

A Patient Registry Characterizing the Natural History and Long-term Effects on Morbidity and Mortality in Patients Diagnosed with Metabolic Syndrome or Nonalcoholic Fatty Liver Disease

Protocol Date: April 27, 2020

**Sponsor:
Arizona Liver Health**

INVESTIGATOR SIGNATURE

By signing this protocol, the investigator agrees to conduct the study in accordance with the protocol, generally accepted International Conference of Harmonization standards of Good Clinical Practice, as well as all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical study. In addition, where applicable, the investigator agrees to provide the sponsor with accurate financial information to allow the sponsor to submit complete and accurate certification and disclosure statements as required by FDA regulations.

I agree to abide by all provisions set forth in protocol 2020-ALH-NAFLD-0.1.

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Signature

Date

1. INTRODUCTION/RATIONALE

The incidence of obesity in the US population has dramatically increased over the past several decades. The term “metabolic syndrome” (MetS) has been used to describe the suite of conditions that, in addition to obesity as determined by BMI and visceral adiposity, contribute to increased risk of cardiovascular and liver disease in one’s lifetime.

Based on the guidelines from the National Heart, Lung and Blood Institute (NHLBI) and the American Heart Association (AHA), the following five conditions are metabolic risk factors. An individual must have at least 3 of the 5 risk factors to be diagnosed with metabolic syndrome.

- Serum triglycerides: >150 mg/dL
- HDL cholesterol: <40 mg/dL in men or <50 mg/dL in women
- Blood pressure: >130/85
- Fasting blood glucose: >100 mg/dL
- Abdominal obesity: waist circumference >102 cm (40 in) for men and >88 cm (35 in) for women. For Asian Americans, the cutoff values are >90 cm (35 in) for men and >80 cm (32 in) for women.

Although nonalcoholic fatty liver disease (NAFLD) is not one of the defining criteria for metabolic syndrome, it is closely associated with obesity and a common hepatic manifestation. NAFLD includes a spectrum of histological findings ranging from hepatic steatosis to nonalcoholic steatohepatitis (NASH). In addition to being a highly common condition affecting close to 70 million Americans, several lines of evidence suggest that NAFLD, and in particular NASH, is associated with significant liver-related morbidity and mortality. Moreover, the presence of NAFLD has been proposed as a key indicator of metabolic status and NAFLD has been shown to be a good predictor for development of metabolic syndrome, and diabetes.

2. STUDY OBJECTIVES

2.1 Primary Objectives

The primary objective of this study is to:

- Characterize the natural progression of metabolic risk factors and sequela, including non-alcoholic fatty liver disease and other comorbid and secondary conditions, in children and adults. The impact of diet, exercise, weight loss medications, and/or bariatric/endoscopic weight loss procedures will be evaluated over a 10-year period.

2.2 Secondary Objectives

The secondary objectives (will be assessed where data is available based on standard of care procedures) of this study are to characterize the degree of progression of liver disease, weight change, liver cancer and all cancers over time.

The impact of patient baseline characteristics and modifiable risk factors including use of alcohol, tobacco and/or marijuana use will be analyzed to measure any impact on long-term patient outcomes. Impact on cost of therapy with successful management of MetS/ NAFLD risk factors

Detailed analyses of demographics, concomitant medications, metabolic and cardiovascular comorbidities, staging of liver disease and outcomes for patients with metabolic syndrome will be performed.

Additionally, the economic impact of MetS/ NAFLD will be analyzed by including:

- Impact of cost of therapy with successful management of MetS/ NAFLD risk factors
- Assessment of time missed from work due to MetS/ NAFLD
- Assessment of the impact of MetS/ NAFLD on employment potential and the need for disability

3. STUDY METHODOLOGY/ DESIGN

This is a non-interventional registry in which medical data from patient's standard of care visits is recorded in an electronic database over time. No protocol mandated tests or procedures will be performed.

Patients who are enrolled in this registry will also be invited to participate in the Biorepository Specimen Bank. Blood samples and a liver biopsy sample (if available) will be collected on a voluntary basis and lack of participation in that project will not impact participation in the main registry. These samples will be stored in a central repository for future analyses and will only be identified by a unique identifier.

3.2. Study Population Inclusion/Exclusion Criteria:

Inclusion Criteria:

1. Age \geq 10 years of age.
 2. Diagnosed with metabolic syndrome or NAFLD
- >16 years of age the diagnosis of MetS will be based on the documentation of at least 3 of the 5 conditions:
- Serum triglycerides: >150 mg/dL or on treatment for dyslipidemia
 - HDL cholesterol: <40 mg/dL in men or <50 mg/dL in women

- Blood pressure: >130/85 or on treatment for hypertension
 - Fasting blood glucose: >100 mg/dL or on treatment for diabetes
 - Abdominal obesity: Waist circumference >102 cm (40 in) for men and >88 cm (35 in) for women. For Asian Americans, the cutoff values are >90 cm (35 in) for men and >80 cm (32 in) for women.
- 10-15 years of age the diagnosis of MetS will be based on the documentation of abdominal obesity and at least 2 of the other conditions:
- Abdominal obesity defined as waist circumference > 90% for age and gender.
 - Serum triglycerides: >150 mg/dL or on treatment for dyslipidemia
 - HDL cholesterol: <50 mg/dL
 - Blood pressure: > 90% for age or on treatment for hypertension
 - Fasting blood glucose: >100 mg/dL or on treatment for diabetes

3. Informed Consent

- For those 18 years of age and above, ability to read and understand English or Spanish and willingness to sign Informed Consent.
- For those 10-17 years of age, ability of a parent/guardian to read and understand English or Spanish and willingness to sign Informed Consent.

