LIVER FAILURE/CIRRHOSIS/PORTAL HYPERTENSION

Intravenous N-acetylcysteine in Pediatric Patients With Nonacetaminophen Acute Liver Failure: A Placebo-Controlled Clinical Trial

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N-acetylcysteine (NAC) was found to improve transplantation-free survival in only those adults with nonacetaminophen (non-APAP) acute liver failure (ALF) and grade 1-2 hepatic encephalopathy (HE). Because non-APAP ALF differs significantly between children and adults, the Pediatric Acute Liver Failure (PALF) Study Group evaluated NAC in non-APAP PALF. Children from birth through age 17 years with non-APAP ALF enrolled in the PALF registry were eligible to enter an adaptively allocated, doubly masked, placebo-controlled trial using a continuous intravenous infusion of NAC (150 mg/kg/day in 5% dextrose in water [D5W]) or placebo (D5W) for up to 7 days. The primary outcome was 1-year survival. Secondary outcomes included liver transplantation-free survival, liver transplantation (LTx), length of intensive care unit (ICU) and hospital stays, organ system failure, and maximum HE score. A total of 184 participants were enrolled in the trial with 92 in each arm. The 1-year survival did not differ significantly ($P = 0.19$) between the NAC (73%) and placebo (82%) treatment groups. The 1-year LTx-free survival was significantly lower ($P = 0.03$) in those who received NAC (35%) than those who received placebo (53%), particularly, but not significantly so, among those less than 2 years old with HE grade 0-1 (NAC 25%; placebo 60%; $P = 0.0493$). There were no significant differences between treatment arms for hospital or ICU length of stay, organ systems failing, or highest recorded grade of HE. *Conclusion*: NAC did not improve 1-year survival in non-APAP PALF. One-year LTx-free survival was significantly lower with NAC, particularly among those <2 years old. These results do not support broad use of NAC in non-APAP PALF and emphasizes the importance of conducting controlled pediatric drug trials, regardless of results in adults. (HEPATOLOGY 2013;57:1542-1549)

Pediatric acute liver failure (PALF) is a rare and devastating syndrome in which previously healthy children rapidly lose hepatic function due to a variety of causes and become critically ill within days.[1,2] Management is largely supportive unless conditions that are amenable to directed therapy, such as acute acetaminophen toxicity, herpes virus, and potentially treatable causes such as Wilson's disease and autoimmune hepatitis are identified and treated.[3,4] Early referral to a liver transplantation (LTx) center,

improvements in medical management, and LTx are associated with improved survival in PALF.[5] Long-term outcomes following LTx for PALF are poor compared to other indications for LTx,[6,7] hence the need to identify treatments that improve survival. N-acetylcysteine (NAC), used for treating acute acetaminophen (APAP) toxicity, has been used to treat non-APAP PALF but has not been rigorously tested in a multicenter placebo-controlled clinical trial in children.[8,9]

NAC replenishes mitochondrial and cytosolic glutathione stores and is the treatment of choice for acute APAP toxicity.[10,11] Intravenous NAC became incorporated into the general management of acute liver failure in a number of sites in Europe and North America following a small uncontrolled study suggesting improved cardiovascular hemodynamics and oxygen transport in both APAP and non-APAP liver failure in adults.[12] A retrospective single-site review in children affirmed the view that NAC may provide benefit in PALF.[8] In a randomized trial of intravenous NAC versus placebo in adults with non-APAP liver failure, NAC did not improve survival at 21 days.[13] However, an analysis of the secondary outcomes revealed improved LTx-free survival at 3 weeks for those with grade 1-2 hepatic encephalopathy (HE). The primary objective of this study was to determine if a continuous intravenous infusion of NAC for up to 7 days would improve overall survival compared to placebo 1 year following treatment allocation in non-APAP PALF.

Patients and Methods

Study Oversight. This study was funded by the Division of Digestive Diseases and Nutrition within the National Institute of Diabetes and Digestive and Kidney (NIDDK) Diseases of the National Institutes of Health (NIH) and was registered with www.ClinicalTrials.gov (NCT00248625). Study design and management of the NAC trial was accomplished by academic clinical investigators and site coordinators associated with the Pediatric Acute Liver Failure Study Group (PALFSG). A Data and Safety Monitoring Board (DSMB) consisting of experts in pediatric hepatology, pediatric LTx, and statistical analysis was appointed by the NIH/NIDDK and did not have any conflicts of interest with members or institutions that constituted the PALFSG or the suppliers of NAC. NAC was initially provided by Apothecon/Geneva Pharmaceuticals (Princeton, NJ), a division of Bristol Myers Squibb, and after April 2003 was supplied by Cumberland Pharmaceuticals (Nashville, TN). The Investigational New Drug application (IND) was held by the Principal Investigator for the trial (R.H.S.). The PALFSG consisted of 20 pediatric sites in North America and the United Kingdom and a Data Coordinating Center (DCC).

