Cardiopulmonary-Bypass Glial Fibrillary Acidic Protein Correlates With Neurocognitive Skills

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Background. **Neurocognitive deficits at school starting age may affect as many as 50% of children who underwent cardiac surgery for complex congenital heart disease (CHD). The aim of this study was to identify which phases of cardiopulmonary bypass (CPB) are associated with an increased risk of impaired neurodevelopmental skills in children with complex CHD. This was assessed by means of glial fibrillary acidic protein (GFAP) plasma levels during CPB for CHD surgery, as a marker of neurologic insult. We correlated GFAP levels with clinical parameters and neurodevelopmental outcome.**

Methods. **We studied 45 children undergoing surgery for complex CHD. We measured plasma GFAP levels by enzyme-linked immunosorbent assay at the following steps: anesthesia induction, CPB start, end of hypothermia, end of rewarming, and end of CPB. Neurologic assessment and Vineland Adaptive Behavior Scales (VABS-I) were administered to patients at least 18 months after surgery.**

Results. **GFAP was undetectable before surgery and it peaked at the end of hypothermia or rewarming. Multiple regression analyses showed that GFAP peak level and preoperative neurologic comorbidity were significant independent predictors of neurologic impairment, as showed by VABS-I communication domain intelligence quotient (IQ). Receiver operating characteristic curve showed that the model was highly significant.**

Conclusions. **Impaired neurodevelopment was associated with increase of GFAP plasma levels during cardiac surgery in infants. The identification of the neurologic high-risk phases of CPB run could support the application of new neuroprotective strategies for CHD repair.**

(Ann Thorac Surg 2018;106:792–8)

In the last decades, the exponential reduction of the mortality rate in children with congenital heart disease (CHD) has revealed that neurologic and neurodevelopmental complications are key issues in children with CHD, because they may affect functional outcome, peer-interaction, and overall quality of life. Neurocognitive deficits at school starting age affect nearly 50% of children who underwent surgery for complex CHD requiring cardiopulmonary bypass (CPB) and aortic cross clamping (ACC), with or without deep hypothermic circulatory arrest (DHCA) [1]. Altered brain development and newly acquired brain injuries during CHD surgery influence the overall neurologic outcome in a multifactorial, cumulative, and synergistic manner [2]. According to brain magnetic resonance imaging findings, brain injuries occur in 41% of infants preoperatively and in 30% of infants postoperatively [3]. Preoperative brain insults are mainly due to anomalies of cerebral blood flow during pregnancy, and thus they are not preventable. Therefore, research has focused on the potential onset of brain injury during and post cardiac surgery, trying to develop neuroprotective strategies to improve neurologic outcome.

To identify ongoing brain injuries, several biomarkers have been studied [4–8]. Among them, glial fibrillary acidic protein (GFAP), the main intermediate filament in

Accepted for publication March 29, 2018.

Presented at the Poster Session of the Fifty-fourth Annual Meeting of The Society of Thoracic Surgeons, Fort Lauderdale, FL, Jan 27-31, 2018.

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The Supplemental Material can be viewed in the online version of this article [https://doi.org/10.1016/j.athoracsur.2018.03.083] on http://www.annalsthoracicsurgery.org.

0003-4975/$36.00
https://doi.org/10.1016/j.athoracsur.2018.03.083

mature astrocytes, seems to fit the requirements of specificity, readiness of release, and ease of assaying, all required to identify an acute brain injury [9].

Generally, there is still a lack of data on assessment of the predictive role of such biomarkers. Several methods are available to evaluate neurologic adaptive functioning. This can reveal how individuals are able to apply cognitive skills to everyday tasks and requirements. In particular, this ability can be effectively evaluated in infancy by means of the Vineland Adaptive Behavior Scales (VABS-I) [10].

In this study, we analyzed plasma GFAP concentration during preset phases of CPB in patients undergoing surgery for CHD, to correlate plasma GFAP values to clinical parameters, neurological development, and VABS-I evaluation at follow-up.

