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Serum α-NH_2-Butyric Acid May Predict Spontaneous Survival in Pediatric Acute Liver Failure

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Abstract

Acute liver failure (ALF) is a serious, often fatal condition. Up to half of pediatric ALF (PALF) patients do not survive without liver transplantation; however, early identification of those least likely to survive spontaneously remains difficult. Clinical experience suggests that recovery from ALF depends on the ability of the liver to regenerate. Based on this, we hypothesized that bio-markers of hepatic regeneration could have utility as predictors of recovery from PALF. In the studies reported here, we used comprehensive amino acid analysis to search for novel metabolomic markers of liver regeneration in mice subjected to partial hepatectomy. This analysis identified α-NH_2-adipic acid and α-NH_2-butyric acid as significantly increased in liver and plasma samples from mice subjected to partial hepatectomy compared to controls. Next, we tested whether serum levels of these markers were associated with clinical outcomes in PALF patients. This examination, performed on the initially collected serum samples from 40 randomly selected patients enrolled in the PALF Study Group, showed increased α-NH_2-butyric-acid (Aab) and Aab:leucine (Aab:Leu) ratio in patients who survived without transplantation compared to those who were transplanted or died. These data indicate that Aab and the Aab:Leu ratio may predict clinical outcomes in PALF.

Keywords

liver; acute liver failure; biomarkers; animal; model

Introduction

Acute liver failure (ALF) is a severe, frequently fatal disorder in which previously healthy individuals present with significant acute liver injury and dysfunction characterized by varying degrees of hypertransaminasemia, coagulopathy, and encephalopathy (1;2). Clinical management of ALF is primarily supportive until recovery or death occurs or until orthotopic liver transplantation is performed. Approximately half of all adult (1) and pediatric (2) ALF patients do not recover without liver transplantation. However, early identification of those least likely to survive spontaneously remains extraordinarily challenging. As liver transplant list waiting times increase and suitable donor livers remain

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in short supply, timely recognition of ALF patients most likely to require transplantation in order to survive has become increasingly important (3) Currently, adult ALF patients are generally considered for liver transplantation based on King's College Hospital (KCH) Criteria (4). Such prognostication is based on etiology, age, plasma creatinine, and degrees of encephalopathy, acidosis, and coagulopathy. Various other predictive strategies have also been examined, including the Clichy criteria (5), serum phosphate (6;7), the MELD (Model for End-Stage Liver Disease) score (8), the APACHE II measurements (9;10), coagulation factors V and VII (11;12), histological evidence of liver regeneration on biopsy (13;14), hepatic volume on CT scan (15), serum α-fetoprotein (16–18), and serum actin-free Gc-globulin (19). Each of these models has significant limitations, and improved strategies for predicting outcomes in ALF continue to be sought (20). In addition, a pediatric ALF (PALF) risk-stratifying algorithm based on peak prothrombin time (PT), bilirubin, and ammonia has been reported (21). Nevertheless, the clinical value of KCH Criteria and each of these other models in predicting clinical outcomes in pediatric patients with ALF remains untested (22;23).

Spontaneous recovery from ALF depends, in part, on the ability of the liver to regenerate following acute injury. Furthermore, low rates of spontaneous recovery from ALF in adult patients with idiopathic and drug-induced liver failure is thought to result from a low capacity for such regeneration (17). Therefore, we hypothesized that the presence of serum bio-markers of liver regeneration detectable at the time of clinical presentation might distinguish PALF patients most likely to survive without liver transplantation from those whose native livers are not recovering. In the experiments reported here, we performed comprehensive amino acid analysis on liver and corresponding plasma samples from mice subjected to partial hepatectomy (24) to identify novel metabolomic bio-markers of liver regeneration. Next, we tested whether levels of these markers determined on archived samples of serum from a randomly selected subset of patients enrolled in the NIH-sponsored PALF Study Group were predictive of clinical outcome.

Materials and Methods

Animal Husbandry and Surgery

8–12 week old C57Bl/6J mice (Jackson Laboratory, Bar Harbor, ME), maintained on 12 h dark-light cycles and standard mouse chow, were subjected to two-thirds partial hepatectomy, allowed to recover, and then sacrificed for plasma and tissue harvest as previously described (25–27). Three to six animals were examined at each time point. These experiments were approved by the Animal Studies Committee of Washington University and conducted in accordance with institutional guidelines and the criteria outlined in the “Guide for Care and Use of Laboratory Animals” (NIH publication 86-23).

