Title

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Journal

The American Journal of Gastroenterology, 114(10)

ISSN

0002-9270

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Publication Date

2019-10-01

DOI

10.14309/ajg.0000000000000388

Peer reviewed

HHS Public Access

Author manuscript

Am J Gastroenterol. Author manuscript; available in PMC 2020 October 01.

Published in final edited form as:

Am J Gastroenterol. 2019 October ; 114(10): 1626–1635. doi:10.14309/ajg.0000000000000388.

Histologic findings of advanced fibrosis and cirrhosis in patients with NAFLD who have normal aminotransferase levels

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Abstract

Background and aims: Patients with nonalcoholic fatty liver disease (NAFLD) and normal aminotransferase levels may have advanced liver histology. We conducted a study to characterize the prevalence of and factors associated with advanced liver histology in patients with histologically characterized NAFLD and normal aminotransferase levels.

Methods: We evaluated 534 adults with biopsy-proven NAFLD and ALT and AST < 40 U/L within 3 months of their liver biopsy. Histological phenotypes of primary interest were NASH with stage 2-3 fibrosis (NASH F2-3) and cirrhosis. Using multiple logistic regression models with Akaike's Information Criteria (AIC), we identified variables associated with these histological phenotypes. We developed and internally validated their clinical prediction models.

Results: The prevalence of NASH F2-F3 and cirrhosis were 19% and 7%, respectively. The best multiple regression AIC model for NASH F2-3 consisted of type 2 diabetes, White race, lower

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Author's contributions: Study concept, data analysis, manuscript preparation (SG, NC, LW, and JT); Study concept and critical review of manuscript (OWC, JMC, RL, BH, MFA, SD, BNT, KK, DK, ED, and AJ).

LDL, lower platelet count, higher AST/ALT ratio, higher serum triglycerides, and hypertension. The best AIC model for cirrhosis consisted of lower platelet count, lower AST/ALT ratio, higher BMI, and female sex. The area under the receiver operator curves of the prediction models were 0.70 (95% CI: 0.65-0.76) for detecting NASH-F2-3 and 0.85 (95% CI: 0.77-0.92) for detecting cirrhosis. When models were fixed at maximum Youden's index, their positive and negative predictive values were 35% and 88% for NASH F2-F3 and 30% and 98% for cirrhosis, respectively.

Conclusion: Clinically significant histological phenotypes are observed in patients with NAFLD and normal aminotransferase levels. Our models can assist the clinicians in excluding advanced liver histology in NAFLD patients with normal aminotransferase levels.

Keywords

Normal liver enzymes; NAFLD; NASH; Cirrhosis

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a global health problem. It is estimated to affect nearly a quarter of the world population[1]. A significant number of patients with NAFLD have normal aminotransferase levels. Depending on the upper limits of the reference range used ("normal value") and the population studied, the percent of patients with NAFLD and normal alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels vary widely. It ranges from 14% in Italian patients with NAFLD selected for liver biopsies[2], to 23% in NAFLD patients retrospectively identified through pathology database at a U.S. academic center[3], and exceeding 65% of morbidly obese patients undergoing bariatric surgery[4–7]. In large scale epidemiological studies, normal aminotransferase levels were noted in 79% of patients with NAFLD in the Dallas Heart study and 55% of patients with NAFLD in the Dionysis study[8].

These studies have consistently shown that when patients with NAFLD and normal aminotransferase levels underwent liver biopsy, clinically significant histological findings and levels of fibrosis were commonly observed and not significantly different than those in NAFLD patients with elevated aminotransferase levels . In 103 consecutive patients with type 2 diabetes and normal aminotransferase levels [9], NAFLD was diagnosed in 50% of the patients by magnetic resonance spectroscopy criteria. Nonalcoholic steatohepatitis (NASH) was diagnosed in 56% of NAFLD patients who underwent liver biopsy in that study. The largest prior non-bariatric study with liver histology data evaluated 80 obese Italian patients with normal liver tests referred to abdominal surgery for uncomplicated gall stones, gastric or large bowel cancer[10]. Patients underwent intraoperative core liver biopsy in the first part of the operative procedure if they met the diagnostic criteria for the metabolic syndrome, had a body mass index (BMI) ≥35 kg/ m^2, chronically normal aminotransferase levels, and no evidence of alcohol abuse or other liver diseases. That study reported NAFLD was observed in 97.5%, NASH in 72.5%, any fibrosis in 62.5%, and advanced fibrosis in 22.5% of these patients.

Previous studies to characterize NAFLD with normal aminotransferase levels originated mostly from single centers[3,4,9–11], and were limited by retrospective design[3,11], selected patient population[9,10], and relatively small study size[2–4,9–11]. Furthermore, they have not systematically characterized the variables associated with important histological phenotypes, especially NASH with stages 2 and 3 fibrosis, which recently has emerged as the primary target for clinical trials.

In this study, we characterized the spectrum of liver histology in a large cohort of individuals with NAFLD and normal aminotransferase levels prospectively enrolled in the NASH Clinical Research Network (CRN) studies. We identified demographic, clinical, and laboratory variables associated with key histological phenotypes and subsequently developed and validated their clinical prediction models.

Methods

Study participants' identification

Individuals 18 years or older with biopsy proven NAFLD and enrolled into studies undertaken by the NASH CRN (Database 1, Database 2, PIVENS, and FLINT) were eligible for inclusion in this study if they met the following criteria: 1) ALT and AST < 40 U/L, 2) liver biopsy within 3 months of aminotransferase levels measurement, and 3) central pathology review of the liver biopsy by the NASH CRN Pathology Committee.

