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Hepatic Encephalopathy in Children with Acute Liver Failure – Utility of Serum Neuromarkers

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Abstract

Background: Pediatric acute liver failure (PALF) is a public health burden, often requiring prolonged hospitalization and liver transplantation. Hepatic encephalopathy (HE) is a complication of PALF with limited diagnostic tools to predict outcomes. Serum neurological markers (neuron specific enolase (NSE), S100 β , and myelin basic protein (MBP)) can be elevated in traumatic or ischemic brain injury. We hypothesized that these neuromarkers would be associated with the development of HE in PALF.

Methods: PALF study participants enrolled between May 2012 and December 2014 by 12 participating centers were the subjects of this analysis. Daily HE assessments were determined by study investigators. Neurological and inflammatory markers were measured using ELISA and MILLIPLEX techniques, respectively. To model encephalopathy, these markers were log₂ transformed and individually examined for association with HE using a generalized linear mixed model with a logit link and random intercept.

Results: 82 children had neurological and inflammatory marker levels and HE assessments recorded, with the majority having assessments for 3 days during their illness. An indeterminate diagnosis (29%) was most common and the median age was 2.9 years. Significant associations

were observed for HE with S100 β (OR 1.16, 95% CI [1.03 – 1.29], $p=0.04$) and IL-6 (OR 1.24 [1.11 – 1.38], $p=0.006$).

Conclusion: Serum S100 β and IL-6 are associated with HE in children with PALF. Measuring these markers may assist in assessing neurological injury in PALF, impacting clinical decisions.

Keywords

hepatic encephalopathy; pediatric acute liver failure; acute liver failure; neuromarkers; inflammatory markers

Introduction

Pediatric acute liver failure (PALF) is an acute form of liver injury manifested by a rapid onset of symptoms, coagulopathy and encephalopathy. Clinical outcomes of PALF include (i) recovery of liver function after extensive medical treatment(s), (ii) survival after successful liver transplant, or (iii) death - with or without liver transplant^{1–3}. In a recent observational study of PALF, we found that 53% recovered, 32% underwent transplantation and 14% died⁴. Furthermore, we have demonstrated that survivors of PALF have decreased motor abilities, attention, executive function and quality of life compared to age-matched controls⁵. Thus, although rare, PALF has high mortality and morbidity for an individual child and a large burden on families and for the public health.

Hepatic encephalopathy (HE) is often clinically evident in PALF, although its pathophysiology is not fully understood. Presence of HE influences decision making for medical therapies as well as decisions to list for and proceed with liver transplantation⁶. Clinical assessment of HE currently relies upon the experience of the individual examining the patient and significant variability in observation and manifestation of HE may exist. Therefore, objective tools to assess HE would be welcomed.

Serum neuromarkers are structural, brain-related proteins measurable in serum following neurological injury. Individual neuromarkers are derived from specialized cell types within the brain with neuron-specific enolase [NSE] present in neurons, S100 β present in astrocytes, and myelin basic protein [MBP] in oligodendrocytes. Recent evidence determined that brain-derived proteins from within the neurovascular unit can escape into the peripheral circulation despite an intact blood-brain barrier^{7–9}. Many neuromarkers have been demonstrated to be increased after brain injuries such as hypoxic/ischemic injury after cardiac arrest, traumatic brain injury, stroke and others^{10–17}. Most have been used to either diagnose an injury or to provide some evidence for injury severity – yielding a more objective assessment of neurological injury. While reports of neurological markers to detect brain injury associated with intracranial hypertension in patients with traumatic brain injury^{18, 19} are noted, similar studies have not been performed in children with ALF.

In this exploratory study, we intend to test the hypotheses that serum neuromarkers (NSE, S100 β and MBP) are detectable in children with PALF and that these neuromarkers are associated with the degree of HE. Additionally, we will utilize circulating inflammatory

mediators previously reported²⁰ to determine if an association between inflammatory markers and encephalopathy is present in PALF participants.

Methods

Patient Enrollment

The PALF Study is a multi-center, observational cohort study of infants and children with acute liver failure. All medical decisions for the care of children, including medical, surgical, and transplant decisions, were made by the clinical teams of each institution. Approval from the Institutional Review Board at each participating site was secured. Informed consent for the child to participate in the PALF study was obtained from the parent/caregiver (and assent from study subjects, as appropriate). Participants for this analysis were enrolled between May 2012 and December 2014 by 12 participating centers. Consent included permission to collect and store biospecimens for future research. This report represents one such investigation with measurement of neuromarkers and inflammatory markers from stored specimens.