Patients and Methods

Patients

This is a prospective, observational, single-center study in children with complex CHD undergoing a cardiac surgical procedure with CPB from 2014 to 2016. The study was approved by the institutional review board and by the ethics committee of the Padova University Hospital. Inclusion criteria were: children with complex CHD requiring elective cardiac surgery; CPB time greater than 60 minutes on hypothermia; ACC greater than 20 minutes, when performed; and written informed consent. All infants underwent a neurological evaluation and head ultrasound before surgery. Exclusion criteria were age greater than 3 years, previous heart surgery, hemodynamic instability, factor V less than 20%, creatinine clearance less than 30%, or chromosomal and neurological abnormalities before surgery. In addition, we excluded patients undergoing a reoperation.

We defined neurological risk time-interval (NRTI) as the timeframe during CPB in which the patient had selective regional cerebral perfusion (RCP) and/or DHCA, whenever performed, as previously reported [11].

For each study infant we collected birth weight, gestational age, sex, weight and age at the time of surgery, presence of preoperative cyanosis (defined as arterial oxygen saturation $< 90\%$), plasma arterial lactate before and during surgery, duration of CPB and of surgical intervention, core temperature, cerebral oxygen saturation, intensive care unit stay, hospital length of stay, survival, and neurological complications during the hospital stay.

Surgery and Sample Collection

Anesthesia and CPB management were described previously [11]. Briefly, after anesthesia induction and heparin administration, patients were cannulated and CPB was initiated with a hematic prime, to keep hematocrit between 25% and 30%. DHCA or selective RCP were applied according to surgeon choice; CPB flows were set according to body surface area, cardiac index, and temperature nadir. Cerebral regional oxygen saturation by near infrared spectroscopy (NIRS; INVOS, Somanetics, Troy, MI) was recorded every minute. A single NIRS probe was positioned on an area of non-hair-bearing scalp, near the right frontal hairline, to encompass both hemispheres. Blood gas analysis and metabolic parameters were measured at least every 20 minutes. At the end of surgery modified ultrafiltration was applied and all patients underwent rewarming to 36°C measured by rectal probe and then they were allowed to rewarm spontaneously in the intensive care unit.

Blood samples were collected in EDTA-containing tubes (1.5 mL) from the superior vena cava at anesthesia induction, CPB start, end of hypothermia, end of rewarming, and end of CPB before modified ultrafiltration.

Sample Analysis

Blood tubes were centrifuged at 1,400 x *g* for 10 minutes to obtain plasma. Plasma was divided in 150-μL aliquots and stored at −80°C until analysis; GFAP was measured using ELISA kit RD192072200R (BioVendor, Brno, Czech Republic).

Calculations

Plasma GFAP was expressed as concentration (ng/mL) at each study point and as the maximum GFAP concentration (GFAP Max) reached by each patient. Lastly, VABS-I scores for each domain were log(10)-transformed prior to the statistical analysis.

Neurologic and Neurodevelopmental Follow-Up

After hospital discharge, all infants received a cardiologic follow-up at least every 6 months. At 18 months after surgery, all study infants underwent neurologic and behavioral assessments. Neurologic evaluation included detailed medical and neurodevelopmental history, evaluation of muscular tone, strength, and position asymmetry, sensory assessment (tactile and vibration), osteotendinous reflexes, cerebellar and cranial nerve integrity, language skills, and head circumference. All neurological items were recorded as normal/not normal when appropriate. We defined neurologic comorbidity as the presence of an abnormal neurologic or neurodevelopmental finding not associated with the cardiac surgery, but with other clinical events or risk factors identified from the child's medical history.

Vineland Adaptive Behavior Scales

Adaptive functioning was assessed by VABS-I [10], a psychometrically validated parent interview administered by a trained psychological examiner who assesses adaptive behaviors at developmental levels from birth through adulthood. Several domains are evaluated, yielding index scores for socialization, communication, daily living, and motor skills (for children up to age 5 years). All index scores have an age-referenced mean of 100 and a SD of 15, where higher scores reflect better skills. Each domain includes several subdomains with developmentally sequenced items, starting with skills typically observed in infancy.

We considered intelligence quotient (IQ) values for each domain as normal when greater than 80, borderline

between 70 and 80, and impaired when IQ is less than 70 (−2 SD from the mean).

