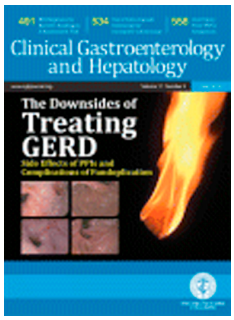
Accepted Manuscript



Clinically Significant Fibrosis is Associated With Longitudinal Increases in Fibrosis-4 and NAFLD Fibrosis Scores

Preya Janubhai Patel, Johnson Chieh-yu Cheng, Xuan Banh, Lucy Gracen, Daniel Radford-Smith, Fabrina Hossain, Leigh Ula Horsfall, Kelly Lee Hayward, Suzanne Williams, Tracey Johnson, Nigel Neil Brown, Nivene Saad, Katherine Anne Stuart, Anthony William Russell, Patricia Casarolli Valery, Andrew Donald Clouston, Katharine Margaret Irvine, Anne Bernard, Elizabeth Ellen Powell

PII: S1542-3565(19)30780-3
DOI: https://doi.org/10.1016/j.cgh.2019.07.036
Reference: YJCGH 56642

To appear in: *Clinical Gastroenterology and Hepatology*
Accepted Date: 19 July 2019

Please cite this article as: Patel PJ, Cheng JC-y, Banh X, Gracen L, Radford-Smith D, Hossain F, Horsfall LU, Lee Hayward K, Williams S, Johnson T, Brown NN, Saad N, Stuart KA, Russell AW, Valery PC, Clouston AD, Irvine KM, Bernard A, Powell EE, Clinically Significant Fibrosis is Associated With Longitudinal Increases in Fibrosis-4 and NAFLD Fibrosis Scores, *Clinical Gastroenterology and Hepatology* (2019), doi: https://doi.org/10.1016/j.cgh.2019.07.036.

Clinically Significant Fibrosis is Associated With Longitudinal Increases in Fibrosis-4 and NAFLD Fibrosis Scores

Short Title: Longitudinal changes in fibrosis biomarkers

Preya Janubhai Patel[1,2], Johnson Chieh-yu Cheng[2], Xuan Banh[2], Lucy Gracen[2], Daniel Radford-Smith[3], Fabrina Hossain[4], Leigh Ula Horsfall[1,2], Kelly Lee Hayward[2], Suzanne Williams[4], Tracey Johnson[4], Nigel Neil Brown[5], Nivene Saad[3,6], Katherine Anne Stuart[1], Anthony William Russell[3,7], Patricia Casarolli Valery[8], Andrew Donald Clouston[2], Katharine Margaret Irvine[2,9], *Anne Bernard[10], *Elizabeth Ellen Powell[1,2]

[1]Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, Australia; [2]Centre for Liver Disease Research, Translational Research Institute, Faculty of Medicine, The University of Queensland, Brisbane, Australia; [3]Faculty of Medicine, University of Queensland, Brisbane Australia; [4]Inala Primary Care, Brisbane, Australia; [5]Pathology Queensland, Brisbane, Australia; [6]Department of Radiology, Princess Alexandra Hospital, Brisbane, Australia; [7]Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Australia; [8]QIMR Berghofer Medical Research Institute, Brisbane, Australia; [9]Mater Research, Translational Research Institute, The University of Queensland, Brisbane, Australia; [10]QFAB Bioinformatics, Institute for Molecular Bioscience, Queensland Bioscience Precinct, The University of Queensland, Brisbane, Australia.

***Equal contribution as senior authors**

Corresponding author:

Prof Elizabeth Powell, e.powell@uq.edu.au

Department of Gastroenterology and Hepatology

Princess Alexandra Hospital, Brisbane

Ipswich Rd

Woolloongabba, QLD 4102

Funding:

This study was funded by the Pathology Queensland - Study, Education and Research Trust Fund.

PC Valery was supported by the Australian National Health and Medical Research Council (Career Development Fellowship #1083090). Unrestricted educational grant and ELF test provided by Siemens Healthineers

Word Count:

Abstract: 279

Main Text: 3098 (excluding references and figure legends)

Conflicts of interest:

The authors have no conflicts of interest to declare.

Author Contributions

PJP LUH EEP designed the study. PJP, JCC, XB, LG LUH EEP collated patient data. PJP, DRS, AB EEP analysed the data and drafted the manuscript. All authors contributed to manuscript review and revision.

Abbreviations:

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; ELF, enhanced liver fibrosis; CSF, clinically significant fibrosis; FIB-4, Fibrosis-4; HCV, hepatitis C virus; HDL, high density lipoprotein; ICCs, intraclass correlation coefficients; IFG, impaired fasting glucose; IQR, inter-quartile range; LSM, liver stiffness measurements; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD Fibrosis Score; NASH, non-alcoholic steatohepatitis; NPV, negative predictive value; SD, standard deviation; T2DM, type 2 diabetes mellitus.

Abstract:

Background & Aims: There is limited knowledge regarding the longitudinal utility of biomarkers of fibrosis, such as the non-alcoholic fatty liver disease (NAFLD) fibrosis score (NFS) or the fibrosis-4 score (FIB-4) score. We examined longitudinal changes in the NFS and the FIB-4 score in patients with NAFLD, with and without clinically significant fibrosis (CSF).

Methods:

We performed a retrospective study of 230 patients with NAFLD, collecting clinical and laboratory records to calculate NFS and FIB-4 scores at 6 monthly intervals for 5 years prior to hepatology assessment of fibrosis. Linear mixed models with random intercept and slope and adjusted for age at baseline were used to assess the progression of NFS and log-transformed FIB-4 scores over time in subjects with and without CSF, determined by liver stiffness measurements ≥8.2kPa.

Results: Patients had a median of 11 (minimum, 10 and maximum, 11) retrospective observations over a median time period of 5 years (minimum, 4.5 years and maximum, 5 years). Of patients with low baseline NFS and FIB-4 scores, 31.11% and 37.76%, respectively, had CSF at the time of hepatology assessment. There was a correlation between NFS and Log_{10}FIB-4 over time (repeated measure r=0.55; 95% CI, 0.52–0.59). The rate of increase in NFS and Log_{10}FIB-4 was significantly higher in patients with than without CSF (both *P*<.001). Predicted NFS increased by 0.17 and 0.06 units per year in subjects with and without CSF, respectively. Predicted Log_{10}FIB-4 score increased by 0.032 and $3x10^{-4}$ units per year in subjects with and without CSF respectively.

Conclusions: Non-invasively measured fibrosis scores increase progressively in patients with NAFLD and CSF. Further studies are needed to determine whether repeated measurements can identify patients at risk for CSF.

Keywords:

non-invasive biomarkers, fibrosis, nonalcoholic fatty liver disease, repeated measurements.

Non-alcoholic fatty liver disease (NAFLD) is reported to have a global prevalence of approximately 25%, with higher prevalence in people with severe obesity (>95%), type 2 diabetes (>76%) and dyslipidemia (50%).[1, 2] People with NAFLD have increased overall mortality when compared to a matched control population,[3] and the most important predictor of mortality is the extent of liver fibrosis.[4] In particular, the presence of advanced fibrosis (Brunt stage 3-4) is associated with increased risks of overall and liver-related mortality[5, 6] and these patients may benefit from specialist care and surveillance for liver cancer and liver decompensation. Therefore, once NAFLD is identified, assessment of the extent of liver fibrosis is crucial in order to make decisions about management and referral.