Patients from birth through 18 years of age were eligible for enrollment if they met the following entry criteria for the PALF study: (i) no known evidence of chronic liver disease, (ii) evidence of acute liver injury, and (iii) hepatic-based coagulopathy not corrected by vitamin K with the follow parameters: prothrombin time (PT) ≥ 15 seconds or international normalized ratio (INR) ≥ 1.5 in the presence of clinical hepatic encephalopathy (HE) or a PT ≥ 20 seconds or INR ≥ 2.0 regardless of the presence or absence of clinical HE [4]. For this ancillary study, an additional inclusion criterion was the availability of a research serum sample for analyzing neurological and other markers with concomitant assessment of hepatic encephalopathy on at least one of the days a sample was available. Exclusion criteria for the PALF study were: (i) known chronic underlying liver disease; (ii) multi-organ system failure following heart surgery or Extracorporeal Membrane Oxygenation (ECMO); (iii) solid organ or bone marrow transplantation; (iv) acute trauma; (v) previous enrollment in the PALF Study

Study Procedures

Data for the PALF study were collected daily from enrollment until discharge with the native liver, liver transplant, or death. Data extracted from the medical records included diagnostic studies, morning laboratory tests obtained as part of standard care to assess the child's clinical condition, and documentation of medical/surgical therapies administered to the child. All children were assessed for hepatic encephalopathy with a score of 0 – 4, with 0 corresponding to no encephalopathy, using the Whittington scale²¹ for infants and children under 3 years of age and the West Haven criteria²² for those 3 to 18 years. The study investigator assessed participants each day at the earliest morning encounter to approximate clinical and research data with bedside clinical assessment.

Measurements of Markers and Data Analysis

Weight-based daily and monthly blood volume restrictions for obtaining additional blood samples for research purposes were observed. If allowable, a daily serum sample for

research purposes was collected at the time of the first morning blood draw, stored locally at minus 80 degrees, and then batch-shipped for long-term storage at the National Institute of Diabetes and Digestive and Kidney Diseases funded Biorepository. Serum samples from three time points that met criteria for analysis were shipped to the investigators. Given the variability of the interval among participants and the dynamic clinical course of PALF, preference for serum selection of the three qualifying study days was for one to be nearest to enrollment, one day nearest the outcome, and the third day nearest the middle of the interval for the participant. Serum concentrations of NSE, S100 β and MBP were measured using commercially-available enzyme-linked immunosorbent assay (ELISA) kits. In general, serum was applied to 96-well kits along with samples of known concentrations and controls (both negative and positive). Sandwich ELISAs were performed using biotin-labelled antibodies for primary antigen detection. Optical density was measured at 450 nm by a microplate reader (Thermomax Microplate Reader, Molecular Devices, Sunnyvale, CA), and data were analyzed with Soft Max Pro software, version 5.0.1 (Molecular Devices, Sunnyvale, CA). Standard curves were determined from known standards and sample neuromarker concentrations were calculated.

Cytokine measurements were performed as previously reported.²³ Briefly, the human inflammatory MILLIPLEX MAP Human Cytokine/Chemokine Panel-Premixed 24 Plex (Millipore, Billerica, MA) and a Luminex 100 IS apparatus (Luminex, Austin, Tx) were used to measure serum levels of Interleukin (IL) –1 β , IL-1 receptor antagonist (IL-1RA), IL-2, soluble IL-2 receptor- α (sIL-2R α), IL-4, IL-5, IL-6, IL-7, IL-8 (CCL8), IL-10, IL-13, IL-15, IL-17, interferon (INF)- γ , INF- γ inducible protein (IP)-10 (CXCL10), monokine induced by gamma interferon (MIG; CXCL9), macrophage inflammatory protein (MIP)-1 (CCL2), granulocyte-macrophage colony stimulating factor (GM-CSF), Eotaxin (CCL11) and tumor necrosis factor (TNF).

For all but one participant, serum samples were available for three days; one patient had four samples available. For a participant to be included in this analysis they had to have at least one serum sample and an evaluation of HE on the same day. Participants were followed for the interval between enrollment and the outcome of hospital discharge, liver transplant, or death. Laboratory personnel (NAT and MJB) were masked with respect to HE results and all PALF study staff were masked with respect to neuromarker concentrations until they were generated by the lab personnel. Due to the observed distribution of the HE assessments, and the difficulty distinguishing HE grade 1 from grade 0, HE grading was dichotomized to “No HE” (grades 0 and 1) and “HE” (grades 2 – 4). If the child’s encephalopathy grade could not be assessed because the child was on a respirator, “HE” (grades 2 – 4) was assigned *a priori*. We chose this approach with the assumption that participants on the respirator at the time clinical assessment of HE was to be determined were highly likely to have HE that was at least stage 2. If unassessable for other reasons, such as use of sedative medications, HE was considered missing.

Descriptive statistics are shown using frequencies and percentages for categorical data and medians, 25th and 75th percentiles, minima and maxima for continuous data. Differences in distributions between participants in the PALF cohort who were included in the analysis and those who were not were tested using the Wilcoxon test for continuous data or the Pearson

chi-square test of association for categorical data. If the expected value in any cell for categorical data was less than 5, an exact test was used. Gray's test was used to test whether the cumulative incidences of death and transplantation differed significantly between those included in the analyses and the others in the PALF cohort. The proportional hazards assumption for both death and liver transplantation were tested by assessing the statistical significance of the interaction between time and the indicator variable for inclusion in the analysis.

