FATTY LIVER FOUNDATION

SUNN STUDY

Screening for Undiagnosed NAFLD and NASH

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FibroScan screening, using a mobile device, of an at risk population to measure the liver status in asymptomatic people with no diagnosis of fibrotic liver disease

IRB submittal flf-screen 1   date 9/27/2018

Hypothesis

This study is foundational to the screening strategy proposed so it seeks to define data points from which future programs can be developed.

The hypothesis of this study is that a patient subset, unknowingly at risk for asymptomatic liver disease, can be drawn from patients with other chronic diseases. Those patients can be educated and motivated to self-select and engage in screening for liver disease. Post testing, they will be willing to state their perception of what the value of the test is to them.

That population will yield a subset with advancing but asymptomatic disease. Within that sub-population a group will exist who will proceed to diagnosis and be willing to learn about clinical trials. By measuring this response the effectiveness of the messaging about engaging diagnosis and follow up can be shown. There is not comparable data so this will establish a baseline.

A separate track will be to measure the number of participants who enter the diet and lifestyle education programs. Measuring this response will also establish a baseline measure for the effectiveness of this messaging and inform future efforts.

Overview

Research has shown that there is a high incidence of undiagnosed fibrosis within comorbid populations as a result of the frequently asymptomatic course of NASH

development.   (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4542082/   )
Liver disease is a dangerous and expensive problem to manage medically with the cost of NAFLD estimated to exceed $32 billion per year.
(https://www.sciencedaily.com/releases/2018/07/180703105956.htm)

Liver disease in the form of Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are rapidly increasing population health threats driven primarily by diet and lifestyle. Fibrotic liver disease, culminating in cirrhosis, is frequently asymptomatic so it is common for a patient to first learn of what is a life threatening condition by being told that they have cirrhosis. Management and treatment of cirrhosis is complex and very costly with few treatment options and the only current therapy being a very expensive transplant for end stage liver disease.

The position of the Fatty Liver Foundation is that this problem is magnified by the current American Association for the Study of Liver Disease, (AASLD), practice guidelines which do not advise routine screening for NAFLD. This is the policy:

> Excerpts from the AASLD Practice Guidelines 2017:
> > (AASLD Diagnosis Mgt NAFLD Chalasani et al 2017 Hepatology)

- It can be argued that there should be systematic screening for nonalcoholic fatty liver disease, (NAFLD), at least among higher-risk individuals attending diabetes and obesity clinics.
- Routine Screening for NAFLD in high-risk groups attending primary care, diabetes, or obesity clinics is not advised at this time because of uncertainties surrounding diagnostic tests and treatment options, along with lack of knowledge related to long-term benefits and costeffectiveness of screening.

The issue of screening is complex as the result of a variety of competing needs and forces within society broadly and healthcare specifically. Powerful entrenched societal practices and counterproductive aspects of human nature are producing a health crisis with serious generational impact. Technology is moving at ever increasing speed and our systems, political and healthcare, are ill prepared to respond even given the best of intentions. The result is increased morbidity, cost, and stress.

Research has established the health risks associated with liver disease and comorbidity with a wide range of other illnesses. (Comorbidities and Metabolic Derangement of NAFLD
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4608226/   )   FibroScan has been shown to be a useful test to identify asymptomatic liver disease and is

recommended by the European Association for the Study of the Liver, (EASL) in the EASL CLINICAL PRACTICE GUIDELINES,
(http://www.easl.eu/research/our-contributions/clinical-practice-guidelines/detail/non-invasive-tests-for-evaluation-of-liver-disease-severity-andprognosis/report/4)

but it has not yet been recommended by AASLD.

