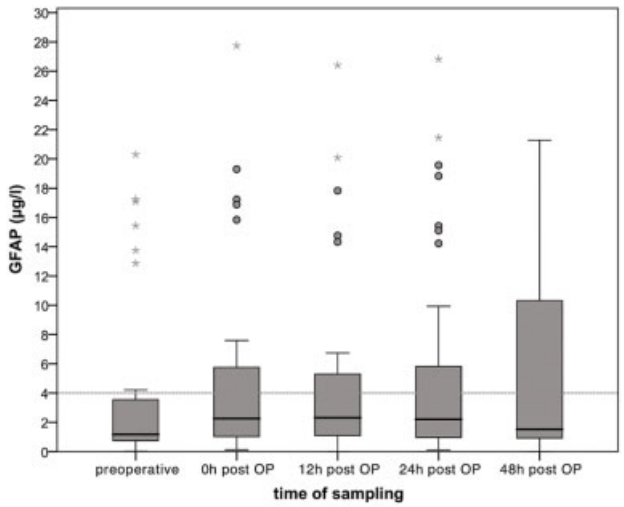

Fig. 1 Pre- and postoperative glial fibrillary acid protein (GFAP) serum concentrations ($n = 35$). Whiskers above and below the box represent the largest and smallest data points that are <1.5 box lengths (interquartile range) away from the end of the box; circles highlight data points >1.5 box lengths (outliers) and asterisks data points >3 box lengths away (extreme values). The red line represents the 95th percentile of preoperative GFAP values after exclusion of outliers and extreme values.

glial fibrillary acid protein and those in the normal range ($60 \pm 12\%$ vs. $67 \pm 9\%$, $p = 0.163$).

There was no association between elevated preoperative GFAP and clinical factors including the need for septostomy in patients with transposition of the great arteries.

Elevated Postoperative Glial Fibrillary Acid Protein Concentrations

Elevated postoperative glial fibrillary acid protein concentrations were found after 18 surgeries, including all seven cases with elevated preoperative values. Glial fibrillary acid protein was elevated after the arterial switch operation in 7 of 12 cases, after 5 of 15 Norwood procedures, and after other operations in 6 of 8 cases ($p = 0.144$). Median age at surgery was higher in patients with elevated postoperative glial fibrillary acid protein. The duration of cardiopulmonary bypass, aortic cross clamp, and selective cerebral perfusion were not different. No differences in variables of the postoperative course were noticed (►**Table 4**).

►**Fig. 2** compares pre-, intra-, and postoperative cerebral oxygen saturation readings between cases with elevated postoperative glial fibrillary acid protein and those with concentrations in the normal range. Preoperative cerebral oxygen saturation values were not different. Mean intraoperative cerebral oxygen saturation was lower in patients with elevated glial fibrillary acid protein (►**Table 5**). Specifically, cerebral oxygen saturations during cooling ($72 \pm 13\%$ vs. $80 \pm 8\%$, $p = 0.045$) and while on bypass until rewarming ($76 \pm 15\%$ vs. $85 \pm 7\%$, $p = 0.029$) were lower in cases with elevated glial fibrillary acid protein (►**Fig. 2**). No differences were observed in postoperative cerebral and somatic tissue oxygen saturations (►**Fig. 2**, ►**Table 4**). Routine monitoring data showed no differences between groups (►**Table 5**).

Subgroup Analysis

Patients undergoing the Norwood procedure or the arterial switch operation were analyzed separately. The arterial switch operation was performed earlier compared with the Norwood procedure (median age 4 [2–5] days vs. 6 [4–7] days, $p = 0.032$). Duration of cardiopulmonary bypass was not different, but aortic cross-clamp time was longer for the arterial switch operation (158 ± 23 vs. 151 ± 21 minutes, $p = 0.445$ and 101 ± 16 vs. 46 ± 11 minute, $p < 0.001$). The temperature nadir was higher during the arterial switch operation (24.8 ± 2.4°C vs. 18.5 ± 1.2°C, $p < 0.001$). Mean intraoperative cerebral oxygen saturation was not different between subgroups (Norwood: $75 \pm 8\%$ vs. arterial switch operation: $75 \pm 10\%$, $p = 0.923$). However, cerebral oxygen saturation during hypothermic bypass was lower ($78 \pm 12\%$ vs. $87 \pm 6\%$, $p = 0.040$), while cerebral oxygen saturations after termination of cardiopulmonary bypass were higher in the arterial switch operation group ($79 \pm 10\%$ vs. $62 \pm 12\%$, $p = 0.001$). The frequency of cases with elevated postoperative glial fibrillary acid protein was not different between subgroups (Norwood: 5/15 vs. arterial switch operation: 7/12, $p = 0.194$).

