AlcHepNet Consortium
Clinical Study Protocol

Protocol Title

ALCOHOLIC HEPATITIS NETWORK OBSERVATIONAL STUDY

Protocol Number

AlcHepNet 01

Version 3: March 9, 2021

Primary Sponsor

National Institute of Alcohol Abuse and Alcoholism

Participating Sites

Indiana University, IN (PI: Kavish Patidar)
University of Louisville affiliated hospitals in Kentucky (PI: Craig McClain),
Beth Israel Deaconess Medical Center (PI: Gyongyi Szabo),
Mayo Clinic in Rochester, Minnesota (PI: Vijay Shah),
Cleveland Clinic Foundation, Ohio (PI: Srinivasan Dasarathy),
University of Pittsburgh Medical Center in Pennsylvania (PI: Ramon Bataller),
University of Texas at Southwestern in Dallas, Texas (PI: Mack Mitchell),
Virginia Commonwealth University in Richmond, Virginia (PI: Arun Sanyal)

GCP Statement: This study will be performed in compliance with GCP, including the archiving of essential documents.

SPONSOR'S approval of the protocol

Reviewed and Approved by:

_____ _____

Samer Gawrieh, MD Date

Primary Investigator (Indiana University)

Investigator's Affirmation

I have received and read the current version of the AlcHepNet-01 protocol. I understand that no data are to be made public or published without prior knowledge and written approval by the AlcHepNet Steering Committee. By my signature below, I hereby attest that I have read, understood and agreed to abide by all the conditions, instructions and restrictions contained in protocol AlcHepNet- 01 and in accordance with Good Clinical Practice (CPMP/ICH/135/95), 21CFR Part 312 and all applicable regulatory requirements. I acknowledge that the Steering Committee of the AlcHepNet Consortium has the right to discontinue this observational trial at any time.

_____

Investigator's Name (Printed)

_____ _____

Investigator's Signature Date

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Tables

Table 1: Abbreviations

AASLD	American Association for the Study of Liver Disease
AAH	Acute alcoholic Hepatitis
AE(s)	Adverse event(s)
AGA	American Gastroenterological Association alcoholic hepatitis
AH	Alcoholic Hepatitis
AKI	Acute kidney injury
ALT	Alanine aminotransferase
ALP	Alkaline Phosphate
AST	Aspartate aminotransferase
AUDIT	Alcohol use disorder identification test
BL	Baseline
BMP	Basic metabolic panel
BP	Blood pressure
BUN	Blood urea nitrogen
CBC	Complete blood count
CFR	Code of federal regulations
CLDQ	Chronic liver disease questionnaire
CRA	Clinical research associate
CRF	Case report form
DCC	Data coordinating center
DCC-IU	Data coordinating center-Indiana University
dL	Deciliter(s)
DSMB	Data and safety monitoring board
ER	Emergency room
FDA	Food and Drug Administration

GCP	Good clinical practice
HIV	Human immunodeficiency virus
HIV RNA	Human immunodeficiency virus ribonucleic acid
ICF	Informed consent form
ICU	Intensive care unit
IgM	Immunoglobulin M
IRB	Institutional review board
IU/L	international units per liter
IV	Intravenous
MAP	Mean arterial pressure
MELD	Model for end stage liver disease
mg	Milligram(s)
mL	Milliliter(s)
mm Hg	Millimeter of mercury
mmol/l	Millimoles per liter
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NDB	Nutritional database
NIAAA	National Institute of Alcohol Abuse and Alcoholism
NIH	National Institute of Health
PBC	Primary biliary cirrhosis
SAE	Serious adverse event
U/L	Units per liter
ULN	Upper limit(s) of normal
US	United States (of America)
Vs	Versus
WBC	White blood cell

WIRB	Western Institutional Review board

1. ETHICAL CONDUCT OF THE STUDY AND REGULATORY REQUIREMENTS

1.1 Institutional Review Board (IRB)

The study protocol and any amendments will be reviewed by Western Institutional Review Board (WIRB). WIRB will review the informed consent form, their updates (if any), and any written materials given to the subjects. A list of all IRB and contact information will be included in the study report.