NASH CRN Database 1 observational study enrolled patients with suspected or biopsy proven NAFLD between 2004 and 2009 from 8 U.S. medical centers[12]. NASH CRN Database 2 observational study is an extension of the NASH CRN Database 1 study and uses similar inclusion and exclusion criteria, except for requiring histological proof of NAFLD as an inclusion criterion[13]. The study enrolled patients with biopsy proven NAFLD between 2009 and 2018 from 8 U.S. medical centers. The PIVENS study was a randomized placebo-controlled trial that evaluated the efficacy of vitamin E and pioglitazone for biopsy proven NASH[14]. Individuals with cirrhosis or diabetes were excluded. The FLINT study was a randomized placebo-controlled trial that evaluated the efficacy of obeticholic acid for biopsy proven NASH[15]. In the FLINT trial, patients with diabetes were included whereas those with cirrhosis were excluded. For individuals enrolled in the clinical trials (PIVENS and FLINT), baseline pre-treatment variables and liver biopsies were used for this analysis. All studies were approved by the institutional review boards of participating institutions. All participants provided written informed consent prior to enrollment. The data for these NASH CRN studies were stored, monitored, and analyzed at the Data Coordinating Center at the Johns Hopkins Bloomberg School of Public Health.

Justification for using 40 U/L as a cutoff for normal aminotransferase levels

Although the upper limits of normal (ULN) for aminotransferase levels vary by local laboratories, 40 U/L is a common upper reference range value used by clinical laboratories. Laboratories establish their reference ranges for transaminases using local populations. The characteristics of local population but not laboratory or analyzer variability has a major influence on the ALT ULN in different laboratories, which prompted investigators to suggest

that laboratories should rely on healthy individuals without metabolic risk factors to derive local “healthy” ALT ULN[16]. An earlier study of Italian blood donors by Prati et al observed a relationship between ALT level, BMI, and metabolic indices[17]. After excluding donors with obesity and other risks for metabolic disease, they calculated the upper limits for “healthy” normal ALT at 30 U/L for men and 19 U/L in women and this increased the sensitivity of ALT for detecting NAFLD or hepatitis C. However, this has not been widely accepted, even in Italian studies[2,10]. A cutoff of 43 U/L for ALT level, which was the ULN of the National Health and Nutrition Examination Survey (NHANES) reference laboratory, was used for NHANES based studies[18–20]. A cutoff of 40 U/L for the ULN of aminotransferase levels has been used previously in research studies[2,6,9,10], and therefore it was used for the primary analysis in this study. To address the question of how the Prati based cutoffs for ULN for ALT level affect the study findings, we also conducted a secondary analysis using the Prati suggested ALT ULN cutoffs for men and women.

Characterization of study participants

Extensive demographic, anthropometric, clinical, and laboratory data were systematically collected on all participants as part of the individual study protocol. Routine laboratory tests were performed on fresh samples in Clinical Laboratory Improvement Amendments-certified laboratories at each participating clinical site according to standard clinical protocols. The fibrosis-4 (FIB-4) score was calculated using on age, AST, ALT and platelets[21]. Liver biopsies were evaluated and scored by the NASH CRN Pathology Committee using the NASH CRN scoring system[22]. A diagnosis of definite NASH is made by the Pathology Committee based on a pattern recognition, which requires the presence of classic hepatocyte ballooning. Borderline NASH largely fell into two broad categories: a majority with steatosis, lobular inflammation and fibrosis, but no ballooning and a minority with steatosis, lobular inflammation and borderline quality ballooning but no fibrosis.

Statistical Methods

Characteristics of patients with NAFLD and ALT<40 U/L and AST<40 U/L were described using means (SD) or N (%). Demographic, anthropometric, medical comorbidities, and laboratory data collected within three months of the biopsy were compared for four histologic phenotypes: definite steatohepatitis, definite steatohepatitis and fibrosis stage 2-3 (NASH F2-3) (patients with cirrhosis were excluded from this analysis), advanced fibrosis (stage 3 or 4), and cirrhosis. In this paper we focus on the characteristics of and factors associated with NASH F2-3 and cirrhosis as these two NAFLD phenotypes are immediately actionable: patients with NASH F2-3 are the primary focus for phase 2 and phase 3 clinical trials, and NAFLD patients with cirrhosis receive variceal and hepatocellular carcinoma screening surveillance[23]. We also include in the supplementary material the characteristics of and factors associated with definite NASH and advanced fibrosis.

To determine the statistical significance of the comparisons, p-values were derived from Fisher’s exact test for categorical measures and t tests for continuous measures. Clinical prediction models were developed for the histological phenotypes using multiple logistic regression models with Akaike’s Information Criteria (AIC), a penalized likelihood method

that is a trade-off between goodness of fit vs. model size, with smaller AICs corresponding to models with more information about the outcome[24,25]. This approach is not based on p-values and avoids the need to adjust p-values for the multiplicity of candidate models. The candidate set of variables for the clinical prediction models were: age, sex, race, Hispanic/Latino ethnicity, BMI, waist circumference, type 2 diabetes, hypertension, hypothyroidism, metabolic syndrome, AST/ALT ratio, platelets, triglycerides, HDL-cholesterol, and LDL-cholesterol. The model selection method does not include highly co-linear variables. Rather, it picks one or more members of a co-linear set of variables that give the most information (best AIC) about the outcome. There may be some residual collinearity among the selected variables, but the combination selected gives the best model for the outcome based on information contained in the variables selected. The odds ratios, 95% confidence intervals, and p-values are presented, as well as the clinical prediction equations.

Performance characteristics for the prediction models, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were estimated for sensitivity fixed at 90%, specificity fixed at 90%, and Youden's Index (sensitivity +specificity-1) fixed at the maximum value. We then validated the models using cross-validation, split-sample validation (in which the prediction model was applied to 80% of the sample selected on calendar time, and the performance characteristics were calculated in the 20% validation sample using the predicted probabilities from the 80% training sample), and a random split-sample validation, in which the split-sample validation was repeated with a randomly selected 20% of the sample. Area under the receiver operating curves (AUROCs) and 95% confidence intervals were compared for each model. We then compared the performance of our models [named, Low ALT Clinically Significant NAFLD (LACSNA)] to that of FIB-4[21] and NAFLD fibrosis score (NFS)[26]. We created an online LACSNA calculator, which is publicly available on the website http://gihep.com.

