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Original Article

Glial fibrillary acidic protein in children with congenital heart disease undergoing cardiopulmonary bypass

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Abstract *Objective:* To determine whether blood levels of the brain-specific biomarker glial fibrillary acidic protein rise during cardiopulmonary bypass for repair of congenital heart disease. *Methods:* This is a prospective observational pilot study to characterise the blood levels of glial fibrillary acidic protein during bypass. Children <21 years of age undergoing bypass for congenital heart disease at Johns Hopkins Hospital and Texas Children's Hospital were enrolled. Blood samples were collected during four phases: pre-bypass, cooling, re-warming, and post-bypass. *Results:* A total of 85 patients were enrolled between October, 2010 and May, 2011. The median age was 0.73 years (range 0.01–17). The median weight was 7.14 kilograms (range 2.2–86.5). Single ventricle anatomy was present in 18 patients (22%). Median glial fibrillary acidic protein values by phase were: pre-bypass: 0 ng/ml (range 0–0.35); cooling: 0.039 (0–0.68); re-warming: 0.165 (0–2.29); and post-bypass: 0.112 (0–0.97). There were significant elevations from pre-bypass to all subsequent stages, with the greatest increase during re-warming ($p = 0.0001$). Maximal levels were significantly related to younger age ($p = 0.03$), bypass time ($p = 0.03$), cross-clamp time ($p = 0.047$), and temperature nadir (0.04). Peak levels did not vary significantly in those with single ventricle anatomy versus two ventricle repairs. *Conclusion:* There are significant increases in glial fibrillary acidic protein levels in children undergoing cardiopulmonary bypass for repair of congenital heart disease. The highest values were seen during the re-warming phase. Elevations are significantly associated with younger age, bypass and cross-clamp times, and temperature nadir. Owing to the fact that glial fibrillary acidic protein is the most brain-specific biomarker identified to date, it may act as a rapid diagnostic marker of brain injury during cardiac surgery.

Keywords: Brain biomarker; glial fibrillary acidic protein; cardiopulmonary bypass

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SURGICAL REPAIR FOR CONGENITAL HEART DISEASE was pioneered over 60 years ago. Mortality rates for repair of congenital heart disease have drastically fallen and focus has turned to quality of life, especially neurologic outcomes, as surgical techniques have been refined. Neurologic injury occurs during surgical repair in 30–70% of children with congenital heart disease.[1–3] During cardiopulmonary bypass, patients are at risk of embolic phenomenon, brain hypoperfusion, and inflammation from exposure of the blood to plastic surfaces. Efforts to improve cardiopulmonary bypass safety and post-operative neurocognitive outcomes have

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been hampered by the ability to detect and measure peri-operative brain injury. Efforts to diagnose peri-operative brain injury have utilised magnetic resonance imaging changes, near-infrared spectroscopy trends, electroencephalogram changes, intelligence quotients, and results of developmental testing.[4–6] The goal of all of these areas of study is to quickly identify brain injury and to alter management in order to improve patient outcomes.

Blood-based brain biomarkers are a promising area of study related to neurologic injury. The ideal biomarker would be specific to brain tissue, released into the bloodstream quickly after neurologic injury, accurately reflect areas of injury and degree of injury as detected by imaging and neurocognitive outcomes, and easily assayed.

Neuron-specific enolase was first described in the 1970s and is an enzyme involved in the glycolytic pathway. It was originally thought to be specific to neurons. Further studies showed, however, that this enzyme is also present in platelets and red blood cells. Attempts to use neuron-specific enolase as a biomarker of brain injury in children undergoing surgical repair have proven unsuccessful, as neuron-specific enolase levels have not correlated with neurodevelopmental outcomes.[7–9]

S100β was also first described in the 1970s and is a calcium-binding protein present in glial cells and involved in many cell functions. S100β has also been found in adipose tissue, muscle, marrow and thymus tissue, however,[10] making interpretation of elevations during cardiac surgery difficult because of the trauma to these tissues. Although S100β has been found to correlate with neurologic injury in adults, studies in children have thus far been inconclusive and further studies are needed.[11,12]

