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MRE combined with FIB-4 (MEFIB) Index in detection of candidates for pharmacologic treatment of NASH related fibrosis

Jinho Jung¹, Rohan R Loomba¹, Kento Imajo², Egbert Madamba¹, Sanil Gandhi¹, Ricki Bettencourt¹, Seema Singh¹, Carolyn Hernandez¹, Mark A. Valasek³, Cynthia Behling⁴, Lisa Richards¹, Kathryn Fowler⁵, Claude Sirlin⁵, Atsushi Nakajima², Rohit Loomba^{1,6}

¹NAFLD Research Center, Division of Gastroenterology and Hepatology, Department of Medicine, University of California at San Diego, La Jolla, United States

²Yokohama City University Graduate School of Medicine, Department of Gastroenterology, Yokohama, Japan

³University of California at San Diego, Department of Pathology, La Jolla, United States

⁴Sharp Medical Group, Department of Pathology, San Diego, United States

⁵University of California at San Diego, Liver Imaging Group, Department of Radiology, La Jolla, United States

⁶University of California at San Diego, Department of Family Medicine and Public Health, La Jolla, United States

Abstract

Please address correspondence to: Rohit Loomba, MD, MHSc, ACTRI Building, 1W202, 9452 Medical Center Drive, La Jolla, CA 92037, Ph: 858-246-2201, Fax: 858-246-2255, roloomba@ucsd.edu Web: <http://fattyLiver.ucsd.edu>.

Guarantor(s) of the article: Rohit Loomba

AUTHOR CONTRIBUTIONS

Jinho Jung: Analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript, approved final submission.

Rohan R Loomba: Analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript, approved final submission.

Kento Imajo: data analysis, critical revision of the manuscript, approved final submission

Egbert Madamba: patient visits, critical revision of the manuscript, approved final submission

Sanil Gandhi: data collection, critical revision of the manuscript, approved final submission.

Ricki Bettencourt: data analysis, critical revision of the manuscript, approved final submission

Seema Singh: data collection, critical revision of the manuscript, approved final submission.

Carolyn Hernandez: patient visits, critical revision of the manuscript, approved final submission

Mark A. Valasek: data analysis, critical revision of the manuscript, approved final submission

Cynthia Behling: data analysis, critical revision of the manuscript, approved final submission

Lisa Richards: patient visits, critical revision of the manuscript, approved final submission

Kathryn Fowler: data analysis, critical revision of the manuscript, approved final submission

Claude Sirlin: data analysis, critical revision of the manuscript, approved final submission

Atsushi Nakajima: data analysis, critical revision of the manuscript, approved final submission

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Conflict of interests: Dr. Rohit Loomba serves as a consultant or advisory board member for Arrowhead Pharmaceuticals, AstraZeneca, Bird Rock Bio, Boehringer Ingelheim, Bristol-Myer Squibb, Celgene, Cirus, CohBar, Conatus, Eli Lilly, Galmed, Gemphire, Gilead, Glympse bio, GNI, GRI Bio, Intercept, Ionis, Janssen Inc., Merck, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Pfizer, Prometheus, Sanofi, Siemens, and Viking Therapeutics. In addition, his institution has received grant support from Allergan, Boehringer-Ingelheim, Bristol-Myers Squibb, Cirus, Eli Lilly and Company, Galectin Therapeutics, Galmed Pharmaceuticals, GE, Genfit, Gilead, Intercept, Grail, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, NuSirt, Pfizer, pH Pharma, Prometheus, and Siemens. He is also co-founder of Liponexus, Inc.

Objective: Patients with non-alcoholic fatty liver disease (NAFLD) with stage 2 fibrosis are at increased risk for liver related mortality and are candidates for pharmacologic therapies for treatment of NAFLD. The aim of this prospective cohort study to examine the diagnostic accuracy of magnetic resonance elastography (MRE) combined with FIB-4 in diagnosing stage 2 fibrosis (candidates for pharmacologic therapies).

Design: This is a cross-sectional analysis of a prospective cohort (UCSD-NAFLD) including 238 consecutive patients with contemporaneous MRE and biopsy-proven NAFLD. NASH-CRN-Histologic Scoring System was used to assess histology. The radiologist and pathologist were blinded to clinical, pathological, and imaging data, respectively. Receiver operating characteristics were determined to examine the diagnostic accuracy of MRE and FIB-4 for diagnosis of stage 2 fibrosis in NAFLD. We then validated these findings in an independent validation cohort derived from Yokohama City University in Japan (Japan-NAFLD Cohort; N=222 patients).

