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Methods to Improve the Noninvasive Diagnosis and Assessment of Disease Severity in Children with Suspected Nonalcoholic Fatty Liver Disease (NAFLD): Study Design

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is strongly associated with obesity and is the most common liver disease in the developed world. In children with suspected NAFLD, present guidelines suggest consideration of alternative diagnoses via extensive blood testing, though the yield of this work up is unknown. Furthermore, the gold standard diagnostic test for NAFLD remains liver biopsy, making the development of non-invasive tests critically important.

Objectives: Our objectives are: 1) to determine the accuracy of elastography and multiple serum biomarkers – each assessed individually and as algorithms (including those previously tested in adults) – for the diagnosis of nonalcoholic steatohepatitis (NASH) and early fibrosis in children and (2) to examine the utility of extensive testing for rare alternative diagnoses in overweight or obese children with elevated alanine aminotransferase (ALT) suspected to have NAFLD.

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Design: This is an ongoing, cross-sectional study in children 2 – 18 years of age with up to 2 years of prospective follow up. Eligible patients are asymptomatic, overweight or obese, and have an ALT ≤ 35 U/L upon enrollment. Two forms of elastography are obtained serially along with anthropometric data and routine laboratory tests. Elastography and serum biomarkers are also performed immediately prior to any clinically-indicated biopsy.

Methods: Between April 2015 and April 2018, 193 children have been enrolled in this ongoing study and 71 have undergone liver biopsy. Here we carefully report the rationale, methodology, and preliminary data for this study.

Keywords

Non-Alcoholic Fatty Liver Disease; Nonalcoholic Steatohepatitis; Elasticity Imaging

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), an obesity-related condition, is the most common liver disease in the United States. More than a third of U.S. children are overweight or obese and, of those, up to a third have NAFLD (nearly 10% of all U.S. children).^{1–3} NAFLD is an umbrella term for two distinct conditions occurring in the absence of alcohol intake: nonalcoholic fatty liver (NAFL), characterized by large fat droplets (termed macrovesicular steatosis) within hepatocytes, and nonalcoholic steatohepatitis (NASH), which is defined by macrovesicular steatosis and inflammation. Fibrosis is variably present and may occur with either condition.^{4,5} It is important to address NAFLD early in life since treatment (weight management and lifestyle modification) can effectively reverse disease.

A liver biopsy with expert histologic evaluation is currently needed to definitively diagnose NAFLD, differentiate between NAFL and NASH, and stage fibrosis. However, biopsy is invasive and carries a small risk of serious morbidity. It is also limited by sampling bias and restrictions on the use of repeat biopsy to follow disease over time. Accurate, non-invasive tests for the diagnosis of NAFLD and stratification between disease states are therefore needed.

And while several non-invasive serum biomarkers or biomarker panels can identify specific histologic findings like steatosis,⁶ inflammation,⁷ and fibrosis,^{8–18} or disease states such as NASH,^{7–9,16,19–23} with moderately good predictive value, these data come from a limited number of studies largely in adults. Other diagnostic modalities such as transient elastography and acoustic radiation force impulse (ARFI) imaging correlate reasonably well with significant ($\geq$ F2) hepatic fibrosis, with an area under the receiver operator curve (AUROC) of 0.86 and 0.87 respectively, but cannot readily differentiate between stages of fibrosis.²⁴ In two European cohorts, transient elastography was able to identify early fibrosis ($\geq$ F1) in children with an AUROC of 0.98.^{25,26} No published study has examined ARFI in children with biopsy-proven NAFLD or transient elastography among a NAFLD-specific, pediatric, U.S. cohort, however.

A second issue is that NAFLD is often clinically treated as a diagnosis of exclusion. Pediatric professional society position statements suggest, based largely on expert opinion,

that alternative etiologies of hepatitis – such as autoimmune hepatitis, Wilson’s disease, and others – be considered in children with suspected NAFLD prior to obtaining a liver biopsy.^{27,28} While a recent retrospective analysis demonstrated that the yield of this testing is low, the present study includes over 3-times the number of biopsied patients, prospective follow up, and more extensive covariate information.²⁹

The current study is designed to address these clinical challenges. Our aims, specifically, are as follows: 1) to determine the accuracy of elastography and serum biomarkers – each assessed individually and as algorithms (including those previously tested in adults) – for the diagnosis of childhood NAFL, NASH, and early (F1) fibrosis and (2) to examine the utility of extensive testing for rare, alternative diagnoses in overweight or obese children with an elevated ALT suspected to have NAFLD. This paper describes the overall study design and methods for data collection in this ongoing research.

2. METHODS

2.1. Design overview

Our recently initiated longitudinal study is enrolling 450 (150 with a biopsy) overweight or obese children, aged 2–18 years, with asymptomatic ALT elevation (≥ 35 U/L). Routine laboratory testing is subsequently performed to rule out non-NAFLD etiologies of hepatitis (e.g. Wilson’s disease, autoimmune hepatitis, etc.) in all enrolled patients (group 1). Transient elastography (TE, or FibroScan™) and acoustic radiation force impulse (ARFI) imaging, two forms of elastography, are obtained at baseline. Subjects are followed every 3 months for up to 2 years, with routine laboratory testing and serial elastography. A liver biopsy is performed in children with a persistently elevated ALT after 6 months, as clinically indicated. Repeat elastography testing and blood for serum biomarker analysis are obtained at the time of biopsy. Alternatively, patients who had previously undergone a work up are offered enrollment prior to any clinically indicated biopsy (group 2). That is, there are two pathways to enrollment (Figure 1). Patients are screened by a board certified Pediatric Gastroenterologist or Hepatologist and enrolled by a trained research coordinator. Consent, and assent where age appropriate, was obtained in all patients. This study is approved by our Institutional Review Board and follows all guidelines for data collection and privacy protection.

2.2. Participant selection

Patients were enrolled from the Pediatric Gastroenterology, Pediatric Hepatology, or Pediatric Fatty Liver clinics if they met inclusion and exclusion criteria, as outlined below, starting in April of 2015. All children were enrolled at a single center, the Children’s Hospital at Montefiore in the Bronx, New York, which is the teaching hospital for Albert Einstein College of Medicine.

