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ORIGINAL ARTICLE

Demographic and nutritional factors associated with prolonged cholestatic jaundice in the premature infant

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Objective: The primary aim of this study was to determine if an association exists between amino-acid levels and development of cholestasis. The secondary aim of our amino-acid dose comparison trial was to identify factors associated with the development of prolonged cholestatic jaundice.

Study Design: We compared demographic characteristics and amino-acid levels in neonates who developed cholestasis with those who did not. Parenteral-associated cholestatic liver disease was defined as a direct serum bilirubin above 5 mg per 100 ml any time during the first 28 days after birth in neonates with no history of biliary atresia or viral hepatitis. We obtained filter paper blood spots for amino acid and acylcarnitine measurements on the day of randomization and days 7 and 28 of age to identify a profile of values that could be used to identify neonates with evidence of abnormal liver function.

Result: We enrolled 122 neonates in our study; 13 (10.7%) developed cholestasis. Neonates who developed cholestasis were more immature, had lower birth weight, were exposed to parenteral nutrition for a longer period, had a higher cumulative dose of amino acids, were less often on enteral nutrition by day 7 of age, more often had a patent ductus arteriosus and severe intraventricular hemorrhage and were more commonly treated with steroids by 28 days of age. Amino acid and acylcarnitine values were not different for the two groups on the day of randomization. On day 7 (parenteral phase of nutrition), blood urea nitrogen, citrulline, histidine, methionine and succinyl carnitine were higher, and serine, glutamate and thyroxine levels were lower in the neonates who developed cholestasis than in who did not.

Conclusion: Cholestasis remains an important complication of parenteral nutrition, and several clinical and biochemical factors may be helpful in identifying high-risk patients.
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Introduction

A frequently identified risk factor for neonatal cholestatic jaundice is prolonged administration of parenteral nutrition.[1,2] Parenteral nutrition-associated cholestasis is a multifactorial disease process, the pathogenesis of which remains uncertain. By definition, cholestasis is a pathological decrease in bile formation and bile flow. Newborn infants and, to a greater extent, preterm infants have a functionally and structurally immature biliary system with reduced bile flow. There is a decreased synthesis of bile salts and inefficient uptake, or recycling, of bile salts from portal blood. Physiologic or toxic insults to the liver pose additional risks for immature enterohepatic circulation.[3]

Parenteral nutrition-associated liver dysfunction was first described by Peden *et al*.,[4] in 1971. Despite advances in the understanding of parenteral nutrition-associated liver dysfunction, there is still no specific test that will diagnose the liver injury that may occur following parenteral therapy.[5] The diagnosis is made by eliminating other causes of cholestasis. Typically, neonates with parenteral nutrition-associated cholestasis have a serum-conjugated bilirubin level greater than 2.0 mg per 100 ml and more than 15% of the total bilirubin level after receiving parenteral nutrition for a minimum of 14 consecutive days.[3] A stricter definition of cholestasis, namely a direct serum bilirubin above 5 mg per 100 ml in the first 28 days of life, was used in the present research study to identify neonates with significant liver dysfunction.

In a previous randomized clinical trial, our research group measured the effect of two distinct strategies of parenteral nutrition supplementation on blood amino-acid profiles and on growth from birth to 28 days of life.[6] In our clinical trial, the use of a higher dose (higher initial dose, faster advancement and higher maximum dose) of amino acids in parenteral nutrition did not promote improved growth (weight gain or changes in length and head circumference) compared to a lower dose of amino-acid supplementation. In addition, several blood amino-acid levels and the blood urea nitrogen were higher by day 7 in patients treated with the higher-dose approach. There was no difference in the incidence of cholestasis between the two treatment groups.

A secondary objective of our clinical trial was to examine factors associated with the development of prolonged cholestatic jaundice. The purpose of this study was to report on the characteristics of patients in the study cohort that developed severe parenteral nutrition-associated cholestasis.

