PEDIATRICS OPERATIONS MANUAL

ACUTE LIVER FAILURE STUDY GROUP

3rd Edition

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PURPOSE

Acute liver failure (ALF) is a unique illness of multiple etiologies, unusual severity and a rapid clinical course. Even when the etiology is known, the reasons for the fulminant nature of the disease in a given individual (hepatitis B, for example) remain unknown. The Adult Acute Liver Failure Study Group (ALFSG) was organized in 1997 around the idea that a disease this devastating and this infrequent (estimated 2,000 cases per year in the U.S.) can only be studied effectively by gathering data and evaluating treatments using multiple centers around the United States. The initial two year project was funded by an RO3 planning grant from the National Institutes of Health, grant # RO3-DK52827-01, with the goals of 1) establishing the group itself, 2) gathering careful prospective data on patients from the 14 sites selected, and 3) launching a pilot therapeutic trial of the use of N-acetylcysteine (NAC), a sulfhydryl group donor, in the treatment of acute liver failure not caused by acetaminophen. NAC is an effective antidote in cases of acetaminophen overdose, and is said to have beneficial effects on blood pressure and outcome in non-acetaminophen cases, but this has not been subjected to a controlled study. We are currently being funded by NIH grant 1RO1 DK58369-01 from September 1, 2000 through August 31, 2005 to continue the program in place. The interested number of sites has increased from the original 14 to 24 adult sites (5 new sites were invited to join since September 1, 2000) and 22 pediatric sites.

The NAC pilot study began enrolling patients in February 1999. While the RO3 grant expired on August 31, 1999, we successfully competed for further funding for the pilot study via the FDA Orphan Product Development program, FD-R-001661-01. Funding was awarded for 12 months, beginning September 1999. This was intended to support up to 50 patients. Since we have not met that enrollment goal, we have asked for this money to roll over into other expenses. In the latter part of 1999, 7 additional adult sites and 18 pediatric sites were asked to participate in the ALF study group. Sites have been collecting data and serum since January/February 2000, and ultimately some of these (both adult and pediatric) will take part in the NAC study. The RO1 application submitted to NIDDK on October 1, 1999 was successful in securing funds for 5 years starting September 1, 2000 for the full NAC protocol to

extend over an estimated 4 years to enroll 200 patients. Data and serum collection will be continued over this period and spin-off studies are being planned.

The Pediatric Acute Liver Failure Study Group met for the first time in November, 1999. This is the first large multicenter collaboration aimed to identify infants, children, and adolescents who present with acute liver failure. The first goal is to better define this condition as it relates to children. Then, once an acceptable definition is agreed upon, develop a patient registry for this condition. Through this registry, we will be able to determine patient demographics, presenting symptoms, clinical course, suspected etiology, and outcome from center throughout the country. A single, daily blood sample will be obtained for 7 consecutive days which will be set aside for further analysis. While the NAC protocol for adults might be adapted for children, our initial focus is to determine whether defects in fatty acid metabolism might account for the large percentage of infants with liver failure of unclear etiology. A project committee will serve as a clearing house for future proposals in the evaluation and treatment of children with acute liver failure.

This is an exciting time in hepatology, with new treatments and the identification of new viruses occurring almost yearly. It seems likely that there is another virus yet to be identified which is responsible for many, if not most, of the indeterminate cases of ALF. Studies such as ours can be instrumental in identifying this virus. It is a particularly exciting time for *clinical* research, and this project is an outgrowth of that excitement. This operations manual will introduce you to and take you through all aspects of the study. No manual can foresee every question, so it is important that you feel free to contact the study office in Dallas to get your questions answered. Contact information can be found at the beginning of this manual.

BACKGROUND AND SIGNIFICANCE

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. Many etiologies of ALF

are recognized. Traditionally, the most frequent cause in the US has been viral hepatitis with drug-induced liver injury second, and smaller numbers of cases due to a variety of etiologies. In nearly 20%, no clear-cut cause can be determined. Mortality figures in the range of 80-94% were observed in the pre-transplant era. 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 infants and children, acute liver failure can occur without clear evidence of encephalopathy. One of the goals of our group is to agree upon a set of guidelines and descriptors that might help us better define hepatic encephalopathy in infants and children. Unlike adults, the majority of cases of acute liver failure are unknown. Up to 40% of cases are presumed to have a viral etiology, yet are Non-A-E. As diagnostic tools improve and our index of suspicion is enhanced, an increasing number of infants have been found to have a variety of metabolic conditions including disorders of mitochondria and peroxisomes.

Orthotopic liver transplantation (OLT) improved 1 year survival in ALF, for ~65% of those receiving a transplant. However, many patients never receive a graft because they fail to reach a transplant facility in time, develop complications prior to transplantation, or lack donor organ availability. Additionally, after a liver transplant, patients must remain on immunosuppression for life. Therefore, therapies which might improve survival without transplantation should be tested. At the same time, identification of a metabolic defect that can be managed medically might save the child from the need for a transplant.

Multi-organ 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 (per definition) the brain. A unique feature of ALF in

comparison to other causes of MODS is cerebral edema. 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-α), are involved. The severe circulatory disturbance is characterized by a hyperdynamic circulation and tissue hypoxia similar to the sepsis syndrome, with impaired tissue oxygen extraction or altered peripheral cellular metabolic pathways.