Study Population. Eligible participants for the NAC trial were drawn from a registry of children with PALF established at PALFSG sites. Each site was a pediatric liver transplantation center and no donor organs were obtained from executed prisoners or other institutionalized persons. The goal of the registry was to collect detailed information from children with PALF. Entry criteria for the PALF registry included children who were under the age of 18 years, informed consent and assent when appropriate, absence of a known chronic liver disease, biochemical evidence of acute liver injury, and a liver-based coagulopathy, not corrected by parenteral vitamin K. Clinical evidence of HE was required if the prothrombin time (PT) was between 15-19.9 seconds or the International Normalization Ratio (INR) was between 1.5-1.9. HE was not required for a PT of at least 20 seconds or an INR at least 2.0, given the challenges of assessing clinical HE in infants and children. Detailed clinical and biochemical information was collected prospectively for up to 7 days following enrollment.[2,14,15]

From February 2001 through September 2009, 635 children enrolled in the PALF registry after site-specific Institutional Review Board (IRB) approvals for the NAC trial were obtained. Eligibility criteria for the NAC trial included enrollment in the PALFSG registry and completion of a separate informed consent for the NAC study from a parent or guardian and an informed assent from the patients who were 14-17 years of age,

developmentally able to sign his or her name, and were without clinical HE.

Exclusion criteria included known acute acetaminophen toxicity, prior exposure to N-acetylcysteine during the course of the presenting illness, pregnancy, malignancy, sepsis, signs of cerebral herniation, being on a liver support device, intractable hypotension defined as systolic blood pressure less than 85 mmHg or hypotension that required treatment with inotropic drugs, other than renal dosing dopamine, and other issues that in an investigator's opinion made a potential participant unsuitable for the study. We defined "sepsis" in the manual of operations as a having bacteremia and/or a temperature of 39.5°C at the time of enrollment. If the patient was known to have a specific infection or if, in the opinion of the site principal investigator, the patient was suspected of having sepsis, prior to enrollment into the NAC trial, they were excluded.

Study Design. The NAC study was doubly masked. Eligible children were adaptively allocated within strata defined by age (less than 2 years of age or at least 2 years old) and HE (grade 0-1 or 2-4) to receive NAC (150 mg/kg/d) in 5% dextrose (D5W) and water or placebo consisting of an equal volume of D5W alone. Volumes were adjusted for small children. Study medications were infused over 24 hours for up to 7 consecutive days in a dedicated line without other medications. Treatment was stopped earlier than 7 days in the case of hospital discharge, LTx, or death within 7 days of randomization. Although multiple different dosing regimens have been used in previous studies,[8,12,13,16,17] a recent retrospective study[8] utilized a continuous infusion to support the evolving and dynamic nature of ALF in children.

Clinical parameters including vital signs, diagnostic and clinical laboratory tests, need for ventilatory, renal or other supportive measures, coma, and adverse events were recorded. With the exception of study medication, patient management conformed to the standard of care at each participating site, including decisions related to LTx.

The primary outcome was 1-year survival following treatment allocation. Secondary outcomes included LTx, survival without LTx, lengths of intensive care unit (ICU) and hospital stay, maximum degree of HE, and number of organ systems failing. Organ systems and definitions of failure were: (1) cardiovascular failure: the patient requires treatment with inotropic drugs such as norepinephrine, epinephrine, or dopamine (the latter >5 μg/kg/min) at a time prior to the terminal 24 hours before death to attribute cardiovascular failure to liver failure and not terminal events; (2) renal failure: serum creatinine greater than 2 times the upper limit of normal for age and urine output less than 0.5 cc/kg/hour; (3) intracranial hyperten-

sion: intracranial pressure >25 mmHg if ICP monitored or clinical signs such as decerebrate posturing or abnormal pupillary reflexes; (4) pulmonary failure: need for mechanical ventilation for HE or respiratory failure, defined as an inspired oxygen fraction (FiO2) above 0.40, the first 12 hours after intubation and the terminal 12 hours before death excluded; and (5) infection: defined as identification, by culture, serologies, polymerase chain reaction (PCR) or immunofluorescence, of a specific microorganism or virus present in blood, tracheal aspirate, urine, intravenous catheter, wound, liver tissue, stool, naso-pharynx, cerebral spinal fluid, bone marrow, or ascites.