Methods

In a multicenter ($n = 11$ sites) trial, we randomly allocated infants to one of the two parenteral nutrition approaches.[6] Eligible infants had an estimated gestational age between 23 and 0/7 weeks and 29 and 6/7 weeks, were less than 48 h of age at randomization, were inborn and had parental consent obtained for participation in the study. Investigational review boards of each hospital ($n = 11$) approved the protocol. Patients were excluded if they had a major chromosomal or congenital anomaly. There were two treatment groups. In one group, the maximum dose was 2.5 g kg^{-1} day^{-1} group (2.5 group). Amino-acid supplementation was started at 1.0 g kg^{-1} day^{-1} and advanced 0.5 g kg^{-1} day^{-1} to a maximum of 2.5 g kg^{-1} day^{-1} on day 4 of treatment. Amino-acid supplementation decreased to 1.0 g kg^{-1} day^{-1} once feedings reached 80 to 100 ml kg^{-1} day^{-1}. In the second group, the maximum dose was 3.5 g kg^{-1} day^{-1} group (3.5 group). Amino-acid supplementation started at 1.5 g kg^{-1} day^{-1} of amino acids and advanced 1 g kg^{-1} day^{-1} to a maximum of 3.5 g kg^{-1} day^{-1} by day 3 of treatment. Amino-acid supplementation decreased to 2.0 g kg^{-1} day^{-1} once feedings reached 80 to 100 ml kg^{-1} day^{-1}. In both treatment groups, the amino-acid supplementation stopped once feedings reached 100 to 130 ml kg^{-1} day^{-1} and the patient was considered to have completed the treatment. Subsequent parenteral nutrition was given at the discretion of the health-care team.

We compared the demographic characteristics and amino-acid levels in neonates who developed cholestasis with those that did not. In our clinical trial, parenteral-associated cholestatic liver disease was prospectively defined as a direct serum bilirubin above 5 mg per 100 ml any time during the first 28 days after birth in neonates with no history of biliary atresia or viral hepatitis. We obtained filter paper blood spots for amino-acid measurement on the day of randomization and on days 7 and 28 of age to monitor laboratory values.

Numeric data are presented as median and quartiles (25th and 75th) because the data were not normally distributed. We compared the differences between treatment groups using univariate and multivariate techniques. Continuous variables (estimated gestational age, birth weight and total protein dose) were evaluated with two-tailed Student's *t*-tests, and nonparametric continuous data were assessed with a Kruskal–Wallis analysis of variance. Categorical variables (for example, race and gender) were evaluated with a two-tailed χ^2 test and Fisher's exact test. After univariate analysis, we used multivariate logistic regression to calculate the adjusted odds ratio for the development of cholestasis. In the logistic regression analysis, we incorporated the variables found by univariate analysis to be $P<0.1$. Birth weight and gestational age were entered into the model as continuous variables and included in all models. All statistical analysis was performed using JMP Release 5.0.1a (SAS Institute Inc, Cary, NC, USA).

Results

We enrolled 122 neonates in our study; 13 (10.7%) developed cholestasis. Neonates who developed cholestasis were more immature (26 vs 27 weeks, $P = 0.02$) and had a lower birth weight (800 vs 990 g, $P = 0.04$) compared with neonates who did not develop cholestasis (Table 1). Neonates who developed cholestasis were exposed to parenteral nutrition for a longer period (27 vs 16 days, $P = 0.001$), received a higher cumulative dose of parenteral amino acids (91.5 vs 38.5 g kg^{-1}, $P = 0.001$) and were less often on enteral nutrition by day 7 of age (30.8 vs 73.4%, $P = 0.003$) than neonates who did not develop cholestasis (Table 1).

Neonates with cholestasis also more often had a report of a patent ductus arteriosus (76.9 vs 42.4%, $P = 0.04$), severe intraventricular hemorrhage (53.8 vs 7.5%, $P = 0.001$), steroid treatment by 28 days of age (53.8 vs 22.9%, $P = 0.04$) and a blood transfusion (100 vs 72.2%, $P = 0.04$) than neonates who did not develop cholestasis (Table 1).

Metabolic evaluation of the study neonates showed no differences between the two groups on the day of randomization in any of the measured amino acid or acylcarnitine values (data not shown). On day 7 (parenteral phase of nutrition), neonates with cholestasis had higher blood urea nitrogen (50 vs 23 mg per 100 mg, $P = 0.003$), citrulline (11.4 vs 8.2, $P = 0.021$), histidine (49.5 vs 39 μmol l^{-1}, $P = 0.007$), methionine (35 vs 27.2 μmol l^{-1}, $P = 0.04$) and succinyl carnitine (0.127 vs 0.08 μmol l^{-1}, $P = 0.009$) levels than neonates who did not develop cholestasis. In contrast, glutamate (97 vs 124 μmol l^{-1}, $P = 0.018$), serine (84 vs 115 μmol l^{-1}, $P = 0.01$) and thyroxine (3.5 vs 6.2 μg per 100 ml, $P = 0.005$) levels were lower in the neonates who developed cholestasis compared with those who did not (Figure 1 and Table 2).