More recently, glial fibrillary acidic protein has been shown to be specific to the nervous system and has been found to be a specific predictor of neurologic outcome in traumatic brain injury,[13–15] cardiac arrest,[16] and stroke.[17] It was first described in the late 1960s and is the main intermediate filament protein that composes the major part of the cytoskeleton of astrocytes. Animal studies have shown that glial fibrillary acidic protein expression is upregulated in reactive gliosis after cerebral injury.[18] Using a high-sensitivity assay developed at Johns Hopkins, we have found circulating glial fibrillary acidic protein levels to be a significant predictor of (1) neurologic injury and survival in children on extracorporeal membrane oxygenation,[19] (2) abnormal magnetic resonance imaging and neurodevelopmental outcomes in neonates with birth-related hypoxic ischaemic encephalopathy,[20] and (3) periventricular white matter injury on 6-week head ultrasound in premature infants by the fourth day of life.[21] Although we have shown that glial fibrillary acidic protein is a promising biomarker of brain injury in paediatrics, no studies have been published investigating glial fibrillary acidic protein in children with heart disease.

The purpose of this pilot study was to explore the blood levels of glial fibrillary acidic protein and to analyse patient characteristics associated with elevation of glial fibrillary acidic protein in children undergoing cardiopulmonary bypass for repair of congenital heart disease.

Materials and methods

We performed a prospective, observational study of children undergoing cardiopulmonary bypass for repair of congenital heart disease at the Johns Hopkins Hospital and Texas Children's Hospital between October, 2010 and April, 2011. The study was approved by the Institutional Review Board at each institution. All neonates, infants, and children up to 21 years of age undergoing cardiopulmonary bypass were eligible. Exclusion criteria were repair of congenital heart disease without use of cardiopulmonary bypass.

After induction of anesthesia in the cardiac operating room and before commencing bypass, waste blood from the activated clotting time sample was collected as a baseline. Each syringe of waste blood from activated clotting time samples was collected throughout bypass. Once bypass was discontinued, the remaining syringes of waste blood from activated clotting time samples were collected as post-bypass samples. Each blood sample was centrifuged for 8 minutes at 3000 rpm and the plasma layer separated. Plasma was placed into cryotubes and stored at −80°C until assayed. Assays were run in a blinded, duplicate manner. All patients received standard of care during the peri-operative and recovery periods. Cardiopulmonary bypass was managed as per the routine of the surgical teams at each institution. At both institutions, goal haematocrit is generally 30% and blood gas management is pH-stat. The Johns Hopkins Hospital uses modified ultrafiltration at the end of bypass for fluid removal before decannulation. Texas Children's Hospital uses conventional ultrafiltration throughout the bypass period. Assay results were not available to the clinical team and had no impact on clinical decision making.

Glial fibrillary acidic protein was assayed using an electrochemiluminescent sandwich immunoassay as previously described.[19–22] Briefly, the assay had a detection range of 0.04–40.0 ng/ml. Our glial fibrillary acidic protein assay had a lower limit of detection of 0.011 ng/ml as defined by two standard

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deviations above the background of blank wells (n = 19 experiments) and the signal to noise ratio was 1.17 at 0.01 ng/ml (n = 19 experiments). The lower limit of quantification, defined as the lowest dilution with a calculated concentration ±20% of a known concentration, was 0.04 ng/ml (n = 3 experiments).

For analysis, the operative period was divided into four phases: pre-bypass, cooling on bypass, re-warming on bypass, and post-bypass. There were often multiple samples from each phase, and thus the results of each sample were averaged together for the respective phase. Chart reviews were performed to gather clinical data. Patient demographics were recorded including age, weight, sex, single ventricle anatomy, and confirmed chromosomal anomalies. Operative characteristics recorded included cardiopulmonary bypass time (≤120 minutes and >120 minutes), aortic cross-clamp time (≤60 minutes and >60 minutes), use of deep hypothermic circulatory arrest (present or absent); intra-operative ultrafiltration (yes or no), temperature nadir, near-infrared spectroscopy nadir, pressor score, and conversion to extracorporeal membrane oxygenation for failure to come off cardiopulmonary bypass. Post-operative data recorded included intensive care unit and hospital length of stay, complications – for example, cardiac arrest, seizures, intracranial hemorrhage, stroke, or cerebral oedema diagnosed by brain imaging, etc. – and radiologic reports of any imaging obtained during the inpatient stay.