Nominal, 2-sided p-values were considered significant if $P<0.05$. Analyses were performed using SAS software (version 9.4, SAS Institute, Cary, NC) and Stata (Release 15.1, Stata Corporation, College Station, TX).

Results

Characteristics of study participants

There were a total of 1,235 unique adult patients with a liver biopsy and liver enzymes within 3 months of enrollment into NASH CRN Database 1, Database 2, PIVENS, and FLINT. Of those, 534 (43%) patients had normal aminotransferase levels and represented this study cohort: 90% were recruited through the NASH CRN Database 1 and 2 studies and 10% through the PIVENS and FLINT trials. Their mean (SD) for ALT and AST levels were 27.9 (7.3) U/L and 24.7 (5.9) U/L, respectively. The median (IQR) time between liver panel and biopsy was 30 days (14-56 days).

The distribution of NAFLD histological lesions and phenotypes for the entire cohort and per ALT ranges is shown in Table 1. Remarkably, 99% of the patients in the entire cohort had some degree of lobular inflammation, 47% had hepatocyte ballooning and 61% had fibrosis. Further, 35% of the patients had definite NASH, 19% had NASH F2-F3, 20% had advanced

fibrosis, and 7% had cirrhosis. The frequency and severity of NAFLD histological features, advanced fibrosis and definite NASH increased with rising ALT value (Table 1). Nearly a quarter of the patients with ALT<20 U/L had definite NASH and 20% of them had advanced fibrosis.

Factors associated with NASH F2-3 in NAFLD patients with normal aminotransferase levels

Definite NASH with stage 2-3 fibrosis was present in 19% of the patients. Compared to other NAFLD patients in the cohort excluding those with cirrhosis (Table 2), individuals with NASH F2-3 and normal aminotransferase levels were older, more commonly White, and more frequently had type 2 diabetes, hypertension, and the metabolic syndrome. They were more insulin resistant with higher insulin, HOMA-IR, HgA1c, and triglycerides than other NAFLD patients in the cohort excluding those with cirrhosis. Further, these individuals had lower LDL and platelets and higher AST/ALT ratio and FIB-4 score than other NAFLD patients in the cohort excluding those with cirrhosis. In a multiple logistic regression model using AIC to select the best model (Table 3), type 2 diabetes (OR 2.1, 95% CI 1.3 – 3.4, p=0.004), lower LDL (OR 1.1 per 10 mg/dL decrement, 95% CI 1.0 – 1.2, p=0.02), White race (OR 2.1, 95% CI 1.1 – 4.2, p=0.03), higher AST/ALT (OR 2.5, 95% CI 1.0 – 6.6, p=0.06), lower platelets (OR=1.0 per 10 unit (10^9/L) decrement, 95% CI 1.0 – 1.2, p=0.07), lower triglycerides (OR=1.0 per 10 mg/dL decrement, 95% CI 1.0 – 1.1, p=0.09), and history of hypertension (OR 1.5, 95% CI 0.9 – 2.5, p=0.13) were associated with higher probability of NASH F2-3. Of these factors, type 2 diabetes and lower platelets were also associated with higher probability of definite NASH and any fibrosis.

Factors associated with cirrhosis in NAFLD patients with normal aminotransferase levels

Compared to other NAFLD patients in the cohort (Table 4), NAFLD patients with cirrhosis and normal aminotransferase levels were less commonly Hispanic, and more frequently had type 2 diabetes. They were more insulin resistant with higher insulin, HOMA-IR, and HgA1c than other NAFLD patients in the cohort. Further, these patients had lower platelets and higher AST/ALT ratio and FIB-4 score than other NAFLD patients in the cohort.

In a multiple logistic regression model (Table 5), lower platelets (OR=1.2 per 10 unit (10^9/L) decrement, 95% CI 1.2 – 1.3, p<0.001), higher AST/ALT ratio (OR 5.3, 95% CI 1.7 – 16.5, p=0.004), higher BMI (OR=1.1 per 2 kg/m^2 increment, 95% CI 1.0 – 1.2, p=0.06), and female sex (OR 2.1, 95% CI 0.8 – 5.5, p=0.12) were associated with the highest probability of cirrhosis.

Clinical and laboratory factors associated with definite NASH and with advanced fibrosis and their multiple logistic regression AIC models are shown in Supplementary Tables 1–4.

LACSNA Clinical prediction models for detecting selected histological phenotypes in patients with NAFLD and normal aminotransferase levels

The AUROCs (95% CI) for definite NASH was 0.70 (0.65-0.74), for NASH-F2-3 fibrosis 0.70 (0.65-0.76), for NAFLD with advanced fibrosis 0.79 (0.74-0.84), and for NAFLD 0.85 (0.77-0.92) (Table 6). The performance of these models remained stable after three internal

validation methods with cross-validation, split-sample validation and random split-sample validations (Table 6).

The diagnostic statistics of the LACSNA clinical prediction models for identifying histological phenotypes of interest are shown in Table 7. In general, their positive predictive values (PPV) were low, but negative predictive values (NPV) were very high to excellent. For example, when the models were fixed at the maximum Youden's index, their PPV and NPV were 35% and 88% for NASH F2-F3 and 30% and 98% for cirrhosis, respectively (Table 7). The LACSNA models have better AUROC than that of FIB-4 or NFS for predicting the NAFLD phenotypes of interest (Table 7).

Characterization of the cohort and performance of simple clinical prediction models based on the Prati criteria for normal ALT

When we evaluated the cohort using the more stringent values for normal ALT (<30 U/L for men and <19 U/L for women) identified in a study of Italian blood donors by Prati et al[17], the number of patients meeting these criteria dropped to 143 individuals (11.5%) out of the 1,235 unique adult patients with a liver biopsy and liver enzymes within 3 months of enrolment into NASH CRN studies. Still, a significant number of patients in this group had definite NASH (24%), NASH F2-F3 (28%), advanced fibrosis (20%) or cirrhosis (10%) (Supplementary Table 5).