Statistical Analysis

Normal distribution was assessed by Shapiro-Wilk and Kolmogorov-Smirnov tests and group comparisons by univariable Kruskal-Wallis analysis of variance. Multivariable regression analysis was performed by the forced-entry method of the independent predictors and by stepwise-backward regression analysis. Continuous independent predictors were screened with a model of bivariate linear correlation (Pearson's p) against the logarithmic transformation of each VABS-I domain score. Predictors were selected based on a p value less than 0.1 at the univariate analysis or if considered clinically relevant. Multicollinearity was assessed with the variance inflator factor (<10), the tolerance statistic (>0.1), and the correlation matrix (<0.8). Durbin-Watson ($1 < \text{Resid} < 3$) test and Cook's distance (<1) were used for residues evaluation. Logistic regression was assessed with the same independent predictors tested by the forced-entry method, against the VABS-I score less than 70, as outcome. Receiver operating characteristic (ROC) analysis was calculated from the predicted probability of the logistic regression model. Statistical significance was set at p less than 0.05. We used SPSS 21.0 (IBM Corp, Armonk, NY) for the analysis.

We calculated that a sample size of 40 patients would detect a 64% standardized difference in GFAP measured before CPB and at the end of rewarming with a power of 80% and a two-sided alpha error of 0.05, based on previous plasma GFAP values in 32 infants with CHD. Taking into account a possible drop-out rate of 10%, we determined that 45 patients with CHD would have to be enrolled.

Results

Patients

We enrolled 45 pediatric patients undergoing surgery for CHD with CPB (alpha-stat strategy). Six patients presented with a main diagnosis of univentricular heart (UVH; 5 hypoplastic left heart syndrome, 1 double-outlet right ventricle), 24 patients had conal defects (9 transposition of the great arteries [TGA], 15 tetralogy of Fallot [TOF]), and 15 patients had septal defects (11 ventricular, 2 atrial, 1 truncus arteriosus and 1 cor triatriatum). Clinical and surgical characteristics are reported in Table 1. No neurologic injuries were reported before surgery and at hospital discharge. There were no deaths during surgery.

GFAP Analysis

Plasma concentrations of GFAP at the various CPB stages are reported in Table 2. They were very low or undetectable in all patients at anesthesia induction and they

Table 1. Baseline and Surgical Characteristics

Variable	All (n = 45)	Univentricular Heart (n = 6)	Conal Defect (n = 24)	Septal Defect (n = 15)
Baseline characteristics				
Age, months	3.0 (0.4–8.1)	1.0 (0.1–7.5)	4.9 (3.3–6.4)	4.4 (3.0–32)
Weight, kg	5.8 ± 3.7	3.8 ± 1.0	5.0 ± 2.4	7.8 ± 5.1
Sex, male/female	24/21	4/2	16/8	4/11
Term/preterm	40/5	5/1	21/3	14/1
SaO_2, %	91 ± 8	84 ± 10[a]	88 ± 7[a]	96 ± 6[b]
Lactate, mmol/L	1.2 ± 0.6	1.1 ± 0.4	1.4 ± 0.8	1.1 ± 0.5
Age at VABS-I, months	42 (28–50)	41 (21–47)	42 (29–51)	42 (27–50)
Neurological comorbidities	5/45	2/6	2/24	1/14
Surgical characteristics				
Surgery time, minutes	240 ± 60	291 ± 94	244 ± 48	211 ± 50
CPB time, minutes	137 ± 53	148 ± 57	152 ± 83[a]	111 ± 63[b]
ACC time, minutes	68 ± 29	33 ± 19[a]	83 ± 20[b]	55 ± 30[a]
Hypothermia time, minutes	62 ± 31	33 ± 27[a]	80 ± 28[b]	46 ± 21[a]
Rewarming rate, °C/min	0.3 ± 0.2	0.5 ± 0.4[a]	0.2 ± 0.1	0.2 ± 0.1[b]
Temperature nadir, °C	29.5 ± 4.2	24.7 ± 4.1[a]	29.6 ± 3.5	31.4 ± 4.1[b]
Minimum temperature time, minutes	54 ± 31	23 ± 15[a]	72 ± 28[a]	37 ± 21[b]
Basal cerebral saturation, %	57 ± 13	51 ± 14	57 ± 12	59 ± 13
CPB cerebral saturation, %	52 ± 10	49 ± 11	54 ± 12	51 ± 8
Lactate during CPB, mmol/L	2.6 ± 1.6	3.6 ± 1.8	2.4 ± 1.2	2.6 ± 2.0
NRTI, performed	9/45	5/6	3/24	1/15
NRTI, minutes	41 (22–56)	41 (35–85)	10 (4–51)	46

[a,b] Different superscripts within a row indicate significant differences among congenital heart diseases ($p < 0.05$).