The FibroScan device (Echosens) works by measuring shear wave velocity. The FibroScan device is cleared by the FDA using this technique (see attached 510(k) K123806). In the test, a 50-MHz wave is passed into the liver from a small transducer on the end of an ultrasound probe. The probe also has a transducer on the end that can measure the velocity of the shear wave (in meters per second) as this wave passes through the liver. The shear wave velocity can then be converted into liver stiffness, which is expressed in kilopascals. Essentially, the technology measures the velocity of the sound wave passing through the liver and then converts that measurement into a liver stiffness measurement; the entire process is often referred to as liver ultrasonographic elastography. The procedure is non-invasive and presents no bio-chemical hazards as it uses only well understood ultrasound technology.

FibroScan is available and has been shown to detect advancing levels of fibrosis, and it is clear that nonalcoholic fatty liver disease is increasing in prevalence throughout the western and developing worlds. Advancing liver disease leads to fibrosis, cirrhosis, and early death in a substantial portion of the population who could be helped with earlier lifestyle and medical interventions. Vibrationcontrolled transient elastography (VCTE) is a point-of-service non-invasive technology that measures tissue elasticity and steatosis and can identify individuals at risk for disease progression and may therefore serve as an effective screening tool to alter the natural history of this disease and improve outcomes.

Changing a foundational strategy, such as responding to symptoms of liver disease, which is the current recommendation, to one of proactively intervening by screening for asymptomatic disease carries a large cost burden and is difficult to accomplish.

Objectives
The goal of this screening study is to gather FibroScan data from the at-risk but undiagnosed population on a self-selection basis so that perceived incidence rates can be calculated and the economics of a single function screening service can be evaluated.

The objective is to develop evidence based information to determine if noninvasive screening of at risk, comorbid people is feasible with this modality.

Furthermore, a secondary objective is to educate all participants about liver disease, lifestyle, healthy diets, and the role of clinical trials as part of the Foundation's larger mission of being patient advocates.

The value and necessity of clinical trials for the development of effective therapies is not well understood by the public. A goal of the foundation is to provide information about the clinical trial process to screening participants. The information is of a general nature (examples are attached). No specific trials will be mentioned. The participants will also be offered the opportunity to receive additional general education about trials and provided with links to systems in which they can explore available trials should they wish to do so. That information is of a general nature and no specific trials will be promoted. Participation in the screening will not automatically enroll them in further education as that decision is post testing and opt-in.

Questions Posed by this Study:

- What population demographics justify routine screening on a cost benefit basis?
- The de-identified, but self-described, chronic ailments will be used with the scan results to broadly identify cohorts that may benefit from screening for asymptomatic disease.
- What operating cost burden is required to sustain a mobile screening project and what is the minimum utilization required to justify it?
- The operating costs of the study will be captured in order to inform an analysis of the financial constraints for deployment of mobile screening systems into local underserved communities.
- What portion of the asymptomatic patients with advanced disease will be willing to learn about clinical trials?
- Analysis of the posttest behavior of participants by their enrolling to learn more about clinical trials will measure the efficacy of using this method to educate the at risk population about clinical trials and ultimately inform decisions about how trial participants may be recruited in the future.
- What portion of the patients take advantage of free online diet support?
- Lifestyle changes are challenging as it is difficult to engage patients in the weeks following any testing. Tracking of the number who take advantage of the follow-up support will help guide future efforts to develop educational materials.

Patient Selection Criteria

The screening will be available to adults who self-select and who meet the inclusion criteria. The project will stop at 3 months or 1,000 patients, whichever occurs first. The goal is to

examine whether a self-referral design can serve as a cost effective patient centered wellness screening model.

Who may participate

- o Adults 18 and over
- o Patients with no diagnosis of liver disease
- o Patients with any of these self-identified chronic conditions
    - Type 2 diabetes
    - Metabolic syndrome
    - Cardiovascular disease
    - Pre Diabetes
    - Sleep apnea
    - Digestive disorders
    - Osteoporosis
    - Polycystic ovarian syndrome
    - Psoriasis

Who may not participate

- o Age less than 18
- o Pregnant women
- o Anyone with an implanted electronic device such as a defibrillator o Morbidly obese, BMI 40 or greater
- o Anyone not personally able to give informed consent
    o Non English speakers, no translator is available

Trial Candidate Outreach

The primary patient outreach efforts will be to focus on high risk comorbid patients. We will communicate with local physicians to inform them that the service exists and invite them to refer appropriate patients to the screening facility or alternatively invite us to visit their offices with a portable unit.

