Published in final edited form as:
J Pediatr Gastroenterol Nutr. 2013 March ; 56(3): 311–315. doi:10.1097/MPG.0b013e31827a78b2.

Immunophenotype predicts outcome in pediatric acute liver failure

John Bucuvalas, MD[1], **Lisa Filipovich, MD**[2], **Nada Yazigi, MD**[1], **Michael R. Narkewicz, MD**[3], **Vicky Ng, MD**[4], **Steven H. Belle, PhD**[5,6], **Song Zhang, MS**[5], and **Robert H. Squires, MD**[7]

[1]Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital, Cincinnati, OH, United States

[2]Division of Bone Marrow Transplantation and Immunodeficiency, Cincinnati Children's Hospital, Cincinnati, OH, United States.

[3]Section of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, University of Colorado School of Medicine and Children's Hospital Colorado, Aurora, CO, United States.

[4]Division of Gastroenterology, Hepatology and Nutrition, Hospital for Sick Children, Toronto, ON, Canada

[5]Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States.

[6]Department of Biostatistics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States.

[7]Division of Gastroenterology, Hepatology and Nutrition, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States.

Abstract

Objectives—We sought to determine if markers of T cell immune activation, including soluble interleukin 2 receptor alpha (sIL2Rα) levels predict outcome in pediatric acute liver failure (PALF) and might target potential candidates for immunomodulatory therapy.

Methods—We analyzed markers of immune activation in 77 patients with PALF enrolled in a multi-national, multi-center study. The outcomes were survival with native liver, liver transplantation, and death without transplantation within 21 days after enrollment.

Results—Adjusting for multiple comparisons, only normalized serum sIL2Rα level differed significantly among the 3 outcomes, and was significantly higher in patients who died (p=0.02) or underwent liver transplantation (p=0.01) compared to those who survived with their native liver. The 37 patients with normal sIL2Rα levels all lived, 30 with their native liver. Of the 15 subjects with markedly high sIL2Rα (≥5000 IU/mL), 5 survived with their native liver, 2 died, and 8 underwent liver transplantation.

Corresponding Author: John C. Bucuvalas M.D. Professor of Pediatrics Cincinnati Children's Hospital Medical Center 3333 Burnet Avenue Cincinnati, Ohio 45229 phone: 513-803-1791 fax: 513-636-7805 john.bucuvalas@cchmc.org.

Conflicts of Interest: None

Conclusions—Evidence of immune activation is present in some patients who die or undergo liver transplantation. Patients with higher sIL2Rα levels were more likely to die or undergo liver transplantation within 21 days than those with lower levels. Identifying a subset of patients at risk for poor outcome may form the foundation for targeted clinical trials with immunomodulatory drugs.

Keywords

acute liver failure; sIL2Rα; immune activation; outcome

Introduction

Pediatric acute liver failure is a devastating life-threatening condition, particularly for those children with acute liver failure of indeterminate etiology who comprise almost 50% of the disease population (1). Medical decision-making for patients with ALF is complex and requires repeated assessment over time. The physician must regularly estimate the probability of survival with native liver compared to the risks of liver transplantation (LT). Existing liver failure scoring systems (2-7) fall short of the ideal prognostic tool (8-10) since outcomes vary among children with similar disease severity scores. Therefore, a better tool is needed to stratify patients by prognosis and target potentially life-saving treatment.

Emerging evidence suggests a subgroup of patients with ALF have clinical findings and plasma cytokine profiles consistent with natural killer (NK) cell dysfunction (11) or systemic inflammatory response syndrome (12). One such example is hemophagocytic lymphohistiocytosis (HLH), a disorder of immune regulation often occurring in infancy and early childhood. Here, we sought to determine if markers of T cell immune activation were associated with patient outcome.

Materials and Methods

Organization

The PALF Study Group was funded by the National Institute of Diabetes and Digestive and Kidney (NIDDK) Diseases of the National Institutes of Health (NIH). At the time these data were collected, the PALF Study Group consisted of 20 pediatric liver transplant sites, 17 within the United States, 1 in Canada, and 2 in the United Kingdom. Only the 17 of the 20 sites in the United States contributed to this study.