ALF studies elsewhere

In adults, a large amount of the research in ALF was conducted between 1972 and 1997 at Kings College Hospital, London which has a dedicated Acute Liver Failure Intensive Care Unit. Much of the information concerning etiology, pathogenesis, prognosis and treatment modalities over the past 24 years has come from this unit. This unit pioneered use of activated charcoal perfusion columns (shown in a controlled trial to be of little efficacy), and most recently performed a pilot study using one of the early extracorporeal liver assist devices (ELAD). This same unit developed the prognostic criteria most commonly used around the world for determining the need for liver transplantation in ALF patients. The extensive experience at Kings has given this unit the edge in honing critical care skills, and their outcomes figures, which have improved considerably over recent years, cannot be extrapolated to smaller, less experienced intensive care unit settings. However, it is difficult to extrapolate the experience of the London group to the US, in part because the etiology of more than 50% of cases in the UK is acetaminophen (suicidal) overdoses, a problem commonly identified in our study, but in differing proportions and presentations. Etiologies differ around the world considerably, with drug-induced acute liver failure playing a very small role in developing countries in contrast to Europe or the US, highlighting the need to collect data specific to the United States. India, a developing country, has no drug-induced hepatotoxicity other than the 5% shown which is associated with isoniazid and there is no acetaminophen-related

toxicity. Most of India's Other category is hepatitis E. By contrast, developed countries have highly variable rates of acetaminophen and other drug-related toxicity, and less viral hepatitis.

Fulminant hepatic failure in children has not received the attention it deserves. Published studies are few and the numbers of patients are small. Investigators from Los Angeles, London, and Chile in separate reports identified only 125 patients over many years time. Studies of children with liver failure that present to a transplant center include patients with known chronic liver disease. We plan to focus upon causes of acute hepatic failure in children without a know chronic liver disease.

The ALF Multi-Center Study Group addresses a definite need: lack of significant research in acute liver failure in the United States in both adults and children, due in part to overall decreased population density and to different referral patterns in the US, resulting in fewer cases of ALF being seen at any one US center. Having established a widely-based multi-center study group through which information concerning the natural history, etiologies and outcome of ALF in the United States can be drawn, we intend to facilitate collection of clinically-derived samples, and use the group's strength in numbers to perform collaborative therapeutic trials in ALF.

ALF STUDY GROUP INFRASTRUCTURE AND STUDY DESIGN

The initial grant application was submitted in October 1, 1996, and funded on September 1, 1997 for a two-year period. The award stipend was $50,000, plus indirect costs for each of the two years. This stipend was divided into salary for the PI, the administrative assistant, Academic Computing Consultative Services (form design, data management), and limited support of meetings and travel. Unfortunately, no money was available to individual sites during the planning grant period. Funding from the FDA has provided $1800 per patient enrolled in the NAC pilot study (for up to 50 patients) and $5000 for data entry and analysis. The RO1 grant, funded September 1, 2000 will provide adequate support for all the study sites by paying $400 per patient enrolled in the data and serum collection study and an additional $1800 per NAC patient. Recruitment of study patients will continue for 4

years. The current funding will allow us to send out a monitor to your site on a yearly basis. The final year will be dedicated to the completion of data collection and analysis. At the end of the first phase of the study (2 years of data and serum collection), a number of other satellite and collaborative projects are under way including the development and testing of new prognostic models, and provision of serum samples to investigators searching for new viruses or metabolic diseases. To date tests run have included SEN V virus, hepatitis B, alpha-fetoprotein and Gc protein. Later studies might be directed toward the testing of new liver assist devices. The future is limitless in terms of the variety of studies in which the group may participate, and it is ultimately up to the group to decide its direction and project choices.

William M. Lee, MD is the principal investigator for the adult study; Robert H. Squires, Jr., MD is the principal investigator for the pediatric study. The initial grant was written by Will, with help from a Danish visiting fellow, Frank Schiødt, MD. George Ostapowicz, MD currently acts as the "chief operating officer" of the study. George can be contacted by telephone: **(214) 648-2516**; fax: **(214) 648-2070**; or email: gostap@mednet.swmed.edu. Grace Samuel is currently the adult research coordinator coordinating shipment of serum tubes and samples and serum bank cataloging. Grace can be contacted on **(214) 648-2665** or by email: grace.samuel@utsouthwestern.edu. Nancy Simonds, RN is the pediatric study coordinator handling the IRB setup, grants contract, billing issues and may be reached at 214-456-8038 or nsimon@childmed.dallas.tx.us. Other personnel involved in the study at UT Southwestern in Dallas include Joan Reisch, PhD, Linda Hynan, PhD, Joe Webster and Janet Smith in the Department of Academic Computing Services and Rehanna Mohammed, Dr. Lee's secretary. Dr. Reisch and Ms. Smith have played a crucial role in the design of the forms for data collection. Both have extensive experience in management of clinical studies. The Academic computing group will be active in data management, allowing the PI to remain blinded in the controlled trials. In addition, an independent review and safety monitor group will be appointed by the NIH to oversee the blind study from a safety and efficacy standpoint.

Time Line of the ALFSG

Initial group organizational meeting DDW San Francisco	May 12, 1996
Data collection for 2 year retrospective study	July 1, 1996
RO3 grant submitted	October 1, 1996
Grant award announced	May 1, 1997
Actual grant period began	September 1, 1997
Form design, IRB submissions	October/November, 1997
Prospective data collection began	January 1, 1998
Site selection for pilot study	April 1, 1998
FDA FD-R-001661-01 grant submitted	September 1, 1998
Pilot study of NAC treatment began	February 1, 1999
RO3 funding ceased	August 31, 1999
FDA funding began	September 1, 1999
RO1 grant application submitted	October 1, 1999
RO1 funding began	September 1, 2000
Begin larger NAC study (all sites)	September 1, 2000

The Multi-Center Group

The following sites have agreed to participate in the study:

(A list of investigator/coordinator emails is attached in **Appendix 1**)