Glial Fibrillary Acid Protein after the Arterial Switch Operation

Preoperative cerebral ($55 \pm 9\%$ vs. $64 \pm 4\%$, $p = 0.048$) and somatic tissue oxygen saturations ($59 \pm 8\%$ vs. $70 \pm 6\%$, $p = 0.025$) as well as arterial oxygen saturations ($86 \pm 5\%$ vs. $92 \pm 4\%$, $p = 0.03$) and arterial partial pressure of oxygen (38 ± 5 mm Hg vs. 45 ± 5 mm Hg, $p = 0.046$) were lower in cases with elevated glial fibrillary acid protein. Median age at surgery was 2 (2–3) days compared with 4 (3–6) days in patients with elevated glial fibrillary acid protein ($p = 0.149$). Duration of cardiopulmonary bypass and aortic cross-clamp and intraoperative tissue oxygen saturations was not different between

Table 3 Perioperative GFAP serum concentrations (µg/L)

Time of sampling	Single ventricle ($n = 16$)		Transposition ($n = 12$)		Others ($n = 7$)		p-Value
Preoperative	0.97	(0.33–1.36)	1.39	(0.75–12.87)	7.34	(0.89–17.09)	0.246
0h postoperative	1.73	(0.75–5.53)	2.30	(1.14–6.95)	4.21	(1.02–19.30)	0.484
12h postoperative	1.48	(0.85–4.61)	3.20	(1.09–5.01)	3.86	(1.73–20.09)	0.356
24h postoperative	1.48	(0.63–9.53)	3.92	(1.67–5.26)	2.06	(1.43–19.57)	0.440
48h postoperative	1.46	(0.25–12.86)	2.25	(0.95–14.30)	1.26	(0.90–7.78)	0.876

Abbreviation: GFAP, glial fibrillary acid protein.

Table 4 Surgical data and postoperative course

	GFAP normal ($n = 17$)		GFAP elevated ($n = 18$)		p-Value
Surgical data					
Age at surgery (d)	4	(2–5)	6	(4–7)	0.009
Weight at surgery (kg)	3.51	±0.45	3.32	±0.41	0.205
Cardiopulmonary bypass (min)	143	±38	140	±35	0.807
Aortic cross-clamp[a](min)	61	±27	76	±30	0.127
Selective cerebral perfusion[b] (min)	42	±9	46	±15	0.574
Temperature nadir (°C)	21.7	±4.8	22.4	±3.8	0.624
Postoperative course					
Mechanical ventilation (h)	88	(58–116)	80	(58–116)	0.961
Inotropic support (h)	22	(16–55)	46	(18–88)	0.143
Intensive care unit stay (d)	22	(8–38)	11	(6–29)	0.245
Hospital stay (d)	42	(13–60)	20	(11–49)	0.365

Abbreviation: GFAP, glial fibrillary acid protein.
[a]Aortic cross clamp, $n = 16$ versus 17;
[b]selective cerebral perfusion, $n = 10$ versus 7.