Due to extreme values, when examining the association of HE with neuromarkers, the neuromarkers were first transformed using $\log_2$. They were individually examined for association with HE using a simple generalized linear mixed model with a logit link (binomial) and random intercept to estimate the odds ratio for encephalopathy. These models were used to account for the repeated measures of the neuromarkers in individual participants. The model assumes that data are missing at random. Overdispersion was tested using the ratio of the Pearson chi-square test and degrees of freedom. The logit of the probability of HE was plotted against each neuromarker. When there was evidence that the assumption of a linear relationship was not met, then quadratic and, in some cases, higher order polynomial terms were included in the model. Multiple generalized linear mixed models were used to examine the association of HE and each neuromarker, adjusting separately for age at sample draw, sex, and race. All available data were used in this analysis for the 82 participants with at least one neuromarker measure and encephalopathy grade on the same day. Due to the multiple comparisons (3 neuromarkers, 28 cytokines and other analytes), the probability of incorrectly finding a statistically significant result (type I error) is inflated. Therefore, to reduce the probability of finding a significant association due to the many comparisons performed, Holm's stepdown method was used. An adjusted p-value of 0.05 was used to determine statistical significance. Odds ratios for encephalopathy per unit change in $\log_2$ biomarker (i.e., doubling the value) are presented. All statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary, North Carolina) and figure was drawn using R 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Of the 158 participants in this phase of PALF, 82 had serum samples available for analysis on the same day HE assessment was recorded. The median age was 2.9 years, ranging from infants (6 days old) through 17.8 years (25th-75th percentiles 0.1–13.7 years). Most children were male (63%) and nearly $\frac{3}{4}$ were Caucasian (74%). At the time of enrollment, 11% of subjects had HE grade > 1. Demographic, clinical, and outcome information for those meeting criteria for the neuromarker study as well as those not included are provided in Table 1. There was not evidence of nonproportionality of hazards for either death or liver transplantation. There were no significant differences between those eligible for the neuromarker study and those who were not.

The etiology of acute liver failure and laboratory analysis on study entry are also provided in Table 1. Consistent with previous findings⁴, an indeterminate diagnosis (29%) was the most common final diagnosis, with acetaminophen intoxication being the second most common (11%). Children were quite icteric at study entry (total bilirubin; 7.6 mg/dl [3.8 – 15.5],

median [25th-75th percentile]), but largely had normal renal function (Cr; 0.4 mg/dl [0.2 – 0.7]). ALT (1598 IU/l [500 – 3817]) and INR (2.7 [2.2–3.9]) values are consistent with severe hepatic injury and diminished synthetic function of the children at the time of study entry.

Of the 82 participants in the study, 81 had 3 serum samples available for analysis and 1 child had 4 samples that were analyzed yielding a total of 247 samples. There were 80 samples (32% of the total) obtained on the day of, or the day after, study entry and 36 samples (15%) were obtained 10 or more days after study entry. HE was present during the study period in 37/82 (45%) of participants. The distribution of HE scores on the day of the serum sample is shown in Table 2. For 20 (8%) time points there were missing HE scores, either because they were not collected or because the HE could not be assessed, most often due to concurrent administration of sedatives or barbiturates. Of those that were measured, 161 (71%) time points were “No HE” while 66 (29%) scores corresponded to “HE” group.

Table 3 segregates those who were never, ever, or always on the ventilator at a time point when HE assessment and sample collection were paired to examine age and clinical characteristics of participants. Participants who were always on the ventilator were younger and more likely to have a viral etiology and die, while those never on the ventilator were more likely to have an indeterminate diagnosis and receive a liver transplant.

Progression of HE is associated with a higher incidence of liver transplantation and death.²⁴ Clinical assessment of HE progression can only occur in individuals who are neither sedated nor ventilated. However, HE cannot be assessed for participants on the ventilator. Therefore, we assumed those on the ventilator were highly likely to have HE grade 2–4. As noted in Table 3, 10/18 (66%) of the participants who were never ventilated had clinical HE grade 0–1 at study entry, but progressed to grade 2–4 and this was associated with a high frequency of liver transplantation in the never ventilated cohort. For those always ventilated at a study timepoint, 3/17 had HE 0–1 assessed at study entry, but all progressed to being on the ventilator and the ventilated group had a high rate of mortality. The dynamic nature of HE among PALF participants, coupled with the high mortality rate among those always on the ventilator supports our assumption to classify ventilated participants as having HE grade 2–4.