2.3. Inclusion and Exclusion Criteria

Asymptomatic children between the ages of 2 and 18 years with a body mass index (BMI) $\geq 85^{\text{th}}$ percentile for age and an elevated ALT (≥ 35 U/L) were eligible for enrollment. Children were excluded for any of the following: an elevated bilirubin (direct bilirubin

0.5); known history of liver disease; chronic alcohol abuse, total parenteral nutrition or hepatotoxic medication use within the previous year. For purposes of this study, alcohol use was based on patient report and defined as > 21 drinks/week in males and >14 drinks/week in females. Patients were eligible for enrollment if the screening physician did not think a patient's medication was responsible for the serum ALT elevation based on clinical expertise and by consulting the National Institutes of Health online database (livertox.nih.gov) as necessary.

2.4 Liver Biopsy

Professional society recommendations suggest obtaining a liver biopsy before initiating pharmacologic therapy, to exclude other treatable diseases, and/or as a part of a clinical research study.^{28,30,31} Moreover, a position paper from the American Association for the Study of Liver Diseases specifically recommends that a biopsy be performed in all pediatric biomarker studies, noting that ALT is an "inadequate" disease surrogate.³² Clinically, biopsies are obtained in children with suspected NAFLD if there is no response to a 6 month period of life style modification, as per published position statements.^{27,30,32} All biopsies are obtained per these guidelines, for clinical indications, at the sole discretion of the treating physician; no additional biopsies or passes are obtained as a part of this research protocol.

Once obtained, liver tissue is affixed and stained per standard laboratory techniques and the specimen is read by an institutional surgical pathologist for routine clinical management. Specimens are then de-identified and re-interpreted by two board-certified pathologists expert in liver histology and NAFLD, who review the specimens together and grade the slides according to the NAFLD Activity Score (NAS). Specimen length; number of portal tracts; results of copper staining; iron quantification (0–4 scale); and the presence or absence of interface hepatitis, alpha-one antitrypsin globules, Mallory's hyaline, and glycogenated nuclei are all recorded.

2.5 Abdominal Ultrasound

A right upper quadrant ultrasound is performed after the enrollment visit, at which time the liver and biliary tree is carefully inspected for any abnormalities. The size of the right and left hepatic lobes as well as the echogenicity (compared to kidney echogenicity) is measured. Color and spectral Doppler of the main portal vein is performed, and peak velocity recorded.

The right and left lobes of the liver are measured with the subject lying in the supine position. The measurement of the skin to liver capsule distance, the width of the intercostal space in the right upper abdomen, and dimensions of the spleen are also measured.

2.6 ARFI

ARFI is performed at the same time as ultrasound by the study radiologist or trained technician in a mid-intercostal space while the patient is in the standard biopsy position (supine with the right arm in maximum abduction). A region of interest (ROI) is selected 2 cm from the capsule in the right hepatic lobe. All ROIs are free of large blood vessels. Once

a ROI is identified, 10 shear wave scores are recorded during breath holding, if the child is cooperative. Values are obtained during quiet breathing if child is unable to hold his or her breath.

Standard ultrasonography tests and ARFI results reinterpreted by one of the study's two board certified pediatric radiologists. All patients are fasting for at least 4 hours prior to ARFI testing, as per manufacturer suggestions.

2.7 Transient Elastography

TE (FibroScan™) is also performed in a mid-intercostal space in the standard biopsy position. The transducer is placed on the skin, between the ribs, as close to the middle of the parenchyma as possible. The operator, assisted by an ultrasonic time motion image, located a portion of the liver free of large vascular structures prior to triggering a measurement. For each patient, 10 valid measurements are recorded along with the following data: time to last meal; probe used; median and interquartile range for elasticity; median and interquartile range for CAP; number of attempted measurements; and the number of valid measurements. All patients are fasting for at least 3 hours prior to FibroScan™ testing, as per manufacturer suggestions.

2.8 Serum/Plasma Biomarkers

Blood is drawn via standard technique for biomarker analysis and a programmatic biorepository immediately prior to biopsy. In a small number of children – such as those with difficult airway access, anesthesia complications, very young children, or those with anxiety – the sample is instead obtained after the liver biopsy. In such cases, blood is drawn within 5 minutes of the biopsy. Samples for plasma are then centrifuged at 3000 x g for 10 min at 4°C while the samples for serum are allowed to clot for 30 minutes prior to centrifugation. Biomarker samples are stored at –80°C prior to being run in batches at the Biomarker Analytic Research Core at Albert Einstein College of Medicine. The following biomarkers are measured: cytokeratin 18, cytokeratin18 fragments, fibroblast growth factor 21, α 2-macroglobulin, hyaluronic acid, interleukin 6, plasminogen activator inhibitor-1, adiponectin, leptin, amino-terminal propeptide of type III collagen, retinol binding protein-4, soluble receptor for advanced glycation end products, tissue inhibitor of metalloproteinase 1, tumor necrosis factor- α , human cartilage glycoprotein-39 (see Table 1). Biomarker panels or biomarker algorithms previously identified in adult populations will also be calculated utilizing appropriate pediatric data (see Table 2) and validated where appropriate.

2.9 Nutritional Support

All subjects receive individualized recommendations for lifestyle management by the treating physician and are offered nutritional counseling by a registered dietician. After an initial assessment, families are offered follow up with a nutritionist at regular intervals. Patients filled out a WAVE screening form on enrollment, which is a validated screening tool designed to assess weight perception, physical activity level, food variety, and excess food intake.⁴¹

2.10 Statistical Analysis

The proposed study uses the paired design where each patient undergoes a battery of tests or procedures to detect NAFLD. To test accuracy of various diagnostic measures, we will use regression model framework where each biomarker will be tested using logistic regression model with biopsy (gold standard results) as an outcome and test result as exposure. The continuous biomarkers will be modeled to get the cut points with high sensitivity and specificity using logistic regression models based receiver operative curve analysis. In all the proposed methods we will consider relative true positive fraction and relative false positive fraction as important indices.

