HHS Public Access

Author manuscript

Ann Neurol. Author manuscript; available in PMC 2022 August 01.

Published in final edited form as:

Ann Neurol. 2021 August ; 90(2): 217–226. doi:10.1002/ana.26133.

Longitudinal CSF Iron Pathway Proteins in Posthemorrhagic Hydrocephalus: Associations with Ventricle Size and Neurodevelopmental Outcomes

Jennifer M. Strahle, MD[1], **Kelly B. Mahaney, MD**[2], **Diego M. Morales, MS**[1], **Chandana Buddhala, PhD**[1], **Chevis N. Shannon, DrPH, MPH, MBA**[3], **John C. Wellons III, MD, MSPH**[3], **Abhaya V. Kulkarni, MD, PhD**[4], **Hailey Jensen, MStat**[5], **Ron W. Reeder, PhD**[5], **Richard Holubkov, PhD**[5], **Jay K. Riva-Cambrin, MD, MSc**[6], **William E. Whitehead, MD**[7], **Curtis J. Rozzelle, MD**[8], **Mandeep Tamber, MD, PhD**[9], **Ian F. Pollack, MD**[10], **Robert P. Naftel, MD**[3], **John R.W. Kestle, MD, MSc**[11], **David D. Limbrick Jr., MD, PhD**[1], **Hydrocephalus Clinical Research Network**

[1]Department of Neurosurgery, Washington University St. Louis, St. Louis, MO

[2]Department of Neurosurgery, Stanford University, Palo Alto, CA

[3]Department of Neurosurgery, Vanderbilt University Medical Center, Nashville, TN

[4]Department of Neurosurgery, University of Toronto, Toronto, ON, Canada

[5]Data Coordinating Center, University of Utah, Salt Lake City, UT

[6]Department of Clinical Neurosciences, University of Calgary, Calgary, AB, Canada

[7]Department of Neurosurgery, Baylor College of Medicine, Houston, TX

[8]Department of Neurosurgery, University of Alabama – Birmingham, Birmingham, AL

[9]Department of Surgery, University of British Columbia, Vancouver, BC, Canada

[10]Department of Neurosurgery, University of Pittsburgh Medical Center, Pittsburgh, PA

[11]Department of Neurosurgery, University of Utah, Salt Lake City, UT

Abstract

Objective.—Iron has been implicated in the pathogenesis of brain injury and hydrocephalus after preterm germinal matrix hemorrhage-intraventricular hemorrhage, however it is unknown how external or endogenous intraventricular clearance of iron pathway proteins affect outcome in this group.

Corresponding Author: Jennifer M. Strahle, MD, Associate Professor of Neurosurgery, Orthopedic Surgery and Pediatrics, Washington University in St. Louis, St. Louis Children's Hospital, One Children's Place, St. Louis, MO 63110, Phone: 314-454-2816, Fax: 314-454-2818, strahlej@wustl.edu.

Author Contributions:

JMS, KBM, DLL contributed to the conception and design of the study; JMS, KBM, DMM, CB, HJ, RWR, RH, CNS, JCW, AVK, JKR, WEW, CJR, MT, IFP, RPN, JRK, DDL contributed to the acquisition and analysis/interpretation of the data; JMS, KBM, DMM, CNS, JCW, AVK, JKR, WEW, CJR, MT, IFP, RPN, JRK, DDL3 contributed to drafting and reviewing a significant portion of the manuscript. All authors approved the final version of the manuscript.

Potential Conflicts of Interest:

None to report.

Methods.—This prospective multicenter cohort included patients with posthemorrhagic hydrocephalus (PHH) who underwent (1) temporary and permanent cerebrospinal fluid (CSF) diversion and (2) Bayley Scales of Infant Development-III testing around 2 years of age. CSF proteins in the iron handling pathway were analyzed longitudinally and compared to ventricle size and neurodevelopmental outcomes.

Results.—Thirty-seven patients met inclusion criteria with a median estimated gestational age at birth of 25 weeks; 65% were boys. Ventricular CSF levels of hemoglobin, iron, total bilirubin, and ferritin decreased between temporary and permanent CSF diversion with no change in CSF levels of ceruloplasmin, transferrin, haptoglobin, and hepcidin. There was an increase in CSF hemopexin during this interval. Larger ventricle size at permanent CSF diversion was associated with elevated CSF ferritin ($p = 0.015$) and decreased CSF hemopexin ($p = 0.007$). CSF levels of proteins at temporary CSF diversion were not associated with outcome, however, higher CSF transferrin at permanent CSF diversion was associated with improved cognitive outcome ($p = 0.015$). Importantly, longitudinal change in CSF iron pathway pro-teins, ferritin (decrease), and transferrin (increase) were associated with improved cognitive ($p = 0.04$) and motor ($p = 0.03$) scores and improved cognitive ($p = 0.04$), language ($p = 0.035$), and motor ($p = 0.008$) scores, respectively.

Interpretation.—Longitudinal changes in CSF transferrin (increase) and ferritin (decrease) are associated with improved neurodevelopmental outcomes in neonatal PHH, with implications for understanding pathogenesis of poor outcomes in PHH.

Introduction

Germinal matrix hemorrhage-intraventricular hemorrhage (GMH-IVH) is a significant cause of morbidity and mortality in preterm infants. Thirty percent of those with high grade GMH-IVH develop posthemorrhagic hydrocephalus (PHH),[1] which is responsible for the worst neurodevelopmental outcomes in patients with preterm brain injury.[2] PHH requires lifelong treatment beginning in the weeks after hemorrhage, first with temporary and later permanent cerebrospinal fluid (CSF) diversion, which is associated with failure rates of 40%[3]. Despite treatment for hydrocephalus, infants with PHH have overlapping debilitating neurodevelopmental outcomes with 85% experiencing cognitive deficits and 70% with motor deficits[4]. Importantly, there is a critical window between the occurrence of GMH-IVH and permanent CSF diversion where 1) the brain undergoes a period of significant neurodevelopment and 2) there is clearance and resolution of intracranial blood products associated with GMH-IVH. In 70% of patients after high grade IVH (grades III and IV) the response to the initial GMH-IVH and subsequent clearance of blood does *not* result in hydrocephalus[1]. Similarly, within patients who develop PHH, there is a range of neurodevelopmental outcomes [2].