Adult sites:

Site No.	Principal Investigator/co-investigator	Center
10.	Lee/Ostapowicz	UT Southwestern, Dallas
11.	Larson	U. Washington, Seattle
12.	Santyanarayana/Crippin	Washington U., St Louis
13.	Davern/Bass	UC San Francisco
15.	McCashland	U. Nebraska, Omaha
16.	Hay	Mayo Clinic, Rochester
17.	Murray/Weinstein	Baylor, Dallas
18.	Shakil	U. Pittsburgh, Pittsburgh
19.	Blei/Flamm	Northwestern, Chicago
20.	Benner/Flora	OHSU, Portland
21.	Han/Martin	UCLA
22.	Schiff/Torres	University of Miami
23.	Fontana	U. Michigan, Ann Arbor
25.	McGuire	U. Alabama, Birmingham
26.	Trotter/Everson	U. Colorado, Denver
27.	Chung/Dienstag	Mass. General, Boston
28.	Aranda-Michel/Sherman	U. Cincinnati, Cincinnati
31.	Brown/Russo	Columbia-Pres., New York
32.	Korula/Selby	U. Southern Calif., Los Angeles

New Sites joining adult ALF Study Group:

Site No.	Principal Investigator/co-investigator	Center
14.	Schilsky	Mount Sinai, New York
30.	Muir	Duke U., Durham
33.	Harrison/Rakela	Mayo Clinic, Scottsdale
34.	Ricci	Emory Clinic, Atlanta
35.	Munoz	Albert Einstein, New York

37.	Shiffman	Medical College of Virginia

Pediatric sites:

Site No.	Principal Investigator/co-investigator	Center
10P.	Squires	UT Southwestern, Dallas
11P.	Murray	U. Washington, Seattle
13P.	Rosenthal	UC San Francisco
14P.	Shneider/Emre	Mt Sinai, new York
15P.	Horslen/Kaufman	U. Nebraska, Omaha
16P.	Freese	Mayo Clinic, Rochester
19P.	Soriano/Alonso	Northwestern, Chicago
20P.	Terry	OHSU, Portland
21P.	Vargas	UCLA, Los Angeles
22P.	Mittal	University of Miami
23P.	Holmes/Olson	U. Michigan, Ann Arbor
24P.	Moyer	Yale U., New Haven
25P.	McGuire	U. Alabama, Birmingham
26P.	Sokol/Narkewicz	U. Colorado, Denver
27P.	Jonas	Children's Hospital, Boston
28P.	Bucuvalas/Balistreri	U. Cincinnati, Cincinnati
29P.	Schwarz/Scheimann	John Hopkins U., Baltimore
30P.	Treem	Duke U., Durham
31P.	Lobritto	Columbia-Pres., New York
32P.	Thomas	U. Southern Calif., Los Angeles

New Sites joining pediatric ALF Study Group:

18P.	Khan	U. of Pittsburg, Pittsburg
36P.	Piccoli	U. of Pennsylvania, Philadelphia

The 5 sites currently participating in the NAC pilot study are:

10.	Lee/Ostapowicz	UT Southwestern, Dallas
11.	Larson	U. Washington, Seattle

12.	Santyanarayana/Crippin	Washington U., St Louis
23.	Fontana	U. Michigan, Ann Arbor
27.	Chung/Dienstag	Mass. General, Boston

Sites planning to join the NAC pilot study in early 2001 are:

17.	Murray/Weinstein	Baylor, Dallas
22.	Schiff	U. of Miami
30.	Muir	Duke U., Durham
32.	Korula/Selby	U. Southern Calif., Los Angeles
33.	Harrison/Rakela	Mayo Clinic, Scottsdale
34.	Ricci	Emory Clinic, Atlanta
35.	Munoz	Albert Einstein, New York

Grants Contract and Payments

Finally payment for the work being done! The NIH grant 1RO1 DK58369-01 that started September 1, 2000 allows us as the coordinating site to set up sub-contracts with the individual sites. This process was started late October, 2000 after money was received and appropriately established in an account at UT Southwestern. Our grants contract specialist, Deborah Price, will negotiate the contract with each site.

Once you have your contract in place you can begin billing us. Payments will be made for cases enrolled after September 1, 2000. There are several steps involved in the billing process. In the contract you will be assigned a Grants Management Office number (GMO#). Please make note of that number, as you will need it when you submit the invoice **(Appendix 2)**. An electronic copy of the invoice is available from Nancy Simonds. You must fill in the appropriate information on the invoice, i.e., the name of the PI and the address where the check should be sent, GMO#, etc. Please mail or fax your invoice to Grace.

Committee Structure

The purpose of the committees is to provide advice and guidance to the PI concerning matters of mutual interest affecting the study as a whole. Ultimately, it is

the PI's responsibility to manage the study with help from NIH staff regarding certain issues, but in a multi-center study of this nature, committees can help provide governance and direct and manage aspects of the study. The committees as of 12/8/00 are:

Steering
Lee, Chair
Blei
Larson
Squires
Korula
Olson
Treem
Shiffman
Munoz

Pediatric Steering
Squires, Chair
Bucuvalas
Soriano
Rosenthal
Shneider
Schwarz
Sokol

Serum Bank
McCashland, Chair
Torres
Bass
Chung
Brown
Freese
Harrison

Research/ Ancillary
Fontana, Chair

Flamm

Davern

Han

Trotter

Terry

Piccoli

Publications
Shakil, Chair

Crippin

Hay

Satyanarayana

Aranda-Michel

McGuire

Jonas

Ricci

Meeting dates

Brief organizational meetings have taken place during DDW and the AASLD fall meetings. The annual December meeting at DFW airport represents a departure from this and has involved a full day. The morning has been used for presentations of topics related to ALF, including an update of the study results, while during the afternoon organizational issues have been discussed at length and the committees have had an opportunity to meet. Expenses incurred in attending the meeting are reimbursed. In 1998, 1999 and 2000 site study co-ordinators also attended, reflecting their importance in the smooth running of this study. Future annual meetings are planned. Whenever possible, committee recommendations will be brought to the group as a whole.