Exclusion Criteria:

1. Any patient with a life expectancy <2 years
2. Any patient with an active malignancy requiring chemotherapy

4. STUDY PROCEDURES

Patients seen in an outpatient setting will be followed as standard of care, with appointments set based on patient's medical requirements. Data collection will occur from standard chart information, including laboratory, imaging and clinical progress notes.

The investigator will make certain that an informed consent is in place to ensure that patients are fully informed about the collection of their health information.

The investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of consented patients are addressed.

5. DATA COLLECTION

The following will be collected when available from standard of care evaluations:

- Patient demographics and baseline characteristics including
 - Baseline only: date of birth, gender, race, ethnicity
 - Throughout the study: body mass index (BMI), waist circumference, blood pressure
- Social behavior practices (baseline and throughout the study)
 - Alcohol, marijuana and tobacco use
 - Number of meals eaten out (etc., fast food, take out, dine in restaurants)/ Exercise and activity level
- Laboratory and imaging results relating to the comorbid conditions (baseline and throughout the study when available):
 - AST, ALT, BILI, ALP, GGT, ALB, PLT, HGB, WBC, SCR, total protein
 - CHOL, TG, HDL, LDL, VLDL
 - Glucose, Insulin, HbA1C
 - HepA (total Ab), HepB (surface Ab), HepC (Ab), HIV antibody, transferrin saturation, ceruloplasmin, ferritin, iron, ANA, SMA
- Comorbid conditions (baseline and throughout the study)
 - Metabolic syndrome, HOMA-IR, IR/pre-diabetes, diabetes, hypertriglyceridemia, low HDL, hypertension, coronary artery disease, asthma, obstructive sleep apnea (OSA)
 - Fibromyalgia, psychiatric disorders (eg, anxiety, depression, other)
- Concomitant medications for the following conditions (includes dose and frequency)
 - Dyslipidemia
 - Diabetes
 - Hypertension
 - Vitamin E
 - Weight loss meds
 - Asthma meds
 - Depression
 - Anxiety
 - Herbal/OTC meds for weight loss or control of comorbid conditions
 - Other relevant concomitant medications
- Lifestyle modifications for weight loss/metabolic syndrome
 - Physical exertion (exercise program)
 - Diet (if patient is managed by a dietitian, the type of diet the patient is assigned)
 - Work/school missed due to MetS complications

- Procedures/interventions relating to the comorbid conditions (when applicable and data available)
 - CPAP (for OSA treatment)
 - Bariatric surgery (type of procedure and date)
 - Endoscopic surgery for weight loss (type of procedure and date)
 - Other procedures/interventions such as cardiac angioplasty relevant to MetS (type of procedure and date)
 - Transient elastography/other non-interventional measurements for fatty liver disease (type of test, probe used, LSM by TE, CAP score (dB) and IQR/M (%))
 - Liver biopsy (date, path-NAFLD, fibrosis, lobular inflammation, portal inflammation, steatosis, ballooning, NAS).
- Death (if applicable): date and cause of death
- OPTIONAL (Separate consent): Blood samples and liver biopsy sample (if available) to store for future analyses (see Appendix I for procedures)
 - Specimen type (serum, plasma, buffy coat, liver biopsy)
 - Date of sample collection

Since this is a long-term observational study, data will be collected during 6-month windows in order to capture any change in medical conditions during that time.

All information will be identified using a limited data set and will be reported in aggregate.

6. DATA EVALUATION AND PUBLICATION

6.1. Sample Size/ Statistical Consideration

The study is not powered since it is an observational registry with no comparative arm. Interim analyses will be performed on a regular basis; however, these analyses will have no impact on the power of the study.

6.2. Primary Endpoint

- Rate of natural progression of metabolic risk factors, including NAFLD and other comorbid and secondary conditions, in children (10 years and older) and adults.

6.3. Secondary Endpoint

- Rate of progression of liver disease over time
- Rate of weight change over time
- Rate of liver cancer over time

- Rate of all cancers over time
- Change in cost of therapy with successful management of MetS risk factors
- Change in time missed from work due to MetS
- Change in employment potential and the need for disability due to MetS

The impact of patient baseline characteristics and modifiable risk factors including use of alcohol, tobacco and/or marijuana use will be analyzed to measure any impact on long-term patient outcomes.

Detailed analyses of demographics, concomitant medications, metabolic and cardiovascular comorbidities, staging of liver disease and outcomes for patients with MetS/NAFLD will be performed.

All endpoints will be analyzed for subpopulations such as age, ethnicity and gender as well as presence/absence of each independent feature of MetS.

