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Serum bile acids as a prognostic biomarker in biliary atresia following Kasai portoenterostomy

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BA, biliary atresia; BASIC, Biliary Atresia Study in Infants and Children; BSEP, bile salt export pump; CA, cholic acid; CDCA, chenodeoxycholic acid; CEPH, clinically evident portal hypertension; ChiLDReN, Childhood Liver Disease Research Network; GGT, gamma-glutamyltransferase; GI, gastrointestinal; KP, Kasai portoenterostomy; NTCP, sodium-taurocholate cotransporting polypeptide; OH, hydroxy; OST, organic solute and steroid transporter; PROBE, Prospective Database of Infants With Cholestasis; START, steroids in BA randomized trial; UDCA, ursodeoxycholic acid;

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Abstract

Background and Aims: In biliary atresia, serum bilirubin is commonly used to predict outcomes after Kasai portoenterostomy (KP). Infants with persistently high levels invariably need liver transplant, but those achieving normalized levels have a less certain disease course. We hypothesized that serum bile acid levels could help predict outcomes in the latter group.

Approach and Results: Participants with biliary atresia from the Childhood Liver Disease Research Network were included if they had normalized bilirubin levels 6 months after KP and stored serum samples from the 6-month post-KP clinic visit ($n = 137$). Bile acids were measured from the stored serum samples and used to divide participants into ≤ 40 μmol/L ($n = 43$) or > 40 μmol/L ($n = 94$) groups. At 2 years of age, the ≤ 40 μmol/L compared with >40 μmol/L group had significantly lower total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl-transferase, bile acids, and spleen size, as well as significantly higher albumin and platelet counts. Furthermore, during 734 person-years of follow-up, those in the ≤ 40 μmol/L group were significantly less likely to develop splenomegaly, ascites, gastrointestinal bleeding, or clinically evident portal hypertension. The ≤ 40 μmol/L group had a 10-year cumulative incidence of liver transplant/death of 8.5% (95% CI: 1.1%–26.1%), compared with 42.9% (95% CI: 28.6%–56.4%) for the >40 μmol/L group ($p = 0.001$).

Conclusions: Serum bile acid levels may be a useful prognostic biomarker for infants achieving normalized bilirubin levels after KP.

INTRODUCTION

Biliary atresia (BA) is a serious liver disease of infancy characterized by extrahepatic bile duct obstruction. As a result, bile flow is impaired, which leads to bile retention, liver injury, and end-stage liver disease typically within the first year of life.[1] The Kasai portoenterostomy (KP) can be performed to try to restore bile flow and slow BA's rapid course.[2] This operation removes the obstructed extrahepatic bile ducts, connects the liver hilum directly to the intestine, and attempts to create a conduit for bile to flow.

Clinicians often follow serum levels of bilirubin, an important component of bile, to predict disease progression after KP.[3–12] High serum bilirubin levels reliably predict poor outcomes, including complications from progressive liver disease and the invariable need for liver transplant. Normalized serum bilirubin levels, in contrast, do not reliably predict good outcomes. For example, of the infants with normalized serum bilirubin levels after KP, approximately 71% still develop splenomegaly, 45% develop thrombocytopenia, 18% develop ascites, and 18% need a liver transplant in the first 2 years of life.[13] An additional 50% of infants require liver transplant before adulthood.[14] To address the limitations of bilirubin measurements, we hypothesized that serum levels of another component of bile—bile acids—could help predict near-term and long-term outcomes in infants who achieve normalized bilirubin levels after KP.

METHODS

Participant selection

Participants in this study were enrolled in one of two prospective observational studies supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)–sponsored multicenter Childhood Liver Disease Research Network (ChiLDReN) after parental/guardian written informed consent was obtained. The first, Prospective Database of Infants With Cholestasis (PROBE; NCT00061828), enrolls infants with neonatal cholestasis ≤ 180 days old. The second, Biliary Atresia Study in Infants and Children (BASIC; NCT00345553), enrolls participants with BA > 180 days old. Both studies collect clinical data,

laboratory results, and biological specimens at enrollment, throughout the first 2 years of life (for PROBE participants), and annually afterward until liver transplant, death, age > 20 years, or loss to follow-up. These studies have been approved by a single institutional review board that covers all ChiLDReN sites and the ChiLDReN Scientific and Data Coordinating Center. Each site conforms to the ethical guidelines of the 1975 Declaration of Helsinki and does not obtain donor organs from executed prisoners or other institutionalized persons.

Of the PROBE and BASIC participants with BA, those included in this analysis met two criteria. The first criterion was achieving normalized serum bilirubin levels by the 6-month post-KP study visit (which occurred within a 4.5–9.0-month postoperative window in study participants). "Normalized" serum bilirubin levels were defined as total bilirubin level < 1.5 mg/dl or, if total bilirubin was not drawn, conjugated bilirubin ≤ 0.2 mg/dl. Bilirubin levels were not included for analysis if they were total bilirubin estimates (by summing conjugated and unconjugated levels) or direct bilirubin levels (which have reference intervals that vary across sites).[15] The second criterion was having nonfasting serum samples from the 6-month post-KP study visit available for analysis. These samples were collected as part of PROBE and BASIC protocols and stored at −70°C in an NIDDK biosample repository.