The Foundation will also perform educational outreach through social media, communication through internet advertising, online forums, interaction with local support groups, media interviews and other educational efforts emphasizing the problem of our growing societal health crisis with obesity in general and liver disease in particular.

It is important to emphasize that during this study the FibroScan test will be free to qualified patients but they will be interviewed to ask what price they would be willing to pay on a private pay basis. This information will be used to perform the analysis of the viability of a private pay model. There will be no insurance or third party payment. Following testing the patient will be asked what payment amount, ranging from zero to more than $400, would seem fair to them as a charge. The goal is to develop perceived value measures which can inform the financial modeling for the development of the fully deployed business plan.

Outputs of the project for the patient

All patients will receive education about liver disease as part of the intake process. Following the test, the patient will receive a printed output from their FibroScan test and instructions about next steps. Interpretation of test results require professional medical knowledge which is not provided by the study. Patients will be directed to a telemedicine system where they can discuss their results with professional staff and determine any next steps. Patients may elect to confer with their personal physician instead as is their right. Patients with test results indicating no active disease will be offered connections to nutrition and dietary support. In all cases patients will be offered enrollment in online educational tools about diet and lifestyle.

Patients will also be told about clinical trials and receive information about that process and offered access to online educational information and trial access tools if they are interested. Part of the outreach from the study is to educate patients about the need for clinical trials and to encourage learning more about it. The core message is that therapies will not be successful in the future without a cohort of patients who have been educated about clinical trials and are willing to consider participating. We will not recruit for any particular trials but will discuss their value and importance to society and potentially to individual patients.

By focusing on asymptomatic and undiagnosed liver disease the project will identify a currently unavailable population ideally suited for the upcoming clinical trials required for the development of therapies for liver fibrosis in NAFLD, NASH, and cirrhosis.

Study procedures

Testing will be done by appointment. Since the test itself is very fast, with a planned test throughput of 15 minutes, most of the visit will be taken up by the consent procedure, explanation of the test itself, discussion of liver disease, and distribution of literature.

When the test schedule is created, patients will be instructed not to eat within 3 hours of their appointment time so that a convenient time can be chosen.

There will be four steps in the process.

Step 1: Explanation and Consent
- At the intake point a staff members will explain the purpose for the study, provide information about liver disease, fatty liver, NASH, and fibrosis.
- The staff member will review the inclusion/exclusion rules and confirm that the patient has met the 3 hour fasting rule and has not been diagnosed with a liver disease.
- If inclusion criteria are met, the staff member will describe non-invasive testing process using the FibroScan, give the patient printed information, and explain that it measures both liver stiffness and fat content which their doctor can use to help them with any diagnosis that may be needed. It will be explained that FibroScan does not provide a diagnosis but is part of the information a doctor would use to understand the health of their liver. A sample test report will be used as a visual aid. Any questions will be answered.

- o The staff member will explain what information will be gathered and explain that it is demographic information only and that no personally identifiable information will be collected.
- o When any questions have been answered, the staff member will explain consent, provide the patient with the document, and answer any other questions.
- o Upon signing of the consent the staff member will assign a random number to the consent and to the patient questionnaire.

Step 2: Data Capture

o After signing the consent form the patient will be asked to fill out the demographic form which will record basic health information, height, weight, sex, age, ethnicity, and identify any comorbid conditions of which they are aware from a list of common liver related comorbidities.