Data Collection for the PALF longitudinal cohort

This was an ancillary study to the Pediatric Acute Liver Failure (PALF) study (1). To be enrolled in the PALF study cohort, informed c onsent was obtained from a parent or legal guardian. Subsequently, demographic, clinical and laboratory information were recorded daily for up to 7 days, starting on the date of enrollment into the study. Diagnostic evaluation and medical management were consistent with the standard of care at each site. The NIH provided a Certificate of Confidentiality to the study and IRB approval was secured at each site before patient enrollment.

Sample collection

Blood samples to assess markers of T-cell immune activation were collected within 48 hours of enrollment into the PALF cohort. Participants were excluded if they were reported to have received corticosteroids or rituximab prior to sample collection or were less than 10 kg to avoid excessive blood withdraw in smaller children. We did not collect data regarding

administration of blood products. Consequently, we did not exclude subjects based on the timing of administration of blood products.

Blood was collected in ethylenediaminetetraacetic acid (EDTA) and sodium (Na) heparin tubes. For children weighing 10 to 20 kg, 1 mL of blood in an EDTA tube and 4 mL blood in a Na-heparin tube were collected and for children more than 20 kg, 3 mL blood in an EDTA tube and 7 mL blood in Na-heparin tube were collected. Collection days were Monday through Thursday with samples shipped at room temperature by priority overnight for delivery on weekdays only with Friday deliveries received by 11:00 a.m. Eastern Time. Samples were shipped to the Diagnostic Immunology Laboratory at Cincinnati Children's Hospital. Blood samples were processed within 2 hours of receiving the specimen. If blood collected in the EDTA tube was insufficient, then measurement of sIL2Rα was considered a priority. Specimen processing occurred within 24 hours of collection from the patient according to standard procedure, which was why PALF study sites outside of the United States were not included in this ancillary study. The assays were done under the supervision of one of the authors (LF) in a laboratory accredited by the College of American Pathologists and by Clinical Laboratories Improvement Amendments. Results were compared to established age-specific reference ranges. Markers of immune activation were categorized to permit comparisons among subjects in different age-groups.

Analysis of T-cell activation

The T-cell immune activation profile assembled in this study included NK cell function, flow cytometry, analysis of cytotoxic lymphocytes, and measurement of sIL2Rα levels. Markers of immune activation included sIL2Rα, perforin expressing NKT cells and CD56 bright immunoregulatory NK cells. NK cell cytolytic function was used as the primary marker of NK cell function (13-16). Cytolytic function of NK cells was measured by release of radioactive chromium from labeled target cells. In addition, granzyme B and perforin expression in lymphocytes were determined by four-color flow cytometry after staining with both surface and intracellular monoclonal antibodies (15). Levels of sIL2Rα were measured using the Immunlite 1000 method, a solid phase, two-site chemoluminescent immunometric assay. Normal ranges for sIL2Rα levels analyzed in pediatric populations are well-defined (17). In contrast to adults, sIL2Rα levels are physiologically high in normal children. Levels are highest in children less than 4 years of age and taper to near adult levels in adolescents. For this analysis, age-normalized levels for sIL2Rα are reported unit-free (e.g, 2 × upper limit of normal) and measured sIL2Rα levels that are not normalized for age are reported with units (e.g., 5,000 IU/mL). sIL2Rα levels greater than 2 times the upper limit of normal for age were considered to have potential clinical importance. These cut-off levels were based on confidence intervals from normal populations (17). A sIL2Rα level greater than or equal to 5,000 IU/mL is considered to be markedly elevated, regardless of age.

Study Design

Final diagnoses were determined by each site's principal investigator. Diagnostic categories for PALF based on previously defined criteria[1] included (1) acetaminophen associated ALF (2) autoimmune ALF (3) drug-induced ALF (4) ALF due to viral hepatitis (5) metabolic disease including Wilsons disease, mitochondrial disease, or disorders of fat metabolism diagnosis (6) other or (7) indeterminate ALF.