Data collection

Clinical data, laboratory results, and medication use were collected from PROBE and BASIC case report forms. Baseline information included race and ethnicity as reported by parents, whether BA was accompanied by splenic malformations, and participation status in the Steroids in Biliary Atresia Randomized Trial (START; NCT 00294684).[16,17] Laboratory results at 2 years of age included total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT), albumin, internationalized normal ratio, total bile acids, platelets, and 25-hydroxy (OH) vitamin D levels. Liver parameters included spleen size (measured by ChiLDReN investigators during the physical examination and recorded as centimeters below the left costal margin) and height/weight measurements (which were used to obtain age-adjusted and sex-adjusted *z* scores, using SAS macros provided by the Centers for Disease Control and Prevention).[18]

Sentinel events were recorded at study visits using definitions specified in the PROBE and BASIC protocols. Cholangitis was defined as fever > 38°C with elevation in total or conjugated bilirubin, new-onset acholic stools, right upper quadrant pain, and/or liver enzyme elevations. Thrombocytopenia was defined as a platelet count < 150,000/μl, splenomegaly defined as spleen palpable > 2 cm below the left costal margin, and ascites defined as peritoneal fluid accumulation requiring diuretics. Gastrointestinal (GI) bleeding was defined as hematemesis, hematochezia, or melena with endoscopic confirmation of varices. Clinically evident portal hypertension (CEPH) was assessed as probable or definite using a previously published ChiLDReN definition, which includes at least one of the following: thrombocytopenia, splenomegaly, ascites, and/or esophageal or gastric varices.[19]

Bile acid measurements

Serum bile acid concentrations were measured from nonfasting samples collected at the 6-month post-KP visit and stored for 2–174 months at −70°C. An enzymatic immunoassay was used to quantify total bile acids, and stable-isotope dilution liquid chromatography–tandem mass spectrometry was used to quantify individual bile acid species.[20]

Statistical analysis

For all analyses, participants were divided into two groups using the measured 6-month post-KP measured total serum bile acid levels (≤ 40 μmol/L and > 40 μmol/L groups). The 40-μmol/L cutoff was based on an earlier listing of reference intervals for infants at various ages in the first year of life, in conjunction with inspecting the range of nonfasting values measured in the study (see Discussion).[21] Clinical data at baseline (6 months after KP) and concentrations of bile acid species were compared using Fisher exact tests for discrete variables and Wilcoxon rank-sum tests for continuous variables. To obtain CIs, Spearman correlations were calculated using Fisher's z transformation. The 2-year physical exam and laboratory outcomes were analyzed with univariate linear regression models. Variables with nonnormal distributions, including total bilirubin, AST, ALT and GGT, were modeled on the log scale and then reverse-transformed into fold-change for interpretation. Time to transplant or death and time to CEPH (starting from the 6-month post-KP sample collection) were compared between bile acid groups using Kaplan–Meier curves, and significance was assessed using the log-rank test. For other sentinel events, in which there was a competing risk of transplant or death, analyses were performed using Gray's test to account for the competing risk. Event-free survival and cumulative incidence curves were graphed starting at date of KP for ease of interpretation; *p* values were calculated using the 6-month post-KP sample date as time 0. For all analyses, there was no imputation of missing data and therefore analyses were limited to complete cases (the number of cases in each analysis is listed in the

tables and figures; “n/N" indicates cases affected/total cases available for analysis). Calculations were performed using SAS version 9.4.

RESULTS

Between the years 2004 and 2019, 756 children with BA were enrolled in ChiLDReN studies by the 6-month post-KP time point (Figure 1). Of these, 279 children did not have a 6-month post-KP serum bilirubin value due to already undergoing liver transplantation ($n = 118$), death ($n = 16$), or missing data ($n = 145$). Another 232 children failed to achieve normalized serum bilirubin levels by 6 months following KP. Of the remaining 245 children, 137 children had serum samples available from the 6-month post-KP visit and were included in this study. The study group was 51.8% female ($n = 71$), 56.7% White ($n = 76$), and 32.8% Hispanic ($n = 45$). Ursodeoxycholic acid (UDCA) was administered in 61.5% ($n = 83$) of study participants at the time of serum sample collection (Table 1).

The concentrations of total bile acids measured from the stored serum samples ranged from 2 to 322 μmol/L (median 70 μmol/L) and were distributed with a positive skew (Figure 2). The levels varied even in samples from participants with a conjugated bilirubin of 0.0 mg/dl (Figure S1A). Importantly, bile acids did not appear to degrade appreciably with storage, because the concentrations measured from stored samples correlated with levels that were obtained in a subset of patients at time of blood draw as part of routine clinical care ($r = 0.94$, 95% CI 0.89–0.96, $p < 0.001$, $n = 49$) (Figure S1B).

The measured total serum bile acid levels were then used to dichotomize participants into ≤ 40 μmol/L ($n = 43$) and > 40 μmol/L ($n = 94$) groups. These groups did not differ in sex, race, ethnicity, presence of splenic malformation, or UDCA use (Table 2, Figure S2). The groups were also similar in previous occurrence of cholangitis, fractures, ascites, or GI bleed. The groups differed in age at KP (median age 57 vs. 69 days in the ≤ 40 μmol/L and > 40 μmol/L groups, respectively; $p = 0.009$) and development of CEPH by the 6-month post-KP time point (37.2% vs.