STUDY FORMAT

Institutional Review Boards

It is the responsibility of each site to submit timely applications to its institutional review board, including renewals and amendments to current protocols. Failure to do so will disqualify a center from participation "until the paperwork is done." UTSW IRB submission and consent forms were forwarded on disc or by email from Dallas to each site. Each site must have an IRB approval letter on file in Dallas (fax is OK), citing the study and its approval date from the board itself. Please also send for our records a copy of the consent forms and a copy of the entire submission as it looked when received by the local board. Also send along a biographical sketch or CV of principal investigator involved in the study. No site can begin data or serum collection without the proper paper work on hand in Dallas.

A second submission to the IRB has been necessary regarding the NAC pilot study. This study began with only a few sites for simplicity, to get some early experience prior to going to all sites. A similar approach will be used for pediatric studies such as the one proposed by Dr. Ben Shneider to study fatty acid oxidation defects in children with ALF.

Confidentiality

Needless to say, it is paramount that confidentiality regarding patient identities be maintained at all times. Because of the frequently fatal outcomes observed in acute liver failure, and the possibility of litigation surrounding the occasional case (toxins, drug reactions, etc.), it is imperative that we maintain good rapport with patient families, emphasizing that confidentiality is being maintained. Patient numbers assigned by each site will be the only numbers used to identify patients at the central site, and will be recorded in a Project Number Assignment Log Sheet. A “P” on the pediatric form will distinguish a child from an adult that comes from the same site. For example, a patient from site no. 10 who is patient number 13 would be listed as 10-P013 at the central site. Serum samples will only be coded by day and patient number. The two main data forms used will be compared to each other as to the date of birth which appears on all forms to confirm that the study number is correct

on both forms. Hospital ID numbers and names will be kept separately at each site on the Project Number Assignment Log Sheet in case retrospective information is sought. It is important that each site keep this log carefully for obvious reasons! See further discussion of the forms below.

Phase I: Data Collection

Central to this study is the prospective collection of data on all clinical and demographic aspects of the acute liver failure patients that we see. We have tried to make this as easy and effective as possible. This will require hard work at each site by a number of people.

Who is the Data Collector?

In the beginning, the person who fills out the data form should ideally be the physician-investigator (usually the PI) who is closest to the ALF Study. At some sites this may be a single junior faculty member, at others two or three different faculty members. It may be necessary to assign this task to a clinical coordinator and this is certainly acceptable. However, we are interested in feedback concerning the forms under actual combat conditions, so it is probably best in the beginning if you each try it a few times before assigning it to others. We would also like the principal investigator or the sub-investigator reviewing cases and countersigning them before faxing them to UTSW or they will not be accepted. **The investigator at each site is responsible for the quality of the data.** As the saying goes "garbage in, garbage out." The data is only as good as the way in which it is collected. Figure out ahead of time who at your site is the principal investigator responsible for submitting the forms or seeing that the form is submitted. It may be necessary to assign the job on a weekly or monthly basis or to use your call schedule. We will rely on each site to deal with this and assume that few if any cases will fall through the cracks. A mechanism for recording excluded cases will be used employing the Project Number Assignment Log Sheet (see below and **Appendix 3**). It is understood that since most sites and most patients will be listed for transplantation regardless of the way in which the patient reaches your hospital, you or your service will be called directly upon admission to see the patients. Have the forms, consents and the log sheet

readily available in your office or in the ICU itself for easy access. We have also provided a synopsis of the protocol as a quick reference when consenting the patient and collecting the data. If you are out of town, see that your covering partner knows what to do and where to find the forms. **This is vital.** Nothing will sink this project like poor data collection!

When you get the call to see a patient, bring the form with you, complete it immediately after you finish your initial evaluation and then fax it on to us. At the same time, collect the initial blood sample, once the permit is signed, and see that it is stored properly.

Project Number Assignment Log Sheet

In addition to the Admission and Clinical Assessment and Outcome forms, each site will keep a Project Number Assignment Log Sheet (PNALS) as mentioned above, to allow for confidentiality and blinding of the Dallas data center and to keep track of cases not included in the study. This is a master log for each site of patients entered into the study, listed by the study site number followed by the consecutive numbers P001, P002, P003, etc. Also list on this sheet all the patients who were <u>not</u> enrolled and the reason for not enrolling such patients, **and assign them a consecutive study number**. Reasons might include: coagulopathy but no coma, family refused consent, etc. This will give us important data on the completeness of our sample. The PNALS also ensures that each site can track patients for retrospective data collection if a specific point about the patient's blood samples, or a specific historical fact is desired at a later date. This allows other investigators or the central site to request data without their having direct access to the patient's medical record or identity. This log needs to be kept in a safe and accessible place for all who might be called upon to enter data on any given day, at any given time. Carefully record the patient's name, date of birth, study number, medical record number at your hospital, date of entry into study and comments. The comment section allows you to qualify the reason for exclusion.