Data are expressed as mean ± SD or median (interquartile range).

ACC = aortic cross-clamp; CPB = cardiopulmonary bypass; NRTI = neurological risk time interval; VABS-I = Vineland Adaptive Behavior Scales.

Table 2. GFAP Levels at Various CPB Phases

GFAP	All (n = 45)	Univentricular Heart (n = 6)	Conal Defect (n = 24)	Septal Defect (n = 15)
Pre-CPB, ng/mL	0.05 ± 0.08	0.05 ± 0.04	0.03 ± 0.02	0.06 ± 0.12
Start of CPB, ng/mL	0.10 ± 0.10	0.06 ± 0.10	0.08 ± 0.07	0.14 ± 0.14
End of hypothermia, ng/mL	1.25 ± 1.51	2.06 ± 2.98	1.50 ± 1.28[a]	0.56 ± 0.52[b]
End of rewarming, ng/mL	1.45 ± 1.11	1.76 ± 1.51	1.75 ± 1.16[a]	0.88 ± 0.60[b]
End of CPB, ng/mL	1.26 ± 1.07	1.01 ± 1.10	1.59 ± 1.24	0.89 ± 0.60
Maximum, ng/mL	1.71 ± 1.56	2.51 ± 2.83	1.98 ± 1.42[a]	0.98 ± 0.71[b]

[a,b] Different superscripts within a row indicate significant different median among congenital heart diseases ($p < 0.05$).

Data are expressed as mean ± SD.

CPB = cardiopulmonary bypass; GFAP = glial fibrillary acidic protein.

did not increase at the beginning of CPB. The highest mean GFAP concentration was recorded at the end of rewarming. Before and at the beginning of CPB, plasma GFAP levels were significantly lower than values measured at all other time points ($p < 0.0001$). GFAP Max was significantly different between septum and conal defects (Fig 1). The highest GFAP value was measured at the end of rewarming in a Norwood stage 1 procedure. We also measured GFAP in 16 age-matched operated non-CPB control patients and in 7 healthy adults. In all these patients GFAP was not detectable (<0.044 ng/mL). See Supplemental Material for details.

Regression Analysis

Median and interquartile ranges of VABS-I IQ domains are reported in Table 3. Communication IQ was predicted significantly by GFAP Max ($p = 0.009$), and by the presence of a neurological comorbidity (epilepsy, acquired microcephaly, stroke, etc) (Table 4). The other predictors of the model (which were nonsignificant), were cerebral oxygen saturation during CPB, age at VABS-I, presence of UVH, temperature nadir during CPB, occurrence of a period of NRTI during CPB.

Age at repair, CHD group, preoperative cyanosis, and surgical variables (mean hemoglobin concentration during CPB, hypothermia duration, CPB duration, rewarming rate, etc) were included in the stepwise model but subsequently discarded as nonsignificant. Other VABS-I domains used as dependent variables in the same regression generated nonsignificant models. Nomogram of the regression is shown in Figure 2.

ROC Analysis

The same predictors used for the multiple regression model were used in a logistic regression to test the ability of the model to predict a pathological value of VABS-I Communication IQ (<70). The predicted probabilities obtained were used to construct a ROC curve (Fig 3) that was highly significant ($p = 0.001$, Area = 0.9, 95% CI, 0.8–1.0, SE = 0.058).

