A Multi-center Study of the Safety and Efficacy of N-acetylcysteine In the Treatment of Acute Liver Failure in Pediatric Patients Not Caused by Acetaminophen

Clinical Center:
"Robert H. Squires, Jr., MD"
"Children's Hospital of Pittsburgh"
"3705 Fifth Ave."
"Pittsburgh, PA 15213-2583"

Clinical Coordinating Center:
Principal Investigator: Robert H. Squires, Jr., MD
Children's Hospital of Pittsburgh
3705 Fifth Avenue
Pittsburgh, PA 15213-2583
(412) 692 5180
(412) 692 7355 FAX
Email: Robert.squires@chp.edu

Data Coordinating Center:
Epidemiology Data Center
Graduate School of Public Health
University of Pittsburgh
130 DeSoto Street, 127 Parran Hall
Pittsburgh, PA 15261

Note: Items in quotes will be different for each treatment site.

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Primary Objective: To test the safety and efficacy of a 7 day course of intravenous N-acetylcysteine in children with acute liver failure not due to acetaminophen toxicity.

Inclusion Criteria:
1. Enrollment in PALF registry (criteria listed below, use PT INR results from the most recent tests performed prior to plasma therapy, but no more than 72 hours prior to enrollment in the registry.)
2. Informed consent obtained from the patient (when patient is 14-17 years of age or <17 and developmentally able to sign his or her name and is without clinical encephalopathy), parent, or guardian

Exclusion Criteria:
1. Acute Acetaminophen toxicity
2. Patient on N-acetylcysteine or received N-acetylcysteine during the course of this illness.
3. Pregnancy
4. Known malignancy
5. Patient is on a liver support device
6. Sepsis
7. Signs of cerebral herniation
8. Intractable hypotension defined as systolic BP < 85 mm Hg or hypotension that requires treatment with ionotropic drugs, other than renal dosing dopamine

PALF Registry Criteria
Inclusion Criteria:
1. Evidence of acute liver injury: defined as severe liver dysfunction occurring within 8 weeks of onset of illness, with no known underlying chronic liver disease.
2. INR ≥ 1.5 or PT ≥ 15 with encephalopathy OR INR ≥ 2.0 or PT ≥ 20 with or without encephalopathy.
3. Patient < 18 years of age at the time of enrollment.
4. Informed consent obtained from the patient (when patient is 14-17 years of age or <17 and developmentally able to sign his or her name), parent, or guardian

Exclusion Criteria:
1. Known chronic underlying liver disease
2. Coagulopathy corrected with Vitamin K

Type of Study: A double blind, placebo-controlled, randomized clinical trial.

Primary outcome measure: Overall survival rate (spontaneous survival without transplant plus survival following transplantation) at one year following treatment allocation.

Secondary outcome measures:
1. Spontaneous survival (survival without transplant)
2. Transplantation rate
3. Length of ICU and hospital stay
4. Number of organ systems failing:

a) Cardiovascular failure: defined as treatment with inotropic drugs: norepinephrine, epinephrine or dopamine (the latter >5 µg/kg/min), the terminal 24 hour period before death excluded.
b) Renal failure: defined as serum creatinine greater than 2 x the upper limit of normal for age and urine output less than 0.5 cc/kg/hour.
c) Intracranial hypertension: defined as intracranial pressure > 25 mmHg (if ICP monitored) or clinical signs of this condition (decerebrate posturing, abnormal pupillary reflexes).
d) Pulmonary support: defined as the need for mechanical ventilation for encephalopathy 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. The FiO2 criterion is important for the definition for two reasons: firstly, mechanical ventilation is used as a standard in the treatment of patients with cerebral edema to induce hyperventilation and thereby hypocapnia, and secondly, the medullary respiratory center may be depressed in patients with hepatic encephalopathy grade 4 and may thus cause a 'non-pulmonary' need for mechanical ventilation.
e) Infection: defined as detection of pathogenic microbial species in blood, tracheal, or urine cultures.

5. Degree of hepatic encephalopathy. The maximal hepatic coma grade (I - IV), and the number of days until recovery or death.

Therapy: Intravenous N-acetylcysteine

Total Duration of Therapy: 7 days. Duration may be less in the event of spontaneous recovery or liver transplantation.

Introduction

Acute liver failure (ALF) is a dramatic syndrome in which previously healthy individuals rapidly lose hepatic function due to a variety of causes, develop hepatic encephalopathy, and become critically ill within days [1, 2]. Even in the era of liver transplantation, many patients die either due to complications of this devastating disease or because of lack of available donor organs in a timely fashion [3]. Since ALF occurs relatively infrequently, no one center in the US has a large enough experience to conduct important clinical studies, either of natural history or treatment. The incidence of acute liver failure in children is unknown, but likely approaches 100 cases per year.

The Pediatric Acute Liver Failure (PALF) Study Group met for the first time in November 1999. This is the first multi-center, multi-national collaborative study aimed at identifying, characterizing, and developing management strategies for infants, children, and adolescents who present with acute liver failure (ALF). Initial funding and support from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) came through the Acute Liver Failure Study Group (NIH 1RO1-DK58369-01) with a Data Coordinating Center (DCC) at the University of Texas Southwestern in Dallas, TX. Due to the successful growth of this international pediatric network and the separate scientific questions posed by the pediatric investigators, PALF established its independence from the adult project with the Principal Investigator, Dr. Robert Squires, moving from Dallas, TX to the University of Pittsburgh. The registry is computerized and maintained by a Data Coordinating Center (DCC) which has moved to the Epidemiology Data Center (EDC) of the Graduate School of Public Health at the University of Pittsburgh.

The PALF study group, comprised of 20 clinical centers and a DCC, is conducting a randomized, placebo-controlled trial of N-acetylcysteine (NAC) for the treatment of non-acetaminophen ALF in children. The purpose is to test the safety and efficacy of a 7 day course of intravenous N-acetylcysteine in children with acute liver failure not due to acetaminophen toxicity.