Neuromarker concentrations at each of the three time points are summarized in Table 4. There was a significant relationship between S100 β and HE (OR 1.16, 95% confidence interval [1.03 – 1.29], adjusted $p = 0.04$), (Table 5). With a doubling of S100 β levels there was a 16% increased odds of having HE (grades 2–4). There were no significant associations between HE and either MBP or NSE. Cytokine concentrations from each of the three sampling time points are summarized in Supplemental Table 1. Only IL-6 was significantly associated ($p=0.003$) with HE after adjusting for multiple comparisons. The relationship was nonlinear such that the odds of encephalopathy was 35% higher ([1.18–1.54]) when the level of IL-6 doubled from the median value of 13 to 26 picograms/ml (pg/ml), increasing to 71% higher ([1.34–2.17]) when IL-6 level doubled from the mean value of 127 to 254 pg/ml.-).

Discussion

In this exploratory analysis from a multi-center, observational PALF study, we are the first to test the utility of measuring serum neuromarkers specific to neurons, astrocytes and oligodendrocytes during the acute period of illness. We demonstrated that HE was significantly associated with both the astrocyte marker S100 β and the proinflammatory cytokine IL-6. To our knowledge, this is the first report that has demonstrated these associations in children and may lead to improved monitoring methods in the future.

The pathophysiology of ALF, and the manifestation of HE during the course of the illness, is complex^{25, 26}. Any number of mechanisms including alterations of cerebral blood flow/ cerebrohemodynamics with intracranial hypertension, effects of metabolic derangements (including hyperammonemia among others), oxidative stress, inflammatory cascades have been implicated in HE development. Despite these disparate mechanisms, the interaction of neurons and glia is critical as glial elements play important roles in water homeostasis, neurotransmitter metabolism and clearance of byproducts as neurons may become dysfunctional during HE. For this reason, we endeavored to categorize the neurological markers related to the main subtypes of cells within the CNS (neurons, astrocytes and oligodendrocytes) and found that the astrocyte marker was associated with HE.

Clinically, HE remains a main driving force for adverse outcomes for ALF. Ciocca and colleagues demonstrated that HE and coagulopathy were associated with death or need for transplantation in 215 children from Argentina, and Ng and colleagues demonstrated that severe HE (grade III or IV) or progression of encephalopathy was associated with 21 day mortality in 769 children in the US^{6, 24}. In a smaller series with 19 children, Hussain and colleagues demonstrated some utility of EEG monitoring of such children as those with the moderate-to-severe abnormalities were more likely to die or require transplantation²⁷. However, they acknowledge additional neurological assessment strategies will be useful in medical and transplant decisions.

Although this is the first study to investigate the role of neuromarkers in PALF, several reports have been published regarding the association of neuromarkers in adults with liver disease. Several decades ago, Kimura and Budka demonstrated astrocyte activation in brains of patients with HE at autopsy²⁸. Shiotani and colleagues demonstrated that S100 β was associated with perioperative brain edema in 13 adults undergoing liver transplantation for fulminant hepatic failure²⁹, while Isobe-Harima and colleagues demonstrated that S100 β was increased at the onset of HE in 9 adults with hepatitis³⁰. Similar to our findings, Saleh and colleagues found that S100 β was increased in 29 cirrhotic adults with stage 1 and 2 HE, while NSE was not changed³¹. In the two largest series, Strauss and colleagues found that S100 β was increased in most patients with fulminant hepatic failure (n = 35) and acute-on-chronic liver disease (n = 6), while NSE was increased in subjects who developed cerebral herniation³². And using samples from 54 subjects in the US Acute Liver Failure Study Group, Vaquero and colleagues found S100 β was increased in subjects with (i) stage 1–2 HE who did not progress, (ii) stage 1–2 HE who progressed to a deeper encephalopathy, (iii) stage 3–4 HE who survived and (iv) stage 3–4 HE who died or required transplantation³³. However, they failed to find a difference among these 4 groups – thereby arguing that S100 β

is not useful in this condition. Our data from 82 children is the largest of these studies and does demonstrate an association between S100 β and the presence of HE. Given that the assessment of children and infants with severe liver disease may be more difficult than adults, we believe that there may be more utility in this neuromarker in children with PALF than in these previous studies.

As stated previously, one of PALF's goals was to derive a model of inflammation and repair that would predict the need for liver transplantation²⁰. The link between neuroinflammation with acute liver failure has long been postulated³⁴, from evidence of experimental models demonstrating that inflammation can precipitate HE³⁵ to evidence of microglial proliferation in patients with HE³⁶. In our study, we tested the relationship between selected inflammatory mediators with HE and found that only IL-6 had a significant association. Similar to our study, Tsai and colleagues found that IL-6 was associated with mild HE in 94 adults with cirrhosis³⁷, while Srivastava and colleagues demonstrated that both TNF and IL-6 were increased in 20 adults with minimal HE – along with MRI findings and ammonia concentrations³⁸. Should the association between HE in PALF and elevations in S100 β and IL-6 be consistently demonstrated in other studies, it is possible that use of these simple blood tests could prove useful in evaluating children with PALF and HE.