Within the indeterminate group are those who were fully evaluated and did not meet specific diagnostic criteria or those were incompletely evaluated, often due to death or LT prior to a diagnosis being established. Laboratory studies and clinical assessment following site specific standard of care included white blood cell count, hemoglobin, platelet count, serum aminotransferases, international normalized ratio (INR), prothrombin time (PT), bilirubin,

ammonia, and coma stage. These parameters provided assessment of bone marrow function, liver cell injury, and disease severity scores. Coma stage was determined by established criteria (1). Elements of the immune activation profile were assessed to determine their association with death without transplantation, liver transplantation, and survival with native liver at 21 days.

Statistical Analysis

For demographic, clinical characteristic and severity scores, percentages were reported if categorical and medians (25^{th} and 75^{th} percentiles) were reported if continuous. NK measures were age-normalized by dividing assay results by the upper limit of the age-specific normal range. Kruskal –Wallis tests were used for comparing each of 15 age-specific normalized NK function measurements among those who died without LT, received liver transplantation or survived with native livers. To adjust the error rate in multiple testing (15 normalized NK measures) and multi-group comparisons (pairwise comparisons when the Kruskal-Wallis met criterion for statistical significance), the Bonferroni-Holm step-down procedure was applied. An exact chi-square test was used to test equality of the probabilities of the three outcomes for the four sIL2Rα groups.

p-values less than 0.05 (adjusted for multiple comparisons as above) were used to determine statistical significance. Analyses were conducted using SAS Statistical Software (SAS Institute, Cary, NC).

Results

Study Cohort

Samples for T-cell immune activation analysis were obtained from 86 participants. Nine were excluded because samples were not received and processed by the testing laboratory within 24 hours after they were drawn so the final study group included 77 subjects. Outcomes at day 21 were known for 75; two participants discharged alive at days 9 and 10 following enrollment did not have further follow-up and were assumed to be alive at day 21.

Patient Characteristics

The distribution of diagnoses and outcomes are shown in Table 1. Subjects with a defined diagnosis accounted for 65% (n=50) of the cohort. Three subjects (6%) with a defined diagnosis died and 10 (20%) underwent LT. Of the 27 subjects with indeterminate ALF, two (7%) died and 11 (41%) underwent LT. Thirty one percent of the study population was younger than 5 years (Table 2). At presentation, 11% of the subjects had coma grade III or IV. Peak coma grade was grade III or IV in 16% of subjects.

T-cell Immune Activation Markers

sIL2Rα levels were not determined for 2 of 77 subjects due to lab errors; 1 of these subjects died and the other survived with native liver. Of the 75 subjects with measured sIL2Rα levels, 50 (67%) survived with their native liver, 21 (28%) received a LT, and 4 (5%) died (Table 3). Note that, among all the NK function measurements, only sIL2Rα levels differed significantly across the three outcomes groups after accounting for the multiple comparisons.

Outcome differed significantly (p=0.04) by level of sIL2Rα (Table 4). Among the 37 subjects with normal sIL2Rα levels, 30 (81%) were alive with native liver, 7 (19%) underwent LT, and none died within 3 weeks of entry into the study. At the other extreme, only 5 of the 15 subjects with markedly elevated sIL2Rα (≥5000 IU/mL) were alive with

their native liver, whereas 8 (53%) underwent LT, and 2 died (13%) by 21 days after enrollment.

For the 15 subjects with sIL2Rα levels at least 5000 IU/mL, the final clinical diagnosis was indeterminate for 10 (67%) subjects, hemophagocytic lymphohistiocytosis (HLH) for 3 subjects, drug-induced disease for one subject and autoimmune disease for one subject. For the 60 patients with sIL2Rα levels less than 5000 IU/mL, 16 (27%) were indeterminate.

No markers of T-cell immune activation other than sIL2Rα differed significantly among the outcomes when adjusting for the multiple comparisons. The associated between sIL2Rα and the number of CD8 cells was weak (Kendalll's $\tau = 0.16$, $p=0.07$).

Discussion

sIL2Rα was associated with outcome in this group of children with PALF. While none of the 37 children with normal sIL2Rα died, 9% of those with values more than normal but less than 5000 IU/ml, and 13% of those with values at least 5000 IU/ml died. LT was also more common among the groups with higher sIL2Rα.