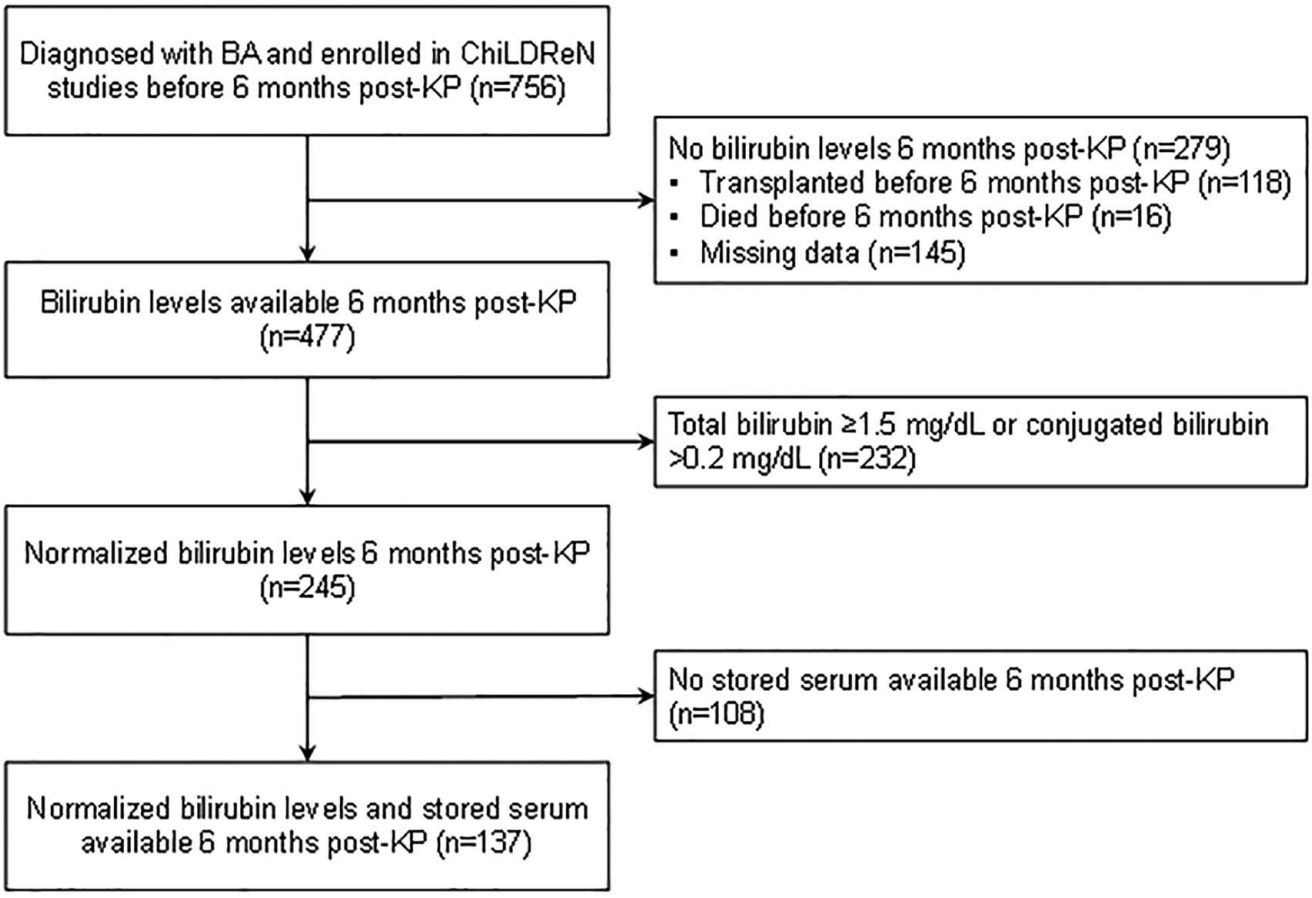

FIGURE 1 Participant flow. There were 756 participants in Childhood Liver Disease Research Network (ChiLDReN) with biliary atresia (BA) enrolled in Prospective Database of Infants With Cholestasis (PROBE) or Biliary Atresia Study in Infants and Children (BASIC) before or at the 6-month post–Kasai portoenterostomy (KP) visit. Of these, 137 had (i) a total bilirubin level < 1.5 mg/dl or, if total bilirubin was not drawn, conjugated bilirubin ≤ 0.2 mg/dl by 6 months after KP; and (ii) stored serum from the 6-month post-KP visit.

TABLE 1 Demographic and clinical features of participants

	All participants (n = 137)
Sex, % (n/N)	
Female	51.8 (71 of 137)
Male	48.2 (66 of 137)
Race, % (n/N)	
Asian	9 (12 of 134)
Black	10.4 (14 of 134)
Multiracial[a]	14.2 (19 of 134)
White	56.7 (76 of 134)
Other[b]	9.7 (13 of 134)
Hispanic, % (n/N)	32.8 (45 of 137)
BASM, % (n/N)	8.8 (12 of 137)
KP age (days), median (range) [n]	64 (17, 133) [137]
START arm, % (n/N)	
Placebo	46.9 (23 of 49)
Steroid	53.1 (26 of 49)
Ursodeoxycholic acid use, % (n/N)	61.5 (83 of 135)
Total serum bile acids (μmol/L), median (range) [n]	70 (2, 322) [137]

Abbreviations: BASM, biliary atresia splenic malformations; START, Steroids in Biliary Atresia Randomized Trial.
[a]More than 1 of American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, or Other.
[b]American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, or Other.