Our small sample size precluded detailed multivariate analysis, but in logistic modeling the most important and only independent variable associated with an increase risk of cholestasis was the total protein dose.

Discussion

In our multicenter study comparing two strategies of parenteral nutrition supplementation, 10.7% of the enrolled preterm infants developed cholestasis. Consistent with reports of previous researchers, we found that the affected infants were more immature

Table 1 Demographics and outcomes

	No cholestasis	*Cholestasis*	*All patients*	P-*value**
	109	13	122	
Gestational age (week), median (25–75th percentile)	27 (26–28)	26 (24–27)	27 (25–28)	0.02
Birth weight (g), median (25–75th percentile)	990 (804–1220)	800 (739–884)	930 (787–1193)	0.04
Birth length (cm), median (25–75th percentile)	35.5 (32.8–38)	33.5 (32.1–34.4)	35.25 (32.5–38)	0.10
Head circumference (cm), median (25–75th percentile)	25 (23.5–26.5)	23.7 (22.8–24.8)	25 (23–26.5)	0.10
Apgar 1 min, median (25–75th percentile)	5 (4–7)	4 (3–6)	5 (3–7)	0.33
Apgar 5 min, median (25–75th percentile)	8 (7–8)	7 (6–8)	8 (7–8)	0.14
Total days on parenteral nutrition, median (25–75th percentile)	16 (11–25)	27 (24.5–27.5)	18 (12–25)	0.001
Total parenteral amino acids during first 28 days ($g\,kg^{-1}$), median (25–75th percentile)	38.5 (23–59)	91.5 (64–92)	44 (27–63)	0.001
Type of amino acids, n *(%)*				
Aminosyn	8 (7.3)	0	8 (6.6)	
TrophAmine	101 (92.7)	13 (100)	114 (93.4)	0.68
Day 7 nutrition, median (25–75th percentile)				
Glucose infusion (mg/kg/min)	11.3 (8.6–13.6)	11.4 (7.7–13.0)	11.3 (8.6–13.5)	0.75
Lipids infusion (gm/kg/d)	2.3 (1.6–2.9)	2.3 (1.7–2.8)	2.3 (1.6–2.9)	0.96
IV fluids (cc/kg/d)	132 (105.25–159)	147 (125–159)	134 (108–158)	0.27
Enteral feedings (cc/kg/d)	19.2 (0–55)	0 (0–2.6)	12.8 (0–52.5)	0.003
Total fluids (cc/kg/d)	154 (141–174)	149 (132–172)	154 (140–173)	0.44
Randomized to 3.5 group, *n* (%)	53 (48.6)	5 (38.5)	58 (47.5)	0.57
Antenatal steroids, *n* (%)	89 (81.6)	13 (100)	102 (83.6)	0.27
Cesarean section, n (%)	68 (62.4)	10 (76.9)	78 (63.9)	0.37
Male gender, *n* (%)	63 (57.8)	7 (53.8)	70 (57.4)	0.78
Race, n *(%)*				
Black	32 (29.4)	5 (38.5)	37 (30.3)	0.96
Hispanic	12 (11)	1 (7.7)	13 (10.7)	0.96
Other	12 (11)	1 (7.7)	13 (10.7)	0.96
White	53 (48.6)	6 (46.2)	59 (48.4)	0.96
Surfactant given, *n* (%)	89 (81.7)	12 (92.3)	101 (82.8)	0.46
Outcomes to 28 days				
Steroids in first 28 days, *n* (%)	25 (22.9)	7 (53.8)	32 (26.2)	0.04
Growth in first 28 days, *n* (%)	106	12	118	
Weight gain, median (25–75th percentile)	11.9 (8.35–14.6)	13.1 (10.4–22.5)	12.2 (8.5–14.9)	0.097
Head growth, median (25–75th percentile)	0.5 (0.38–0.75)	0.25 (0.19–0.56)	0.5 (0.28–0.75)	0.06
Length growth, median (25–75th percentile)	0.75 (0.38–1)	0.5 (0.32–0.75)	0.75 (0.375–1)	0.10
PDA treatment reported, *n* (%)	46 (42.2)	10 (76.9)	56 (45.9)	0.04
Positive blood or CSF culture, *n* (%)	22 (20.2)	4 (30.8)	26 (21.3)	0.60
Intraventricular hemorrhage grade 3 or 4, *n* (%)	8 (7.5)	7 (53.8)	15 (12.6)	0.001
Cystic periventricular leukomalacia, *n* (%)	3 (2.9)	1 (9.1)	4 (3.5)	0.34
Any report of bowel disease (NEC or SIP), *n* (%)	9 (8.2)	2 (15.3)	11 (9.0)	0.74
Received a blood transfusion, *n* (%)	78 (72.2)	13 (100)	91 (75.2)	0.04
Died, *n* (%)	2 (1.8)	1 (7.7)	3 (2.5)	0.73
Died or chronic lung disease, *n* (%)	68 (62.3)	12 (92.3)	80 (65.6)	0.07