Statistical Analysis. Differences in baseline characteristics of participants in each treatment arm were tested using Pearson's chi-square test or Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. Product-limit estimates were used to obtain the cumulative percentages of participants surviving 1 year following randomization and the percentages of participants surviving 1 year following randomization with their native liver. A log-rank test was used to assess statistical significance of the difference in survival curves. The proportional hazards model[18] was used to estimate the relative risk for both outcomes adjusting for age (less than 2 years, at least 2 years) and HE grade at randomization (0-1, 2-4), which were used as strata in the minimization scheme. Differences between treatment arms for length of hospital stay and length of ICU stay were tested for statistical significance using the Kruskall-Wallis test and the Cochran-Armitage test for trend, respectively. Tests of ordinal measures were used to enable us to account for deaths or transplantations that occurred prior to discharge from the hospital or ICU, respectively. Transplantations that occurred prior to discharge were assigned a value greater than the maximum stay and deaths occurring prior to discharge were assigned a value greater than transplantation. Pearson's chi-square test or Fisher's exact test were used to test the null hypothesis of no difference in the proportion of participants in each treatment arm who had each organ system fail, and the Cochran-Armitage test for trend was used to test for treatment arm differences in the number of organ systems failing and for maximum grade of HE. Due to two interim analyses, the *P*-value for statistical significance of each outcome was 0.0490, overall and in subgroups. For all other comparisons, *P* < 0.05 was used to determine statistical significance.[19]

Results

Baseline Characteristics. Of the 607 participants in the PALF registry eligible for screening for the NAC
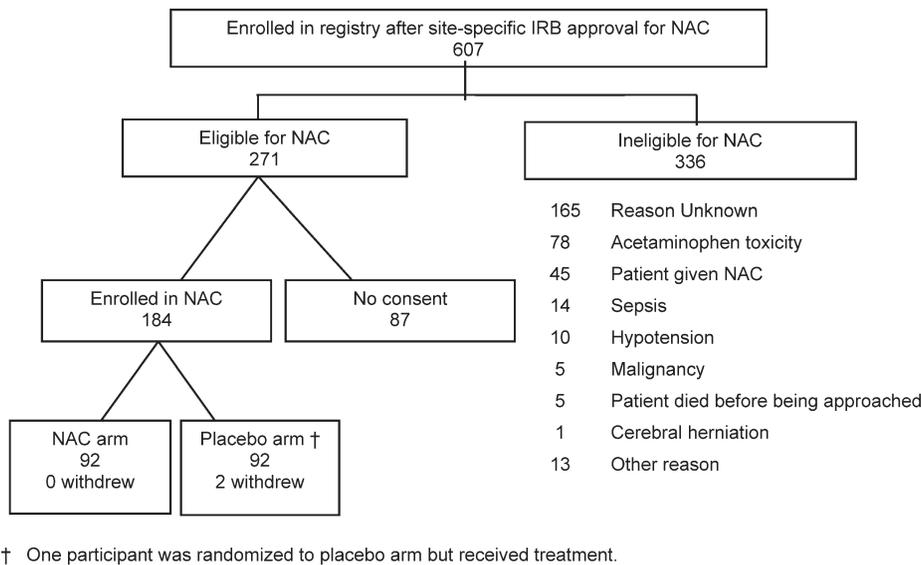
Fig. 1. Enrollment in the NAC trial. Between June 2003 and September 2009, 607 patients enrolled in the PALF longitudinal study were screened following site-specific IRB approval for the NAC protocol; 336 were ineligible. Of the 271 patients eligible for enrollment, 87 refused consent, leaving 184 enrolled in the NAC trial; there were 92 patient in each arm. Only two patients withdrew following enrollment, and they were both in the placebo arm.

† One participant was randomized to placebo arm but received treatment.

study, 336 were ineligible (Fig. 1). Ineligibility criteria remained constant throughout the study, but reasons for ineligibility were not recorded on the initial case report form for 165 participants and are shown as "reason unknown." Of the remaining 171, reasons for ineligibility were recorded. In addition to those who failed to meet inclusion criteria, or who met at least one exclusion criterion, five died in the interval between entry into the PALF longitudinal study and the 24 hours allotted to enroll into the NAC trial. Another 13 were excluded for other reasons: seven had preexisting conditions that, in the opinion of the investigator, excluded them from the trial, two were demonstrating clinical recovery from PALF, two were excluded because they presented when the trial was briefly suspended to ensure all sites were in agreement with the statistical analyses plan, one patient was not able to be randomized due to technical difficulties with computer entry, and in for one patient liver transplantation was imminent. Of the 271 potentially eligible, consent was not obtained from 87, leaving 184 participants with 92 allocated to each treatment. Two participants withdrew from the placebo arm and no one withdrew from the NAC arm. One participant was randomized to placebo but received NAC. One randomized participant in the placebo arm had APAP toxicity that was determined during the hospitalization but after treatment allocation; this patient exited the study and was treated with NAC clinically. One patient was found to have biliary atresia after treatment allocation. All of these participants were analyzed in the treatment arm to which they were assigned (intention-to-treat analysis).

