NON-INVASIVE BIOMARKERS OF METABOLIC LIVER DISEASE (*NIMBLE*)

AN FNIH BIOMARKERS CONSORTIUM STUDY

Protocol Number: NIMBLE Study 1.1

Sponsor: FNIH

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and applicable United States Code of Federal Regulations (CFR). The Principal Investigator at each site will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participant(s). All personnel involved in the conduct of this study have completed Human Subjects Protection and CITI GCP Training.

The protocol and informed consent form(s) will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title	NON-INVASIVE BIOMARKERS OF METABOLIC LIVER DISEASE (NIMBLE); AN FNIH BIOMARKERS CONSORTIUM STUDY: Study 1.1
Study Description (NIMBLE)	NIMBLE is a comprehensive, five-year collaborative effort to standardize, compare, validate, and advance the regulatory qualification of imaging and circulating biomarkers to diagnose and stage nonalcoholic steatohepatitis (NASH), and to predict and assess response to therapeutic intervention (*https://fnih.org/what-we-do/biomarkers-consortium/programs/nimble*)
Study Description (Study 1.1)	This study, Study 1.1, is a prospective, observational, two-center, short-term cross-sectional study to assess the reproducibility and repeatability of a set of specified ultrasound-based quantitative imaging biomarkers. The primary focus will be on imaging biomarkers of the liver fibrosis component of nonalcoholic fatty liver disease (NAFLD), rather than the steatosis or inflammation component. The rationale is that the fibrosis component is linked most closely to survival and other clinical outcomes. Study 1.1 will also collect data to explore vendor- or device-specific investigational biomarkers on other components of NAFLD such as steatosis and possibly inflammation. The data collected will be used to inform a decision of which of these biomarkers have sufficient precision to be advanced to NIMBLE Stage 2.
Objectives (NIMBLE)	The primary objectives of NIMBLE are to: • Standardize, compare, validate, and advance the regulatory qualification of a set of non-invasive imaging biomarker(s) for the diagnosis and staging of NASH. • Standardize, compare, validate, and advance the regulatory qualification of a set of non-invasive imaging biomarker(s) to predict and assess response to therapeutic intervention in patients with NASH.
Objectives (Study 1.1)	The primary objectives of Study 1.1 are:

- To evaluate the pooled different-day, different-operator reproducibility coefficient of ultrasound measurements of shear wave speed (SWS).
- To evaluate the different-day, different-operator reproducibility coefficient of vibration controlled transient elastography (VCTE) on the measurement of SWS.

Endpoints (Study 1.1)

Primary endpoints:

- Evaluation of pooled different-day, different-operator reproducibility coefficient of ultrasound measurements of SWS.
- Evaluation of different-day, different-operator reproducibility coefficient of VCTE on the measurement of SWS.

Secondary endpoints:

- Evaluation of pooled same-day, same-operator repeatability coefficient of ultrasound-based measurements of SWS.
- Evaluation of pooled different-scanner, same-day reproducibility coefficient of ultrasound-based measurements of SWS.
- Evaluation of same-day, same-operator repeatability coefficient of VCTE on the measurement of SWS and Young's modulus.
- Evaluation of different-day, different-operator reproducibility coefficient of VCTE on the measurement of Young's modulus.

Exploratory endpoints

- Precision and other reliability metrics of vendor- or device-specific investigational measurements of other components of NAFLD such as steatosis and possibly inflammation.

Study Population

This study will enroll patients with suspected or confirmed diagnosis of NAFLD. Based on protocol-specified FIB-4 values, about one-third are expected to have low, one-third to have intermediate, and one-third to have high likelihood of advanced fibrosis. The sample size of 40 participants was determined to provide sufficient sample to provide >80% power to qualify SWS (or equivalent) as an ultrasound (US)-based biomarker for advancement to Stage 2. The threshold for automatic qualification for advancement to Stage 2 will be an upper bound of the 95% confidence interval (CI) of the reproducibility coefficient less than 35%. Dropouts and technical failures were included in this sample size determination.

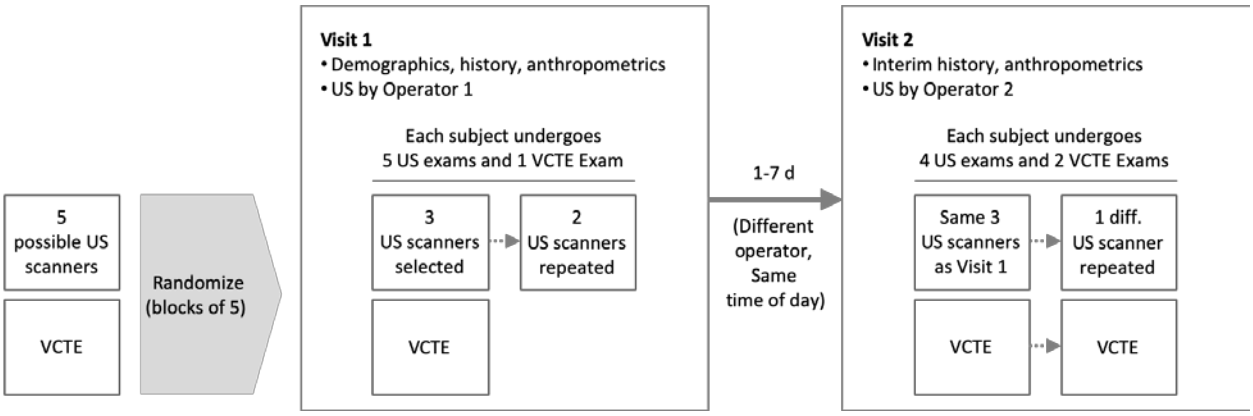
Sex: ~ 50:50 – Note- no stratification will be done based on sex
Age: ≥ 18 yrs
Demographic group: Patients with a high probability of NAFLD based on the eligibility criteria
General health status: Patients with suspected or confirmed diagnosis of NAFLD
Geographic location: San Diego, CA and Boston, MA (greater metropolitan areas)

Phase

N/A

Description of Sites/Facilities Enrolling Participants	Planned facilities/participating sites enrolling participants: University of California- San Diego (UCSD) and Massachusetts General Hospital (MGH) Number of sites: 2
Description of Study Intervention	Study will not have any therapeutic intervention. The goal of the study will be evaluating the variability of ultrasound-based measures for the diagnosis and monitoring of NAFLD/NASH.
Study Duration	12 months
Participant Duration	< 1 month