Data Forms

Overall design of these forms **(Appendix 4)** was based on the goal of collecting useful information which can be interpreted effectively, incorporated into a database and accessed easily. The information to be obtained should include demographic and clinical features of ALF (with particular attention to etiology, rapidity of onset, risk factors), and outcomes. These are not all-inclusive forms, because it was felt that it was most important to obtain the basic facts and that the forms be user-friendly, not cumbersome. None of these forms takes more than 20-30 minutes to complete. They should be faxed upon completion to the central pediatric site in Dallas (214 456-8006) where they will be screened and then directly entered into a database program. It is vital that you write (print) carefully and complete the forms thoroughly. Most fields have a place for "data not available" which should be used. If not, line through or write “UNK” in the data field (Ex. Ht UNK). Please correct any mistakes by drawing a single line through the incorrect entry, dating and initialing it (Ex. ~~11/15/99~~ 11/15/00 *ns11/16/00).* Filling in the forms by hand allows for speed and for the minimum of equipment at each site. It also allows the data collector to simply take the form to the bedside, and then return to the fax machine to complete the task. If glaring errors or omissions arise, we will be reviewing each form before it is entered into the computer and will prompt you.

Two forms are required for each ALF patient, one at the beginning and one at the end of the admission. The first, the Admission to Study form, is to be submitted within 24 hours of study entry. This time interval is different from the adult group, many of whom are participating in a randomized NAC trial that requires initiating a treatment protocol. We may need to shorten the “time to submission” if we enter a similar trial. Please fill out the form as completely as possible. After the form is submitted, we will review the form and may contact you for additional missing data. Once the Admission form is as complete as possible, it will be submitted for analysis. The **Clinical Assessment and Outcome form can be completed daily during rounds and should be completed at time of hospital discharge, death, or transplant or at 3 weeks following admission to the study, whichever comes first.** Fax each form immediately upon completion to the pediatric study center in

Dallas at 214 456 8006. This allows us to review the forms before entering the data automatically into the computer. This technique also gets site personnel into the habit of completing the study form on admission which will be crucial to randomization for entry of patients into the later NAC study.

All forms are a compromise, and none is perfect. Do the best you can. Fill these in as carefully as possible with the information available at the time. Missing data can be supplied on the Clinical Assessment and Outcome form, in which many of the fields are repeated. The exact timing of the Clinical Assessment and Outcome form is less crucial than the Admission form. You need not repeat the data from the Admission form (except the initial demographics) on the Clinical Assessment and Outcome form. *When in doubt, fill it out.* Do not modify the Admission form at any later time point. Simply put the new information on the Clinical Assessment and Outcome form in the appropriate place. Each form has a comment section where you can elaborate on the circumstances and peculiarities of each case. We expect you to do so and that these will be if not written, read and edited by the PI.

Serum Samples

We wish to collect a single daily serum sample. Plasma is acceptable should no serum be available. The amount of extra blood to be taken is based upon the weight of the patient and is outlined in the following table:

PATIENT WEIGHT	AMOUNT OF EXTRA BLOOD
Less than 10 kg (22 pounds)	2.5 cc (1/2 teaspoon)
10 kg to 20 kg (44 pounds)	5.0 cc (1.0 teaspoon)
20 kg to 30 kg (66 pounds)	10.0 cc (2.0 teaspoons)
Over 30 kg (66 pounds)	15.0 cc (3.0 teaspoons)

We supply both the blood collection tubes (3 and 5 ml) and the cryo tubes (1.8 ml) used for freezing the serum. The blood will need to be spun as soon as possible after phlebotomy, and placed at -70 degrees within two hours, although less time at room temperature would be even better. We will be looking for viral RNA which would be degraded by RNAses if serum remains for a prolonged time unseparated

from the cells or if the time at room temperature is prolonged. Using a sharpie marker, label each specimen with the site number, patient number, date of collection and day (1-7) of collection. Decide at your own site how you will handle the daily collections. You may use existing samples (**serum collected at the first a.m. blood draw**) or add the SST tubes to the daily orders with the additional tubes to be collected and held (or spun) for you. Be creative--work out a way which will be as easy as possible and as fail-safe, so that we have these all-important sera available as a resource for you and other investigators. Set up your collection system before the first sample comes in. Serum should be aliquoted into 200-250 μl (0.2-0.25 ml) samples (if possible) prior to freezing at each site. If this exceeds 8 cryovials then aliquot into 2 ml samples. We will arrange for shipping to Dallas at roughly 3-month intervals depending on the need at each site. We have provided step by step directions for processing and shipping the specimens **(Appendix 5)**. We have also provided a **serum bank log sheet (Appendix 6)** where you can write in comments.

A serum sample is to be collected through the first seven days of the study if possible. Each sample ideally should be the morning collection, spun within two hours and frozen at -70°. The log sheet will be similar to the PNALS sheet. Simply record the center number, patient's number and sample, representing the hospital day after study entry. For example, a sample from patient 13 from site 10, from the 3rd study day becomes 10-13-03. It is advisable to write on the tube the actual date as well. The log sheet allows for comments on the sample. Please record as nearly as can be determined whether the patient had received plasma prior to the initial and subsequent samples if known; if unknown, check this box as well. This will serve as a log of samples in the freezer at your site. Keep in a safe place. This may be faxed to Dallas as necessary so do not use patient's names on this sheet.

Tissue samples

All sites are encouraged to send liver tissue samples to Zach Goodman, MD at AFIP from all patients who have a liver biopsy, liver transplant or autopsy. This is a unique opportunity to acquire a large collection of liver biopsy samples from patients with acute liver failure. These specimens will be available to the ALFSG for research

purposes e.g. describing the pathological changes in liver failure of different etiology. Tissue blocks are preferred, however if these are not available, six unstained slides are a suitable option. A tissue submission form is attached (**Appendix 7**). Copy it at your site and submit it with each specimen. Funding is not available for this step, however, sending the tissue samples on a consultation basis to AFIP should not incur any charges to you. You must signify on the submission form that this is an ALF study case and indicate the patient's study number for Dr. Goodman to waive the fees.