Results: In the UCSD-NAFLD (training) Cohort, MRE demonstrated a clinically significant diagnostic accuracy for the detection of stage 2 fibrosis with an AUROC of 0.93 (95% CI: 0.90-0.97) versus FIB-4 with an AUROC of 0.78 (0.71-0.85), which was both clinically and statistically significant ($p<0.0001$). We then combined MRE with FIB-4 (MRE 3.3kPa and FIB-4 1.6) to develop a clinical-prediction-rule to rule in stage 2 fibrosis patients which had positive predictive value (PPV) of 97.1% ($p<0.02$) in the UCSD-NAFLD Cohort [AUROC of 0.90(0.85-0.95)] which remained significant at PPV of 91.0% ($p<0.003$) in the Japan-NAFLD Cohort [AUROC of 0.84(0.78-0.89)].

Conclusion: MRE combined with FIB-4 (MEFIB) index may be used for non-invasive identification of candidates for (stage 2 fibrosis) pharmacologic therapy among patients with NAFLD with a high positive predictive value.

Keywords

NASH; liver stiffness; fibrosis progression; MRE

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an increasingly common liver disease that affects worldwide population and is also prevalent in the United States (US) [1, 2, 3]. NAFLD is broadly categorized into two clinical subtypes including nonalcoholic fatty liver (NAFL), which is the non-progressive form of liver disease, and nonalcoholic steatohepatitis (NASH), which is the progressive form of liver disease. NASH is a clinical-pathologic diagnosis characterized by presence of hepatic steatosis, lobular inflammation, ballooning with or without peri-sinusoidal fibrosis in individuals who consume little or no alcohol and do not have a secondary cause for hepatic steatosis. NASH can lead to progressive liver injury, significant fibrosis, cirrhosis and end-stage liver disease leading to liver related morbidity and mortality [4]. NASH is now the second leading indication for liver transplant in the US [5]. However, currently there are no FDA- approved therapies for the treatment of NASH and NASH related fibrosis.

Among patients with NAFLD, the stage of fibrosis portends a worse prognosis [6, 7, 8]. Recent meta-analyses suggest that NAFLD patients with stage 2 fibrosis have significantly

increased risk of progression to cirrhosis and liver related mortality [9, 10, 11]. Guidance by FDA and EMA requires inclusion of patients who have stage 2 fibrosis for the purposes of registration trials for assessment of therapeutic response among patients with NAFLD. Resolution of NASH on histology or reversal of fibrosis by 1 or more stages is required for subpart H approval of medications for the treatment of NASH-related fibrosis. Currently there are five drugs in Phase 3 trials for the treatment of NASH related fibrosis [12]. In the near future, newer therapies will become available for the treatment of NASH.

To determine whether a patient is a candidate for a pharmacologic therapy for NASH related fibrosis, we currently require a liver biopsy assessment as the gold standard [13]. However, liver biopsy is limited due to sampling errors, intra-, and inter-observer variability [14]. Furthermore, it is an invasive procedure and associated with adverse effects such as pain, risk of infection, bleeding, perforation and extremely rarely even death [15]. Given the burden of NAFLD afflicting approximately 80-100 million Americans, liver biopsy assessment for the detection for candidacy for the treatment of NASH related fibrosis is impractical [16, 17].

To address these problems, various non-invasive techniques have been proposed such as FIB-4. FIB-4 is a blood test which utilizes widely available and simple variables such as age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and platelet count to detect patients with advanced fibrosis (stage 3 & 4) [18]. However, FIB-4 is suboptimal in detecting lower stages of fibrosis and does not help in ruling in who needs to be treated but rather helpful in ruling out who does not need to be treated [19, 20].

Liver stiffness assessment by ultrasound-based modalities including vibration-controlled-transient elastography (VCTE), acoustic radiation impulse force imaging (ARFI) and shear wave elastography have also been used to risk stratify patients with NAFLD [18, 21]. Magnetic resonance imaging (MRI) based assessment has also been proposed such as magnetic resonance elastography (MRE). MRE is a noninvasive, imaging based, diagnostic tool that allows quantitative measures of liver stiffness as it correlates with fibrosis stage on liver histology [6, 22, 23]. Previous studies have also shown that MRE is more accurate than VCTE and ARFI in the detection of liver fibrosis among patients with NAFLD [23, 24, 25, 26, 27, 28].

As biopsy has its limitations, noninvasive identification of candidates with high positive predictive value is a major unmet need for pharmacologic treatment [29]. The advantage of combining two unrelated methods, such as imaging and serum biomarkers, has been proposed for staging liver fibrosis in chronic liver diseases [30, 31]. In application to NAFLD, serial and paired combinations have shown promising results in diagnosing stage 3 fibrosis [32]. However, there are limited data for whether these may be utilized for identification of patients with stage 2 fibrosis, and what cut-points may be used [18].

Current non-invasive tests (such as FIB-4 and VCTE) have a high negative predictive value but a low positive predictive value so are unable to rule-in a patient who needs to be treated. Therefore, there is a major unmet need to examine if a combination of advanced imaging tests (such as MRE) and serum markers will yield a high enough positive predictive value

for a clinician to rule in clinically significant disease that needs pharmacologic treatment in NAFLD.