In the future, this study group will be available to test new therapies including pharmaceutical agents or liver assist machines for ALF, and should enhance the development of better understanding of the pathogenesis of ALF, and improved prognostic models for determining outcome of this complex condition.

1.0 Objective and Specific Aims

The purpose of the NAC trial is to test the safety and efficacy of a 7 day course of intravenous N-acetylcysteine in children with acute liver failure not due to acetaminophen toxicity. The primary end-point for determining efficacy will be overall survival (survival following transplantation plus spontaneous survival) at one year following treatment allocation.

Secondary endpoints will be length of intensive care unit and hospital stay, number of organ systems showing failure, maximum degree of hepatic encephalopathy, and spontaneous survival (survival without liver transplantation).

2.0 Background and Significance

2.1 Background

2.1.1 Acute Liver Failure

In adults, acute liver failure is best defined as the onset of altered mental status (hepatic

encephalopathy) and coagulopathy (prolonged prothrombin time) occurring within 8 weeks of initial symptoms of a hepatitis-like illness. A broader definition used by many investigators extends the interval between the onset of symptoms and liver failure to 26 weeks. Many etiologies of ALF are recognized. Traditionally, the most frequent causes in the US have been viral hepatitis and drug-induced liver injury with smaller numbers of cases due to a variety of etiologies. In nearly 20%, no clear-cut cause can be determined [4-6]. Mortality figures in the range of 80-94% were observed in the pre-transplant era, but still are approximately 35%, even with liver transplantation. Prognosis in ALF has been thought to depend on a variety of factors including age, etiology, length of illness and the availability of specialized critical care facilities, but up-to-date studies and the development of better methods to predict outcome (need for transplantation) are needed.

In children, however, acute liver failure may be present without clinical evidence of encephalopathy [7]. Coagulopathy appears to be an important, consistent, and reliable finding in children with ALF. The incidence of acute liver failure in children is unknown, but likely approaches 1-200 cases per year in North America and the United Kingdom. For those in whom a diagnosis is identified, the causes are typically related to infection, inherited metabolic defects, autoimmune hepatitis, or drug-induced liver injury, and treatment can be directed to the underlying condition. However, with over half of the cases of ALF in children found to be indeterminate, treatment strategies are primarily supportive [3, 8].

Orthotopic liver transplantation can be a life saving procedure for children with ALF [9]. Given the shortage of available organs, however, many will die prior to receiving a liver transplant. For those who receive an organ, long term graft and patient survival is diminished when compared to those receiving liver transplants for other causes [10, 11]. Therefore, therapies that might improve survival with or without transplant should be tested.

2.1.2 Multiorgan Failure in ALF

ALF is not merely a disease involving the liver. The sudden loss of hepatocyte function sets in motion a complex process referred to as the multiple organ dysfunction syndrome (MODS), involving failure of kidneys, lungs, bone marrow, the circulatory system, and the brain [12]. A unique feature of ALF in comparison to other causes of MODS is cerebral edema [13]. Patients with ALF are also more susceptible to severe infections. Cerebral edema and systemic sepsis are the most frequent causes of death in ALF, but intractable arterial hypotension and the adult respiratory distress syndrome (ARDS) also account for significant morbidity and mortality in these patients. The pathogenesis of MODS in ALF is complex and not well elucidated. In addition to the loss of hepatocyte function, mediators including interleukin (IL)-1, endotoxin, IL-6, and tumor necrosis factor alpha (TNF-a), are involved [14]. The severe circulatory disturbance is characterized by a hyper-dynamic circulation and tissue hypoxia similar to the sepsis syndrome [15], with impaired tissue oxygen extraction or altered peripheral cellular metabolic pathways.

2.1.3 Treatments for ALF

ALF is estimated to affect 2,000 individuals annually in the United States, only 200-300 of whom receive a liver transplant. Previous treatment trials for the overall condition of ALF have proved futile. In the 1960’s and 1970's, a US-based multi-center study group (the Acute Hepatic Failure Study Group) made important observations regarding use of corticosteroids and hyperimmune globulin (for hepatitis B viral infection), both negative studies [16]. With the advent of transplantation no further multi-center trials were conducted. However, survival for ALF patients

following OLT is still suboptimal (~65%, generally less than that for chronic liver disease which is usually >85%). Evidence from the adult data and serum study which began in 1998, suggest that only 28% of ALF patients undergo transplantation. Since many patients never receive a liver graft, renewed efforts to tackle acute liver failure itself make sense, with the goal of preserving remaining hepatocytes or improving regeneration of the failing liver. Specific therapies for the various causes of ALF have been limited to antidotes for mushroom poisoning and N-acetylcysteine (NAC) for acetaminophen overdose. There is no known effective therapy for the overall condition ALF (or for MODS in ALF) capable of reducing the high mortality in such patients [4]. However, NAC has recently been studied and advocated in the United Kingdom as such a treatment. NAC is a potent antioxidant and vasodilator thought to be beneficial for acetaminophen-induced ALF or for septic shock [17, 18].

Since severe, acute lack of hepatocyte function is the 'lesion' of ALF, substitution of hepatocytes in various forms as liver assist or support devices has been developed over the last ten years [19]. These techniques employ cultured immortalized human hepatocellular cancer lines or fresh, isolated porcine hepatocytes maintained in cartridges through which patient blood or plasma is allowed to flow. Transgenic pig livers modified to prevent hyperacute rejection may also serve as a bridge to transplantation until a human organ suitable for grafting becomes available. Transplantation of isolated hepatocytes into the portal circulation has also been attempted [20]. To date, there is no convincing evidence that hepatocyte-based systems improve ALF outcome, either as primary therapy or as a bridge for eventual transplantation. A trial of a bio-artificial liver (BAL) was undertaken in the last 3 years at 16 centers around the U.S. including several centers involved in this study. The study was recently halted on the grounds that no efficacy could be detected. Needless to say, hepatocyte-based systems are remarkably expensive and have little applicability beyond a few highly specialized academic transplant centers. Research in this area may bear fruit in the future either via the development of successful hepatocyte replacement therapy for acute or chronic liver conditions or, in the case of the transgenic pigs, success in xenotransplantation. However, current studies do not suggest that the techniques are effective or will have wide application for patients with ALF in the foreseeable future.