The diagnostic statistics and the AUROCs of the models for identifying various histological phenotypes of interest are shown in Supplementary Tables 6 and 7. Their performance worsened with the 3 methods we used for internal validation, likely due to the smaller cohort size (Supplementary Table 7).

Discussion

This study shows that normal serum aminotransferase levels are common in the patients with the spectrum of NAFLD and observed in 43% of patients with NAFLD enrolled in the NASH CRN studies. The full spectrum of NAFLD histological lesions and phenotypes is seen in these patients. Normal aminotransferase levels provide no assurance of absence of clinically significant NAFLD as NASH, advanced fibrosis, and cirrhosis were observed in 35%, 20%, and 7% of these individuals. To facilitate identifying patients with normal aminotransferase levels but clinically significant NAFLD, we created clinical predication models (which we named LACSNA), based on readily available clinical and laboratory data that can be used to reliably exclude NASH-F2-3, advanced fibrosis and cirrhosis in these patients.

This study confirms the findings of prior studies reporting a high prevalence of NASH and advanced fibrosis as well as an increased risk of NASH with insulin resistance and diabetes in NAFLD patients with normal aminotransferase levels[2–4,9–11]. It further shows that even individuals with NAFLD and ALT<20 U/L can have clinically significant liver disease, as almost a quarter of these patients had definite NASH and 20% of them had advanced fibrosis. Further, this study shows that the frequency and severity of NAFLD histological features, advanced fibrosis and definite NASH increased with increasing ALT value from

<20 U/L to the 20-39 U/L range (Table 1). This highlights the importance of closer follow up of patient with low ALT values who are classified by clinical prediction models.as high risk for having clinically significant NAFLD.

In this study, White race and lower LDL-cholesterol levels were also associated with increased risk of fibrosing NASH. The AST/ALT ratio and lower platelets counts are risk factors for advanced fibrosis and cirrhosis in all patients with NAFLD, including those with elevated aminotransferase levels [26–28]; findings this study confirms to be applicable to patients with NAFLD and normal aminotransferase levels. Lower platelets count is possibly a correlate with early portal hypertension and indicator of more advanced liver disease, as it was a risk factor for all the major phenotypes we evaluated in this study, whereas type 2 diabetes, higher AST/ALT ratio, and female sex were associated with increased risk of the majority of these phenotypes.

Clinicians are commonly faced with the challenge of determining whether a patient with NAFLD has a clinically significant phenotype or not. A common approach is to consider a patient's comorbidities and routinely available laboratory tests such as aminotransferase levels to stratify risk and determine the need for further evaluation. Patients suspected of having a clinically significant NAFLD phenotype may be referred to specialty care for further definition of their phenotype, implementation of interventions to slow or reverse disease progression, and when available, enrollment in therapeutic clinical trials. NAFLD patients with cirrhosis are enrolled into surveillance programs for gastroesophageal varices and hepatocellular carcinoma[23]. Despite the advent of a plethora of serum based non-invasive markers and magnetic resonance- or ultrasound-based elastography to identify clinically significant NAFLD[29–40], these useful clinical tools are not readily or widely available. The LACSNA clinical prediction tools we developed have fair to good performance for detection of NASH, NASH-F2-3, advanced fibrosis and cirrhosis. The LACSNA models have better AUROC than that of FIB-4 or NFS for predicting the NAFLD phenotypes of interest. However, to be useful in identifying patients with clinically significant NAFLD, clinical prediction models have to have high positive predictive value (PPV), to reduce the likelihood of misclassification and unnecessary subsequent confirmatory testing (e.g., abdominal imaging, elastography or liver biopsies). Our models, similar to other reported models, have poor PPV and thus are poorly suited for this task[12,27,29,41,42].

Alternatively, clinical predictions models could be used to reassure the patient and clinician of absence of clinically significant NAFLD phenotype. This could obviate the need for additional testing and specialty care referral at the time of evaluation. For this use, our models and similar other models do well. With our model fixed at 90% sensitivity, the highest NPV is for cirrhosis (98%), followed by advanced fibrosis (94%), and NASH-F2-3 (91%). Therefore, the best utilization of these clinical predication models in practice may be to exclude these phenotypes.

This study has several limitations. Patients in this study were recruited from NASH CRN sites, and had histologically confirmed NAFLD as an entry criterion into these studies. This implies that clinically significant NAFLD was suspected by managing clinicians at the time

of ordering a clinically indicated liver biopsy, thus creating a selection bias. No external validation of the findings was done. However, external validation in a similar cohort with ALT<40 U/L with liver biopsy data is not practically feasible. Taking advantage of the large sample size, we used three methods to internally validate the clinical prediction models we developed. The utility of liver stiffness measurement or controlled attenuation parameter measurements in risk stratifying these patients was not evaluated in this study as these results were available only on a small subset of included patients. The clinical prediction models were developed on patients with NAFLD in this study, so they should only be applied to those with known NAFLD. It would be interesting to test these models in the general population suspected to have NAFLD. It would, however, be extremely difficult do large liver histology-based studies in the general population. Finally, given the known variability in ALT and AST levels, one measurement of normal ALT and AST within 3 months of the liver biopsy (our study entry criterion) may not reflect persistently normal transaminases values.

This study also has several strengths. It is the largest systematic study of non-bariatric patients with NAFLD and normal aminotransferase levels as defined by stringent criteria to date. Patients were prospectively enrolled and thus complete clinical and laboratory data were available on all participants. Additionally, liver histology was centrally evaluated by the NASH CRN Pathology Committee using a standardized protocol.

In conclusion, there is high prevalence of NASH with clinically significant fibrosis, advanced fibrosis, and cirrhosis among NAFLD patients with normal aminotransferase levels enrolled in NASH CRN studies. The variables we observed to be associated with these clinically important histological phenotypes and their clinical prediction models can be useful for clinicians to exclude advanced histological phenotypes when they encounter NAFLD patients with normal aminotransferase levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement:

The authors wish to thank Dr. Timothy D. Imler for his help in building the online calculator for the clinical prediction models.