Study Form A: Admission to Study

This form provides basic demographic information, confirms the presence of features of ALF appropriate for study entry, and reviews the admission history, physical, and laboratory data. An admission diagnosis is made, although this may later be altered on the Clinical Assessment and Outcome form. Pediatric forms became available in March 2000.

PAGE 1:

Fill in your center number and the patient study number for each consecutive patient fully entered into the study. Include on the top line the initial hospital admission date and time (first hospital entered). Write carefully and legibly in large block letters and numbers. Note the spacing lines, particularly with regard to dates and times. If the patient was transferred from another hospital, indicate the date of transfer to your hospital. If the patient was admitted de novo from home, then this is not a transfer from another hospital. We have made provision for some laboratory values from an outlying hospital to be included on the Outcome form. Summary sheets or discharge summaries from the referring hospital may be faxed to Dallas along with your Admission sheet, but be sure to redact (white out) names on the record and medical record numbers and place the patient ID on the sheet.

Item 1. This deals with qualifying features for inclusion in the study. In the analysis of the data, patients will be divided into fulminant (<8 weeks illness) and sub-fulminant (8-26 weeks illness). Each study patient must have **all three** features to qualify as having ALF (fulminant or sub-fulminant):

1. Evidence of a liver related illness

2. No history of known chronic liver disease
3. Coagulopathy, that is uncorrected by vitamin K, that fulfills either of the following conditions:
    a. INR > or = 1.5 PLUS encephalopathy OR;
    b. protime > or = 15 PLUS encephalopathy OR;
    c. INR > or = 2.0 WITH or WITHOUT encephalopathy OR;
    d. protime > or = 20 WITH or WITHOUT encephalopathy

Item 2. Once these criteria are met, a consent should be obtained from a parent or guardian. All patients entered into the study must have an informed consent available for review. Keep these in a file near the PNALS clipboard. It is not necessary to forward the consents to UTSW. The consent authorizes examination of the record and the collection of serum as well. It is imperative that all eligible patients be enrolled. For patients where consent cannot be obtained, it is possible to appeal to an ombudsman or ethics committee which usually has someone on call over 24 hours.

Item 3,4. Now record the time of admission to study. This will be defined as the day and time the consent form is signed. But remember, the time of admission to the initial hospital and the time of study entry may differ. Study entry may occur several days into the hospital admission for a patient who enters with elements of severe acute hepatitis but does not meet entry criteria. Record time on a 24 hour time scheme (Army time), i.e., 1500 rather than 3 pm.

Item 5. Record whether at study admission the patient had received sedatives or FFP. These facts are important in interpretation of level of consciousness and whether initial serum samples are uncontaminated with plasma from others.

Item 6,7. Date of birth and age. Should be self-explanatory.

Item 8. Gender. A no brainer.

Item 9. Years of education. This may not be obtained easily. List as unknown if you do not know rather than guess.

Item 10. Race/ethnicity. In general, list one, but you may list more than one.

Item 11. Try to obtain an accurate date of onset for the first symptom, usually this will be fatigue or malaise, but other symptoms have been listed separately.

Item 12. Onset of jaundice has been used as a separate criterion so we wished to collect this piece of data. This item will not be applicable in those few individuals who are not jaundiced.

Item 13. Dates of onset of coma grades. This will depend on the stage of illness at entry to study. Those who are grade one will have only (presumably) one date filled in and the other lines blank. For those patients who are grade IV on admission to study, try to back track and determine when the other stages intervened. Ordinarily, this may be known, since the patient will usually have been in a referring hospital.

Item 14. Previous health and illnesses checklist. Please mark all the disease/system categories which apply to the patient and specify the diagnosis if known. There is a space at the end of each column that indicates whether the history was obtained and negative or whether the history was not obtained or unknown. We tried to save space with additional “yes and no” columns and hope this will allow us to identify familial problems.

PAGE 2:

Don't forget to bring forward the patient's study number and date of birth from the first page.

Item 15. Has the patient used injection drugs in the past six months? Alcohol?

Item 16. Checklist of medication(s) used in the last 6 months. The listed drug groups are known to have been associated with liver damage (although this list is not exhaustive). Please inquire specifically about all the groups and mark all that apply. Specific medications are to be recorded under “Item 17” (below).

Item 17. Medications. This includes all herbs, or any xenobiotic ingested as a toxin (mushrooms, acetaminophen, vitamins, etc.), anesthetics, as well as prescription drugs. Try to be specific with the patient or family members as to quantity, strength, last date ingested, and for how long. When compound drug containing acetaminophen and another agent has been used, please be specific with its “Trade name” and record the dose of acetaminophen taken per day. Note that we have not included a long list of peculiar features such as environmental exposures, but these may be covered in the comment section.

Item 18. We wish to track acetaminophen overdoses. In most instances, there is a clear story of either a purposeful overdose with suicidal intent, or an accidental overdose in an attempt to relieve pain. Try to specify the total dose and the date and time of the ingestion, as well as whether it was a single time point. If accidental then indicate the reason for acetaminophen use. Please mark “unknown”, if you are unsure, don’t guess.

Item 19. The admission physical examination is to be performed/recorded based on the ***time of entry to study***, not to hospital. These may, of course, occur at the same time or be vastly different. Characterize the patient's nutritional status by height (**in cm**) and weight (**in kg**), from which a BMI can be calculated. Encephalopathy in children is often difficult to assess. Therefore, a Table follows to provide a uniform application of terminology:

Table to assess encephalopathy:

- For patients where it can be done use the standard clinical scales. Certainly children over 10 years of age will be able to use these criteria and may be useful down to 3 years or so.