1.2 SCHEMA



1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Screening (Day -7 to 1[a])	Visit 1 (Day 1)	Study Visit 2 (Day 2 to 7)
Informed consent [b]	X		
Demographics	X		
Medical history	X		
Interim medical history		X	X
Medication review	X		
Change in medication review		X	X
Physical activity review	X		
Change in physical activity review		X	X
AUDIT questionnaire	X		
Vital signs	X	X	X
Height	X		
Weight	X	X	X
Fasting blood collection [c]	X		
Ultrasound Imaging		X	X
Complete Case Report Forms (CRFs)	X	X	X

a. Screening visit can occur on the same day as Visit 1. If visits occur on the same day, repeat tests (i.e. vital signs) and nonapplicable tests (e.g., change in medication review) do not need to be collected.
b. Informed consent to be collected first at Screening Visit before all other procedures. Informed consent can be collected up to 45 days prior to Visit 1.
c. CBC, platelet count, complete metabolic panel (albumin, alkaline phosphatase, gamma glutamyl transferase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, AST, ALT, sodium), lipids (triglycerides, total cholesterol, HDL, LDL). If the labs have been collected within the last 3 months the PI may opt to not repeat. Blood collected at MGH will be analyzed by the MGH clinical laboratory. Blood collected at UCSD will be analyzed by the UCSD clinical laboratory

2 INTRODUCTION

2.1 STUDY RATIONALE

Nonalcoholic fatty liver disease (NAFLD) is a common liver problem which affects 30% of the United States population. Liver biopsy is accepted as the most accurate technique to detect patients at risk of developing serious liver conditions. However, liver biopsy is an invasive test with risk for complications and risk of mortality. Alternative imaging and blood-based biomarkers are needed to replace liver biopsy for screening and longitudinal assessment, to assist drug development process and to detect patients at high risk of developing liver-related complications.

2.2 BACKGROUND

NAFLD is defined as fat accumulation in liver, when other fat-accumulating factors like alcohol consumption and steatogenic medications are excluded. NAFLD can be classified into two sub-types; 1) Nonalcoholic Fatty Liver (NAFL), and 2) Nonalcoholic Steatohepatitis (NASH). Both NAFL and NASH can progress to cirrhosis, however, NASH patients progress more frequently and more rapidly when compared to NAFL. NASH-related cirrhosis is currently the second most common indication for liver transplantation in the United States and is projected to become the leading cause in the near future [1,2,3]. NASH is associated with a vast economic burden, estimated at $103 billion in medical costs each year in the United States alone[4].

Currently, liver biopsy is the reference standard for diagnosis and risk stratification of NASH, as it can evaluate both the inflammatory and fibrosis components of NAFLD. However, biopsy is limited by sampling error, and high intra- and inter-observer variability[5]. Moreover, liver biopsy is invasive, painful, costly, and associated with morbidity and even mortality[6]. Considering the large disease burden with high prevalence, it is not feasible to screen, diagnose, or monitor all suspected patients with non-focal liver biopsy. As a result, only a small minority of NASH patients typically undergo liver biopsy and an estimated 95% of patients remain undiagnosed[7, 8]. Non-invasive and inexpensive methods are needed to diagnose, risk-stratify, and monitor NASH.

Noninvasive tests including imaging techniques and blood biomarkers might be useful in management of high-risk patients. Liver-specific ultrasound-based measurements including shear wave speed, or equivalently Young's modulus, and sonographic fat assessment methods have the potential to provide complementary information, improving diagnostic confidence and guiding clinical and research practice [9, 10].

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

There are no known physical risks to study participants from the imaging procedures, as shear wave elastography (SWE), vibration controlled transient elastography (VCTE), and any exploratory imaging procedures are non-invasive ultrasound-based imaging tests. These techniques are performed using FDA-cleared devices that comply with United States Food and Drug administration (FDA) safety requirements, have no known bioeffects, and cause no patient discomfort. Images will be obtained on multiple ultrasound devices over 1.5 to 3 hours. If the patient becomes fatigued during this period, breaks will be provided. Inadvertent disclosure of protected health information is a potential risk that will be mitigated by storage of paper records in a locked filing cabinet and de-identification of clinical data. There may be mild discomfort or pain from blood collection during screening. The study is therefore considered a minimal risk study.

There is a small but non-negligible risk of incidental findings. An incidental finding is one unknown to the subject that has potential health or reproductive importance, which is discovered unexpectedly in the course of a research study but is unrelated to the purpose and beyond the aims of the study. This is a potential risk since the discovery of incidental findings may cause anxiety and lead to additional workup and even treatment, with attendant costs and risks, possibly without health benefit. Management of incidental findings is discussed in **Section 8.2**.

2.3.2 KNOWN POTENTIAL BENEFITS

There are no known direct benefits to study participants. The benefit of participation in this study is the contribution to the advancement of non-invasive biomarkers for NAFL and NASH, a contribution which could benefit the participant in their own future clinical surveillance or screening exams.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Non-invasive biomarkers could be valuable tools for advancing the care of patients with NAFL and NASH. Participation in NIMBLE could advance the knowledge of imaging biomarkers to allow for more routine use in clinical trials and routine clinical care. The broader benefits of biomarker development are as follows:

Drug and Biomarker Development

Difficulty in finding high-risk patients and risks of repetitive liver biopsy as a tool to assess treatment response, are major limitations in NASH drug development. By advancing the qualification of candidate ultrasound biomarkers and selecting reliable techniques, this limitation will be addressed.

Regulatory agency approval process

Acquiring ultrasound data, analysis and research results will be helpful to obtain clearance and permission from regulatory agencies.