Individual or small pairings of cytokines have been tested as biomarkers that might inform disease severity or outcome. For example, elevated serum levels of alpha-fetoprotein, a markers of liver regeneration, has been associated with a favorable outcome in patients with acetaminophen induced liver injury (18), but the rate of rise early in the course may be more important than the absolute value (19). Soluble CD154 (sCD154), a marker of immune activation expressed on activated CD4+ T-cells and platelets, was associated with death in adult patients with acute liver failure (20). A panel of pro- and anti-inflammatory markers were assessed in adults with acute liver failure before and after treatment with the molecular absorbent recirculating system (MARS) (21). While MARS did not alter circulating cytokine levels, investigators found IL-6 and IL-8 levels were decreased in patients who survived with their native liver, IL-6 was increased in those who either died or received LT, and initial values for sIL-2Rα were higher in patients who died or received a liver transplant relative to those who survived with their native liver.

The rarity and patient heterogeneity of PALF leads to practical and logistical difficulties related to predicting outcomes in PALF. Establishing reliable predictive models are confounded by liver transplantation, an intervention that is influenced by local experience and organ availability. Inclusion of death and LT into a single outcome is problematic as the two outcomes are not similar. Those receiving LT constitute a mixed cohort of participants who would have died or survived had LT not interrupted the natural history of PALF. The ideal predictive model will include markers that will reliably predict meaningful outcomes that include survival with native liver, death with native liver, and futility to inform LT decisions.

Our examination of a variety of markers of T-cell immune activation in a small PALF cohort identified only sIL2Rα to be discriminatory between survival with native liver, death, and LT. Acute liver failure is associated with myriad processes that variably affect outcome including immune activation, immune inactivation, apoptosis, and liver regeneration. We have previously reported a biomarker profile of hepatic regeneration associated with survival with native liver (22). Given the complex physiologic components associated with outcome in PALF, a mechanistic modeling platform that incorporates these and other components should be pursued to develop a more reliable predictive model for outcomes in PALF (23).

This study has several weaknesses. This exploratory work was done as a cross-sectional study with sIL2Rα measured at a single point in time and consequently the findings cannot reflect dynamic changes in immune activation. Those participants who died or underwent transplantation following enrollment in PALF but prior to the blood sample taken for NK cell analysis were not available for analysis. From these data, we cannot determine if sIL2Rα levels reflect a response to liver injury or reflect a role in the etiopathogenesis of PALF.

These preliminary data suggest patients with higher sIL2Rα levels were more likely to die or undergo liver transplantation within 21 days than those with lower levels. Identifying a subset of patients with evidence of immune activation and at risk for poor outcome may form the foundation for targeted clinical trials with immunomodulatory drugs. In addition, sIL2Rα should be considered as a potential component in future PALF disease severity scores when they are developed. Improved ability to predict hepatic recovery may spare children from unnecessary LT, while identifying those who are likely to die will allow more timely decisions to move to LT.

Acknowledgments

The authors give special thanks to Andre Hawkins, the research coordinator at Cincinnati Children's Hospital who coordinated the efforts across centers. The project was funded by NIH-NIDDK 1UO1-DK072146. The work was made possible by the collaborative effort of principal investigators and research coordinators at the University of Pittsburgh, Mt. Sinai Medical Center, Cincinnati Children's Hospital, Children's Memorial Hospital (Chicago), Kings College-London (England),University of Texas Southwestern, Texas Children's Hospital, Seattle Children's Hospital, Children's Hospital Colorado (Aurora) Children's Hospital Medical Center (Boston), St Louis Children's Hospital, Johns Hopkins University, Columbia University, University of California at San Francisco, Hospital for Sick Children (Canada), Columbia University, Riley Hospital for Children (Indianapolis). Supported in part by NIH/NCRR Colorado CTSI Grant Number UL1 RR025780. Contents are the authors' sole responsibility and do not necessarily represent official NIH views.

Grant Support Supported by the Division of Digestive Diseases and Nutrition within the National Institute of Diabetes and Digestive and Kidney (NIDDK) Diseases of the National Institute of Health (NIH) (5U01 DK072146).