GMH-IVH results in the release of red blood cell breakdown products (hemoglobin, iron, bilirubin) which have been implicated in the pathophysiology of PHH, while other blood components such as thrombin and fibrin have been associated with down-stream inflammation[5,6]. We and others have shown hemoglobin and iron mediated pathogenesis of both neonatal and adult Posthemorrhagic hydrocephalus in animal models [7–15]. Our group recently reported that lumbar CSF levels of hemoglobin shortly after GMH-IVH in preterm

infants predicted the later development of PHH, further supporting our preclinical studies[16]. In addition, elevated CSF hemoglobin, ferritin and bilirubin levels were also associated with larger ventricle size. While these iron pathway proteins have been implicated in the pathophysiology of PHH and ventricle size, their longitudinal profiles and relationship to neurodevelopmental outcome is not known. We hypothesize that specific iron pathway proteins interact with the brain ventricles (CSF, ventricular ependyma, subependymal/subventricular zone and choroid plexus) to alter neurodevelopment and that longitudinal CSF levels of these proteins may impact long-term outcome and ependymal development/ventricle size.

Therefore, the aim of this study evaluating a multi-institution prospectively recruited cohort of patients with PHH is to (1) determine the longitudinal profile of key iron pathway proteins after GMH-IVH in patients treated with temporary and then permanent CSF diversion, and (2) determine the association of these proteins with ventricle size and neurodevelopmental outcomes. Understanding the relationship of these proteins to ventricle size and outcome will help guide specific interventions to interrupt the pathogenesis of PHH and brain injury in neonates with GMH-IVH.

Methods

Research subjects

Research subjects were recruited from 7 different centers of the Hydrocephalus Clinical Research Network (HCRN), including Vanderbilt University/Monroe Carell Jr. Children's Hospital, University of Alabama-Birmingham/Children's of Alabama, University of Utah/Primary Children's Hospital, University of Toronto/The Hospital for Sick Children, Baylor College of Medicine/Texas Children's Hospital, University of Pittsburgh/UPMC Children's Hospital of Pittsburgh, and Washington University/St. Louis Children's Hospital. Each individual site maintained their own institution-specific Institutional Review Board approval for the study; written consent was obtained from the parents/guardians of the children who participated in the study. Washington University housed the CSF for the entire study and was solely responsible for the analysis. Individual site neurosurgeons and coordinators were responsible for screening and enrolling eligible participants. Inclusion criteria included preterm neonates (≤ 34 weeks pre-menstrual age (PMA)), < 1500 grams at birth weight, Papile's grade III or IV IVH[17], Frontal Occipital Horn Ratio (FOHR) ≥0.50, and a >72 hour life expectancy. This study followed the standardized HCRN Shunting Outcomes in PostHemorrhagic Hydrocephalus (SOPHH) parameters for PHH diagnosis and treatment[18]. For this study, patients meeting the above criteria with sufficient CSF for analysis and who underwent formal neurodevelopmental testing were ultimately recruited from 4/7 HCRN enrollment sites (Vanderbilt University/Monroe Carell Jr. Children's Hospital, University of Alabama-Birmingham/Children's of Alabama, University of Utah/Primary Children's Hospital, and Washington University/St. Louis Children's Hospital).

FOHR measurements

Cranial ultrasound scans closest to PMA of the CSF sample (median of 2 days for temporary and permanent procedure) were digitized and measurements of bi-frontal horn width (A), bi-

occipital horn width (B) along with interparietal diameter (C) were determined. Estimations of ventricular size were calculated using the frontal/occipital horn radio [FOHR = (A+B)/2C][19].

CSF Acquisition

CSF was collected in the operating room following a stan-dardized protocol, either at the time of the temporizing procedure (reservoir [n = 18], subgaleal shunt [n = 13], or hybrid [n = 1]), and/or at the time of the permanent procedure (shunt [n = 21]; endoscopic third ventriculostomy [ETV], [n = 3]; ETV-choroid plexus cauterization [n = 11]). CSF was collected from the ventricular catheter and placed into polypropylene microcentrifuge tubes labeled with HCRN codes. The sample was then transported on ice to a −80°C freezer for storage until shipment time. Specimens were shipped in dry ice, every 4–6 months, to Washington University's Tissue Procurement Core. Samples remained at −80°C until they were slowly thawed for experimental analysis.

CSF Assays

Total CSF protein measurements were estimated using the Pierce Bicinchoninic Acid proteins assay (Thermo Scientific, Waltham, MA) according to the manufacturer's protocol as previously described[16]. Bovine serum albumin standards and CSF samples were pipetted into duplicate wells (R&D systems; Minneapolis, MN); working reagent then added and the entire plate incubated at 37°C for 30 minutes. Plates were then cooled and absorbance levels in each well were measured at 562 nm on a Versamax microplate reader (Molecular Devices; Sunnyvale, CA). Estimated total CSF proteins were extrapolated using a four-parameter logistic standard curve.

Commercially available sandwich ELISA assays were used to measure the concentrations of iron metabolism markers (Total Iron, Hemoglobin, Total Bilirubin, Ceruloplasmin, Ferritin, Transferrin, Haptoglobin, Hemopexin, and Hepcidin). Assays were all run in accordance to the manufacturer's protocol with CSF samples and protein standards placed in duplicate into the plate wells. As previously described, assays were piloted to determine the appropriate [16]CSF dilutions. ELISA kit manufacturer details and dilutions used for each assay are listed in Supplementary Table 1. CSF dilutions were optimized based on the optical density measurements that fell in the middle of the range of standard curve of the pilot assays. Individual protein levels were determined using a four-parameter logistic standard curve as detailed by the manufacturer, except for total iron and total protein, where a linear standard curve was used.

Neurodevelopmental Testing

Each site had a trained psychometrician perform the Bayley Scales of Infant Development-III testing at 15–30 months corrected age. The Bayley III is a standard, validated testing paradigm to assess neurobehavioral development (cognition, language, and motor) in infants and toddlers [20]. The cognitive subtest assesses sensorimotor development, object exploration, manipulation, relatedness, memory and concept formation. The language subtest assesses receptive and expressive language development, and the motor subtest assesses the fine and gross motor function. These individual subtests were then combined

and a composite score for the three main categories were derived and used to compare performance versus age-matched, typically developing children (composite score equal to 100).

Statistical Analysis

Continuous variables were summarized as median and interquartile range while categorical variables were summarized with counts and percentages. Cohort demographics were summarized. Wilcoxon signed-rank test was used to compare the change in CSF antigen values from temporizing to permanent procedure (Table 2). Multivariable linear regression models were created to assess the relationship of the change in CSF antigen values (from temporizing and permanent procedure) with Bayley III scores, adjusting for gestational age at birth and IVH grade (III, IV). Additional multivariable linear regression models were developed to assess the relationship between CSF antigen values and FOHR at temporizing procedure and permanent procedure (Table 3). Statistical analysis was performed using SAS 9.4 (SAS Institute; Cary, NC). All p-values were based on testing with a two-sided alternative and considered significant if $p<0.05$. No adjustment was made for multiple comparisons, and all results should be considered exploratory.

