Prevalence and Prognostic Value of Abnormal Liver Test Results in Critically Ill Children and the Impact of Delaying Parenteral Nutrition*

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Objectives: In the Early versus Late Parenteral Nutrition in the Pediatric ICU randomized controlled trial, delaying parenteral nutrition to beyond day 7 (late parenteral nutrition) was clinically superior to supplemental parenteral nutrition initiated within 24 hours (early parenteral nutrition), but resulted in a higher rise in bilirubin. We aimed to document prevalence and prognostic value of abnormal liver tests in the PICU and the impact hereon of withholding early parenteral nutrition.

Design: Preplanned secondary analysis of the Early versus Late Parenteral Nutrition in the Pediatric ICU randomized controlled trial. Total bilirubin, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase plasma concentrations were measured systematically in PICU. Liver test analyses were adjusted for baseline characteristics including severity of illness.

Setting: Three PICUs in Belgium, the Netherlands, and Canada.

Patients: As neonatal jaundice was considered a confounder, only the 1,231 of the 1,440 Early versus Late Parenteral Nutrition in the Pediatric ICU-patients 28 days to 17 years old were included.

Interventions: Late parenteral nutrition as compared with early parenteral nutrition.

Measurements and Main Results: During the first seven PICU days, the prevalence of cholestasis (> 2 mg/dL [34.2 μmol/L] bilirubin) ranged between 3.8% and 4.9% and of hypoxic hepatitis (≥ 20-fold upper limit of normality for alanine aminotransferase and aspartate aminotransferase) between 0.8% and 2.2%, both unaffected by the use of parenteral nutrition. Throughout the first week in PICU plasma bilirubin concentrations were higher in late parenteral nutrition patients ($p < 0.05$), but became comparable to early parenteral nutrition patients as soon as parenteral nutrition was started on day 8. Plasma concentrations of gamma-glutamyl transpeptidase, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were unaffected by parenteral nutrition. High day 1 plasma concentrations of gamma-glutamyl transpeptidase, alanine aminotransferase, and aspartate aminotransferase ($p \leq 0.01$), but not alkaline phosphatase, were independent risk factors for PICU mortality. Day 1 plasma bilirubin concentrations displayed a U-shaped association with PICU mortality, with higher mortality associated with bilirubin less than 0.20 mg/dL and greater than 0.76 mg/dL (< 3.42 μmol/L and > 13 μmol/L) ($p \leq 0.01$).

Conclusions: Overt cholestasis and hypoxic hepatitis were rare and unrelated to the nutritional strategy. However, withholding parenteral nutrition up to 1 week in PICU increased plasma bilirubin. A mild elevation of bilirubin on the first PICU day was associated with lower risk of death and may reflect a stress response, rather than true cholestasis. (*Pediatr Crit Care Med* 2018; 19:1120–1129)

Key Words: cholestasis; critical illness; liver tests; parenteral nutrition; pediatric intensive care unit; total bilirubin

In critical illness, liver test abnormalities are associated with a poor outcome independently of other organ dysfunctions. Plasma concentrations of total bilirubin, gamma-glutamyl

transpeptidase (γGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are often above the normal range in critically ill adults, the severity of which has been associated with a more complicated course of critical illness, a longer duration of ICU stay and a higher risk of death (1–6). However, despite its common occurrence and its association with poor outcome in critically ill adults, the prevalence and prognostic value of liver test abnormalities in pediatric critical illness remain largely unexplored.

Timely identification of true liver dysfunction may lead to prevention or attenuation of its consequences (7). Grossly, deranged liver test results often indicate severe liver injury as a result of hepatic ischemia and/or a true cholestatic liver dysfunction (8). However, the precise etiology and clinical relevance of milder cholestatic abnormalities in response to critical illness are not completely understood. It has been suggested that the presence of mild hyperbilirubinemia in response to critical illness may merely reflect a biochemical epiphenomenon or could be part of an adaptive and beneficial stress response (7, 9). Such a possibility was recently corroborated by findings from a large randomized controlled trial (RCT) in adult ICU patients (the Early Parenteral Nutrition Completing Enteral Nutrition in Adult Critically Ill Patients [EPaNIC] trial) (10). The EPaNIC trial investigated the impact of delaying initiation of parenteral nutrition (PN) to beyond the first week of critical illness (late PN) and found fewer infections, less organ failure, and faster recovery as compared with early PN (10, 11). Remarkably, total bilirubin plasma concentrations were significantly higher in adult patients who did not get early PN, whereas biochemical markers of hepatocyte lysis and cholestasis did not rise to the same extent as compared with patients who had received early PN (12). Furthermore, withholding PN during the first week of adult critical illness lowered the occurrence rate of biliary sludge (12). Also in critically ill children, withholding PN in the first week in the PICU showed to be clinically superior to providing early PN with fewer infections, less organ failure and faster recovery (13). During this first week in the PICU, late PN was also associated with lower peak plasma concentrations of γGT and ALP, whereas plasma total bilirubin peaked higher (13).