1.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, in compliance with the approved protocol, GCP and applicable regulatory requirements.

1.3 Subject Information and Consent

The investigator will obtain a freely given written consent from each subject or authorized representative after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other aspects of the study that are relevant to the subject's decision to participate. The consent form must be signed and dated by the subject before he/she is exposed to any protocol-specific procedure.

The investigator will explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, without any consequences for their further care and without the need to justify.

The patient will receive a copy of the patient information and the signed informed consent.

The patient will be informed if information becomes available that may be relevant to his/her willingness to continue participation in the study.

Each subject will be informed that a monitor or a health authority inspector, in accordance with applicable regulatory requirements, may review the portions of their source records and source data related to the study. Data protection and confidentiality will be handled in compliance with local laws.

2. EXECUTIVE SUMMARY

Title
ALCOHOLIC HEPATITIS NETWORK OBSERVATIONAL STUDY

Study Type
Prospective, observational study

Investigational Sites
Indiana University in Indianapolis, IN, University of Louisville affiliated hospitals in Louisville, KY, Beth Israel Deaconess Medical Center (BIDMC) in Boston, MA, Mayo Clinic in Rochester, MN, Cleveland Clinic Foundation, in Cleveland, OH, University of Pittsburgh Medical Center in Pittsburgh, PA, University of Texas at Southwestern in Dallas, TX, and Virginia Commonwealth University in Richmond, VA.

Planned Number of Patients
Approximately 1260 (720 subjects with alcoholic hepatitis (AH), 360 heavy drinkers without significant liver disease, and 180 healthy controls) will be enrolled.

2.1 Objectives

Primary Objective: To collect and store clinical data to facilitate investigations of the epidemiology, diagnosis, pathophysiology, natural history, and treatment of alcoholic hepatitis.

Secondary Objective: To develop a bio-specimen bank comprised of plasma, serum, PBMC, saliva, DNA, urine, stool, liver biopsy and other biological specimens obtained from patients with alcoholic hepatitis, heavy drinkers without clinical liver disease, and healthy subjects to support translational research in the pathophysiology of alcoholic hepatitis.

2.2 Methodology

Screening phase: Subjects will be assessed for the eligibility criteria and a written informed consent will be obtained from the eligible subjects.
Study phase: Subjects will undergo history taking, physical examination, questionnaire administration, and laboratory tests. Biosamples including serum/plasma, peripheral mononuclear cells (PBMC) (at select sites), genomic DNA, stool samples (when available), urine, saliva (until an adequate number of samples are collected from all sites), and liver tissue (when available) will be obtained.
Follow up phase: Alcoholic hepatitis subjects will be followed for 24 weeks in study and then every 24 weeks for a maximum of 5 years, Heavy drinking controls for 24 weeks, and healthy controls subjects for 1 day (at initial meeting for baseline).

2.3 Duration of the study

Up to 5 years

2.4 Biosample Repository

Plasma/serum
Peripheral blood mononuclear cells (PBMC) (collected at select sites)
Genomic DNA
Urine (when available)
Saliva (until an adequate number of samples are collected from all sites)
Liver tissue (when available)
Stool (when available)

3. KEY INCLUSION/EXCLUSION CRITERIA

CASES: Heavy drinkers with alcoholic hepatitis

Inclusion criteria

1. A clinical diagnosis of alcoholic hepatitis as defined by the NIAAA pan-consortia for AH:
   a) Onset of jaundice (defined as serum total bilirubin >3 mg/dL) within the prior 8 weeks to screening visit
   b) Regular consumption of alcohol with an intake of > 40 gm daily or >280gm weekly on average for women and > 60 gm daily or >420gm weekly on average for men for 6 months or more, with less than 8 weeks of abstinence before onset of jaundice
   c) AST > 50 IU/l
   d) AST:ALT > 1.5 and both values < 400 IU/l
   e) and/or histological evidence of AH*
2. Serum total bilirubin >3 mg/dL
3. Subject or guardian ability to understand and willingness to provide written consent
4. Age greater or equal to 21 years
5. Re-enrolment of an alcoholic hepatitis donor is permissible up to 4 times if the donor presents with a new episode of alcoholic hepatitis 24 weeks or longer after the most recent enrolment in the study