Results

Subject Characteristics

A total of 37 subjects were included in our analysis (Table 1). The median PMA at birth was 25 weeks and the median birthweight was 800 grams. Sixty five percent of subjects were male; 54% were White, and 38% were African American. Thirty-eight percent presented with grade III IVH and 62% with grade IV IVH. Thirty percent of subjects presented with cardiovascular complex chronic conditions (cardiomyopathy, conduction disorder, dysrhythmias, heart and great vessel malformations), while none had any renal, gastrointestinal or congenital/genetic defect. Median PMA at the time of CSF sample acquisition corresponded with time of temporary and permanent CSF diversion, and was 31 (mean, 31) and 41 (mean, 46) weeks, respectively. Median occipitofrontal circumference was 28 cm at time of temporizing procedure and median FOHR was 0.68. The median time between temporary and permanent CSF diversion was 11 weeks (range = 7-16 weeks). FOHR was 0.64 at time of permanent procedure. All subjects underwent the Bayley III neurodevelopmental testing at a median of 23 months corrected age with a median cognitive composite score of 60 (range = 55 – 75), language composite score of 65 (range = 53 – 80), and motor composite score of 52 (range = 46 – 71.5).

CSF Iron Pathway Markers: Temporary vs. Permanent CSF Diversion

CSF levels of hemoglobin, iron, ferritin, total bilirubin and total protein decreased significantly between time of temporary CSF diversion and permanent CSF diversion (Table 2). There was no change in CSF levels of ceruloplasmin, transferrin, haptoglobin, or hepcidin between time points. The heme scavenger, hemopexin, was the only marker with a significant increase in CSF levels between treatment time points (4.3 vs. 16.1 mg/ml, $p=0.018$) in this study.

FOHR and CSF Iron Pathway Markers

As we previously showed a significant relationship between lumbar puncture CSF levels of hemoglobin, ferritin and bilirubin and ventricle size in IVH and PHH, we evaluated ventricular CSF levels of these proteins at temporary and permanent CSF diversion. The median rate of progression of ventricular enlargement from birth until temporary CSF diversion (FOHR at temporary CSF diversion/days since birth) is 0.021 [Q1 = 0.016, Q3 = 0.030].. There was no association between CSF levels of any of the iron pathway markers analyzed and FOHR at the time of temporary CSF diversion. However, at the time of permanent CSF diversion, higher CSF levels of ferritin were associated with larger FOHR (p=0.015) (Fig 1) and higher CSF hemopexin levels were associated with smaller FOHR (p=0.007) (Table 3, Fig 2).

CSF markers of Neurodevelopmental Outcome

After controlling for PMA at birth and IVH grade, CSF levels of iron metabolism markers at time of temporary CSF diversion were not associated with cognitive, motor or language composite scores of the Bayley's examination at 2 years of age. At the time of permanent CSF diversion, higher CSF levels of transferrin were significantly associated with improved cognitive composite scores of the Bayley III examination (Fig 3), 2.17 (95% confidence interval [CI] = 0.47–3.87, p = 0.015). No other CSF iron metabolism proteins at time of permanent procedure were associated with Bayley III scores.

Longitudinal CSF response to IVH and Neurodevelopmental Outcome

We analyzed the change in levels of CSF markers (Table 2) between the time of temporary and permanent CSF diversion in order to assess if clearance of iron pathway proteins or response to hemorrhage by iron handling proteins was associated with long term neurodevelopmental outcomes. After controlling for PMA at birth and IVH grade, all proteins were evaluated for the association of longitudinal change with neurodevelopmental outcome. We found that a larger decrease in ventricular CSF ferritin from the time of temporary to permanent CSF diversion was associated with improved cognitive (3.33, 95% CI = −6.55, −0.12, p = 0.043) and motor (−4.45, 95% CI = −8.42, −0.48, p = 0.03) composite scores. In this same model controlling for PMA at birth and IVH grade, an increase in CSF transferrin between temporary and permanent CSF diversion was significantly associated with improved Bayley III composite cognitive (p=0.04), language (p=0.035) and motor (p=0.008) scores (Fig 3). Finally, as we found a significant relationship between ferritin and FOHR at the time of permanent procedure, in addition to PMA at birth and IVH grade, we also controlled for change in FOHR from temporizing to permanent procedure and found that the significant relationship between change in CSF ferritin and cognitive (p=0.04) and motor (p=0.028) outcome remained.

Discussion

This is the first study to directly evaluate and compare red blood cell breakdown and iron handling in patients undergoing early treatment for PHH, during a critical period of brain development. We show that there are significant decreases in CSF levels of the iron pathway proteins, hemoglobin, iron, total bilirubin, and ferritin with a significant

increase in CSF levels of the heme scavenger, hemopexin between temporary and permanent CSF diversion. We show that ventricle size is associated with CSF ferritin (increase) and hemopexin (decrease). Finally, longitudinal changes in CSF transferrin (increase) and ferritin (decrease) were associated with improved neurodevelopmental outcomes. These findings have implications for direct interaction of these proteins with the ventricular system including the ependyma, choroid plexus, and subependymal regions (subventricular zone, hippocampus and additional brain resions subjacent to the epedyma) in the setting of IVH to result in hydrocephalus and alter neurodevelopment.

Ferritin is an intracellular protein which stores iron in the ferric (3+) state while also functioning as a ferroxidase reducing ferrous (2+) iron to ferric (3+) iron, preventing ferrous iron from participating in the Fenton reaction with H2O2, which produces a free radical. Ferritin also functions as an acute phase reactant and is upregulated in the presence of infection, stress and chronic disease states. We show here that a larger decrease in ferritin levels between temporary and permanent CSF diversion is associated with improved cognitive and motor outcomes. Finally, higher CSF ferritin levels at time of permanent CSF diversion were associated with larger ventricle size. The mechanisms underlying modulation of ventricular CSF ferritin may involve a direct response to elevated iron (and thereby blood products) after hemorrhage as we have previously shown that peripheral treatment with iron chelation decreases ventricle size in a rat model of IVH[7]. However, CSF ferritin may also be acting as an acute phase reactant in response to inflammation, and this deserves further study.

We previously showed in CSF obtained via lumbar puncture in preterm infants that elevated CSF ferritin levels were associated not only with high-grade IVH and PHH, but with early ventriculomegaly. Animal models of IVH and PHH have shown both periventricular and hippocampal ferritin localization (3, 5, 6) which is consistent with autopsy studies of PHH[21]. The germinal matrix and subventricular zone, located immediately subjacent to the ventricle, actively contribute to neuro- and glio-genesis during the critical developmental time period between temporary and permanent CSF diversion. There may be direct effects of local iron neurotoxicity and subsequent ferritin levels or from inflammatory-mediated pathways which contribute to worse cognitive and motor outcomes. Further study into the exact role of ventricular CSF ferritin and the relationship to ventricle size and neurodevelopmental outcomes is needed, particularly in light of long-term data from the DRIFT trial where those treated with CSF irrigation and fibrinolysis had a significant improvement in long-term cognitive outcomes, but no differences in rates of shunt placement[22,23].