*Kruskal–Wallis ANOVA for continuous data elements that were non-pararnetric.

and had lower birth weight than the non-affected group.[5,7–9] Other investigations have also described an association between cholestasis and the quantity and formulation of amino acids administered parenterally, excess caloric intake of fats and carbohydrates,[10,11] toxicity of trace minerals,[12–16] male gender,[17] perinatal asphyxia,[18] phototoxicity of multivitamin

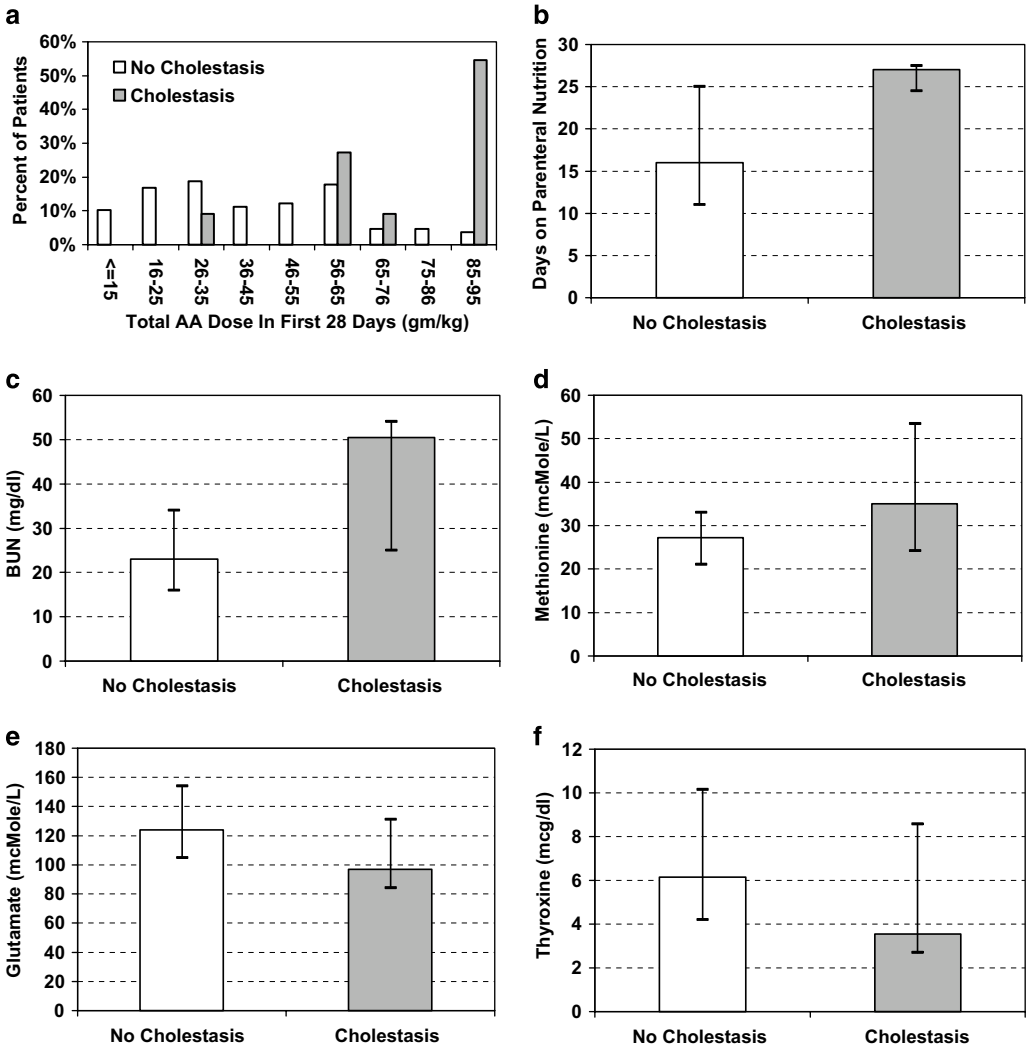

Figure 1 (**a**) Histogram plot of the proportion of neonates (*y* axis) who received a specified amount of amino acids (*x* axis) through parenteral nutrition. The amount of amino acids reported on the *x* axis is the total amount order for the 28 days of the clinical study. The *y* axis is the proportion of patients in each specified group. Neonates who developed cholestasis fall to the right side of the graph, which suggests that neonates with cholestasis were exposed to higher doses of parenteral amino-acid supplements. (**b**) Median and quartiles (25–75th) for the number of days on parenteral nutrition for neonates with cholestasis compared to those without cholestasis. (**c**) Median and quartiles (25–75th) for blood urea nitrogen (mg per 100 ml) on day 7 for neonates with cholestasis compared with those without cholestasis. (**d–f**) Selected examples of laboratory findings: (**d**) methionine, (**e**) glutamate and (**f**) thyroxine. All values reported in $\mu mol\,l^{-1}$. *P*-values are listed in Table 2. Graphs present median and 25–75th percentile for measure values.

supplements,[19–21] toxicity from plant phytosterols[22,23] and genetic predisposition.[2]

Aggressive administration of parenteral amino acids to improve protein accretion rates in very preterm neonates has been supported in the literature.[24–27] Although tolerance of high-dose amino acids has been described, researchers acknowledge that sensitive tests to monitor amino-acid toxicity are not readily available in the clinical setting.[25,28] Our study results are consistent with previous reports that associate cholestasis with increased duration of parenteral nutrition and higher cumulative dose of amino acids.[5,8,29] However, within the context of our randomized clinical trial, early higher doses of amino acids were not associated with an increase in the risk of cholestasis, suggesting that the daily dose of amino acids may not be as important as the total cumulative dose of amino acids.[6]