Abbreviations

sIL2α	Soluble interleukin 2 receptor alpha
PALF	Pediatric acute liver failure
LT	Liver Transplantation
NK	Natural Killer
HLH	Hemophagocytic lymphohistiocytosis
NIDDK	National Institute of Diabetes and Digestive and Kidney
NIH	National Institutes of Health
EDTA	Ethylenediaminetetraacetic acid
Na	Sodium
INR	International normalized ratio
PT	Prothrombin time
MCF	Mean Channel Fluorescence

References cited

1. Squires RH Jr. Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr. 2006; 148(5):652–8. [PubMed: 16737880]
2. Dhiman RK, Seth AK, Jain S, Chawla YK, Dilawari JB. Prognostic evaluation of early indicators in fulminant hepatic failure by multivariate analysis. Dig Dis Sci. 1998; 43(6):1311–6. [PubMed: 9635624]
3. Liu E, MacKenzie T, Dobyns EL, Parikh CR, Karrer FM, Narkewicz MR, et al. Characterization of acute liver failure and development of a continuous risk of death staging system in children. J Hepatol. 2006; 44(1):134–41. [PubMed: 16169116]
4. Lu BR, Gralla J, Liu E, Dobyns EL, Narkewicz MR, Sokol RJ. Evaluation of a scoring system for assessing prognosis in pediatric acute liver failure. Clin Gastroenterol Hepatol. 2008; 6(10):1140–5. PMCID: 2581795. [PubMed: 18928939]
5. Pelaez-Luna M, Martinez-Salgado J, Olivera-Martinez MA. Utility of the MAYO End-Stage Liver Disease score, King's College Criteria, and a new in-hospital mortality score in the prognosis of in-hospital mortality in acute liver failure. Transplant Proc. 2006; 38(3):927–9. [PubMed: 16647512]
6. Rhee C, Narsinh K, Venick RS, Molina RA, Nga V, Engelhardt R, et al. Predictors of clinical outcome in children undergoing orthotopic liver transplantation for acute and chronic liver disease. Liver Transpl. 2006; 12(9):1347–56. [PubMed: 16741901]
7. Sindhi R, Soltys K, Bond G, Marcos A, Mazariegos G. PELD allocation and acute liver/graft failure. Liver Transpl. 2007; 13(5):776–7. [PubMed: 17457898]
8. Katoonizadeh A, Decaestecker J, Wilmer A, Aerts R, Verslype C, Vansteenbergen W, et al. MELD score to predict outcome in adult patients with non-acetaminophen-induced acute liver failure. Liver Int. 2007; 27(3):329–34. [PubMed: 17355453]
9. Polson J, Lee WM. AASLD position paper: the management of acute liver failure. Hepatology. 2005; 41(5):1179–97. [PubMed: 15841455]
10. Shakil AO, Kramer D, Mazariegos GV, Fung JJ, Rakela J. Acute liver failure: clinical features, outcome analysis, and applicability of prognostic criteria. Liver Transpl. 2000; 6(2):163–9. [PubMed: 10719014]
11. Yazigi N, Tial G, Filipovich A, Bucuvalas JC. Natural Killer Dysfunction in Pediatric Acute Liver Failure. Am J Transplant. 2008; 8(Supp s2):327A.
12. Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. Hepatology. 2000; 32(4 Pt 1):734–9. [PubMed: 11003617]
13. Filipovich AH. Life-threatening hemophagocytic syndromes: current outcomes with hematopoietic stem cell transplantation. Pediatr Transplant. 2005; 9(Suppl 7):87–91. [PubMed: 16305623]
14. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007; 48(2):124–31. [PubMed: 16937360]
15. Kogawa K, Lee SM, Villanueva J, Marmer D, Sumegi J, Filipovich AH. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. Blood. 2002; 99(1):61–6. [PubMed: 11756153]
16. Sullivan KE, Delaat CA, Douglas SD, Filipovich AH. Defective natural killer cell function in patients with hemophagocytic lymphohistiocytosis and in first degree relatives. Pediatr Res. 1998; 44(4):465–8. [PubMed: 9773832]
17. Filipovich AH. Hemophagocytic lymphohistiocytosis and other hemophagocytic disorders. Immunol Allergy Clin North Am. 2008; 28(2):293–313. viii. [PubMed: 18424334]
18. Schmidt LE, Dalhoff K. Alpha-fetoprotein is a predictor of outcome in acetaminophen-induced liver injury. Hepatology. 2005; 41(1):26–31. [PubMed: 15690478]
19. Schiodt FV, Ostapowicz G, Murray N, Satyanarana R, Zaman A, Munoz S, et al. Alpha-fetoprotein and prognosis in acute liver failure. Liver Transpl. 2006; 12(12):1776–81. [PubMed: 17133565]
20. Zheng YB, Gao ZL, Zhong F, Huang YS, Peng L, Lin BL, et al. Predictive value of serum-soluble CD154 in fulminant hepatic failure. J Int Med Res. 2008; 36(4):728–33. [PubMed: 18652769]