Disclosures: Drs. Clark, Doo, Wilson, Tonascia, and Dasarathy declare no conflicts of interest. **Dr. Gawrieh** consulting: TransMedics, research grant support: Cirius, Galmed and Zydus. **Dr. Cummings** has service contract with Novo-Nordisk. **Dr. Loomba** serves as a consultant or advisory board member for Bird Rock Bio, Celgene, Enanta, GRI Bio, Metacrine, NGM, Sanofi, Arrowhead Research, Genfit, Galmed, NGM, Novo Nordisk, Metacrine, Pfizer, Seal Rock, and Viking Therapeutics. In addition, his institution has received grant support from Allergan, BMS, BI, Daiichi-Sankyo Inc., Eli Lilly, Galectin, Galmed, GE, Genfit, Gilead, Intercept, Janssen Inc, Merck, NGM, Pfizer, Prometheus, Shire, Siemens, and Cirius. He is also co-founder of Liponexus Inc. **Dr. Hameed** serves as a consultant or advisory board member for Gilead, Surrozen, Mallinckrodt. His institution has received grant support from Gilead, Intercept, Conatus, Dova , Salix, Genfit. **Dr. Abdelmalek** declares that her consulting activities and industry research support are not relevant for this paper. For full disclosure, she has advisory or consulting agreements with the following companies in the last 12 months: Allergan, Madrigal, BMS, TaiwanJ, NGM Bio. She has received research support from Intercept, Allergan, Genfit, Galactin, BMS, NGM Bio, Galmed, TaiwanJ, Excelanz, Prometheus, Madrigal, Conatus, TARGET-NASH, Progenity and Boehringer-Ingelheim. **Dr. Neuschwander-Tetri** has advisory or consultant agreements with Allergan, Arrowhead, Blade, Boehringer Ingleheim, BMS, Coherus, Consynance, Cymabay, Enanta, Gelesis, Gilead, Intercept, Karos, Lexicon, Lipocine,

Madrigal, Medimmune, Merck, Metacrine, NGM, pH-Pharma, Prometheus. **Dr. Kowdley** serves as a consultant or advisory board member for Conatus, Corcept Therapeutics, Enanta, Gilead and Intercept. He is on speakers' bureau for Gilead and Intercept. In addition, his institution has received grant support from Allergan, Enanta, Galectin, Gilead, Immuron, Intercept, Prometheus and Zydus Discovery DMCC. **Dr. Sanyal** is President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect, Exhalenz and Hemoshear. He has served as a consultant to Astra Zeneca, Nitto Denko, Ardelyx, Conatus, Nimbus, Amarin, Salix, Tobira, Takeda, Fibrogen, Jannsen, Gilead, Lilly, Poxel, Artham, Cymabay, Boehringer Ingelhiem, Novo Nordisk, Birdrock, Novartis, Pfizer, Jannsen and Genfit. He has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Syntlogic, Affimune, Chemomab, Nordic Bioscience and Bristol Myers Squibb. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Intercept, Merck, Astra Zeneca, Malinckrodt, Cumberland and Novartis. He receives royalties from Elsevier and UptoDate. **Dr. Chalasani** had paid consulting activities with following companies in last 12 months: Abbvie, Shire, NuSirt, Afimmune, Axovant, Allergan, Madrigal, Coherus, and Genentech. He has received research support from Lilly, Galectin, Gilead, Exact Sciences, and Cumberland.

Source of funding: The Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) is supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (grants U01DK061718, U01DK061728, U01DK061731, U01DK061732, U01DK061734, U01DK061737, U01DK061738, U01DK061730, U01DK061713). Additional support is received from the National Center for Advancing Translational Sciences (NCATS) (grants UL1TR000439, UL1TR000436, UL1TR000006, UL1TR000448, UL1TR000100, UL1TR000004, UL1TR000423, UL1TR000058).

References

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73–84. [PubMed: 26707365]
2. Fracanzani AL, Valenti L, Bugianesi E, et al. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology. 2008;48(3):792–798. [PubMed: 18752331]
3. Verma S, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). Liver Int. 2013;33(9):1398–1405. [PubMed: 23763360]
4. Kunde SS, Lazenby AJ, Clements RH, Abrams GA. Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. Hepatology. 2005;42(3):650–656. [PubMed: 16037946]
5. Abrams GA, Kunde SS, Lazenby AJ, Clements RH. Portal fibrosis and hepatic steatosis in morbidly obese subjects: A spectrum of nonalcoholic fatty liver disease. Hepatology (Baltimore, Md). 2004;40(2):475–483.
6. Ulitsky A, Ananthakrishnan AN, Komorowski R, et al. A noninvasive clinical scoring model predicts risk of nonalcoholic steatohepatitis in morbidly obese patients. Obesity Surgery. 2010;20(6):685–691. [PubMed: 20336392]
7. Campos GM, Bambha K, Vittinghoff E, et al. A clinical scoring system for predicting nonalcoholic steatohepatitis in morbidly obese patients. Hepatology (Baltimore, Md). 2008;47(6):1916–1923.
8. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis. 2010;28(1):155–161. [PubMed: 20460905]
9. Portillo-Sanchez P, Bril F, Maximos M, et al. High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels. J Clin Endocrinol Metab. 2015;100(6):2231–2238. [PubMed: 25885947]
10. Sorrentino P, Tarantino G, Conca P, et al. Silent non-alcoholic fatty liver disease-a clinical-histological study. J Hepatol. 2004;41(5):751–757. [PubMed: 15519647]
11. Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology (Baltimore, Md). 2003;37(6):1286–1292.
12. Neuschwander-Tetri BA, Clark JM, Bass NM, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology. 2010;52(3):913–924. [PubMed: 20648476]