Recent clinical guidance recommends using first-line, non-invasive tests, such as Fibrosis-4 (FIB-4)[7, 8] and NAFLD Fibrosis Score (NFS)[9] to identify NAFLD patients with advanced fibrosis.[10] These simple scoring systems combine routine biochemical tests with clinical risk factors for fibrosis such as age or diabetes, and have high negative predictive values for excluding advanced fibrosis.[11] It is recommended that people with a low FIB-4 (<1.3) and low NFS (<-1.455) are managed in primary care with reinforcement of lifestyle advice and ongoing assessment and management of metabolic risk factors.[1, 10, 12, 13] People with indeterminate or high FIB-4/NFS scores require further assessment with second-line biomarkers such as serum Enhanced Liver Fibrosis (ELF) test[14] or liver stiffness measurements (LSM).[15] They may require referral to a hepatology clinic for investigation of liver disease or management of advanced fibrosis.[10]

Despite their recommendation, there is limited evidence as to the utility of non-invasive tests to monitor NAFLD patients for evolution of fibrosis over time. Guidance suggests repeating the NFS/FIB-4[16] or ELF test[13] every 2 or 3 years respectively. The ELF test is

calculated from combining 3 measured direct markers of liver matrix metabolism in serum. Although it is commercially available, to date there is no reimbursement for the test in Australia. In contrast the NFS/FIB-4 tests are low to no cost as they are calculated from routine blood tests, age and body mass index (BMI) and can be run repeatedly without additional costs. In a study of 108 NAFLD patients with 2 liver biopsies (median interval 6.6 years), a significant relationship was seen between change in NFS and change in fibrosis stage, and the NFS was effective at identifying patients with advanced fibrosis on the follow-up liver biopsy (area under the receiver operating characteristic curve (AUROC): 0.83).[17] It remains unclear however, whether individuals' FIB-4/NFS scores change in a linear fashion over time, and whether the trajectories of the scores vary based on the severity of fibrosis.

The purpose of this study was to retrospectively examine the longitudinal change in FIB-4 and NFS scores in a cohort of NAFLD patients with and without clinically significant fibrosis (CSF). We also examine whether other factors including age, gender, severe obesity, presence of diabetes, or the presence of poorly controlled diabetes (HbA1c>53mmol/mol (7%)) influence the progression of these scores across time.

Methods

Subjects

This is a retrospective study of patients identified with NAFLD between October 2015 and August 2017 who attended the liver clinic at the Princess Alexandra Hospital, Brisbane for clinical assessment (n=252). The source population has previously been described.[18] Informed written consent was obtained from each eligible patient and the protocol was approved by the Metro South Health and The University of Queensland Human Research Ethics Committees (HREC/15/QPAH/301; UQ2015001047).

Clinical assessment

Clinical assessment included anthropometric measurements, routine hematological, biochemical and serological tests, ELF test, LSM and liver ultrasound. Metabolic syndrome was defined according to International Diabetes Federation guidelines.[19] Transient elastography was performed using FibroScan technology (Echosens, Paris, France) as previously described.[18] For the purposes of this study we used a cut-off value of 8.2kPa for CSF,[15] for both standard M and XL probes.[20, 21]

Predictor and Outcome measures

The primary outcome was rate of hepatic fibrosis progression, defined by change in FIB-4 and NFS units per year, over the 5-year period prior to baseline hepatology assessment. The NFS was calculated using the algorithm: −1.675 + 0.037 × age (years) + 0.094 × body mass index (BMI) (kg/m^2) + 1.13 × IFG/diabetes (yes=1, no=0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×10^9/L) – 0.66 × albumin(g/dL).[9] The FIB-4 score was calculated using the algorithm: (age × AST)/(platelets × (√ALT)).[8]

The primary predictor of interest was presence or absence of CSF (≥ stage 2 fibrosis) assessed by LSM ≥ or <8.2kPa respectively at initial hepatology assessment.

For each patient, observation commenced at the time of initial hepatology assessment and clinical data required to calculate the FIB-4 and NFS scores, along with diabetic control (HbA1c) were retrieved from clinical records at 6 monthly time-point intervals over the preceding 5 years. Time point 0 was five years prior to the initial hepatology assessment. Laboratory results were carried forward for a maximum of 365 days if required in order to calculate the NFS and FIB-4. BMI was documented annually for each patient; if missing, this was imputed annually by calculating the change between the earliest available BMI and the BMI at the time of hepatology assessment.

Statistical analysis

Descriptive statistics are presented using mean and standard deviation (SD) for normally distributed continuous data or median and inter-quartile range (IQR) when normality was not met. Categorical variables are described using frequencies and percentages. The relationship between categorical variables was assessed using Pearson's Chi-square test, or Fisher's exact test as appropriate. The comparison of a continuous variable between two groups was tested using the independent samples T-test or Mann-Whitney U test when the assumption of normality was not met. The distribution of FIB-4 was right-skewed therefore the log-transformed variable was considered for all statistical analyses to reduce skewness of the distribution.

The intraclass correlation coefficients (ICCs) were calculated using a one-way random-effects ANOVA to estimate the correlation between repeated measures taken from the same patient at different times and evaluate the reproducibility of the test. ICC should be

equal to 1 for a perfectly reproducible test and ICC higher than 0.6 are usually stated as acceptable reproducibility.[22] The evolution of the outcomes of interest across time was assessed using linear mixed model analyses with time, fibrosis status and corresponding interaction as fixed effects and patient and time as random intercept and slope, respectively, and was adjusted for patient age at baseline. Potential confounders were tested for NFS (gender, HbA1c≥7) and Log_{10}FIB-4 (gender, BMI, HbA1c≥7). Assumptions of normality of residuals, collinearity and influential points were checked and validated for each model. Analyses were performed using the R statistical software. All p values <0.05 were considered as significant.

Results

Study population

A total of 230 patients (91.3%) were evaluated in the hepatology clinic with LSM meeting quality criteria. Overall, the mean age of subjects included in the study was 56.84±12.27 years, 55.22% were male, with a mean BMI of 34.38±7.72kg/m^2 and mean girth of 115.56±17.87cm. Patients had a high prevalence of metabolic syndrome (83.91%), T2DM (82.17%), and 18.34% of the cohort had ≥ class 3 obesity (BMI≥40kg/m^2). Median LSM was 6.10kPa (IQR 4.8-9.2) with a range from 2.6 to 63.9kPa and required use of the XL probe in 76.96% of subjects. LSM≥8.2kPa, consistent with CSF, were present in 31.30% of patients; 23.91% had LSM≥9.5kPa (consistent with advanced fibrosis)[21]; and 16.09% had LSM>13kPa (concerning for cirrhosis).[20, 21]

The demographic and clinical characteristics of the patients with and without CSF (assessed by LSM ≥ or <8.2kPa) at the time of hepatology assessment (time point 10) are summarized in Table 1. In comparison with the cohort without CSF, patients with CSF had a lower platelet count (p=0.027), higher serum GGT (p<0.001), AST (p<0.001), ALT (p=0.021) and a greater proportion had metabolic syndrome (p=0.003) and type 2 diabetes (p<0.001).