Exclusion criteria

1. Liver disease significantly caused by hemochromatosis, autoimmune liver disease, Wilson disease, and acute viral hepatitis (NOTE: The presence of chronic hepatitis C, hepatitis B, HIV, or stage 1 (one lesion <2 cm) HCC is not exclusion to participation)
2. Pregnant or breast feeding
3. Received liver transplant
4. Based on the judgment of the investigator, subject is not capable of understanding or complying with the study requirements

CONTROLS: Heavy drinkers without significant liver disease

Inclusion criteria

1. History of chronic alcohol consumption sufficient to cause liver damage. Generally, this is considered to be >40 g/day or >280g/week on average for women and >60 g/day or >420 g/week on average for men, for 6 months or more, with less than 8 weeks of abstinence. Judgement about chronic alcohol consumption will be made by the site investigator.
2. Subject or guardian ability to understand and willingness to provide written consent
3. Age greater or equal to 21 years

Exclusion criteria

1. Past evidence of alcoholic liver disease, defined as a bilirubin > 2.0 mg/dL, an AST > 1.5 ULN, and any hospital admission for liver disease, or the presence of

esophageal varices or ascites (at any time in the past)
2. Liver disease significantly caused by hemochromatosis, autoimmune liver disease, Wilson disease, NAFLD, and acute viral hepatitis (NOTE: The presence of chronic hepatitis C, hepatitis B, or HIV is not exclusion to participation.)

Individuals with a diagnosis of Gilbert's can have total bilirubin up to 3.0 mg/dL and still be eligible for participation.

3. Alcohol intake at **less than** 40 g/day or 280g/week on average for women and 60 g/day or 420 g/week on average for men for longer than the past 28 days
4. If liver stiffness has been assessed within the prior 90 days, then stiffness suggesting fibrosis of F1 or greater is excluded. For Fibroscan, this is a fibrosis score >7.0 kPa.
5. Received liver transplant
6. Pregnant or breast feeding
7. Any of the following laboratory abnormalities within 90 days prior to signing the consent.
   a) Total bilirubin: >ULN*
   b) INR: > 1.4

Healthy Controls

Inclusion criteria

1. AUDIT-C scores of <4 for men and <3 for women (signifying no alcohol misuse)
2. Abstinent (consumption of less than one standard drink/week) during the 6 months prior to enrolment
3. Ability to understand and willingness to provide written consent.
4. Age greater or equal to 21 years

Exclusion criteria

1. Clinical history or laboratory evidence of liver disease including alcoholic liver disease, NAFLD, hemochromatosis, alcoholic hepatitis, autoimmune liver disease, Wilson disease, hepatitis C, or hepatitis B.
2. Presence of diabetes (requiring treatment with oral agents or insulin).
3. Significant heart disease (prior history of heart disease, other than hypertension)
4. Chronic lung disease (requiring chronic treatment)
5. Immune related conditions (such as Crohn's disease, rheumatoid arthritis, ulcerative colitis, systemic lupus erythematosus, severe psoriasis, etc.)
6. Known infection with HIV
7. Presumed infection, or use of antibiotics or other medications (e.g., corticosteroids) that would affect immune function, within the past 14 days
8. BMI>35
9. Current or known history of cancer (except *in situ* carcinoma of the cervix or adequately treated basal or squamous cell carcinoma of the skin) within 5 years prior to enrollment
10. Received liver transplant

11. Pregnant or breast feeding
12. Any of the following laboratory abnormalities within 90 days prior to signing the consent.
    a. Hemoglobin: <10 g/dL
    b. Conjugated bilirubin: > ULN
    c. INR: > 1.4
    d. AST: >40 IU/mL
    e. ALT: >40 IU/mL
13. Based on the judgment of the investigator, subject is not capable of complying with the study requirements