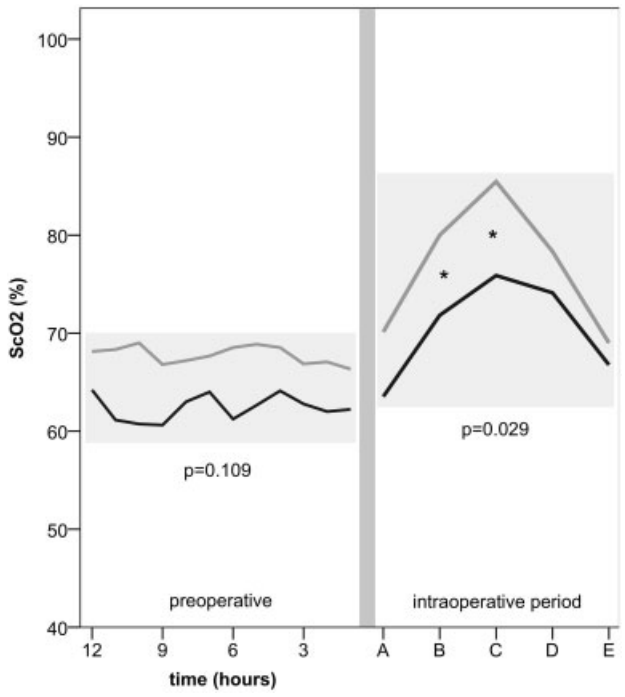

Fig. 2 Comparison of pre-, intra-, and postoperative cerebral tissue oxygen saturations (ScO_2) between patients with normal ($n = 17$, green line) and elevated ($n = 18$, red line) postoperative glial fibrillary acid protein (GFAP) concentrations. The intraoperative course was divided into five periods: pre-bypass (**A**), cooling (**B**), low-flow (**C**), rewarming (**D**), and off-pump (**E**). p-Values refer to the comparison of mean values between groups in the outlined perioperative period (highlighted in yellow). Mean ScO_2 values during cooling and low-flow were significantly lower in cases with postoperative elevated GFAP (asterisks).

cases with normal or elevated glial fibrillary acid protein values (cerebral oxygen saturation: $73 \pm 13\%$ vs. $77 \pm 5\%$, $p = 0.507$ and somatic oxygen saturation: $86 \pm 6\%$ vs. $85 \pm 4\%$, $p = 0.905$). Routine monitoring and near-infrared spectroscopy data of the postoperative course showed no differences.

Glial Fibrillary Acid Protein after the Norwood Procedure

Median age at Norwood procedure was 7 (6–8) days in cases with elevated glial fibrillary acid protein compared with 5 (4–6) days with serum levels in the normal range ($p = 0.028$). The duration of selective cerebral perfusion tended to be longer in patients with elevated glial fibrillary acid protein (53 ± 10 vs. 42 ± 9 minutes, $p = 0.056$). There were no differences in duration of cardiopulmonary bypass (146 ± 11 vs. 154 ± 24 minutes, $p = 0.463$). The mean intraoperative cerebral tissue oxygen saturation was $72 \pm 3\%$ compared with $77 \pm 8\%$ in cases with glial fibrillary acid protein in the normal range ($p = 0.208$). There were no differences in perioperative routine monitoring or near-infrared spectroscopy data.

Discussion

Perioperative hypoxemia may be an important modifiable risk factor for developmental impairment in children with complex CHD requiring corrective or palliative surgery during the neonatal period. Our pilot study evaluated the relation between cerebral oxygenation derived by near-infrared spectroscopy and serum concentrations of the astrocyte protein glial fibrillary acid protein, a potential biomarker for brain injury. In our cohort, postoperative glial fibrillary acid protein concentrations were elevated in about every second patient. Overall, neonates with elevated glial fibrillary acid protein were operated later and had lower intraoperative cerebral tissue oxygen saturations. In neonates with transposition of the great arteries undergoing arterial switch operation, preoperative cerebral and somatic oxygen saturations, as well as arterial oxygen saturations and partial pressures of oxygen were lower in those with elevated postoperative glial fibrillary acid protein. In neonates undergoing the Norwood procedure, only older age at surgery was associated with elevated glial fibrillary acid protein. For cases