Pressor score was calculated according to the formula developed by Gaies et al[23] with a modification to add phenylephrine to the calculation.

The primary outcome was change in glial fibrillary acidic protein concentrations during bypass.

Statistical analysis

We calculated summary statistics of all patient demographic and operative data and summarised the distribution of glial fibrillary acidic protein during the four phases of the operative period. We compared demographic data between the two clinical sites to determine whether there were differences in patient composition between sites. Continuous variables were compared using Wilcoxon rank-sum test. Categorical variables were compared using χ^2 test or Fisher's exact test wherever appropriate. To determine whether patient demographic or operative data were associated with elevated glial fibrillary acidic protein values post bypass, we performed longitudinal analyses using generalised estimating equations to control for multiple glial fibrillary acidic protein measures within a patient. We also conducted collinearity analyses to help guide any additional modelling. In exploratory analyses, one potential outlier was identified. In order to ensure that the potential outlier did not have undue influence on the results, we conducted a sensitivity analysis excluding the potential outlier. All analyses were conducted using STATA (STATA Intercooled, version 11.0; STATA Corporation, College Station, Texas, United States of America). Statistical significance was determined by a p-value <0.05 and, in linear modelling, a 95% confidence interval that did not cross zero.

Results

Intra-operative glial fibrillary acidic protein samples were collected from 91 consecutive patients: 62 patients at the Johns Hopkins Hospital and 29 patients at Texas Children's Hospital. Owing to an incomplete number of samples, six patients were excluded, leaving 85 patients for analysis. Patient demographics are shown in Table 1. Median patient age was 0.7 years and median patient weight was 7.14 kg. There was a male predominance in the study population. In all, 22% had single ventricle anatomy and 23.5% had confirmed chromosomal anomalies. The median cardiopulmonary bypass time was 126 minutes and median cross-clamp time was 70 minutes. Deep hypothermic circulatory arrest was used in 14 patients (16.5%) with a median deep hypothermic circulatory arrest time of 18 minutes. Regional cerebral perfusion was used in only seven patients. Temperature nadir was 28°C for the population with a median near-infrared spectroscopy nadir of 52%. There were three patients (3.5%) who required conversion to extracorporeal membrane oxygenation for failure to wean from bypass. The median intensive care unit length of stay was 4 days. The median hospital length of stay was 9 days. Analyses of the patient and operative characteristics by clinical site suggested differences in the patient populations between the two sites, including statistically significant differences in presence of confirmed chromosomal anomaly, length of cardiopulmonary bypass time, aortic cross-clamp time, and pressor score (Table 1).

Glial fibrillary acidic protein was assayed in 636 patient samples in duplicate with a range of 3–13 samples per patient depending upon the length of the surgical procedure. Assay performance was excellent with an inter-assay CV% of 3.2% and an inter-plate CV% of 2.3%. Figure 1 shows the distribution of glial fibrillary acidic protein concentrations during the four phases of bypass. Glial fibrillary acidic protein levels increased rapidly during bypass and peaked during re-warming. Median glial fibrillary acidic protein for pre-bypass was 0 ng/ml

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Table 1. Patient characteristics by clinical site, Johns Hopkins Hospital and Texas Children's Hospital, among children undergoing cardiopulmonary bypass for repair of congenital heart disease, October, 2010 to April, 2011 (n = 85).