Step 3: The FibroScan test

- o The patient will take the completed intake form and the educational materials with them to the FibroScan test room where the test will be performed.
- o A certified FibroScan technician will double check the intake form and answer any additional questions that the patient may have.
- o The technician will then administer the test according to the established standard test procedure and will identify it with the random number assigned to this test in step 1.

Step 4: Posttest process and instructions

- o When the test is complete the test results will be printed out and given to the patient.
- o The patient will also be given detailed information about how FibroScan is used and what it measures in a format that they can use to inform themselves so that they can better discuss the results and next steps with a physician. This will include explanations of how liver stiffness and fatty infiltration are defined and measured by the device.
- o The patient will be asked to assess their perception of the value of the test to them personally by indicating what they perceive the value is of the test to them personally on a scale from nothing to over $400.
- o The patient will also be given printed information providing ways to contact resources and information about lifestyle changes, free support services, and information about how to access additional online tools.
- o Most importantly, the patient will be instructed on how to access telemedicine support for interpretation of the test results and next steps. The technician will explain that the study provides the test, but interpretation must be done by a physician. Some patients may elect to discuss it with their existing doctor but

they will be encouraged to utilize the hepatology services, particularly if their test results are elevated.

Confidentiality

Under this protocol, no personally identifiable information will be captured. The consent form does require that it be personally signed but that information will not be digitized. Upon completion of the study the paper copy of the completed consent forms will be placed in secure storage.

The test will be assigned a randomly generated ID code which will be assigned to the consent form and to the demographic form. That code will connect the demographic information to the results of the test for analysis. In the event that a competent authority required the retrieval of the consent for the test, it would be possible, through the use of the ID code, to connect the information. At no time will the information be stored together or be in a compatible format.

The ID code will be keyed to the digitally stored FibroScan results database which the patient can provide to their physician at their discretion. The scans will not be personally identified and can only accessed by the unique code. The dataset will be stored and encrypted and individual test results can only be retrieved using the random key code. In this way the patient can have access to the test result but only the patient will be in possession of the key that accesses the information.

Employees will be trained in the expectations of confidentiality and will have signLinda Lied an agreement to keep any patient information private as part of the hiring procedure.

The FibroScan Test Procedure

FibroScan is notable for its fast, safe, non-invasive design. This is the procedure:

A certified FibroScan trained technician will administer the test.

- A pain-free diagnostic test, FibroScan® requires no sedation of any kind.
- The procedure itself usually takes about 5 minutes.
- For the procedure, the patient will lie on their back with their right arm raised behind their head and their right abdominal area exposed.
- The technician will apply a water-based gel to the skin and then place a non-invasive probe over the liver.
- During the exam, the patient may feel a slight vibration on the skin at the tip of the probe as it delivers ultrasound waves to the area for measuring purposes.

Risk/Safety Information

There are no uncommon risks or safety concerns resulting from this procedure. There will be the normal office space risks when accommodating patients with the common comorbidities

such as falls, nausea, paper cuts, and other normal space management concerns. None, however, derive from the FibroScan screening process itself.

Study Oversight

The principal investigator and the co-investigators will provide oversight for the project and assure that all procedures are followed. The PI will assure that the staff will be properly qualified and trained to deliver the study information in a proper way according to the study protocol. The staff will be instructed on the informed consent process, privacy policies, data systems, instructional materials, and patient care as examples of site management.

Data Management

Data collected for study purposes will be stored on a HIPAA compliant system for statistical analysis as part of the economic modeling of this screening process for these high risk populations. The anonymized data will be used by the study sponsor or a designee for analysis but will not be made publically accessible. This information is not part of the patient medical record at this time, however, the test report the patient receives will have an ID which they could provide to a physician who could use it to copy the scan information into the formal medical record at the patient's request. The test data will be maintained for a period of at least 5 years to determine its value in future research study.

IRB Review/Ethics/Informed Consent

As part of the intake and education process, informed consent will be explained and the patient will be provided with an IRB approved informed consent form describing the FibroScan process and what the entire process will be. Since the FibroScan is a well-known device, the consent form will be consistent with non-invasive testing of this kind.