The aim of this study was to examine whether a combination of MRE plus FIB-4 may be used for non-invasive identification of candidates for (stage 2 fibrosis) pharmacologic therapy among well-characterized patients with NAFLD with liver biopsy assessment using NASH CRN histologic scoring system as the reference standard. We then validated these findings in a geographically and ethnically diverse external validation cohort.

METHODS

UCSD-NAFLD Cohort

Study Design—This study includes a prospective cohort (UCSD-NAFLD) including 238 well-characterized, consecutively recruited patients with biopsy-proven NAFLD who had a clinical indication for a liver biopsy assessment (Fig 1). After a careful exclusion of other causes for liver diseases (see inclusion and exclusion criteria section), the study participants underwent a detailed standardized research visit that included history, physical examination, biochemical validation and an advanced MRI assessment including 2D-MRE at the UCSD NAFLD Research Center. These patients were enrolled from January 2012 through January 2020. All patients provided written informed consent prior to enrollment. The study was approved by UCSD Institutional Review Board as well as the UCSD Clinical and Translational Research Institute.

Inclusion and Exclusion Criteria—Patients who are at least 18 years of age with biopsy-proven NAFLD or NAFLD-related cirrhosis were included in the study. Written informed consent was required to participate in the study. Patients with evidence of alcoholic liver disease were excluded based histological or clinical evidence. History or conditions that are associated with hepatic steatosis were used to screen patients such as short bowel syndrome, bariatric surgery, hepatitis B&C, and HIV infection. Patients with any other diseases determined by clinical investigator to confound the purpose of the study have been excluded. As the study requires MR usage, patients with claustrophobia, metallic implants, or who were not able to fit in MR chambers were excluded. Detailed exclusion criteria are provided in Supplementary materials.

Magnetic Resonance Elastography—Magnetic Resonance Elastography (MRE) was performed at UCSD MR3T Research Laboratory using GE 3T research scanner (E Signa EXCITE HDxt, GEHealthcare, Waukesha, WI) as previously described [33, 34]. All patients were fasting for at least four hours before the visit. An acoustic passive driver was placed on the patient's body anterior to the liver. It was held in place with an elastic band and a plastic tube connected the passive driver to an active driver outside the MRI room. By producing shear waves from active to passive driver of 60 Hz, liver vibrations were measured. Specialized software processed the image for the whole liver to calculate an elastogram. Radiologists were blinded to clinical and pathology data.

Histological Evaluation—All patients with a clinical indication for a liver biopsy underwent a liver biopsy procedure using a 16 gauge Biopince needle under strict aseptic

precautions. All histologic assessment for the training cohort were systematically assessed by an expert liver pathologist who was blinded to both imaging and clinical data. Biopsy results were scored based upon the NASH CRN Histologic Scoring System [35]. Steatosis was scored from 0 to 3 (0,1,2,3). Lobular inflammation was scored from 0 to 3 (0,1,2,3). Hepatocellular ballooning was scored from 0 to 2 (0,1,2). Steatosis, lobular inflammation, and ballooning scores were combined to obtain the total NAFLD Activity Score (NAS) score ranging from 0 to 8. NASH was defined by presence of hepatic steatosis with lobular inflammation and definite ballooning with or without fibrosis. Fibrosis was staged from 0 to 4 [35]. Patients with stage 0 had no fibrosis. Patients with stage 1 fibrosis included those with either mild-moderate peri-sinusoidal or peri-portal fibrosis. Patients with stage 2 fibrosis included those with both peri-sinusoidal and portal/peri-portal fibrosis, stage 3 included those with bridging fibrosis, and stage 4 included those with cirrhosis. These categories were employed prior to statistical evaluation.

Japan-NAFLD Cohort

Study Design—Japan-NAFLD Cohort patients were recruited from Yokohama City University Hospital, Yokohama, Japan. Total of 222 patients enrolled from March, 2013 to March, 2019. All patients had received a biopsy paired with MRE within 6 month. Patients with history of excessive alcohol consumption (>140g for male and >70g for women per week), and other liver diseases such as chronic hepatitis, drugs that are associated with fatty liver, weight reduction, renal disease or thyroid disease were excluded. Ethics Committee of Yokohama City University Hospital approved the study protocol and all subjects were provided with written consent prior to examination.

Histological Evaluation—Liver biopsy samples were obtained from all patients diagnosed with NAFLD. Patients diagnosed with NASH-associated cirrhosis were defined clinically and pathologically [36]. 16 gauge needle biopsy was used according to standard protocol to obtain 2 specimen. An adequate liver sample was defined as >20mm in length and/or >10 portal tracts. Pathologists were blinded to clinical and radiology data.

Magnetic Resonance Elastography—3.0T scanner was used for MRE for all included patients (GE Healthcare, Milwaukee, WI). 2-dimensional (2D) MRE protocol was used in multiple clinical sites on Yokohama City University Campus [37] between November, 2013 to March, 2019. Abdominal radiologists interpreted 2D- MR elastograms following protocols established in the department [38]. Radiologists were blinded to clinical and pathology data.