The clinical characteristics of these subjects at baseline (time point 0) are summarized in Supplementary Table 1. In comparison with the cohort without CSF, patients with CSF had a higher BMI (p<0.001) and a higher serum GGT (p=0.005).

Risk Stratification Scores at time point 0 (five years prior to initial hepatology assessment).

Patients' NFS, FIB-4 and Log_{10}FIB-4 scores at time point 0 (five years prior to the initial hepatology assessment) are illustrated in Figure 1. There was a significant difference in the

NFS level at time point 0 between patients with (mean NFS -0.590 ± 1.394) and those without (-1.245 ± 1.245) CSF at hepatology assessment (p=0.006). In contrast, there was no significant difference in Log_{10}FIB-4 scores at time point 0 between patients with (mean -0.01 ± 0.55) and those without (-0.09 ± 0.48) CSF at hepatology assessment (p=0.422). Importantly, 14 of 45 (31.11%) patients with low risk NFS at time point 0 had CSF at hepatology assessment. Thirteen (92.86%) of these 14 patients either had diabetes or developed diabetes prior to hepatology assessment, compared to 19 of the 31 (61.29%) patients without CSF (p=0.038). Similarly, 37 of 98 (37.76%) patients with low risk FIB-4 scores had CSF at the time of hepatology assessment. Thirty-four of these 37 (91.89%) patients had or developed diabetes prior to hepatology assessment, compared to 46 of 61 (75.41%) without CSF (p=0.059).

Within-subject correlations for FIB-4 and NFS measurements

Patients had a median of 11 (Min 10, Max 11) retrospective observations over a median time period of 5 (Min 4.5, Max 5) years. Over this time period, the within-subject correlations for NFS and Log_{10}FIB-4 measurements (ICCs) were 0.87 (95%CI 0.85-0.89), and 0.75 (95%CI 0.71-0.78), respectively, indicating similar and good reproducibility of each noninvasive algorithm over time. The repeated measure correlation between NFS and the Log_{10}FIB-4 measures over time was moderate (repeated measure correlation r=0.55, 95%CI 0.52-0.59).

Longitudinal trends in FIB-4 and NFS measurements

The progression of NFS, log-transformed FIB-4 scores and serum HbA1c levels across time was estimated in subjects with and without CSF. Age at study baseline was included in the

regression model as a potential confounder, since older age is an independent predictor of severe fibrosis in patients with NAFLD.[24]

A significant interaction effect between time and CSF status (Yes/No) and effect of age at baseline on NFS level was detected (Table 2). The rate of increase in NFS was significantly higher in patients with CSF than in those without ($p<0.001$), with an estimated NFS level increase of 0.17 units and 0.06 units per year in subjects with and without CSF, respectively (Figure 2). The potential effects of gender and HbA1C≥7 on progression of NFS level across time were also tested but were not significant ($p=0.42$ and $p=0.30$, respectively).

Likewise, there was a significant interaction effect of time and fibrosis status and effect of age at baseline on the progression of Log_{10}FIB-4 (Table 3). The rate of increase in Log_{10}FIB-4 was significantly higher in patients with CSF than without CSF ($p<0.001$), with an estimated Log_{10}FIB-4 score increase of 0.032 units and 3e-4 units per year in subjects with and without CSF, respectively (Figure 3). The potential effects of gender, BMI and HbA1C≥7 on progression of Log_{10}FIB-4 level across time were also tested but were not significant ($p=0.86$, $p=0.47$ and $p=0.18$, respectively).

Higher rates of increase in NFS and Log_{10}FIB-4 levels across time were also observed in subjects with, compared to those without advanced fibrosis (LSM >9.5 kPa, 23.91% in this study) and in subjects with, compared to those without cirrhosis (LSM>13kPa, 16.09% in this study). (see Supplementary Figure 1, $p<0.001$ for all comparisons).

In contrast to NFS and FIB-4, there was no significant difference in the trend for HbA1c measurements over the 5-year period prior to initial hepatology assessment between patients with and without CSF ($p=0.19$). Observed mean and standard error across time is presented in Supplementary Figure 2.

Longitudinal trends in Log_{10}FIB-4 and NFS measurements in patients with low risk NFS or FIB-4 scores at time point 0

A subgroup analysis was performed in patients with low fibrosis scores at baseline, to estimate the progression of NFS and log-transformed FIB-4 scores across time in subjects with and without clinically significant fibrosis at the time of hepatology assessment (the proportion of patients with a change in low risk NFS or FIB-4 scores over time is presented in Supplementary Table 2). The estimated rate of increase of both NFS and Log_{10}FIB-4 scores was significantly higher in patients with clinically significant fibrosis than in those without ($p=0.030$ and $p<0.001$ respectively, Supplementary Figure 3). See Supplementary Text and Supplementary Table 3 for further information regarding changes in NFS and FIB-4 risk stratification groups over time.

Discussion

To our knowledge this is the first study to use a time-dependent analysis of repeated simple scores to determine their role in fibrosis monitoring during follow-up of patients with NAFLD. In our cohort, we observed significant longitudinal changes in NFS and FIB-4 scores that varied by severity of fibrosis. In patients with CSF, the simple scores progressively increased over a 5-year period prior to assessment of liver disease. In contrast, NFS and FIB-4 scores remained relatively stable over time in subjects without CSF. These findings support current recommendations to repeat NFS and FIB-4 scores every 1-2 years to monitor patients and identify those at high risk of progressive fibrosis.

Few prior longitudinal or interventional studies have examined dynamic changes in NFS and FIB-4 scores and their ability to predict NAFLD fibrosis severity. McPherson and colleagues

calculated NFS and FIB-4 scores from clinical and laboratory data collected within 6 months of paired liver biopsies from 108 NAFLD patients.[17] Over a median interval of 6.6 years (range 1.3 – 22.6), 42% of their patients had fibrosis progression while the remainder had no change in fibrosis (40%) or fibrosis regression (18%). The authors identified a significant relationship between the change in fibrosis stage between biopsies and the change in both NFS (r_s=0.24, p=0.035) and FIB-4 score (r_s=0.24, p=0.033). Another study examined the relationships between changes in serum biomarkers and histological fibrosis in paired liver biopsies from 261 NAFLD patients who completed a 12-month lifestyle intervention.[25] In patients with fibrosis improvement (20%), NFS was significantly reduced (mean −1.00 points). In contrast, NFS increased (mean +0.02 points) in patients with fibrosis progression (17%) and slightly decreased (mean −0.42 points) when fibrosis remained stable (63% of patients) (p<0.01 between the 3 groups). By multivariate analysis, change in NFS was independently associated with fibrosis improvement (OR=0.27, 95%CI 0.14-0.52, P<0.01), as well as fibrosis progression (OR=1.81, 95%CI 1.19-2.76, P<0.01). Our study extends these findings by showing a clear difference in the longitudinal change in repeated NFS and Log_{10}FIB-4 scores in patients with and without CSF. These data imply that the incremental increase in scores from baseline may be beneficial in identifying patients at risk of progressive fibrosis, even when the scores remain within a low or indeterminate risk category.