As a preplanned secondary analysis of the Early versus Late Parenteral Nutrition in the Pediatric ICU (PEPaNIC) RCT (14), we here investigate the prevalence and prognostic value of abnormal liver test results in critically ill children treated in the PICU and the impact hereon of late PN versus early PN. Neonates less than 28 days old (n = 209) were excluded from this analysis to avoid confounding induced by neonatal jaundice (15). In the 1,231 PEPaNIC patients between 28 days and 17 years old, plasma concentrations of total bilirubin and of the liver enzymes ALT, AST, γGT, and ALP were quantified systematically. With use of currently accepted criteria, we documented the prevalence of cholestasis (plasma total bilirubin > 2 mg/dL [34.2 μmol/L] [16–21]) and of hypoxic hepatitis (≥ 20-fold the upper limit of normality for plasma ALT and AST concentrations [4, 6, 17, 22]) and assessed the change in liver test results during the first week

in PICU. In addition, we assessed the predictive value for mortality of liver test results on the first day in PICU.

MATERIALS AND METHODS

Patients

This study is a preplanned secondary analysis of the previously published PEPaNIC RCT, performed in three PICUs located in Belgium, the Netherlands, and Canada, of which the detailed study protocol and primary results have been published elsewhere (13, 14).

In brief, 1,440 patients (from term newborns to children 17 yr old) were randomized either to receive early supplementation of insufficient enteral nutrition with PN to reach the estimated caloric target (early PN), or to withhold supplemental PN in the first 7 days in the PICU, even if enteral intake was well below estimated caloric targets (late PN). Clinicians in Edmonton used measured or estimated resting energy expenditure to define individual caloric targets whereas in Leuven and Rotterdam, caloric and macronutrient targets were based on weight and age, according to published guidelines. For a further detailed description of feeding strategies that were used during the study, we refer to the original publications (13, 14). Withholding PN for up to 7 days in pediatric critical illness showed to be beneficial, with fewer new infections, an earlier weaning from mechanical ventilation, and a shorter PICU-stay. Written informed consent had been obtained from next of kin. The study protocol and consent forms were approved by the institutional ethical review boards. Of the 1,440 PEPaNIC-patients, neonates less than 28 days old (n = 209) were excluded from the current analysis to avoid confounding induced by neonatal jaundice (**Supplementary Fig. 1**, Supplemental Digital Content 1, http://links.lww.com/PCC/A770).

Plasma Concentrations of Bilirubin, C-Reactive Protein, and Liver Enzymes

For all patients, plasma concentrations of total bilirubin, γGT, ALP, ALT, AST, and C-reactive protein (CRP) had been measured systematically at the participating PICUs as part of the routine performed laboratory tests, using standard routine automated laboratory assays (Roche/Hitachi cobas 8000 c702 modular analyzer; Roche, Vilvoorde, Belgium). Blood samples were collected in vacutainers by experienced ICU nurses via an arterial catheter, or, in those rare cases were the arterial catheter was no longer in place, through a venous catheter, limiting risk of hemolysis. Plasma total bilirubin concentrations were quantified by the colorimetric diazo-method (lower detection limit 0.1 mg/dL). Liver enzymes ALT and AST were quantified using kinetic colorimetric ALT and AST assays without pyridoxal phosphate activation according to the International Federation of Clinical Chemistry (IFCC) recommendations (lower detection limit ALT and AST 5 U/L). Plasma γGT concentrations were quantified with the Szasz kinetic colorimetric assay (lower detection limit 3 U/L) and plasma ALP concentrations using the adenosine monophosphate kinetic colorimetric assay (lower detection limit 5 U/L), both according to the IFCC recommendations. However, as admission

plasma total bilirubin had not been measured during the trial period, as reference, we also quantified plasma total bilirubin concentrations with use of the colorimetric total bilirubin assay (Thermo Scientific, Waltham, MA) in stored admission samples from 100 randomly selected PEPaNIC-patients. This random selection of 50 late PN patients and 50 early PN patients were comparable for demographics, Severity of Illness scores, and admission features.

No standardized definitions for critical illness-induced cholestasis or hypoxic hepatitis are available for the pediatric critically ill population. We therefore defined critical illness-induced cholestasis in our study population as a plasma total bilirubin concentration higher than 2 mg/dL (34 μmol/L), which is the commonly used cutoff in critically ill adults and has been identified as a cutoff for increased morbidity in children with biliary atresia (18, 20, 21). Hypoxic hepatitis was defined as a plasma concentration of ALT and AST of greater than or equal to 20-fold higher than the upper limit of normality, again, based on the commonly used adult and pediatric cutoffs (4, 6, 17, 22).

Statistical Analyses

As liver tests were performed as part of routine laboratory measurements in the participating centers, liver test data were available for 60–70% of all patients on each day in the PICU. To detect, with 95% certainty and at least 80% power, a difference of about 20% in bilirubin plasma concentrations (12), 400 patients suffice. Furthermore, as withholding early PN has previously shown to shorten PICU-stay (13), fewer late PN patients were still in the PICU at later time points than was the case for early PN patients, and these were likely to have a higher disease severity upon admission. This could induce bias when analyzing the effect of late PN versus early PN on liver test results at later time points. To correct for missing liver test results and in order to avoid bias evoked by shortened PICU-stay in late PN patients, all analyses of liver tests were adjusted for baseline demographics and severity of illness characteristics. On each study day, patients with available liver test results in the early PN and late PN groups were matched with use of propensity scores obtained by logistic regression (one-to-one nearest neighbor matching without replacement and with a caliper of 0.2) for demographics (gender, age, weight), Severity of Illness scores (Pediatric Index of Mortality 2 probability of death at admission, Pediatric Logistic Organ Dysfunction [PELOD] score at first PICU day), admission features (diagnostic group, elective/emergency admission, presence of infection at admission, center of inclusion), and the nutritional risk score (Screening Tool for Risk on Nutritional Status and Growth kids score quantified at PICU admission) (**Supplementary Fig. 2**, Supplemental Digital Content 1, http://links.lww.com/PCC/A770; and **Supplementary Table 1A-N**, Supplemental Digital Content 1, http://links.lww.com/PCC/A770, for baseline characteristics of all propensity matched subsets). Comparisons between groups were made with chi-square tests, Student's *t* test, and Mann-Whitney *U* tests, as appropriate.