Clinical practice

Qualified ultrasound-based biomarkers might replace or reduce the need for liver biopsy, which is an invasive technique with limitations.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The primary objectives of Study 1.1 are: • To evaluate the pooled different-day, different-operator reproducibility coefficient of ultrasound measurements of shear wave speed (SWS). • To evaluate the different-day, different-operator reproducibility coefficient of VCTE on the measurement of SWS.	• Evaluation of pooled different-day, different-operator reproducibility coefficient of ultrasound measurements of SWS. • Evaluation of different-day, different-operator reproducibility coefficient of VCTE on the measurement of SWS. **Pooled diff-day, diff-operator reproducibility coefficient (pooled $RDC_{diff\text{-}day,\ diff\text{-}operator}$)**	Different-day and different-operator reproducibility are needed to inform the context of use of ultrasound and VCTE measurements for future clinical trials and clinical care.
Secondary		
Secondary objectives of Study 1.1 build on the understanding of repeatability and reproducibility of shear wave elastography and VCTE ultrasound-based measurements of shear wave speed (SWS) or, equivalently, Young modulus. The secondary objectives of Study 1.1 are: • To evaluate the pooled same-day, same-operator repeatability coefficient of ultrasound-based measurements of SWS. • To evaluate the pooled different-scanner, same-day reproducibility coefficient of	• Evaluation of pooled same-day, same-operator repeatability coefficient of ultrasound-based measurements of shear wave speed (SWS). • Evaluation of same-day, same-operator repeatability coefficient of VCTE on the measurement of SWS. **Pooled same-day, same-operator repeatability coefficient (pooled $RC_{same\text{-}day,\ same\text{-}operator}$)** • Evaluation of pooled different-scanner, same-day reproducibility coefficient of ultrasound-based measurements of shear wave	These endpoints will help refine the context of use of ultrasound and VCTE measurements for future clinical trials and clinical care.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
ultrasound-based measurements of SWS. • To evaluate the same-day, same-operator repeatability coefficient of VCTE on the measurement of SWS.	speed (SWS) and Young's modulus. **Pooled Diff-scanner, same-day reproducibility coefficient (pooled $RDC_{diff\text{-}scanner,\ same\text{-}operator}$)** • Evaluation of different-day, different-operator reproducibility coefficient of VCTE on the measurement of Young's modulus.	
Exploratory		
Exploratory objectives may include the evaluation of vendor- or device-specific ultrasound-based measurements of other tissue properties.	Precision and other reliability metrics of vendor- or device-specific investigational measurements of other components of NAFLD such as steatosis and possibly inflammation.	Exploratory analyses may contribute to the development of new ultrasound-based biomarkers.

4 STUDY DESIGN

4.1 OVERALL DESIGN

Hypothesis. The upper bound of the 95% Confidence Interval (CI) of the pooled different-day, different-operator reproducibility coefficient (true $RDC_{diff\text{-}operator,\ diff\text{-}day}$) of shear-wave speed values for the five participating ultrasound vendors (Canon, GE, Philips, Siemens, and Supersonic) will be less than 35%. The conservative assumption underlying this hypothesis is that the true $RDC_{diff\text{-}operator,\ diff\text{-}day}$ values for the five ultrasound vendors will be 23, 25, 25, 27, and 30%, with no assumption made about how those true values correspond to the five vendors.

Design. This is a prospective, low-interventional, two-center, short-term cross-sectional precision study.

Study interventions. Ultrasound exams, blood collection, physical measurements, clinical history and medication reviews.

Methods. At each of the two participating clinical sites (MGH and UCSD), approximately equal numbers of eligible participants will be enrolled, for a projected total enrollment of 40 participants across both sites (about 20 at each site). To ensure adequate representation of the disease severity spectrum, participants will be enrolled into three categories according to the likelihood for advanced fibrosis based on Fibrosis-4 (FIB-4) values:

- low likelihood of advanced fibrosis (FIB-4 ≤ 1.3)
- intermediate likelihood of advanced fibrosis (1.3 < FIB-4 < 2.67)
- high likelihood of advanced fibrosis (FIB-4 ≥ 2.67)

About one-third of total participants (about 13 participants total -minimum 8, maximum 18), about 6 at each site (minimum 3, maximum 10) will be enrolled in each category.

The rationale for using FIB-4 is that it is independent of the ultrasound-based measurements being tested in this Study. Although developed for assessment of liver fibrosis in Hepatitis C viral infection, it has been shown to stratify patients with NAFLD into the three likelihood levels with reasonable accuracy [11] [12]. Perfect accuracy is not needed for this study because the aim of the stratification is to ensure a reasonable distribution of fibrosis severity. The FIB-4 levels will not be used as reference standard for fibrosis stage, and the accuracy of the ultrasound-based measurements to diagnose advanced fibrosis will not be tested in Study 1.1. The components of FIB-4 are age (years), AST level (U/L), platelet count (10^9/L), and ALT (U/L). The FIB-4 value is derived from a simple algebraic formula of these four components.

Participants will be consecutively assigned to their corresponding category until the category is closed (reaches maximum of 11). Once a category is closed at a given site, participants will no longer be enrolled into that category at that site.

Each participant will undergo ultrasound imaging at Visit 1 (by operator 1) and at Visit 2 (by operator 2), separated by 1 to 7 days. The 7-day window for Visit 2 is selected to allow flexibility in scheduling while helping to ensure that there is no substantial biological change in the interim. Ultrasound exams at Visits 1 and 2 will be performed at about the same time of day (target is ± 2 hours) and with identical fasting and other preparation instructions. For each visit, each participant will be assigned to three of the five ultrasound scanners plus VCTE. As shown in the Schema (**Section 1.2**), for Visit 1, two of the three ultrasound exams will be repeated; the third ultrasound exam and the VCTE exam will be performed once. For Visit 2, the third ultrasound exam and the VCTE exam will be repeated; the other two ultrasound exams will be performed once. The selection of exams to be repeated will be based on a block assignment strategy to ensure an approximately equal number of scans are performed by each ultrasound system, in addition to VCTE. Thus, each subject will undergo a total of six exams at each of their two visits, for a total of 12 exams across both visits. For the repeated exams, subjects will get off the table with a short break (target about 5 minutes) before getting back on the table. Subjects will not be required to leave the room. The machine will not be turned off and powered up again to save time

Ultrasound operators will follow the acquisition procedures outlined in the imaging manual. The imaging manual will address patient positioning, image acquisition and quality control with scanning protocol input from the relevant ultrasound vendors. Additional investigational ultrasound data may be acquired, including with use of non-FDA-approved vendor proprietary software.

As outlined in the Schedule of Activities (SOA), screening data will include demographic, medical history, medication review, physical activity review, vital signs, height, and weight. Body mass index (BMI) will be calculated. Fasting blood samples will be collected if needed at PI's discretion based on availability of previous blood samples. The Alcohol Use Disorders Identification Test (AUDIT) questionnaire will be administered to assess alcohol consumption, drinking behaviors, and alcohol-related problems, information useful for assessing eligibility.