Our data identified that around one-third of patients with baseline low risk stratification using both NFS and FIB-4 scores developed CSF, suggesting that a single score has limited long term prognostic value. A recent prospective study assessing the role of patients' baseline non-invasive risk stratification scores in predicting liver-related outcomes, found that the presence of diabetes impacted the accuracy of these models.[26] Of 284 subjects

(53% diabetic, 15% cirrhotic) followed for a median of 51.4 months, up to 21% of diabetic patients with a low fibrosis score developed liver decompensation and up to 27% developed HCC at 5 years.[26] In the latter study, liver-related outcomes were not seen in subjects with low fibrosis scores who were not diabetic.[26] In our retrospective study, a higher proportion of patients with low baseline NFS scores and CSF at the time of hepatology assessment had diabetes, compared to those subjects without CSF (NFS p=0.038). These data support the role of diabetes as a risk factor for the development of significant liver disease.

In contrast to NAFLD, multiple studies have used time-course changes in non-invasive tests to estimate fibrosis progression in chronic hepatitis C infection (HCV).[27, 28] In a paired liver biopsy study of non-responders to interferon therapy (n=314), the change in FIB-4 score per year was an independent predictive factor for the progression of fibrosis stage (OR=3.7, 95%CI 1.07-12.5, p=0.03).[28] In subjects with a change in FIB-4 score per year ≥0.4, the cumulative incidence of fibrosis progression to cirrhosis was 34% and 59% over 5 and 10 years respectively, compared to 0% and 3% in those with a change in FIB-4 score per year <0.4.[28] Additional studies have used longitudinal evaluation of FIB-4 to identify and monitor liver disease in HIV-infected youth[29] and following HCV seroconversion.[27]

Although there was a significant difference in average NFS at time point 0 between our patients with and without CSF, at an individual level there was substantial overlap in these scores. Variations in levels of fibrosis at baseline were accounted for in the linear mixed model analysing fibrosis progression across time. Similarly, individual variation in rates of fibrosis progression that may have occurred due to environmental and lifestyle changes during the study period, were accounted for by considering random slopes. In our study there was no relationship between glycemic control over time and CSF, consistent with a

previous study of 1527 diabetic subjects that showed no association between HbA1c levels and progression of FIB-4 score after 3 years of follow-up.[30] Due to the retrospective data collection design of the study we were unable to collect reliable data on patients' medications or compliance and therefore we are unable to assess the impact of the different classes of hypoglycemic agents on HbA1c control or fibrosis progression.

Strengths of our study include the availability of repeated results over a 5-year time period with a clear fixed end point enabling us to assess potential rate of increments in each non-invasive biomarker for patients with and without CSF. A limitation of the study is the lack of liver histology to confirm the severity of fibrosis in the subjects with LSM ≥8.2kPa. In addition, this was a small single-center study with preferential recruitment of patients with metabolic syndrome and diabetes. Therefore, our findings may not be representative of the wider population with NAFLD.

Since fibrosis severity is a major factor predicting all-cause and liver-related mortality in NAFLD, assessment of fibrosis is fundamental in the management of these patients. First-line fibrosis biomarkers such as NFS and FIB-4 are derived from commonly measured serum markers that indirectly assess liver injury and impaired liver function occurring during the development of fibrosis. In this study we have shown that these low-cost simple scores increase progressively in patients with CSF. Development of a predictive model using fibrosis status as the outcome is an important future step, in order to provide guidance for how this knowledge may be applied clinically, to identify patients at risk of developing advanced fibrosis over time.

Figure Legends:

Figure 1: Scatterplot illustrating A) NFS B) FIB-4 and C) Log_{10}FIB-4 scores at time point 0 (five years prior to initial hepatology assessment) according to fibrosis status at time of hepatology assessment.

Figure 2: Mean ± standard error NAFLD Fibrosis Score across time for patients with and without CSF.

Figure 3: Mean ± standard error Log_{10}FIB-4 scores across time for patients with and without CSF.

Supplementary Figure 1: Mean ± standard error NAFLD Fibrosis Scores across time for patients with A) LSM < and ≥9.5kPa B) LSM < and ≥13.0kPa and mean ± standard error Log_{10}FIB-4 scores across time for patients with C) LSM < and ≥9.5kPa D) LSM < and ≥13.0kPa.

Supplementary Figure 2: Mean ± standard error HbA1c level observed across time for patients with and without CSF.

Supplementary Figure 3: Longitudinal progression of A) NAFLD Fibrosis Scores and B) Log_{10}FIB-4 Scores in the cohort identified as “low risk” for fibrosis at time point 0.

References

1. 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):328-57.
2. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84.
3. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology. 2005;129(1):113-21.
4. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2015;149(2):389-97 e10. Epub 2015/05/04.
5. Ekstedt M, Hagstrom H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015;61(5):1547-54. Epub 2014/08/16.
6. Younossi ZM, Stepanova M, Rafiq N, et al. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. Hepatology. 2011;53(6):1874-82. Epub 2011/03/02.
7. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2009;7(10):1104-12.
8. 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. 2006;43(6):1317-25. Epub 2006/05/27.
9. 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):846-54. Epub 2007/03/30.
10. Newsome PN, Cramb R, Davison SM, et al. Guidelines on the management of abnormal liver blood tests. Gut. 2018;67(1):6-19.
11. McPherson S, Stewart SF, Henderson E, et al. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59(9):1265-9. Epub 2010/08/31.
12. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Diabetologia. 2016;59(6):1121-40. Epub 2016/04/08.
13. Glen J, Floros L, Day C, et al. Non-alcoholic fatty liver disease (NAFLD): summary of NICE guidance. BMJ. 2016;354:i4428.
14. 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):455-60. Epub 2007/11/27.
15. Cassinotto C, Boursier J, de Ledinghen V, et al. Liver stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. Hepatology. 2016;63(6):1817-27. Epub 2015/12/15.
16. 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):305-15.
17. McPherson S, Hardy T, Henderson E, et al. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol. 2015;62(5):1148-55. Epub 2014/12/06.