If any unanticipated problems were to occur during the course of the research study, the PI will report these issues to the IRB within 10 working days.

Intended Use of the Data

It is the investigators belief that current standards of care guidelines of the AASLD, which do not, as a result of inadequate data, advise routine wellness screening for liver fibrosis in high risk populations likely constitutes a risk to the lives of those patients.

Data to support a change in guidance from the AASLD does not yet exist. It is the goal of this study to develop data which can guide the design of a strategy which can be implemented privately and lend support to a change of guidance from the AASLD to recommend wellness screening.

The data from the study will be used to support future financial modeling and to provide baselines for programs to educate patients about clinical trials, diet, and lifestyle changes delivered electronically. Since there is no existing measure, an a-priori power analysis is not overly instructive, but based on an assumed normal distribution the study seeks to enroll no fewer than 400 participants to yield a minimum theoretical power of around 0.8 and may include up to a maximum of 1,000 patients.

The study will also produce data on the incidence and staging of NAFLD and NASH, as indicated by their FibroScan scores, and will track, in aggregate, their response to information about clinical trials and dietary support delivered through online resources. This data will not be informed by the patient record as no personally identifiable data will be collected.

The dataset will include demographic information and general ethnicity so aggregate subtotals of the various populations may be examined for additional insights to inform future investigations.

DATA COLLECTION:

Demographics:
After signing the consent form the research subjects will fill out a basic form to provide demographic information, including sex, age, and race/ethnicity.   We will measure height and weight prior to CAP/FibroScan testing.

Metabolic Syndrome:
Subjects will use a form to check YES or NO for comorbid components of the metabolic syndrome that they may or may not have:

- Obesity/overweight
- Diabetes mellitus
- Hypertension
- Hypercholesterolemia
- Hypertriglyceridemia
- Gout or elevated uric acid

Other Health Conditions:
Subjects will use a form to check YES or NO for other health conditions they may or may not have:

- Heart disease
- Heart attack
- Arrhythmia
- Stroke
- Arthritis
- Osteopenia/osteoporosis
- Hypothyroidism
- Low Vitamin D
- Low testosterone
- Menopausal state
- Inflammatory bowel disease (ulcerative colitis or Crohn's disease)
- Cancer o Skin: melanoma o Skin: basal cell or squamous cell cancers
    - o Other type of cancers: lymphoma; leukemia; head or neck; thyroid; brain; breast; lung; stomach; colon; liver; ovary; uterus; prostate; bone

Diet:

Subjects will fill out a form using a 5-point scale of the frequency of their average weekly dietary intake of specific food types: (A. 0 days, B. 1-2 days, C. 3-4 days, D. 5-6 days, E. 7 days per week)

- o Proteins: eggs, cheese, beans, fish, chicken, turkey, beef, pork
- o Starchy carbohydrates: breads, rolls, pasta, cereals, potatoes, rice, corn, peas, lima beans
- o Non-starchy vegetables: lettuces, kale, carrots, green beans, broccoli, peppers, onions, cucumber
- o Fats: butter, margarine, avocado, oils (olive, safflower, cottonseed, peanut, sesame, walnut)
- o Nuts: peanuts, walnuts, cashews, almonds, macadamias, peanuts o Sugars or Syrups: sugar, high fructose corn syrup (sodas), maple or corn syrups o Fast food meals o Eating out at restaurants

Exercise:

Subjects will fill out a form using a 5-point scale of the frequency of their average weekly exercise:

(A. 0 days, B. 1-2 days, C. 3-4 days, D. 5-6 days, E. 7 days per week) o Cardiovascular: brisk walking, jogging, biking, treadmill, stationary bike, stair stepper, elliptical, swimming laps o Strength: weight lifting, resistance machines or cords

Alcohol:

Subjects will fill out a form quantifying their average weekly total of alcoholic drinks:

(1 drink= 12 oz. beer, 6 oz. wine, 1.5 oz. hard liquor; 3 oz. brandy, sherry, liqueur):

o None o 1-2 drinks/week o 3-4 drinks/week o 5-6 drinks/week o 7 -8 drinks/week o 9-10 drinks/week o 10-12 drinks/week o 13-14 drinks/week o 14-20 drinks/week

o 21 or more drinks/week

Other Liver Diseases:

Subjects will check YES or NO for other liver-related diseases that they may or may not have:

o Hepatitis B alone o Hepatitis B with Hepatitis D o Hepatitis C o Hepatitis E o Autoimmune hepatitis o Primary biliary cholangitis o Primary sclerosing cholangitis o Iron overload

o Alpha-1-antitrypsin deficiency

Reporting of CAP-FibroScan Results to Participating Subjects:

A trained individual will perform the CAP-FibroScan according to the FDA-approved, manufacturer's protocol. A licensed hepatologist will determine the degree of fibrosis and will review the results and the steatosis based on the values in the following tables:

Transient Elastography (TE):

Disease	F0-F1	F2	F3	F4
NAFLD/NASH	≤ 7.0	≤ 7.5	≤ 10.0	≤ 14.0

Reference: Current Gastroenterology Reports. 2014; 16: 372

TE Interpretation: The subject's liver stiffness score in kPa units shows:
- **F0:** No liver fibrosis
- **F1-2:** Mild-Moderate liver fibrosis
- **F3:** Severe liver fibrosis
- **F4:** Cirrhosis

Controlled Attenuation Parameter:

Steatosis Grade	% Steatosis on Liver Biopsy	CAP Value
Mild (S1)	5-33%	225-275 dB/M
Moderate (S2) to Severe (S3)	>33% >66%	275-400 dB/M

Reference: World J Gastroenterol. 2016; 22: 7236

CAP-FibroScan Experience and Satisfaction Survey:

Subjects will be asked to complete a survey about their experience after having the CAP-FibroScan and hearing their results, using a 5-point scale: very likely—likely—neither likely nor unlikely—unlikely--very unlikely.

1. Having completed the CAP-FibroScan and obtained your results, how likely are you to:
- recommend this test to a friend or family member?
- share your results with your doctor?
- consult with a liver specialist?
- seek information about FDA-approved clinical trials of therapies for non-alcoholic fatty liver disease?

Subjects will be asked to assess their level of satisfaction with the CAP-FibroScan procedure after being informed of their results, using a 5-point scale: very satisfied—satisfied—neither satisfied nor dissatisfied—dissatisfied—very dissatisfied.

2. Having had the CAP-FibroScan and obtained your results, how satisfied are you with the procedure?
   a. If you answered very satisfied or satisfied, please check all the reasons for your answer:

- Ease of scheduling appointment for procedure.
- Scheduled procedure occurred on time.
- Cleanliness of the facility.
- Professionalism and friendliness of staff.
- Understandable explanation of the procedure and sufficient answers to my questions.
- Quality of explanation of the results and quality of the answers to your questions about the results.
- Comprehensive discussion of options my future care.

b. If you answered dissatisfied or very dissatisfied, please check all the reasons for your answer:
- Lack of ease of scheduling appointment for procedure.
- Procedure did not occur on time.
- Problems with the cleanliness of the facility.
- Problems with the professionalism or friendliness of staff.
- Inadequate explanation of the procedure and deficient answers to my questions.
- Inadequate explanation of the results and deficient answers to my questions about the results.
- Inadequate discussion of options for my future care.