The two treatment groups did not differ significantly with respect to age, gender, race, coma grade, biochemical measures of liver injury, and time from admission to hospital until enrollment into the trial (Table 1). The distribution of final diagnoses did not differ significantly between the two groups ($P = 0.09$) (Table 2). Although an indeterminate diagnosis was the most common, other diagnoses such as infection and autoimmune disease were all comparably common in the two treatment groups. Metabolic disease was more common in the NAC arm (13 NAC versus five placebo) with Wilson's disease (seven NAC versus three placebo) being more common in the NAC arm than the placebo arm. "Other" diagnoses were more common in the placebo arm (seven NAC versus 17 placebo) with hemophagocytic syndrome (none NAC versus four placebo) and drug-induced hepatitis (one NAC versus three placebo) showing the greatest differences within the "other" rare diagnostic category.

Study Outcomes. For the primary outcome (Fig. 2), there was no significant difference ($P = 0.19$) in the survival 1 year following randomization between those receiving NAC (73%) compared to placebo (82%). There was no significant difference ($P = 0.37$) in

Table 1. Baseline Characteristics

	NAC N = 92	Placebo N = 92	P
Male, N (%)	47 (51.1)	54 (58.7)	0.30
Not Hispanic or Latino, N (%)	75 (81.5)	66 (71.7)	0.12
Caucasian	68 (73.9)	65 (70.7)	0.62
Age (years)			
Median (25%, 75%)	3.7 (0.8, 10.5)	4.5 (1.0, 9.5)	0.53
< 2, N (%)	33 (35.9)	29 (31.5)	0.53
Coma grade at randomization, N (%)			0.62
0 – 1	65 (70.7)	68 (73.9)	
2 – 4	27 (29.3)	24 (26.1)	
Initial admission to study enrollment (days)			0.47
Median (25%, 75%)	3 (1, 7)	3 (1, 5)	

Table 2. Diagnosis at Hospital Discharge

	NAC N=92 N (%)	Placebo N=92 N (%)
Indeterminate	55 (59.8)	54 (58.7)
Autoimmune	8 (8.7)	11 (12.0)
Infection	9 (9.8)	6 (6.5)
Metabolic	13 (14.1)	5 (5.4)
Wilson's disease	7	3
Tyrosinemia	1	2
Galactosemia	2	0
Mitochondrial	1	0
Glycosylation defect	1	0
Niemann-Pick type C	1	0
Other	7 (7.6)	16 (17.4)
Hemophagocytic syndrome	0	4
Drug-induced hepatitis	0	3
Neonatal iron storage disease	1	2
Acetaminophen overdose	0	1
Extra-hepatic biliary atresia	0	1
Hemagioendothelioma	0	1
Intraventricular hemorrhage	1	0
Methanol	0	1
Systemic lupus erythematosis	0	1
Drug-induced and sepsis	1	0
Influenza A	1	0
Influenza B	1	0
Sepsis	0	1
EBV + hemophagocytic syndrome	0	1
Shock + adenovirus infection	1	0
Shock ischemia	1	0

P-value = 0.09 from chi-square test.

survival between the 65 children with HE grade 0-1 who received NAC and the 68 who received placebo. Stratification of results by age above and below 2 years, by HE grade 0-1 and grade 2-4, and the combinations of age and HE grade did not reveal significant treatment differences within any of the subgroups (Supporting Table 1).

Of the secondary outcomes that were examined, the 1-year LTx-free survival (Fig. 3) was significantly ($P = 0.03$) lower in children who received NAC (35%) compared to placebo (53%). Children less than 2 years of age were significantly ($P = 0.03$) less likely to survive with their native liver at 1 year if they received NAC (n = 34, LTx-free survival = 29%) than if they received placebo (n = 31, LTx-free survival = 58%). Despite a large difference in the point estimate, children less than 2 years of age with HE grade of 0-1 did not have a significantly lower LTx-free survival ($P = 0.0493$) if they received NAC (n = 20; LTx-free survival = 25%) than did children in the same age and HE grade categories who received placebo (n = 20; LTx-free survival = 60%). There were no significant differences between the treatment groups with respect to length of ICU or hospital stay, type or number of organ systems failing, or maximum grade of HE recorded following treatment allocation.