On the subsequent visit, interim medical history, and changes in medications, physical activity, vital signs, and weight will be collected.

Methods that will be used to minimize bias. The following six methods will be used to minimize bias.

1. Enrollment into fibrosis likelihood categories to ensure adequate representation of disease severity. The determination of fibrosis likelihood will be made by FIB-4, which is independent of any investigational ultrasound biomarker.

2. None of the ultrasound operators will be involved in participant screening, or in the assignment of enrolled participants into the above three enrollment categories.

3. The Visit 2 operator will be instructed to not review the Visit 1 exam results.

4. The ultrasound operators will be blinded to clinical and laboratory data, and to enrollment category.

5. On-site quality control, central quality control, and central reading. Analysis will be performed by analysts and investigators who are not employed by vendor companies, and who have completed conflict of interest documentation for review and management by the Project Team.

6. Central analysts will be blinded to clinical and laboratory data.

Interim analysis. None planned.

Name(s) of sub-studies. None planned.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

All study participants will be scanned on a subset of ultrasound-based scanners. All study participants will contribute data to the primary and secondary outcomes while minimizing burden on the patient.

4.3 JUSTIFICATION FOR DOSE

Not applicable; this is not a clinical trial and there is no drug administration.

4.4 END OF STUDY DEFINITION

A participant will be considered to have completed this study if:

- all scheduled Visit 1 and Visit 2 ultrasound exams have been performed, and
- all requested demographic, history, vital signs, and anthropometric information have been collected.
- blood has been collected to measure the needed laboratory values

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

1. Adult (age ≥ 18 years)

2. Known or suspected NAFLD based on

   - Prior biopsy ≤ 36 months before enrollment consistent with NAFLD

     OR

   - Clinical and laboratory data ≤ 3 months before enrollment consistent with NAFLD: abnormal ALT (>30 U/L for men, > 19 U/L for women) without other common causes such as HCV, HBV AND meets criteriaf or ATP III criteria (2005 revision) for metabolic syndrome with any 3 of the 5:
     i. Waist circumference (WC) > 102 cm (M) or > 88 cm (F)
     ii. Fasting glucose ≥ 100 mg/dL or Rx
     iii. TG ≥ 150 mg/dL or Rx
     iv. Elevated blood pressure (SBP ≥ 130 mmHg or DBP ≥ 85 mmHg)
     v. Reduced HDL-C < 40 mg/dL (M) or < 50 gm/dL (W)

3. Able and willing to participate, including maintaining steady-state: physical activity, alcohol use, medications

4. Classifiable into one of the following enrollment categories by FIB-4 (ALT, AST, platelets, date of birth) collected at screening visit if not available already within 3 months prior:

   - Low likelihood of advanced fibrosis: FIB-4 ≤ 1.3 (about one-third of enrolled participants, minimum 8, maximum 18)
   - Intermediate likelihood of advanced fibrosis: 1.3 < FIB-4 < 2.67 (about one-third of enrolled participants, minimum 8, maximum 18)
   - High likelihood of advanced fibrosis: FIB-4 ≥ 2.67: (about one-third of enrolled participants, minimum 8, maximum 18)

5.2 EXCLUSION CRITERIA

1. Liver disease other than NAFLD

2. Excess alcohol consumption (≥ 2 units/day for women and ≥ 3 units/day for men)

3. Current diagnosis of drug induced liver injury

4. Receiving drug or placebo in treatment trial now or within 30 days

5. Weight loss or gain of ≥ 5 kg in prior 3 months

6. Other factors that in the judgment of the principal investigator might preclude study completion

7. Women who state they are pregnant. Women who state they are pregnant will be excluded in an abundance of caution, since pregnancy might increase intra-abdominal pressure which in turn might affect the assessment of the different-day reproducibility coefficient of ultrasound and VCTE measurements.

8. Patients with active implants such as pacemakers or defibrillators or any other contraindication to ultrasound or VCTE scanning.

5.3 LIFESTYLE CONSIDERATIONS

Participants will be asked to maintain their lifestyle over the course of their involvement in this study (i.e., no changes in medication; levels or types of exercise; amounts, types, or frequency of alcohol intake, etc.).

5.4 SCREEN FAILURES

Screen failures will only be allowed in exceptional circumstances. Potential participants who screen-failed based on the Inclusion Criteria will not be allowed to re-screen. Only potential participants who screen-failed based on the Exclusion Criteria 4, 5 or 6 will be allowed to re-screen if they no longer meet the Exclusion Criteria.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Achieving target enrollment. It is not anticipated that there will be difficulty screening enough participants to meet the enrollment target of a total of 40 participants in this study. The goal will be to enroll approximately equal numbers of males and females, and to enroll a participant population representative of the sex, racial and ethnic mix of patients with NAFLD at the two clinical sites participating in this study. No special stratification will be done based on sex or race. Our effective screen-fail rate is expected to be up to about 25**%**, taking into account failure to satisfy inclusion and exclusion criteria, loss of participants due to possible uneven filling of our three enrollment categories, dropout, and technical failure. Thus, we expect to need to screen 50 participants to achieve our target enrollment of 40 participants.

Anticipated accrual rate. We anticipate being able to enroll one participant at each site every 2 weeks, and therefore expect to be able to complete enrollment in about 40 weeks.

Number of sites. There will be two clinical sites in this study (MGH and UCSD), and both have agreed to participate.

Source of participants. Participants at MGH will be recruited from EMR review and the Fatty Liver Disease Clinic. Participants at UCSD will be recruited from the UCSD NAFLD Research Center.

Methods of identification and approach for potential participants. At both sites, recruitment may be by informational flyers placed at the recruitment venues and/or by physicians at those venues discussing possible participation in this study with potential participants. Patients identified through EMR review may be approached via opt-out mailing sent on behalf of their primary physician. If subjects do not opt-out they will be contacted by study staff.

Recruitment strategies. It is not anticipated that additional recruitment methods or strategies will be needed to achieve our planned target enrollment within one year.

Participant retention. Given the short participation period for each participant (i.e., about a week or less to complete both imaging visits), it is not anticipated that participant retention will be a problem in this study.

Recruitment and retention of participants from historically under-represented populations. Given the expected strong ability to recruit fairly from the outpatient clinic populations at both sites, the disproportionate prevalence of NAFLD in Latino minorities, and the short duration of participant involvement in this study, we do not expect that recruitment or retention of participants from historically under-represented populations will be a problem for this study.