2.1.4 Why Choose N-acetylcysteine?

After acetaminophen overdose, N-acetylcysteine replenishes mitochondrial and cytosolic glutathione stores, and has been shown in patients and in experimental animals to prevent or ameliorate the degree of injury and cell death [21]. Intravenous or oral NAC improves survival in patients with acetaminophen-induced ALF [18]. Evidence that NAC may benefit the patient, even when given as late as 72 hours after overdose, led to its use in non-acetaminophen ALF cases [22]. A small uncontrolled study showed improvement in cardiovascular hemodynamics and oxygen transport in both acetaminophen (n=12) and non-acetaminophen (n=8) patients [23]. Mean arterial pressure, oxygen consumption, and oxygen delivery increased, as did the oxygen extraction ratio. As a result, intravenous NAC has been incorporated into the management strategy used at Kings and elsewhere as treatment for all acute liver failure, but has never been subjected to a controlled trial. A second study confirmed the earlier findings concerning hemodynamics, and showed a significant increase in cyclic guanine monophosphate (cGMP) levels suggesting that NAC may cause vasodilatation by increasing soluble guanylate cyclase activity [24]. However, a more recent study from Edinburgh failed to demonstrate improved hemodynamics with NAC: patients with ALF in Grade IV coma on a ventilator were given intravenous NAC with no improvement in blood pressure or cardiac output [25]. However, all patients were in an advanced disease state and were

ventilated and sedated, factors which may have precluded a beneficial effect. Investigators differ regarding methodology for measuring oxygen consumption, but the Edinburgh methodology seems more accurate. Nevertheless, the Edinburgh patients did not represent a cross-section of ALF. While NAC is widely used in the US in an oral preparation as Mucomyst® for acetaminophen overdoses, intravenous NAC infusion has not been available until recently, when it was approved by the US Food and Drug Administration for use in patients with acute acetaminophen hepatotoxicity. Intravenous NAC is not approved for use in patients with non-acetaminophen ALF in the United States because the limited data on its use for this condition was derived from uncontrolled observations. .

Studies on the use of intravenous NAC in children in the United States are limited to patients with acetaminophen overdose. Using a regimen that consisted of a loading dose of 140 mg/kg followed by 12 maintenance doses of 70 mg/kg every 4 hours, researchers from Boston demonstrated that intravenous NAC was as effective as 72 hours of oral NAC for acetaminophen overdose in children [26]. Two patients in this series were younger than 5 years. An alternative approach, accepted by both adult and pediatric hepatologists at Kings College Hospital in London, utilizes a continuous infusion of NAC at a dose of 150 mg/kg/day [23, 27]. Little information is available on the use of intravenous NAC in infants. However, a report from the Rocky Mountain Poison Control Center (RMPCC) in 1997 identified 3 newborns whose mothers received NAC for acetaminophen overdose at the time of delivery. The cord blood level was within the range associated with a therapeutic dose of NAC. None of the infants had evidence of acetaminophen-related toxicity nor did they experience an adverse reaction to the NAC [28]. A review from the RMPCC on the use of the oral NAC preparation given intravenously for acetaminophen overdose included 35 children under 18 years of age as well as 2 newborns and concluded that this preparation had limited adverse effects and was potentially lifesaving in circumstances that precluded oral administration of NAC [29].

The Pediatric ALF Study Group addresses a definite need: the lack of a multi-center effort to address the complex nature of acute liver failure in children. Information gained from this research could lead to improved medical care for children with ALF.

We will study the use a continuous infusion of NAC for a period of 7 days. Our rationale for the continuous infusion is based upon the presumption that patients with ALF not due to acetaminophen toxicity likely have an evolving liver injury. Therefore, the brief 48-hour regimen used for acute acetaminophen ingestion to replenish glutathione stores may be insufficient. A continuous infusion over a 7 day period may better support the evolving nature of ALF in children [7].

2.1.5 Other Studies of N-acetylcysteine

NAC has been tested in preliminary fashion in other MODS settings including ARDS and sepsis, both in patients and in animal models, where NAC improved pulmonary compliance and oxygen consumption with variable results on overall patient survival [30]. NAC suppresses the production of TNF-α [31] in vitro and improves survival and lung function in pigs and rats with endotoxin-induced ARDS [32]. Since the ALF and MODS syndromes overlap, these non-specific beneficial effects might well apply to ALF.

2.2 Significance

The NAC trial is part of the PALF study group effort to study the natural history, etiologies and potential therapies of ALF in childhood. Through this coordinated effort, we will advance our understanding of ALF in children which will enhance healthcare delivery, conserve healthcare resources and improve overall outcome for these desperately ill children.

3.0 Research Design and Methods

3.1 Drug Information: Intravenous N-acetylcysteine

Intravenous NAC is a very safe agent with no significant side effects found during extensive use in Europe as well as sporadic use in the U.S. Since its efficacy has only been suggested by uncontrolled trials, NAC is an ideal candidate for our initial controlled randomized double blind trial by the PALF Study Group. Intravenous NAC is not approved by the US Food and Drug Administration for use in patients with non-acetaminophen related hepatotoxicity. Results of this trial could lead to an expanded indication of IV NAC to include non-acetaminophen acute liver failure and include children. Prior to its approval by the FDA, intravenous NAC preparations were made by pharmacists and used occasionally for non-acetaminophen cases in the US, but this has not gained general acceptance [29]. Cumberland Pharmaceuticals will provide the intravenous N-acetylcysteine for the study. The current IND number is 73,010. IV NAC will be prepared in the research pharmacy of the clinical center.

The main adverse event reported in previous studies of NAC was a rare hypersensitivity reaction, manifested by rash or wheezing, and occurring with increased frequency in patients with a previous asthmatic diathesis. In each instance, use of intravenous anti-histamine (diphenhydramine) or corticosteroids aborted the attack and no instances of severe reactions have been reported. In most instances, the infusions were not discontinued and all symptoms resolved once diphenhydramine was instituted and the rate of infusion slowed.

