Study title: Quantitative Ultrasound with Velacur for Evaluation of Non-Alcoholic Fatty Liver Disease

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Background

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide [1]. It is characterized by increased hepatic fat accumulation in the absence of significant alcohol intake. NAFLD has a wide spectrum that starts with simple accumulation of triglycerides droplets in hepatocytes (macrosteatosis) without associated inflammation, hepatocyte injury or fibrosis, a phenotype called non-alcoholic fatty liver (NAFL) [2]. A more severe NAFLD phenotype is nonalcoholic steatohepatitis (NASH), which in addition to the presence of macrosteatosis is characterized by hepatocyte ballooning, lobular inflammation with or without fibrosis. While NAFL generally has a benign course, NASH may progress to cause cirrhosis, liver failure and hepatocellular carcinoma (HCC) [3, 4].

The global prevalence of NAFLD in the general population is estimated at 25% and it increases with age [1]. There is geographic variation in NAFLD prevalence probably due to differences in populations' genetic compositions, dietary habits and environmental aspects. About a quarter of the populations in Europe and North America are estimated to have NAFLD, whereas populations in South America and the Middle East are estimated to have the highest rates of NAFLD globally (range 30-32%)[1]. NASH is estimated to affect 2% - 6% .of the general population, 7% - 30% of NAFLD patients who had a liver biopsy without a specific clinical indication (e.g. evaluation for liver organ donation), and 59% in NAFLD patients with clinical indication for liver biopsy (e.g. elevated liver enzymes) [1].

Current NAFLD guidelines by the American Association of study of Liver Diseases (AASLD) do not recommend routine screening for NAFLD among patients with diabetes or obesity in the setting of primary care and obesity clinics [2]. Use of non-invasive clinical prediction models and detection methods such as vibration-controlled transient elastography by FibroScan® to evaluate the presence and severity of fibrosis is proposed [5]. Several serum-based non-invasive clinical prediction models can be used to screen patients with NAFLD for NASH or advanced fibrosis [6, 7]. For example, the NAFLD fibrosis score (NFS) allows a non-invasive evaluation of the extent of fibrosis using weight, and age and laboratory data which include liver enzymes and serum glucose level [8, 9]. Another useful clinical tool to estimate fibrosis is Fibrosis-4 score which relies solely on laboratory data [10]. When available, tools such as transient elastography (FibroScan®) with controlled attenuation parameter (CAP) allow simultaneous estimation amount of hepatic fat (using CAP) and fibrosis [using the liver stiffness measurement (LSM)] [5, 11]. MR based methods can also offer simultaneous assessment of hepatic fat and fibrosis, albeit it is not widely available and is more expensive [12].

While the use of FibroScan® in liver clinics is attractive as a point of care exam that can provide immediate indirect data about hepatic steatosis and fibrosis in the clinic, advancement in quantitative ultrasound technology may now allow even more detailed and directed non-invasive assessment of these measures of liver disease.

Velacur offers the ability to simultaneously and non-invasively measure Attenuation Coefficient Estimate (ACE) as a quantitative measure of ultrasound attenuation[13] and detection of hepatic steatosis in addition to LSM which measures deep volumetric liver elasticity using steady state shear waves, known as shear wave absolute viboelastography (SWAVE). This technology enables measurements of liver elasticity to a depth equal to the B-mode image, or in excess of 15 cm. The system consists of an ultrasound machine, a vibration device to excite shear waves in the patient and a ultrasound transducer. The vibration device induces shear waves in the liver at four different frequencies between 40Hz and 70Hz simultaneously. The ultrasound volume is acquired through the ribs at the same location as a typical transient elastography (TE) measurement. Volumes are collected in a fan of approximately 15 degrees and taken at a depth of 15cm, which much deeper than FibroScan® measurement (6 cm), and allows for much larger sample measurement (100,000 mm^3) versus that measured by Fibroscan (3,140 mm^3).

Velacur Probe

Rationale and Specific Aims

Hypothesis and Specific Aims: *Velacur* with ACE and LSM measurements is a novel ultrasonic device that can estimate both liver steatosis and fibrosis using a sophisticated process. The availability of *Velacur* would allow a health care provider to make a diagnosis of NAFLD with or without advanced fibrosis quickly without a need for liver biopsy.

Hypothesis: The diagnosis and severity of NAFLD with or without advanced fibrosis can be defined using *Velacur.*

Specific Aims: To prospectively evaluate the utility of *Velacur* in assessing NAFLD with or without advanced fibrosis in patients seen in liver clinics for suspected NAFLD diagnosis.

Specific Aim #1: To prospectively evaluate the performance of *Velacur* for detecting hepatic steatosis and fibrosis in patients with suspected NAFLD by assessing correlation of ACE and LSM measured by *Velacur* to grade of steatosis and stage of fibrosis on liver histology of patients with NAFLD who undergo standard of care liver biopsy.

Specific Aim #2: To compare the performance of *Velacur* to that of FibroScan® in the same patient population to determine if either test is more accurate in correlating with NAFLD histology.

Specific Aim #3: To compare the performance of *Velacur* to that of non-invasive blood based markers such as APRI, FIB4, and NAFLD fibrosis score in predicting advanced fibrosis in biopsy proven NAFLD.