Vulnerable populations. No subjects from vulnerable populations will be recruited for this study.

Participant compensation. Participants will be given up to $50 per hour for their participation in this study to compensate them for their time, any inconvenience, and parking. Compensation will be made upon completion of all study procedures or after completion of screening if participant screen fails.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Study interventions will include questionnaires, anthropometric measurements, blood collection, and non-invasive ultrasound-based imaging exams. All ultrasound-based devices use energies below FDA limits for diagnostic ultrasound.

6.1.2 DOSING AND ADMINISTRATION

There will be no dosing or administration of investigational products as part of this study.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

There is no study intervention other than ultrasound-based exams. All ultrasound-based exams will be acquired by trained operators.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

This section is not applicable as there is no study interventional product.

6.2.3 PRODUCT STORAGE AND STABILITY

This section is not applicable as there is no study interventional product.

6.2.4 PREPARATION

This section is not applicable as there is no study interventional product.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Study participants will not be randomized to individual treatment groups or be blinded. In order to ensure a uniform distribution of scanner combinations, participants will follow a block-randomization pattern with different scanner combinations for different participants, however, patients and operators will not be blinded to ultrasound scanners being used.

All efforts will be made to keep ultrasound operators blinded to clinical and laboratory data, however, it is not believed that this will significantly affect the ultrasound acquisition. The Visit 2 operator will be asked to not review the Visit 1 exam results.

Central analysts will be blinded to key clinical and laboratory findings to minimize potential bias. Blinding of the central analysts will be outlined in a separate Image Review Charter.

6.4 STUDY INTERVENTION COMPLIANCE

Acquisition and analysis of ultrasound exams will be captured in a separate Acquisition Manual and Image Review Charter. These documents will outline the acquisition procedures and ultrasound analysis procedures.

Participant weight will be measured and interim medical history, medication use, alcohol use, physical activity will be ascertained at each visit to assess compliance by participants with instructions to maintain steady-state weight, exercise, alcohol use, and medications.

6.5 CONCOMITANT THERAPY

Current medications will be collected as part of a participant's medical history.

6.5.1 RESCUE MEDICINE

As there is no study intervention, no rescue medication will be used.

7 STUDY INTERVENTION

7.1 DISCONTINUATION OF STUDY INTERVENTION

If participants become intolerant to ultrasound scanning they may discontinue. If they have not completed Visit 2 they will be withdrawn from the study.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study if, in the opinion of the investigator, there have been significant lifestyle changes or other factors that could affect study results.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for Visit 1 and/or Visit 2, and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the specified time frame and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

If a participant is lost to follow-up and has only completed one study visit, they may or may not be replaced as part of the targeted enrollment. This decision will be based on the number of subjects who have completed the study to date.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING AND EFFICACY ASSESSMENTS

Several assessments will be completed across Screening, Visit 1 and Visit 2. These are listed below and specific ultrasound image acquisition will be outlined in the 'NIMBLE 1.1 Ultrasound Imaging Manual'.

- **Demographics, anthropometrics and medical history will be collected at the Screening Visit**
- **Physical examination**
    - Height will be recorded at Screening.
    - Weight and vital signs will be recorded at each visit.
    - Body mass index will be calculated at each visit.
    - All measurements will be made by trained coordinators using standard and calibrated instruments.

- **Imaging assessments will be collected at Visit 1 and Visit 2**

  Ultrasound based imaging parameters will be collected from all patients in two visits. These will include but may not be limited to:
    - **Shear wave elastography results in m/s and/or kPa**
    - **Quantitative ultrasound parameters, where available, including:**

        - Attenuation Coefficient
        - Backscatter Coefficient
        - Shear Wave Dispersion
        - Speed of Sound
        - Ultrasound derived fat fraction
    - **Conventional B mode (gray-scale) and Doppler ultrasound images, including:**
        - An image of the liver and right kidney on the same image for hepato-renal index calculation
        - Right liver lobe for skin to liver capsule distance calculation
        - Portal vein Doppler for portal vein pulsatility index measurement
    - **VCTE and Controlled Attenuation Parameter measurements with the Fibroscan system**

  Measurements will be collected using the following systems:
    - Ultrasound devices:
        - GE LOGIQ E10
        - Siemens Sequoia
        - Canon Aplio i800
        - Philips EPIQ 7
        - SuperSonic Aixplorer Mach 30
    - VCTE
        - Fibroscan 530 Compact

  For detailed ultrasound image acquisition protocol, please refer to the 'NIMBLE 1.1 Ultrasound Imaging Manual'. Note: Ultrasound-based exams will be reviewed centrally and results will not be shared with study participants.

- **Biological specimen collection and laboratory evaluations will be collected at the Screening Visit**.
    - Blood will be collected by trained phlebotomists at each site using routine methods and standard collection tubes.
    - Total volume is expected to be about 10 mL or less
    - Blood collected at MGH will be analyzed by the MGH clinical laboratory.
    - Blood collected at UCSD will be analyzed by the UCSD clinical laboratory.
    - Blood results obtained within the 3-month interval prior to the screening visit at the MGH or UCSD laboratories will be considered acceptable for study analyses and may be used at PI discretion.
    - For each laboratory, the normal ranges for each blood test will be recorded and filed

- **Special assays or procedures required**
    - None

- **Administration of questionnaires or other instruments**
    - The following questionnaires will be administered at Screening:
        - Medical history questionnaire
        - Medication use questionnaire
        - Alcohol consumption questionnaire (AUDIT questionnaire)
        - Physical activity questionnaire
    - The following questionnaires will be administered at Visit 1 and Visit 2:

- Interim medical history questionnaire
- Change in medication use questionnaire
- Change in physical activity questionnaire

8.2 SAFETY AND OTHER ASSESSMENTS

Laboratory values will be reviewed by the PI at each site. The PI will report clinically important incidental findings to the referring physician or, if there is no referring physician, to the participant.

If a lesion is identified by the operator during real-time scanning the site PI will be alerted by study staff and will review the images for safety.

Additionally, ultrasound images will be reviewed by a central reviewer. The purpose of this review is to evaluate images for liver stiffness and other exploratory biomarkers relevant to NAFLD and NASH. Central image review is not a complete medical review of the subject. If during the central review process, an unexpected observation is identified and this finding could, in the opinion of the central reviewer, have a significant health or reproductive consequence, this finding may be shared with the principal investigator.