Primary Outcome—The primary outcome of the study was defined as presence of significant fibrosis (stage 2 fibrosis). The study cohort was dichotomized into two sub-groups stratified by significant fibrosis status including stage 0-1 vs stage 2-4 fibrosis.

Patients with stage 0-1 fibrosis would be classified as lower risk and may not qualify for NASH treatment in Phase 3 trials. Subsequently, any patient with stage 2 fibrosis would be classified as higher risk and may qualify for NASH treatment in Phase 3 trials. Histology was assessed using NASH CRN Histologic Scoring System as previously noted.

Statistical Analysis—Participants' anthropometric, demographic, histologic, laboratory, and imaging results were summarized in a table below for both UCSD-NAFLD and Japan-NAFLD Cohort. Receiver operating characteristic (ROC) curve analyses were used to assess the diagnostic accuracy of several predictors for the prediction of stage 2 fibrosis, calculating the area under the ROC curve (AUROC), the optimal thresholds, positive predictive value (PPV), and negative predictive value (NPV). The Delong test was used to compare the AUROCs. The optimal threshold of each modality was determined using the Youden's index. Logistic regression model was created to determine factors associated with stage 2 fibrosis and its diagnostic accuracy. A 2-tailed P value .05 was considered statistically significant. An experienced biostatistician analyzed the data using SAS software 9.4 (SAS Institute, Cary, NC).

RESULTS

Patient characteristics of UCSD-NAFLD Cohort

The UCSD-NAFLD Cohort had 238 patients (54.2% female) with mean ($\pm$ standard deviation) age and BMI of 51.0 years ($\pm$ 13.7) and 31.3kg/m²($\pm$ 4.4), respectively. Patient characteristics are summarized in Table 1. 170 patients diagnosed with stage 0, 1 fibrosis were aged 48.9 years ($\pm$ 13.7) with BMI of 31.0 kg/m²($\pm$ 4.2). There were 101 patients with stage 0 fibrosis and 69 patients with stage 1 fibrosis. 68 patients diagnosed stage 2 fibrosis were aged 56.4 years ($\pm$ 12.4) and had a BMI of 32.3kg/m²($\pm$ 4.8). 27 patients were diagnosed with stage 2 fibrosis, 23 patients with stage 3 fibrosis, and 18 patients with stage 4 fibrosis. The median time interval (interquartile range) between MRE and biopsy in UCSD-NAFLD cohort was 34.0 days (57.0). MRI-PDFF, MRE imaging data collected are shown in Table 1. Blood tests were done to all enrolled patients and with age, platelet count, AST, and ALT, FIB-4 score was calculated.

Patient characteristics of Japan-NAFLD Cohort

The Japan-NAFLD Cohort had 222 patients (44.1% female) with mean ($\pm$ standard deviation) age and BMI of 55.5 years ($\pm$ 15.1) and 28.8kg/m²($\pm$ 5.1), respectively. Patient characteristics are summarized in Table 1. 87 patients diagnosed with stage 0-1 fibrosis who were aged 49.3 years ($\pm$ 14.9) with BMI of 28.8 kg/m²($\pm$ 5.0), respectively. There were 7 patients with stage 0 fibrosis and 80 patients with stage 1 fibrosis. 135 patients diagnosed with stage 2 fibrosis were aged 59.5 years ($\pm$ 13.9) and had a BMI of 28.8kg/m²($\pm$ 5.3), respectively. 46 patients were diagnosed with stage 2 fibrosis, 60 patients with stage 3 fibrosis, and 29 patients with stage 4 fibrosis. The median time interval (interquartile range) between MRE and biopsy in Japan-NAFLD Cohort was 54.5 (68.0) days.

MRE accurately diagnoses patients with stage 2 fibrosis compared to FIB-4

Diagnostic accuracy was compared between MRE and FIB-4 in diagnosing stage 2 fibrosis UCSD-NAFLD (training) Cohort and Japan-NAFLD (validation) Cohort. AUROC was calculated and compared for statistical significance using p value (Fig. 2). In both training and validation cohort, MRE outperformed FIB-4 in assessing fibrosis stage. In the UCSD-NAFLD (training) Cohort, MRE demonstrated a robust and clinically significant diagnostic accuracy for the detection of stage 2 fibrosis with an AUROC of 0.93 (95%

CI: 0.90-0.97) versus FIB-4 alone with an AUROC of 0.78 (95% CI: 0.71-0.85), which was both clinically and statistically significant ($p < 0.0001$). The diagnostic accuracy of MRE remained statistically significant ($p < 0.005$) in the Japan-NAFLD (validation) Cohort with an AUROC of 0.89 (95% CI, 0.85-0.93) compared to diagnostic accuracy of FIB-4 (AUROC, 0.79; 95% CI, 0.73-0.85) (Fig. 2). The differences in the MR elastogram and liver histology of representative patients are shown in Figure 3.