21. Ilonen I, Koivusalo AM, Repo H, Hockerstedt K, Isoniemi H. Cytokine profiles in acute liver failure treated with albumin dialysis. Artif Organs. 2008; 32(1):52–60. [PubMed: 18181803]

22. Rudnick DA, Dietzen DJ, Turmelle YP, Shepherd R, Zhang S, Belle SH, et al. Serum alpha-NH-butyric acid may predict spontaneous survival in pediatric acute liver failure. Pediatr Transplant. 2009; 13(2):223–30. [PubMed: 18643912]

23. Mi Q, Li NY, Ziraldo C, Ghuma A, Mikheev M, Squires R, et al. Translational systems biology of inflammation: potential applications to personalized medicine. Per Med. 2010; 7(5):549–59. PMCID: 3041597. [PubMed: 21339856]

Table 1

Distribution of final diagnosis and 21-day outcome

Diagnosis	Total n	Alive* n (%)	Died n (%)	Transplanted n (%)
Acetaminophen	13	13 (100.0)	0 (0.0)	0 (0.0)
Autoimmune marker positive ALF	8	7 (87.5)	0 (0.0)	1 (12.5)
Drug-induced (non-APAP)	2	2 (100.0)	0 (0.0)	0 (0.0)
Viral	6	4 (66.7)	1 (16.7)	1 (16.7)
Metabolic	7	3 (42.9)	0 (0.0)	4 (57.1)
Other	14	8 (57.1)	2 (14.3)	4 (28.6)
Shock/ischemia	3	2	1	0
HLH	3	0	1	2
Veno-occlusive disease	1	1	0	0
Sepsis	1	1	0	0
Meningoencephalitis	1	1	0	0
Influenza A	1	0	0	1
Possible autoimmune related	1	0	0	1
Shock/Ischemia & drug induced hepatitis	1	1	0	0
Drug-induced hepatitis & Sepsis	1	1	0	0
Unspecified multiple diagnoses	1	1	0	0
Indeterminate	27	14 (51.9)	2 (7.4)	11 (40.7)
All categories	77	51	5	21

*Discharged alive within 21 days of enrollment or known to be alive without transplantation at least 21 days following enrollment

Table 2

Demographic, Clinical Characteristic and Severity Scores

	n (%)
Age	
< 5 yrs old	24 (31.2)
5 – 9 yrs old	19 (24.7)
> 9 yrs	34 (44.2)
Median (yrs)	9.0
25th , 75th (yrs)	3.5, 16.0
Sex	
Male	34 (44.2)
WBC at presentation (1000/mm^3)	
N	67
Median	8.1
25th , 75th	5.6, 11.1
ALT at presentation (IU/l)	
N	71
Median	2166.0
25th , 75th	719.0, 3973.0
Total bilirubin at presentation (mg/dl)	
N	56
Median	8.9
25th , 75th	2.4, 19.1
INR at presentation	
N	75
Median	2.3
25th , 75th	2.0, 3.1
Coma Grade at presentation	
Missing	6
0-II	63 (88.7%)
III- IV	8 (11.3%)
Peak Coma Grade during first 7 days	
Missing	3
0-II	62 (83.8%)
III-IV	12 (16.2%)
Admission LIU score	
N	55
Median	220.5

	n (%)
25th , 75th	141.1, 297.5

J Pediatr Gastroenterol Nutr. Author manuscript; available in PMC 2014 March 01.