As previously reported, a log binomial model that incorporates a generalized estimating equation (GEE) will be used to take into account correlations between multiple different test results in the same subject.⁴² A log link is used to allow direct estimation of the relative sensitivity and false positive rate (FPR). Note that: $1 - \text{FPR} = \text{Sp}$. A vector Z is included to account for additional covariates. The model (1) will be:

$$\log P(\text{test is positive}) = \beta_0 + \beta_1 * \text{NAFLD}_+ + \beta_2 * \text{Test}_2 + \beta_3 * \text{NAFLD}_+ * \text{Test}_2 + \beta_4 * Z$$

The anti-log of β_0 (i.e., e^{β_0}) is the probability that test 1 is positive given there is no disease = FPR for test 1, $e^{\beta_0 + \beta_2}$ is then the probability that test 2 is positive when there is no NAFLD = FPR test 2. Controlling for covariates the adjusted FPR for test 1 = $e^{\beta_0 + \beta_4 Z}$ and for test 2 = $e^{\beta_0 + \beta_2 + \beta_4 Z}$. β_1 reflects the presence of disease. Therefore, the adjusted Se of test 1 is the probability that test 1 is positive given there is disease = $e^{\beta_0 + \beta_1 + \beta_4 Z}$ and the adjusted Se of test 2 = $e^{\beta_0 + \beta_1 + \beta_2 + \beta_3 + \beta_4 Z}$ where β_3 allows the relative Se and relative FPR to have different values. That is, the relative Se is then $e^{\beta_2 + \beta_3}$, the relative FPR is e^{β_2} .

Note that multiple biomarker and imaging can be included and compared in a single model. That is, while model (1) was developed for the estimation and comparison of performance for two tests, the model can be extended to concurrently examine and compare more than two tests by including more indicator variables for test type. Further, several methods can be combined (an algorithm) to determine an overall result. Specifically, to estimate the Se or Sp of 2+ methods in combination, a single binary variable (+/-) will be defined based on the algorithm of their results and, thus, is analyzed like an individual +/- test result. All confidence intervals will be estimated based on the robust variance estimator derived from the GEE model.

Using a similar approach we can simultaneously estimate both PPV and NPV for a given assay or algorithm in a single statistical model by using “agreement” (i.e., whether or not the assay is positive in the presence of disease and negative in the absence of disease) as the binary outcome variable. Model (2) will therefore be:

$$\log P(\text{agreement}) = \alpha_0 + \alpha_1 * \text{test_positive} + \alpha_2 * \text{test_type} + \alpha_3 * \text{test_positive} * \text{test_type} + \alpha_4 * Z$$

It can be used to estimate and compare PPVs of Test 1 and 2, $e^{\alpha_0 + \alpha_1 + \alpha_4 Z}$, and $e^{\alpha_0 + \alpha_1 + \alpha_2 + \alpha_3 + \alpha_4 Z}$ as well as the NPVs of Test 1 and 2, $e^{\alpha_0 + \alpha_4 Z}$ and $e^{\alpha_0 + \alpha_2 + \alpha_3 + \alpha_4 Z}$. The relative PPV is $e^{\alpha_2 + \alpha_3}$ and the relative NPV is e^{α_2} . We will compare different estimated Se and Sp obtained from the above-described method to assess its usefulness. In order to address potential over-fitting issue, we will cross validate the proposed techniques via K-fold cross validation procedure.

In secondary analyses, we will calculate the Se, Sp, PPV, NPV, and area under the receiver operating curve (AUROC) of each pre-established algorithm. Moreover, we will use a tree-based method to agnostically explore more complex algorithms for optimally detecting NAFLD.⁴³ Specifically, the data will be randomly split into training and test sets (2/3 vs 1/3 of the sample size). Cross-validation will be used to determine the optimal level of tree complexity. Then, an “optimal” algorithm to differentiate NAFLD severity (i.e. NAFL, NASH, and fibrosis) can be obtained based on a best pruned tree identified using this cross-validation. The pruned-tree not only produces a more interpretable tree but also minimizes the misclassification error. The misclassification error will then be examined on the test set. However, because the misclassification error is defined as the proportion of true positives and true negatives, it gives equal weight to a missed positive and to a missed negative. If sensitivity is considered more important than specificity then a false negative would be of greater concern. Therefore, we will vary the cost ratio between a false positive and false negative (for example, 1:2 vs 1:1) to re-calculate the optimal trees.

An advantage of using a tree method as compared to the traditional regression methods proposed above is that it presents a clearly defined, easy to understand algorithm for clinical practice to detect NAFLD severity. More importantly, this can include combinations that may not have seemed obvious based on the summary statistics provided by regression methods. Thus, these tree methods are hypothesis generating. On the other hand, tree methods may also present many algorithms that are impractical (e.g., too many serial assays, too costly), nor do they provide a rigorous statistical measure of the relative benefits of one algorithm versus another.

The limitation of the development of a single tree also includes that the result may not be robust. In other words, a small change of the data can cause a large change in the final estimated tree. Therefore, both a random forest approach and a boosting approach will be applied to the training set to construct the best prediction model that minimizes case-control misclassification error averaged over multiple bagged (i.e. bootstrapped) samples of the training set. These methods can produce a best prediction of case and control status but do not produce individual trees. However, these methods will allow us to assess the relative importance of each diagnostic measure in detecting NAFLD. These information can then be used by clinicians to construct an algorithm in order to achieve the “best” composite screening test tool. The tree-based approaches will be conducted using R functions `rpart` and

cforest in the *party* package (R 3.4.0: R Foundation for Statistical Computing, 2017, <https://www.R-project.org>).

Overall, a combination of both regression and tree methods will be used, with tree methods for generating novel hypothetical algorithms and regression methods to compare these algorithms to one another and to single tests, using standard concepts of Se, Sp, PPV, NPV. Cost-benefit analyses will also subsequently be used to assess the real-world practicality of the several best screening approaches generated using our analytic approach.