Univariate predictors of stage 2 fibrosis in UCSD-NAFLD training Cohort

We not only analyzed MRE and FIB-4 but also clinically relevant parameters such as ALT, AST, platelet counts, and age to see which parameters show significance in predicting stage 2 fibrosis in the training cohort (Table 2). Each predictor's AUROC and diagnostic odds ratio were obtained and compared to see if there are any additional factors associated with predicting stage 2 fibrosis. Diagnostic odds ratio assists binary classification as being a measure for effectiveness of a diagnostic test [39].

MRE had an odds ratio of 13.60 (95% CI: 6.71 – 27.56) and FIB-4 4.36 (2.72-7.00). Other factors such as AST, age, platelet count, ALT had lower odds ratio respectively (≈ 1.0). Thus, we chose MRE and FIB-4 to calculate the Youden's Index to determine cut-points to predict stage 2 fibrosis. The cut-points for MRE was 3.3kPa and FIB-4 1.6 respectively for the training cohort.

Combination of MRE and FIB-4 for ruling in stage 2 fibrosis (MEFIB Index)

Using these cut-points, we looked at the diagnostic accuracy of MRE alone, FIB-4 alone, and both together in a model to predict stage 2 fibrosis in the UCSD-NAFLD Cohort (Table 3). In the training cohort, we observed our multivariable model with MRE 3.3 kPa and FIB-4 1.6 to have a higher AUROC of 0.90(0.85-0.95) compared to each diagnostic tool alone and it was statistically significant ($p < 0.02$). MRE coupled with FIB-4 has high positive predictive value (PPV) of 97.1% ruling in patients with stage 2 fibrosis for candidacy of treatment.

We then applied these cut-points to the Japan-NAFLD Validation Cohort (Table 3). The results remained significant in Japan-NAFLD Cohort that yielded a robust PPV of 91.0% and the results were both clinically and statistically significant ($p < 0.003$). Supplementary material: Table 2 shows recalculated AUROC, PPV, and NPV based on the Youden's Index from the Japan-NAFLD Cohort.

DISCUSSION

Main findings

Utilizing a uniquely well-characterized contemporaneous liver biopsy and MRE study cohort, we demonstrated clinical utility of MRE and FIB-4 in detecting stage 2 fibrosis as a *non-proprietary* biomarker panel for high risk NASH patients and those who need to be treated in registrational trial based upon FDA guidance. In multivariable-adjusted models, a combination of MRE 3.3 kPa and FIB-4 1.6 called the MEFIB index provided a robust positive predictive value (PPV) of 97.1% ruling in patients with stage 2 fibrosis who are

candidates for treatment among patients with NAFLD. The results remained significant after validation in a geographically and ethnically distinct cohort.

The study provides specific cut-points for clinical use that may have an impact in clinical care and would be easy to use. It is important to note that these results stayed consistent between geographically and ethnically diverse cohorts. With these thresholds, this study helps providers to rule in patients for pharmacologic registration trial without needing a liver biopsy. High PPV of 97.1% helps by giving providers a much needed non-invasive guideline to screen patients for candidacy for treatment of NASH related fibrosis. Liver biopsy assessment for detecting NASH related fibrosis patient candidates for pharmacologic therapy is impractical due to its unavoidable risks and complications and these data suggest MRE paired with FIB-4 may help reduce the burden for patients in avoiding the risk in a subset of patients [18]. Further studies are needed to noninvasively assess change in liver histology post pharmacologic treatment.

In Context of Published Literature

Liver fibrosis stage has been clinically important as higher stages of fibrosis were shown to be linked with liver related morbidity and mortality [9, 37]. Various noninvasive biomarkers have been proposed and evaluated to diagnose advanced fibrosis as a result such as MRE, FIB-4, and VCTE. As FDA accepted critical inclusion criteria to have fibrosis score greater than 1 for purposes of registration trial, the importance of assessing not only advanced fibrosis but also stage 2 fibrosis rose. Previously, it has been shown MRE is accurate in diagnosing liver fibrosis not only in mild fibrosis but also for any, advanced fibrosis, and cirrhosis [27]. However, limited research has been done in using FIB-4 to diagnose patients other than advanced fibrosis but has been shown to be less accurate compared to MRE [40]. This study confirms that MRE is more accurate than FIB-4 in diagnosing patients with stage 2 fibrosis and provides new data that combination of MRE with FIB-4 can be clinically actionable. MEFIB index correctly ruled in 34 out of 68 patients in the UCSD-NAFLD Cohort and 81 out of 135 patients in the Japan-NAFLD Cohort with 56% who were correctly classified, and 44% who were missed (Supplementary Material: Table 1). The patients who were missed would still need a liver biopsy assessment. Hence, one can avoid 56% of liver biopsies in patients who need to be treated. This appears to be better than FAST score where sensitivity was 48% with a significant lower PPV of 83% [41]. Further studies with head-to-head comparison are needed to compare MEFIB index versus FAST score in future. In a recent cost-effectiveness study comparing various non-invasive modalities for detection of cirrhosis, Vilar-Gomez and colleagues found that FIB-4 followed by MRE yielded the highest diagnostic accuracy versus FIB-4 followed by VCTE in order to avoid liver biopsy for the diagnosis of cirrhosis. [42]. Our study provides prospective validation with an actionable cut-point for its clinical application using the MEFIB index (defined as FIB-4 ≥ 1.6 and MRE ≥ 3.3 kPa) to rule-in patients who have stage 2 fibrosis in patients with NAFLD.