Previous researchers have suggested that the composition of the amino acids used in the parenteral solution may be a risk factor for parenteral nutrition-associated cholestasis.[29–31] In our study, 93.4% of the preterm infants received Trophamine and 6.6% received Aminosyn. There was no significant difference in the occurrence of cholestasis in the infants receiving these two forms of amino-acid supplementation.

Certain amino acids (for example, methionine, phenylalanine, tyrosine and tryptophan) are hepatotoxic, whereas other amino

Table 2 Metabolic panel on day 7

	No cholestasis	*Cholestasis*	P-*value**
	105	12	
Amino-acid			
Alanine	148 (110–189)	130 (92–142)	0.148
Arginine	14.3 (10.3–21.9)	14.6 (8.3–29)	0.936
Aspartate	15.3 (12.4–18.5)	13.6 (11.7–16.4)	0.273
Citrulline (119)	8.2 (6.6–9.7)	11.4 (7.7–14.3)	0.021
Glutamate	124 (105–154)	97 (84–131)	0.018
Glycine	114 (96.8–137)	116 (82.8–154)	0.872
Histidine	39 (32.4–45.8)	49.5 (40.7–55.1)	0.007
Leucine–isoleucine	162 (141–190)	179 (142–215)	0.374
Methionine	27.2 (21.1–33)	35 (24.2–53.4)	0.040
Ornithine	45.5 (38.7–56.9)	51.8 (39–78.8)	0.281
Phenylalanine	55.7 (46.6–63.2)	51.7 (47.6–65.2)	0.686
Serine	115 (88.5–148)	84 (71.7–113)	0.010
Tyrosine	51.4 (33.1–72.9)	36.4 (23–57.5)	0.070
Valine	103 (86.7–124)	117 (98.8–146)	0.181
Acylcarnitines			
Acetyl (C2)	12.4 (9.2–17.1)	12 (6.2–18.5)	0.553
Butyryl (C4)	0.28 (0.21–0.38)	0.35 (0.26–0.59)	0.105
Free-carnitine	15.4 (10.6–28.5)	13.1 (7.2–42.5)	0.660
Glutaryl (C5DC)	0.024 (0.018–0.041)	0.026 (0.014–0.065)	0.861
Isovaleryl (C5)	0.29 (0.21–0.39)	0.27 (0.16–0.81)	0.993
3-Hydroxyisovaleryl (C5OH)	0.15 (0.11–0.19)	0.18 (0.14–0.25)	0.077
Linoleyl (C18:2)	0.57 (0.38–0.81)	0.59 (0.44–0.89)	0.706
Oleyl (C18:l)	0.63 (0.43–0.81)	0.54 (0.38–0.67)	0.094
Palmitoyl (C16)	0.63 (0.45–0.94)	0.57 (0.38–0.71)	0.152
Palmitoleoyl (16:l(N–7))	0.038 (0.028–0.067)	0.041 (0.03–0.052)	0.886
Propionyl (C3)	0.81 (0.59–1.3)	0.92 (0.46–1.6)	0.872
Stearoyl (C18)	0.55 (0.43–0.74)	0.57 (0.4–0.66)	0.812
Succinyl (C4DC)	0.08 (0.053–0.13)	0.127 (0.093–0.18)	0.009
Other laboratory data on day 7			
Blood urea nitrogen (mg per 100 ml)	23 (16–34)	50.5 (25–54)	0.003
Thyroxine (μg per 100 ml)	6.2 (4.2–10.1)	3.5 (2.7–8.6)	0.005