18. Patel PJ, Hossain F, Horsfall LU, et al. A pragmatic approach identifies a high rate of NAFLD with advanced fibrosis in diabetes clinics and at-risk populations in primary care. Hepatology Communications. 2018;2(8):893-905. Epub 6th August 2018`.
19. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469-80. Epub 2006/05/10.
20. Koehler EM, Plompen EP, Schouten JN, et al. Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: The Rotterdam study. Hepatology. 2016;63(1):138-47. Epub 2015/07/15.
21. Roulot D, Roudot-Thoraval F, G NK, et al. Concomitant screening for liver fibrosis and steatosis in French type 2 diabetic patients using Fibroscan. Liver Int. 2017;37(12):1897-906.
22. Chinn S. Statistics in respiratory medicine. 3. Scale, parametric methods, and transformations. Thorax. 1991;46(7):536-8.
23. Bakdash JZ, Marusich LR. Repeated Measures Correlation. Front Psychol. 2017;8:456.
24. Angulo P, Keach JC, Batts KP, et al. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology. 1999;30(6):1356-62.
25. Vilar-Gomez E, Calzadilla-Bertot L, Friedman SL, et al. Serum biomarkers can predict a change in liver fibrosis 1 year after lifestyle intervention for biopsy-proven NASH. Liver Int. 2017;37(12):1887-96.
26. Bertot LC, Jeffrey GP, de Boer B, et al. Diabetes impacts prediction of cirrhosis and prognosis by non-invasive fibrosis models in non-alcoholic fatty liver disease. Liver Int. 2018;38(10):1793-802. Epub 2018/03/27.
27. Butt AA, Yan P, Lo Re V, 3rd, et al. Liver fibrosis progression in hepatitis C virus infection after seroconversion. JAMA Intern Med. 2015;175(2):178-85.
28. Tamaki N, Kurosaki M, Tanaka K, et al. Noninvasive estimation of fibrosis progression overtime using the FIB-4 index in chronic hepatitis C. J Viral Hepat. 2013;20(1):72-6.
29. Kapogiannis BG, Leister E, Siberry GK, et al. Prevalence of and progression to abnormal noninvasive markers of liver disease (aspartate aminotransferase-to-platelet ratio index and Fibrosis-4) among US HIV-infected youth. AIDS. 2016;30(6):889-98.
30. Giorda CB, Forlani G, Manti R, et al. Trend over time in hepatic fibrosis score in a cohort of type 2 diabetes patients. Diabetes Res Clin Pract. 2018;135:65-72.

Table 1: Demographic and clinical characteristics of the subjects with and without clinically significant fibrosis (assessed by LSM ≥ or <8.2kPa) at initial hepatology assessment (N=230)

		No clinically significant fibrosis LSM <8.2kPa N = 158	Clinically significant fibrosis LSM ≥8.2kPa N = 72	p-value
Age	Overall*	56.65±12.36	57.25±12.15	0.733
	Males*	56.92 (±13.22)	56.88 (±12.67)	0.968
	Females¥	56.00 (49.00-64.00)	59 (53.50-66.00)	0.490
Male Gender, N (%)		86 (54.43%)	41 (56.94%)	0.722
Metabolic syndrome, N (%)†		125 (79.11%)	68 (94.44%)	0.003
Type 2 diabetes, N (%)†		121 (76.58%)	68 (94.44%)	<0.001
Body mass index (kg/m^2)¥		31.25 (28.23-35.89)	37.47 (32.90-43.08)	<0.001
Waist (cm)¥		109.00 (98.25-120.00)	128.0 (117.50-136.50)	<0.001
Serum Liver enzymes*	**ALT (IU/mL)**	39.66±33.17	50.14±28.17	0.021
	AST (IU/mL)	25.44±18.28	38.24±21.45	<0.001
	GGT (IU/mL)	41.42±48.27	88.38±93.35	<0.001
Platelet count (x10^9)¥		246.00 (208.00-295.00)	228.0 (184.00-278.50)	0.027
Serum Albumin (g/L)¥		42.00 (40.00-44.00)	41.00 (38.00-43.00)	0.013
LSM (kPa)¥		5.35±1.29	17.79±11.71	<0.001
ELF test¥		9.00 (8.40-9.51)	9.89 (9.26-10.50)	<0.001
NAFLD Fibrosis Score¥		-0.74 (-1.83--0.02)	0.34 (-0.73-1.12)	<0.001
FIB-4*		0.98±0.49	1.58±1.23	<0.001

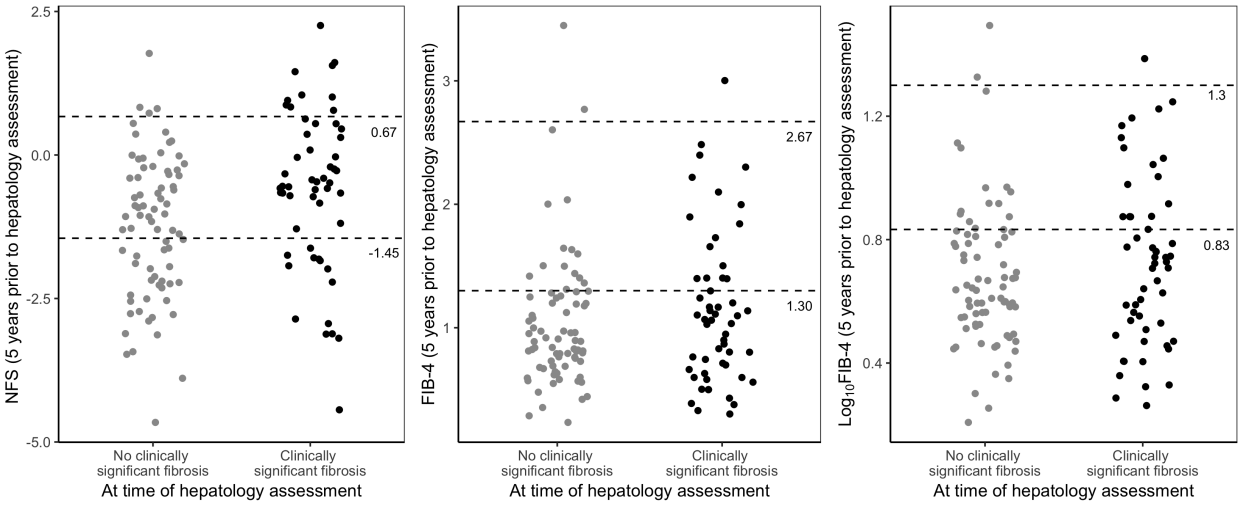
*Continuous data (mean ± SD) analysed using an Independent T Test. ¥Continuous data (median (IQR)) analysed using a Mann-Whitney U test. †Categorical data analysed using the Fisher's Exact test. All other categorical data analysed using the Pearson's χ^2

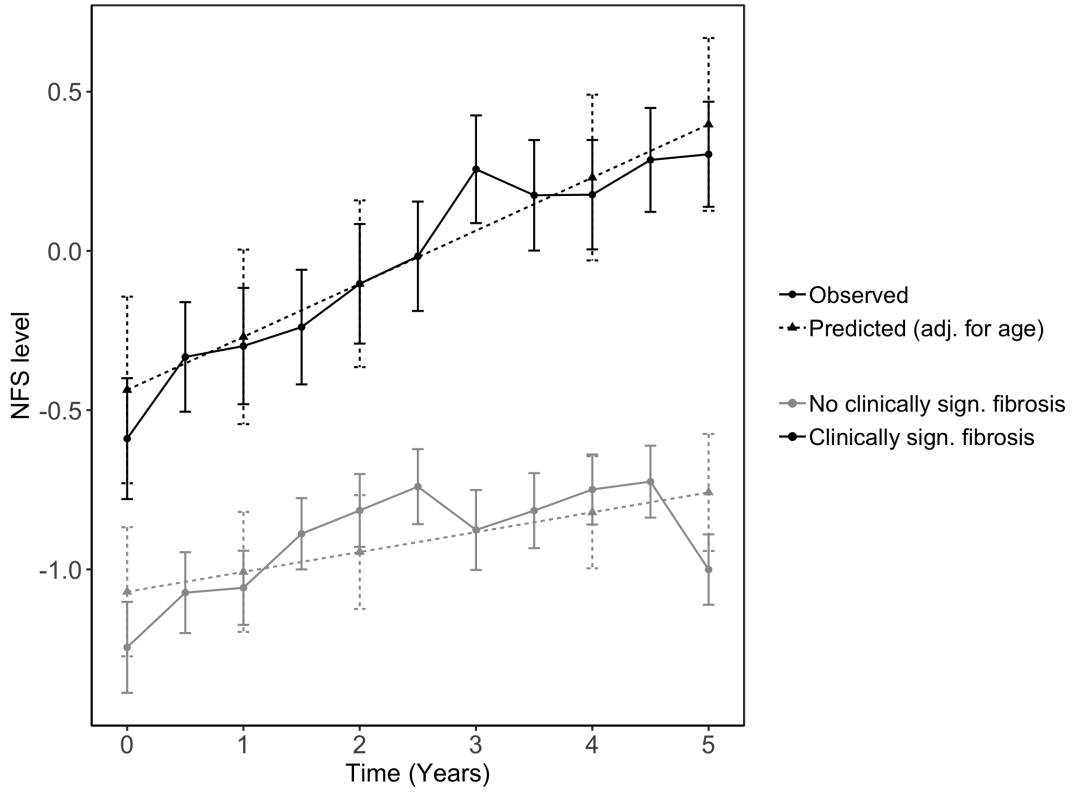
Table 2. Linear mixed model results for NFS level with fixed effects of time, fibrosis status, age at baseline and interaction time*fibrosis status and with patient as random intercept and time as random slope