4. RESEARCH STRATEGY

4.1 Background and Significance

Alcoholic liver disease **(ALD)** is a complex disorder and its pathogenesis is a multi-step and multi-factorial process that progresses through a series of histopathological changes [1]. More than 90% of drinkers develop alcoholic steatosis which is reversible upon abstinence.
However, if alcohol abuse continues, the disease may progress to alcoholic hepatitis **(AH)**, advanced fibrosis, and cirrhosis in up to 10-15% of heavy drinkers [2;3]. It is completely unknown why some heavy drinkers develop AH and what determines the severity of the condition. These questions can only be answered by studying sufficient numbers of patients with AH as well as heavy drinkers at risk for AH, and developing clinical studies testing basic pathophysiological mechanisms.

While alcoholic steatosis is a reversible condition, AH is associated with significant morbidity and mortality [4;5]. In mild cases, patients may recover with conservative medical management and alcohol abstinence. Those with more severe cases of AH; however, have a high mortality rate [6;7]. Using the National Inpatient Sample data, we reported that AH accounted for 0.7% of all inpatient admissions in the United States. The average length of stay (LOS) was 7 days and nearly 7% died during hospitalization. Of importance, hospitalized AH patients result in significant healthcare cost and utilization [8]. The average total charges during hospitalization for AH were $37,769; which was higher than that from acute myocardial infarction (~$16,200), acute cerebrovascular disease (~$11,100), and acute pancreatitis ($9,870). Costs per hospitalization and cost adjusted for LOS were higher in those who died during the hospital stay, presumably from more severe forms of AH ($84,642 and $11,754/day, respectively) [8]. These data were obtained during a period of time when current best therapy (corticosteroids or pentoxifylline) was in broad use [9]; thus, they confirm the disease burden of AH in the US and attest that better understanding of pathogenesis and treatment of AH are urgently needed to improve patients' outcome.

The collection of clinical information and biosamples in subjects with AH are very crucial to pursue research and advance the science in the field of alcoholic liver disease. The current protocol provides a system to uniformly obtain demographics, clinical information, and biological samples from AH subjects and well-matched controls with heavy alcohol use without clinical presentation of AH and heathy controls for future investigations. In addition, they will serve as the potential subjects to be enrolled in the future clinical trials of the AlcHepNet consortium.

4.2 Study Objectives

Primary Objectives: To collect and store clinical data to facilitate investigations of the epidemiology, diagnosis, pathophysiology, natural history, and treatment of alcoholic hepatitis.

Secondary Objectives: To develop a bio-specimen bank comprised of plasma, serum, PBMC, saliva, stool, urine, liver biopsy, DNA, and other biological specimens obtained from patients with alcoholic hepatitis, heavy drinkers without clinical liver disease, and healthy subjects to support translational research in the pathophysiology of alcoholic hepatitis.

4.3 Research and study procedures

4.3.1 Target enrollment:

90 patients with AH, 45 Heavy drinkers without significant liver disease and 22-23 healthy controls will be enrolled at each site. They will be followed for 24 weeks following their enrollment. The cohort will be characterized demographically, anthropometrically, clinically, through laboratory tests, and histologically wherever applicable.

4.3.2 Definitions*:*

Subjects with AH: We plan to enroll 720 patient with AH. The diagnosis of AH will be established on published criteria [6;10] and this is based on history of heavy alcohol consumption (defined as >40 g/day or >280g/week on average for women and >60 g/day or >420 g/week on average for men for 6 months or more, with less than 8 weeks of abstinence before onset of jaundice), clinical evaluation and appropriate laboratory testing (as defined as total bilirubin > 3 mg/dL and AST > 40 U/L). When the diagnosis of AH remains in question, a liver biopsy (if clinically feasible and subject has no contra-indications) will be required. A sizable proportion of individuals with AH have co-existing viral hepatitis or HIV, and there is an unmet need in terms of understanding the nature of their liver disease and to develop suitable therapies. Therefore, we plan to enroll patients with AH in special population infected with chronic HBV, chronic HCV, or HIV.