63.8% in the ≤40 μmol/L and >40 μmol/L groups, respectively; p = 0.005). The ≤40 μmol/L group also had a higher proportion of participants receiving steroids versus placebo in the START trial, although this difference was not statistically significant.

To determine whether serum bile acid levels at 6 months following KP were associated with future events, liver parameters at 2 years of age were studied first. The ≤40 μmol/L group had significantly lower total bilirubin, AST, ALT, GGT and spleen size, as well as significantly higher albumin and platelet counts at 2 years of age (Table 3). In addition, infants with bile acid levels > 40 μmol/L had 4.9-fold (95% CI 2.8–8.7) higher total serum bile acid concentrations at 2 years compared to those with bile acids levels ≤ 40 μmol/L. Serum bile acid levels at 6 months following KP accounted for 47% of the variation in serum bile acid concentrations at 2 years, which was reflected by the high correlation between bile acid levels at the two time points in individual patients (r = 0.79, 95% CI 0.63–0.89, p < 0.001, n = 35) (Figure S3).

The occurrence of sentinel events was then examined over 734 person-years of follow-up (median 4.7 years/participant, range 0–13.9 years). New-onset splenomegaly, new-onset ascites, GI bleeding, and new-onset CEPH were significantly less common in the ≤40 μmol/L group (Table 4, Figure S4, Figure 3). In addition, 2 participants underwent liver transplant or died before liver transplant in the ≤40 μmol/L group (4.7%) compared with 30 participants (31.9%) in the >40 μmol/L group (log-rank p = 0.002). The 10-year cumulative incidences of liver transplant/death before liver transplant were 8.5% (95% CI 1.1–26.1%) and 42.9% (95% CI 28.6–56.4%) in the ≤40 μmol/L and >40 μmol/L groups, respectively (Gray's test p = 0.001).

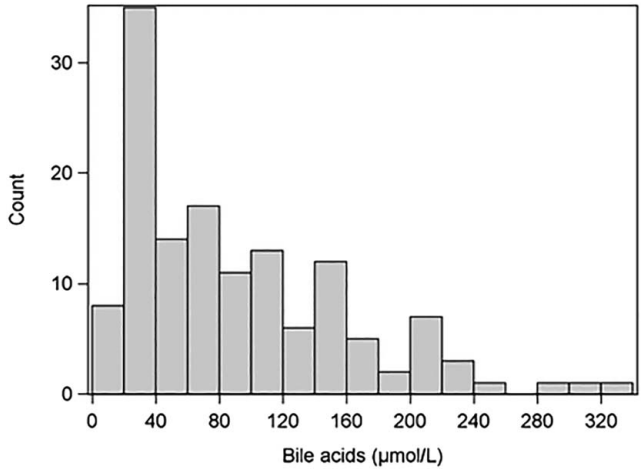

FIGURE 2 Total serum bile acid levels varied widely despite normalized serum bilirubin levels 6 months after KP. x-axis: bile acid concentrations grouped in 20-μmol/L bins; y-axis: number of measurements.

Finally, to identify differences in bile acid composition, the concentrations of individual bile acid species in the stored samples were determined. For the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA), as well as for UDCA, concentrations of the unconjugated forms were similar in the ≤40 μmol/L and >40 μmol/L groups (Table 5). In contrast, concentrations of conjugated CA, CDCA, and UDCA were significantly lower in the ≤40 μmol/L group compared with the >40 μmol/L group (3.5 μmol/L vs. 45.3 μmol/L, 7.2 μmol/L vs. 39.9 μmol/L, and 6.9 μmol/L vs. 28.0 μmol/L, respectively; p < 0.001 for all comparisons). Furthermore, of the conjugated bile acids, there were significantly lower ratios of taurine-conjugated versus glycine-conjugated CA, CDCA, and UDCA in the ≤40 μmol/L group. The secondary bile acids were not studied further, because their unconjugated and conjugated concentrations were too low for additional analyses (see Discussion).

DISCUSSION

This study examines whether serum bile acid levels are associated with outcomes in infants achieving normalized serum bilirubin levels after KP. Infants with total serum bile acid levels ≤40 μmol/L 6 months after KP had better liver parameters at 2 years of age, including significantly lower markers of liver injury such as AST, ALT, and GGT. Infants with total serum bile acid levels

TABLE 2 Demographic and clinical features of participants based on bile acid group