Posttransplant survival was not significantly different between the two groups. Duration of posttransplant
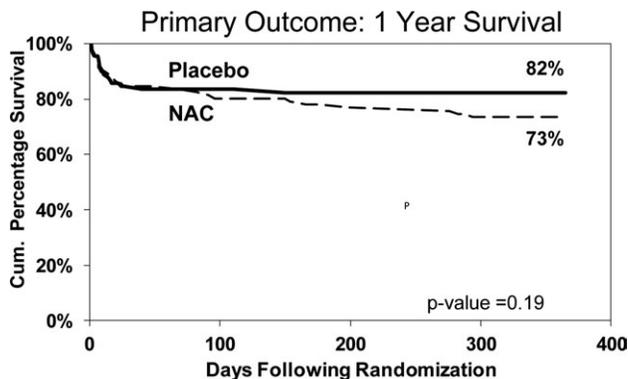
Fig. 2. Primary outcome: 1 year survival. Product-limit estimates were used to obtain the cumulative percentages of participants surviving 1 year following randomization. A log-rank test was used to assess statistical significance of the difference in survival curves. The cumulative percentage of children who were alive 1 year following randomization to NAC (dashed line) or placebo (solid line) is depicted. The percent surviving 1 year was higher in patients receiving placebo at 82% than NAC at 73%, but the differences were not significant with a P-value of 0.19.

follow-up varied depending on the timing of LTx after randomization. The cumulative probability of survival 9 months (274 days) postrandomization was 0.88 in the NAC arm and 0.84 in the placebo arm ($P = 0.59$). This is due to five post-LTx deaths among 41 in the NAC arm who underwent transplantation, and five deaths in the placebo arm among the 32 in the placebo arm who underwent transplantation.

Acetaminophen Adducts. Eighty-four participants had a sufficient amount of serum collected on day 1 or 2 following enrollment to be analyzed by a sensitive and specific serum assay for acetaminophen-cysteine
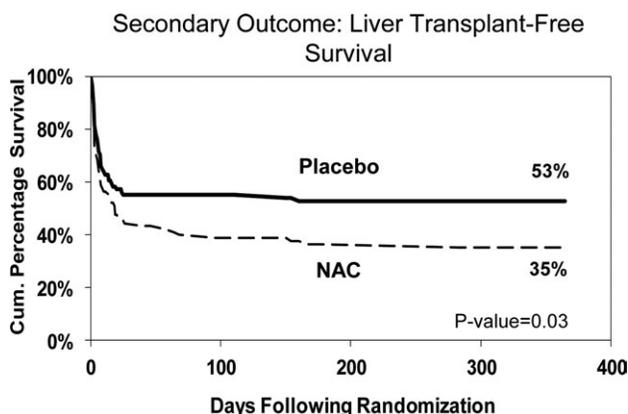
Fig. 3. Product-limit estimates were used to obtain the cumulative percentages of participants with 1-year transplantation-free survival. A log-rank test was used to assess statistical significance of the difference in survival curves. The cumulative percentage of children with liver transplantation-free survival 1 year following randomization to NAC (dashed line) or placebo (solid line) is depicted. The cumulative percentage of patients with liver transplantation-free survival was 53% when given placebo versus 35% when given NAC, with a P-value of 0.03.

adducts (A-CA).[20,21] Testing for A-CA was performed for research purposes; results were neither available, nor used, for clinical purposes. The final diagnosis was provided by the site principal investigator without knowledge of the results of the A-CA. The presence of A-CA is a biomarker for patients with acetaminophen-related liver injury. There were no statistically significant differences in the characteristics (Supporting Table 2) or outcomes (Supporting Table 3) examined of the 84 who were tested for A-CA compared to the other 100 in the trial who did have sera available for testing. Of those who were tested, 9/84 (11%) tested positive for A-CA, with three of those having positive A-CA randomized to NAC and the other six randomized to placebo; all survived 1 year after randomization; in the NAC arm, two underwent LTx whereas none of the six in the placebo arm underwent LTx. Final diagnoses of the A-CA-positive patients were three indeterminate cases for those randomized to NAC and three indeterminate, one APAP, one viral hepatitis, and one sepsis randomized to placebo. The patient with the final diagnosis of APAP was determined during the hospitalization when a participant who initially denied APAP exposure subsequently gave a positive history. This participant was given NAC clinically when the history became known and exited the study.