Heavily Drinking Controls: We plan to enroll 360 heavy drinking controls without significant evidence of liver disease at the time of recruitment. For this application, heavy alcohol drinking will be defined as >40 g/day or >280g/week on average for women and >60 g/day or >420 g/week on average for men for 6 months or more, with less than 8 weeks of abstinence before onset of jaundice. Heavy drinkers, who have just become abstinent within prior 2 weeks, including those we convince to seek alcohol abuse/addiction treatment as part of the recruiting process, are eligible for enrollment. The study team (investigators-coordinators) will emphasize to both

alcoholic hepatitis subjects and heavy drinking subjects the importance of abstinence from alcohol and encourage them to seek an alcohol abuse/addiction treatment. We envision that some of these subjects will maintain sobriety and can serve to demonstrate the results of withdrawal from alcohol; some will relapse to drinking, and resemble non-treatment seeking heavy drinkers over 24 weeks. Control subjects must meet the following criteria: (INR < 1.4 and total bilirubin levels must ≤ULN (If bilirubin is increased due to a suspected Gilbert's Syndrome, patient may be still be enrolled; (2) no prior history of known alcoholic liver disease; and (3) absence of hepatosplenomegaly (from physical examination or radiographic imaging) or stigmata of liver disease.

Healthy Controls: Approximately 180 healthy donors will be recruited to serve as a comparison group.

4.3.3 Recruitment of Subjects with AH:

Subjects with AH will be site specific.

4.3.3 Recruitment of controls:

Heavy drinking controls and Healthy Controls will be site specific.

4.3.4 Matching strategies

We will perform approximate frequency matching for recruiting control subjects using age, gender and race. The purpose of frequency matching is to ensure similar enough distributions between case and control groups to allow for appropriate statistical adjustment in the analyses. For every 30 heavy drinking controls the Data Coordinating Center (DCC) will evaluate the distribution of age (categories: 20-40, 41-60, 61-80, >80), gender, race (whites, blacks, others) overall and at each site. We will then compare that to the distributions of already recruited cases to determine if the distributions in age, gender, and race are similar. If the controls differ markedly, the DCC will instruct the coordinators to concentrate on recruiting the underrepresented groups in the control cohorts. The distribution of matching variables in the case and control groups will be re-examined periodically (approximately every 30 heavy drinking controls) and the control subjects will be recruited based on the updated distributions of demographic variables, as needed.

4.3.5 Variables and Data to be collected:

We will collect the following clinical details from subjects with AH and their controls (as appropriate). The detailed data collection and the questionnaires that we will use are outlined in the case report form (CRF).

a) Demographics and anthropometric measures: These variables characterize the study cohort.
b) Medical History: Detailed past medical history including prior history of AH. Specific

attention will be paid to collecting family history of alcohol abuse and alcoholic liver disease and history of bariatric surgery.

c) Medicinal history: A medication review of prescription and over the counter medications will be completed
d) Detailed alcohol consumption history: We will use AUDIT, AUDIT-C and Time Line Follow-Back Questionnaires (TLFB) for quantifying alcohol consumption [11;12].
e) Quality of life: We will use the Chronic Liver Disease Questionnaire (CLDQ) for quantifying quality of life.
f) Signs and symptoms associated with liver disease and routine laboratories. These include the presence of ascites, jaundice, varices, and hepatic encephalopathy. Additionally, the subject's HBV, HCV and HIV status will be recorded, if available.
g) Discriminant factor (DF), Model for End Stage Liver disease (MELD) score, and Child- Pugh score will be calculated [7].

5. STUDY PROCEDURES

The study consists of two parts, a baseline phase and a follow-up phase.

5.1 Baseline Phase

All subjects:
Patients will sign the consent and data will be collected on demographics, ethnicity, clinical characteristics, alcohol history, anthropometric measures, biosamples (Plasma/serum, peripheral blood mononuclear cells [at select sites], genomic DNA, urine [when available], stool [when available], saliva and liver tissue [where available] will be collected and stored).