3.2 Research Design and Methods

N-acetylcysteine is being tested in a double blind, placebo-controlled, randomized trial as a treatment for non-acetaminophen ALF in children. The study drug or placebo will be given as a 7 day continuous intravenous infusion to children with acute liver failure not due to acute acetaminophen toxicity.

Vials of NAC will be available at each clinical center in the research pharmacy. Upon identification of a patient, study personnel (investigator or coordinator) will obtain informed consent, and then notify the research pharmacist of the patient's body weight, coma grade, and age. Orders for the solutions and the informed consent will be submitted to the clinical center’s pharmacy, confirming that the patient is part of the study and outlining the timing for the infusion. The pharmacist will then log onto a web-based treatment allocation system maintained at the DCC which assigns therapy using a minimization scheme designed to balance treatment groups within a site by age (less than 2 years vs. at least 2 years) and coma score (0,1 vs. 2-4). The research pharmacist will prepare either a NAC solution as outlined below or 5% dextrose (placebo). The N-acetylcysteine will be added to 5% dextrose. The solution containing N-acetylcysteine will be administered as a continuous infusion at a dose of 150 mg/kg bodyweight in 5% dextrose over 24-hours for 7

consecutive days. Volumes will be adjusted for small children. The solution will be delivered to the clinical center with study labels which do not disclose the specific contents. Patients allocated to placebo will receive volume-matched 5% dextrose infusions. The NAC solution is colorless, so there should be no difficulty with inadvertent product identification. It may be possible to identify the solution by its smell, but all reasonable efforts will be made to exclude investigators from awareness of this information. A standard order form for the infusions will be inserted in the patient's chart to provide uniform orders at the study onset. Infusion of the study solution should take place as soon as possible following study entry (consent signed).

Participation in the NAC trial will not interfere with standard treatment for ALF including liver transplantation, if it is considered appropriate.

3.3 Data Collection and Statistical Considerations

3.3.1 Sample Size Calculation:

It is anticipated that 90 children with PALF will be admitted to the clinical centers each year. Of those, approximately 20% will likely be due to acetaminophen overdose leaving 72 patients eligible for the study. We anticipate 25% of the eligible patients will not be enrolled, leaving an estimated 54 patients per year to be randomized. The one year death rate without transplant of 15% is lower than in the adult population. However, while a greater percentage of children receive a liver transplantation (40%), 40% of these children die, leaving an estimated overall survival with or without a liver transplant of 69% in the placebo arm. See diagram provided in Appendix 1 on page 20.

Based on these data, the estimated sample size to evaluate the efficacy of IV-NAC in the treatment of ALF in children was calculated. The original sample size was calculated assuming a one year survival rate for the patients in the treatment arm is 86.5% and 6 planned interim analyses. .This comparison would need 92 patients in each arm (184 total) to achieve 80% power (likelihood of rejecting the hypothesis of equal survival rates) with a two-sided test of survival (log-rank test) at $\alpha=0.054$. The external Data and Safety Monitoring Board has accepted a revised interim monitoring plan for efficacy consisting of a single interim analysis when half of the sample of 184 reaches the one-year follow-up. Keeping the sample size at 184, this effectively lowers the type I error to 0.05 while maintaining 80% power.

Due to preliminary findings in a similar study, an additional interim analysis was performed. There will be 3 looks at the data; the first when 79 participants are enrolled (which was shown to the DSMB on August 30, 2007) among whom 42 participants were enrolled on or before July 1, 2006, i.e., enrolled for at least 1 year, the second when 92 participants reach the 1 year follow-up and the last when all 184 participants reach the 1 year follow-up.

With this revised plan, the power remains at 85%. The new O'Brien-Fleming boundaries will be:

-4.550, 4.550 corresponding to a nominal α of 0.00005 at the first look
-2.963, 2.963 corresponding to a nominal α of 0.00305 at the second look
-1.969, 1.969 corresponding to a nominal α of 0.04900 at the third look

3.3.2 Endpoints of the study

Primary outcome measures:
1. Overall survival rate (spontaneous survival without transplant plus survival following transplantation) at one year following treatment allocation.

Secondary outcome measures:
1. Spontaneous survival (survival without transplantation)
2. Transplantation rate
3. Length of ICU and hospital stay
4. Number of organ systems failing:
   a) Cardiovascular failure: defined as treatment with inotropic drugs: norepinephrine, epinephrine or dopamine (the latter >5 µg/kg/min), the terminal 24 h before death excluded.
   b) Renal failure: defined as serum creatinine greater than 2 x the upper limit of normal for age and urine output less than 0.5 cc/kg/hour.
   c) Intracranial hypertension: defined as intracranial pressure > 25 mmHg (if ICP monitored) or clinical signs of this condition (decerebrate posturing, abnormal pupillary reflexes).
   d) Pulmonary support: defined as the need for mechanical ventilation for encephalopathy 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. The FiO2 criterion is important for the definition for two reasons: firstly, mechanical ventilation is used as a standard in the treatment of patients with cerebral edema to induce hyperventilation and thereby hypocapnia, and secondly, the medullary respiratory center may be depressed in patients with hepatic encephalopathy grade 4 and may thus cause a 'non-pulmonary' need for mechanical ventilation.
   e) Infection: defined as detection of pathogenic microbial species in blood, tracheal, or urine cultures.
5. Degree of hepatic encephalopathy. The maximal hepatic coma grade (I - IV), and the number of days until recovery or death.

3.3.3 Study Data Collection

All participants of the NAC trial must first be enrolled in the PALF registry and therefore all data collected as part of the registry will be available for these patients. Additional data collected for the NAC trial include:

- A form listing the patient's match to inclusion/exclusion criteria
- A daily log to capture the date and time infusion was started, the amount of the intravenous infusion, clinical measures, and adverse events. The date of treatment allocation, patient's weight and coma grade are also recorded on the log.