All follow-up testing and final diagnosis will be left to the discretion of the medical professionals at the site or those with an existing physician-patient relationship. The principal investigator will be responsible for reporting any adverse events identified from incidental findings as described in the Adverse Event Reporting section. Identification of such incidental findings during the central review process should not be expected, and the site maintains responsibility for performing a general safety review of all images as per site protocols.

There are no dedicated Safety Assessments as part of this study as this study does not involve study medication.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal physical exam or certain unexpected abnormal laboratory finding, symptom, or disease, temporally associated with a subject's participation in the research.

In this clinical study, we anticipate only minimal risk of adverse events because the investigational procedure is limited to noninvasive diagnostic ultrasound imaging of the abdomen using the vendor specific investigational liver fat quantification and shear wave elastography/VCTE software. All ultrasound systems that will be used in this clinical trial use ultrasound energies below FDA limits for diagnostic ultrasound. At these energies, these ultrasound systems and software have no known bioeffects and are not expected to cause risk to participants.

Anticipated nonserious AEs may include;

- Potential discomfort during the ultrasound scans due to body positioning, and

- Potential discomfort during the ultrasound scans due to ultrasound transducer pressure.
- Potential discomfort related to IV stick following blood draw at screening

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A SAE is any AE that:

1. Results in death, or
2. Is life-threatening, or
3. Results in hospitalization or prolongation of existing hospitalization, or
4. Results in a persistent or significant disability/incapacitation, or
5. Results in a congenital anomaly/birth defect, or
6. May jeopardize the participant's health and may require medical or surgical intervention to prevent one of the other outcomes listed above.

No SAEs are anticipated in this clinical trial.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs), the following guidelines are used to describe severity.

Mild - Events require minimal or no treatment and do not interfere with the participant's daily activities.

Moderate - Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe - Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term 'severe' does not necessarily equate to 'serious'.

AEs will be assessed by site PIs using the criteria mentioned above. Site PIs are responsible for reporting and managing AEs.

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All AEs will have their relationship to study procedures assessed by the site principal investigator. The following criteria will be used to identify causality; (0) Not related, (1) Unlikely to be related, (2) Potentially related, (3) Potentially related, (4) Probably related, (5) Definitely related.

8.3.3.3 EXPECTEDNESS

Site PIs will be responsible for determining whether an adverse event (AE) is expected or unexpected in this NIMBLE clinical trial. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with known ultrasound risks and complications, which are very limited.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during ultrasound scans or screening visits. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

8.3.5 ADVERSE EVENT REPORTING

The site investigators will record adverse events and report them to the NIMBLE leadership, and to MGH and UCSD IRBs according to the timetable for reporting specified in the protocol. Any AE regarding research participants' health will be reported in 10 working days after the AE becomes known.

Abnormal liver function tests and elevated blood lipids can be accepted as Disease-Related Events (DRE). These DREs are common in the target study population, patients with NAFLD. High liver function test and blood lipids are not associated with ultrasound exam. These factors will not be reported as adverse events.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

In case of a serious adverse event, the site investigator will inform NIMBLE leadership and the MGH or UCSD IRBs as soon as possible, but in no event later than 10 working days after the site investigator first learns of the effect. NIMBLE leadership is responsible for conducting an evaluation of an unanticipated adverse device effect and shall report the results of such evaluation to the FNIH and MGH or UCSD IRB and participating investigators within 10 working days after NIMBLE first receives notice of the effect. Thereafter, the sponsor or designee (i.e. NIMBLE Leadership) shall submit such additional reports concerning the effect as FDA requests.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Any AE regarding research participants' health will be reported to participant’s physician, or in the absence of a physician directly to the participant, via phone call or secure e-mail in 72 hours or less after the PI becomes aware of the AE. AE management strategies and next steps will be handled by the participant’s physician.

8.3.8 EVENTS OF SPECIAL INTEREST

This section is not applicable to this protocol.

8.3.9 REPORTING OF PREGNANCY

Women who state they know they are pregnant will be excluded from the study. Pregnancy might increase the intra-abdominal pressure which in turn might affect the reproducibility of elastographic measurements.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

In this clinical study, UP will be accepted as any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) ultrasound scans that are described in this protocol and NIMBLE 1.1 Ultrasound Imaging Manual; and (b) the characteristics of the NAFLD population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the ultrasound scan); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

In this clinical study, unanticipated adverse device effects will be accepted as, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, ultrasound systems, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in this protocol or any other unanticipated serious problem associated with ultrasound that relates to the rights, safety, or welfare of participants.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The site primary investigators will report unanticipated problems (UPs) to the reviewing MGH and UCSD IRBs and to NIMBLE leadership. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the site IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to MGH and UCSD IRBs and to the NIMBLE leadership within 24 hours of the investigator becoming aware of the event.

- Any other UP will be reported to MGH and UCSD IRBs and to the NIMBLE leadership within 72 hours of the investigator becoming aware of the problem.

An investigator shall submit to the sponsor and NIMBLE Leadership and to the site IRB a report of any unanticipated adverse device effect occurring during ultrasound scans as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect (21 CFR 812.150(a)(1)), A sponsor who conducts an evaluation of an unanticipated adverse device effect under 812.46(b) shall report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing

IRBs and participating investigators within 10 working days after NIMBLE consortium first receives notice of the effect. Thereafter the sponsor or designee (i.e. NIMBLE Leadership) shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150(b)(1)).

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Any UP regarding research participants' health (i.e. SAE) will be reported to participant in 24 hours. UP management strategies and next steps will be explained by site PI.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s):

  Different-day, different-operator reproducibility coefficient of ultrasound measures

- Secondary Efficacy Endpoint(s):

  Same-day, same-operator repeatability coefficient of ultrasound measures;
  Different-scanner, same-day reproducibility coefficient of ultrasound measures;
  Precision and other metrics measured by investigational analyses of ultrasound measures; and
  Vendor- or device-specific metrics of ultrasound measures

9.2 SAMPLE SIZE DETERMINATION

The following assumptions were made in determining sample size for the primary study objective:

1. Repeat measurements on the same participant follow a normal distribution.
2. $RDC_{\text{diff-day, diff-operator}}$ is in the range of 20-30% (wCV of 7.2-10.8%).
3. A balanced design is planned (i.e. equal number of participants per scanner) where the following scenarios describe the possible RDCs across the five scanners:
   - 25, 25, 25, 25, 25%
   - 24, 25, 25, 26, 27%
   - 23, 25, 25, 27, 29%
   - 23, 25, 25, 27, 30%
4. A study with 80% power is desired.