The predictive value of day 1 PICU liver test results on PICU mortality was analyzed with use of a multivariate logistic regression analysis, adjusting for demographics, Severity of Illness scores, admission features, and study randomization (early/late PN). In brief, in an initial visual exploratory univariable analysis, we compared deciles of plasma total bilirubin, ALP, γGT, ALT, and AST concentrations and PICU mortality and identified potential thresholds for further evaluation in multivariable analyses (23). With a bootstrap approach consisting of 1,000 samples (24), we evaluated the range of 10% available concentration values centered at the visually identified cutoffs for each marker. Concentration values were dichotomized as greater than or less than or equal to the threshold, and entered as a covariate in a multivariable regression analysis, adjusting for demographics, Severity of Illness scores, admission features, nutritional risk score, and randomization to early or late PN as described above. The threshold with the minimum *p* value was identified in the multivariable model for each bootstrap sample, and the most frequently occurring in the 1,000 samples was selected as the final threshold for that marker (25)

Data are presented as numbers with percentages, mean ± SEM, or median (interquartile range), as is also indicated in the figure legends. All statistical analyses were performed with SPSS (IBM, North Castle, Armonk, NY) including the R based plugin for propensity score matching, JMP (SAS Institute, Cary, NC) and MATLAB 2014b (The MathWorks, Natick, MA). For all comparisons, a *p* value of less than or equal to 0.05 was considered significant.

RESULTS

The Prevalence of Cholestasis and Hypoxic Hepatitis in Pediatric Critical Illness and the Effect of Nutritional Strategy

Total daily caloric intake during the first 10 days in PICU and PICU baseline characteristics of the 1,231 patients between 28 days and 17 years old are shown in **Figure 1** and **Table 1**, respectively. In this subset of patients, excluding neonates less than 28 days old, patients randomized to late PN received significantly less calories during their first week in the PICU (Fig. 1). As was the case for the total population, randomization to late PN resulted in a better clinical outcome also in this subset (Table 1). Indeed, patients randomized to late PN acquired fewer new infections and required a shorter PICU- and hospital stay (Table 1). Although there were fewer new infections with late PN, the peak plasma concentrations of CRP were higher with late PN during the first 7 days in ICU (Table 1).

The daily prevalence of cholestasis increased with time in the PICU, with the proportion of patients fulfilling the predefined criterion during the first 7 days in the PICU ranging between 3.8% and 4.9%. The daily prevalence of hypoxic hepatitis during the first week in the PICU ranged between 0.8% and 2.2%. The prevalence of both cholestasis and hypoxic hepatitis was not different for patients in the late PN and the early PN group (**Table 2**). Also, daily PELOD scores remained comparable throughout the first 7 days in ICU (**Table 3**).
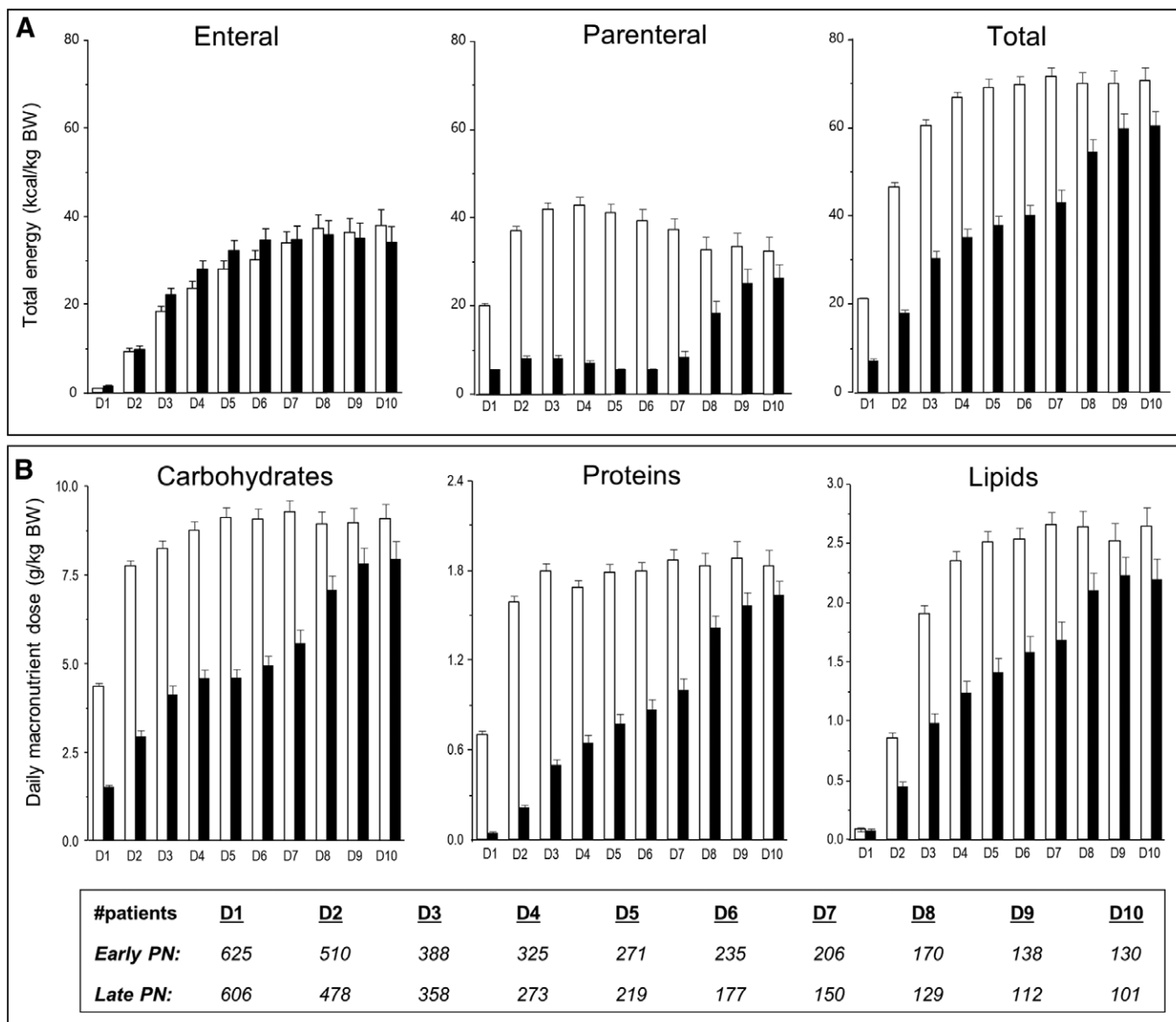
Figure 1. Daily caloric intake. **A**, Present daily total caloric intake in kilocalories per day per kg bodyweight (BW) provided by the enteral route, the parenteral route, or both (total). **B**, Present daily macronutrient intake split by carbohydrates, proteins, and lipids. *White bars* represent patients with early parenteral nutrition (PN) supplementation, *black bars* present values of late PN patients. Number of patients still in ICU per day are presented below the panels. Data are presented as mean ± SEM.