2.11 Sample Size and Power Calculation:

The target sample size of this study is 450 patients. This assumes that a third of all enrolled patients will go on to receive a liver biopsy (n=150 biopsied patients). We calculated our power for the study based on a single method, especially focusing on the ARFI procedure. As there are no prior studies among the children, we assume that under null hypothesis the sensitivity and specificity of ARFI is 60% , which is a conservative estimate as compared to data compiled in adults.²⁴ Assuming a 30% prevalence of fibrosis and a total sample size of 150 (which includes 45 subjects with fibrosis), we will achieve 83% power to detect a change in sensitivity from 0.6 to 0.8 and 99% power to detect a change in specificity from 0.6 to 0.8 using a two-sided binomial test at 5% level of significance. The actual significance level achieved by the sensitivity test is 0.03 and achieved by the specificity test is 0.04.

3. CONDUCT OF THE STUDY

3.1 Study Visit Overview

For the purpose of data analysis, patients are stratified into two distinct groups. Subjects in group 1 met all inclusion/exclusion criteria but *had not been tested* for alternative etiologies of hepatitis prior to enrollment. Subjects in group 2 met all inclusion/exclusion criteria but *did have previous testing* for non-NAFLD etiologies of liver disease (such as Wilson's disease, autoimmune hepatitis, viral hepatitis, etc.). By stratifying subjects into two distinct groups, we will be able to identify the yield of testing children with suspected NAFLD for other etiologies of liver disease (aim 2). Data from any biopsied patient, in either group, will be used to generate a diagnostic algorithm (aim 1).

3.2 Visit 1

All enrolled patients undergo a standard history and physical exam, paying particular attention to any “red flags” – such as rashes, chronic arthritis, splenomegaly, etc. – which might suggest a diagnosis other than NAFLD. The physical exam always includes an assessment for hepatosplenomegaly, jaundice, ascites, peripheral manifestations of liver disease and acanthosis. Anthropometric and demographic data are captured. Anthropometric data are defined as height, weight, BMI, BMI percentile, BMI z-score, age, waist circumference and blood pressure and demographic data as gender, race, ethnicity, the parents' country of origin, age at menstruation (as appropriate) and tanner stage..

Upon enrollment, all subjects undergo routine testing to rule out other etiologies of hepatitis or ALT elevation, if not previously performed, including the following: α -1 antitrypsin

(A1A) phenotype, ceruloplasmin, total immunoglobulin A (IgA), tissue transglutaminase (tTG), anti-nuclear antibody (ANA), anti-liver kidney microsomal antibody (anti-LKM Ab), anti-smooth muscle antibody (anti-SM Ab), hepatitis A immunoglobulin M (Hep A IgM), hepatitis A immunoglobulin G (Hep A IgG), hepatitis B surface antigen (Hep BsAg), hepatitis B surface antibody (Hep BsAb), hepatitis C antibody (Hep C Ab), gamma-glutamyl transferase (GGT), cytomegalovirus immunoglobulin M (CMV IgM), Epstein-Barr virus IgM (EBV IgM), prothrombin time (PT), partial prothromboplastin time (PTT), international normalization ratio (INR), complete blood count (CBC), creatine phosphokinase (CPK), thyroid stimulating hormone (TSH), free thyroxine (FT4), fasting glucose, fasting insulin, fasting lipid panel, and hemoglobin A_{1C}. All testing is performed via standard assays at our central institutional laboratory. A leukocyte lysosomal acid lipase (LAL) activity level was later added to this routine panel after commercial testing became available through Alexion Pharmaceuticals. LAL testing is sent overnight in prepared kits to a contracted laboratory at Drexel University. Patients with any positive testing for any condition undergo additional work up, testing, and treatment at the physician's discretion.

Subjects in both groups have a routine abdominal ultrasound, TE, and ARFI examination within 3 months of enrollment, though most tests were performed on the same day of enrollment. Group 2 patients complete the study upon undergoing a liver biopsy and no additional imaging tests are therefore obtained.

3.3. Follow-up Visits

Group 1 patients are followed clinically at 3-month intervals, at which time liver tests and GGT are obtained. After 6 months of follow up, FibroScan™ is repeated and additional laboratory testing are sent: iron studies (serum iron, total iron binding capacity, percent iron saturation and ferritin), erythrocyte sedimentation rate (ESR), c-reactive protein (CRP), vitamin E level, 25-OH vitamin D level, fasting insulin, fasting glucose, and fasting lipids. A liver biopsy is discussed with all families at 6 months of follow up if the ALT remained elevated, patients did not lose weight, and/or there are specific clinical concerns (e.g. elevated autoimmune markers, newly developed symptoms suggesting an alternative diagnosis, etc.). Liver biopsies are only performed for clinical indications and are not considered a research test. If no biopsy is performed, data collection continued for up to 2 years from the initial enrollment visit; otherwise, enrollment ends at the time of biopsy. The visit and data collection schedule is summarized in Table 3.

3.4 Specimen Banking

During the biopsy, a small piece of liver tissue less than 0.5 cm in length is collected for storage in our Fatty Liver Center Biorepository if extra tissue is left over. A total of 11 mL of blood is obtained at the time of liver biopsy but only half that amount (roughly 6 mL) is required for the biomarkers listed in Table 1. Extra serum and plasma (approximately 5 mL) are stored in the Fatty Liver Center Biorepository at Montefiore Medical Center for future research use at -80°C in 0.25 mL aliquots. All specimens are stored in the biorepository only after obtaining appropriate consent from the patient (as applicable) and family.

3.5 Data Collection and Storage

All data – clinical and anthropometric parameters, laboratory tests, ultrasound results, biomarker, biopsy and elastography results – are captured on paper forms and entered into REDCap, an online institutional database. This database is maintained by the Research Informatics Core (RIC) of the Harold and Muriel Block Institute for Clinical and Translation Research at Einstein and Montefiore.

Data are collected via electronic data capture forms and transferred to the database using an encrypted data transfer protocol. At the data center, hard disks are encrypted by industrial standards. Data access is role-based and the privileges of each role are defined by the authorized committee and enforced by the project owner. The RIC backs up its data periodically for safety and users are authenticated with each data accessing. Their activities, such as data entry, revision, and retrieving are audited at all times and a user activity report is available upon request. Among other roles, the RIC provides a central database to host the data, provide web-based data collection forms for data capture, and enforce the HIPAA compliance.