The null hypothesis for the primary study objective is H_o: $RDC_{\text{diff-day, diff-operator}} \geq 35\%$, versus the alternative hypothesis that $RDC_{\text{diff-day, diff-operator}} < 35\%$, where RDC is the reproducibility coefficient for different day and different operator. The upper 95% confidence bound for the RDC will be constructed.

A Monte Carlo simulation study was conducted to determine the sample size needed. For each simulated participant, two observations were generated from a normal distribution with the specified variance. Samples of varying size were simulated. From each sample $RDC_{diff\text{-}day,\ diff\text{-}operator}$ was estimated, along with its 95% CI. This process was repeated 10,000 times.

In the table below the sample size is reported such that 80% of the samples of that size met the precision qualification (i.e. upper 95% confidence bound for RDC <35%). The results suggest that by pooling across the five scanners, up to 55 independent observations are needed. With each subject scanned on three different scanners at the two time points and assuming moderate correlation between observations from the same subject (r=0.25), we consider the design effect to account for the clustering of the data (i.e. 3 observations/subject). The design effect is 1+(s-1)r, where r=correlation and s=# of observation/subject, i.e. 1+(3-1)0.25=1.5. Thus, (55 x 1.5)/3=27.5 so a study with 28 total participants would provide 80% power.

For estimating the $RDC_{diff\text{-}day,\ diff\text{-}operator}$ of VCTE, assuming an $RDC_{diff\text{-}day,\ diff\text{-}operator}$ of 25%, 32 subjects provides 80% power to show that the RDC<35%. Taking into consideration the possibility of a drop-out or technical failure rate of no more than 25%, a study with 40 total participants (20 per site) is proposed.

Number of Independent Observations needed for ≥80% power as a function of the five scanners' $RDC_{diff\text{-}day,\ diff\text{-}operator}$

RDCs across 5 scanners:	25, 25, 25, 25, 25%	24, 25, 25, 26, 27%	23, 25, 25, 27, 29%	23, 25, 25, 27, 30%
Total # independent observations	40	45	50	55

9.3 POPULATIONS FOR ANALYSES

All participants completing Visit 1 and Visit 2 with evaluable ultrasound images as defined during central review will be included in the analysis.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Means, medians, standard deviations (SDs), coefficients of variation (CVs), and relative frequencies will be used to describe the study sample's characteristics and to report the observed imaging measurements. 95% CIs will be constructed for measures of precision. Log transformations of imaging measurements will be performed, as appropriate.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

$RDC_{diff\text{-}day,\ diff\text{-}operator}$ will be estimated as follows:

$$\widehat{RDC}_{diff-day,diff-operator} = 2.77 \times \sqrt{\sum_{j=1}^{5}\sum_{i=1}^{N_j}(Y_{1ij} - Y_{2ij})^2/2\bar{Y}_{ij}^2 N}$$

where Y_{1ij} is the biomarker measurement at timepoint 1 and Y_{2ij} is the biomarker measurement on the same scanner *j* by a different operator at timepoint 2 for the i^{th} participant. $\bar{Y}_{ij}$ is the mean of Y_1 and Y_2 for participant *i* for scanner *j*. N_j is the total number of participants per scanner, and N is the total number of observations. The 95% upper bound for $RDC_{diff\text{-}day,\ diff\text{-}operator}$ will be constructed as follows:

$$\widehat{RDC}\sqrt{\frac{N}{\chi^2_{N,\alpha}}}$$

where $\chi^2_{N,\alpha}$ is the αth percentile of the chi square distribution with N degrees of freedom and α is 0.95. A bootstrap 95% CI will also be constructed for RDC, where sampling with replacement will be performed at the subject level. If the upper 95% confidence bound is < 0.35 (< 35%), then it will be concluded that the biomarker meets the prespecified precision criterion.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

For each biomarker and each scanner, $RC_{same\text{-}day,same\ operator}$ will be estimated as follows:

$$\widehat{RC}_{same-day,same\ operator} = 2.77 \times \sqrt{\sum_{i=1}^{N}(Y_{1i} - Y_{2i})^2/2\bar{Y}_i^2 N_j}$$

where Y_{1i} is the biomarker measurement at timepoint 1 and Y_{2i} is the replicate biomarker measurement on the same scanner by the same operator at timepoint 1 for the i^{th} participant. $\bar{Y}_i$ is the mean of Y_1 and Y_2 for participant i. N_j is the total number of participants per scanner.

For each biomarker and each scanner, $RC_{diff\text{-}day,diff\text{-}operator}$ will be estimated as follows:

$$\widehat{RC}_{diff-day,diff-operator} = 2.77 \times \sqrt{\sum_{i=1}^{N}(Y_{1i} - Y_{2i})^2/2\bar{Y}_i^2 N_j}$$

where Y_{1i} is the biomarker measurement at timepoint 1 and Y_{2i} is the biomarker measurement on the same scanner by the same operator at timepoint 2 for the i^{th} participant. $\bar{Y}_i$ is the mean of Y_1 and Y_2 for participant i. N_j is the total number of participants per scanner.

For each biomarker and a pair of scanners, $RDC_{scanner}$ will be estimated as follows:

$$\widehat{RDC}_{scanner} = 2.77 \times \sqrt{\sum_{i=1}^{N_{j,j'}}(Y_{1i} - Y_{2i})^2/2\bar{Y}_i^2 N_{j,j'}}$$

where Y_{1i} is the biomarker measurement at timepoint 1 and Y_{2i} is the biomarker measurement on a different scanner for the i^{th} participant. This RDC will be estimated both with the same and different operators, and on the same and different days. $\bar{Y}_i$ is the mean of Y_1 and Y_2 for participant i. $N_{j,j'}$ is the number of participants scanned on the scanners *j* and *j'*.

For each biomarker and each scanner, 95% upper bounds for $RC_{\text{same-day,same-operator}}$, $RC_{\text{diff-day,diff-operator}}$, and RDC_{scanner} will be constructed as follows:

$$\widehat{RC}\sqrt{\frac{N}{\chi^2_{N,\alpha}}}$$

where $\chi^2_{N,\alpha}$ is the αth percentile of the chi square distribution with N degrees of freedom and α is 0.95.