Safety. At least one adverse event was reported for 19 participants given NAC (21 events) and 16 given placebo (17 events). The most common adverse event reported was infection (11 in the NAC arm versus eight in the placebo arm). Other adverse events reported among those given NAC versus those given placebo were rash (four versus two), bronchospasm (one versus none), and arrhythmia (one versus four). There were no significant differences in adverse event rates between the treatment arms. The other events listed for participants in the NAC arm were aspiration, dilated and fixed pupils, hypertension, and pleural effusion. One participant each in the placebo arm had sleepiness, bradycardia, and high fever.

There were five participants in the NAC arm with serious adverse events (SAEs); four had a single SAE (respiratory distress, *Staphylococcus aureus* bacteremia, bradycardiac episode, hypoglycemia) and the other participant had three SAEs (aplastic anemia; Epstein-Barr virus [EBV] posttransplantation lymphoproliferative disease; and fever, chills, and sinusitis). Four participants in the placebo arm had a single SAE (small intestine ulcerations, fever, sensorineural hearing loss, and bigeminy).

Discussion

NAC, given as a continuous intravenous infusion at a dose of 150 mg/kg/d for up to 7 days to children with non-APAP acute liver failure, did not achieve the primary outcome, which was to improve survival 1 year following the infusion compared to placebo. Nor did NAC improve 1-year survival in any of the subgroups defined by stratifying variables for the minimization scheme (age group, HE grade). Interestingly, analysis of secondary outcomes revealed that survival at 1 year with their native liver was significantly lower in children treated with NAC compared to placebo. In addition, we found that children with minimal HE of grade 0-1 did not benefit from the NAC infusion. This finding differs from secondary outcomes analysis of adults, which found NAC to benefit those with grade 0-II HE.[13] Adverse events were similar between NAC and placebo arms in our study. The striking difference between the effects of NAC in children as compared to adult patients is likely related to a number of factors that include differences in etiology, underlying pathophysiology, and age.

Acute liver failure in children is a heterogeneous, dynamic clinical entity with etiologies that differ from adults.[4,22] Although etiologic categories of nonacetaminophen acute liver failure in children are similar to adults and include, metabolic, infectious, and immune-mediated conditions, medications, and other toxins as well as an indeterminate group, the proportion of children within those categories differs substantially from adults. Although an indeterminate diagnosis accounted for 24% in the adult study,[13] 54% of the pediatric cases had such a diagnosis. Similar proportions were noted in the larger cohort of PALF participants not specifically included in this clinical trial.[2,23] A portion of the pediatric indeterminate group likely constitutes an evaluation interrupted by death, LTx, or clinical improvement. In addition, the indeterminate cohort may include unexpected acetaminophen toxicity,[21] a novel or unrecognized virus, metabolic or xenobiotic injury. Undiagnosed immune dysregulation may also result in ALF.[24]

The presence of A-CA has been associated with known and occult acetaminophen-related liver disease.[21,25] The percent of patients with a positive A-CA in the NAC trial is similar to previous studies in children.[21] Although the numbers are very small, all of the A-CA-positive patients randomized to placebo survived at least 1 year without LTx. Reasons for this finding might include APAP toxicity that was not the primary driver of liver failure or, perhaps, adducts were generated when the injured liver was exposed to APAP.

In previous studies, NAC was evaluated for the treatment of multiorgan system failure (MOSF) including acute respiratory distress syndrome (ARDS)

and sepsis,[26] and was found to improve pulmonary compliance and oxygen consumption with variable results on overall patient survival.[12,16,17] NAC suppresses the production of tumor necrosis factor alpha (TNF-α) in vitro[27] and improves survival and lung function in pigs with endotoxin-induced ARDS.[28] As clinical features of ALF overlap with those seen in MOSF syndromes, we hypothesized that the nonspecific beneficial effects of NAC in MOSF might well apply to ALF. Unfortunately, with respect to 1-year survival, NAC did not provide the anticipated benefit.

The observation that NAC failed to improve and possibly worsened the likelihood of LTx-free survival at 1 year suggests pathobiological mechanisms of non-APAP acute liver failure may differ between children and adults. These findings provide an opportunity to identify novel mechanisms of hepatocellular injury in PALF such as immune dysregulation[29] or an altered inflammatory response[30] and investigate targeted treatment strategies in children with acute liver failure.