Strengths and Limitations

There are following notable strengths of this study. This is a prospective study derived from a uniquely well-characterized cohort residing in San Diego, California, United States, and

these data have been validated in an ethnically and geographically distinct cohort residing in Yokohama, Japan. This underscores the generalizability and clinical applicability of these data across both Western and Eastern population. This *non-proprietary* biomarker panel and its cut-points are available to all without any cost and freely available to the world for clinical use.

Although this study is performed by experienced investigators with expertise in non-invasive MRI assessment, there are some notable limitations. As our study aimed to develop a combination of non-invasive tests to rule-in patients with significant fibrosis, low sensitivity may have rooted from a trade-off in reducing false-positives by inevitably increasing false-negatives. However, high PPVs in both cohorts provide evidence for clinicians to use the MEFIB index to identify patients with stage 2 fibrosis without needing a liver biopsy assessment. These thresholds are recommended for usage in a hepatology clinic setting to determine potential candidates for treatment of NASH. It is likely that cut-points may be different in primary care population. Therefore, further studies are needed in patients in the primary care setting and those derived from diabetes clinic. It is important to note that patients were enrolled consecutively which underscores the entire spectrum of liver disease in a liver clinic and provides a context of use for these cut-points. While we excluded clinically relevant biomarkers, such as AST, ALT, platelet count, and age, based on univariate analysis, there might be a more comprehensive multivariate logistic model that could further improve its accuracy. However, the MEFIB index utilizes readily available FIB-4 and MRE results so it is clinically actionable without deriving yet another score. Moreover, this study uses MRE which might not be readily available at all centers. Hence, further studies are needed to examine the role of combination of other elastography methods along with clinical prediction rules [26].

Conclusions

There is a major unmet need to identify patients who have stage 2 fibrosis and are candidates for pharmacologic therapy without needing a liver biopsy assessment. All currently available clinical prediction rules such as FIB-4 and elastography methods that are routinely available have a high negative predictive value and there is a need for a combination of biomarkers that have a high positive predictive value. Further studies are needed to establish cut-points for VCTE, ARFI and SWE along with FIB-4 in risk stratification of patients with NAFLD. This study provides evidence that MEFIB index (MRE 3.3 kPa and FIB-4 1.6) can yield a high PPV to rule in patients with stage 2 fibrosis without needing a liver biopsy assessment in the context of a liver clinic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BMI	body mass index
MRE	magnetic resonance elastography
MRI-PDFF	magnetic resonance imaging proton density fat fraction
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
UCSD	University of California at San Diego
EMA	European Medicines Agency
FDA	Food and Drug Administration
NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network
MEFIB	MRE combined with FIB-4

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Summary Box:**What is already known about this subject?**

Patients with non-alcoholic fatty liver disease (NAFLD) with stage 2 fibrosis are at increased risk for liver related mortality and are candidates for pharmacologic therapies for treatment of NAFLD. However, current non-invasive tests (such as FIB-4 and VCTE) have a high negative predictive value but a low positive predictive value so are unable to rule-in a patient who needs to be treated. Therefore, there is a major unmet need to examine if a combination of advanced imaging tests (such as MRE) and serum markers will yield a high enough positive predictive value for a clinician to rule in clinically significant disease that needs pharmacologic treatment in NAFLD.

What are the new findings?

In multivariable-adjusted models, a combination of MRE ≥ 3.3 kPa and FIB-4 ≥ 1.6 (MEFIB index) provided a robust positive predictive value (PPV) of 97.1% ruling in patients with stage 2 fibrosis who are candidates for treatment among patients with NAFLD. The results remained significant after validation in a geographically and ethnically distinct cohort.

How might it impact on clinical practice in the foreseeable future?

MRE combined with FIB-4 (MEFIB) index may be used for non-invasive identification of candidates for (stage 2 fibrosis) pharmacologic therapy among patients with NAFLD with a high positive predictive value.