The Effect of Withholding PN for 1 Week in PICU on the Daily Profile of Plasma Total Bilirubin Concentrations and Other Liver Tests

From the first day after randomization onward, withholding PN in the first week of critical illness elevated plasma total bilirubin concentrations throughout the 7-day study intervention window as compared with early PN administration (**Fig. 2**; and Supplementary Table 1A-G, Supplemental Digital Content 1, http://links.lww.com/PCC/A770). However, from day 8 onwards, as soon as the total caloric intake became comparable for both groups, plasma total bilirubin concentrations were no longer different between late PN and early PN patients (Fig. 2; and Supplementary Table 1A-G, Supplemental Digital Content 1,

http://links.lww.com/PCC/A770). Also, peak plasma total bilirubin concentrations during the first week was higher in late PN patients as compared with early PN patients (Table 1).

Although peak plasma concentrations of ALP and γGT during the first week of critical illness were significantly lower in children receiving late PN compared with those receiving early PN (Table 1), there were no significant differences when analyzed on a daily basis (Supplementary Table 1H-N, Supplemental Digital Content 1, http://links.lww.com/PCC/A770). Also, daily plasma concentrations of ALT and AST did not differ significantly between the two treatment groups (Supplementary Table 1H-N, Supplemental Digital Content 1, http://links.lww.com/PCC/A770).

TABLE 1. Baseline and Outcome Characteristics of 1,231 Early Parenteral Nutrition and Late Parenteral Nutrition Patients

Baseline and Outcome Characteristics	Early PN group, n = 625	Late PN group, n = 606	p
Baseline characteristics			
Gender, male, n (%)	357 (57.1)	353 (58.3)	0.68
Age, yr, median (IQR)	2.4 (0.46–7.3)	2.6 (0.48–8.5)	0.31
Weight, kg, median (IQR)	12.0 (6.5–22.0)	13.0 (6.2–25.2)	0.26
Screening Tool for Risk on Nutritional Status and Growth kids risk level[a], median (IQR)	2 (2–3)	2 (2–3)	0.11
Center, n (%)			0.56
Leuven	343 (54.9)	347 (57.3)	
Rotterdam	229 (36.6)	216 (35.6)	
Edmonton	53 (8.5)	43 (7.1)	
Emergency admission, n (%)	303 (48.5)	309 (51.0)	0.37
Pediatric Index of Mortality 2 probability of death[b], median (IQR)	0.05 (0.02–0.18)	0.06 (0.02–0.16)	0.99
Pediatric Logistic Organ Dysfunction score[c] first ICU day, median (IQR)	21 (11–31)	21 (11–31)	0.71
Diagnostic category, n (%)			0.90
Surgical			
Abdominal	23 (3.7)	26 (4.3)	
Cardiac	245 (39.2)	229 (37.8)	
Neurosurgery-traumatic brain injury	63 (10.1)	53 (8.7)	
Thoracic	25 (4)	20 (3.3)	
Orthopedic-trauma	28 (4.5)	26 (4.3)	
Other	33 (5.3)	46 (7.6)	
Medical			
Cardiac	23 (3.7)	23 (3.8)	
Neurologic	51 (8.2)	49 (8.1)	
Respiratory	84 (13.4)	82 (13.5)	
Other	50 (8)	52 (8.6)	
Infection at admission, n (%)	246 (39.4)	233 (38.4)	0.74
Outcome characteristics			
ICU mortality, n (%)	21 (3.4)	26 (4.3)	0.39
ICU length of stay, median (IQR)	4 (2–8)	3 (2–7)	0.05
Hospital mortality, n (%)	33 (5.3)	32 (5.3)	1,00
Hospital length of stay, median (IQR)	12 (6–25)	10 (6–24)	0.07
New infection, n (%)	104 (16.6)	59 (9.7)	< 0.01
Peak C-reactive protein during first week, mg/L, median (IQR)	51 (24–103)	61 (31–129)	< 0.01
Liver variables, median (IQR)			
Peak total bilirubin first week, mg/dL	0.43 (0.27–0.76)	0.52 (0.31–0.83)	< 0.01
Peak alkaline phosphatase first week, IU/L	157 (119–208)	148 (114–194)	0.04
Peak gamma-glutamyl transpeptidase first week, IU/L	20 (11–47)	16 (10–34)	< 0.01
Peak alanine aminotransferase first week, IU/L	22 (13–50)	21 (13–47)	0.60
Peak aspartate aminotransferase first week, IU/L	67 (40–135)	68 (42–133)	0.82