3. Having had the CAP-FibroScan and obtained your results, how satisfied are you that the procedure provided useful information about your health?
   a. If you answered very satisfied or satisfied, please check all the reasons for your answer:
   - I understand what the CAP-FibroScan measures.
   - I understand how much fat I have within my liver.
   - I understand if I have or do not have evidence of scarring (fibrosis) of my liver.
   - The results gave me a better understanding of my liver health.
   - The discussion provided useful information about FDA-approved clinical trials of therapies.
   - The discussion of my liver health will help me obtain better health care for my liver.

   b. If you answered dissatisfied or very dissatisfied, please check all the reasons for your answer:
   - I do not understand what the CAP-FibroScan measures.
   - I do not understand how much fat I have within my liver.
   - I am not sure if I have or do not have scarring (fibrosis) of my liver.
   - The results did not give me a better understanding of my liver health.
   - The discussion did provide useful information about FDA-approved clinical trials of therapies.
   - The discussion of my liver health is unlikely to help me obtain better health care for my liver.

SUNN STUDY

STATISTICAL ANALYSIS PLAN

PRIMARY OUTCOME MEASURE – DETERMINE THE INCIDENCE AND STAGING OF UNDIAGNOSED NAFLD AND NASH IN A HEALTH CONSCIOUS SELF SELECTED POPULATION AS MEASURED WITH A FIBROSCAN

The study will measure the incidence and severity of the fat content and liver stiffness in an at large, self selected population who do not, at the time of testing, have a diagnosis of NAFLD, NASH or other liver disease. This will measure the incidence of undiagnosed liver disease and the statistical distribution of the disease staging in this population.

A key secondary outcome measure will be the incidence and disease distribution as a function of ethnicity.

We will analyze data using a standard statistical software package, Stata® 12.1 (Stata Corp, College Station, TX). We will express continuous variables using the mean ± standard deviation, and we will test the null-hypothesis using the Student t-test.   We will compare categorical variables using contingency table analysis. For all statistical tests, we will define a two-sided p-value of ≤0.05 as statistically significant. We will perform multivariate analysis of all risk factors found to be statically significant (p ≤0.05) using univariate analysis.

Data Analysis

We will record all data in a Microsoft Excel worksheet. We will sequentially number each participating subject. We will not record any identifying information (e.g., names, initials, DOB, SSN, insurance, EPIC MRNs) in the database. Thus, all data assessments will maintain the anonymity of all participating subjects.

1. The primary goal is to calculate the proportion of men and women that when tested have CAP-FibroScan evidence of:

- No fat or liver stiffness
- No fat but evidence of liver stiffness
- Fat without liver stiffness
- Fat with liver stiffness
  - o Stiffness values indicative of cirrhosis
  - o Stiffness values indicative of fibrosis without cirrhosis

NOTE: no individual follow up or track will be done as not personally identifiable information will be captured or retained.

2. The secondary goal is to determine factors that are statistically significantly associated with the presence or absence of fat with or without increased liver stiffness.

We will analyze data using a standard statistical software package, Stata® 12.1 (Stata Corp, College Station, TX). We will express continuous variables using the mean ± standard deviation, and we will test the null-hypothesis using the Student t-test. We will compare categorical variables using contingency table analysis. For all statistical tests, we will define a two-sided p-value of ≤0.05 as statistically significant. We will perform multivariate analysis of all risk factors found to be statically significant (p ≤0.05) using univariate analysis.

This exploratory goal seeks to determine the degree of satisfaction of subjects who undergo a screening CAP-FibroScan free of charge.

We will calculate the frequency of answers for each of the five items reflecting subjects' likelihood of:
- recommending this test to a friend or family member.
- sharing their results with their doctor.
- consulting with a liver specialist.
- seeking information about FDA-approved clinical trials of therapies for non-alcoholic fatty liver disease.
-

We will not perform statistical analyses for these descriptive data.

Disease Staging
The disease staging to be analyzed will be based upon these breakpoints from the FibroScan test. The test scores will be translated into these staging ranges for analysis.