The daily log will be faxed upon completion to the DCC.

A parent may withdraw consent for participation in this research study at any time. In addition, a site principal investigator may withdraw a child from the study in circumstances related to either the parent or child's failure to cooperate fully with the conduct of the study, or the recognition of significant medical risks associated with the child's continued participation in this study. If a child's participation in this study is stopped, the reasons will be discussed with the parents and the child. Any research or medical information recorded for, or resulting from, the child's participation in this research study prior to the date that consent for child's participation was formally withdrawn may continue to be used by the investigators for the purposes described above.

3.3.4 Biospecimen Collection

No additional samples will be collected for participants who are enrolled into this trial on the same day that they are enrolled in the PALF registry, since the registry includes the collection of daily samples for 7 consecutive days. If for some reason the patient is enrolled in this trial at least one day after entry into the registry, blood samples will be collected for up to 7 days following enrollment in this trial. The maximum blood volume collected over the 7 day period will not exceed the maximum whole blood volume for a single blood draw or the cumulative volume, as stated in the clinical centers IRB guidelines.

3.3.5 Statistical Analysis

The primary outcome for this study is to compare survival at one year (with or without transplantation) for patients in the two treatment arms. Due to potential missing data, survival techniques to account for censored data will be used. Product-limit estimates of survival for the two treatment arms will be compared using a log-rank test.

In addition, demographic and patient characteristics will be analyzed for trends. Two proportion comparisons or Chi Square contingency table analyses will be performed when the data to be compared are from 2 x 2 tables (e.g. gender by year 1997 vs. 1999). When the data are measured at several different levels (e.g. race) across two or more years, the Cochran-Armitage Test for Trend or Jonckheere-Terpstra Test will be performed. To examine the association in these tables, Phi or Cramer's V will be utilized for 2 x 2 or higher way tables. If the data have ordered categories, Goodman and Kruskal's Gamma (G) is appropriate.

Univariate statistical analyses will be performed for each of the patient demographic and illness characteristics at baseline for descriptive purposes and to determine their possible relationship with the primary outcome. Baseline characteristics will be compared between patients in the two treatment arms to determine whether treatment allocation balanced the groups with respect to measured variables. Chi square analysis will be used to examine group differences for dichotomous variables, independent group Student t-tests will be performed for normally distributed, continuous variables, and Mann-Whitney U tests for the other continuous measures.

Cox's proportional hazards regression model will be used to model the primary outcome and secondary outcomes of spontaneous survival and transplantation rate. The predictor variables in each of the equations will include the variables used in the minimization scheme (age, coma score). In addition, analyses will examine whether treatment effect changes when adjusting for a measure of patient illness, e.g., number of organ systems failing at baseline, APACHE II, or King's criteria.

3.3.6 Interim Analysis

For the Data Safety and Monitoring Board (DSMB), interim analyses will be performed by the DCC. There will be 3 looks at the data; the first when 79 participants are enrolled (which was shown to the DSMB on August 30, 2007) among whom 42 participants were enrolled on or before July 1, 2006, i.e., at least 1 year ago, the second when 92 participants reach the 1 year follow-up and the last when all 184 participants reach the 1 year follow-up.

With this revised plan, the power remains at 85%. The new O'Brien-Fleming boundaries will be:

-4.550, 4.550 corresponding to a nominal α of 0.00005 at the first look
-2.963, 2.963 corresponding to a nominal α of 0.00305 at the second look
-1.969, 1.969 corresponding to a nominal α of 0.04900 at the third look

3.3.7 Efforts to Ensure Compliance with Protocol

Every effort will be made by study personnel to ensure protocol compliance. This includes enrollment of all eligible patients, providing data in a timely fashion, prompt notification of the pharmacist to obtain the treatment allocation, and proper administration of study treatment.

Site visits serve as important quality control checks and provide a means to become familiar with personnel and basic practices and procedures at each center. Clinical centers will have at least one site visit over the course of the study. The CCC PI and DCC personnel will visit each center and review source documents for a sample of patients, including patients with an exceptional number of inconsistencies identified through the data monitoring processes. At each site visit, the quality of data collection procedures will be assessed, the responsibilities of study personnel will be reviewed and visitors will confirm that data are collected in a timely and accurate manner. Problems with adhering to study protocols, data collection, and management within or between centers and within or between data collection personnel will be identified and addressed. In addition, site visitors will review subject recruitment and retention procedures, and other study specific administrative and organizational procedures. Site visits provide a means for the DCC to become familiar with personnel and basic practices and procedures at each center.

4.0 Human Subjects

4.1 General Characteristics – Minority Inclusion and Non-Discriminatory Statements

In general, ALF patients comprise somewhat more females than males, but there is no preponderance of any racial group, other than that expected on the basis of geographic differences. No exclusion will be made on the basis of race, ethnic group or gender. Criteria for inclusion of females and minorities will be those established in the NIH guidelines.

4.2 Inclusion of Children in Research

This research is restricted to children.

Intravenous NAC is a very safe agent with no significant side effects found during extensive use in Europe as well as sporadic use in the U.S. Individual children may or may not benefit from participation in this research.

In the future, other young people with acute liver failure could benefit from the results of this research. Information gained from this research could lead to improved medical care for them. However, your child's study doctor will not know whether there are benefits to other young people with acute liver failure until all of the information obtained from this research has been collected and analyzed.

4.3 Inclusion/Exclusion Criteria – Pregnancy and Birth Control Statements

INCLUSION CRITERIA
1. Enrollment in PALF registry (use PT INR results from the most recent tests performed prior to plasma therapy, but no more than 72 hours prior to enrollment in the registry.)
2. Informed consent obtained from the patient (when patient is 14-17 years of age or <17 and developmentally able to sign his or her name and is without clinical encephalopathy), parent, or guardian

EXCLUSION CRITERIA
1. Acute Acetaminophen toxicity
2. Patient on N-acetylcysteine or received N-acetylcysteine during the course of this illness.
3. Pregnancy
4. Known malignancy
5. Patient is on a liver support device
6. Sepsis
7. Signs of cerebral herniation
8. Intractable hypotension defined as systolic BP < 85 mm Hg or hypotension that requires treatment with ionotropic drugs, other than renal dosing dopamine

All female subjects of childbearing potential will receive a pregnancy test prior to enrollment. Pregnant females are not eligible for participation in the NAC trial.