We found a strong relationship between an increase in ventricular CSF transferrin levels between temporary and permanent CSF diversion and improved outcomes in all components of the Bayley III examination (cognition, language, and motor). Transferrin is a protein that binds ferric iron, and whereas most transferrin is produced by the liver, there is significant endogenous transferrin production in the brain by the choroid plexus and oligodendrocytes, with more transferrin production from the choroid plexus by weight than the liver[24]. The exact role of transferrin in the brain is not known. Unlike iron regulatory protein 1, iron regulatory protein 2 and divalent metal transporter 1, which are expressed in high levels from birth in the ventricular ependyma, the transferrin receptor is absent at birth until

it is expressed at high levels at postnatal day 15[25,26]. Transferrin is likely to function in distributing iron through the brain interstitium, particularly in the developing brain, with cellular uptake independent of the transferrin receptor[26], however it may have a role independent from iron[27]. Transferrin is also important in the myelination and functioning of oligodendrocytes and has been shown to promote myelinogenesis along with IGF-1 in a myelin deficient rat model[28]. With respect to CSF transferrin, after intraventricular injection in a rat model, transferrin was present in the periventricular regions as well as the anterior thalamic and medial habenular nuclei and regions with synaptic communication to these areas[26]. In a separate study, transferrin was found to be present in the choroid plexus and ependyma without detectable iron or ferritin and suggests these periventricular regions may be a regulatory site of CSF transferrin levels[29]. Similar to our findings, CSF transferrin levels increased after shunt placement for normal pressure hydrocephalus and were associated with cognitive recovery[30]. As transferrin is normally secreted by the choroid plexus, higher CSF levels, as seen in association with improved outcomes in our study, may indicate a return to normal function of the choroid plexus, and could be a baseline marker of less severe brain injury. Alternatively, the significance of CSF transferrin levels may directly relate to iron handling at the CSF-choroid plexus and CSF-ependymal surfaces. Further studies are needed to more clearly define the role of transferrin in choroid plexus function, transferrin's role in iron distribution within the brain and neurocognitive outcomes.

In this cohort, we found a significant increase between the time of temporary and permanent CSF diversion in CSF levels of the heme scavenger, hemopexin, which binds met-heme and is then taken up by the CD91/LRP-1 cell surface receptor. Hemopexin was the only CSF marker that was increased between time points and CSF levels at time of permanent CSF diversion inversely correlated with ventricle size. CSF hemopexin has been studied in CNS diffuse B cell lymphoma [31] and degenerative disk disease [32] where in both cases it was elevated in the disease state. In a mouse intracerebral hemorrhage (ICH) model, increased endogenous brain hemopexin resulted in improved outcomes[33]. Hemopexin expression in one study was limited to ventricular ependymal cells and to a lesser extent the hippocampus[34] and may play an important role in CSF and brain heme levels. Our finding of the inverse relationship between CSF hemopexin levels and ventricle size could be related to retained function of ependymal cells in the setting of more normal ventricular morphology, and possibly *function* in the setting of response to IVH, although this deserves further study.

Limitations.

Our study was limited to patients who ultimately underwent permanent CSF diversion and therefore does not capture patients who were initially treated for PHH and never went on to require permanent CSF diversion or patients who did not participate in Bayley III testing. The patients in our study therefore may vary from those who were excluded and our results may not be generalizable beyond this specific group. Ventricular tapping protocols vary between centers and treating neurosurgeons and therefore, we were not able to control for the amount of CSF that was drained between temporary and permanent CSF diversion. Given our small sample size we were unable to control for age at time of either procedure which may be an important factor in outcome. In addition, our sample size is small and

given our multiple statistical analyses there is the potential for false discovery. Our findings need to be validated in a larger cohort of infants. The CSF samples used for this study were collected prospectively under the same protocol by four of seven enrolling HCRN centers and stored in a single biobank (Washington University in St. Louis), however, given the multicenter nature of the study there may have been slight differences in the way the samples were handled which may have contributed to variability in the results. Finally we were able to include patients from four of the seven enrolling sites due to limited CSF availability for patients recruited from three of the sites.

Conclusions

This is the first study to show the longitudinal profiles of iron pathway proteins in the CSF of preterm infants treated for PHH. We show that larger ventricle size is associated with increased CSF ferritin and decreased CSF hemopexin levels. Furthermore, longitudinal changes in CSF transferrin (increase) and ferritin (decrease) are associated with improved neurodevelopmental outcomes. The direct interaction of these ventricular CSF proteins with the ependymal surface lining the ventricles, subependymal/subventricular zone and choroid plexus deserves further study, where hemopexin, transferrin and ferritin handling in the brain ventricles in response to IVH is a potentially modifiable outcome determinant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to acknowledge the patients and families who generously participated in this study and the members of the individual HCRN clinical teams who participated in their care (Supplementary Online Table 2).

Sources of funding for this study: This project was supported by the Doris Duke Fund to Retain Clinical Scientists (JMS), NIH R01 NS110793 (JMS), K23 NS075151-01A1 (DL), Neurosurgeon Research Career Development Program (JMS), the Gerber Foundation Ref. 1692-3638 (C.N.S.), NIH/NINDS 1RC1NS068943, private philanthropy, and the Hydrocephalus Association. No industry funding was used during the completion of this study. None of the sponsors participated in design and conduct of this study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of this manuscript.

Sources of funding for the HCRN: The HCRN is thankful for the following sources of funding: National Institute of Neurological Disorders and Stroke (NINDS grant nos. 1U01NS107486-01A1 and 1RC1NS068943-01), Patient Centered Outcome Research Institute (PCORI grant no. CER-1403-13857), The Gerber Foundation (reference no. 1692-3638), private philanthropy and the Hydrocephalus Association.

Reference:

1. Christian EA, Jin DL, Attenello F, et al. Trends in hospitalization of preterm infants with intraventricular hemorrhage and hydrocephalus in the United States, 2000–2010. J Neurosurg Pediatrics 2016;17(3):260 269.
2. Strahle JM, Triplett RL, Alexopoulos D, et al. Impaired hippocampal development and outcomes in very preterm infants with perinatal brain injury. Neuroimage Clin 2019;22(Pediatr. Res. 55 2004):101787.
3. Riva-Cambrin J, Kestle JRW, Holubkov R, et al. Risk factors for shunt malfunction in pediatric hydrocephalus: a multicenter prospective cohort study. J Neurosurg Pediatrics 2016;17(4):382–390.