There are several limitations to the study. First, the sample size is modest, though larger than others cited above, and the sampling was done at various times during the child's illness. The study design attempted to minimize this limitation by having HE and neuromarkers measured on the same day. While sites were instructed to draw research samples with the earliest morning clinical lab draw and principal investigators were instructed to obtain HE scores in their first morning assessment of the day, actual times for both were not collected. Definitions of HE scoring were available and the principal investigator was encouraged to limit the number of physicians performing the daily neurological examination, however the scoring of HE was performed by more than one individual during hospitalization and assignment of scores from children with mechanical ventilation was challenging. Reliability cross-centers would be somewhat mitigated by collapsing categories as we did for this analysis. As an observational study across multiple centers, it was not possible to perform detailed neurological assessments other than the HE scoring, which is a standard metric used by all participating sites as part of their clinical practice. Furthermore, it was not possible to intervene within this clinical study to decrease sedative and/or neuromuscular blockade medications to obtain these scores in a more systematic manner. *A priori* scoring of HE 2–4 for all participants on a ventilator may have been incorrect for individual participants.

In conclusion, this study demonstrates the potential utility of neuromarkers in the neurological assessment of children with acute liver failure and suggests associations between neurological injury with proinflammatory cytokines that are responsible for regeneration and repair. If future studies confirm these associations, it may lead to novel ways to monitor patients with acute liver failure as they are recovering or awaiting transplantation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ALF	acute liver failure
ECMO	Extracorporeal Membrane Oxygenation
ELISA	enzyme-linked immunosorbent assay
HE	hepatic encephalopathy
IL	Interleukin
MBP	myelin basic protein
NSE	neuron specific enolase

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References

1. Cochran JB, Losek JD. Acute liver failure in children. *Pediatr Emerg Care* 2007;23:129–35. [PubMed: 17351416]
2. D'Agostino D, Diaz S, Sanchez MC, et al. Management and prognosis of acute liver failure in children. *Curr Gastroenterol Rep* 2012;14:262–9. [PubMed: 22528660]
3. Treem WR. Fulminant hepatic failure in children. *J Pediatr Gastroenterol Nutr* 2002;35 Suppl 1:S33–8. [PubMed: 12151819]
4. Squires RH Jr., Shneider BL, Bucuvalas J, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. *J Pediatr* 2006;148:652–658. [PubMed: 16737880]
5. Sorensen LG, Neighbors K, Zhang S, et al. Neuropsychological functioning and health-related quality of life: pediatric acute liver failure study group results. *J Pediatr Gastroenterol Nutr* 2015;60:75–83. [PubMed: 25250681]
6. Ciocca M, Ramonet M, Cuarterolo M, et al. Prognostic factors in paediatric acute liver failure. *Arch Dis Child* 2008;93:48–51. [PubMed: 17872939]
7. Aspelund A, Antila S, Proulx ST, et al. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J Exp Med* 2015;212:991–9. [PubMed: 26077718]
8. Iliff JJ, Wang M, Liao Y, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. *Sci Transl Med* 2012;4:147ra111.
9. Plog BA, Dashnaw ML, Hitomi E, et al. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. *J Neurosci* 2015;35:518–26. [PubMed: 25589747]
10. Yardan T, Cevik Y, Donderici O, et al. Elevated serum S100B protein and neuron-specific enolase levels in carbon monoxide poisoning. *American Journal of Emergency Medicine* 2009;27:838–842. [PubMed: 19683113]
11. Zurek J, Fedora M. The usefulness of S100B, NSE, GFAP, NF-H, secretagogin and Hsp70 as a predictive biomarker of outcome in children with traumatic brain injury. *Acta Neurochir (Wien)* 2012;154:93–103; discussion 103. [PubMed: 21976236]
12. Studer M, Goeggel Simonetti B, Heinks T, et al. Acute S100B in serum is associated with cognitive symptoms and memory performance 4 months after paediatric mild traumatic brain injury. *Brain Inj* 2015;29:1667–73. [PubMed: 26502808]
13. Varrica A, Satriano A, Tettamanti G, et al. Predictors of Ominous Outcome in Infants who Undergo Cardiac Surgery and Cardiopulmonary By-Pass: S100B Protein. *CNS Neurol Disord Drug Targets* 2015;14:85–90. [PubMed: 25957579]
14. Rodriguez-Rodriguez A, Egea-Guerrero JJ, Gordillo-Escobar E, et al. S100B and Neuron-Specific Enolase as mortality predictors in patients with severe traumatic brain injury. *Neurol Res* 2016;38:130–7. [PubMed: 27078699]
15. Usui A, Kato K, Murase M, et al. Neural tissue-related proteins (NSE, G0 alpha, 28-kDa calbindin-D, S100b and CK-BB) in serum and cerebrospinal fluid after cardiac arrest. *J Neurol Sci* 1994;123:134–9. [PubMed: 8064306]
16. Soares H, Bednar M, Mennti F, et al. S100 and NSE correlate with clinical outcomes in severe traumatic brain injury. *J Neurotrauma* 2003;20:1080.
17. Olivecrona Z, Bobinski L, Koskinen LO. Association of ICP, CPP, CT findings and S-100B and NSE in severe traumatic head injury. Prognostic value of the biomarkers. *Brain Inj* 2015;29:446–54. [PubMed: 25518864]
18. Petzold A, Green AJ, Keir G, et al. Role of serum S100B as an early predictor of high intracranial pressure and mortality in brain injury: a pilot study. *Crit Care Med* 2002;30:2705–10. [PubMed: 12483062]
19. Stein DM, Lindell A, Murdock KR, et al. Relationship of serum and cerebrospinal fluid biomarkers with intracranial hypertension and cerebral hypoperfusion after severe traumatic brain injury. *J Trauma* 2011;70:1096–103. [PubMed: 21610426]