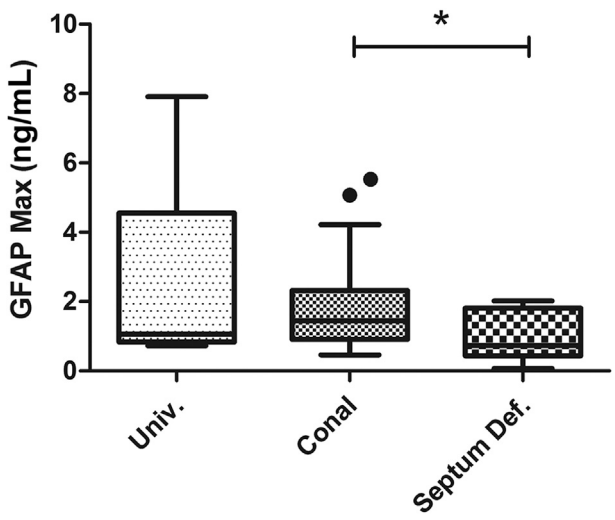

*Fig 1. Overall maximum glial fibrillary acidic protein concentrations (GFAP Max) during different cardiopulmonary bypass stages. GFAP Max is significantly different between the conal defect and septum defect groups (*significant difference, p < 0.05). (Septum Def. = septum defect; Univ. = univentricular.)*

Comment

To the best of our knowledge, this is the first report demonstrating the association of a plasma acute marker of brain injury (in particular white matter, namely GFAP) with the neurodevelopmental outcome assessed by VABS-I in children after CHD surgery.

We previously demonstrated that GFAP is not constitutively present at induction of anesthesia, and it increases only during surgery with CPB, ACC, and aortic arch reconstruction [11, 12]. We confirmed this observation in other complex CHD, where we found a nonsignificant increase of GFAP in patients with UVH and TOF/TGA compared with those with septum defects. We suggest that a combination of age, CHD, and surgery complexity forms a key cluster of variables influencing brain insult during CPB.

GFAP plasma level in UVH patients was not significantly different from the other 2 groups of CHD, even though there was a tendency toward a higher GFAP concentration in these patients. Notably, in this study the number of patients with UVH was limited, and we excluded a newborn with hypoplastic left heart syndrome who needed a second CPB run. Interestingly, in that

Table 3. Vineland Adaptive Behavior Scales Score

VABS-I IQ	All (n = 45)	Univentricular Heart (n = 6)	Conal Defect (n = 24)	Septal Defect (n = 15)
Communication	100 (83–122)	87 (59–114)	101 (85–123)	106 (81–152)
Daily living	82 (69–92)	78 (62–97)	82 (69–92)	83 (66–97)
Socialization	82 (67–90)	80 (62–103)	80 (69–88)	88 (61–94)
Motor skills	90 (71–109)	72 (61–94)	90 (75–106)	95 (60–114)

Data are expressed as median (interquartile range).

Univentricular heart children had the lowest median intelligence quotient (IQ) in the Communication domain. There were no significant differences among domains of congenital heart disease infants.

VABS-I = Vineland Adaptive Behavior Scales.

patient, GFAP at the end of the second hypothermia was similar to the other UVH patients. For the analysis, we clustered our study infants by the binary variable "Univentricular Yes/No" that did not yield an independent predictor of VABS-I scores. However, UVH patients are cyanotic, they are usually the youngest at the time of cardiac surgery, they have the longest operations, and they have the highest risk of neurologic dysfunction. Thus, we cannot exclude the possibility that a higher number of patients would have made UVH a significant risk factor by multiple regression analysis.

As demonstrated by our study, plasma GFAP, together with preoperative neurological comorbidity and age at neurodevelopmental evaluation, were significant predictors of outcome after surgery for CHD in infancy.

Severe neurological deficits, such as cerebral palsy, mental retardation, and epilepsy are uncommon events after surgery for CHD with CPB, representing 5% to 9% of recent cohorts, when UVH infants were not included. Nevertheless, previous research on children with CHD revealed an increased risk for neurodevelopmental delay and a wide spectrum of behavioral, cognitive, and motor abnormalities [13–15], which may deeply affect quality of life. Early markers of these sequelae are not yet available in the absence of overt brain damage on neuroimaging or perioperative severe EEG abnormalities.