4. Adams-Chapman I, Hansen NI, Stoll BJ, et al. Neurodevelopmental Outcome of Extremely Low Birth Weight Infants With Posthemorrhagic Hydrocephalus Requiring Shunt Insertion. Pediatrics 2008;121(5):e1167 e1177. [PubMed: 18390958]
5. Garton T, Hua Y, Xiang J, et al. Challenges for intraventricular hemorrhage research and emerging therapeutic targets. Expert Opin Ther Tar 2017;00(00):1 12.
6. Davalos D, Ryu JK, Merlini M, et al. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nat Commun 2012;3(1):1227. [PubMed: 23187627]
7. Garton TP, He Y, Garton HJL, et al. Hemoglobin-induced neuronal degeneration in the hippocampus after neonatal intraventricular hemorrhage. Brain Res 2016;1635:86 94. [PubMed: 26772987]
8. Garton T, Keep RF, Wilkinson DA, et al. Intraventricular Hemorrhage: the Role of Blood Components in Secondary Injury and Hydrocephalus. Transl Stroke Res 2016;7(6):447 451. [PubMed: 27358176]
9. Strahle JM, Garton T, Bazzi AA, et al. Role of Hemoglobin and Iron in Hydrocephalus After Neonatal Intraventricular Hemorrhage. Neurosurgery 2014;75(6):696 705-discussion 706. [PubMed: 25121790]
10. Chen Z, Chen Z, Gao C, et al. Role of iron in brain injury after intraventricular hemorrhage. Stroke 2011;42(2):465 470. [PubMed: 21164132]
11. Gao C, Du H, Hua Y, et al. Role of red blood cell lysis and iron in hydrocephalus after intraventricular hemorrhage. J Cereb Blood Flow Metabolism 2014;34(6):1070 1075.
12. Chen Q, Tang J, Tan L, et al. Intracerebral Hematoma Contributes to Hydrocephalus After Intraventricular Hemorrhage via Aggravating Iron Accumulation. Stroke 2015;46(10):2902 2908. [PubMed: 26265129]
13. Meng H, Li F, Hu R, et al. Deferoxamine alleviates chronic hydrocephalus after intraventricular hemorrhage through iron chelation and Wnt1/Wnt3a inhibition. Brain Res 2015;1602:44 52. [PubMed: 25152462]
14. Gram M, Sveinsdottir S, Cinthio M, et al. Extracellular hemoglobin - mediator of inflammation and cell death in the choroid plexus following preterm intraventricular hemorrhage. J Neuroinflamm 2014;11(1):200.
15. Savman K, Nilsson UA, Blennow M, et al. Non-protein-bound iron is elevated in cerebrospinal fluid from preterm infants with posthemorrhagic ventricular dilatation. Pediatr Res 2001;49(2):208 212. [PubMed: 11158515]
16. Mahaney KB, Buddhala C, Paturu M, et al. Intraventricular Hemorrhage Clearance in Human Neonatal Cerebrospinal Fluid: Associations With Hydrocephalus. Stroke 2020;51(6):1712–1719. [PubMed: 32397930]
17. Papile L-A, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: A study of infants with birth weights less than 1,500 gm. J Pediatrics 1978;92(4):529–534.
18. Wellons JC, Shannon CN, Holubkov R, et al. Shunting outcomes in posthemorrhagic hydrocephalus: results of a Hydrocephalus Clinical Research Network prospective cohort study. J Neurosurg Pediatrics 2017;20(1):1 11.
19. O'Hayon BB, Drake JM, Ossip MG, et al. Frontal and occipital horn ratio: a linear estimate of ventricular size for multiple imaging modalities in pediatric hydrocephalus. karger.com [date unknown];
20. Bayley N. Bayley Scales of Infant and Toddler Development. 2006.
21. Fukumizu M, Takashima S, Becker LE. Glial reaction in periventricular areas of the brainstem in fetal and neonatal posthemorrhagic hydrocephalus and congenital hydrocephalus. Brain Dev 1996;18(1):40 45. [PubMed: 8907341]
22. Luyt K, Jary S, Lea C, et al. Ten-year follow-up of a randomised trial of drainage, irrigation and fibrinolytic therapy (DRIFT) in infants with post-haemorrhagic ventricular dilatation. Health Technol Asses 2019;23(4):1–116.
23. Whitelaw A, Jary S, Kmita G, et al. Randomized trial of drainage, irrigation and fibrinolytic therapy for premature infants with posthemorrhagic ventricular dilatation: developmental outcome at 2 years. Pediatrics 2010;125(4):e852 8. [PubMed: 20211949]

24. Aldred AR, Dickson PW, Marley PD, Schreiber G. Distribution of transferrin synthesis in brain and other tissues in the rat. J Biological Chem 1987;262(11):5293–7.
25. Siddappa AJM, Rao RB, Wobken JD, et al. Developmental changes in the expression of iron regulatory proteins and iron transport proteins in the perinatal rat brain. J Neurosci Res 2002;68(6):761 775. [PubMed: 12111837]
26. Moos T, Morgan EH. Kinetics and distribution of [59Fe-125I]transferrin injected into the ventricular system of the rat. Brain Research 1998;790(1–2):115 128. [PubMed: 9593852]
27. Papanastasiou DA, Vayenas DV, Vassilopoulos A, Repanti M. Concentration of iron and distribution of iron and transferrin after experimental iron overload in rat tissues in vivo Study of the liver, the spleen, the central nervous system and other organs. Pathology - Res Pract 2000;196(1):47–54.
28. Espinosa-Jeffrey A, Kumar S, Zhao PM, et al. Transferrin Regulates Transcription of the MBP Gene and Its Action Synergizes with IGF-1 to Enhance Myelinogenesis in the md Rat. Dev Neurosci-basel 2002;24(2–3):227–241.
29. Benkovic SA, Connor JR. Ferritin, transferrin, and iron in selected regions of the adult and aged rat brain. J Comp Neurol 1993;338(1):97 113. [PubMed: 8300902]
30. Murakami Y, Matsumoto Y, Hoshi K, et al. Rapid increase of 'brain-type' transferrin in cerebrospinal fluid after shunt surgery for idiopathic normal pressure hydrocephalus: a prognosis marker for cognitive recovery. J Biochem 2018;164(3):205–213. [PubMed: 29701803]
31. Zheng W, Song Y, Xie Y, et al. Cerebrospinal Fluid Proteins Identification Facilitates the Differential Diagnosis of Central Nervous System Diffuse Large B Cell Lymphoma. J Cancer 2017;8(17):3631–3640. [PubMed: 29151949]
32. Lim TKY, Anderson KM, Hari P, et al. Evidence for a Role of Nerve Injury in Painful Intervertebral Disc Degeneration: A Cross-Sectional Proteomic Analysis of Human Cerebrospinal Fluid. J Pain 2017;18(10):1253–1269. [PubMed: 28652204]
33. Leclerc JL, Santiago-Moreno J, Dang A, et al. Increased brain hemopexin levels improve outcomes after intracerebral hemorrhage. J Cereb Blood Flow Metabolism 2016;38(6):1032–1046.
34. Morello N, Tonoli E, Logrand F, et al. Haemopexin affects iron distribution and ferritin expression in mouse brain. J Cell Mol Med 2009;13(10):4192–4204. [PubMed: 19120692]