5.2 Follow up visits

Alcoholic hepatitis cases: will be seen at (week 4), (week 12), (week 24). During these follow-up visits, data on clinical characteristics, alcohol use, and biosamples will be obtained. If a patient misses a follow-up visit, the patient will be called and the data will be collected from their medical records (if available). Cases will then be followed until the end of study after their final visit (week 24) by checking their medical record. Vitality status will also be checked via National Death Index to ensure survival status could be collected for subjects who could not be followed through medical record review.

Heavy drinking controls without significant liver disease: The second part consists of a follow-up visit during week 24, data on clinical characteristics, alcohol use, and biosamples will be obtained.

Healthy Controls: There will be no follow-up visits

Table 2. Study procedures during the baseline and follow up visit.

Procedures	Baseline[1]	Week 4 (D15 - D56)	Week 12 (D57 - D 126)	Week 24 (D127 - D 252)	Every 24 weeks for a maximum of 5 years
Source of data	**Patient visit & medical records**	**Patient visit, medical records ±phone call**	**Patient visit, medical records ±phone call**	**Patient visit, medical records ±phone call**	**Medical Records**
Group	**AH, Drinking Controls, and Healthy Controls**	**AH**	**AH**	**AH and Drinking Controls**	**AH**
Informed Consent	X				
Demographics	X				
Anthropometric measurements	X	X	X	X	
Vital Signs	X	X	X	X	
Medical history	X				
Alcohol use History	X	X	X	X	
Questionnaires[3]	X	X	X	X	
Con-Med Review	X	X	X	X	
Events of Special Interest[5]		X	X	X	
General PE	X				
PE (liver-related)		X	X	X	
Blood tests and other procedures					
Hepatic Function	X	X	X	X	
Basic metabolic panel	X	X	X	X	
Hematology/CBC	X	X	X	X	
Coagulation/INR	X	X	X	X	
Infection screen[2]	X				
Imaging/X-ray[2]	X				
Endoscopy[2]	X				
Fibroscan or other[4]	X			X	
Pregnancy Test, Urine	X	X	X	X	
Research Specimen Collection+					
Plasma	X	X	X	X	
Serum	X	X	X	X	
Stool (when available)	X	X	X	X	
Saliva[6]	X		X		
Urine (when available)	X	X	X	X	
Liver Biopsy (when available)	X	X	X	X	
PBMC (optional by site)	X	X	X	X	
DNA (if patient consents)	X				
Survival, from medical records, phone call or death indices					X

[1]For patients with AH, data obtained prior to start of treatment for AH. If patient did not receive specific treatment for AH, then data obtained as early as possible after admission to hospital.
[2]Data will be recorded only if it has been obtained as standard of care.
[3] Questionnaires include timeline follow back (TLFB), AUDIT- C, AUDIT, and Chronic Liver Disease Questionnaire. TLFB will be the only questionnaire done at follow-up visits
[4]Assessment of liver fibrosis includes Fibroscan, ARFI, MRE and others, and will be recorded if they were performed as SOC.

[5] Events of special interest include complications of liver disease, such as variceal bleeding, new/worsening hepatic encephalopathy, acute kidney injury, new/worsening ascites, and infections.
[6] Saliva will only be collected until an adequate number of samples are collected from all sites.
+ Specimen collection at baseline can be collected and processed 24-48 hours after enrollment

5.2.1 Baseline Evaluations:

Prospectively enrolled patients will undergo baseline **evaluations**. The below procedures/evaluations will be performed during the baseline phase:

- Obtain Consent
- Verify Inclusion/Exclusion Criteria
- Obtain Background Information
  - Patient Profile (age, gender, race, ethnicity, etc.)
  - Complications of Liver Disease
  - Physical Examination
  - Medication Review
  - Family history of alcohol use/ complications
  - Liver Biopsy History
  - History of alcoholism counselling/treatment
- Anthropometric Measurements
- Vitals
- Timeline Follow back
- AUDIT/AUDIT-C
- Chronic Liver Disease Questionnaire
- Collect all samples below as outline in Table 2
- Collection of 60 mls of blood for research (Plasma/Serum and peripheral blood mononuclear cells [at select sites])
- Collection of 15 mls of blood for standard biochemistry tests (CBC, Platelet count, INR and CMP) if not already completed as standard of care.
- Collection of saliva
- Collection of a urine sample (when available)
- Collection of stool sample (when available)
- Collection of liver tissue (when available)
- Perform a Laboratory Examination All Subjects:

An examination of the medical record for laboratory results of the following tests will be conducted. If the laboratory tests have been completed as standard of care within the timeframe specified, those results will be used. If laboratory results are not available, these tests will be completed as part of the baseline phase.

Within 30 days of visit:
- Complete Blood Count (CBC)
- Hepatic Function
- International Normalized ratio (INR)
- Basic metabolic panel (BMP)

Optional tests: (data collected only if completed as standard of care within the timeframe specified):
- Infection screen
- Imaging/X-ray

- Endoscopy
- Fibroscan

5.2.2 Follow-up visits evaluations:

Alcoholic hepatitis subjects and heavy drinkers without clinical liver disease controls will also be seen for a follow-up visit at week 4, 12, and 24 from the date of their "baseline visit". At the follow-up visit the below procedures/evaluations will be performed.

- Physical Examination
- Medication Review
- History of alcoholism counselling/treatment
- Review of complications of liver disease
- Laboratory examination
- Anthropometric Measurements
- Vitals
- Timeline Follow back
- Collect all samples below as outlined in Table 2
- Collection of 60 mls of blood for research(Plasma/Serum and peripheral blood mononuclear cells [at select sites])
- Collection of 15 mls of blood for standard biochemistry tests (CBC, Platelet count, INR and CMP) if not already completed as standard of care.
- Collection of saliva
- Collection of a urine sample (when available)
- Collection of stool sample (when available)
- Collection of liver tissue (when available)
- Perform a Laboratory Examination <u>All Subjects:</u>

An examination of the medical record for laboratory results of the following tests will be conducted. If the laboratory tests have been completed as standard of care within 30 days of the visit, those results will be used. If laboratory results are not available, these tests will be completed as part of the follow up visit.

- Complete Blood Count (CBC)
- Hepatic function
- International Normalized ratio (INR)
- Basic metabolic panel (BMP)

<u>Optional tests</u>: (data collected only if completed as standard of care within the timeframe specified):

- Infection screen
- Imaging/X-ray
- Endoscopy
- Fibroscan

5.2.3 Exemptions to the Eligibility Criteria:

Exemptions may be allowed on a case by case basis for individuals narrowly missing the eligibility criteria. Exemptions may be sought either for cases or controls. Requests for exemptions should be initiated by the clinical center PI using an Exemptions Form which explains the rationale for seeking the exemption. Each request for the exemption will be reviewed by two investigators not associated with the site requesting the exemption. An exemption will be granted only if both reviewers agree to allow the exemption.

1. MANAGEMENT AND STORAGE OF BIOSAMPLES:

Plasma/serum, peripheral blood mononuclear cells, genomic DNA, saliva, urine (when available), stool samples (when available), and liver tissue (where available) will be collected at individual sites and shipped on a quarterly basis to be stored at the AH Tissue Bank at the University of Massachusetts until they are needed for translational studies, or the study is complete.

2. PARTICIPANT COMPENSATION

Participant compensation is site specific.

3. PARTICIPANT RECRUITMENT

Avenues of recruitment will be site specific for example: website, flyers, social media, craigslist, studykik, newspaper, and newsletters.