Figure 1. Derivation of UCSD – NAFLD Cohort

Abbreviations: MRE, magnetic resonance elastography

*NASH CRN Histologic Score System was used [35]

Figure 2. MRE is more accurate than routinely available clinical prediction rule, FIB - 4
 Comparison of diagnostic accuracy between MRE and FIB-4 in detecting stage 2 fibrosis in UCSD-NAFLD Cohort and Japan-NAFLD Cohort. Left: In UCSD -NAFLD Cohort, the AUROC for MRE was 0.93 which was statistically significant compared to FIB-4 which was 0.78 ($p < 0.0001$). Right: The diagnostic accuracy of MRE (AUROC=0.89) in Japan – NAFLD Cohort was also statistically significant compared to FIB-4 (AUROC=0.79). The p value is provided.

Abbreviations: MRE, magnetic resonance elastography; FIB – 4, fibrosis -4

Figure 3. Representative patient characteristics by Magnetic Resonance Elastography (MRE) image and liver biopsy. Patient A had a FIB-4 of 1.03; patient B had a FIB-4 of 2.62.

Abbreviation: MRE, magnetic resonance elastography; FIB -4, fibrosis – 4

*Trichrome stain highlights normal collagen surrounding the central hepatic venule (no significant fibrosis, 40X magnification, patient A) and bridging fibrosis (10X magnification, patient B).

Table 1.

Demographic, histologic, biochemical, and imaging results at baseline visit in UCSD-NAFLD Cohort and Japan-NAFLD Cohort.

	UCSD-NAFLD Cohort			Japan-NAFLD Cohort		
	Total Patient Number (N=238)	Patients with stage 0-1 fibrosis (N=170)	Patients with stage 2 fibrosis (N=68)	Total Patient Number (N=222)	Patients with stage 0-1 fibrosis (N=87)	Patients with stage 2 fibrosis (N=135)
Demographic Profile						
Age (yr), mean (SD)	51.0 (13.7)	48.9 (13.7)	56.4 (12.4)	55.5 (15.1)	49.3 (14.9)	59.5 (13.9)
Female, n (%)	129 (54.2%)	80 (47.1%)	49 (72.1%)	98 (44.1%)	31 (35.6%)	67 (49.6%)
Diabetes Mellitus, n (%)	82 (34.5%)	39 (22.9%)	43 (63.2%)	123 (55.4%)	37 (42.5%)	86 (63.7%)
BMI (kg/m ²), mean (SD)	31.3 (4.4)	31.0 (4.2)	32.3 (4.8)	28.8 (5.1)	28.8 (5.0)	28.8 (5.3)
Race, n (%)						
White	109 (46.6%)	87 (51.8%)	22 (33.3%)	0	0	0
Hispanic	75 (32.0%)	49 (29.1%)	26 (39.4%)	0	0	0
African American	5 (2.1%)	4 (2.4%)	1 (1.5%)	0	0	0
Asian	39 (16.7%)	24 (14.3%)	15 (22.7%)	222 (100%)	87 (100%)	135 (100%)
Other	6 (2.6%)	4 (2.4%)	2 (3.1%)	0	0	0
Histologic Results *						
NASH, n (%)						
Not NAFLD †	9 (4.3%)	8 (5.4%)	1 (1.6%)	5 (2.2%)	1 (1.2%)	4 (3.0%)
NAFLD	68 (32.2%)	66 (44.6%)	2 (3.2%)	32 (14.4%)	16 (18.4%)	16 (11.8%)
Borderline/Suspicious	15 (7.1%)	12 (8.1%)	3 (4.7%)	132 (59.5%)	51 (58.6%)	81 (60.0%)
Definite NASH	119 (56.4%)	62 (41.9%)	57 (90.5%)	53 (23.9%)	19 (21.8%)	34 (25.2%)
Fibrosis Stage, n (%)						
0	101 (42.4%)	101 (59.4%)	0 (0%)	7 (3.2%)	7 (8.1%)	0
1	69 (29.0%)	69 (40.6%)	0 (0%)	80 (36.0%)	80 (91.9%)	0
2	27 (11.3%)	0 (0%)	27 (39.7%)	46 (20.7%)	0	46 (34.1%)
3	23 (9.7%)	0 (0%)	23 (33.8%)	60 (27.0%)	0	60 (44.4%)
4	18 (7.6%)	0 (0%)	18 (26.5%)	29 (13.1%)	0	29 (21.5%)
Steatosis, n (%)						
0	14 (6.0%)	11 (6.5%)	3 (4.7%)	5 (2.3%)	1 (1.2%)	4 (3.0%)
1	101 (43.4%)	73 (43.2%)	28 (43.7%)	99 (44.6%)	26 (29.9%)	73 (54.1%)
2	83 (35.6%)	59 (34.9%)	24 (37.5%)	79 (35.6%)	39 (44.8%)	40 (29.6%)
3	35 (15.0%)	26 (15.4%)	9 (14.1%)	39 (17.6%)	21 (24.1%)	18 (13.3%)
NAS Score, mean (SD)	3.7 (1.5)	3.4 (1.4)	4.5 (1.3)	3.7 (1.3)	3.5 (1.3)	3.8 (1.3)
Biochemical Data						
AST (u/L), mean (SD)	43.8 (29.5)	36.7 (20.0)	61.8 (40.1)	51.3 (29.6)	49.1 (36.2)	52.7 (24.5)
ALT (u/L), mean (SD)	59.7 (41.5)	56.2 (40.2)	68.4 (43.8)	72.9 (49.9)	84.3 (57.3)	65.6 (43.1)
FIB-4, mean (SD)	1.5 (1.4)	1.1 (0.5)	2.5 (2.3)	2.1 (1.7)	1.3 (1.0)	2.7 (1.9)
plt (1000/mm ³), mean (SD)	243.9 (74.2)	254.5 (68.7)	217.5 (81.1)	205.9 (66.5)	240 (59.6)	184 (61.4)