9.4.4 SAFETY ANALYSES

There are no safety analyses planned for this study. All AEs, including their severity and relatedness to study interventions, will be recorded, however, there are no further planned analyses.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Means, medians, standard deviations (SDs), coefficients of variation (CVs), and relative frequencies will be used to describe the study sample's characteristics. There are no intervention groups for comparison.

9.4.6 PLANNED INTERIM ANALYSES

No interim analysis is planned for this study.

9.4.7 SUB-GROUP ANALYSES

There are no sub-groups planned for this study. Any additional analyses will be outlined in the Statistical Analysis Plan.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data may be listed by measure and time points.

9.4.9 EXPLORATORY ANALYSES

There are no exploratory analyses that are planned as part of this study protocol other than exploratory endpoints.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the ultrasound scans, blood sampling, and risks will be given to the participant and written documentation of informed consent will be required prior to starting ultrasound scans.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent forms will be MGH and UCSD IRB approved for each institution, and the participant will be asked to read and review the document at the start of the Screening visit. The investigators, or their designees (e.g., coordinators), will explain the research study to the participant and answer any questions that may arise. This explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the ICF and ask questions prior to signing. The participants will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate.

The participant will sign the ICF prior to any procedures being done specifically for the NIMBLE 1.1 study. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. Both investigator and study participant will sign the informed consent form (ICF). A copy of the signed informed consent form will be given to participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures.

The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. Appropriate efforts will be made at an institutional level to provide adequate interpretative services for participants who are not English speaking. Children or other vulnerable population will not be included in the study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

NIMBLE 1.1 study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided to study participants, investigators, site IRBs, the NIMBLE consortium, and FNIH. Study participants will be contacted, as applicable, and be informed of changes to ultrasound scan visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the FNIH, NIMBLE and the IRBs.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, MGH and UCSD staff, NIMBLE consortium and FNIH. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the NIMBLE 1.1 study or the data will be released to any unauthorized third party without prior written approval of the sponsor and/or NIMBLE Leadership. Data may be included in the participant's EMR as determined by the site PI.

All research activities will be conducted in as private a setting as possible. Study participant's contact information and PHI will be securely stored at each clinical site for internal use during the study. At the end of the study, all records, including signed consent form copies, will continue to be kept in a secure location.

Study participant de-identified ultrasound imaging data, lab and clinical background information, will be transmitted to and stored in secure servers. Copy of the data will be stored at NIMBLE 1.1 Ultrasound Image Analysis Center (MGH). Investigators at MGH will be responsible for storing this data copy in secure server. Participants and their research data will be identified by a unique study identification number. The data entry and management in an image management system will be secured and password protected. Access will be limited to authorized personnel.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at UCSD and MGH. After the study is completed, the de-identified, archived ultrasound images, ultrasound data and clinical data will be transmitted to and stored at a secure location. Permission to transmit data to the data storage center will be included in the informed consent.

During the conduct of the study, an individual participant can choose to withdraw consent to have ultrasound images and clinical data stored for future research.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator (MGH)	**Principal Investigator (UCSD)**
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10.1.6 SAFETY OVERSIGHT

There will be no Safety Oversight committee for this study as there is no intervention and all analyses will be conducted using FDA approved non-invasive imaging devices.

10.1.7 CLINICAL MONITORING

There will be no external clinical monitoring as part of this study. Clinical monitoring will be the responsibility of the PI and appropriate team members at MGH and UCSD respectively.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

MGH and UCSD will perform internal quality management of study conduct, data collection, documentation and management. An external auditor as a representative of the NIMBLE 1.1 team may perform independent assessments on an as-needed basis.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of MGH and UCSD staff under the oversight of the PI at each site. Coordinators under PI oversight will ensure accurate, complete, legible, and contemporaneous data entry in source documents.

10.1.9.2 STUDY RECORDS RETENTION

All study records, de-identified data and research findings will be retained for 7 years after the study completion. No records will be destroyed without the written consent of FNIH and NIMBLE consortium. It is the responsibility of the FNIH and NIMBLE consortium to inform the investigators when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 10 working days of identification of the protocol deviation. All deviations will be reported to the NIMBLE leadership team. Protocol deviations will be sent to MGH and UCSD IRBs per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

As per National Institutes of Health (NIH) Public Access Policy, final peer-reviewed journal manuscripts will be submitted to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the FNIH Data Sharing and result dissemination policy. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

NIMBLE consortium leadership will be responsible for developing publication procedures and resolving authorship issues

10.1.12 CONFLICT OF INTEREST POLICY

Any potential conflicts of interest will be managed by FNIH.

10.2 ADDITIONAL CONSIDERATIONS

None.

10.3 ABBREVIATIONS

AE	Adverse Event
AUDIT	Alcohol Use Disorders Identification Test
BMI	Body Mass Index
CFR	Code of Federal Regulations
CI	Confidence Interval
CV	Coefficient of Variation
CRF	Case Report Form
DRE	Disease-Related Event
eCRF	Electronic Case Report Forms
EMR	Electronic Medical Record
FDA	United States Food and Drug Administration
FIB-4	Fibrosis-4
FNIH	Foundation for the National Institute of Health
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
MGH	Massachusetts General Hospital
NAFL	Nonalcoholic fatty liver
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NIMBLE	Non-Invasive Biomarkers of Metabolic Liver Disease
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RC	Repeatability Coefficient
RDC	Reproducibility Coefficient
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOA	Schedule of Activities
SOP	Standard Operating Procedure
SWE	Shear Wave Elastography

SWS	Shear wave speed
UCSD	University of California,San Diego
U/L	Units per Liter
UP	Unanticipated Problem
US	Ultrasound
VCTE	Vibration Controlled Transient Elastography
WC	Waist Circumference

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
Version 1.0	24-Jan-2020	Original document	Original Version
Version 2.0	03-Nov-2020	Removed 'Alcohol follow-back' questionnaire.	Due to the short time between Visit 1 and Visit 2 this questionnaire did not seem necessary.
Version 3.0	23 June 2021	Clarified metabolic syndrome criteria and minor site operation updates	Inconsistency between protocol language and published criteria.
Version 4.0	11 August 2021	Remove pre-screening to allow sites to manage this individually, removed urine pregnancy test requirement at screening and extended the time before scanning that sites can collect ICDs.	Updated to align with study site operations.

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