All data presented as median (25–75th percentile). Serum amino-acid and acyl carnitine levels are reported in $\mu mol\,l^{-1}$.
*Kruskal–Wallis ANOVA for continuous data elements that were non-pararnetric.

acids (for example, glutamine and taurine) may reduce liver injury.[7] The use of parenteral nutrition solutions, which are lower in methionine and aromatic amino acids and higher in taurine, have been associated with a decreased incidence in liver dysfunction.[7] Glutamate and glutamine are important for cellular metabolism, and supplementation with glutamine in the parenteral solution has been recommended as a possible management strategy for neonates with prolonged cholestatic jaundice.[1,32–35] We found that levels of blood urea nitrogen, citrulline, histidine, methionine and succinyl carnitine were higher on day 7 in neonates who developed cholestasis. In contrast, glutamate and serine levels were lower in neonates who developed cholestasis than in those who did not. Our data do not allow us to determine whether the difference in amino-acid levels that we observed is the cause or the consequence of ongoing liver injury. They do lend credence, however, to the concept that monitoring amino-acid levels may identify neonates at risk for developing cholestasis and that avoiding high levels of potentially hepatotoxic amino acids might reduce the occurrence of cholestasis in premature neonates.

Enteral feedings, even small volumes with minimal caloric value, appear to be important for the stimulation of enterohepatic function.[5,36,37] In the absence of feedings, hormone levels of gastrin and cholecystokinin are reduced, decreasing bile transport. Our data supported the importance of early enteral feedings. Preterm infants with cholestasis received more parenteral nutrition

and less enteral nutrition by 7 days of age than infants who did not develop cholestasis.

Finally, we found that preterm infants with cholestasis were more often treated with steroids in the first 28 days after birth. The use of steroids is a marker for degree of illness (dexamethasone for treatment of chronic lung disease, and hydrocortisone for cardiovascular support). An interesting finding of our research was that preterm infants with cholestasis more often had severe intraventricular hemorrhage, were more likely to have received treatment for a patent ductus arteriosus and more often received a blood transfusion. Neonates with cholestasis also had lower thyroxine levels. Although an association between hypothyroidism and prolonged jaundice has been documented in the literature,[38] the role of low thyroxine levels in premature infants and the risk of cholestasis have not been adequately studied. Exposure to steroids, severe intraventricular hemorrhage, treatment for ductus arteriosus, need for blood products and low thyroxine levels may all be covariate markers for the degree of immaturity or severity of illness. Additional research is needed, however, to determine if these associations are reproducible in a larger population of preterm infants and to what degree each of these factors contributes to the development of cholestasis in premature neonates.

Summary

Cholestasis remains an important and common complication of parenteral nutrition in premature neonates. Our data suggest that there may be metabolic markers that identify at-risk patients (high blood urea nitrogen, citrulline, histidine, methionine, serine and succinyl carnitine, and low thyroxine and glutamate). Further research is urgently needed to define safe and effective strategies for providing parenteral nutrition to critically ill premature neonates.

Pediatrix Amino-Acid Study Group

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Conflict of interest: All of the authors are employees of Pediatrix Medical Group that owns Pediatrix Screening, a company that offers newborn screening for inborn errors of metabolism and hearing loss.

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