4. RESULTS

Enrollment in this study is ongoing, with 233 subjects having been screened. A total of 23 children/families (9.9%) have declined participation, leaving 210 enrolled subjects. Of these, 71 subjects have undergone liver biopsy (47% of target enrollment). A total of 27 patients (12.9%) have been lost to follow up, 32 (15.2%) completed the 2 year follow up period without a biopsy, and 75 (35.7%) are still being actively followed.

The mean age at enrollment is 12.2 (range 5.8 – 17.9) years and 68.6% are male. The majority of patients identified themselves as Hispanic (89%) and, of those, 96 (45%) have at least one parent of Mexican descent. The mean presenting ALT is 88.7 U/L (range 35 – 412) and BMI z-score 2.2 (range 1.1 – 3.4). A total of 25 children (12%) are overweight and 185 (88%) are obese (See Table 4).

Though FDA-approved for use in children, ARFI is not validated for use in a population of children with NAFLD and FibroScan™ was validated just recently – though this mixed study population contained relatively few patients with NAFLD.⁴⁴ Moreover, there are no set cutoff values for TE or ARFI in children with NAFLD. ARFI and TE values will therefore be reported in future publications after properly assessed.

5. DISCUSSION

Liver biopsy is an imperfect diagnostic test for NAFLD given its invasiveness, risks for serious morbidity, sampling bias, and relatively high cost. Furthermore, liver biopsy cannot be readily repeated to monitor disease progression and is an inadequate population-level screening test.

For these reasons, the development of non-invasive tests to diagnose NAFLD or at least to stratify risk – that is, the identification of children with NASH or hepatic fibrosis – is of

paramount importance. And although there have been considerable work developing non-invasive tests in adult populations, similar data in pediatrics are significantly more limited.

None of the non-invasive, commercially available tests in the United States are sensitive or specific enough to replace biopsy. Elastography, perhaps the most commonly used, is an imaging modality in which an external force is used to quantitate tissue stiffness (i.e. elasticity). There are several types of elastography but all work by applying a non-painful force to the liver, thereby producing a shear wave within the hepatic parenchyma. The velocity of the shear wave can subsequently be measured and the tissue elasticity calculated via the formula $E=3rV_s^2$ (whereas E is elasticity, V_s is the shear velocity, and r is the mass density). The mechanism by which force is applied and the shear wave velocity measured separate elastography types but, in general, this approach has demonstrated strong ability to “rule out” fibrosis or “rule in” cirrhosis. Elastography cannot easily differentiate between intermediate fibrosis stages, however, and few studies have examined the use of elastography in children with NAFLD. Moreover, elastography types differ in their ability to detect hepatic steatosis.

Two of the most common forms of elastography are used in this study: TE and ARFI. In adults, TE has a sensitivity of 67–88% and a specificity of 70–84% in identifying significant (F2) fibrosis.^{25,26,45–49} TE can also simultaneously assess hepatic steatosis via the Controlled Attenuation Parameter (CAP) module, though this has been largely unstudied in large pediatric NAFLD cohorts. ARFI has a reported sensitivity and specificity of 79% and 85%, respectively, for diagnosing significant fibrosis.^{24,50} Only one study has been published utilizing ARFI in children with NAFLD but, in this European cohort, the NAFLD diagnosis was not made via biopsy.⁵¹ Another form of elastography, magnetic resonance elastography (MRE), was not analyzed in this study. While MRE has somewhat comparable accuracy to ultrasound-based elastography, it has some significant drawbacks such as cost and increased test time.⁵² And though it's becoming more widely available, MRE is still performed only at specialized centers, which limits accessibility to relatively few patients.

This study also utilizes the largest number of serum biomarkers within a single cohort published to date. Doing so allows us to examine multiple biomarkers or biomarker panels head-to-head without cross-study comparisons. In several cases, this may be the first serum biomarker data published in pediatrics. Second, combining biomarkers that are individually insufficient in stratifying disease may lead to the development of new algorithms better capable of doing so. And third, we will be able to study the use of biomarker panels generated in adult cohorts or validate those previously studied in children.

This will be one of the largest studies utilizing liver biopsy in children with NAFLD and the most comprehensive such study to examine non-invasive tests ever published. In short, this work is a critical step towards generating a non-invasive diagnostic algorithms and, hopefully, limiting the use of biopsy in children. Moreover, this work will provide the first prospective data assessing the yield of testing children with suspected NAFLD for alternative etiologies of liver disease, which are needed to guide future professional society guidelines and research.

6. SUMMARY

This cross-sectional study, with up to 2-years of prospective follow-up, is one of the largest studies utilizing biopsy in children with NAFLD and the most comprehensive such study examining non-invasive tests published.

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SOURCES

1. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118(4):1388–1393. [PubMed: 17015527]
2. Welsh JA, Karpen S, Vos MB. Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988–1994 to 2007–2010. *J Pediatr* 2013;162(3):496–500.e491. [PubMed: 23084707]
3. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* (Baltimore, Md.) 2004;40(6):1387–1395.
4. Bedossa P, Poitou C, Veyrie N, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology* (Baltimore, Md.) 2012;56(5):1751–1759.
5. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* (Baltimore, Md.) 2005;41(6):1313–1321.
6. Lebensztejn DM, Wojtkowska M, Skiba E, Werpachowska I, Tobolczyk J, Kaczmarek M. Serum concentration of adiponectin, leptin and resistin in obese children with non-alcoholic fatty liver disease. *Advances in medical sciences* 2009;54(2):177–182. [PubMed: 20022856]
7. Tanwar S, Trembling PM, Guha IN, et al. Validation of terminal peptide of procollagen III for the detection and assessment of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease. *Hepatology* (Baltimore, Md.) 2013;57(1):103–111.
8. Malik R, Chang M, Bhaskar K, et al. The clinical utility of biomarkers and the nonalcoholic steatohepatitis CRN liver biopsy scoring system in patients with nonalcoholic fatty liver disease. *Journal of gastroenterology and hepatology* 2009;24(4):564–568. [PubMed: 19378390]
9. Abdelaziz R, Elbasel M, Esmat S, Essam K, Abdelaaty S. Tissue Inhibitors of Metalloproteinase-1 and 2 and Obesity Related Non-Alcoholic Fatty Liver Disease: Is There a Relationship. *Digestion* 2015;92(3):130–137. [PubMed: 26329758]
10. Nobili V, Alisi A, Torre G, et al. Hyaluronic acid predicts hepatic fibrosis in children with nonalcoholic fatty liver disease. *Translational research : the journal of laboratory and clinical medicine* 2010;156(4):229–234. [PubMed: 20875899]
11. Lebensztejn DM, Wierzbicka A, Socha P, et al. Cytokeratin-18 and hyaluronic acid levels predict liver fibrosis in children with non-alcoholic fatty liver disease. *Acta biochimica Polonica* 2011;58(4):563–566. [PubMed: 22140659]
12. Kumagai E, Mano Y, Yoshio S, et al. Serum YKL-40 as a marker of liver fibrosis in patients with non-alcoholic fatty liver disease. *Scientific reports* 2016;6:35282. [PubMed: 27739482]
13. Chen J, Zhu Y, Zheng Q, Jiang J. Serum cytokeratin-18 in the diagnosis of non-alcoholic steatohepatitis: A meta-analysis. *Hepatology research : the official journal of the Japan Society of Hepatology* 2014;44(8):854–862. [PubMed: 23834322]