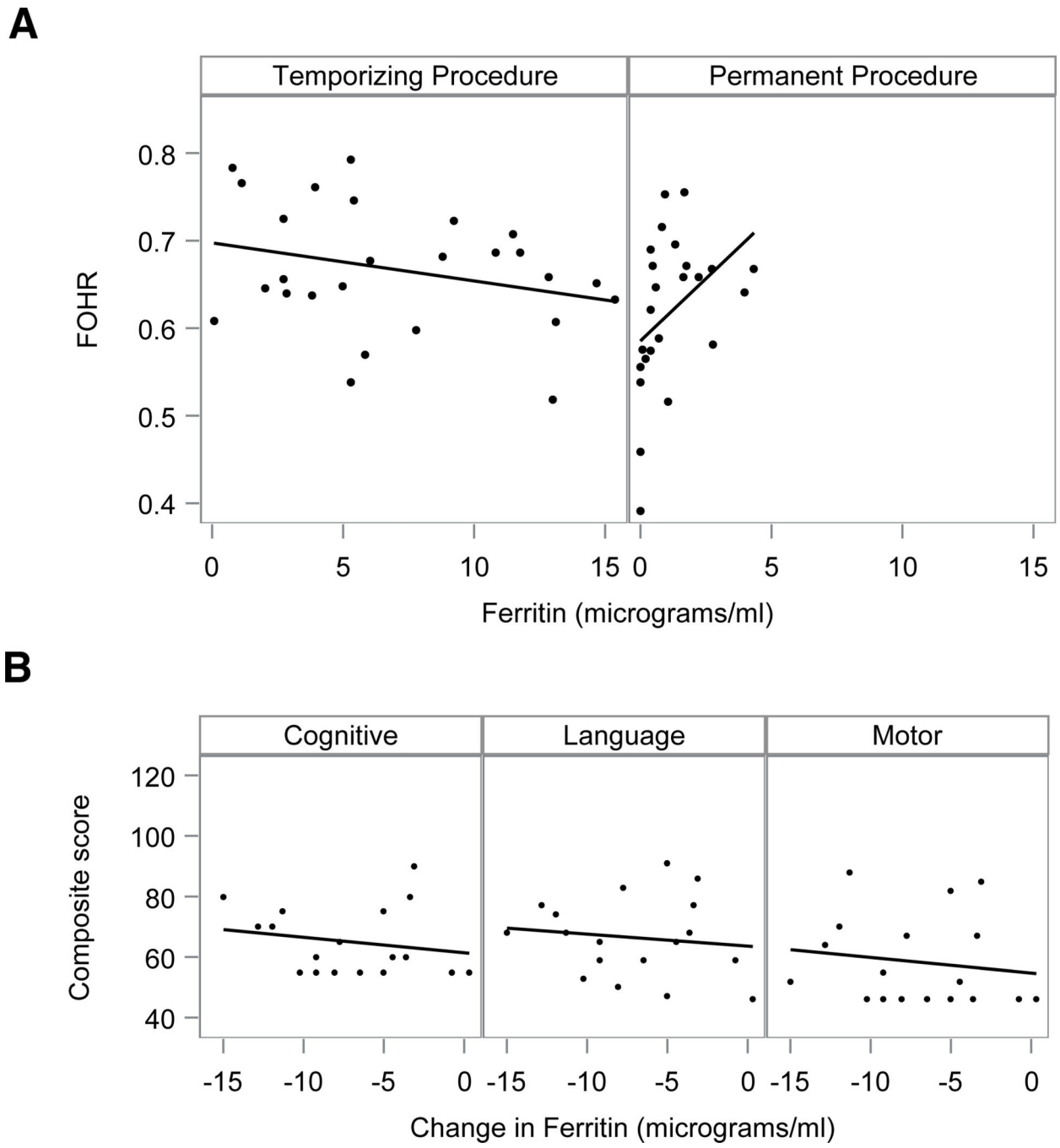

Figure 1.

Top panel (A). Relationship between FOHR and ventricular CSF ferritin levels at temporizing and permanent procedure. At the time of permanent CSF diversion, higher CSF levels of ferritin were associated with larger FOHR (p=0.015) when controlling for PMA at birth and IVH grade. Graph lines represent best fit between FOHR and ferritin at each time point not controlling for other factors. Bottom Panel (B). Relationship between Bayley III composite scores and change in ventricular CSF ferritin levels from temporizing to permanent procedure. Ventricular CSF ferritin levels at time of permanent CSF diversion

are associated with Bayley III composite cognitive (p=0.04) and motor (p=0.028) when controlling for change in FOHR from temporizing to permanent procedure. Graph lines represent best fit between composite scores and change in ferritin levels not controlling for other factors.