Patient Sample Analysis and Study Design

The NIH-sponsored PALF Study Group is collecting and maintaining a database of clinical information and a repository of serially collected serum samples on consenting children with PALF. At the time samples were drawn for this study 556 patients were enrolled in the study, including 312 who survived at least 21 days without liver transplantation and 244 who did not. For this case-control study, we obtained from the PALF study group an aliquot of the initially collected serum sample and corresponding clinical data from 20 randomly selected patients in each of 2 outcomes groups: (a) survival without transplantation, and (b) either transplantation or death. The number of samples analyzed was chosen based on a power calculation using plasma amino acid analysis data obtained during standard clinical management of 10 PALF patients cared for at St. Louis Children’s Hospital from 2002–2006, the results of which indicated that 20 patients in each outcomes group had at least

80% power to detect significant differences ($p<0.05$) between groups in levels of markers identified as associated with hepatic regeneration in the mouse model. Random sample selection was conducted by the PALF Study Group Data Coordinating Center, and serum amino acid analysis was performed without knowledge of outcome group. Patient demographic and clinical data, including age, gender, initial encephalopathy grade, etiology, and number of positive KCH Criteria at the time of presentation were compared between patient outcomes groups. These experiments were conducted in accordance with institutional guidelines and those set forth in the 1975 Declaration of Helsinki, and were approved by the Human Studies Institutional Review Board of Washington University.

Amino acid analysis

Amino acid analyses on mouse liver and plasma and human serum was performed by the St. Louis Children's Hospital Metabolic Genetics Laboratory. Whole tissue lysates of mouse liver were prepared at a density of 100 mg/mL as previously described (25). Blood and tissue samples were deproteinated using 0.7% sulfosalicylic acid. Free amino acids were quantified by cation exchange chromatography using a LiCl step gradient and s-aminoethylcysteine as internal standard on a Beckman 7300 (Beckman-Coulter: Brea, CA). Amino acid-ninhydrin conjugates were identified by retention time and quantified using integrated peak areas. Imprecision of measurement was 5–7% across a concentration range of 4–650 μmol/L. The limit of detection (concentration distinguishable from zero) for each metabolite was 1 μmol/L.

Statistical Analysis

SigmaStat (SPSS, Chicago, IL), SAS (SAS Institute Inc., Cary, NC), and Stata (StataCorp LP, College Station TX) were used for all analyses. Data are reported as mean ± standard error. For evaluating the amino acid analysis data obtained on mouse liver and plasma, Student's t test was used to compare detectable metabolite levels between un-operated, sham operated, and regenerating livers and also between corresponding plasma samples (with $p<0.05$ used as threshold for significance). In cases where a specific metabolite was un-detectable in all replicates from one group, *i.e.* where a test of normality failed, the nonparametric Wilcoxon Rank Sum test was used to compare these groups ($p<0.05$). For evaluating human serum, median and range are reported in addition to mean ± standard error and the Wilcoxon Rank Sum test was used to compare levels of metabolites between patients who survived without transplantation and those who either died or were transplanted ($p<0.05$). Pearson's chi-square test for association (without continuity correction) or Fisher's exact test was used to compare categorical patient demographic, clinical, and amino acid data between outcomes and to compare patient outcomes at α-NH_2-butyric acid:leucine ratio cutoff points selected to maximize discrimination. Receiver-Operator-Characteristic (ROC) curves for α-NH_2-butyric acid:leucine, PT, and bilirubin were generated and areas under the fitted curve compared using a nonparametric test.

Results

Novel Metabolomic Markers of Murine Liver Regeneration

Results of amino acid analysis on liver tissue and plasma recovered from mice subjected to partial hepatectomy were compared to those from un-operated and sham-operated animals. This analysis identified two amino acids that were significantly increased in murine samples of both liver and plasma recovered 6 hours after partial hepatectomy (compared to controls, Table 1): α-NH_2-adipic acid (Aaa) and α-NH_2-butyric acid (Aab). Subsequent analysis of duplicate liver samples at serial times from 0–72 hours after partial hepatectomy or sham surgery verified the specificity of these changes and demonstrated the kinetics of appearance and disappearance of each of these metabolites in regenerating liver (Figure 1).