Variable		Estimate	Std.Error	df	t.value	P-value
(Intercept)		-4.09	0.33	246.92	-12.56	<0.001
Time		0.06	0.02	219.83	3.77	<0.001
Age at baseline		0.06	0.01	231.88	9.67	<0.001
Fibrosis status	No clinically significant fibrosis	reference				
	Clinically significant fibrosis	0.63	0.18	212.80	3.49	<0.001
Time* Fibrosis status	No clinically significant fibrosis	reference				
	Clinically significant fibrosis	0.10	0.03	208.20	3.67	<0.001

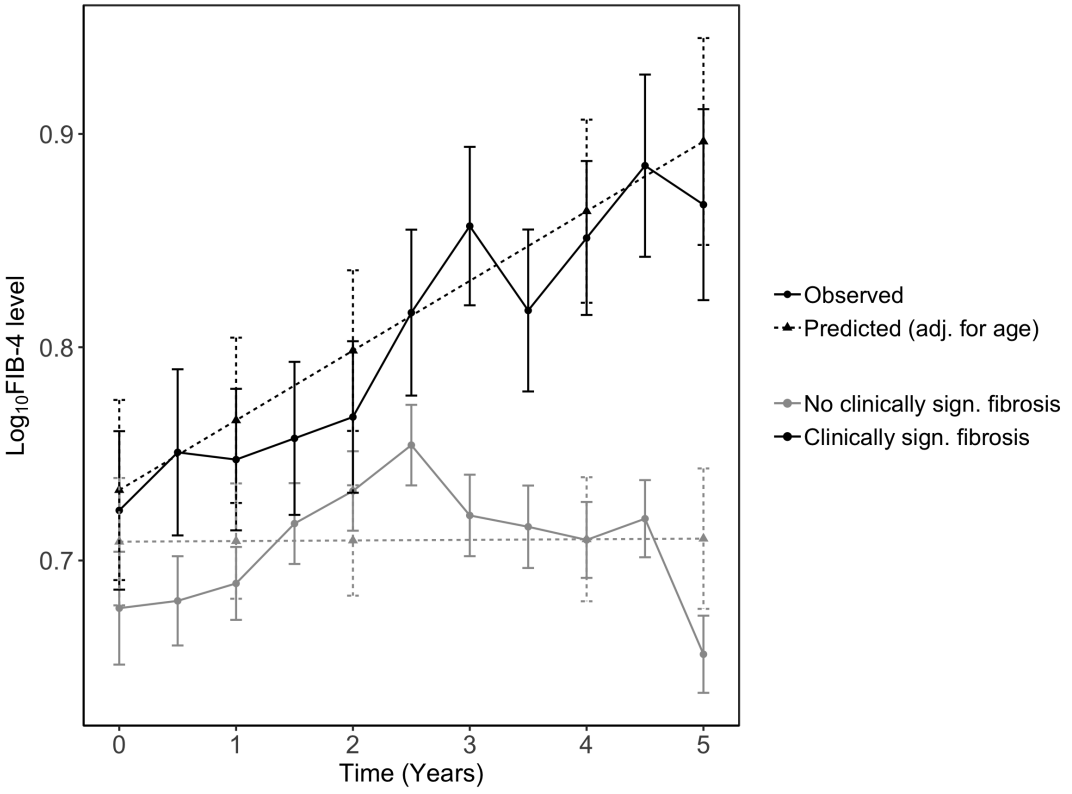
Table 3. Linear mixed model results for logFIB-4 level with fixed effects of time, fibrosis status, interaction time*fibrosis status, age at baseline and with patient as random intercept and time as random slope

Variable		Estimate	Std.Error	df	t.value	P-value
(Intercept)		0.08	0.05	253.40	1.55	0.12
Time		0.0003	0.004	223.90	0.08	0.94
Age at baseline		0.01	0.0009	235.10	13.49	<0.001
Fibrosis status	No clinically significant fibrosis	reference				
	Clinically significant fibrosis	0.02	0.03	211.90	0.92	0.36
Time* Fibrosis status	No clinically significant fibrosis	reference				
	Clinically significant fibrosis	0.03	0.006	209.90	5.21	<0.001





No clin. sign. fibrosis	76	103	121	124	123	125	127	133	142	149	158
Clin. sign. fibrosis	54	55	60	62	60	58	62	64	70	69	72



No clin. sign. fibrosis	77	105	122	124	123	125	127	133	142	149	158
Clin. sign. fibrosis	54	55	60	62	60	58	62	64	70	69	72

Supplementary text

A total of 36 patients with CSF had low (n=7) or indeterminate (n=29) risk NFS at time point 10. Among the 7 CSF patients with low risk NFS at time point 10, 5 (71.4%) were also low risk at time point 0 (within those, all experienced an increase in NFS but remained low risk) and 2 (28.6%) were indeterminate risk. Among the 29 CSF patients with indeterminate risk NFS at time point 10, 18 (62.1%) were also indeterminate risk at time point 0 (within those, 88.2% experienced an increase in NFS level but remained indeterminate risk), 9 (31.0%) were low risk at time 0 and therefore showed an increase in NFS and two patients (6.9%) were high risk at time 0.

A total of 46 patients with CSF had low (n=30) or indeterminate (n=16) risk FIB-4 scores at time point 10. Among the 30 CSF patients with low risk FIB-4 at time point 10, 26 (86.7%) were also low risk at time point 0 (53.8% experienced an increase in FIB-4 but remained low risk) and 4 (13.3%) were indeterminate. Among the 16 CSF patients with indeterminate risk FIB-4 at time point 10, 5 (31.3%) were also indeterminate at time point 0 (20% increased their FIB-4 level but remained indeterminate risk) and 11 (68.8%) were low risk at time point 0 and therefore showed an increase in FIB-4. These data (summarized in Supplementary Table 3) illustrate that although NFS and FIB-4 scores may increase significantly over time in patients with CSF, they may remain within low or indeterminate risk categories, and therefore the rate of increase may be more important than a single time point analysis.