Patient characteristics	Overall (n = 85)	Texas Hospital (n = 29)	Johns Hopkins Hospital (n = 56)	p-value
Age [median (range)] (years)	0.725 (0.01–17)	0.4 (0.02–17.2)	0.875 (0.01–17)	0.295
Weight [median (range)] (kg)	7.14 (2.2–86.5)	6.35 (2.7–73.3)	7.4 (2.2–86.5)	0.266
Gender [n (%)]				
Male	49 (60)	17 (63)	32 (59)	0.748
Female	32 (40)	10 (37)	22 (41)	
Single ventricle anatomy [n (%)]	18 (22)	6 (21)	12 (22)	0.934
Confirmed chromosomal anomaly [n (%)]	19 (23)	10 (36)	9 (17)	0.053
Cardiopulmonary bypass time [median (range)] (minutes)	126 (37–313)	154 (81–293)	119 (37–313)	0.005
Aortic cross-clamp time [median (range)] (minutes)	70 (9–195)	84 (35–185)	56 (9–195)	0.025
Deep hypothermic circulatory arrest [median (range)] (minutes)	18 (4–43)	20 (5–34)	11 (4–43)	0.841
Temperature nadir (°C)	28.05 (16.1–37)	27.8 (17.1–34.5)	28.45 (16.1–37)	0.275
NIRS nadir (%)	51.5 (24–78)	52.71 (33–71)	50.2 (24–78)	0.333
Pressor score	4.25 (0–30)	3.75 (0–12.85)	5 (0–30)	0.011
Conversion to ECMO [n (%)]	3 (4)	1 (4)	2 (4)	0.768
Length of ICU stay [median (range)] (days)	4 (2–141)	4 (2–78)	4 (2–141)	0.929
Length of hospital stay [median (range)] (days)	8.5 (3–279)	8 (4–106)	9 (3–279)	0.861

ECMO = extracorporeal membrane oxygenation; ICU = intensive care unit; NIRS = near-infrared spectroscopy

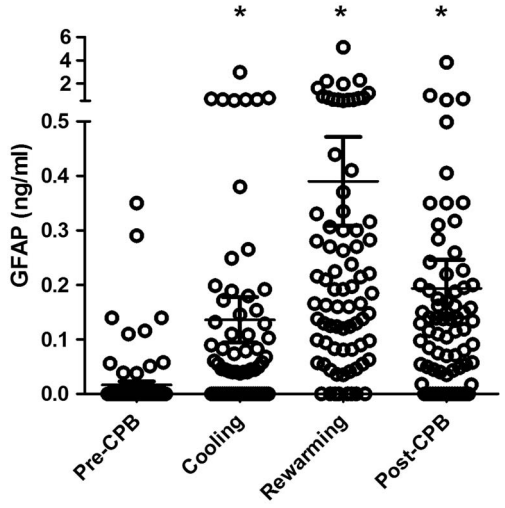

Figure 1.
*Distribution of individual GFAP values during the four phases of cardiopulmonary bypass. Bars are mean ± SEM. *p < 0.05 versus pre-cardiopulmonary bypass. GFAP = glial fibrillary acidic protein.*

Table 2. Comparison of median GFAP values in those with and without chromosomal anomalies by phase of surgery.

Chromosomal anomaly	GFAP median (range)	p-value
Pre-CPB		
No	0.01 (0–0.24)	0.26
Yes	0.01 (0–0.35)	
Cooling		
No	0.03 (0–0.68)	0.41
Yes	0.04 (0.01–2.98)	
Re-warming		
No	0.17 (0.02–2.29)	0.84
Yes	0.17 (0.03–5.12)	
Post-CPB		
No	0.11 (0.01–0.69)	0.38
Yes	0.13 (0.01–3.83)	

CPB = cardiopulmonary bypass; GFAP = glial fibrillary acidic protein

(range 0–0.35). Only nine out of 85 patients had a quantifiable level of glial fibrillary acidic protein before bypass. Median glial fibrillary acidic protein for the cooling phase was 0.039 (range 0–0.677). Median glial fibrillary acidic protein for the re-warming phase was 0.165 (range 0–2.285) and median glial fibrillary acidic protein during the post-bypass phase was 0.112 (range 0–0.974). In all, 80% of patients had a decline in glial fibrillary acidic protein during the post-bypass phase. Of note, although the surgical routines vary at the two participating institutions, there were no differences in distribution of glial fibrillary acidic protein between centres.