Table 3

Age-specific normalized[#] NK function measurements by 21-day outcome

	LT (n=21)		Death (n=5)		Survival with native liver (n= 51)		P-value from Kruskal-Wallis test [&]
NK measurements	**N of available samples**	**Median (Minimum, Maximum)**	**N of available samples**	**Median (Minimum, Maximum)**	**N of available samples**	**Median (Minimum, Maximum)**	
Normalized sIL2Rα levels	21	1.44 (0.30, 13.72)	4	5.20 (1.10, 18.56)	50	0.83 (0.12, 37.69)	0.001
Normalized CD56 bright	20	0.23 (0.05, 2.08)	3	0.13 (0.03, 0.31)	47	0.43 (0, 2.95)	0.06
Normalized NK lytic activity	18	0.77 (0, 5.55)	4	0.02 (0, 2.10)	47	0.97 (0, 7.03)	0.27
Normalized NK Perforin +	20	0.91 (0.43, 1.07)	3	0.96 (0.89, 0.99)	47	0.87 (0.35, 1.07)	0.64
Normalized NK Perforin MCF	20	0.88 (0.37, 1.69)	3	0.71 (0.69, 0.80)	48	0.64 (0, 1.25)	0.07
Normalized Perforin + CD8	20	0.38 (0, 7.97)	5	1.10 (0.10, 2.88)	50	0.13 (0, 4.15)	0.06
Normalized NKT Perforin	15	0.33 (0, 1.82)	1	0.12 (--, --)	30	0.25 (0, 2.61)	0.67
Normalized Granzyme B NK% +	20	0.92 (0.62, 1.03)	3	0.78 (0.77, 0.95)	47	0.89 (0.41, ,1.02)	0.50
Normalized Granzyme B NK MCF	20	1.29 (0.55, 2.63)	3	1.25 (0.80, 2.86)	47	0.85 (0.50, 3.38)	0.04
Normalized Granzyme B + CD8	19	0.97 (0.06, 3.03)	5	0.16 (0.06, 2.00)	49	0.29 (0, 2.81)	0.048
Normalized Granzyme B + NKT	16	0.60 (0.25, 1.48)	1	0.36 (--, --)	31	0.60 (0, 1.40)	0.79
Normalized %CD8	20	1.07 (0.44, 2.55)	5	0.91 (0.49, 2.38)	51	0.67 (0.32, 2.24)	0.048
Normalized CD8 absolute	17	0.38 (0.09, 1.51)	5	0.27 (0.02, 1.07)	40	0.33 (0.06, 1.90)	0.48
Normalized %CD16/56	20	0.19 (0, 0.52)	5	0.15 (0.11, 0.42)	51	0.19 (0.03, 0.91)	0.99
Normalized CD16/56 absolute	17	0.08 (0, 0.34)	5	0.03 (0.007, 0.27)	40	0.10 (0.007, 0.69)	0.47

[&]P-value from Kruskal-Wallis test to compare the groups of died subjects, subjects with LT and those alive with native liver;

[#]Normalized result: assay results divided by the upper limit of the age-specific normal range.

Table 4

Outcomes by sIL2Rα groups (n=75)

	LT	Death	Survival with native liver
sIL2Rα in the age-specific normal range (n=37)	7 (19%)	0 (0%)	30 (81%)
sIL2Rα greater than the upper limit of age-specific normal and less than 2 times the upper limit of age-specific normal (n=15)	4 (27%)	1 (7%)	10 (67%)
sIL2Rα greater than or equal to 2 times the upper limit of age-specific normal and less than 5000 IU/ml (n=8)	2 (25%)	1 (13%)	5 (63%)
sIL2Rα at least 5000 IU/ml (n=15)	8 (53%)	2 (13%)	5 (33%)

P-value from Pearson Chi-square exact test for association is 0.04