IQR = interquartile range, PN = parenteral nutrition.

[a]Screening Tool for Risk on Nutritional Status and Growth kids, ranging from 0 to 5, with a score of 0 indicating low risk, 1 to 3 indicating medium risk, and 4 to 5 indicating high risk on malnutrition.

[b]Probability of death assessed by the Pediatric Index of Mortality 2 score, estimating mortality risk at admission.

[c]Pediatric Logistic Organ Dysfunction score of the first 24 hr, ranging from 0 to 71, with the highest scores indicating more severe illness. To convert plasma total bilirubin concentrations in mg/dL to μmol/L, multiply by 17.1.

TABLE 2. Daily Prevalence of Critical Illness-Induced Cholestasis and Hypoxic Hepatitis in PICU

PICU Day	1	2	3	4	5	6	7
Prevalence of cholestasis (> 2 mg/dL [34.2 μmol/L] plasma total bilirubin)							
Total prevalence (%)	3.9	3.8	4.3	4.1	3.6	4.9	4.7
Patients with bilirubin data/total patients in PICU, *n*	913/1,229	713/988	509/746	413/598	335/490	287/412	235/356
Number of patients per group in propensity matched set	441	323	222	179	133	116	85
Prevalence in early PN patients (%)	4.5	2.8	1.8	3.4	1.5	1.7	3.5
Prevalence in late PN patients (%)	3.6	4.6	6.8	4.5	5.3	6.0	5.9
p[a] (late vs early PN)	NS	< 0.01	NS	NS	NS	NS	NS
Prevalence of hypoxic hepatitis (≥ 20 × upper limit of normality of plasma ALT and AST)							
Total prevalence (%)	0.8	1.7	1.9	2.2	2.0	1.0	1.3
Patients with ALT/AST data/total patients in PICU, *n*	982/1,229	747/988	527/746	415/598	350/490	287/412	233/356
Number of patients per group in propensity matched set	478	341	231	180	141	118	86
Prevalence in early PN patients (%)	0.6	0.9	0.4	1.1	0.7	0.0	1.2
Prevalence in late PN patients (%)	1.0	2.3	2.6	3.3	3.5	2.5	2.3
p[a] (late vs early PN)	NS	NS	NS	NS	NS	NS	NS

ALT = alanine aminotransferase, AST = aspartate aminotransferase, NS = not significant, PN = parenteral nutrition.

[a]The comparison of late vs early PN patients was corrected for baseline characteristics including severity of illness by performing the analyses in propensity score matched subsets per day (Supplementary Table 1A-N, Supplemental Digital Content 1, http://links.lww.com/PCC/A770.

TABLE 3. Daily Pediatric Logistic Organ Dysfunction Scores of Early and Late Parenteral Nutrition Patients

dPELOD	Early PN, Median (IQR)	Late PN, Median (IQR)	*p*	No. of Patients in ICU (Early PN/Late PN)
dPELOD day 1	21 (11–31)	21 (11–31)	0.71	625/606
dPELOD day 2	13 (10–31)	13 (10–31)	0.49	510/478
dPELOD day 3	11 (2–31)	12 (1–22)	0.54	388/358
dPELOD day 4	12 (1–22)	12 (1–22)	0.47	325/273
dPELOD day 5	12 (2–22)	11 (1–21)	0.27	271/219
dPELOD day 6	12 (1–21)	12 (2–21)	0.47	235/177
dPELOD day 7	12 (1–21)	12 (2–22)	0.37	206/150

dPELOD = Daily Pediatric Logistic Organ Dysfunction, IQR = interquartile range, PN = parenteral nutrition.