4.4 Recruitment Procedures

All patients enrolled in the pediatric acute liver failure registry will be offered an opportunity to participate in this treatment trial, if potentially eligible. The clinical center principal investigator/co-investigator will inform the parent/guardian about the purpose of the study, procedures involved, the randomization process and measures taken to protect the subject and confidentiality. The parent/guardian will be informed of the risks and potential benefits of the study and of the child's rights as a research subject. All questions will be answered prior to obtaining their signature on the informed consent document. Each site will follow their respective state laws regarding the definition of a legal guardian and the right of the legal guardian to provide consent for research.

4.4.1 Informed Consent:

Informed written consent from the patient's parent or legal guardian is obtained for the collection of data and biospecimens as part of the multi-center PALF registry already in operation. A separate informed consent for the NAC study will be documented by the use of a written consent form approved by the IRB and signed by the patient's parent or legal guardian. Children less than 18 who are developmentally able to sign his or her name, and whose clinical condition, allows will be asked to assent in accordance with local IRB practices. A copy shall be given to the person signing the form. The consent form must embody the legally required elements of informed consent. The clinical center principal investigator shall give the patient's representative adequate time to read the form prior to signature. The parent or legal guardian can withdraw the patient from the study at any time, and this decision will not influence the treatment otherwise offered. It is not anticipated that any special groups will be enrolled in the study in any proportion other than those who are

representative of the US population. The NIH guidelines on the inclusion of females and minorities as subjects in clinical research will be observed.

4.5 Risk/Benefit Ratio

A possible risk is a breach of confidentiality, although steps have been taken to minimize such an occurrence. All information collected for this research study will be kept confidential. Patients' names will be used only for the informed consent form and medical chart reviews. Patients will be given unique study identifiers, which will be written on all data collection forms and biospecimens. In addition, data collection forms will be kept in secured, locked files. Known breaches of confidentiality will be reported to NIDDK.

The main adverse event reported in previous studies of NAC was a rare hypersensitivity reaction, manifested by rash or wheezing, and occurring with increased frequency in patients with a previous asthmatic diathesis. In each instance, use of intravenous anti-histamine (diphenhydramine) or corticosteroids aborted the attack and no instances of severe reactions have been reported. In most instances, the infusions were not discontinued and all symptoms resolved once diphenhydramine was instituted and the rate of infusion slowed.

Unusual events and anaphylactic reactions that occur during the seven day treatment period will be reported on a separate adverse event reporting sheet, and faxed to the Data Coordinating Center within 24 hours of knowledge of event. All Serious Adverse Event (SAE) reports received by the Data Coordinating Center will be forwarded to the Medical Safety Officer, the Data Safety and Monitoring Board (DSMB), the NIDDK project officer, and the IND holder. Serious adverse events that occur during the course of the trial and that meet criteria for expedited reporting will be forwarded to the FDA within 24 hours of receipt.

A DSMB, with members appointed by the NIDDK, consists of individuals who are independent of the institutions and investigators participating in the PALF study and who have no financial ties to the outcome of the trial. The ongoing review of the data by this independent committee assures the investigators and the NIDDK that the trial can continue without jeopardizing patient safety. The DSMB charter was developed by the NIDDK. The data and safety monitoring plan was approved by the DSMB. The roster and charter of the DSMB members will be provided to investigators participating in the study for submission to their IRB.

The DSMB will review the study protocol, recommend recruitment continuation, monitor all aspects of the study (e.g., recruitment, protocol deviations, adverse events, site visit summaries, data quality, attrition, descriptive characteristics), and recommend protocol modifications, which may include early study termination. Quarterly reports will be prepared by the DCC. Tables showing study progress overall and stratified by clinical center will be presented. These will include recruitment, protocol deviations, attrition, adverse events, and data quality. There will also be a table providing descriptive characteristics of the study sample. The DCC will maintain a cumulative summary of adverse events overall and stratified by serious/non-serious status to be forwarded to the DSMB quarterly. The DSMB will meet twice yearly, once in person and once via teleconference to review the data. A closed session will be held to review safety, efficacy, and data quality. Results of the interim data analysis will be viewed in closed session. Based on the data presented, the DSMB will recommend continuation or termination of the study. A summary of the DSMB findings will be forwarded to all investigators for submission to their respective IRBs.

In the future, other young people with acute liver failure could benefit from the results of this research. Information gained from this research could lead to improved medical care for them. However, benefits to other young people with acute liver failure will not be known until all of the information has been collected and analyzed.

5.0 Costs and Payments

5.1 Research Study Costs

The patient's insurance provider will not be billed for any procedures performed solely for the purpose of this research study. Expenses resulting from standard care for medical problems are the responsibility of the patient, patient's guardian, or the patient's insurance provider or government program. There are no funds available to pay for lost time away from work and other activities, lost wages, or child care expenses.

5.2 Research Study Payments

There are no payments or other remunerations to the research subjects.