20. Zamora R, Vodovotz Y, Mi Q, et al. Data-Driven Modeling for Precision Medicine in Pediatric Acute Liver Failure. *Mol Med* 2016;22. [PubMed: 26772775]
21. W PF, A. EM Fulminant hepatitis and acute liver failure. In: K DA, ed. *Paediatric Liver Disease* Oxford: Blackwell, 2003:107–26.
22. Conn HO, Leevy CM, Vlahcevic ZR, et al. Comparison of lactulose and neomycin in the treatment of chronic portal-systemic encephalopathy. A double blind controlled trial. *Gastroenterology* 1977;72:573–83. [PubMed: 14049]
23. Zamora R, Vodovotz Y, Mi Q, et al. Data-Driven Modeling for Precision Medicine in Pediatric Acute Liver Failure. *Mol Med* 2016;22:821–829. [PubMed: 27900388]
24. Ng VL, Li R, Loomes KM, et al. Outcomes of Children With and Without Hepatic Encephalopathy From the Pediatric Acute Liver Failure Study Group. *J Pediatr Gastroenterol Nutr* 2016;63:357–64. [PubMed: 27367788]
25. Butterworth RF. Pathogenesis of hepatic encephalopathy and brain edema in acute liver failure. *J Clin Exp Hepatol* 2015;5:S96–S103. [PubMed: 26041966]
26. Wijdicks EF. Hepatic Encephalopathy. *N Engl J Med* 2016;375:1660–1670. [PubMed: 27783916]
27. Hussain E, Grimason M, Goldstein J, et al. EEG abnormalities are associated with increased risk of transplant or poor outcome in children with acute liver failure. *J Pediatr Gastroenterol Nutr* 2014;58:449–56. [PubMed: 24345828]
28. Kimura T, Budka H. Glial fibrillary acidic protein and S-100 protein in human hepatic encephalopathy: immunocytochemical demonstration of dissociation of two glia-associated proteins. *Acta Neuropathol* 1986;70:17–21. [PubMed: 3727931]
29. Shiotani S, Shimada M, Soejima Y, et al. S100 beta protein: the preoperative new clinical indicator of brain damage in patients with fulminant hepatic failure. *Transplant Proc* 2004;36:2713–6. [PubMed: 15621131]
30. Isobe-Harima Y, Terai S, Segawa M, et al. Serum S100b (astrocyte-specific protein) is a useful marker of hepatic encephalopathy in patients with fulminant hepatitis. *Liver Int* 2008;28:146–7. [PubMed: 18173564]
31. Saleh A, Kamel L, Ghali A, et al. Serum levels of astroglial S100-beta and neuron-specific enolase in hepatic encephalopathy patients. *East Mediterr Health J* 2007;13:1114–23. [PubMed: 18290405]
32. Strauss GI, Christiansen M, Moller K, et al. S-100b and neuron-specific enolase in patients with fulminant hepatic failure. *Liver Transpl* 2001;7:964–70. [PubMed: 11699032]
33. Vaquero J, Jordano Q, Lee WM, et al. Serum protein S-100b in acute liver failure: Results of the US Acute Liver Failure Study group. *Liver Transpl* 2003;9:887–8. [PubMed: 12884207]
34. Butterworth RF. Neuroinflammation in acute liver failure: mechanisms and novel therapeutic targets. *Neurochem Int* 2011;59:830–6. [PubMed: 21864609]
35. Chastre A, Belanger M, Nguyen BN, et al. Lipopolysaccharide precipitates hepatic encephalopathy and increases blood-brain barrier permeability in mice with acute liver failure. *Liver Int* 2014;34:353–61. [PubMed: 23910048]
36. Dennis CV, Sheahan PJ, Graeber MB, et al. Microglial proliferation in the brain of chronic alcoholics with hepatic encephalopathy. *Metab Brain Dis* 2014;29:1027–39. [PubMed: 24346482]
37. Tsai CF, Chu CJ, Huang YH, et al. Detecting minimal hepatic encephalopathy in an endemic country for hepatitis B: the role of psychometrics and serum IL-6. *PLoS One* 2015;10:e0128437. [PubMed: 26039496]
38. Srivastava A, Yadav SK, Yachha SK, et al. Pro-inflammatory cytokines are raised in extrahepatic portal venous obstruction, with minimal hepatic encephalopathy. *J Gastroenterol Hepatol* 2011;26:979–86. [PubMed: 21362045]