Metabolomic Markers of Liver Regeneration in PALF Patient Serum

Levels of Aaa and Aab were determined on the initially collected serum sample from 20 randomly selected PALF patients in each of two outcomes groups (survival with native liver versus death/transplant). Demographic and clinical data such as age, gender, initial encephalopathy grade, etiology, and number of positive KCH Criteria at the time of presentation were comparable and did not distinguish between patients in the two groups (Table 2–Table 4). As previously reported (2), both PT and total bilirubin were significantly lower in serum samples from spontaneous survivors compared to those from patients in the death/transplant group (Figure 2 and Table 3). Comparison of serum metabolite levels between these groups showed no significant differences in Aaa levels (p=0.3), which were undetectable in the majority (37/40) of study patients. In contrast, levels of Aab were significantly greater in patients who survived without transplantation (29.2±5.9 μmol/l; median 22.5 μmol/l) compared to those who died or were transplanted (13.9±3.2 μmol/l, median 10.5 μmol/l, p=0.02, Figure 3). Based on these data, taken together with previously published studies proposing that the ratio of serum Aab to leucine (Aab:Leu) may be a specific indicator of alcoholic (28) and other forms (29) of liver injury, Aab:Leu was compared between outcomes groups. The results showed that this ratio was also significantly greater in PALF patients who survived with their native livers (0.29±0.05, median 0.25) compared to those who died or were transplanted (0.14±0.03, median 0.13, p=0.003, Figure 3). No significant difference in serum leucine levels between groups was observed (data not shown).

Prognostic Value of Aab:Leu, PT, and Bilirubin on PALF Outcomes

Analyses of Aab:Leu thresholds selected to maximize accuracy of prediction of spontaneous survival (for Aab:Leu ≥ threshold), death or transplant (for Aab:Leu < threshold), or both showed that 80% (12 of 15) of patients with an Aab:Leu ratio of at least 0.210 survived without transplantation and 68% (17/25) of patients with a ratio less than 0.210 either died or were transplanted (p=0.006, Table 5), for an overall diagnostic accuracy of 72.5% (29/40) at this threshold. This predictive accuracy is better than that of KCH criteria (4) with which only 55% (22/40) of clinical outcomes are correctly predicted when using a cutoff of 3 or more positive KCH indicators (Table 2 and Table 4). However, the predictive accuracy of KCH criteria in this analysis could be confounded, in part, by absence of any patients with PT > 100 seconds (Table 3) which may be the result of differences in PT determination (30) in North America (39/40 study patients including 19/20 spontaneous survivors) versus the United Kingdom (1/40 study patients).

As noted above, PT and bilirubin were significantly lower in spontaneous survivors of PALF compared to those who died or underwent liver transplantation (Figure 2, Table 3 and (2)). Comparison of ROC analysis areas under the curve for Aab:Leu, PT, and bilirubin indicated no significant differences (Figure 4). Further analysis showed that combinations of Aab:Leu and PT thresholds that maximize the positive (PPV) and negative (NPV) predictive value of each individual parameter (Table 5 and Table 6) can result in overall improvement in outcomes prognostication compared to that provided by either parameter alone (Illustrated in Table 7).

Discussion

In the studies reported here, comprehensive amino acid analysis was used to identify Aaa and Aab as novel metabolomic markers of liver regeneration in the murine partial hepatectomy model. The mechanisms that account for increased hepatic and circulating levels of these amino acids are speculative and remain unknown. Aaa is a metabolic breakdown product of lysine (31), and it is intriguing that our analysis also showed

increased lysine levels in regenerating mouse liver (data not shown). Aab is generated during catabolism of methionine and threonine (31). Together, these data suggest that precise regulation of lysine, methionine, or threonine metabolism may be important for normal hepatic regeneration.

Based on the hypothesis that recovery from ALF is dependent on regeneration of the injured liver, the metabolomic markers of liver regeneration identified in the mouse model were tested for their ability to predict clinical outcomes in PALF. The results showed that levels of Aab but not those of Aaa are significantly correlated with such outcomes. The explanation for the discordance between Aaa and Aab as markers of liver regeneration versus predictors of PALF outcomes is unknown, and may reflect differences in mouse versus human amino acid metabolism. Alternatively, these data raise the intriguing possibility that Aaa and Aab may correlate with distinct stages of the hepatic regenerative response, a consideration supported by data reported here showing more rapid and complete disappearance of Aaa than Aab from regenerating mouse liver 72 hours after partial hepatectomy (Figure 1). These observations also indicate that further investigation for additional (*e.g.* non-amino acid) metabolomic markers of hepatic regeneration may lead to the identification of other novel predictors of ALF outcomes.