Ultrafiltrate was tested to determine whether glial fibrillary acidic protein is depleted from the circulation by ultrafiltration. We found the ultrafiltrate to have no detectable glial fibrillary acidic protein (n = 20 cases). Although we could not estimate volume of distribution for each patient, plasma total protein was assayed in pre-bypass and re-warming samples as a measure of circulating volume dilution (n = 49 cases). The median total protein concentration was not different between the two phases measured (12.9 versus 12.0 μg/μl, p = 0.06).

There was a high number of chromosomal anomalies present in the cohort. Genetic testing

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was done at the discretion of the care teams at each institution. Some chromosomal anomalies detected had uncertain clinical significance. Given the high incidence of anomalies, we performed an additional subgroup analysis comparing glial fibrillary acidic protein elevation in those with and without chromosomal anomalies for all four stages of surgery, as shown in Table 2. There was no significant difference in glial fibrillary acidic protein elevation between these two groups for all stages of surgery (p-values 0.26–0.84.)

Outcome data for the study group (Table 3) showed only one clinical seizure in one patient (1.2%) post-operatively. Greater than 96% survived to intensive care unit and hospital discharge.

To identify characteristics associated with glial fibrillary acidic protein elevation, we performed longitudinal bivariate analyses using generalised estimating equations and controlling for clinical site (Table 4). Patient age, length of bypass, aortic cross-clamp, and temperature nadir were significantly associated with elevations in glial fibrillary acidic protein. Figure 2 shows the rise in glial fibrillary acidic protein by age distribution. Figure 3 shows glial fibrillary acidic protein distribution compared with length of cardiopulmonary bypass.

Table 3. Post-operative characteristics.

Outcome	Overall (n = 85)
Seizures	1/85 (1.2%)
Survival to ICU discharge	83/85 (97.6%)
Survival to hospital discharge	82/85 (96.5%)

ICU = intensive care unit

Glial fibrillary acidic protein values were higher in patients with longer cardiopulmonary bypass times ($p < 0.0001$). Patient weight had a trend towards significance but did not achieve significance. Owing to collinearity between multiple variables, multivariate analysis was not conducted.

We looked more closely at two patients: the patient with clinical signs of neurologic injury and the patient with the highest glial fibrillary acidic protein values. The patient with clinical seizures was a 5-month-old female with tetralogy of Fallot and no other diagnoses. Her operative course was uneventful with cardiopulmonary bypass and cross-clamp times below the medians. Her near-infrared spectroscopy nadirs intra-operative were 52% on the left and 65% on the right. She was admitted to

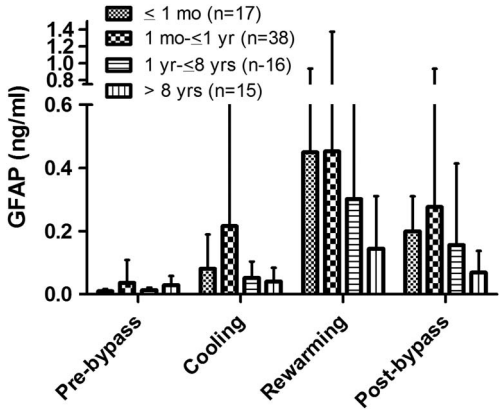

Figure 2.
GFAP values during cardiopulmonary bypass in different age groups. GFAP values are mean. GFAP = glial fibrillary acidic protein.

Table 4. Bivariate associations between patient characteristics and GFAP over time in longitudinal analyses controlling for multiple observations per individual and clinical site among children undergoing cardiopulmonary bypass for repair of congenital heart disease, Johns Hopkins Hospital and Texas Children's Hospital, October, 2010 to April, 2011 (n = 85).