6.0 Appendicies

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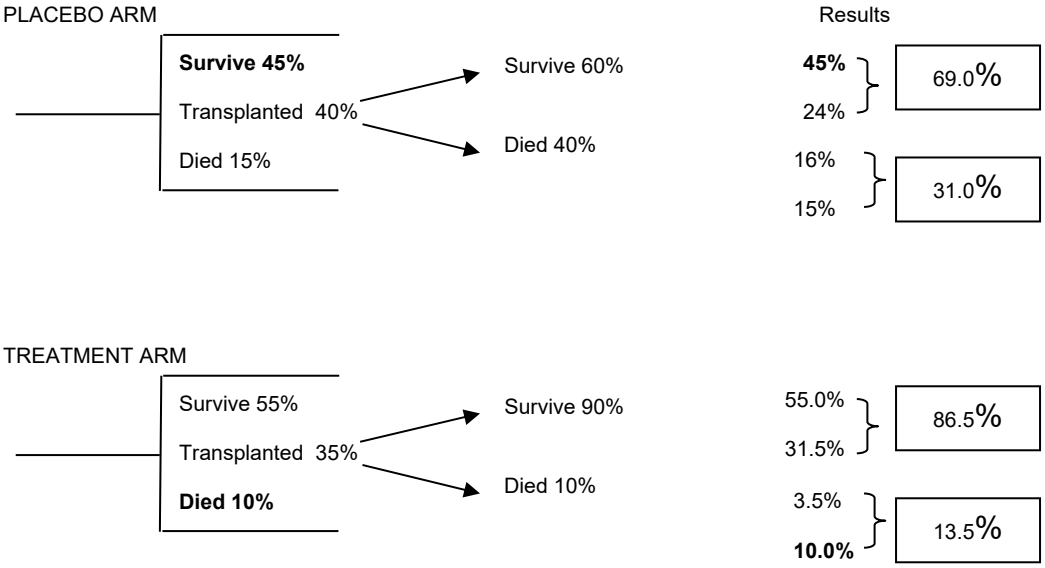
6.2: Pediatric NAC Protocol



The overall survival rate at one year in this model is 69.0% (45.0% + 24.0%) in the placebo arm and 86.5% (55.0% + 31.5%). Estimating the total number of planned analyses at 6 using O'Brien Fleming boundaries for both H_0 and H_1, a minimum of 184 patients (92 in each arm) will provide 80% power using a two-sided log-rank test (assuming proportional hazards) and an overall $\alpha = 0.054$ (if all 6 interim analyses are performed).

6.3 Overall patient management and the NAC trial

Standard treatment for acute liver failure includes placement in an intensive care unit and close observation for infection, bleeding, brain swelling, and other common complication of the condition. Supportive measures include fluids by vein, antibiotics, blood products, and a breathing support machine as necessary.

Initial management will be at the discretion of each institution, but general guidelines have been established during early meetings of the PALF Study Group. Triage for establishing diagnosis and degree of severity will be along standard clinical parameters.

Every effort will be made to establish the diagnosis within eight hours of admission to the hospital at which time informed consent can be sought from the child's parent or legal guardian. Once consent is obtained, patients will be allocated to treatment with NAC or placebo for the following 7 days. The intensive care treatment of acute liver failure patients will otherwise be unaffected. This treatment generally includes:

- Maintenance of blood glucose levels > 90 mg/dl via dextrose infusion (range 5%-20%) that is evaluated by blood glucose tests at regular intervals.
- Adequate fluid replacement and balance, as assessed by clinical inspection, electrolytes, central venous or pulmonary capillary wedge pressure.
- Nasogastric tube placement and treatment with H2-receptor antagonist or proton pump inhibitor.
- Close monitoring of possible infections. Proper antibiotics if infected and prophylactic antibiotics (cephalosporins) to all patients with deep coma grade III or grade IV.
- Head elevation to 20^{o}, if coma grade III or IV.
- Mechanical ventilation and induced hyperventilation in patients with deep coma grade III and coma grade IV.
- Infusion of mannitol if ICP >25 mmHg or clinical signs of intracranial hypertension (unstable mean arterial pressure with hypertension, irregular breathing, pupillary dilatation, decerebrate movements).

In general, clinical and laboratory parameters in the clinical center are measured at least twice daily, and it should be unnecessary to take special blood samples or any samples more often than twice daily in regard to monitoring the NAC study. There are daily assessments of laboratory parameters and intensive care measures such as intubations and the need for circulatory support.

LIVER TRANSPLANTATION:

In general, most patients advancing to grade III-IV encephalopathy are listed for liver transplantation unless there are contra-indications. Criteria for transplantation will be those usually practiced at each transplant center, but essentially conform to those leading to placement as UNOS status 1.

6.4 NAC checklist prior to entering and randomizing a patient

A Multi-center Study of the Safety and Efficacy of N-acetylcysteine in the Treatment of Acute Liver Failure in Pediatric patients not Caused by Acetaminophen

Investigators:	**Phone/Pager:**	**Nights/Weekends**

Patient Name: _____ MRN: _____ Study No: _____

Inclusion Criteria:

1. Enrollment in PALF registry
2. Informed consent obtained from the patient (when patient is 14-17 years of age or <17 and developmentally able to sign his or her name), parent, or guardian.

Exclusion Criteria:

1. Acute Acetaminophen toxicity
2. Pregnancy
3. Known malignancy.
4. Patient is on a liver support device
5. Sepsis
6. Signs of cerebral herniation.
7. Intractable hypotension defined as systolic BP < 85 mm Hg or hypotension that requires treatment with ionotropic drugs, other than renal dosing dopamine.

Needed on each patient prior to randomization

1. Signed consent form
2. Patient weight (kg): _____
3. Coma Grade (0, I, II, III or IV): _____
4. Age: _____

Randomizing patient

1. Fax a copy of the drug order form (with patient weight in kg, coma grade, and age), and if needed, the signed consent form to the Investigational Drug Pharmacy at:_____

2. Contact IDS Pharmacist: Weekdays, after hours & weekends:

6.5: Sample Order Form

CHILDREN'S MEDICAL CENTER OF DALLAS 1935 Motor Street ● Dallas, Texas 75235 ● (214) 456-7000 Physician Orders Generic equivalent drugs may be used unless otherwise specified	MED REC NO._____ ACCT NO._____ PATIENT_____ DATE_____LOCATION_____ DOB_____
ALLERGIES: □ NKA- No known allergies □ (Specify)_____ Weight (kg) _____ _____	