Because the analysis of PALF serum reported here was performed on the initially-collected sample after enrollment into the Study Group, our data indicate that determination of serum Aab and the Aab:Leu ratio at the time of clinical presentation may be useful as an early predictor of outcome in PALF. The methodology used here for serum amino acid analysis can be completed in as little as several hours, and newer techniques utilizing mass spectrometry may permit similar analyses in less than an hour (32) thus allowing this test to be available for clinical decision making. Further investigations are warranted to verify the findings reported here, to characterize the temporal pattern of change in serum Aab:Leu in patients with ALF, and to prospectively evaluate the utility of this marker for predicting outcomes in pediatric and adult ALF in comparison to and in concert with other algorithms. For example, the data reported here show that thresholds of Aab:Leu and PT can be identified with which PALF outcomes can be predicted with greater reliability than is possible using either parameter alone (Table 5–Table 7). Future studies should address whether the predictive value of Aab and Aab:Leu varies with clinical or demographic variables. While plasma Aab:Leu has been reported to be elevated in alcoholic (28) and various metabolic forms (29) of liver injury, the utility of this marker for predicting outcomes in ALF caused by infectious, inflammatory, ischemic, and other toxic and metabolic causes of liver injury is unknown and should be determined. Finally, comparison of initial Aab:Leu levels between ALF patients who die prior to liver transplantation, those who are transplanted, and spontaneous survivors, and examination of levels in patients transplanted earlier with live donor-grafts versus those transplanted later with cadaveric grafts should be performed. Such analyses could lead to strategies for identification of those ALF patients who undergo liver transplantation but might have recovered spontaneously if given the opportunity. If the results of future studies confirm the observations reported here, serum amino acid analyses will become an important diagnostic tool with which to evaluate and direct clinical management of ALF patients.

Acknowledgments

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Medical School (Maureen Jonas MD), Hospital for Sick Children (Toronto, Vick Ng MD), Indiana University Riley Hospital (Girish Subbarao MD), Johns Hopkins University (Kathleen Schwartz MD), King's College Hospital (UK, Anil Dhawan MD), Mt. Sinai School of Medicine (Sukru Emre MD), Northwestern University (Estella Alonso MD), University of California, San Francisco (Philip Rosenthal MD), University of Cincinnati (John Bucuvalas MD, Nada Yazigi MD), University of Colorado (Michael Narkewicz MD), University of Michigan (M. James Lopez MD-PhD), University of Pennsylvania (Liz Rand MD), University of Texas Southwestern (Norberto Rodriguez Baez MD), University of Washington (Seattle, Karen Murray MD), Washington University (St. Louis, David Rudnick MD-PhD, Ross Shepherd MD). The authors are also grateful for support from National Institutes of Health (Patricia R. Robuck PhD-MPH, Director Clinical Trials Program, DDDN-NIDDK) and for assistance from members of the Data Coordinating Center (directed by Steven Belle PhD-MScHyg).

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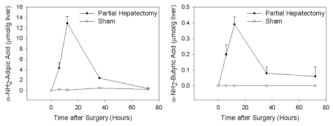

Figure 1. Change of α-NH_2-Adipic Acid and α-NH_2-Butyric Acid in Regenerating Mouse Liver over Time

The mean and range of 2–4 replicate determinations of α-NH_2-adipic acid (left panel) and α-NH_2-butyric acid (right panel) levels on liver extracts for each time point and surgical group is shown.

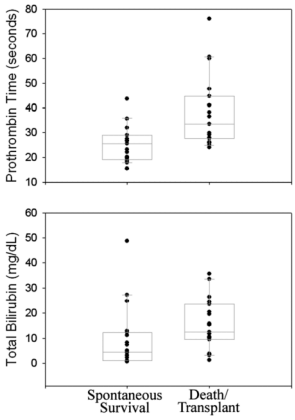

Figure 2. Scatter and Box Plots of PT and Bilirubin in PALF Patient Serum Samples

Results from PALF patients who spontaneously survived are compared to those from patients who died or were transplanted (10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile are indicated).