Hypotheses can be generated to help explain our findings. One hypothesis is that immune or inflammatory dysregulation drives liver injury for many in the indeterminate cohort. Although little is known of the ontogeny of intrahepatic immune responses, evidence suggests systemic inflammatory responses to trauma differ significantly between adults and children.[31] Inflammation is a complex and dynamic process that encompasses both injury, healing, and regeneration through proinflammatory and antiinflammatory responses.[30] A well-regulated inflammatory response is necessary for proper healing and regeneration.[32] Should proinflammatory signals that initiate healing and regenerative responses be blunted at a critical time, an imbalance favoring inflammation may develop. Whether NAC, which serves as an antioxidant[33] and can alter the secretion of a variety of chemokines,[34] could interrupt healing responses is not known. A second hypothesis is that prolonged treatment with NAC might suppress the hepatic regenerative response to injury, as was recently reported in a murine model of APAP-induced liver injury.[35]

Other variables that may have impacted our findings include differences in diagnosis between treatment arms, dosing regimen, and the extended enrollment period. Children with mitochondrial disease suffer from a systemic condition associated with late mortality that is currently without a directed therapy. Although antioxidant therapy with NAC may have theoretical benefit for mitochondrial injury, the opposite might also be true.[36] Treatment of drug-induced liver injury is generally limited to withdrawal of the offending medication. A recent study reported that NAC might provide benefit independent of glutathione repletion in a murine model of lasix-induced liver injury, raising the possibility that NAC might also have such effects in human drug-induced liver.[37] Although uneven distribution of these rare conditions may have a theoretical impact on outcomes for individuals, the small numbers are unlikely to be a major factor in the outcome of the trial.

The NAC dosing regimen of 150 mg/kg/d for no more than 7 days fits within other adult and pediatric dosing regimens that have ranged from 288 mg/kg/d for up to 5 days with MOSF[16] to 100 mg/kg/d for a median of 5 days (range 1-77) in ALF. The study duration, requiring ~9 years to recruit the number of participants required by the study design, speaks to the challenges of studying a rare, acute, severe condition such as PALF. Despite the prolonged period, we have no evidence that significant changes in management or liver transplant decisions occurred during the course of the study that would have impacted the outcome.

In summary, NAC did not improve 1-year survival in children with non-APAP acute liver failure. One-year LTx-free survival was significantly lower in the NAC-treated group, especially among children less than 2 years of age with HE grade 0-1. Although the difference in outcome by treatment arm was large in that small group, the interim analyses and small sample size reduced substantially the statistical power to find a large difference to be statistically significant. This study does not support the broad use of NAC in non-APAP PALF and it emphasizes the importance of conducting prospective pediatric drug trials, regardless of results in adults.

References

1. Dhawan A. Etiology and prognosis of acute liver failure in children. Liver Transpl 2008;14(Suppl 2):S80-S84.
2. Squires RH Jr, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr 2006;148: 652-658.
3. Polson J, Lee WM. AASLD position paper: the management of acute liver failure. HEPATOLOGY 2005;41:1179-1197.
4. Squires RH Jr. Acute liver failure in children. Semin Liver Dis 2008; 28:153-166.
5. Rivera-Penera T, Moreno J, Skaff C, McDiarmid S, Vargas J, Ament ME. Delayed encephalopathy in fulminant hepatic failure in the pediatric population and the role of liver transplantation. J Pediatr Gastroenterol Nutr 1997;24:128-134.
6. Baliga P, Alvarez S, Lindblad A, Zeng L. Posttransplant survival in pediatric fulminant hepatic failure: the SPLIT experience. Liver Transpl 2004;10:1364-1371.
7. Soltys KA, Mazariegos GV, Squires RH, Sindhi RK, Anand R. Late graft loss or death in pediatric liver transplantation: an analysis of the SPLIT database. Am J Transplant 2007;7:2165-2171.

8. Kortsalioudaki C, Taylor RM, Cheeseman P, Bansal S, Mieli-Vergani G, Dhawan A. Safety and efficacy of N-acetylcysteine in children with non-acetaminophen-induced acute liver failure. Liver Transpl 2008;14:25-30.

9. Sklar GE, Subramaniam M. Acetylcysteine treatment for non-acetaminophen-induced acute liver failure. Ann Pharmacother 2004;38:498-500.

10. Marzullo L. An update of N-acetylcysteine treatment for acute acetaminophen toxicity in children. Curr Opin Pediatr 2005;17:239-245.

11. Prescott LF, Illingworth RN, Critchley JA, Stewart MJ, Adam RD, Proudfoot AT. Intravenous N-acetylcystine: the treatment of choice for paracetamol poisoning. Br Med J 1979;2:1097-1100.

12. Harrison PM, Wendon JA, Gimson AE, Alexander GJ, Williams R. Improvement by acetylcysteine of hemodynamics and oxygen transport in fulminant hepatic failure. N Engl J Med 1991;324:1852-1857.