Patient demographics and operative characteristics	Coefficient (95% confidence interval)	p-value
Age (years)	−0.008 (−0.015, −0.001)	0.031
Weight (kg)	−0.002 (−0.004, 0.0002)	0.072
Gender (female)	−0.057 (−0.130, 0.017)	0.130
Single ventricle anatomy, present	−0.027 (−0.114, 0.061)	0.549
Confirmed chromosomal anomaly	−0.004 (−0.090, 0.082)	0.933
Length of cardiopulmonary bypass time (>120 minutes)	0.080 (0.008, 0.152)	0.029
Aortic cross-clamp time (>60 minutes)	0.086 (0.001, 0.171)	0.047
Deep hypothermic circulatory arrest, presence	0.070 (−0.022, 0.163)	0.137
Temperature nadir	−0.007 (−0.014, −0.0003)	0.041
NIRS nadir	−0.002 (−0.005, 0.002)	0.350
Pressor score	0.003 (−0.004, 0.010)	0.450
Conversion to ECMO	−0.006 (−0.210, 0.197)	0.952
Length of ICU stay (days)	0.0002 (−0.002, 0.002)	0.835
Length of hospital stay (days)	−0.0002 (−0.001, 0.0007)	0.685

ECMO = extracorporeal membrane oxygenation; GFAP = glial fibrillary acidic protein; ICU = intensive care unit; NIRS = near-infrared spectroscopy

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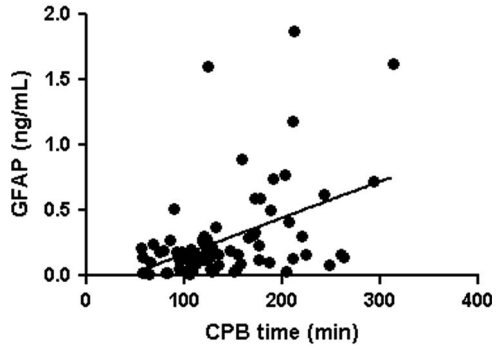

Figure 3.
Glial fibrillary acidic protein distribution during the re-warming phase compared with length of cardiopulmonary bypass.

the intensive care unit on a milrinone infusion only. She was noted to have elevated blood pressures with adequate pain control in the intensive care unit but did not require treatment for hypertension. She was discharged from the intensive care unit on post-operative day 2 and, on that day, experienced a tonic clonic seizure on the general inpatient floor. Head computed tomography was concerning for a right-sided subdural haemorrhage and magnetic resonance imaging confirmed a right-sided subdural haemorrhage with intraparenchymal blood products and evidence of emboli. Although most patients experienced a peak glial fibrillary acidic protein level during the re-warming phase with a decline noted during the post-bypass phase, this patient's glial fibrillary acidic protein levels showed a continued rise in glial fibrillary acidic protein during her operative course, with the peak occurring during the post-bypass phase.

The second patient had the highest elevations in glial fibrillary acidic protein in this study. She was a 3.5-month-old with a genetic diagnosis of translocation of chromosome 8 to 9. She had a perimembranous ventricular septal defect and underwent a patch closure. Her cardiopulmonary bypass time was at the median, but her cross-clamp time was above the median. Her right-sided near-infrared spectroscopy nadir was 52% intra-operative. Left-sided near-infrared spectroscopy was not recorded. She was admitted to the intensive care unit on milrinone and low-dose epinephrine infusions. Her pre-bypass glial fibrillary acidic protein was appropriately low, but her cooling glial fibrillary acidic protein rose to 2.98 ng/ml. Her glial fibrillary acidic protein peaked during re-warming at 5.12 ng/ml and dropped slightly to 3.83 ng/ml post bypass. These are the highest glial fibrillary acidic protein values recorded for the cooling, re-warming, and post-bypass phases in the study population. She had a pre-operative head computed tomography that showed no acute abnormalities, but there was no clinical indication for post-operative imaging so none was obtained. Her intensive care unit length of stay was prolonged at 17 days and hospital length of stay was 58 days.