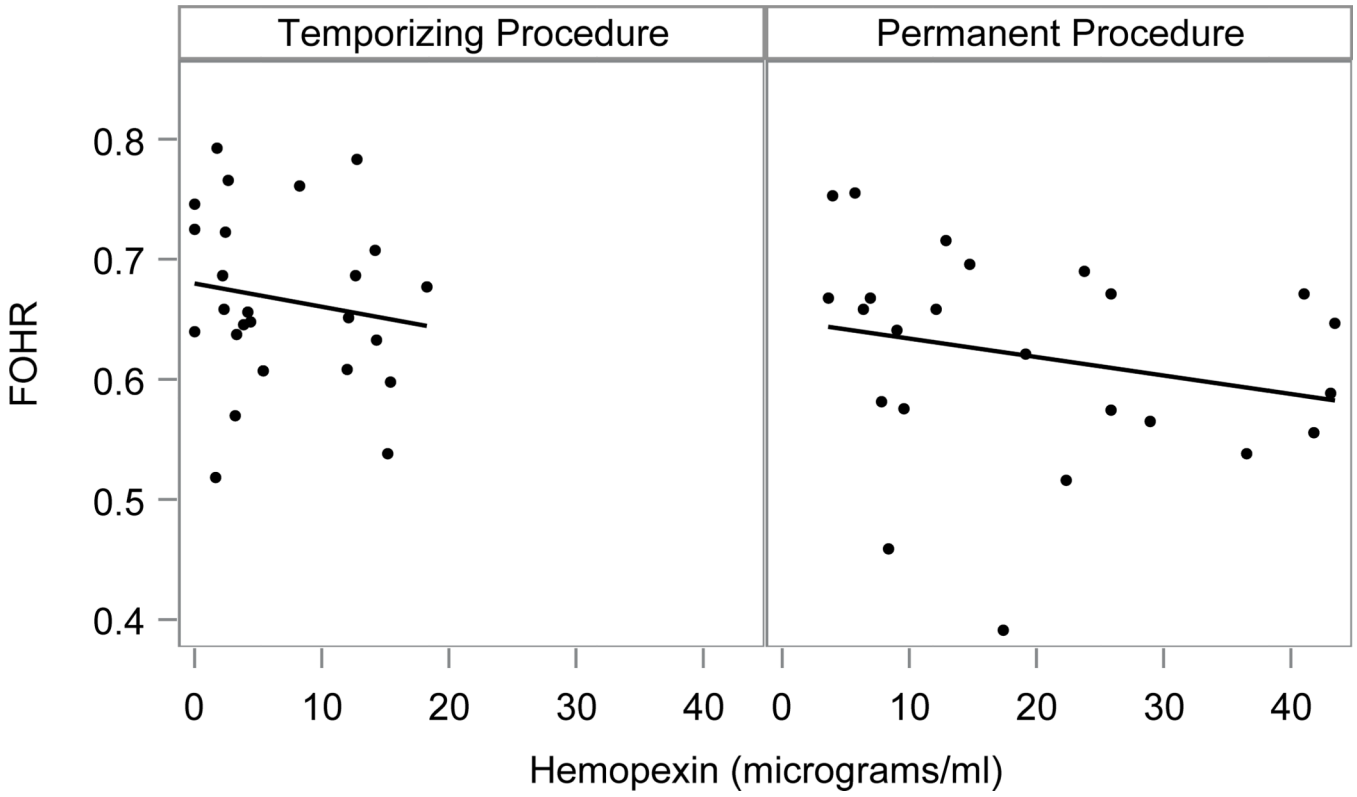

Figure 2.

Relationship between frontal occipital horn ratio (FOHR) and ventricular cerebrospinal fluid hemopexin levels at temporizing and permanent procedure. At the time of permanent CSF diversion, higher CSF levels of hemopexin levels were associated with smaller FOHR (p=0.007) when controlling for PMA at birth and IVH grade. Graph lines represent best fit between FOHR and hemopexin at each time point not controlling for other factors.

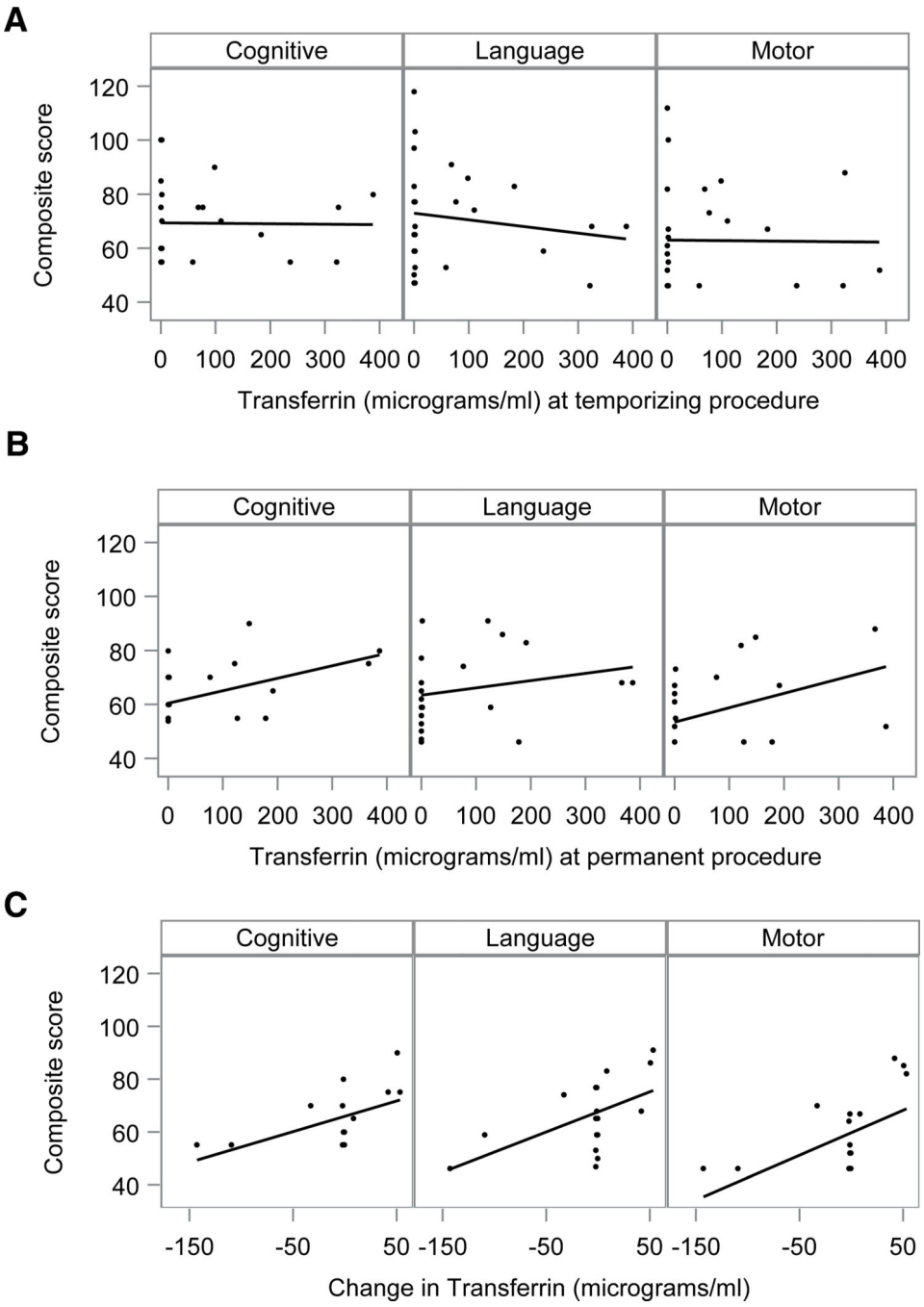

Figure 3.

Longitudinal changes in CSF transferrin are associated with outcome in posthemorrhagic hydrocephalus. Top panel (A). No relationship between ventricular CSF transferrin levels vs. Bayley III composite cognitive, language and motor scores at time of temporary CSF diversion. Middle Panel (B). Ventricular CSF transferrin levels at time of permanent CSF diversion are associated with Bayley III composite cognitive scores. Bottom Panel (C). Change in ventricular CSF transferrin levels between temporary and permanent CSF diversion is significantly associated with improved Bayley III composite cognitive (p=0.04),

language (p=0.035) and motor (p=0.008) scores. Statistical model controls for PMA at birth and IVH grade. Graph lines represent best fit between composite scores and transferrin not controlling for other factors.