Transient Elastography (TE):

Disease	F0-F1	F2	F3	F4
NAFLD/NASH	≤ 7.0	≤ 7.5	≤ 10.0	≤ 14.0

Reference: Current Gastroenterology Reports. 2014; 16: 372

TE Interpretation: The subject's liver stiffness score in kPa units shows:
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Controlled Attenuation Parameter:

Steatosis Grade	% Steatosis on Liver Biopsy	CAP Value
Mild (S1)	5-33%	225-275 dB/M
Moderate (S2) to Severe (S3)	>33% >66%	275-400 dB/M

Reference: World J Gastroenterol. 2016; 22: 7236

DATA COLLECTION

Demographics:
The data for analysis will be captured by these patient completed forms to provide demographic information, including sex, age, and race/ethnicity.   We will measure height and weight prior to CAP/FibroScan testing.

Metabolic Syndrome:
Subjects will use a form to check YES or NO for comorbid components of the metabolic syndrome that they may or may not have:
- Obesity/overweight
- Diabetes mellitus
- Hypertension
- Hypercholesterolemia
- Hypertriglyceridemia
- Gout or elevated uric acid

Other Health Conditions:
Subjects will use a form to check YES or NO for other health conditions they may or may not have:
- Heart disease
- Heart attack
- Arrhythmia
- Stroke
- Arthritis
- Osteopenia/osteoporosis
- Hypothyroidism
- Low Vitamin D
- Low testosterone
- Menopausal state
- Inflammatory bowel disease (ulcerative colitis or Crohn's disease)
- Cancer o Skin: melanoma o Skin: basal cell or squamous cell cancers

       ○ Other type of cancers: lymphoma; leukemia; head or neck; thyroid; brain; breast; lung; stomach; colon; liver; ovary; uterus; prostate; bone

Diet:

Subjects will fill out a form using a 5-point scale of the frequency of their average weekly dietary intake of specific food types: (A. 0 days, B. 1-2 days, C. 3-4 days, D. 5-6 days, E. 7 days per week)

- ○ Proteins: eggs, cheese, beans, fish, chicken, turkey, beef, pork
- ○ Starchy carbohydrates: breads, rolls, pasta, cereals, potatoes, rice, corn, peas, lima beans
- ○ Non-starchy vegetables: lettuces, kale, carrots, green beans, broccoli, peppers, onions, cucumber
- ○ Fats: butter, margarine, avocado, oils (olive, safflower, cottonseed, peanut, sesame, walnut)
- ○ Nuts: peanuts, walnuts, cashews, almonds, macadamias, peanuts ○ Sugars or Syrups: sugar, high fructose corn syrup (sodas), maple or corn syrups ○ Fast food meals ○ Eating out at restaurants

Exercise:

Subjects will fill out a form using a 5-point scale of the frequency of their average weekly exercise:

(A. 0 days, B. 1-2 days, C. 3-4 days, D. 5-6 days, E. 7 days per week) ○ Cardiovascular: brisk walking, jogging, biking, treadmill, stationary bike, stair stepper, elliptical, swimming laps ○ Strength: weight lifting, resistance machines or cords

Alcohol:

Subjects will fill out a form quantifying their average weekly total of alcoholic drinks:

(1 drink= 12 oz. beer, 6 oz. wine, 1.5 oz. hard liquor; 3 oz. brandy, sherry, liqueur):
       ○ None ○ 1-2 drinks/week ○ 3-4 drinks/week ○ 5-6 drinks/week ○ 7 -8 drinks/week ○ 9-10 drinks/week ○ 10-12 drinks/week ○ 13-14 drinks/week ○ 14-20 drinks/week
       ○ 21 or more drinks/week

Other Liver Diseases:

Subjects will check YES or NO for other liver-related diseases that they may or may not have:
       ○ Hepatitis B alone ○ Hepatitis B with Hepatitis D ○ Hepatitis C ○ Hepatitis E ○ Autoimmune hepatitis ○ Primary biliary cholangitis ○ Primary sclerosing cholangitis ○ Iron overload
       ○ Alpha-1-antitrypsin deficiency