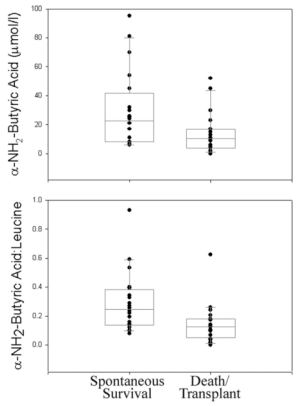

Figure 3. Scatter and Box Plots of α-NH_2-Butyric Acid and the α-NH_2-Butyric Acid:Leucine Ratio in PALF Patient Serum Samples

Results from PALF patients who spontaneously survived are compared to those from patients who died or were transplanted (10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile are indicated).

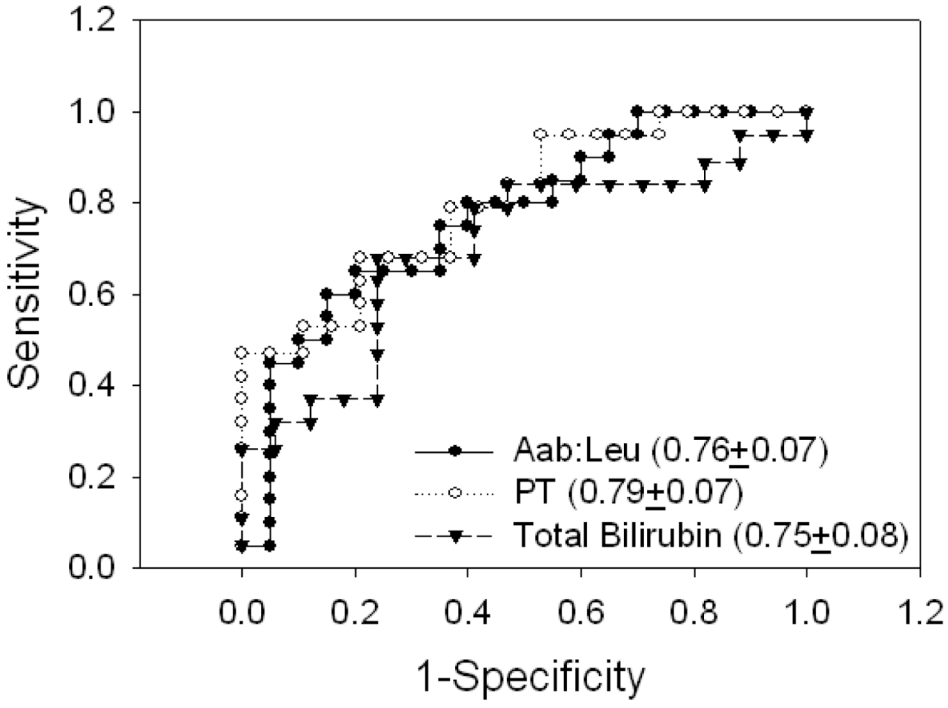

Figure 4. ROC Analysis of α-NH_2-Butyric Acid:Leucine (Aab:Leu), Prothrombin Time (PT), and Total Bilirubin on PALF Outcomes

Areas under the fitted curve (AUC) and estimated standard error are shown.

Table 1

Amino Acids Higher in Both Regenerating Mouse Liver and Corresponding Plasma Recovered Six Hours after Partial Hepatectomy

Amino Acid	Surgery	Liver (μmol/g)	*p* value	Plasma (μmol/l)	*p* value
α-NH_2-adipic acid	None	0.0±0.0	0.03[b]	2.3±2.3	0.04[a]
	Sham	0.2±0.1	0.03[a]	4.0±2.6	0.05[a]
	Partial Hepatectomy	4.3±1.1	-	22.0±5.0	-
α-NH_2-butyric acid	None	0.0±0.0	0.006[b]	1.0±1.0	0.02[a]
	Sham	0.0±0.0	0.03[b]	0.0±0.0	0.02[b]
	Partial Hepatectomy	0.2±0.1	-	12.3±2.6	-

[a] Student's t test versus Partial Hepatectomy

[b] Wilcoxon Rank Sum test versus Partial Hepatectomy (see Materials and Methods)