Table 5 Near-infrared spectroscopy and routine monitoring data

	GFAP normal ($n = 17$)		GFAP elevated ($n = 18$)		*p*-Value
Preoperative course					
ScO_2 (%)	68	±8	62	±10	0.109
SsO_2 (%)	67	±8	62	±7	0.060
$\Delta SacO_2$ (%)	25	±9	27	±9	0.551
SaO_2 (%)	92	±3	89	±6	0.102
MAP (mm Hg)	50	±4	48	±4	0.229
p_aCO_2 (mm Hg)	43	±10	43	±6	0.907
p_aO_2 (mm Hg)	55	±22	52	±21	0.634
Lactate (mmol/L)	1.5	±0.6	1.5	±0.5	0.681
Intraoperative data (entire period)					
ScO_2 (%)	77	±7	70	±10	0.029
SsO_2 (%)	77	±8	78	±11	0.800
Early postoperative course (first 4 h)					
ScO_2 (%)	57	±4	56	±13	0.834
SsO_2 (%)	86	±9	85	±15	0.791
SaO_2 (%)	90	±7	93	±8	0.222
MAP (mm Hg)	54	±6	52	±6	0.422
p_aCO_2 (mm Hg)	37	±6	39	±7	0.540
p_aO_2 (mm Hg)	82	±52	105	±57	0.226
SvO_2 (%)	72	±11	70	±10	0.479
Lactate (mmol/L)	6.7	±2.2	5.6	±1.9	0.120
Entire postoperative course					
ScO_2 (%)	70	±13	72	±10	0.654
SsO_2 (%)	77	±11	77	±11	0.881
$\Delta SacO_2$ (%)	20	±9	23	±7	0.269
SaO_2 (%)	88	±7	93	±7	0.072
MAP (mmHg)	50	±4	49	±3	0.175
p_aCO_2 (mmHg)	42	±4	44	±3	0.273
p_aO_2 (mmHg)	72	±38	99	±47	0.066
SvO_2 (%)	72	±10	72	±7	0.918
Maximum lactate (mmol/L)	7.9	±2.2	6.5	±1.9	0.055

Abbreviations: GFAP, glial fibrillary acid protein; MAP, mean arterial pressure; p_aCO_2, arterial carbon dioxide tension; p_aO_2, arterial oxygen tension; $\Delta SacO_2$, arterial–cerebral saturation difference; SaO_2, arterial oxygen saturation; ScO_2, cerebral tissue oxygen saturation; SsO_2, somatic tissue oxygen saturation;; SvO_2, central venous saturation.

who had a Norwood procedure, no relation between perioperative cerebral oxygenation and postoperative glial fibrillary acid protein was observed.

Glial fibrillary acid protein is part of the astrocyte cytoskeleton and is thought to be specific to the nervous system. After astrocyte injury, glial fibrillary acid protein and its breakdown products can be detected in the peripheral blood. Circulating glial fibrillary acid protein levels were predictive of abnormal brain MRI and neurodevelopmental outcome in neonates with hypoxic ischemic encephalopathy and for the occurrence of periventricular white matter injury in premature infants.[18,19] Glial fibrillary acid protein has also been evaluated in neonates and infants undergoing surgery for CHD in previous studies.[20–26] However, in these reports samples were often limited to the intraoperative period. Up to now, only one study evaluated the relation between intraoperative cerebral oxygenation derived by near-infrared spectroscopy and glial fibrillary acid protein concentrations.[20] The association between pre- or postoperative cerebral oxygenation and postoperative glial fibrillary acid protein release has not been previously studied.

In our cohort, preoperative glial fibrillary acid protein concentrations were found to be elevated in ~20% and postoperative values in ~50%. Similar frequencies have been reported for the prevalence of pre- and postoperative white matter injury seen on MRI in neonates with complex CHD.[5–11] In contrast to our results, preoperative glial fibrillary acid protein levels were usually undetectable or very low in previous studies, which seem to be inconsistent with the prevalence of white matter injury based on MRI data. However, the protein was also detectable in the majority of patients after initiation of cardiopulmonary bypass.[20–26] Highest values were usually noticed at the end of bypass.[20–23] Unfortunately, up to now methodological heterogeneity in glial fibrillary acid protein assessments with different ELISA-platforms hinders comparability of absolute values. Two studies reported postoperative concentrations in the range of 2 ng/mL in patients with transposition of the great arteries or in cases undergoing the Norwood operation.[20,22]

Among others, glial fibrillary acid protein concentrations were related to the duration of cardiopulmonary bypass, lower temperature nadir, lower oxygen delivery while on bypass, and intraoperative cerebral saturations below 45% in previous reports.[20–23] In our cohort, mean intraoperative cerebral oxygen saturations were lower in patients with elevated postoperative glial fibrillary acid protein, especially during cooling and hypothermic cardiopulmonary bypass. During hypothermic bypass relatively high cerebral oxygen saturations are usually achieved, which are well above accepted threshold of 40 to 45%. Prolonged periods of low cerebral oxygen saturation are rarely seen if circulatory arrest is avoided. In a previous study, brain MRI abnormalities in terms of hemosiderin foci were associated with lower mean cerebral oxygen saturation in neonates and infants undergoing corrective surgery for CHD.[11] On average, cerebral oxygen saturations were all well above widely accepted thresholds for both patients with or without imaging abnormalities.[11] Thresholds for adequate cerebral oxygenation may vary according to specific bypass conditions, particularly during hypothermia. It is also likely that additional factors increase the susceptibility to intra- or postoperative injury. Among others, experimental data suggest that preoperative hypoxia increases the vulnerability of developing white matter to ischemia and reperfusion injury.[27] Patients with complex CHD are at risk of cerebral hypoxemia before surgery.[6,28–32] Due to hemodynamic alterations of