	Six-month post-KP bile acid group		
	≤40 μmol/L (*n* = 43)	>40 μmol/L (*n* = 94)	*p*
Sex, % (*n*/*N*)			
Female	44.2% (19 of 43)	55.3% (52 of 94)	0.270
Male	55.8% (24 of 43)	44.7% (42 of 94)	
Race, % (*n*/*N*)			
Black	9.5% (4 of 42)	10.9% (10 of 92)	0.769
Multiracial[a]	9.5% (4 of 42)	16.3% (15 of 92)	
White	64.3% (27 of 42)	53.3% (49 of 92)	
Other[b]	7.1% (3 of 42)	10.9% (10 of 92)	
Hispanic, % (*n*/*N*)	25.6% (11 of 43)	36.2% (34 of 94)	0.245
BASM, % (*n*/*N*)	9.3% (4 of 43)	8.5% (8 of 94)	1.000
KP age (days), median (range) [*n*]	57 (17, 117) [43]	69 (25, 133) [94]	**0.009**
START arm, % (*n*/*N*)			
Placebo	28.6% (4 of 14)	54.3% (19 of 35)	0.125
Steroid	71.4% (10 of 14)	45.7% (16 of 35)	
Ursodeoxycholic acid use, % (*n*/*N*)	58.1% (25 of 43)	63% (58 of 92)	0.705
History of cholangitis, % (*n*/*N*)	41.9% (18 of 43)	27.7% (26 of 94)	0.116
History of fracture, % (*n*/*N*)	0% (0 of 43)	1.1% (1 of 94)	1.000
Ascites, % (*n*/*N*)	11.6% (5 of 43)	14.9% (14 of 94)	0.791
History of GI bleed, % (*n*/*N*)	0% (0 of 43)	5.3% (5 of 94)	0.325
CEPH, % (*n*/*N*)	37.2% (16 of 43)	63.8% (60 of 94)	**0.005**

Abbreviation: GI, gastrointestinal.
[a]More than 1 of American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, or other.
[b]American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, or other.

≤40 μmol/L 6 months after KP were also significantly less likely to experience sentinel events during childhood, such as development of splenomegaly, ascites, GI bleeding, and CEPH. Most importantly, their 10-year cumulative incidence of liver transplant/death was only 8.5%, compared with 42.9% in infants with total serum bile acids levels > 40 μmol/L 6 months after KP.

Our findings begin to address two gaps in the field. First, they provide a practical tool for clinicians to predict which infants will have the best outcomes after a "successful KP", i.e., those who achieve normalized bilirubin levels. Clinicians can potentially incorporate the 40-μmol/L cutoff into routine clinical care, without requiring infants to be fasting or to stop UDCA therapy before blood draws (nonfasting samples from infants both taking and not taking UDCA were used in this study). In addition, clinicians may not need to order specialized tests measuring concentrations of individual bile acids species. Instead, testing only for total bile acids may be sufficient, because serum CA, CDCA, and total bile acid levels behaved similarly in this study.

Second, the findings provide a surrogate endpoint for investigators to consider when conducting therapeutic clinical trials in BA. The most commonly used endpoints currently are bilirubin normalization and transplant-free survival. Bilirubin normalization is assessed in a timely manner (by 3–6 months after KP), but it is limited because it does not necessarily predict good outcomes.[13] In contrast, transplant-free survival may be the most important clinical outcome, but it is limited because it requires years of follow-up to assess. As suggested by results from this study, serum bile acids potentially address both limitations, because bile acid levels not only associate with transplant-free survival and other important outcomes but also can be assessed quickly at the 6-month post-KP time point.

From a mechanistic perspective, infants with serum bile acid levels > 40 μmol/L had significantly higher levels of conjugated versus unconjugated bile acids. In addition, the conjugated bile acid species were more likely to be conjugated with taurine versus glycine, suggesting that hepatocytes were in a more fetal, less-developed state.[22–24] One explanation for high-serum conjugated bile acids is hepatocyte adaptation to cholestasis. For example, with cholestasis, hepatocytes down-regulate the sodium-taurocholate cotransporting peptide (NTCP), which would limit uptake of conjugated bile acids from the blood.[25] Hepatocytes also up-regulate the organic solute and steroid transporter (OSTα/β), which would promote efflux of conjugated bile acids into the blood.[26] Importantly, hepatocytes may maintain these changes in NTCP and OSTα/β even