Additionally, impact of diet, exercise, weight loss medication use and/or bariatric/endoscopic weight loss surgery will also be assessed.

6.5. Definition of analysis population

All patients who meet the inclusion criteria and none of the exclusion criteria will be included in the primary analysis.

Additional analyses will be conducted, including but not limited to, comparing results based on different baseline characteristics as well as site/regional differences.

6.6. Publication

The results of this study will be presented at a major medical conference and/or published in a peer-reviewed journal.

7. ADHERENCE TO ETHICAL, REGULATORY, AND ADMINISTRATIVE CONSIDERATIONS

The study must be conducted in accordance with Good Clinical Practice (GCP) as outlined in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines, E6 Good Clinical Practice: Consolidated Guidance and other applicable laws and regulations. In addition, the study must be conducted in accordance with the US Code of Federal Regulations (CFR).

7.1. Ethical Conduct of the Study

7.1.1 Independent Ethics Committee or Institutional Review Board

Prior to initiation of the study at any site, an appropriate Institutional Review Board (IRB) must approve the study, including the protocol, informed consent, and other study documents. The IRB must be constituted according to applicable regulatory requirements. As appropriate, amendments to the protocol must also be approved by the IRBs before implementation at the sites. The IRB approval should be obtained in writing, clearly identifying the study, the documents reviewed (including the informed consent), and the date of the review. The study as described in the protocol (or amendment), informed consent, and other study documentation may be implemented only after all the necessary approvals have been obtained and the Sponsor has confirmed that it is acceptable for the investigator to do so. In the event that the IRB requires changes in the protocol, the Sponsor shall be advised and must approve the changes prior to implementation. The investigator shall not modify the study described in the protocol once finalized and after approval by the IRB without the prior written approval of Sponsor.

7.1.2 Patient Information and Authorization Form

The details of the protocol must be discussed with each potential patient, and a written informed consent must be obtained for all patients before any health information is collected. In obtaining written authorization, the information must be provided in language and terms understandable to the patient. The patient, or the patient's legal representative, must give their written authorization to participate in the study. The investigator must retain the signed and dated authorization form itself as part of the study records. A copy of the signed and dated authorization form must be given to the patient. Any subsequent changes to the approved informed consent must be reviewed and approved by the appropriate IRB and Sponsor before implementation.

7.1.3 Reporting to Sponsor

The investigator will ensure that there are sufficient time, staff, and facilities available for the duration of the study to conduct the study as described in the protocol and according to all applicable guidance, laws, and regulations. The investigator must maintain records and data during the study in compliance with all applicable legal and regulatory requirements. The investigator is to provide patient data on completed CRFs. CRFs and the electronic database from the study are the exclusive property of the Sponsor. A CRF must be completed for all patients who have given informed consent. All entries into the eCRFs are the responsibility of the investigator and must be completed by the investigator or a qualified designee. The investigator will acknowledge via an electronic signature that he/she has verified the accuracy of the recorded data.

APPENDIX I.

Sample Collection, Labeling and Storage for Biobanking (Applicable if separate consent signed)

Serum Samples

- Collect 1 x 10 mL of blood into a red capped tube and properly label with subject identification number.
- Allow blood to clot at room temperature for 30-45 minutes before proceeding to centrifugation step. Samples should be processed within 2 hours of collection.
- Centrifuge for 15-20 minutes at 2000 RPM at 4°C.
- Carefully remove the tube from the centrifuge. The serum is the top layer.
- Immediately after spinning the tube:
 - Remove the serum layer.
 - Aliquot 100 uL serum into each of 6 plastic screw-cap cryovials.
- Each cryovial tube (not cap) must be labeled with type of sample (serum), subject identification number. Permanent marker must be used.
- Freeze at -80°C (-20°C for short term until samples can be transferred to -80°C freezer).

Plasma and Buffy Coat Samples

- Collect 2 x 10 mL of blood into purple capped EDTA tube and properly label each tube with subject identification number.
- Store at 4°C prior to processing. Samples should be processed within 2 hours of collection.
- Centrifuge for 15-20 minutes at 2200-2500 RPM at 4°C.
- Carefully remove the tubes from the centrifuge. The plasma is the top layer and the buffy coat is the layer between the plasma and red blood cells.
- Immediately after spinning the 2 tubes:
 - Remove the plasma layer without disturbing the buffy coat layer.
 - Aliquot 100 uL plasma into each of 6 plastic screw-cap cryovials (will only need plasma from one purple capped tube).
 - Aliquot 150 uL buffy coat into each of 6 plastic screw-cap cryovials (will need to recover buffy coat sample from both purple capped tubes to harvest 6 x 150 uL cryovials).
- Each cryovial tube (not cap) must be labeled with type of sample (plasma, buffy coat), subject identification number. Permanent marker must be used.
- Freeze at -80°C (-20°C for short term until samples can be transferred to -80°C freezer).

Liver Tissue

1. In patients that will have a liver biopsy done as part of their routine clinical evaluation, a paraffin-embedded liver tissue sample will be labeled with the subject identification number.
2. Samples are for research purposes and will be clearly identified.