DATE AND TIME	ORDERS Copies (Do Not Write Orders If No Copies Remain, Begin New Form) **Remaining**
Acute Liver failure N- Acetylcysteine vs. Placebo Study	
IRB File No: 0502-296	
Principal Investigator:	
Patient Weight:	
Patient age (years and months) at randomization	
Patient's exact coma grade at randomization	
Study Orders:	
Infuse study solution at a rate of cc/hr (5cc/kg/24hrs) for 7 days	
Pharmacy to randomize patient to receive either N- Acetylcysteine 30 mg/ml in	
D5W or Placebo Solution (D5W alone)	
Notify physician for:	
Urticaria	
Respiratory distress	
Meds	
Benadryl mg (1mg/kg) for IV use to be available for bronchospasm, hives	
Continue all other evaluations and treatment unchanged.	
MD Signature	

6.6: Handling Adverse Reactions to N-acetylcysteine (NAC)

NAC is a very safe drug and has been used extensively by mouth and intravenously for the treatment of acetaminophen poisoning. The main adverse event reported in previous studies of NAC was a rare hypersensitivity reaction: rash or wheezing, which occurs with increased frequency in asthmatics.

Suggested treatment:
1-1.25 mg/kg diphenhydramine (Benadryl) intravenously slowly.

1 mg/kg methylprednisolone (Solumedrol) stat intravenously (max dose 1.0 gm)

Epinephrine as treatment of anaphylaxis might be considered, although this has not been reported to be needed in the past. Dose: 0.01 cc/kg (1:1,000) subcutaneously (max 0.3 cc) may repeat every 15 minutes

Resumption of medication:

If minor allergic reaction develops, discontinue medication for 2 hours then cautiously restart at a slow rate, continuing to increase rate to full dose if no reaction observed.

Other pointers:

Continuous EKG monitoring is recommended in light of one report of minor EKG abnormalities. Since acute liver failure carries a high mortality, it will be difficult in one sense to recognize evidence of additional toxicity due to NAC, in light of the multi-organ failure picture that patients typically develop. Please report any suspicious events such as the development of adult respiratory distress syndrome (ARDS), gastrointestinal bleeding, etc.

Serious Adverse Event Reporting

Unusual events and the occurrence of anaphylactic reactions that occur during the seven day treatment period will be reported on a separate adverse event reporting sheet, and faxed to the Data Coordinating Center within 24 hours of knowledge of event. All Serious Adverse Event (SAE) reports received by the Data Coordinating Center will be forwarded to the Medical Safety Officer, the Data Safety and Monitoring Board (DSMB), the NIDDK project officer, and the IND holder. Serious adverse events that occur during the course of the trial and that meet criteria for expedited reporting will be forwarded to the FDA within 24 hours of receipt.

6.7 Cumberland Letter of Authorization

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

IND 73,010

Robert H. Squires, Jr., M.D.
Clinical Director
Children's Hospital of Pittsburgh
5701 Fifth Ave.
Pittsburgh, PA 15213

Dear Dr. Squires, Jr.:

We acknowledge receipt of your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act. Please note the following identifying data:

IND Number Assigned: 73,010

Sponsor: Robert H. Squires, Jr., M.D.

Name of Drug: N-acetylcysteine Intravenous

Date of Submission: August 2, 2005

Date of Receipt: August 3, 2005

Studies in humans may not be initiated until 30 days after the date of receipt shown above. If, on or before September 2, 2005, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies, we will notify you immediately that (1) clinical studies may not be initiated under this IND ("clinical hold") or that (2) certain restrictions apply to clinical studies under this IND ("partial clinical hold"). In the event of such notification, you must not initiate or you must restrict such studies until you have submitted information to correct the deficiencies, and we have notified you that the information you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND. However, if the drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

6.7 Cumberland Letter of Authorization Continued

IND 73,010
Page 2

As sponsor of this IND, you are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the implementing regulations (Title 21 of the Code of Federal Regulations). Those responsibilities include (1) reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]; (2) reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]; and (3) submitting annual progress reports [21 CFR 312.33].

As required by the Food and Drug Modernization Act and the Best Pharmaceuticals for Children Act, you are also responsible for registering certain clinical trials involving your drug product in the Clinical Trials Data Bank (http://clinicaltrials.gov & http://prsinfo.clinicaltrials.gov/). If your drug is intended for the treatment of a serious or life-threatening disease or condition and you are conducting clinical trials to test its effectiveness, then you must register these trials in the Data Bank. Although not required, we encourage you to register effectiveness trials for non-serious diseases or conditions as well as non-effectiveness trials for all diseases or conditions, whether or not they are serious or life-threatening. Additional information on registering your clinical trials, including the required and optional data elements and the FDA Draft Guidance for Industry, "Information Program on Clinical Trials for Serious or Life-Threatening Diseases and Conditions," is available at the Protocol Registration System (PRS) Information Site http://prsinfo.clinicaltrials.gov/.

Please forward all future communications concerning this IND in triplicate, identified by the above IND number, to the following address:

U.S. Postal Service/Courier/Overnight Mail:
Food and Drug Administration
Center for Drug Evaluation and Research
Division of Gastrointestinal & Coagulation Drug Products, HFD-180
Attention: Division Document Room, 8B-45
5600 Fishers Lane
Rockville, Maryland 20857

If you have any questions, call me at (301) 827-9333.

Sincerely,

{See appended electronic signature page}

Monika Houstoun, Pharm.D.
Regulatory Project Manager
Division of Gastrointestinal & Coagulation Drug Products
Office of Drug Evaluation III
Center for Drug Evaluation and Research

6.8: Frequently Asked Questions

1. Does the NAC study alter the patients care?
   No. No other aspect of ALF care should change and listing for and undergoing transplantation are assumed to take place for many patients.

2. Can the patient be involved in any other form of artificial liver support?
   No.

3. What sort of reactions should we be aware of?
   The only significant reactions that have been observed with NAC are mild urticaria and wheezing, suggesting a mild anaphylactic reaction, which can be aborted with diphenhydramine and /or corticosteroids.

4. Is central line or pulmonary artery catheter placement necessary?
   No. If such lines are in place, data may be collected regarding cardiac output, but these lines are not to be established solely for the purpose of the study.