TABLE 3 Liver parameters at 2 years of age in each bile acid group

Two-year liver parameter	Value, median (range) [n]	Estimate > 40 vs. ≤40 μmol/L (95% CI)	*p*	R^2
Total bilirubin (mg/dl)				
≤40 μmol/L group	0.3 (0.1, 0.8) [30]	2.1-fold (1.6, 2.8)	**<0.001**	0.22
>40 μmol/L group	0.6 (0.2, 6.0) [66]			
AST (U/L)				
≤40 μmol/L group	58 (28, 218) [34]	1.8-fold (1.4, 2.3)	**<0.001**	0.18
>40 μmol/L group	106 (40, 860) [72]			
ALT (U/L)				
≤40 μmol/L group	45 (8, 392) [34]	1.9-fold (1.4, 2.6)	**<0.001**	0.13
>40 μmol/L group	114 (17, 828) [72]			
GGT (U/L)				
≤40 μmol/L group	44 (7, 331) [29]	3.7-fold (2.3, 5.9)	**<0.001**	0.26
>40 μmol/L group	187 (17, 2608) [57]			
Albumin (g/dl)				
≤40 μmol/L group	4.4 (3.6, 5.0) [34]	−0.3 (−0.5, −0.2)	**<0.001**	0.14
>40 μmol/L group	4.1 (3.0, 5.0) [71]			
INR				
≤40 μmol/L group	1.0 (0.9, 1.2) [31]	0.009 (−0.038, 0.057)	0.704	0.00
>40 μmol/L group	1.0 (0.8, 1.6) [62]			
Total bile acids (μmol/L)				
≤40 μmol/L group	12 (5, 44) [14]	4.9-fold (2.8, 8.7)	**<0.001**	0.47
>40 μmol/L group	59 (9, 330) [21]			
Spleen size (cm below costal margin)				
≤40 μmol/L group	0.0 (0.0, 7.0) [32]	2.2 (1.2, 3.2)	**<0.001**	0.16
>40 μmol/L group	3.0 (0.0, 10.0) [69]			
Platelet count (10^9/L)				
≤40 μmol/L group	275 (107, 440) [31]	0.7-fold (0.6, 0.9)	**<0.001**	0.11
>40 μmol/L group	178 (68, 579) [69]			
Weight *z* score				
≤40 μmol/L group	−0.06 (−2.86, 2.36) [35]	0.047 (−0.418, 0.513)	0.843	0.00
>40 μmol/L group	0.16 (−3.04, 2.13) [75]			
Height *z* score				
≤40 μmol/L group	−0.24 (−4.29, 2.22) [34]	0.002 (−0.466, 0.470)	0.994	0.00
>40 μmol/L group	−0.48 (−3.25, 2.45) [76]			
25-OH vitamin D (ng/ml)				
≤40 μmol/L group	37.0 (16.0, 62.3) [21]	3.7 (−5.4, 12.8)	0.433	0.01
>40 μmol/L group	38.0 (3.2, 110.0) [41]			

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; INR, internationalized normal ratio; OH, hydroxy.

after cholestasis resolves, which could explain why serum bile acid levels can remain high even after bilirubin levels normalize in BA.[27,28] Persistent changes in NTCP and OSTα/β could also explain why bile acid levels fluctuated little between the 6-month post-KP and 2-year time points in individual patients.

In contrast to conjugated bile acids, unconjugated bile acids were not increased in the >40 μmol/L group. One explanation is that intestinal bacteria were disrupted, from oral antibiotics routinely given for many months following KP as cholangitis prophylaxis.[29,30] Normal intestinal bacteria are required to deconjugate the conjugated bile acids secreted by the liver. Normal intestinal bacteria are also required to metabolize primary bile acids into secondary bile acids, and their disruption could account for the lack of deoxycholic and lithocholic acid detected in this study.[31] An alternative explanation for normal serum unconjugated bile acid

TABLE 4 Occurrence of sentinel events in each bile acid group

Sentinel event	Frequency overall follow-up[a], % (*n*/*N*)	Ten-year cumulative incidence (95% CI)	*p*
Cholangitis			
≤40 μmol/L group	34.9% (15 of 43)	47.9% (25.9%–67.0%)	0.517
>40 μmol/L group	30.9% (29 of 94)	35.4% (24.9%–46.1%)	
Fracture			
≤40 μmol/L group	0.0% (0 of 43)	0%	0.200
>40 μmol/L group	4.3% (4 of 94)	5.9% (1.8%–13.8%)	
New-onset splenomegaly[b]			
≤40 μmol/L group	18.2% (6 of 33)	29.1% (9.4%–52.7%)	**0.001**
>40 μmol/L group	58.5% (24 of 41)	66.5% (46.4%–80.5%)	
New-onset thrombocytopenia[b]			
≤40 μmol/L group	25.0% (9 of 36)	34.7% (15.4%–54.9%)	0.156
>40 μmol/L group	43.1% (25 of 58)	48.4% (33.7%–61.7%)	
New-onset ascites[b]			
≤40 μmol/L group	2.6% (1 of 38)	2.8% (0.2%–12.6%)	**0.048**
>40 μmol/L group	16.3% (13 of 80)	18.6% (10.4%–28.7%)	
GI bleed			
≤40 μmol/L group	2.3% (1 of 43)	5.7% (0.3%–23.7%)	**0.031**
>40 μmol/L group	16.0% (15 of 94)	18.5% (10.3%–28.5%)	
New-onset CEPH[b]			
≤40 μmol/L group	33.3% (9 of 27)	41.2% (19.1%–62.2%)	**0.038**
>40 μmol/L group	64.7% (22 of 34)	68.8% (48.5%–82.4%)	
Transplant/death			
≤40 μmol/L group	4.7% (2 of 43)	8.5% (1.1%–26.1%)	**0.001**
>40 μmol/L group	31.9% (30 of 94)	42.9% (28.6%–56.4%)	

[a]Median follow-up: 4.7 years (range 0–13.9 years).
[b]Participants with CEPH ($n = 76$), splenomegaly ($n = 63$), thrombocytopenia ($n = 43$), or ascites ($n = 19$) at 6 months following KP were excluded from respective analysis of incident events.

levels is that unconjugated species are hydrophobic and do not require membrane transporters such as NTCP for clearance from the blood. Instead, unconjugated bile acids are passively absorbed into the intestine, travel through the portal blood, and passively enter the hepatocyte for conversion into conjugated bile acids.[32]