Table 1:

Patient Demographics

	Overall (N = 37)
Gestational age at temporizing procedure (weeks) [1]	**30.6 [28.6, 32.6]**
Gestational age at permanent procedure (weeks) [2]	**41.0 [38.4, 48.3]**
Gestational age at birth (weeks)	**25.0 [24.0, 27.0]**
Birth weight (kg)	**0.8 [0.7, 1.1]**
Male	**24 (64.9%)**
Race	
Asian	1 (2.7%)
Black or African American	14 (37.8%)
Native Hawaiian or Other Pacific Islander	1 (2.7%)
White	20 (54.1%)
Unknown or Not Reported	1 (2.7%)
Hispanic or Latino	4 (10.8%)
IVH grade (highest)	
III	14 (37.8%)
IV	23 (62.2%)
Occipitofrontal circumference at enrollment (cm)	27.6 [26.0, 31.0]
Split sagittal suture	34 (91.9%)
Bulging fontanelle	30 (81.1%)
Frontal and occipital horn ratio at temporizing procedure [3]	0.68 [0.64, 0.74]
Frontal and occipital horn ratio at permanent procedure [4]	0.64 [0.54, 0.67]
Bayley Scales of Infant Development (BSID) at 2 years of age	
Cognitive composite score	60.0 [55.0, 75.0]
Language composite score	65.0 [53.0, 80.0]
Motor composite score	52.0 [46.0, 71.5]

Continuous variables are summarized as Median [Q1, Q3].

[1] Missing on 5 patients.

[2] Missing on 4 patients.

[3] Missing on 5 patients.

[4] Missing on 2 patients.

Table 2:

CSF iron metabolism at temporizing procedure and permanent procedure

	Temporizing procedure	Permanent procedure	Change from temporizing to permanent procedure	P-value[1]
Total Protein (mg/dl)	393.6 [197.5, 649.1]	271.3 [114.5, 314.5]	−153.9 [−341.9, −53.1]	0.003[1]
Total Iron (ng/μL)	1.7 [0.8, 6.7]	0.1 [0.1, 0.7]	−1.6 [−6.1, −0.8]	<0.001[1]
Hemoglobin (μg/ml)	29.4 [4.1, 127.8]	1.4 [0.2, 7.5]	−42.9 [−127.5, −5.7]	<0.001[1]
Ceruloplasmin (μg/ml)	2.0 [0.9, 3.0]	2.3 [1.3, 4.4]	−0.4 [−1.2, 1.0]	0.702[1]
Ferritin (μg/ml)	5.6 [2.9, 11.5]	0.8 [0.3, 1.7]	−7.1 [−10.2, −3.6]	<0.001[1]
Transferrin (μg/ml)	2.3 [0.0, 97.9]	0.7 [0.0, 124.2]	−1.1 [−2.3, 0.0]	0.421[1]
Total Bilirubin (mg/dl)	1.1 [1.0, 3.4]	0.9 [0.6, 1.0]	−0.3 [−2.1, −0.1]	<0.001[1]
Haptoglobin (μg/ml)	0.0 [0.0, 1.8]	0.6 [0.2, 1.6]	0.3 [0.0, 1.2]	0.196[1]
Hemopexin (μg/ml)	4.3 [2.3, 12.8]	16.1 [8.1, 27.4]	8.9 [−0.7, 19.1]	0.018[1]
Hepcidin (ng/ml)	3.7 [0.0, 33.8]	4.0 [0.0, 29.6]	−0.4 [−7.4, 1.6]	0.097[1]

[1]Wilcoxon signed-rank test

Table 3:

CSF iron metabolism markers in predicting frontal occipital horn ratio at temporary and permanent CSF diversion.

	CSF Protein at temporizing procedure				CSF Protein at permanent procedure			
	N	**Median [Q1, Q3]**	**Effect on FOHR[1] (95% CI)**	**P-value**	**N**	**Median [Q1, Q3]**	**Effect on FOHR[1] (95% CI)**	**P-value**
Total Protein (mg/dl)	31	393.6 [197.5, 649.1]	0.001 (−0.011, 0.013)	0.876	25	271.3 [114.5, 314.5]	0.008 (−0.003, 0.019)	0.153
Total Iron (ng/μL)	31	1.7 [0.8, 6.7]	−0.005 (−0.014, 0.004)	0.258	25	0.1 [0.1, 0.7]	0.019 (−0.011, 0.049)	0.210
Hemoglobin (μg/**ml**)	26	29.4 [4.1, 127.8]	−0.012 (−0.030, 0.006)	0.180	24	1.4 [0.2, 7.5]	−0.003 (−0.013, 0.008)	0.608
Ceruloplasmin (μg/**ml**)	26	2.0 [0.9, 3.0]	0.001 (−0.004, 0.005)	0.792	24	2.3 [1.3, 4.4]	0.002 (−0.018, 0.022)	0.853
Ferritin (μg/**ml**)	26	5.6 [2.9, 11.5]	−0.022 (−0.044, 0.001)	0.056	24	0.8 [0.3, 1.7]	0.021 (0.005, 0.037)	0.015
Transferrin (μg/**ml**)	26	2.3 [0.0, 97.9]	−0.004 (−0.015, 0.007)	0.464	24	0.7 [0.0, 124.2]	−0.003 (−0.021, 0.015)	0.715
Total Bilirubin (mg/dl)	31	1.1 [1.0, 3.4]	−0.001 (−0.003, 0.002)	0.524	25	0.9 [0.6, 1.0]	−0.007 (−0.035, 0.021)	0.616
Haptoglobin (μg/**ml**)	31	0.0 [0.0, 1.8]	−0.001 (−0.004, 0.003)	0.596	25	0.6 [0.2, 1.6]	0.003 (−0.003, 0.008)	0.344
Hemopexin (μg/**ml**)	26	4.3 [2.3, 12.8]	0.001 (−0.006, 0.008)	0.750	24	16.1 [8.1, 27.4]	−0.030 (−0.052, −0.009)	0.007
Hepcidin (ng/ml)	31	3.7 [0.0, 33.8]	−0.012 (−0.031, 0.007)	0.197	25	4.0 [0.0, 29.6]	0.022 (−0.016, 0.059)	0.241

[1]Reported effect estimates are for an increase of one half of the interquartile range, (Q3 - Q1)/2, in the CSF iron metabolism marker.Results are based on multivariable model(s) adjusting for Gestational age at birth (weeks), IVH grade.