The Predictive Value of Day 1 Liver Test Results for PICU Mortality

Plasma total bilirubin concentrations on the first PICU day displayed a U-shaped relationship with PICU mortality (**Fig. 3**). Using a multivariable bootstrap approach, we identified 0.20 mg/dL (3.42 μmol/L) and 0.76 mg/dL (13 μmol/L) as cutoffs in this U-shaped relationship. Indeed, when plasma total bilirubin concentrations were categorized into three categories (< 0.20 mg/dL, between 0.20 and 0.76 mg/dL, and > 0.76 mg/dL [< 3.42 μmol/L, 3.42–13 μmol/L, and > 13 μmol/L]), the group with plasma total bilirubin concentrations between 0.20 and 0.76 mg/dL (3.42–13 μmol/L) had a significantly lower mortality as compared with the patients in the lower category (T2 vs T1: odds ratio [OR] 0.25 [0.10–0.62]; $p = 0.003$), and the highest category (T2 vs T3: 0.34 [0.15–0.79]; $p = 0.01$). The latter remained significant after adjusting for baseline risk factors, severity of illness, and randomization to late PN versus early PN (T2 vs T1: 0.17 [0.04–0.67]; $p = 0.01$ and T2 vs T3: 0.15 [0.04–0.55]; $p = 0.003$).
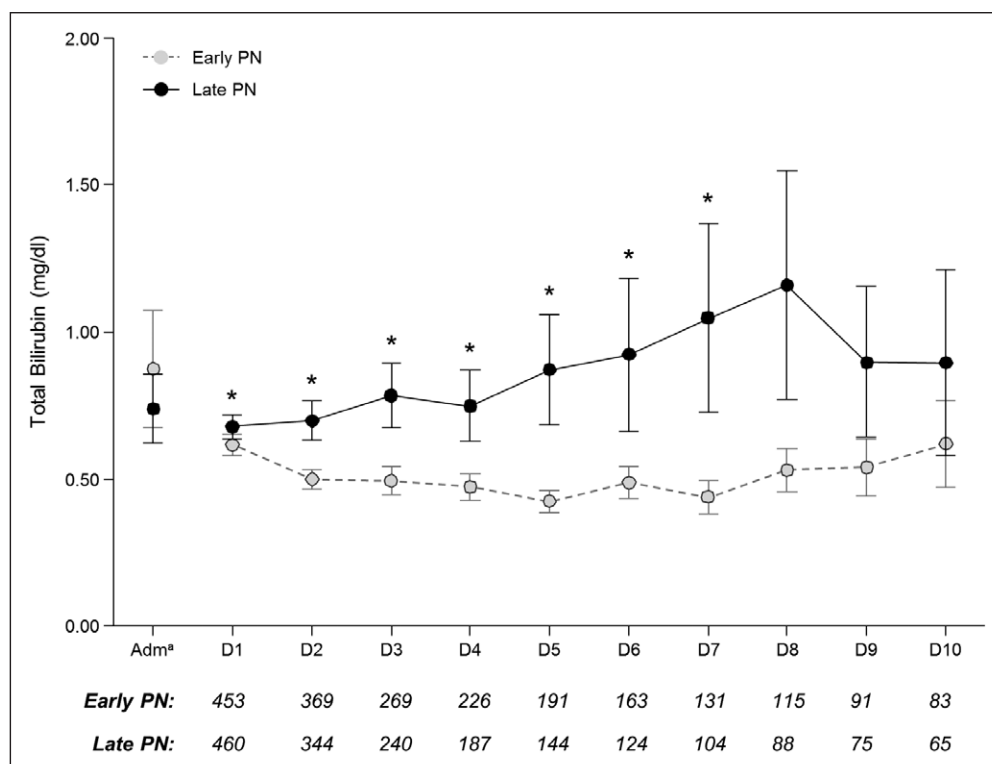
Figure 2. Daily plasma total bilirubin concentrations of all patients still in the PICU. *Black dots* present values of late parenteral nutrition (PN) patients, whereas *gray dots* represent patients with early PN supplementation. Number of patients still in ICU and of which plasma bilirubin concentrations were available at each day are presented below the panels. Data are presented as mean ± SEM. *$p \leq 0.05$ early vs late PN patients, corrected for baseline characteristics including severity of illness by performing the analyses in propensity score matched subsets per day (Supplementary Table 1A-G, Supplemental Digital Content 1, http://links.lww.com/PCC/A770). aAdmission values represent the mean ± SEM of 50 randomly selected patients per intervention arm. To convert plasma total bilirubin concentrations in mg/dL to μmol/L, multiply by 17.1.

The parenchymal lysis enzymes ALT and AST concentrations also displayed a biphasic relation with PICU mortality, using a multivariable bootstrap approach, we identified 27 IU/L for ALT and 99 IU/L for AST as cutoffs (**Supplementary Figs. 5** and **6**, Supplemental Digital Content 1, http://links.lww.com/PCC/A770). PICU mortality was significantly higher in patients with plasma ALT greater than 27 IU/L (OR, 21.95 [9.33–64.38]; $p < 0.001$) and plasma AST greater than 99 IU/L (OR, 9.632 [4.74–21.65]; $p < 0.001$) as compared with patients with plasma concentrations below these thresholds. This remained significant after adjustment for baseline characteristics including severity of illness (ALT: OR 8.22 [2.96–26.97]; $p < 0.001$) and (AST: OR 4.80 [1.78–13.86]; $p = 0.001$).

DISCUSSION

Overt cholestasis, defined as plasma concentrations of bilirubin greater than 2 mg/dL, and hypoxic hepatitis, defined as plasma concentrations of ALT and AST of greater than or equal to 20-fold higher than the upper limit of normality, were found to be rare in this PICU population and were unrelated to nutritional strategy. However, withholding PN during week 1 in the PICU increased plasma total bilirubin concentrations. A mild elevation in plasma total bilirubin on the first PICU day was associated with a lower risk of death.