STAGE	Clinical	Asterixis/Reflexes	Neurological signs	EEG changes
0	None	None/normal	Psych testing only	Normal
I	Confused, mood changes, altered sleep habits, loss of spatial orientation, forgetful	None/normal	Tremor, apraxia, impaired handwriting	Normal or diffuse slowing to theta rhythm, triphasic waves
II	Drowsy, inappropriate behavior, decreased inhibitions	None/ hyperreflexic	Dysarthria, ataxia	Abnormal generalized slowing, triphasic waves
III	Stuporous, obeys simple commands	None/hyperreflexia, up-going toes (+ Babinski)	Rigidity	Abnormal generalized slowing, triphasic waves
IV	Comatose, arouses with painful stimuli (IVa), or no response (Ivb)	Absent	Decerebrate or decorticate	Abnormal, very slow delta activity

- For younger patients, would try the following, adapted from Peter Whittington.

Stage	Clinical	Asterixis/Reflexes	Neurological signs
Early (I and II)	Inconsolable crying, sleep reversal, inattention to task	Unreliable/ normal or hyperreflexic	Untestable
Mid (III)	Somnolence, stupor, combativeness	Unreliable/hyperreflexic	Most likely untestable
Late (IV)	Comatose, arouses with painful stimuli (IVa) or no response (IVb)	Absent	Decerebrate or decorticate

Assessment of admission edema, splenomegaly, ascites, hyper-reflexia, pupillary dilatation (>5 mm and hypo- or unresponsive) using only physical examination techniques.

PAGE 3:

Re-enter study number data at top.

Item 20. Laboratory values at admission. Once again, this is ***at admission to study*****,** not to hospital although these will frequently be the same. These are the basic values which should almost without fail be available on all patients at admission to study: CBC, electrolytes, hepatic studies, arterial blood gasses, and toxin screen, lactate level. Ionized calcium and glucose levels have now been added to the list.

Blood for arterial ammonia can be collected at the same time as the ABGs. If your chemistry lab performs this test on call, the blood needs to go to the lab on ice and be analyzed within **60 minutes** of arterial puncture.

PAGE 4:

Center number and patient study number please!

Item 21. Serological Parameters. This includes all the serological tests for viruses, as well as pregnancy. You may include data from the transferring hospital.

Item 22. Miscellaneous: This includes serum autoantibodies, copper studies, αFP and any other relevant tests performed. Please make note here whether admission ALF Study serum was collected. Please record who the gave patient history (i.e. relative, etc).

Item 23. List the one presumptive diagnosis which best fits the present circumstances. Often this will be completely known, at other times completely unknown or indeterminant. Be as accurate as possible but do not list pure guesses. Remember we are very interested in the indeterminant cases.

Item 24. Comments: Give a little background for the case if this is not elucidated in the data collected so far.

Final Item. Very Important. Give your own name (print), telephone, pager, and when the form was submitted, actually completely finished and faxed. Simple, no?

Study Form B: Clinical Assessment and Outcome Form

This form is completed when the patient has been discharged, transplanted, has died or when 3 weeks have elapsed since admission. The aim is to summarize the outcome and the course in a usable fashion. This form has been changed to reflect the group's desire for more detailed information. The flow sheets should allow maximum data collection with a minimum of work. Take this form to the ward during daily rounds and as close to the time of discharge, death, etc. as possible, since charts often disappear and memories get cloudy if a significant interval elapses. *Take the admission form with you when you fill out the Clinical Assessment and Outcome form and refer to it.* **We will bug you if we don't hear in three weeks!**

PAGE 1:

Enter center number, patient number, and date of this outline summary report, similar to the admission sheet. Refer to the PNALS (the log clipboard) for the study number of the patient.

Item 1. Summarize in outline form the final outcome for the patient. If still hospitalized but not transplanted, this should be evident: (alive yes, transplanted no, discharged, no). Specify date of death, whether an autopsy was performed, presumed cause of death, date of transplant or discharge, and whether to home. Specify whether tissue was saved for storage at AFIP, either as slides, frozen tissue or block. Refer to Tissue Sample section for details on how to collect and send tissue. **Be sure to fill out the brief AFIP form when tissue is being sent. (Appendix 7)**

Item 2. List the apparent etiology of this episode of acute liver failure, as clearly as is known. It is unlikely that there would be two causes, but occasionally there might be (hepatitis B plus acetaminophen, for example). **Uniform guidelines for the different diagnoses are attached (Appendix 8). It is important that other diagnoses are truly excluded in those cases said to be indeterminate.**

Item 3. List the basis for the diagnosis given in Item 2. More than one category may be used, i.e., biopsy plus lab.

Item 4. Medications. Frequently, this may differ from those known and listed on the admission form. Refer to the admission form as needed. **List here only if there are differences from the admission form based on additional data.** Do not list in-hospital medications.

PAGE 2:

Item 5. The first column serves to collect important pre study data. Data may be derived from the referring hospital or your hospital before the child was entered into the study. **The admission labs will be those from admission to study; i.e. SD#1, not hospital day #1.** Please record the date for each “SD”. Fill in as much data as you can. There may be missing values for some days. Fill in circles where indicated.

For uniformity, use the first values of the day. These may not be the worst values, but they will provide a uniform timing across centers.