One strength of this study is that serum bile acids levels were unlikely to have confounded key outcomes such as liver transplantation. This is because bile acid levels for this study were measured from stored samples and compared retrospectively to clinical outcomes. In most cases, clinicians were unaware of 6-month post-KP bile acid levels, as bile acids were not routinely measured in the clinical setting. In contrast, other commonly used biomarkers can be confounding because they not only predict but also may influence outcomes in BA. For example, markers such as high total bilirubin, ascites, and GI bleeding are strong prognostic markers for need for liver transplant, but this is at least partly because clinicians may use them as indications for transplant listing.[13,33]

This study has important limitations. First, it does not measure samples across time to describe how serum bile acid levels change at different points in the disease course. Instead, the 6-month post-KP time point was chosen, because this is the same time point used in a previous ChiLDReN study (START) to assess bilirubin normalization.[17] However, by 6 months after KP, 64% of participants with serum bile acid levels > 40 μmol/L had already developed CEPH. This suggests that additional studies examining serum bile acid levels at earlier time points could be informative.

Second, the study does not adjust cutoffs for feeding and routine UDCA use following KP, both which may be predicted to increase serum bile acid levels.[30,34] Instead, a single cutoff of 40 μmol/L was used, based on a previous report as well as inspecting the distribution of levels in this study.[21] The 40 μmol/L is higher than the 20–30 μmol/L cutoffs derived from healthy fasting infants not taking UDCA, as well as the 10-μmol/L cutoff used for healthy older children and adults (only 3 participants in this study had bile acid levels ≤10 μmol/L).[35–37] Prospective studies controlling for feeding and UDCA

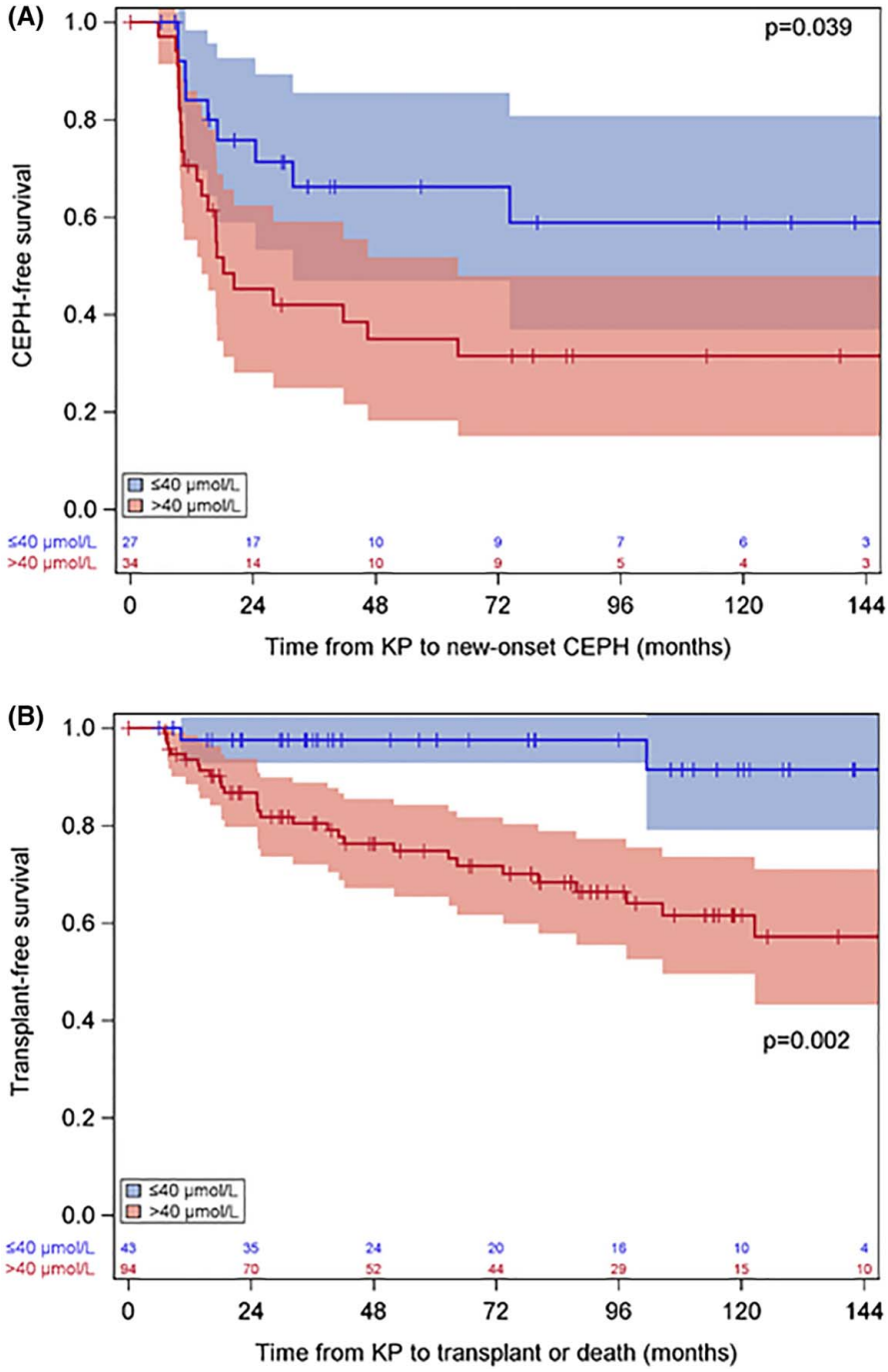

FIGURE 3 Lower occurrence of clinically evident portal hypertension (CEPH) and higher transplant-free survival in infants with total serum bile acids ≤40 umol/L 6 months following KP. New-onset CEPH (A) and transplant/death before death (B). This analysis does not include participants who had already developed CEPH at the 6-month post-KP time point ($n = 76$). For transplant-free survival, there were 2 participants (4.7%) in the ≤40 µmol/L group who did not survive with their native liver compared with 30 participants (31.9%) in the >40 µmol/L group.