Table 2

Summary of Demographic and Clinical Data for Study Patients

Parameter	Survival with Native Liver (n=20)	Death/Transplant (n=20)	*p* value
Age			0.80[a]
<2 years	40%	30%	
2–10 years	25%	30%	
>10 years	35%	40%	
Gender (% female)	65%	40%	0.11[a]
Initial Encephalopathy Grade[d]			0.58[b]
0–1	80%	65%	
2–4	20%	30%	
Diagnosis			0.54[b]
Indeterminate	45%	70%	
Acetaminophen	15%	0%	
Other	40%	30%	
Application of King's College Hospital Criteria on Enrollment			
Acetaminophen[e]	(n=3)	(n=0)	
	0–1 100%	0–1 --	
	2–3 0%	2–3 --	
			0.25[c]
Non-acetaminophen	(n=17)	(n=20)	
(# of positive indicators)	0–1 88%	0–1 70%	
	2 12%	2 20%	
	3 0%	3 10%	

[a] Pearson chi-square test for association

[b] Pearson chi-square exact test

[c] Fisher's exact test

[d] Data not available from 1 patient in 'Death/Transplant' group

[e] pH>7.3 or any 1 of PT> 100s and Serum Cr > 300 μM with grade III or IV encephalopathy

Table 3

Detailed Demographic and Clinical Data for Study Patients

	Survival with Native Live N=20 (%)	Death/Transplant N=20 (%)	*p* value
Outcome			
Death without transplant	0 (0.0)	4 (20.0)	
Death after transplant	0 (0.0)	2 (10.0)	
Transplant only	0 (0.0)	14 (70.0)	
Survival with native liver	20 (100.0)	0 (0.0)	
Demographics			
Age (year)			
Less than 2 years old	8 (40.0)	6 (30.0)	0.80[a]
2 to 10 years old	5 (25.0)	6 (30.0)	
More than 10 years old	7 (35.0)	8 (40.0)	
Median	3.6	6.1	0.99[b]
Min, Max	0.1, 16.8	0.1, 17.0	
Sex			
Male	7 (35.0)	12 (60.0)	0.11[a]
Female	13 (65.0)	8 (40.0)	
King's College Criteria parameters	Survival with Native Liver	Death/Transplant	
Days from hospitalization to study enrollment			
Median	1	4	0.008[b]
Min, Max	0, 10	0, 18	
Days from first symptom to study enrollment			
# missing	0	2	
Median	6	13	0.03[b]
Min, Max	2, 159	2, 40	
pH at study admission			
# missing	11	11	
Median	7.4	7.4	0.99[c]
Min, Max	7.2, 7.5	7.2, 7.6	
Prothrombin time at study admission (sec)			
# missing	1	0	
Median	25.9	36.3	0.004[b]
Min, Max	15.4, 45.6	21.9, 76.0	
Serum creatinine at study admission (mg/dl)			
Median	0.6	0.6	0.65[b]

	Survival with Native Live N=20 (%)	Death/Transplant N=20 (%)	*p* value
Min, Max	0.2, 2.5	0.1, 2.7	
Total bilirubin at study admission (mg/dl)			
# missing	0	2	
Median	4.9	15.5	0.04[b]
Min, Max	0.6, 48.7	1.2, 33.6	
Potassium at study admission (mmol/L)			
# missing	0	2	
Median	3.8	3.8	0.56[b]
Min, Max	2.6, 5.5	3.0, 7.8	
Alpha-fetoprotein at study admission			
# missing	10	12	
Median	164	168	0.90[c]
Min, Max	13.0, 228000.0	0.8, 11345.0	
Encephalopathy grade at study admission			
# missing	0	1	
0	9 (45.0)	7 (36.8)	0.58[d]
I	7 (35.0)	6 (31.6)	
II	4 (20.0)	3 (15.8)	
III	0 (0.0)	2 (10.5)	
IV	0 (0.0)	1 (5.3)	
Initial diagnosis			0.47[d]
Acetaminophen	3 (15.0)	0 (0.0)	
Hemophagocytic syndrome	0 (0.0)	1 (5.0)	
Veno-occlusive disease	1 (5.0)	0 (0.0)	
Wilson’s disease	1 (5.0)	1 (5.0)	
Metabolic, other	2 (10.0)	3 (15.0)	
Hepatitis A	1 (5.0)	0 (0.0)	
Autoimmune hepatitis	2 (10.0)	1 (5.0)	
Drug-induced hepatitis	1 (5.0)	1 (5.0)	
Mushroom toxicity	1 (5.0)	0 (0.0)	
Indeterminate	8 (40.0)	13 (65.0)	