PAGE 3:

Item 5. Flow sheet, cont'd.: As you can see, this is all basically fill in the circles. **Once again, "Admission SD#1" refers to admission to study, not hospital.** This will allow us to keep track of length of ICU stay, number of complications, and when. Should not be too hard to do, once you get the hang of it. For example, In ICU? presumably you will know readily when the patient entered and when was transferred out. Filling in the circles becomes easy at that point. We have added a few new complications (acute renal failure – defined as serum creatinine >300 mmol/L and urine output <300 ml/24 hours) and treatments (lactulose, acid suppressants). This information does not apply to the day of discharge, only to the day of death or transplantation, if after SD#7.

PAGE 4:

Items 6,7. Serological parameters, miscellaneous. Once again, do not enter values here unless they are additive or different from those listed on admission. Check the admission form closely for changes, additions. In the middle right portion of the page, record how many day's serum samples you have collected. This is very important!

Item 8. Scans, EEG monitoring, liver biopsy. A category to capture further diagnostic studies. Specify please if not done. Write in important findings in telegraph form.

PAGE 5:

Item 9. Infections. A common complication, this is the simplest method we could devise for tracking and reporting this data. Specify source of all positive cultures.

Item 10. Antimicrobials used. Please list separately the antibiotics used for prophylaxis of infection, bowel decontamination and therapy of infection (s).

Item 11. Record outcome of the infection(s).

Item 12. Liver Transplantation. We need to capture some basic information about the transplant process itself, when transplants are performed, etc. so that we can provide insight as to outcome based on day of performance, coma grade, etc.

Item 13. The comment section should be used in almost every case to cover the nuances that may be missed by the form itself. Use a separate sheet if more detail is thought necessary. On any supplementary sheet, identify patient by study center and patient number.

Item 14. The pediatric Clinical Assessment and Outcome form has a detailed list of serologies and metabolic studies. Please mark those that were performed and the results. Also, please mark those studies that were not done. This list is not meant to be a laundry list of tests you should do. Certainly, those decisions are left to the attending physician. However, this information will help us, particularly in those cases where the etiology of the liver failure was not identified.

Final Item. Very Important. Once again give your own name (print), telephone, pager, and when the form was submitted, actually completely finished and faxed.

Appendix 8
Specific diagnoses should fulfill the following criteria.

1) Acetaminophen
History of acetaminophen ingestion (either suspected overdose or chronic ingestion, especially in combination with significant alcohol use).
a) Toxic serum acetaminophen level.
or
b) ALT > 3500 U/L with a history of acetaminophen ingestion irrespective of the acetaminophen level.

2) ALF of Pregnancy
a) Acute Fatty Liver - ALF occurring between 26 weeks gestation and the immediate
- postpartum period.
- Liver biopsy c/w diagnosis (ie microsteatosis).
b) HELLP syndrome - ALF occurring between 22 weeks gestation and the
- immediate postpartum period (> 90% cases).
- presence of hemolysis, elevated LFTs (transaminases) and low
- platelets (< 100,000).
- often associated with hypertension/pre-eclampsia

3) Autoimmune Hepatitis
a) Globulins elevated > 1.5X ULN.
b) ANA, ASMA or LKMA positive in titer of at least 1:80.
c) Negative serology for viruses associated with acute or chronic hepatitis
±
d) Liver biopsy showing CAH.

4) Budd-Chiari
a) Obstruction of blood flow of the centrilobular veins at any level as shown by:
- doppler US
- angiography
b) Liver biopsy c/w diagnosis

5) Drug-Induced Hepatitis
a) Temporal relationship between exposure to suspected drug and onset of ALF.
b) Exclusion of other causes.

6) Hepatitis A
a) Positive anti-HAV IgM

7) Hepatitis B
a) Positive anti-HBc IgM
b) Positive HBsAg.

8) Delta Hepatitis

a) Positive HBsAg.
b) Positive anti-HDV
±
c) Positive anti-HBc IgM

9) Hepatitis C
a) Positive anti-HCV (may be absent early in the infection).
b) Positive HCV RNA by PCR.

10) Hepatitis E
a) Positive anti-HEV IgM

11) Mushroom Intoxication
a) Temporal relationship between mushroom ingestion and onset of ALF.
b) Exclusion of other causes.

12) Shock/Ischemia
a) Development of ALF following documented hypotension.
b) Development of ALF in association with a documented low flow state eg severe cardiac failure.
c) Exclusion of other causes.

13) Wilson's Disease
a) Serum ceruloplasmin < 20mg/dl
b) Elevated serum free copper > 25μg/dl
c) Urinary copper excretion > 100 μg/ 24 hours
±
d) Copper concentration in liver biopsy > 250 μg/g of dry weight.

14) Other Viruses
a) HSV - anti-HSV IgM positive and anti-HSV IgG negative
- our fold increase between acute and convalescent sera
or - HSV seen in liver tissue
b) EBV - anti-EBV IgM
or - EBV seen in liver tissue
c) CMV - anti-CMV IgM positive and anti-CMV IgG negative
- four fold increase between acute and convalescent sera
or - CMV seen in liver tissue

15) Indeterminate
Exclusion of all the above diagnoses on
a) History
b) Serology and other laboratory testsAppendix 9

A number of people have asked us about some of the abbreviations used in both the admission and Clinical Assessment and Outcome forms. Here is a key that should help:

St.BC: standard bicarbonate

MAP: Mean arterial pressure. This is given by many electronic BP monitors or in those individuals who have an arterial line in situ. It can be calculated as: diastolic pressure + one third of the pulse pressure (difference between systolic and diastolic pressures).

PEEP: Positive end expiratory pressure

PCWP: Pulmonary capillary wedge pressure

NAC: N-acetylcysteine

ELAD: Extra corporeal liver assist device (including BAL – bioartifical liver).