14. Mandelia C, Collyer E, Mansoor S, et al. Plasma Cytokeratin-18 Level As a Novel Biomarker for Liver Fibrosis in Children With Nonalcoholic Fatty Liver Disease. *Journal of pediatric gastroenterology and nutrition* 2016;63(2):181–187. [PubMed: 26835904]
15. Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology* (Baltimore, Md.) 2009;50(4):1072–1078.
16. Fitzpatrick E, Mitry RR, Quaglia A, Hussain MJ, DeBruyne R, Dhawan A. Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD. *Journal of pediatric gastroenterology and nutrition* 2010;51(4):500–506. [PubMed: 20808246]
17. Das SK, Balakrishnan V. Role of cytokines in the pathogenesis of non-alcoholic Fatty liver disease. *Indian journal of clinical biochemistry : IJCB* 2011;26(2):202–209. [PubMed: 22468051]
18. Yang HR, Kim HR, Kim MJ, Ko JS, Seo JK. Noninvasive parameters and hepatic fibrosis scores in children with nonalcoholic fatty liver disease. *World journal of gastroenterology : WJG* 2012;18(13):1525–1530. [PubMed: 22509085]
19. Feldstein AE, Alkhouri N, De Vito R, Alisi A, Lopez R, Nobili V. Serum cytokeratin-18 fragment levels are useful biomarkers for nonalcoholic steatohepatitis in children. *The American journal of gastroenterology* 2013;108(9):1526–1531. [PubMed: 23752877]
20. Manco M, Marcellini M, Giannone G, Nobili V. Correlation of serum TNF-alpha levels and histologic liver injury scores in pediatric nonalcoholic fatty liver disease. *American journal of clinical pathology* 2007;127(6):954–960. [PubMed: 17509993]
21. Nobili V, Alkhouri N, Alisi A, et al. Retinol-binding protein 4: a promising circulating marker of liver damage in pediatric nonalcoholic fatty liver disease. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2009;7(5):575–579. [PubMed: 19268270]
22. Alisi A, Manco M, Devito R, Piemonte F, Nobili V. Endotoxin and plasminogen activator inhibitor-1 serum levels associated with nonalcoholic steatohepatitis in children. *Journal of pediatric gastroenterology and nutrition* 2010;50(6):645–649. [PubMed: 20400911]
23. Shen J, Chan HL, Wong GL, et al. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. *Journal of hepatology* 2012;56(6):1363–1370. [PubMed: 22314419]
24. Friedrich-Rust M, Nierhoff J, Lupsor M, et al. Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis. *Journal of viral hepatitis* 2012;19(2):e212–219. [PubMed: 22239521]
25. Fitzpatrick E, Quaglia A, Vimallesvaran S, Basso MS, Dhawan A. Transient elastography is a useful noninvasive tool for the evaluation of fibrosis in paediatric chronic liver disease. *Journal of pediatric gastroenterology and nutrition* 2013;56(1):72–76. [PubMed: 22922372]
26. Nobili V, Vizzutti F, Arena U, et al. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. *Hepatology* (Baltimore, Md.) 2008;48(2):442–448.
27. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *Journal of pediatric gastroenterology and nutrition* 2017;64(2):319–334. [PubMed: 28107283]
28. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* (Baltimore, Md.) 2018;67(1):328–357.
29. Rudolph B, Rivas Y, Kulak S, et al. Yield of diagnostic tests in obese children with an elevated alanine aminotransferase. *Acta paediatrica* (Oslo, Norway : 1992) 2015.
30. Vajro P, Lenta S, Socha P, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. *Journal of pediatric gastroenterology and nutrition* 2012;54(5):700–713. [PubMed: 22395188]
31. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American

Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012;142(7):1592–1609. [PubMed: 22656328]