Supplementary Table 1: Demographic and clinical characteristics of the subjects at baseline, with and without clinically significant fibrosis (assessed by LSM ≥ or <8.2kPa at initial hepatology assessment)

		No clinically significant fibrosis LSM <8.2kPa N = 158	Clinically significant fibrosis LSM ≥8.2kPa N = 72	p-value
Age[¥]	Overall	52.00 (44.00-61.00)	54.00 (47.75-61.00)	0.623
	Males	54.00 (44.00-62.00)	55.00 (47.00-60.00)	0.953
	Females	51.00 (44.00-58.25)	54.00 (48.00-61.50)	0.494
Male Gender, N (%)		86 (54.43%)	41 (56.94%)	0.831
Type 2 diabetes, N (%)†		105 (66.46%)	54 (75.00%)	0.251
Body mass index (kg/m^2)[¥^]		**30.75 (28.00-35.20)**	**35.65 (31.42-43.52)**	**<0.001**
Serum Liver enzymes[¥^]	ALT (IU/mL)	32.00(24.00-44.00)	37.50 (27.00-54.50)	0.080
	AST (IU/mL)	25.00 (20.00-33.00)	29.00 (20.00-36.00)	0.188
	GGT (IU/mL)	**36.00 (23.00-52.00)**	**52.00 (28.00-97.50)**	**0.005**
Platelet count (x10^9)*[^]		254.92±57.96	240.57±63.02	0.180
Serum Albumin (g/L)[¥^]		42.00 (40.00-44.00)	41.00 (40.00-44.25)	0.819
NAFLD Fibrosis Score[*^]		**-1.245±1.245**	**-0.590±1.394**	**0.006**
FIB-4[¥^]		0.89 (0.69-1.25)	1.06 (0.67-1.40)	0.395
Log_{10} Fibrosis 4[*^]		-0.09**±0.48**	**-0.01±0.55**	0.422

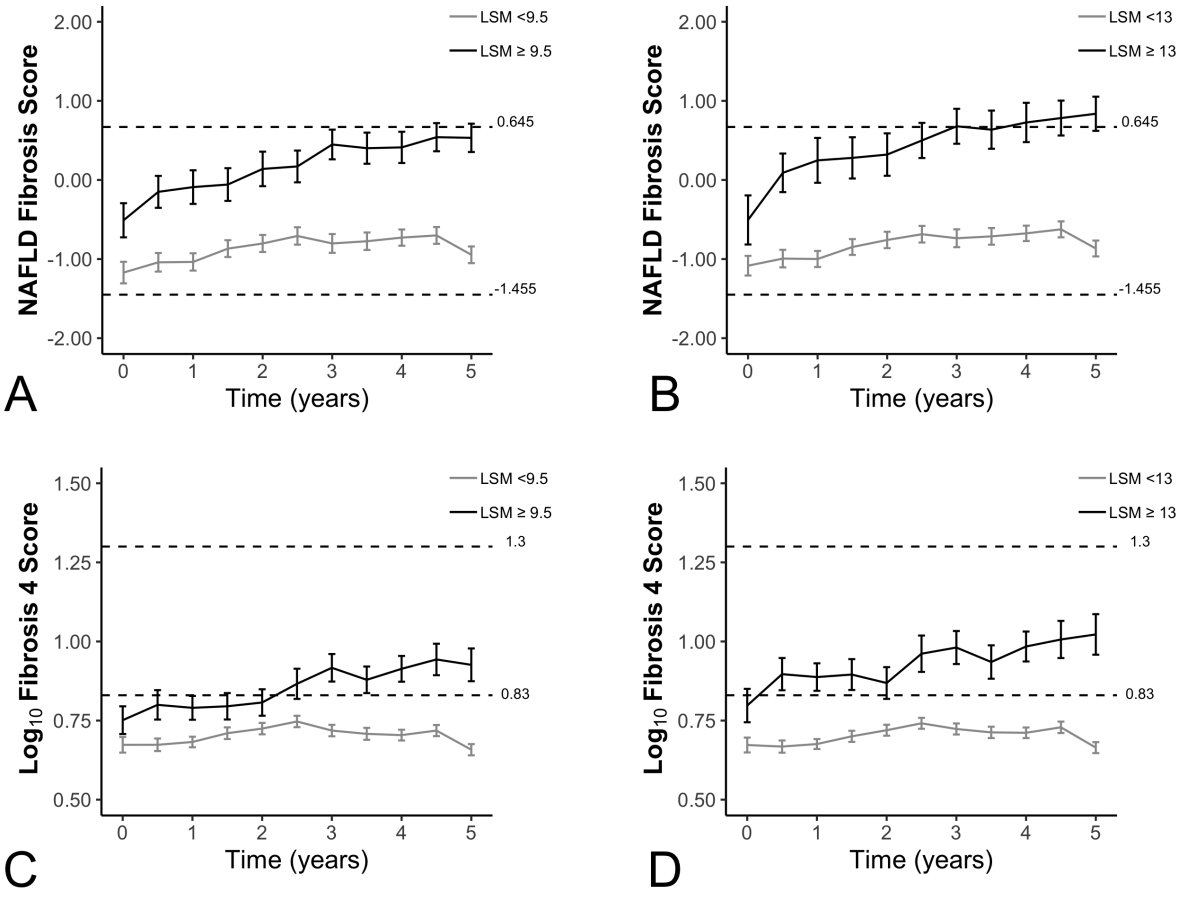
[*]Continuous data (mean ± SD) analysed using an Independent T Test. [¥]Continuous data (median (IQR)) analysed using a Mann-Whitney U test. †Categorical data analysed using the Fisher's Exact test. All other categorical data analysed using the Pearson's χ^2. At time point 0, [^]BMI results were available for 228 patients, liver enzyme results were available for 137 patients, platelet results were available for 135 patients, albumin results were available for 136 patients. NFS results were available for 130 patients, FIB4 and Log_{10} (Fib4) results were available for 131 patients.