13. Vuppalanchi R, Siddiqui MS, Van Natta ML, et al. Performance characteristics of vibration-controlled transient elastography for evaluation of nonalcoholic fatty liver disease. Hepatology. 2018;67(1):134–144. [PubMed: 28859228]
14. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362(18):1675–1685. [PubMed: 20427778]
15. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet. 2014.
16. Neuschwander-Tetri BA, Unalp A, Creer MH. Influence of local reference populations on upper limits of normal for serum alanine aminotransferase levels. Arch Intern Med. 2008;168(6):663–666. [PubMed: 18362260]
17. Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med. 2002;137(1):1–10. [PubMed: 12093239]
18. Ruhl CE, Everhart JE. Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. Gastroenterology. 2005;129(6):1928–1936. [PubMed: 16344061]
19. Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. Gastroenterology. 2003;124(1):71–79. [PubMed: 12512031]
20. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol. 2003;98(5):960–967. [PubMed: 12809815]
21. 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.
22. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313–1321. [PubMed: 15915461]
23. 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. 2018;67(1):328–357. [PubMed: 28714183]
24. Akaike H A new look at the statistical model identification. IEEE Transactions on Automatic Control. 1974;19(6):716–723.
25. Wang Z Model selection using the Akaike information criterion. Stata Technical Bulletin. 2000;9(54).
26. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45(4):846–854. [PubMed: 17393509]
27. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59(9):1265. [PubMed: 20801772]
28. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut. 2008;57(10):1441–1447. [PubMed: 18390575]
29. Guha IN, Parkes J, Roderick P, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology. 2008;47(2):455–460. [PubMed: 18038452]
30. Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology. 2004;127(6):1704–1713. [PubMed: 15578508]
31. Poynard T, Munteanu M, Deckmyn O, et al. Validation of liver fibrosis biomarker (FibroTest) for assessing liver fibrosis progression: proof of concept and first application in a large population. J Hepatol. 2012;57(3):541–548. [PubMed: 22612998]
32. Talwalkar JA, Yin M, Fidler JL, Sanderson SO, Kamath PS, Ehman RL. Magnetic resonance imaging of hepatic fibrosis: emerging clinical applications. Hepatology (Baltimore, Md). 2008;47(1):332–342.

33. Yin M, Talwalkar JA, Glaser KJ, et al. Assessment of hepatic fibrosis with magnetic resonance elastography. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2007;5(10):1207–1213.e1202. [PubMed: 17916548]
34. Ajmera V, Perito ER, Bass NM, et al. Novel plasma biomarkers associated with liver disease severity in adults with nonalcoholic fatty liver disease. Hepatology. 2017;65(1):65–77. [PubMed: 27532276]
35. Caussy C, Reeder SB, Sirlin CB, Loomba R. Non-invasive, quantitative assessment of liver fat by MRI-PDFF as an endpoint in NASH trials. Hepatology. 2018.
36. de Ledinghen V, Vergniol J. Transient elastography for the diagnosis of liver fibrosis. Expert review of medical devices. 2010;7(6):811–823. [PubMed: 21050091]
37. de Ledinghen V, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. Liver Int. 2012;32(6):911–918. [PubMed: 22672642]
38. Siddiqui MS, Vuppalanchi R, Van Natta ML, et al. Vibration-controlled Transient Elastography to Assess Fibrosis and Steatosis in Patients With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol. 2018.
39. Vuppalanchi R, Siddiqui MS, Van Natta ML, et al. Performance characteristics of vibration-controlled transient elastography for evaluation of nonalcoholic fatty liver disease. Hepatology. 2018;67(1):134–144. [PubMed: 28859228]
40. Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. J Hepatol. 2018;68(2):305–315. [PubMed: 29154965]
41. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2009;7(10):1104–1112. [PubMed: 19523535]
42. Younossi ZM, Loomba R, Anstee QM, et al. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. Hepatology. 2018;68(1):349–360. [PubMed: 29222917]

Table 1:

Histological spectrum of NAFLD in patients with normal aminotransferase levels (ALT and AST <40 U/L)

Phenotype	Total (N=534)	ALT<20 U/L (N=89)	ALT 20-39 U/L (N=445)	*p-value
Steatosis grade (%)				0.004
0	3	6	2	
1	55	65	53	
2	30	16	33	
3	12	13	12	
Lobular inflammation (%)				0.003
0	1	4	1	
1	78	84	77	
2	19	9	20	
3	2	2	2	
Hepatocyte ballooning (%)				0.03
0	53	65	50	
1	32	26	34	
2	15	9	16	
NAS (%)				<0.001
1	1	3	1	
2	30	47	27	
3	27	17	29	
4	23	22	23	
5	12	9	12	
6	4	1	5	
7	2	0	2	
Mean (SD)	3.4 (1.3)	2.9 (1.1)	3.5 (1.3)	<0.001
Median (IQR)	3 (2, 4)	2 (2, 4)	3 (2, 4)	<0.001
Fibrosis Stage (%)				<0.001
0	39	52	37	
1a	14	7	15	
1b	6	3	7	
1c	5	10	4	
2	15	8	16	
3	13	8	15	
4	7	12	6	
Steatohepatitis diagnosis (%)				<0.001
NAFLD only	37	58	33	
Borderline	27	18	29	
Definite	35	24	38	

Phenotype	**Total (N=534)**	**ALT<20 U/L (N=89)**	**ALT 20-39 U/L (N=445)**	***p-value**
Definite steatohepatitis with stage2-3 fibrosis (%)	19	13	22	0.09

Abbreviations: NAFLD: Nonalcoholic Fatty Liver Disease; NAS: NAFLD Activity Score; SD: Standard deviation; IQR: Interquartile range.

*comparison is between ALT<20 U/L and ALT 20–39 U/L.