32. Sanyal AJ, Brunt EM, Kleiner DE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology* (Baltimore, Md.) 2011;54(1):344–353.
33. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* (Baltimore, Md.) 2003;38(2):518–526.
34. Forns X, Ampurdanes S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* (Baltimore, Md.) 2002;36(4 Pt 1):986–992.
35. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* (Baltimore, Md.) 2006;43(6):1317–1325.
36. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* (Baltimore, Md.) 2007;45(4):846–854.
37. Nobili V, Alisi A, Vania A, Tiribelli C, Pietrobelli A, Bedogni G. The pediatric NAFLD fibrosis index: a predictor of liver fibrosis in children with non-alcoholic fatty liver disease. *BMC medicine* 2009;7:21. [PubMed: 19409076]
38. Koot BG, van der Baan-Slootweg OH, Bohte AE, et al. Accuracy of prediction scores and novel biomarkers for predicting nonalcoholic fatty liver disease in obese children. *Obesity* (Silver Spring, Md.) 2013;21(3):583–590.
39. Alkhouri N, Mansoor S, Giammaria P, Liccardo D, Lopez R, Nobili V. The development of the pediatric NAFLD fibrosis score (PNFS) to predict the presence of advanced fibrosis in children with nonalcoholic fatty liver disease. *PloS one* 2014;9(8):e104558. [PubMed: 25121514]
40. Cales P, Laine F, Boursier J, et al. Comparison of blood tests for liver fibrosis specific or not to NAFLD. *Journal of hepatology* 2009;50(1):165–173. [PubMed: 18977552]
41. Soroudi N, Wylie-Rosett J, Mogul D. Quick WAVE Screener: a tool to address weight, activity, variety, and excess. *The Diabetes educator* 2004;30(4):616, 618–622, 626–618 passim.
42. Xue X, Kim MY, Castle PE, Strickler HD. A new method to address verification bias in studies of clinical screening tests: cervical cancer screening assays as an example. *Journal of clinical epidemiology* 2014;67(3):343–353. [PubMed: 24332397]
43. Gareth James DW, Hastie Trevor, Tibshirani Robert. *An Introduction to Statistical Learning: with Applications in R* Springer Publishing Company, Incorporated; 2014:265–300.
44. Lee CK, Mitchell PD, Raza R, Harney S, Wiggins SM, Jonas MM. Validation of Transient Elastography Cut Points to Assess Advanced Liver Fibrosis in Children and Young Adults: The Boston Children's Hospital Experience. *J Pediatr* 2018.
45. Lupsor M, Badea R, Stefanescu H, et al. Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis. *Journal of gastrointestinal and liver diseases : JGLD* 2010;19(1):53–60. [PubMed: 20361076]
46. Petta S, Di Marco V, Camma C, Butera G, Cabibi D, Craxi A. Reliability of liver stiffness measurement in non-alcoholic fatty liver disease: the effects of body mass index. *Alimentary pharmacology & therapeutics* 2011;33(12):1350–1360. [PubMed: 21517924]
47. Wong VW, Vergniol J, Wong GL, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* (Baltimore, Md.) 2010;51(2):454–462.
48. Yoneda M, Yoneda M, Mawatari H, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2008;40(5):371–378.
49. Lee MS, Bae JM, Joo SK, et al. Prospective comparison among transient elastography, supersonic shear imaging, and ARFI imaging for predicting fibrosis in nonalcoholic fatty liver disease. *PloS one* 2017;12(11):e0188321. [PubMed: 29176844]

50. Noruegas MJ, Matos H, Goncalves I, Cipriano MA, Sanches C. Acoustic radiation force impulse-imaging in the assessment of liver fibrosis in children. *Pediatric radiology* 2012;42(2):201–204. [PubMed: 22002843]
51. Berna-Serna JD, Sanchez-Jimenez R, Velazquez-Marin F, et al. Acoustic radiation force impulse imaging for detection of liver fibrosis in overweight and obese children. *Acta radiologica* (Stockholm, Sweden : 1987) 2018;59(2):247–253.
52. Schwimmer JB, Behling C, Angeles JE, et al. Magnetic resonance elastography measured shear stiffness as a biomarker of fibrosis in pediatric nonalcoholic fatty liver disease. *Hepatology* (Baltimore, Md.) 2017;66(5):1474–1485.

Figure 1.
Study Design

Table 1:**Biomarkers Measured**

Biomarkers measured in this study. **CK 18** = cytokeratin 18; **CK 18 frag** = CK18 fragments; **CRP** = c-reactive protein; **ELISA** = enzyme-linked immunosorbent assay; **FGF-21** = Fibroblast growth factor 21; **HA** = Hyaluronic acid; **IL-6** = interleukin 6; **PAI-1** = plasminogen activator inhibitor-1; **PIIINP** = Amino-terminal propeptide of type III collagen; **RBP-4** = retinol binding protein-4; **RIA** = Radioimmunoassay ; **sRAGE** = soluble receptor for advanced glycation end products; **TIMP-1** = Tissue inhibitor of metalloproteinase 1; **TNF- α** = Tumor necrosis factor- α ; **YKL-40** = human cartilage glycoprotein-3

Test	Method	Assay Manufacturer/Catalogue #
HA	ELISA	R & D Systems / DHYAL0
PIIINP	ELISA	Cloud Clone / SEA573Hu
TIMP-1	ELISA	R & D Systems / DTM100
YKL-40	ELISA	Quidel / 8020
CK18	ELISA	Diapharma / P10011
CK18 fragments	ELISA	Diapharma / P10020
A2-Macroglobulin	ELISA	Abcam / ab10888
Adiponectin	RIA	EMD Millipore / HADP-61HK
Leptin	ELISA	Mercodia / 10-1199-01
sRAGE	ELISA	R & D Systems / DRG00
FGF-21	ELISA	R & D Systems / DF2100
RBP-4	ELISA	R & D Systems / DRB400
PAI-1	ELISA	BioMedica Diagnostics / 822
TNF- α	ELISA	Alpco / 45-TNFHU-E01
IL-6	ELISA	R & D Systems / Q6000B
High Sensitivity CRP	UV/VIS	Beckman Coulter / OSR6199

Table 2:
Biomarker Algorithms to be Tested with Pediatric Data

Biomarker algorithms or panels utilized (primarily) in adults but unvalidated for use in children