use could more precisely define bile acid cutoffs in BA. In addition, it is possible that larger studies may identify a higher cutoff or a tier of cutoffs as having better predictive value, given that participants had levels as high as 322 µmol/L with a right skew distribution.

Third, the study identifies serum bile acids as a marker but not necessarily as a driver of liver injury and disease progression. Clinical trials with drugs that reduce bile acid pool size will explore this further. For example, there are two ongoing double-blind, randomized, placebo-controlled clinical trials with ileal bile acid transport inhibitors in BA (NCT04524390, NCT04336722). These therapies have previously been shown to lower serum bile acid levels in other causes of neonatal cholestasis such as Alagille syndrome and progressive familial intrahepatic

TABLE 5 Summary of bile acid profiles in each bile acid group

Bile acid species	Six-month post-KP bile acid group		p
	≤40 μmol/L (*n* = 43)	>40 μmol/L (*n* = 94)	
Total (μmol/L), median (range) [*n*]	27 (2, 39) [43]	107 (42, 322) [94]	**<0.001**
Unconjugated (μmol/L), median (range) [*n*]			
CA	0.1 (0.0, 1.7) [43]	0.1 (0.0, 1.5) [94]	0.242
CDCA	0.3 (0.0, 1.4) [43]	0.2 (0.0, 3.3) [94]	0.170
UDCA	2.3 (0.0, 11.0) [25]	2.6 (0.0, 25.7) [58]	0.695
Conjugated (μmol/L), median (range) [*n*]			
CA	3.5 (0.5, 30.1) [43]	45.3 (4.4, 353.6) [94]	**<0.001**
CDCA	7.2 (2.3, 27.8) [43]	39.9 (8.7, 168.5) [94]	**<0.001**
UDCA	6.9 (0.5, 26.5) [25]	28.0 (0.0, 208.3) [58]	**<0.001**
Ratio of taurine/glycine-conjugated, median (range) [*n*]			
CA	0.7 (0.2, 2.9) [43]	1.0 (0.1, 5.7) [94]	**0.003**
CDCA	0.7 (0.1, 3.3) [43]	1.0 (0.2, 5.6) [94]	**<0.001**
UDCA	0.1 (0.0, 0.9) [25]	0.2 (0.1, 1.4) [57[a]]	**0.006**

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid.
[a]One participant had TUDCA and GUDCA concentrations that were below detection, so a ratio was not calculated.

cholestasis, and trials in BA will help determine whether their potential to reduce the bile acid pool size can improve endpoints such as transplant-free survival.[38,39]

Serum bile acids may be a useful prognostic biomarker for infants who achieve normalized serum bilirubin levels after KP. Future prospective studies are needed to explore how clinicians can incorporate serum bile acid measurements into routine post-KP care, as well as how investigators can adopt serum bile acids as a potential endpoint in clinical trials.

AUTHOR CONTRIBUTIONS

Study concept, design, analysis, and data interpretation: Sanjiv Harpavat, Kieran Hawthorne, Kenneth D. R. Setchell, Monica Narvaez Rivas, Lisa Henn, Charlotte A. Beil, Saul J. Karpen, Vicky L. Ng, and Benjamin L. Shneider. *Data acquisition, manuscript draft, and critical revision of the article for important intellectual content*: All authors. All authors were involved with final approval of the version to be published.

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CONFLICT OF INTEREST

Sanjiv Harpavat serves on a Data Safety Monitoring Board coordinated by Syneos Health. Kenneth D. R. Setchell consults for Travere Therapeutics and Mirum

Pharmceuticals. He owns stock in Asklepion Pharmaceuticals and Aliveris SRL. Saul J. Karpen consults for Albireo, Intercept, and Mirum. Simon Horslen consults for Albireo. Kathleen M. Loomes consults for and received grants from Albireo and Mirum. She consults for Travere Therapeutics. Philip Rosenthal consults for and received grants from AbbVie, Arrowhead, and Dicerna. He consults for Albireo, Audentes, Encoded, and Gilead. He received grants from Ambys. Richard J. Thompson consults for and is on the speakers' bureau for Albireo and Mirum. He owns stock in and consults for Generation Bio and Rectify. Ronald J. Sokol advises Albireo and Mirum.

DATA AVAILABILITY STATEMENT

We are currently unable to provide the data used to generate this manuscript. This study is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK); data sets will be provided to the NIDDK repository after completion of study recruitment, which is currently ongoing. After they are deposited, the data will be available through the NIDDK Data Repository (https://www.niddkrepository.org/home/).

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