Table 2:

Characteristics of NAFLD patients with normal aminotransferase levels (ALT and AST <40 U/L) separated by presence of NASH with fibrosis stage 2-3[¶]

	Definite steatohepatitis and fibrosis stage 2-3		
	Yes (N=101)	**No (N=395)**	**P-value**
Demographic			
Age at enrollment (years)	55 (10)	51 (12)	0.002
Sex (%)			0.26
Male	23	28	
Female	77	72	
Race (%)			0.01
White	88	77	
Black	0	6	
Asian	2	6	
Other/Refused	10	11	
Hispanic/Latino (%)	11	13	0.62
Anthropometric			
BMI (kg/m^2)	35.6 (6.1)	34.5 (7.1)	0.16
Weight (kg)	99 (19)	95 (22)	0.17
Waist (cm)	112 (13)	108 (15)	0.01
Comorbidities (%)			
Type 2 diabetes	59	35	<0.0001
Hypertension	72	57	0.006
Dyslipidemia	61	60	0.91
Hypothyroidism	21	21	0.92
Metabolic syndrome	69	56	0.02
Laboratory measures			
Platelet count (10^9/L)	233 (72)	254 (69)	0.01
AST/ALT ratio	0.96 (0.27)	0.90 (0.22)	0.03
FIB-4	1.4 (0.8)	1.0 (0.5)	<0.0001
Hemoglobin A1c (%)	6.4 (1.0)	6.2 (1.2)	0.02

	Definite steatohepatitis and fibrosis stage 2-3		
	Yes (N=101)	**No (N=395)**	**P-value**
Insulin (μU/mL)	28.5 (29.4)	20.2 (17.7)	0.008
HOMA-IR	8.4 (9.8)	5.6 (6.0)	0.007
Total cholesterol (mg/dL)	179 (36)	186 (42)	0.13
Triglycerides (mg/dL)	200 (178)	161 (87)	0.04
HDL-cholesterol (mg/dL)	44 (11)	45 (12)	0.27
LDL-cholesterol (mg/dL)	100 (32)	111 (36)	0.004

¶Patients with cirrhosis excluded from this analysis
Data are presented as number (percent) or means with standard deviation.

Abbreviations: BMI: Body mass index; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **HOMA-IR:** Homeostatic model assessment for insulin resistance; **HDL:** high density lipoprotein; **LDL:** Low density lipoprotein

Table 3:

Multiple logistic regression analysis for definite steatohepatitis with fibrosis stage 2–3 in NAFLD patients with normal aminotransferase levels (ALT and AST <40 U/L)

	Odds ratio Definite steatohepatitis and fibrosis stage 2-3 vs. not	95% CI	P-val
Characteristics (N=480)*			
Type 2 diabetes	2.1	1.3 – 3.4	0.004
LDL – per 10 mg/dL decrement	1.1	1.0 – 1.2	0.02
White race vs. non-white	2.1	1.1 – 4.2	0.03
AST/ALT	2.5	1.0 – 6.6	0.06
Platelets – per 10 unit (10^9/L) decrement	1.0	1.0 – 1.2	0.07
Triglycerides – per 10 mg/dL increment	1.0	1.0 – 1.1	0.09
History of hypertension	1.5	0.9 – 2.5	0.13
Clinical model for P, probability of definite steatohepatitis and fibrosis stage 2–3.			
Coefficients and SEs shown as b(SE): log(P/1-P) = −2.181(0.865) + 0.722(0.250) if type 2 diabetes – 0.008(0.004) X LDL (mg/dL) + 0.762(0.346) if white + 0.931(0.489) X AST/ALT – 0.003(0.002) X platelet count (10^9/L) + 0.003(0.002) X triglycerides (mg/dL) + 0.400(0.263) if hypertension			

*Factors were selected using AIC criteria from a multiple logistic regression model regressing definite steatohepatitis with fibrosis stage 2–3 on a candidate set of variables: age, sex, race, Hispanic/Latino ethnicity, BMI, waist circumference, type 2 diabetes, hypertension, hypothyroidism, metabolic syndrome, AST/ALT, platelets, triglycerides, HDL, and LDL.

Table 4:

Characteristics of NAFLD patients with normal aminotransferase levels (ALT and AST <40 U/L) categorized by the presence of cirrhosis

	Cirrhosis		
	Yes (N=38)	No (N=496)	P-value
Demographic			
Age at enrollment (years)	55 (11)	52 (11)	0.13
Sex (%)			
Male	24	27	0.71
Female	76	73	
Race (%)			
White	92	80	0.29
Black	0	4	
Asian	0	5	
Other/Refused	8	11	
Hispanic/Latino (%)	0	13	0.01
Anthropometric			
BMI (kg/m^2)	36.5 (7.1)	34.7 (6.9)	0.13
Weight (kg)	103 (26)	96 (21)	0.08
Waist (cm)	112 (16)	109 (15)	0.24
Comorbidities (%)			
Type 2 diabetes	63	40	0.006
Hypertension	76	60	0.06
Dyslipidemia	50	61	0.23
Hypothyroidism	24	21	0.68
Metabolic syndrome	73	59	0.12
Laboratory measures			
Platelet count (10^9/L)	170 (58)	250 (70)	<0.0001
AST/ALT ratio	1.21 (0.54)	0.91 (0.24)	0.002
FIB-4	2.2 (1.5)	1.1 (0.6)	<0.0001
HgA1c (%)	7.0 (1.5)	6.2 (1.2)	0.002
Insulin (μU/mL)	37.0 (28.6)	21.9 (20.9)	0.003

	Cirrhosis		
	Yes (N=38)	**No (N=496)**	**P-value**
HOMA-IR			
Mean (SD)	13.3 (10.9)	6.2 (7.1)	<0.001
Median (IQR)	10.2 (5.7, 17.9)	4.0 (2.6, 6.7)	
Total cholesterol (mg/dL)	174 (39)	184 (41)	0.13
Triglycerides (mg/dL)	150 (73)	169 (113)	0.13
HDL-cholesterol (mg/dL)	44 (13)	45 (12)	0.79
LDL-cholesterol (mg/dL)	100 (30)	109 (36)	0.14

[¶]Data are presented as number (percent) or means with standard deviation.