AST/platelet ratio (APRI) ³³	$\frac{\left(\frac{AST (U/L)}{AST \text{ upper limit normal}}\right)}{platelet \text{ count}(10^9/L)} \times 100$
Forns index ³⁴	$7.811 - 3.131 \times \ln[platelet \text{ count}(10^9/L)] + 0.781 \times \ln[GGT (U/L)] + 3.467 \times \ln(\text{age in years}) - 0.104 \times \text{cholesterol (mg/dL)}$
FIB-4 ³⁵	$\frac{Age (years) \times AST (U/L)}{platelet \text{ count}(10^9/L) \times \sqrt{ALT(U/L)}}$
NAFLD fibrosis score ³⁶	$-1.675 + 0.037 \times \text{age (years)} + 0.094 \times BMI(kg/m^2) + 1.13 \times \frac{IFG}{\text{diabetes (yes = 1, no = 0)}} + 0.99 \times \frac{AST}{ALT} - 0.013 \times \text{platelet } (\times 10^9/l) - 0.66 \times \text{albumin(g/dl)}$
Pediatric NAFLD Fibrosis Index (PNFI) ³⁷	$PNFI = \frac{1}{1 + e^{-lp}} \times 10$ where $lp = -6.539 \times \log_e[age (years)] + 0.207 \times \text{waist} + 1.957 \times \log_e[\text{triglycerides (mg/dL)}] - 10.074$
NAFLD Liver Fat Score ³⁸	$-2.89 + 1.18 \times \text{Metabolic Syndrome (yes=1, no=0)} + 0.45 \times \text{Type 2 Diabetes(yes=1, no=0)} + 0.15 \times \text{Insulin (mU/L)} + 0.04 \times \text{AST (U/L)} - 0.94 \times \text{AST/ALT}$
Hepatic steatosis index ³⁸	$\frac{e^{0.315 \times BMI + 2.421 \times \frac{ALT}{AST} + 0.630 \times \text{Diabetes (yes = 1, no = 0)} - 9.960}}{1 + e^{0.315 \times BMI + 2.421 \times (ALT)/AST + 0.630 \times \text{Diabetes (yes = 1, no = 0)} - 9.960}}$
Pediatric NAFLD Fibrosis Score ³⁹	$p = \left[\frac{e^Z}{1 + e^Z} \right] \times 100$ where $z = 1.1 + (0.34 \times \sqrt{ALT}) + (0.002 \times \text{alkaline phosphatase}) - [1.1 \times \log(\text{platelets})] - (0.02 \times GGT)$
Pediatric NAFLD score ³⁸	$\frac{e^{-10.79 + 0.22 \times \frac{waist}{height} + 0.8 \times ALT + 0.82 \times \text{HOMa} - 0.77 \times \text{adiponectin}}}{1 + e^{-10.79 + 0.22 \times \frac{waist}{height} + 0.8 \times ALT + 0.82 \times \text{HOMa} - 0.77 \times \text{adiponectin}}}$
Fibrometer ⁴⁰	$0.4184 \times \text{glucose (mmol/l)} + 0.0701 \times \text{AST} + 0.0008 \times \text{ferritin (ug/L)} - 0.0102 \times \text{platelet(G/l)} - 0.0260 \times \text{ALT} + 0.0459 \times \text{body weight(kg)} + 0.0842 \times \text{age (year)} + 11.6226$

Table 3:
Visit and Data Collection Schedule.

Detailed medical exam included assessment for hepatosplenomegaly, jaundice, ascites, peripheral manifestations of liver disease, and acanthosis. Anthropometric data defined as height, weight, BMI, BMI percentile, BMI z-score, age at enrollment, blood pressure, gender, race, ethnicity, age at menstruation (as appropriate), waist circumference, and tanner stage.

Assessment / Procedure	Enrollment	Follow up Visits (months from enrollment)			Biopsy
		3	6	6-24	
Consent	X	-	-	-	-
Demographic data	X	-	-	-	-
Baseline (B) or interim (I) medical history	B	I	I	I	I
Medication use	X	-	-	-	-
Alcohol use	X	-	-	-	-
Parenteral nutrition use	X	-	-	-	-
Family history	X	-	-	-	-
Detailed physical exam	X	X	X	X	X
Anthropometric data	X	X	X	X	X
Waist circumference	X	-	X	-	X
Tanner stage	X	-	X	-	-
Ultrasound	X	-	-	-	-
ARFI	X	-	-	-	X
TE	X	-	X	-	X
WAVE Screener	X	-	-	-	-
Biomarkers	•	-	-	-	X
<i>Labs</i>					
Liver Tests	X	X	X	X	X
GGT	X	X	X	X	X
PT /INR	X	-	X	-	X
PT T	X	-	-	-	-
AlA phenotype	X	-	-	-	-
Ceruloplasmin	X	-	-	-	-
tTG	X	-	-	-	-
Total IgA	X	-	-	-	-
Total IgG	X	-	-	-	-
ANA	X	-	-	-	-
Anti-LKM Ab	X	-	-	-	-
Anti-SMA Ab	X	-	-	-	-
Hep A IgM	X	-	-	-	-
Hep A IgG	X	-	-	-	-
Hep BsAg	X	-	-	-	-
Hep BsAb	X	-	-	-	-
Hep C Ab	X	-	-	-	-

Assessment / Procedure	Enrollment	Follow up Visits (months from enrollment)			Biopsy
		3	6	6-24	
EBV IgM	X	-	-	-	-
CMV IgM	X	-	-	-	-
CPK	X	-	-	-	-
T SH	X	-	-	-	-
FT 4	X	-	-	-	-
CBC	X	-	X	-	X
Chemistry/fasting glucose	X	-	X	-	X
Fasting insulin	X	-	X	-	X
Fasting lipid	X	-	X	-	X
Hgb A1C	X	-	X	-	-
Vitamin E	-	-	X	-	X
Vitamin D	-	-	X	-	X
Iron studies	-	-	X	-	X
ESR	-	-	X	-	X
CRP	-	-	X	-	X

Table 4:**Patient Characteristics**

Results are expressed as mean (range) unless otherwise specified. The total number of biopsies analyzed at the time of publication is 58.

Characteristic	Result
Age, years	12.2 (5, 17)
Male, N (%)	144 (68.6)
Hispanic, N (%)	187 (89)
Waist Circumference, cm	99.2 (11.9, 137.3)
BMI, kg/m ²	30.8 (20.7, 47.8)
BMI Z-Score	2.2 (1.1, 3.4)
Overweight, N (%)	25 (12)
Obese, N (%)	185 (88)
GGT, U/L	38.6 (7, 154)
ALT, U/L	88.7 (35, 412)
LDL, mg/dL	95.3 (23, 400)
HDL, mg/dL	40.5 (16, 75)
HgbA _{1c} , %	5.8 (4.5, 14.7)
NAS on biopsy	4.35 (2, 7)
Fibrosis stage, N (%)	
0) None	30 (51.7)
1) Perisinusoidal or periportal	22 (37.9)
1A) Mild, zone 3, perisinusoidal	11 (19)
1B) Moderate, zone 3 perisinusoidal	0
1C) Portal/periportal	11 (19)
2) Perisinusoidal and portal/periportal	5 (8.6)
3) Bridging fibrosis	1 (1.8)
4) Cirrhosis	0