Discussion

Our pilot data show that there are significant increases in glial fibrillary acidic protein during cardiopulmonary bypass for neonates and children undergoing surgical repair of congenital heart disease. Small volumes of blood were easily collected and assayed in this population. We were able to detect quantifiable increases of glial fibrillary acidic protein as early as the cooling phase of bypass. Previous studies in adults have studied glial fibrillary acidic protein as a biomarker over a time scale of days.[14,15,24,25] In our study, we demonstrate that glial fibrillary acidic protein levels increase early during cardiopulmonary bypass and has important implications for follow-up studies regarding monitoring for injury in a clinically relevant time period.

Although this was a pilot study, which did not correlate glial fibrillary acidic protein with imaging and neurologic exams, we noted significant trends during the phases of cardiopulmonary bypass. Glial fibrillary acidic protein significantly increased from baseline at all time points, but peaked in the majority of children during re-warming. However, there were a small number of patients with a profound elevation of glial fibrillary acidic protein throughout cardiopulmonary bypass that persisted even after decannulation. It is possible that these different patterns of glial fibrillary acidic protein elevation represent different patterns of injury, as has been demonstrated in adults with stroke.[17,24,25] In our patient population, only one patient had overt, clinically apparent brain injury, manifested as new-onset seizures with computed tomography and magnetic resonance imaging evidence of a subdural haemorrhage and embolic injury. This was a patient who had glial fibrillary acidic protein concentrations that peaked after decannulation.

In our study, cardiopulmonary bypass time and cross-clamp time, lower temperature nadir, and younger age were associated with significant increases in glial fibrillary acidic protein. There was also a trend towards significance for weight. It is interesting that younger patients had higher glial fibrillary acidic protein values, as glial fibrillary acidic protein is a white matter protein and these patients are known to be more vulnerable to white matter brain injury. It is also noteworthy that glial fibrillary acidic protein values were higher in patients with longer bypass times and more

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significant temperature changes. These factors need to be investigated further and correlated with other testing modalities.

It is unlikely that the elevations in glial fibrillary acidic protein levels are related solely to general anesthesia and its effect on the nervous system. McHarg et al[26] found that in >100 children undergoing diagnostic or interventional cardiac catheterisation with general anesthesia, only 6.7% experienced an increase in glial fibrillary acidic protein. The same glial fibrillary acidic protein assay was used by McHarg et al as is used in the current study.

No studies have been previously reported investigating glial fibrillary acidic protein in children undergoing cardiopulmonary bypass for heart disease. There is one study reported in adults undergoing coronary artery bypass grafting for coronary artery disease.[27] The mean age in this study was 62.7 years. A panel of biomarkers, including S100β, neuron-specific enolase, and glial fibrillary acidic protein, were measured. In that study, glial fibrillary acidic protein was below the lower detection limit in all samples measured. There are technical differences in antibody reagents and detection platforms in the report by Missler and our assay, which make these two studies difficult to compare. The assay used in Missler's study was developed while studying adult patients with acute severe head trauma.[28] The glial fibrillary acidic protein assay used in this study has been validated to be diagnostic of brain injury and outcome in children with other types of overt or subclinical brain injury.[19–22] In addition, assays for white matter injury such as glial fibrillary acidic protein may be more sensitive in children because of the increased risk of white matter injury.

Cardiopulmonary bypass is a unique environment for the measurement of biomarkers, given the potential dilutional effect of the bypass circuit and the use of ultrafiltration. Although the volume of distribution could not be determined in this study, plasma total protein was not different in pre-bypass and bypass states, providing some evidence that glial fibrillary acidic protein levels were not artefactually lower at the end of bypass because of dilution. In addition, we demonstrated that glial fibrillary acidic protein is not removed in the ultrafiltrate. Unlike S100β with a mass of 10.7 kDa, glial fibrillary acidic protein is substantially larger at 49 kDa, thereby decreasing loss across the hemofilter.