The prevalence of cholestasis was found to be less than half the prevalence in adult critically ill populations and remained less than 5% throughout the first week in the PICU (1–3, 26). Altered liver physiology, such as reduced metabolic function, a decrease in liver blood flow, and a reduction of the hepatic regenerative capacity in the adult or aging patient may increase susceptibility to liver injury in response to severe illness and could explain the increased risk of illness-induced cholestasis in older subjects (27, 28). Alternatively, the threshold values used for the definition of cholestasis in adults may not be transferable to the pediatric population. Indeed, in our pediatric study population, plasma total bilirubin concentrations above 0.76 mg/dL (13 μmol/L) on the first day in PICU were already independently associated with higher mortality, which is also much below the cutoffs used in the development of

Similarly, plasma ALP concentrations on the first PICU day revealed a U-shaped relation with PICU mortality (**Supplementary Fig. 3**, Supplemental Digital Content 1, http://links.lww.com/PCC/A770). Using a multivariable bootstrap approach, we identified 94 IU/L and 200 IU/L as cutoffs in this U-shaped relationship. When plasma ALP concentrations were categorized into three categories (< 95 IU/L, between 95 and 200 IU/L, and > 200 IU/L), patients with plasma ALP concentrations between 95 and 200 IU/L had a significantly lower mortality as compared with the patients in the lower category (T2 vs T1: OR, 0.38 [0.17–0.90]; $p = 0.02$), and the highest category (T2 vs T3: 0.28 [0.12–0.68]; $p = 0.004$). However, when adjusted for demographics, Severity of Illness scores, admission features, and randomization, plasma ALP was no longer independently associated with PICU mortality. Plasma γGT and PICU mortality displayed a biphasic relation, using a multivariable bootstrap approach, we identified 11 IU/L as cutoff (**Supplementary Fig. 4**, Supplemental Digital Content 1, http://links.lww.com/PCC/A770). Patients with plasma γGT greater than 11 IU/L had a significantly higher mortality as compared with patients with plasma γGT less than or equal to 11 IU/L (OR, 10.17 [3.64–42.41]; $p < 0.001$), also after adjustment for baseline characteristics including severity of illness (OR, 4.92 [1.40–240.12]; $p = 0.01$).
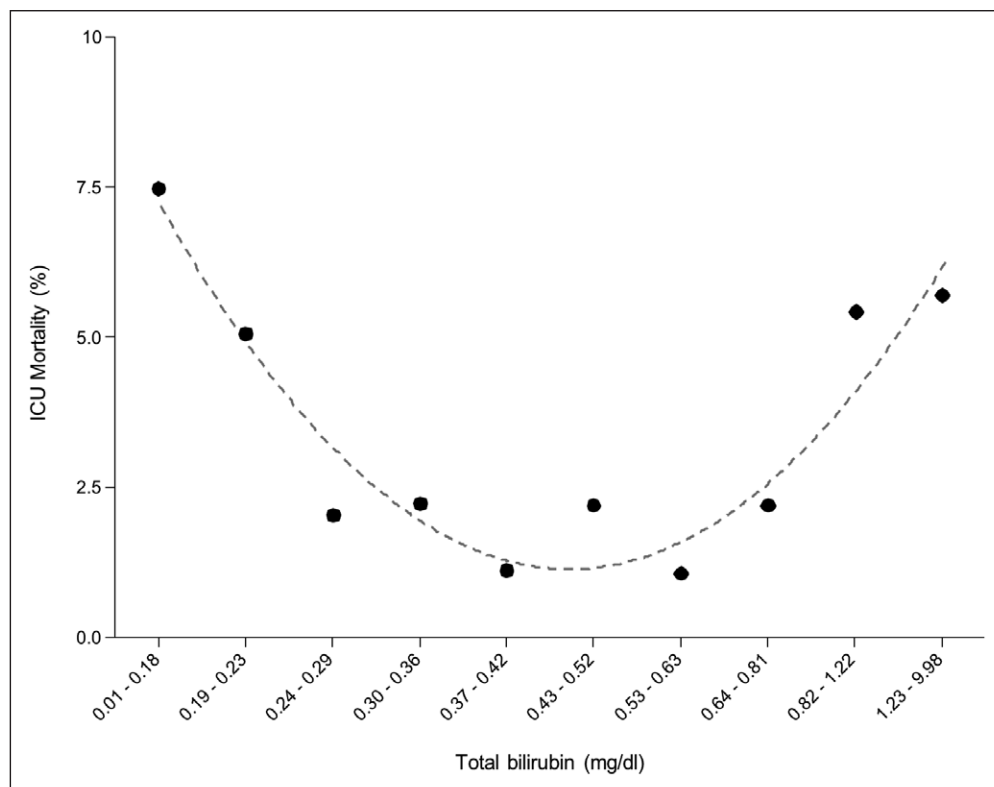
Figure 3. U-shaped relationship between plasma total bilirubin concentrations on day 1 and PICU mortality. Each interval represents 10% of all patients with available data on day 1 (*n* = 913). To convert plasma total bilirubin concentrations in mg/dL to μmol/L, multiply by 17.1.

drugs by inhibition of the hepatobiliary excretion (32, 33). It has indeed been postulated, based on available age- and weight-adjusted guideline recommendations, that early PN might have been clinically inferior to late PN because of glucose and/or lipid overfeeding and protein underfeeding (34–36). However, a post-randomization observational study, testing the independent associations between average doses of administered glucose, lipids, and amino acids and the likelihood of worse clinical outcomes, demonstrated that not the doses of glucose or lipids, but instead the protein doses explained harm caused by early PN (36). In contrast, the observation that early administration of PN actually reduced plasma bilirubin levels argues against nutrition-induced liver injury. Importantly, short-term caloric restriction, both in health and during critical illness, has shown to quickly increase bilirubin and/or bile acids, similar to our current findings (37–41). However, withholding PN also increased CRP values during the first week in ICU, which might be indicative of more inflammation (13). Inflammation is a known contributor to critical-illness induced cholestasis and might as such have contributed to the observed increase in plasma bilirubin in late PN patients (7, 9).