Abbreviations: BMI: Body mass index; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **HOMA-IR:** Homeostatic model assessment for insulin resistance; **HDL:** high density lipoprotein; **LDL:** Low density lipoprotein

Table 5:

Multiple logistic regression analysis for cirrhosis in NAFLD patients with normal aminotransferase levels (ALT and AST <40 U/L)

	Odds ratio cirrhosis vs. not cirrhosis	95% CI	P-val
Characteristics (N=530)*			
Platelets – per 10 unit (10^9/L) decrement	1.2	1.2 – 1.3	<0.001
AST/ALT ratio	5.3	1.7 – 16.5	0.004
BMI - per 2 kg/m^2 increment	1.1	1.0 – 1.2	0.06
Female vs. male	2.1	0.8 – 5.5	0.12
Clinical model for P, probability of cirrhosis. Coefficients and SEs shown as b(SE): log(P/1-P) = −2.284(1.341) – 0.021(0.004) X platelet count (10^9/L) + 1.668(0.578) X AST/ALT + 0.054(0.029) X BMI (kg/m^2) + 0.760(0.485) if female			

*Factors were selected using AIC criteria from a multiple logistic regression model regressing cirrhosis on a candidate set of 15 variables: age, sex, race, Hispanic/Latino ethnicity, BMI, waist circumference, type 2 diabetes, hypertension, hypothyroidism, metabolic syndrome, AST/ALT, platelets, triglycerides, HDL, and LDL.

Table 6:

Comparison of the LACSNA clinical prediction model performance[¶] in prediction and validation samples

	Prediction Model	Validation Models		
		Cross-validated	Split-sample Validation[*]	Repeat Random Split-sample Validation[†]
Definite steatohepatitis	0.70 (0.65-0.74)	0.67 (0.63-0.72)	0.65 (0.54-0.75)	0.71 (0.61-0.82)
Definite steatohepatitis with stage 2-3 fibrosis	0.70 (0.65-0.76)	0.67 (0.61-0.73)	0.69 (0.57-0.80)	0.72 (0.60-0.84)
Advanced fibrosis	0.79 (0.74-0.84)	0.77 (0.72-0.82)	0.76 (0.64-0.88)	0.72 (0.60-0.83)
Cirrhosis	0.85 (0.77-0.92)	0.83 (0.75-0.91)	0.98 (0.95-1.00)	0.80 (0.62-0.99)

LACSNA: **L**ow **A**LT **C**linically **S**ignificant **NA**FLD.

[¶]AUROCs with 95% Confidence intervals

N's for the prediction models ranged from 480 - 530 due to missing values in the outcome (definite steatohepatitis with fibrosis 2-3 & excluding patients with cirrhosis) and covariates. N's for the 20% split-sample validation ranged from 100-107 due to missing values in the outcome (definite steatohepatitis with fibrosis 2-3 & excluding patients with cirrhosis) and covariates.

[*]Split-sample validation, where the prediction model was applied to 80% of the sample selected on calendar time, and the performance characteristics were then calculated in the 20% validation sample using the predicted probabilities from the 80% training sample.

[†]Split-sample validation was repeated with a randomly selected 20% of the sample.

Table 7:

Diagnostic statistics for LACSNA clinical predication models compared to FIB-4 and NFSin the study sample

		Model fixed at 90% specificity			Model fixed at 90% sensitivity			Model fixed at maximum Youden's Index*			
	AUROC (95% CI)	Sens	PPV†	NPV‡	Spec	PPV†	NPV‡	Sens	Spec	PPV†	NPV‡
Definite steatohepatitis											
LACSNA	0.70 (0.65 -- 0.74)	0.25	0.58	0.69	0.35	0.43	0.87	0.76	0.54	0.47	0.80
FIB-4	0.62 (0.57 - 0.67)	0.23	0.56	0.68	0.15	0.37	0.74	0.43	0.77	0.51	0.71
NFS	0.66 (0.61 - 0.71)	0.21	0.54	0.68	0.21	0.39	0.80	0.63	0.65	0.49	0.76
Definite steatohepatitis with stage 2-3 fibrosis											
LACSNA	0.70 (0.65-0.76)	0.24	0.38	0.83	0.27	0.24	0.92	0.63	0.71	0.35	0.88
FIB-4	0.66 (0.60 - 0.72)	0.22	0.36	0.82	0.25	0.23	0.91	0.50	0.74	0.33	0.85
NFS	0.68 (0.62 - 0.73)	0.22	0.36	0.82	0.28	0.24	0.92	0.73	0.58	0.31	0.89
Advanced fibrosis											
LACSNA	0.79 (0.74 -- 0.84)	0.47	0.54	0.87	0.38	0.27	0.94	0.69	0.76	0.43	0.91
FIB-4	0.76 (0.70 - 0.81)	0.37	0.49	0.85	0.31	0.25	0.92	0.66	0.74	0.40	0.89
NFS	0.76 (0.71 - 0.81)	0.38	0.49	0.85	0.38	0.27	0.94	0.65	0.75	0.40	0.89
Cirrhosis											
LACSNA	0.85 (0.77-0.92)	0.68	0.35	0.97	0.41	0.11	0.99	0.74	0.87	0.30	0.98
FIB-4	0.82 (0.74 - 0.89)	0.47	0.27	0.96	0.33	0.10	0.98	0.79	0.78	0.22	0.98
NFS	0.84 (0.78 - 0.92)	0.61	0.32	0.97	0.41	0.11	0.99	0.76	0.83	0.25	0.98

LACSNA**: L**ow **A**LT **C**linically **S**ignificant **NA**FLD, FIB-4: Fibrosis-4, NFS: NAFLD Fibrosis Score.

N's ranged from 480 - 530 due to missing values in the outcome (definite steatohepatitis with fibrosis 2-3 & excluding patients with cirrhosis) and covariates.

*Youden's Index=sensitivity+specificity-1

†PPV = Positive Predictive Value: the probability that the disease is present when the test is positive

‡NPV = Negative Predictive Value: the probability that the disease is not present when the test is negative.