In this prospective study, VABS-I scores were used as an exploratory test. We found them to be easy and cost-effective to administer, but less sensitive when compared with other methods assessing cognitive and behavioral outcomes. We used a conservative threshold of IQ less than 70 to characterize children with a clinically evident neurological deficit in order to avoid the risk of overestimating parents' reports. We found that median VABS-I scores of adaptive functioning were adequate or only mildly impaired. Patients had nonhomogeneous profiles with high intra-subject variability, however, suggesting some areas of vulnerability. The correlation between GFAP and communication skills suggests that this acute-phase marker may predict future development of communication impairment. Communication is a complex ability that comprises both linguistic skills (receptive and expressive) and social abilities. Communication and social skills provide critical tools for learning and for engaging in play and social relationships, and therefore they are important aspects of early development and later functioning [15–19] and seem to persist into adolescence [20]. Furthermore, persistent language impairment is a well-known risk factor for psychiatric and learning disorders later in life [21]. For these reasons, screening and evaluation of neurodevelopmental delay, along with regular follow-up, are essential in order to implement timely interventions and to improve patient's quality of life.

Table 4. Linear Model of Significant Predictors of VABS-I Communication Domain IQ

Communication IQ Log	B	SE of B	Beta	*p*
Constant	2.82 (2.06, 3.58)	0.38		<0.0001
GFAP Max	*−0.04 (−0.07, −0.01)*	*0.01*	*−0.415*	*0.009**
Neurological comorbidity	*−0.21 (−0.36, −0.05)*	*0.08*	*−0.410*	*0.01**

Model parameters were maximum glial fibrillary acidic protein concentration (GFAP Max), cerebral saturation during cardiopulmonary bypass (CPB), age at Vineland Adaptive Behavior Scales (VABS-I), univentricular heart (yes/no), presence of a neurological comorbidity, temperature nadir, occurrence of a period of neurological risk time-interval during CPB. Analysis of variance $p = 0.004$. Asterisks and italics = $p < 0.05$. See Supplemental Material for full regression table.

B = unstandardized coefficients of the regression; IQ = intelligence quotient; SE of B = standard error of B.

Results of this study suggest that GFAP is a potential early marker of future impairments in the communication domain. Interestingly, this correlation, while not significant in our model, was more obvious in older children (data not shown), as described in other studies that showed that neurological deficits emerged gradually and became evident over time. The association between older age at VABS-I test and worse outcome may reflect age-related differences in the sensitivity of the test measures. For instance, VABS-I scores may reflect less impairment at younger ages because expectations for functional skills are minimal in very young children, but deficits relative to peers will become more apparent over time.

Our findings allow for the early identification of children at risk for communication and/or social impairment. This is extremely important in order to provide timely interventions during a crucial phase of brain development, when changes in brain circuitry can still occur. As a promising early marker of neurodevelopmental risk, GFAP offers the unique opportunity to implement dedicated monitoring and interventions to