What is known:

- Children with acute liver failure and hepatic encephalopathy scores that are high or progress are associated with poor outcomes.
- Children who alive one year following their episode of acute liver failure experience significant impairments in neurocognitive skills and quality of life.

What is new:

- Serum neuromarkers (neuron-specific enolase, S100 β and myelin basic protein) are detectable in children with PALF
- Serum S100 β and IL-6, an inflammatory marker, are associated with hepatic encephalopathy in children with acute liver failure.
- Measuring these markers may assist in assessing neurological injury in PALF, impacting clinical decisions.

Table 1.

Comparison between neuromarker analysis subset participants and the PALF study cohort

	In Neuromarker analysis (n = 82)		Not in Neuromarker analysis (n = 76)		comparison p-value
Age at study enrollment(yrs), median, Q1-Q3, (min-max)	2.9, 0.1 – 13.7 (6 days-17.8 years)		3.6, 0.6 – 14.5		0.46*
	n	Percent	n	Percent	
Age at study enrollment (yrs)					0.83**
<1 y	28	34	21	28	
1 – 2 y	14	17	15	20	
3 – 9 y	14	17	13	17	
10 – 17 y	26	32	27	36	
Sex					0.09**
Male	52	63	38	50	
Female	30	37	38	50	
Ethnicity					0.95**
Unknown	2	--	5	--	
Not Hispanic or Latino	69	86	61	86	
Hispanic or Latino	11	14	10	14	
Race					0.69***
Unknown	4	--	4	--	
White	58	74	48	67	
African American	8	10	10	14	
Asian	4	5	4	6	
Other	2	3	5	7	
Mixed	6	8	5	7	
HE Grade at Enrollment					0.70***
0	40	66	37	69	
I	14	23	8	15	
II	4	7	4	7	
III	1	2	3	6	
IV	2	3	2	4	
Not assessable	11	--	5	--	
Missing	10	--	17	--	
Final Diagnosis					0.17***
Indeterminate	24	29	20	26	
Acetaminophen - Acute Toxicity	9	11	15	20	
Acetaminophen - Chronic Exposure	1	1	1	1	
Acetaminophen – Therapeutic Misadventure	0	0	3	4	

	In Neuromarker analysis (n = 82)		Not in Neuromarker analysis (n = 76)		comparison p-value
Adenovirus	1	1	1	1	
Enterovirus/Coxsackie/Echovirus	8	10	0	0	
Hepatitis B	0	0	1	1	
Hepatitis due to Herpes Simplex	3	4	3	4	
Autoimmune marker positive	5	6	2	3	
Hemophagocytic Lymphohistiocytosis	5	6	4	5	
GALD/Neonatal Hemochromatosis	6	7	4	5	
Ischemic hepatopathy/Sepsis/Cardiac	3	4	4	5	
Drug-induced Hepatotoxicity	2	2	6	8	
Galactosemia	0	0	2	3	
Fructosemia	0	0	1	1	
Mitochondrial	4	5	2	3	
Urea Cycle Disorder	1	1	1	1	
Wilson's Disease	1	1	0	0	
Other	9	11	6	8	
	# of events	CI	# of events	CI	
1 year Cumulative Incidence [†]					
Liver Transplantation	22	28%	18	25%	0.93 ^{††}
Death	15	19%	10	15%	0.56 ^{††}

* based on Wilcoxon test

** based on Pearson chi-square

*** based on exact Pearson chi-square

[†] Death is a competing risk for the cumulative incidence of liver transplantation; liver transplantation is a competing risk for death

^{††} based on Gray's Test

CI: cumulative incidence, GALD: gestational alloimmune liver disease, HE: hepatic encephalopathy, Yrs: years,

Table 2:

Distribution of hepatic encephalopathy (HE) scores with serum sampling

HE Score	N	%
Missing	11	4
Not Assessed	9	4
Ventilated	38	15
0	113	46
1	48	19
2	14	6
3	8	3
4	6	2
Total	247	100.0

Table 3.