Plasma total bilirubin concentrations on the first PICU day displayed a U-shaped relationship with PICU mortality. That a mild elevation in plasma total bilirubin on the first PICU day was associated with a lower risk of death, may question the role of bilirubin as a valid marker of liver dysfunction. Possibly, a mild rise in plasma bilirubin may be an adaptive response to critical illness (9, 38). Indeed, antioxidant and antiinflammatory effects have been associated with elevations of plasma bilirubin after challenge with oxidative stressors and lipopolysaccharide (LPS) (42–44). Furthermore, administration of bilirubin has shown to improve survival and attenuated liver injury in response to LPS infusion (45). Additionally, knockout mice that lack heme oxygenase, which is the rate-limiting enzyme in bilirubin formation, were shown to be more likely to die and to suffer significantly more liver and renal dysfunction after LPS challenge, as compared with wild-type mice (46). Other potential protective effects of heme oxygenase induction are a direct modulation of antioxidant genes transcription, an increased production of the tissue mediator carbon monoxide

previously used pediatric organ dysfunction and mortality scores (3.5 mg/dL [60 μmol/L] in the PRISM score and 5 mg/dL [85 μmol/L] in the PELOD score [16, 29, 30]). During the first 7 days in the PICU, the prevalence of hypoxic hepatitis also remained low (< 2.2% of patients), but this low number was overall comparable to that reported for adult ICU patients (4, 6, 26, 31). Of note, we did not assess liver oxygenation to ascertain presence of hypoxia, nor did we exclude presence of potential confounding factors such as medication, blood transfusions, and infections which might have affected liver test results (7).

Both the prevalence of cholestasis and of hypoxic hepatitis were not affected by randomization to either early PN or late PN. Avoiding early administration of PN during the first week of pediatric critical illness did result in higher daily and peak levels of plasma total bilirubin, whereas daily levels of plasma γGT, ALP, ALT, or AST were unaffected, and peak levels of plasma γGT and ALP were lowered. In a previous study in critically ill adults, avoiding early PN administration also increased levels of plasma total bilirubin, but lowered other markers of cholestasis and the prevalence of biliary sludge (10). When PN is administered chronically, in doses that exceed energy expenditure or containing high doses of lipids, this can cause cholestasis outside the context of critical illness (32). This may potentially also be an additional toxic threat to hepatocytes during critical illness. Indeed, PN induced cholestasis can exert toxic accumulation of endogenous toxins and

and a decrease in cellular prooxidant heme (42). Bilirubin might also reduce cellular damage through biliverdin reductase action, which has recently been documented to be increased in septic patients (47). Indeed, when bilirubin reacts with reactive oxygen species, toxicity is neutralized and bilirubin is oxidized to biliverdin. Biliverdin can be converted back to bilirubin through biliverdin reductase, and by repeating this cycle, the antioxidative and cytoprotective effect of small amounts of bilirubin is greatly amplified (44, 47). In our study, however, total plasma bilirubin concentrations on the first day of critical illness were associated with PICU mortality in a "U-shape" relationship, in which both low and high levels of bilirubin were associated with higher risk of death. Also, other studies have described a nonlinear relationship between plasma bilirubin and mortality, both in critically illness (12) and other diseases (48). As expected, high plasma concentrations of the hepatocyte lysis enzymes ALT and AST were, above a certain threshold, independently associated with increased PICU mortality and likely reflect the severity of shock-induced liver injury (4).

Our study has several limitations to take into account. First, admission total bilirubin and other liver test results were not routinely assessed, and the bilirubin values on the first day in PICU were already affected by randomization, which precluded the comparison of changes over time within the same patient in the two intervention groups. Second, because liver tests were measured as part of routine laboratory tests, and not daily, approximately 30% of patient liver test data was missing per day in the PICU. In order to adjust for missing data and to avoid bias as much as possible, propensity score matched subsets were used to analyze liver test data. Third, the low risk of death in this pediatric study population may have limited the relevance of the observed association between admission plasma liver tests and mortality. Fourth, liver ultrasounds were not performed routinely, which would have strengthened the information on prevalence of abnormal liver tests. In addition, the predictive cutoff values that were identified with a multivariable bootstrap approach, are merely illustrative for the association with worse outcome. These cutoffs cannot be used in clinical practice without validation in a properly designed study including information on biliary inflammation, cholestasis, and sludge.

CONCLUSIONS

The overall prevalence of critical illness-induced cholestasis and hypoxic hepatitis was low in the studied critically ill children and was not affected by nutritional strategy. However, withholding PN during the first 7 days of critical illness mildly elevated plasma total bilirubin concentrations. Furthermore, plasma total bilirubin concentration on the first day of critical illness was associated with PICU mortality in a "U-shape" relationship, in which plasma total bilirubin concentrations between 0.20 and 0.76 mg/dL (3.42–13 μmol/L) were associated with lower mortality than either lower or high plasma bilirubin. Whether this may indicate a beneficial role for mildly elevated bilirubin levels as part of the stress response to critical illnesses, requires further research.

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