4. DEVELOPMENT AND MANAGEMENT OF DATA COLLECTION

The data coordinating unit will be operated under the auspices of the Indiana CTSI Dr. Barry Katz from the Department of Biostatistics, with an MS level biostatistician and an experienced data manager will run the data coordinating unit. The data coordinating unit will work with the steering committee and other study personnel, and will be responsible for establishing and/or conducting the following:

- Configure and set up the prospective, multicenter, observational study of patients with well characterized AH and suitable controls, within the CTMS/EDC as well as each of the smaller studies to be carried out by the consortium;
- Design specific case report forms and set up patient calendar and schedule;
- Review and test the system for the large prospective observational study and each of the smaller studies configured within the CTMS/EDC;
- Define and develop Standard Operating Procedures (SOPs) such as a process for paper- based or electronic data capture including instructions for data entry for participating sites, quality control procedures, data dictionary standards, reporting AEs/SAEs, etc.;
- Develop and implement study-specific quality assurance procedures to ensure data integrity;
- Write a data monitoring plan for the observational study and the randomized studies;

- Establish a process to facilitate/conduct audits for participating sites; audits will be done electronically and/or in person at the site, as needed;
- Provide appropriate training and/or educational material to project team members on specific aspects of each clinical study including data management and statistics;
- Prepare an implementation ("go live") plan for each study and facilitate this plan;
- In collaboration with the site study coordinators, provide assistance in the oversight of screening, enrollment, randomization, and data collection; this includes monitoring patient recruitment, compliance with visit schedules and retention.
- Coordinate periodic protocol review visits to sites on as needed basis;
- Monitor of study sites to ensure accurate execution of protocols;
- Prepare and distribute data reports to summarize performance of participating sites;
- Prepare data monitoring, performance and safety reports for interim monitoring by the steering committee, DSMB, or governmental agency;
- Submit DSMB reports of adverse events to IRBs;
- Perform final data audits and any necessary edits and prepare files for analysis;
- Coordinate data analysis with study and/or site deadlines;
- Perform appropriate statistical analyses and provide overall coordination for preparing the analysis and results needed for submitting abstracts and manuscripts
- Implement plans for final disposition of study data
- Monitor for adherence to agreed-upon patient close-out procedures

10. STATISTICAL ANALYSIS

Subjects' demographics, anthropometric measurements, medical history, alcohol consumption, lab test results, MELD score, quality of life, and other variables will be summarized by groups (case vs. control groups) and by visits. Mean and 95% confidence intervals will be calculated for continuous variables for each group. Proportion and frequency will be used to describe the categorical variables by groups. Two-sample t-tests will be used to compare the continuous variables and Chi-square tests will be used to compare the categorical variables by groups at each visit. Appropriate nonparametric test will be used if there is normal assumption violation. All the tests will be performed using SAS 9.3(Cary, NC).

More importantly, this aim serves as the backbone for conducting future clinical, mechanistic, biomarker discovery, therapeutic, and natural history studies. After a

structured process of requesting ancillary study proposals from consortium investigators, submitted proposals will be assessed for feasibility and scientific merit. For studies selected, a formal statistical plan will be developed that will include statistical models and methods to be used to address each specific aim of the study. Methods may be simple parametric or nonparametric methods for two-group comparisons such at t-tests or rank tests or may involve complex multivariable methods such as linear, logistic, or Poisson regression modeling. Each proposal must specify the primary and secondary outcomes. Sample size calculations associated with the primary analysis must be given, including clear specification of the outcome measure, error rates (Type I / II), minimal clinical important difference, and statistical power of at least 0.8. The expect amount and methods for dealing with missing data must be specified. Questions requiring longitudinal data analyses should apply statistical methods that account for, as applicable, time to events, repeated measurements, counts, or other discrete measurements. For time to event data, Cox proportional hazards regression model with appropriate covariates will be used. For hypotheses involving repeated measurements or discrete events, we will use either generalized linear models with generalized estimating equations or multilevel generalized linear mixed models with random coefficients.

11. REPORTING OF ADVERSE EVENTS

Reporting of adverse events, unanticipated problems involving risks to participants or others, and noncompliance will be reported to the Data Monitoring and Safety Board (DSMB), Western Institutional Review Board (IRB) and institutional IRBs when applicable per its Standard Operating Procedures.

12. LENGTH OF STUDY

We anticipate that we can accomplish the target enrollment within the 5 years of funding cycle.