13. Lee WM, Hynan LS, Rossaro L, Fontana RJ, Stravitz RT, Larson AM, et al. Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. Gastroenterology 2009;137:856-864, 64 e1.

14. Baker A, Alonso ME, Aw MM, Ciocca M, Porta G, Rosenthal P. Hepatic failure and liver transplant: Working Group report of the second World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr 2004;39(Suppl 2):S632-S639.

15. Durand P, Debray D, Mandel R, Baujard C, Branchereau S, Gauthier F, et al. Acute liver failure in infancy: a 14-year experience of a pediatric liver transplantation center. J Pediatr 2001;139:871-876.

16. Molnar Z, Shearer E, Lowe D. N-Acetylcysteine treatment to prevent the progression of multisystem organ failure: a prospective, randomized, placebo-controlled study. Crit Care Med 1999;27:1100-1104.

17. Walsh TS, Lee A. N-acetylcysteine administration in the critically ill. Intensive Care Med 1999;25:432-434.

18. Cox DR. Regression models and life tables. J R Stat Soc Ser B 1972; 34:187-220.

19. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics 1979;35:549-556.

20. Davern TJ, 2nd, James LP, Hinson JA, Polson J, Larson AM, Fontana RJ, et al. Measurement of serum acetaminophen-protein adducts in patients with acute liver failure. Gastroenterology 2006;130:687-694.

21. James LP, Alonso EM, Hynan LS, Hinson JA, Davern TJ, Lee WM, et al. Detection of acetaminophen protein adducts in children with acute liver failure of indeterminate cause. Pediatrics 2006;118:e676-81.

22. Lee WM. Etiologies of acute liver failure. Semin Liver Dis 2008;28: 142-152.

23. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002;137: 947-954.

24. Yazigi N, Tial G, Filipovich A, Bucuvalas JC. Natural killer dysfunction in pediatric acute liver failure. Am J Transplant 2008;8(Supp s2):327A.

25. Khandelwal N, James LP, Sanders C, Larson AM, Lee WM. Unrecognized acetaminophen toxicity as a cause of indeterminate acute liver failure. HEPATOLOGY 2011;53:567-576.

26. Rank N, Michel C, Haertel C, Lenhart A, Welte M, Meier-Hellmann A, et al. N-acetylcysteine increases liver blood flow and improves liver function in septic shock patients: results of a prospective, randomized, double-blind study. Crit Care Med 2000;28:3799-3807.

27. Peristeris P, Clark BD, Gatti S, Faggioni R, Mantovani A, Mengozzi M, et al. N-acetylcysteine and glutathione as inhibitors of tumor necrosis factor production. Cell Immunol 1992;140:390-399.

28. Modig J, Sandin R. Haematological, physiological and survival data in a porcine model of adult respiratory distress syndrome induced by endotoxaemia. Effects of treatment with N-acetylcysteine. Acta Chir Scand 1988;154:169-177.

29. Antoniades CG, Berry PA, Wendon JA, Vergani D. The importance of immune dysfunction in determining outcome in acute liver failure. J Hepatol 2008;49:845-861.

30. Mi Q, Li NY, Ziraldo C, Ghuma A, Mikheev M, Squires R, et al. Translational systems biology of inflammation: potential applications to personalized medicine. Per Med 2010;7:549-559.

31. Wood JH, Partrick DA, Johnston RB Jr. The inflammatory response to injury in children. Curr Opin Pediatr 2010;22:315-320.

32. Vodovotz Y. Translational systems biology of inflammation and healing. Wound Repair Regen 2010;18:3-7.

33. Flanagan RJ. The role of acetylcysteine in clinical toxicology. Med Toxicol 1987;2:93-104.

34. Wuyts WA, Vanaudenaerde BM, Dupont LJ, Demedts MG, Verleden GM. N-acetylcysteine reduces chemokine release via inhibition of p38 MAPK in human airway smooth muscle cells. Eur Respir J 2003;22: 43-49.

35. Yang R, Miki K, He X, Killeen ME, Fink MP. Prolonged treatment with N-acetylcystine delays liver recovery from acetaminophen hepatotoxicity. Crit Care 2009;13:R55.

36. Zhang H, Limphong P, Pieper J, Liu Q, Rodesch CK, Christians E, et al. Glutathione-dependent reductive stress triggers mitochondrial oxidation and cytotoxicity. FASEB J 2012;26:1442-1451.

37. Masubuchi Y, Nakayama J, Sadakata Y. Protective effects of exogenous glutathione and related thiol compounds against drug-induced liver injury. Biol Pharm Bull 2011;34:366-370.