Proposed Study Design: The proposed study will evaluate the performance characteristics of LSM as a measure of fibrosis and ACE as a measure of hepatic steatosis by *Velacur* in patients with different stages of NAFLD. In addition, we will compare the diagnostic accuracy of ACE to CAP and LSM measured by *Velacur* to that measured by FibroScan® using liver histology as the reference standard. We will also compare the performance of these two methods to that of non-invasive blood based markers such as APRI, FIB4, and NAFLD fibrosis score in predicting advanced fibrosis in biopsy proven NAFLD.

Inclusion/Exclusion Criteria

Inclusion criteria:

1. Adults aged 18 years or older
2. History of biopsy proven NAFLD or NASH within 6 months prior to enrollment
3. Planned liver biopsy for evaluation of NAFLD within 6 months of enrollment
4. Suspected NAFLD or NASH with planned or recommended standard of care liver biopsy
5. Ability to provide informed consent

Exclusion criteria:

1. Fasting for less than three hours prior to the scan
2. Subject is a pregnant or lactating female
3. Subject with current, significant alcohol consumption or history of significant alcohol consumption for a period of more than 3 consecutive months any time within 1 year prior to screening. Significant alcohol consumption is defined as more than 20 gram per day in females and more than 30 grams per day in males, on average (a standard drink in the US is considered to be 14 grams of alcohol).
4. Subject is unable to reliably quantify alcohol consumption based upon local study physician judgment.
5. Subject uses drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, and other known hepatotoxins) for more than 2 weeks in the year prior to screening
6. Subject with history of cirrhosis and clinical evidence of hepatic decompensation as defined by the presence of any of the following abnormalities at screening:
    a. Serum albumin less than 3.5 grams/deciliter (g/dL).
    b. An INR greater than 1.5.
    c. Total bilirubin greater than 2 milligrams per deciliter (mg/dL).
    d. please note that people with compensated cirrhosis are not excluded from the study protocol
7. Subject has a history of bleeding esophageal varices, ascites or hepatic encephalopathy
8. Subject has history of other forms of chronic liver diseases such as viral hepatitis, autoimmune hepatitis, cholestatic liver disease (primary biliary cirrhosis or primary sclerosing cholangitis).
9. Subject with active substance abuse
10. Acute hepatitis defined as AST/ALT > 500 U/L
11. Patients with a pacemaker or defibrillator

12. Ascites

Enrollment/Randomization

Up to 100 patients will be seen at 3 different sites: Indiana University, Arizona Liver Institute, and Cedars-Sinai Medical Center.

Study Procedures

All patients have to be fasting for 3 hours prior to performance of the study. After subject identification and informed consent, measurement of liver stiffness with *Velacur* will be performed by a certified technician. At that time, subject participation in the protocol is complete. Duration of participation for the subject should be no more than one hour. Medical information from the subjects, including results of the laboratory and imaging tests, liver biopsy results and medical history will be collected from medical records and entered into a database. None of these tests would have been performed as part of this study, rather of as part of routine clinical care the patient receives. Laboratory data to calculate non-invasive blood based markers such as APRI, FIB4, and NAFLD fibrosis score generated within 6 months of the date of the liver biopsy will be collected. Additionally, slides from a standard of care liver biopsy will be made from the tissue collected during the biopsy and will be read centrally according to the NASH CRN system by an experienced pathologist. Digital slides will be created and read via artificial intelligence and the results of the readings will be added to the study database.

Reporting of Adverse Events or Unanticipated Problems Involving Risk to Participants or Others

Participation in this study may cause a loss of privacy. To minimize this risk, all of the patient's personal and medical data will be considered confidential to the extent allowed by law; every effort will be made to keep all of the information strictly confidential. All study information will be stored in locked cabinets and on password-protected computers. Only authorized personnel will have access to the samples, databases, and results. Samples will be labeled with a code. When the study is published, no names or other identifying information will be used.

While there are no direct risks from use of *Velacur,* patients may be uncomfortable lying on their back for a brief period of time. They may experience soreness at the probe site. There is a small risk of an allergic reaction to the gel utilized during the procedure.

Patients will be closely monitored for any evidence of adverse events. Any adverse events associated with the study will be documented and the IRB will be notified per reporting guidelines.

Given this is a minimal risk study, no Data Safety and Monitoring Board (DSMB) will be required.

Study Withdrawal/Discontinuation

Participants are free to withdraw from the study for any reason and at any time without giving a reason for doing so and without penalty or prejudice. The Investigator is also free to terminate a subject's involvement in the study at any time if the subject's clinical condition warrants it.

Statistical Considerations

Descriptive statistics such as mean, standard error, and percentages were used to characterize the cohort. Comparisons will be made between groups by using Student t test or analysis of variance. Pearson correlation coefficient will be used to detect the correlation between continuous variables. Multivariate analysis will be performed to examine the independent association between dependent and several independent variables. Sample size is based on clinical experience and precedent established for studies of similar design.

Privacy/Confidentiality Issues

Participation in this study may cause a loss of privacy. To minimize this risk, all of the patient's personal and medical data will be considered confidential to the extent allowed by law; every effort will be made to keep all of the information strictly confidential. All study information will be stored in locked cabinets and on password-protected computers. Only authorized personnel will have access to the samples, databases, and results. Samples will be labeled with a code. When the study is published, no names or other identifying information will be used.

Follow-up and Record Retention

Use of *Velacur* is an additional tool for diagnostic purposes and provides physicians with additional clinical information to optimize patient care. Participation in this study entails a one-time visit. No direct follow-up with participants is planned.

Study records and documents will be maintained in accordance with federal, state, local, and university laws and guidelines.

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