We have correlated glial fibrillary acidic protein elevations with neurologic injury and outcomes in other paediatric populations. Ennen et al studied neonates with hypoxic ischaemic encephalopathy treated with whole body cooling.[20] Glial fibrillary acidic protein samples were collected after birth and then daily for 7 days. Neonates without neurologic injury served as controls. They found that those with hypoxic ischaemic encephalopathy had significant elevations compared with controls on admission and on days 1, 3, and 4. Interestingly, 17.4% of the cooling patients had abrupt elevations of glial fibrillary acidic protein the day after cooling was discontinued and re-warming was initiated. The hypoxic ischaemic encephalopathy patients had follow-up magnetic resonance imaging before discharge and assessment of functional outcome. Those with consistently elevated glial fibrillary acidic protein levels had abnormal magnetic resonance imaging scans. Time to oral feeding was used to assess functional outcome. Those with abnormal magnetic resonance imaging scans required longer to reach full oral feeds or required gastrostomy tubes for feeding.

Bembea et al studied the association of glial fibrillary acidic protein levels with acute neurologic injury, neurologic outcome, and survival in children undergoing extracorporeal membrane oxygenation.[19] Glial fibrillary acidic protein samples were collected at 6, 12, and 24 hours after initiation of extracorporeal membrane oxygenation and then daily until discontinuation of extracorporeal membrane oxygenation. The median peak glial fibrillary acidic protein levels were significantly higher in children with acute neurologic injury diagnosed during the extracorporeal membrane oxygenation course, in children with poor versus good neurologic outcome as diagnosed by the Pediatric Cerebral Performance Category scale, and in non-survivors.

In ~10% of patients in this study population, baseline glial fibrillary acidic protein was greater than zero. The reasons for this are unclear. These patients represented various ages, including infants and adolescents. They had varied cardiac anatomies, including cyanotic, single ventricle lesions, and acyanotic, two ventricle lesions. In all, 30% of these patients did have documented chromosomal abnormalities. It is possible that the baseline detection of glial fibrillary acidic protein could represent pre-operative brain injury, as magnetic resonance imaging studies have demonstrated that ~30% of neonates have magnetic resonance imaging evidence of brain injury before surgical repair.[2,29,30] It is unknown at this point whether the presence of measureable glial fibrillary acidic protein at baseline indicates a worse long-term outcome.

Strengths of this study include a large number of patients from two different institutions. We found that glial fibrillary acidic protein is a generalisable biomarker, as the pattern of glial fibrillary acidic protein elevation over the course of bypass was

the same at two different clinical sites. We also collected glial fibrillary acidic protein samples in children of various ages to further characterise the glial fibrillary acidic protein response at different stages of central nervous system development. Limitations include the inability to correlate level or persistence of glial fibrillary acidic protein as an indicator of outcome after cardiopulmonary bypass, as imaging and long-term neurologic follow-up were not a component of this study. Another limitation is that the volume of distribution was not known for each patient, which could affect accurate quantification of glial fibrillary acidic protein or any biomarker. To assess for this, however, we did measure total protein and found no difference in circulating total protein between pre-bypass and re-warming phases.

Conclusions

Brain injury in children with congenital heart disease is common and can result in significant cognitive and motor abnormalities. A contributor to the inability to improve outcomes has been the lack of a means to identify to whom and when brain injury is occurring in the intra-operative and peri-operative period. In this regard, circulating biomarkers of brain injury hold great promise, and this study shows the possible application of neuronal biomarkers during cardiac surgery. We demonstrate in the present study that circulating blood levels of glial fibrillary acidic protein are detectable and increase significantly in children during cardiopulmonary bypass for repair of congenital heart disease. Age, length of bypass and aortic cross-clamp, and temperature nadir were associated with significant elevations in glial fibrillary acidic protein. These results provide the evidence for further studies to correlate glial fibrillary acidic protein levels with brain imaging and neurodevelopmental outcomes to further validate glial fibrillary acidic protein as a measure of brain injury in children with congenital heart disease.

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