cerebral blood flow, fetuses with complex CHD might develop brain injury even before birth. Brain development often is delayed and may itself result in greater vulnerability to cerebral white matter injury.[33,34] The majority of patients in our study had the underlying diagnosis transposition of the great arteries or hypoplastic left heart syndrome. For both, abnormal findings on MRI before surgery, particularly white matter injury, have been reported.[5,7,10,31,35] In one study, neonates with transposition of the great arteries diagnosed with periventricular leukomalacia were more hypoxemic and time to surgery was longer compared with those without white matter injury.[32] In addition, Lim et al evaluated 45 infants with transposition of the great arteries using pre- and postoperatively MRI. Surgery beyond 2 weeks of age was associated with impaired brain growth and neurodevelopment. The underlying mechanisms were unclear. However, the authors assumed that extended periods of cyanosis and pulmonary over-circulation may be causative.[35] In our cohort, neonates with transposition of the great arteries and elevated postoperative glial fibrillary acid protein were also exposed to a greater degree of hypoxemia in the preoperative course. They had lower cerebral and somatic oxygen saturation values as well as lower arterial oxygen saturations and partial pressure of oxygen levels on blood gases. For patients with transposition of the great arteries, lower preoperative cerebral saturations were also related to neurodevelopmental outcome in a previous study.[14] Similar observations were made in patients with hypoplastic left heart syndrome. Lynch et al observed a large amount of new or worsened postoperative white matter injury in ~50% of the patients. The risk of injury increased with longer time to surgical repair and cases with white matter injury tended to have lower preoperative cerebral tissue oxygen saturations.[31] In our study, time to surgery was also longer in patients undergoing a Norwood procedure presenting with high postoperative glial fibrillary acid protein.

Postoperative glial fibrillary acid protein levels have not yet been evaluated together with brain imaging in children with CHD. However, one study showed a relation between impaired neurodevelopment assessed 18 months after surgery with the Vineland Adaptive Behavior Scales (VABS-I) and increased glial fibrillary acid protein levels during cardiac surgery.[25] In addition, higher postoperative GFAP levels were independently associated with decreased motor scores in a study evaluating neurodevelopmental outcomes 12 months after neonatal cardiac surgery with the Bayley Scales of Infant and Toddler Development third edition.[26]

Developmental outcomes and the relation to perioperative glial fibrillary acid protein will be determined in our cohort.

Limitations

The sample size of this pilot study was relatively small and statistical power reduced, especially for subgroup analysis. Multivariate analysis was not applicable due to small sample size. The generalizability of our results is therefore limited. Methodological heterogeneity in glial fibrillary acid protein assessments and the lack of reliable reference data for commercially available ELISA-platforms hinder comparability. Normal values were derived from preoperative values from diseased children rather than from a healthy control group. Cerebral MRI was not performed in the perioperative course and no association between glial fibrillary acid protein or cerebral tissue oxygenation and evidence of hypoxic brain injury can be provided. In this observational study, near-infrared spectroscopy monitoring was not used for a goal-directed therapy.

Conclusions

Our pilot study evaluated the astrocyte component glial fibrillary acid protein as a potential biomarker for brain injury in neonates with complex CHD. Elevated postoperative glial fibrillary acid protein concentrations were noted in about every second patient and were related to older age at surgery and intraoperative cerebral oxygenation. In patients with transposition of the great arteries, elevated glial fibrillary acid protein levels were associated with preoperative hypoxemia. Neurodevelopmental outcome still has to be determined, but glial fibrillary acid protein as a brain biomarker after neonatal cardiac surgery warrants further investigation.

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Authors Contribution
All listed authors fulfilled authorship criteria including substantial contributions to research design or the acquisition, analysis, or interpretation of data; drafting the article or revising it critically; and approval of the submitted and final versions.

Conflicts of Interest
None.

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