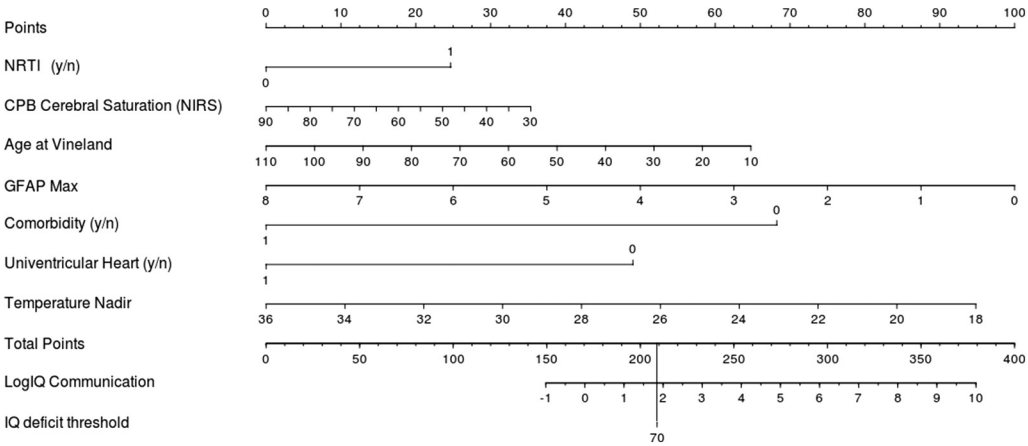

Fig 2. Nomogram of the multiple linear regression analysis. The value of each predictor variable corresponds to a point scale at the top. The sum of each individual predictor variable points corresponds to the total points and the probability of a Communication intelligence quotient (IQ) less than 70 shown at the bottom. For each predictor, the approximate 1st, 5th, 25th, 50th, 75th, 95th, and 99th percentiles are depicted. (CPB = cardiopulmonary bypass; GFAP Max = maximum glial fibrillary acidic protein concentration; NIRS = near infrared spectroscopy; NRTI = neurological risk time-interval; y/n = yes/no.)

subgroups of CHD children scheduled for cardiac surgery. Neuroimaging studies revealed reduced brain volume and incomplete closure of cerebral opercula in fetuses with severe CHD, suggesting delayed fetal brain development [22]. The operculum comprises an area of the brain that includes receptive and expressive language. Abnormalities in this area have been associated with communication impairment [23]. Thus, selective vulnerability of specific brain structures might contribute to a higher risk of developmental impairment in specific brain functions in CHD children.

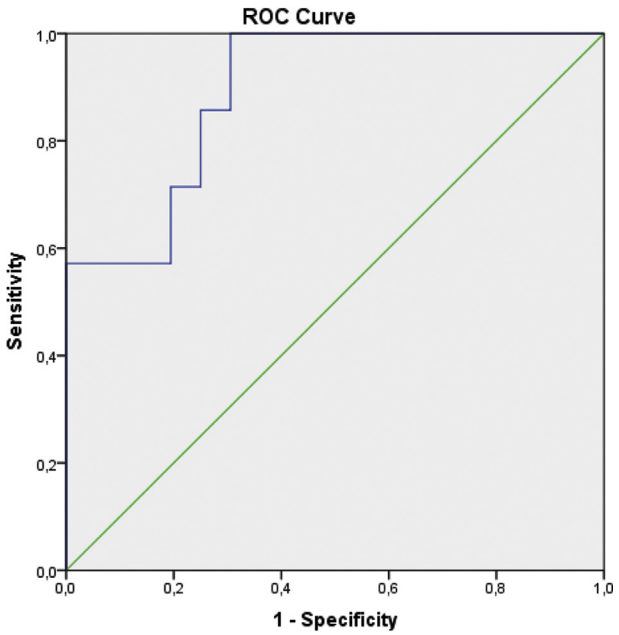

Fig 3. Receiver operating characteristic (ROC) curve of suspected pathological Communication intelligence quotient (IQ) (<70). Predictors were maximum glial fibrillary acidic protein concentration (GFAP Max), cerebral saturation during cardiopulmonary bypass (CPB), age at Vineland Adaptive Behavior Scales (VABS-I), univentricular heart (yes/no), presence of a neurological comorbidity, minimum temperature reached during CPB, and occurrence of a neurological risk time-interval period during CPB (p = 0.001, Area = 0.9, 95% confidence interval = 0.8–1.0, SE = 0.058). See Supplemental Material for full regression table.

Finally, GFAP could be an interesting marker to evaluate perioperative interventions of neuroprotection in children with CHD. Moreover, hypothermia–rewarming seems to be the period most susceptible to neurological risk, given the higher plasma GFAP levels, and thus more detailed investigations into the optimal hypothermia and rewarming protocols are warranted [24].

Study limitations include a lack of neuroimaging workup pre-surgery and post-surgery, and the use of an outcome test (VABS-I) with a relatively low sensitivity, that could have covered a primitive language deficit that we measured as a low IQ.

In conclusion, in children undergoing surgery for CHD, GFAP plasma levels peak during CPB, and independently predict the adverse neurological outcome in the communication domain measured as VABS-I scores.

GFAP seems to be a promising marker of brain injury, originating from a cerebral region (white matter) highly involved in the brain microstructure network responsible for the execution of complex tasks. Further studies are needed to correlate this acute marker with more accurate neurodevelopmental assessments.

This study was supported by the GPYIPR grant, CARIPARO Foundation 2013 (LV) and from the Just Foundation grant 2015 (PC). Funding sources were not involved in any part of the study.

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