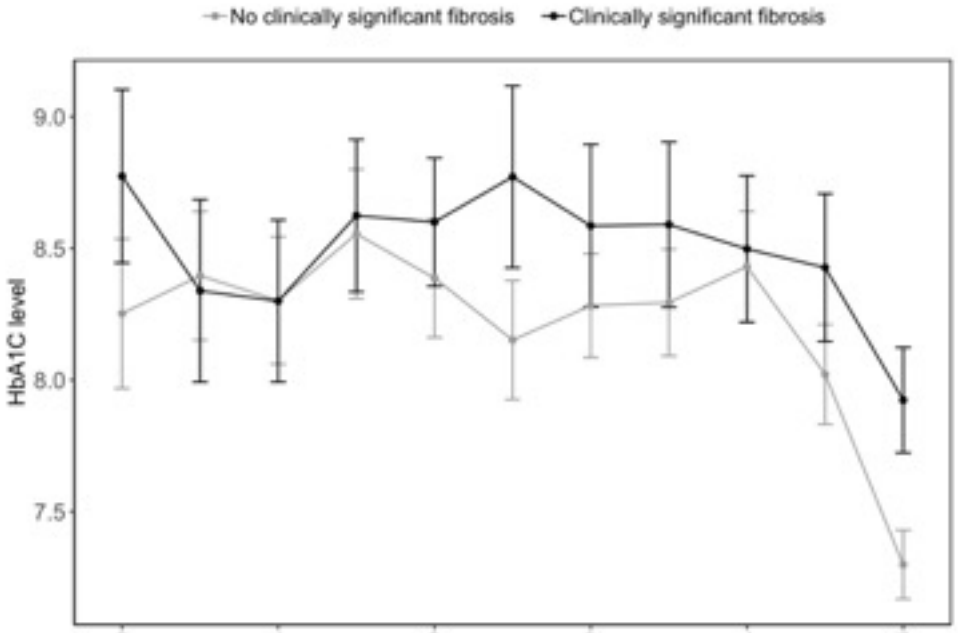
Supplementary Table 2. The proportion of patients with a change in low risk NFS or FIB-4 scores over time

	NFS		FIB4	
Time (Years)	**LSM<8.2**	**LSM≥8.2**	**LSM<8.2**	**LSM≥8.2**
0	0/31	0/14	0/61	0/37
0.5	2/28	2/9	4/58	3/31
1	5/30	3/9	8/60	4/32
1.5	6/24	4/12	6/50	7/36
2	8/26	3/10	7/53	6/32
2.5	12/28	5/10	6/54	7/33
3	10/27	7/10	8/52	9/33
3.5	12/27	6/11	10/53	12/35
4	13/29	7/14	5/58	13/37
4.5	14/30	10/14	7/59	17/37
5	10/31	9/14	11/61	11/37

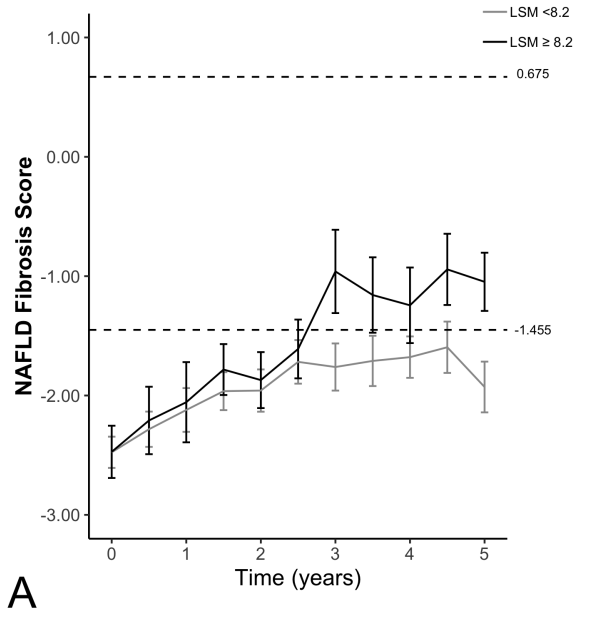
Supplementary Table 3 Changes in NFS and FIB-4 risk stratification group over study period

			NFS Risk Stratification at time point 10		
			Low	Indeterminate	High
LSM<8.2	NFS Risk Stratification at time point 0	Low	21 (84%)	10 (20.4%)	0 (0%)
		Indeterminate	4 (16%)	36 (73.5%)	1 (50%)
		High	0 (0%)	3 (6.1%)	1 (50%)
LSM≥8.2	NFS Risk Stratification at time point 0	Low	5 (71.4%)	9 (31.0%)	0 (0%)
		Indeterminate	2 (28.6%)	18 (62.1%)	10 (55.6%)
		High	0 (0%)	2 (6.9%)	8 (44.4%)
			FIB4 Risk Stratification at time point 10		
			Low	Indeterminate	High
LSM<8.2	FIB4 Risk Stratification at time point 0	Low	50 (89.3%)	10 (52.6%)	1 (50%)
		Indeterminate	5 (8.9%)	9 (47.4%)	0 (0%)
		High	1 (1.8%)	0 (0%)	1 (50%)
LSM≥8.2	FIB4 Risk Stratification at time point 0	Low	26 (86.7%)	11 (68.8%)	0 (0%)
		Indeterminate	4 (13.3%)	5 (31.3%)	7 (87.5%)
		High	0 (0%)	0 (0%)	1 (12.5%)

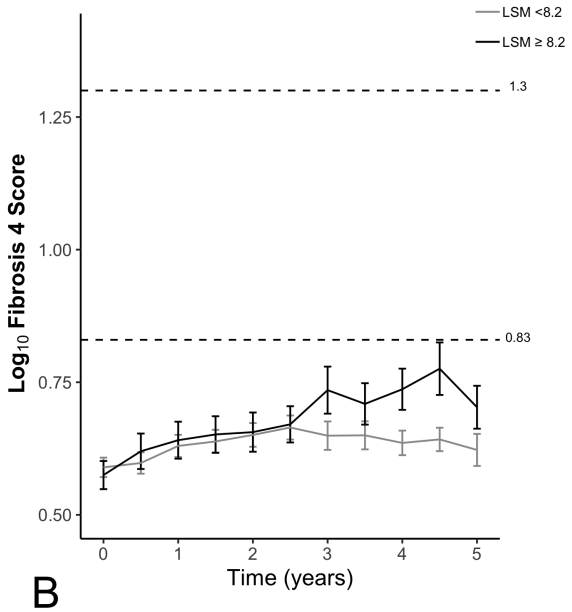

LSM <9.5
LSM ≥ 9.5
NAFLD Fibrosis Score
2.00
1.00
0.00
-1.00
-2.00
0.645
-1.455
0
1
2
3
4
5
Time (years)
A
LSM <13
LSM ≥ 13
NAFLD Fibrosis Score
2.00
1.00
0.00
-1.00
-2.00
0.645
-1.455
0
1
2
3
4
5
Time (years)
B
LSM <9.5
LSM ≥ 9.5
Log10 Fibrosis 4 Score
1.50
1.25
1.00
0.75
0.50
1.3
0.83
0
1
2
3
4
5
Time (years)
C
LSM <13
LSM ≥ 13
Log10 Fibrosis 4 Score
1.50
1.25
1.00
0.75
0.50
1.3
0.83
0
1
2
3
4
5
Time (years)
D



No clin. sign. fibrosis	46	50	51	53	63	52	57	71	68	80	158
Clin. sign. fibrosis	31	31	33	41	39	39	35	42	43	44	72



A



B

"What You Need to Know"

Background:

It is not clear how biomarkers of fibrosis, such as the non-alcoholic fatty liver disease (NAFLD) fibrosis score (NFS) or the fibrosis-4 score (FIB-4) score, change over time. We examined longitudinal changes in the NFS and the FIB-4 score in patients with NAFLD, with and without clinically significant fibrosis.

Findings

Non-invasively measured fibrosis scores increase progressively in patients with NAFLD and CSF.

Implications for patient care

In patients with clinically significant fibrosis, scores from non-invasive tests to measure fibrosis increase progressively. Further assessment of these longitudinal changes is required to identify whether repeated measurements can identify patients at risk for significant hepatic fibrosis.