	UCSD-NAFLD Cohort			Japan-NAFLD Cohort		
	Total Patient Number (N=238)	Patients with stage 0-1 fibrosis (N=170)	Patients with stage 2 fibrosis (N=68)	Total Patient Number (N=222)	Patients with stage 0-1 fibrosis (N=87)	Patients with stage 2 fibrosis (N=135)
Imaging Results						
MRI-PDFF(%), mean (SD) [‡]	13.7 (8.7)	14.4 (9.0)	11.9 (7.6)	13.7 (8.1)	17.2 (8.5)	11.4 (7.0)
MRE(kPa), mean (SD) [§]	2.9 (1.3)	2.4 (0.5)	4.3 (1.5)	4.0 (1.7)	2.8 (0.8)	4.8 (1.7)

Abbreviations: yr, year; NAS, NAFLD Activity Score[35]; AST, aspartate transaminase; ALT, alanine transaminase; FIB-4, fibrosis-4 score; plt, platelet count; MRI-PDFF, magnetic resonance imaging – proton density fat fraction; MRE, magnetic resonance elastography

* NASH CRN histology scoring system was used [35]

[‡] Patients who were histologically diagnosed no NAFLD had clinical evidence of NAFLD prior to biopsy.

[‡] MRI-PDFF was measured as per following manuscript [34]

[§] MRE was measured as per following manuscript [6]

Table 2.

Univariate assessment of continuous predictors in detecting stage 2 fibrosis in UCSD-NAFLD Cohort

Variable:	AUROC (95% CI)	OR[39] (95% CI)
MRE	0.93 (0.90-0.97)	13.60 (6.71-27.56)
FIB - 4	0.78 (0.71-0.85)	4.36 (2.72-7.00)
AST	0.75 (0.68-0.83)	1.03 (1.02-1.05)
Age	0.66 (0.58-0.73)	1.05 (1.02-1.07)
Platelet count	0.64 (0.55-0.72)	0.99 (0.99-1.00)
ALT	0.60 (0.52-0.68)	1.01 (1.00-1.01)

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; AUROC, area under the receiver operating characteristic curve; FIB-4, fibrosis-4 score; MRE, magnetic resonance elastography; NAFLD, non-alcoholic fatty liver disease; UCSD, University of California at San Diego

[39] Glas AS, Lijmer JG, Prins MH, Bossel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. *J Clin Epidemiol* 2003;56:1129-35.

Table 3.

Diagnostic test characteristic of MRE, FIB-4, and MRE+FIB-4 (MEFIB Index) in detecting stage 2 fibrosis in UCSD-NAFLD Cohort and Japan-NAFLD Cohort

Model:	UCSD-NAFLD Cohort					Japan-NAFLD Cohort				
	AUROC (95% CI)	OR (95% CI)	p-value*	PPV [‡]	NPV	AUROC (95% CI)	OR (95% CI)	p-value	PPV [‡]	NPV
MRE 3.3kPa [‡]	0.87 (0.81-0.92)	MRE: 71.55 (28.73-178.18)	ref	86.9	91.5	0.79 (0.74-0.85)	MRE: 14.74 (7.60-28.57)	ref	84.6	72.8
FIB-4 1.6 [‡]	0.72 (0.66-0.78)	FIB-4: 8.23 (4.32-15.65)	0.0002	61.5	83.7	0.73 (0.68-0.79)	FIB4: 8.31 (4.35-15.88)	0.0792	84.6	60.2
MRE 3.3kPa + FIB-4 1.6	0.90 (0.85-0.95)	MRE: 56.41 (21.80-145.94) FIB-4: 5.16 (2.04-13.06)	0.0184	97.1	83.2	0.84 (0.78-0.89)	MRE: 8.96 (4.41-18.22) FIB4: 3.57 (1.69-7.51)	0.0026	91.0	59.4

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; MRE, magnetic resonance elastography, FIB-4, fibrosis – 4

* p-value for comparison to MRE alone

[‡] cut-point was derived from the Youden's Index in the UCSD-NAFLD Cohort

[‡] determined by categorizing a positive response as MRE 3.3kPa and FIB-4 1.6 vs any other combination