Participant ventilator status for participants with HE at time of neuromarker sample HE is defined as either HE 2,3,4 or non-assessable HE and on a ventilator

	Participants with HE 2,3, or 4 or non-assessable HE and ventilated at time of neuromarker sample		
	Ventilator Status		
	Always (n = 17)	Ever (n = 2)	Never (n = 18)
Age at study enrollment (yrs.), median, (Q1-Q3)	0.2 (0.0 – 2.5)	15.4 (14.4 – 16.4)	4.8 (2.3–13.7)
Age at study enrollment (yrs.), n (%)			
< 1 yr	11(65)	0 (0)	3(17)
1– 2 y	2(12)	0 (0)	3(17)
3 – 9y	2(12)	0(0)	6 (33)
10 –17 y	2(12)	2(100)	6 (33)
Sex, n(%)			
Male	14(82)	0(0)	16(89)
Female	3(18)	2(100)	2(11)
Ethnicity			
Not Hispanic or Latino	15(88)	1(50)	15(83)
Hispanic or Latino	2(12)	1(50)	3(17)
Race, n (% of non-missing)			
Unknown	0	1	1
White	12(71)	1(100)	12(71)
African American	3(18)	0(0)	1(6)
Asian	1(6)	0(0)	2(12)
Other	0	0(0)	1(6)
Mixed	1(6)	0(0)	1(6)
HE grade at enrollment			
0	3(75)	0(0)	8(53)
I	1(25)	1(50)	2(13)
II	0(0)	0(0)	3(20)
III	0(00)	0(0)	1(7)
IV	0(0)	1(50)	1(7)
Not assessable	10	0	0
Missing	3	0	3
Final diagnosis			
Indeterminate	2(12)	0(0)	7(39)
Acetaminophen-Acute	0(0)	0(0)	1(6)

	Participants with HE 2,3, or 4 or non-assessable HE and ventilated at time of neuromarker sample					
	Ventilator Status					
	Always (n = 17)		Ever (n = 2)		Never (n = 18)	
Acetaminophen – Chronic	0(0)		0(0)		1 (6)	
Adenovirus	0(0)		0(0)		0(0)	
Enterovirus/Coxsackie/Echovirus	5(29)		0(0)		1(6)	
Hepatitis due to Herpes Simples	1(6)		0(0)		0(0)	
Autoimmune marker positive	0(0)		0(0)		1(6)	
Hemophagocytic Lymphohistiocytosis	1(6)		1(50)		1(6)	
GALD/Neonatal Hemochromatosis	1(6)		0(0)		2(11)	
Ischemic hepatopathy/Sepsis/Cardiac	2(12)		0(0)		0(0)	
Drug-induced Hepatotoxicity	0(0)		1(50)		0(0)	
Mitochondrial	2(12)		0(0)		0(0)	
Urea Cycle Disorder	0(0)		0(0)		0(0)	
Wilson's Disease	0(0)		0(0)		1(6)	
Other	3(18)		0(0)		3(17)	
Outcome, 1 year cumulative incidence	n	CI	n	CI	n	CI
Liver transplantation	1	6%	1	50%	8	45%
Death	6	37%	1	50%	1	6%

Table 4:

Neuromarker concentrations over study time periods

	NSE (ng/ml)	MBP (ng/ml)	S100 β (ng/ml)
1 st Time Point			
N	80	82	82
Median	13.8	0.1	0.02
[25–75%]	8.6, 20.4	0.0, 0.3	0.00, 0.05
(Min, Max)	3.3, 125.9	0.0, 13.3	0.00, 1.08
2 nd Time Point			
N	82	82	82
Median	11.7	0.1	0.02
[25–75%]	7.0, 17.8	0.1, 0.3	0.00, 0.03
(Min, Max)	1.0, 72.5	0.0, 12.3	0.00, 0.83
3 rd Time Point			
N	81	82	82
Median	10.5	0.1	0.01
[25–75%]	6.5, 15.4	0.0, 0.3	0.00, 0.03
(Min, Max)	2.2, 85.4	0.0, 11.8	0.00, 0.59

NSE: neuron specific enolase, MBP: myelin basic protein,

Table 5:**Logistic modeling of hepatic encephalopathy^{*} with neuromarkers**

(n=82)	Estimate	Standard Error	95% CI	Odds Ratio (OR) ^{**}	95% CI for OR	Adjusted p-value
Log ₂ (S100β)	0.14	0.06	0.03 – 0.26	1.16	1.03–1.29	0.04
Log ₂ (MBP)	0.06	0.04	–0.06 – 0.14	1.04	0.94 – 1.15	0.54
Log ₂ (NSE)	0.08	0.13	–0.18 – 0.34	1.09	0.84– 1.41	0.54

^{*} Participants who were on a respirator with non-assessable encephalopathy grade were considered to have encephalopathy grade > 1.

^{**} Odds ratio (OR) estimate and its confidence interval for encephalopathy is shown for the doubling of the neuromarker.

CI: confidence interval, OR: odds ratio, MBP: myelin basic protein, NSE: neuron specific enolase.