[a] Pearson chi-square test for association

[b] Wilcoxon rank sum test

[c] Wilcoxon rank sum exact test

[d] Fisher’s exact test

Table 4

Detailed Application of King's College Hospital Criteria to Study Patients

		Survival with Native Liver	Death/Transplant
For acetaminophen patients		N=3*	N=0*
1) pH < 7.3		0/0	-
or			
2) Any 1 of PT >100 s and serum creatinine >300 umol/L with grade III or IV encephalopathy		0/3	-
For nonacetaminophen patients		N=17*	N=20*
1)	PT >100 s	0/16	0/20
or			
2)	age <10	11/17	12/20
	Etiology: NANB, drug-induced hepatitis	1/17	1/20
	Jaundice >7days before coma onset	0/17	3/20
	PT>50 s	0/16	2/20
	Total bilirubin > 300 umol/L	3/17	7/18
	No positive indicator	4	3
	Any 1 of 5 indicators**	11[b]	11[a]
	Any 2 of 5 indicators**	2	4[c]
	Any 3 of 5 indicators	0	2

*For n/N, n represents the number of study patients positive for the indicator and N represents the number with information available for the indicator.

** *a*includes one patient with missing total bilirubin

*b*includes one patient with missing PT

*c*includes one patient with missing total bilirubin

Table 5

Performance of Aab:Leu for Predicting PALF Outcomes at Various Thresholds

Aab:Leu Threshold	Spontaneous Survival Predictive Value[a]	Death/Transplant Predictive Value[b]	Sensitivity[c]	Specificity[d]
0.05	20/35 (57%)	5/5 (100%)	20/20 (100%)	5/20 (25%)
0.1	18/31 (58%)	7/9 (78%)	18/20 (90%)	7/20 (35%)
0.15	14/21 (67%)	13/19 (68%)	14/20 (70%)	13/20 (65%)
0.19	13/17 (76%)	16/23 (70%)	13/20 (65%)	16/20 (80%)
0.2	12/16 (75%)	16/24 (67%)	12/20 (60%)	16/20 (80%)
0.21	12/15 (80%)	17/25 (68%)	12/20 (60%)	17/20 (85%)
0.25	10/12 (83%)	18/28 (64%)	10/20 (50%)	18/20 (90%)
0.30	7/8 (88%)	19/32 (59%)	7/20 (35%)	19/20 (95%)

[a] number of spontaneous survivors/number with Aab:Leu≥threshold

[b] number dead or transplanted/number with Aab:Leu<threshold

[c] number with Aab:Leu≥threshold/20 spontaneous survivors

[d] number with Aab:Leu<threshold/20 dead or transplanted

Table 6

Performance of PT for Predicting PALF Outcomes at Various Thresholds

PT Threshold (sec)	Spontaneous Survival Predictive Value[a]	Death/Transplant Predictive Value[b]	Sensitivity[c]	Specificity[d]
24	9/9 (100%)	19/29 (66%)	9/19 (47%)	19/19 (100%)
29	15/22 (68%)	12/16 (75%)	15/19 (79%)	12/19 (63%)
34	16/26 (62%)	9/12 (75%)	16/19 (84%)	9/19 (47%)
39	18/30 (60%)	7/8 (88%)	18/19 (95%)	7/19 (37%)
44	19/33 (58%)	5/5 (100%)	19/19 (100%)	5/19 (26%)

[a] number of spontaneous survivors/number with PT≤threshold

[b] number dead or transplanted/number with PT>threshold

[c] number with PT<threshold/20 spontaneous survivors

[d] number with Pt>threshold/20 dead or transplanted

Table 7

Performance of Aab:Leu and PT for Predicting PALF Outcomes

PALF Outcome	Threshold Criteria	Predictive Accuracy
Spontaneous Survival	Aab:Leu>0.25	10/12 (83%)
	PT<24 sec	9/9 (100%)
	Aab:Leu>0.25 OR PT<24 sec	13/15 (87%)
Death/Transplant	Aab:Leu<0.05	5/5 (100%)
	PT>39 sec	7/8 (88%)
	Aab:Leu<0.05 OR PT>39 sec	11/12 (92%)