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ORIGINAL ARTICLE

Association between longitudinal biomarkers and major adverse liver outcomes in patients with non-cirrhotic metabolic dysfunction–associated steatotic liver disease

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Abstract

Background and Aims: Noninvasive biomarkers provide prognostic information for the development of major adverse liver outcomes (MALOs) in patients with metabolic dysfunction–associated steatotic liver disease (MASLD), but the predictive value of longitudinal biomarker measurements has not been evaluated. We assessed whether changes in biomarkers could predict incident MALO in MASLD.

Approach and Results: We analyzed a cohort of 1260 patients (71.7% on biopsy) with non-cirrhotic MASLD between 1974 and 2019. Data at baseline and follow-up visits were obtained from medical charts. MALO was determined through medical charts and linkage to national registers until the end of 2020. A joint modeling approach was used to quantify the associations between the trajectory of biomarkers and the risk of MALO. MASLD was diagnosed at a median age of 52 years (IQR: 39–60), and 59% were male. During a median follow-up of 12.2 years, 111 (8.8%) patients developed MALO. The joint modeling showed that an elevated fibrosis-4 score (HR: 2.60, 95% CI: 1.89–3.50), aspartate aminotransferase (HR: 2.69, 95% CI: 2.57–3.05), and lower platelet count (HR: 0.93, 95% CI: 0.90–0.97) at any time point were associated with an increased risk of MALO, whereas the rate of change in these biomarkers had no association with this risk.

Conclusions: In addition to baseline measurements of noninvasive biomarkers such as fibrosis-4 score, aspartate aminotransferase, and platelets taken at MASLD diagnosis, monitoring their values over time is important, as

Abbreviations: aHR, adjusted Hazard Ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CDR, causes of death register; FIB-4, fibrosis-4 score; ICD, International Classification of Diseases; MALO, major adverse liver outcomes; MASLD, metabolic dysfunction–associated steatotic liver disease; NPR, National Patient Register; VCTE, vibration-controlled transient elastography.

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the latest value of these biomarkers is closely associated with the risk of future MALO. The rate of change may not be as important.

INTRODUCTION

Metabolic dysfunction–associated steatotic liver disease (MASLD) is the most common chronic liver disease, affecting around one-third of the global population.[1,2] MASLD is a significant contributor to the development of major adverse liver outcomes (MALOs), including cirrhosis and HCC,[1,3] and MASLD-related MALO is often diagnosed at a late stage, associated with high mortality.[4] The prognosis and management of patients with MASLD highly depend on the stage of fibrosis, which may also be assessed by noninvasive biomarkers as emphasized by the recent EASL guidelines.[5] Blood-based biomarkers have emerged as an attractive alternative to liver biopsy or vibration-controlled transient elastography (VCTE) in evaluating fibrosis, as biopsy is invasive, and imaging assessments can be costly and may not be available in many clinical settings.

Several biomarkers have been extensively studied for diagnosing advanced liver fibrosis, including the fibrosis-4 score (FIB-4).[6,7] In general, these scores have relatively high negative predictive values. The FIB-4 score is therefore recommended as a first-line test for ruling out advanced fibrosis in patients with a high risk for MASLD.[5] In addition to its diagnostic capacity, FIB-4 may be predictive of future liver events, as many studies showed an association between higher baseline FIB-4 value and incident MALO in patients with MASLD.[8–10] However, measuring such biomarkers longitudinally may provide additional information on the risk for future MALO compared to a single test since changes in, for example, FIB-4 may reflect disease progression over time. Previous studies reported that an increase in FIB-4 over time was associated with a higher risk of MALO in both general and MASLD populations.[11,12] For instance, a 17-fold increased risk of MALO was found in individuals defined as high risk for advanced fibrosis by FIB-4 at 2 separate tests within 5 years compared to individuals consistently in the low-risk group, although half of the events occurred in the low-risk group.[11] The double cutoffs (low and high) of most noninvasive scoring systems, such as FIB-4, result in a gray zone consisting of patients with intermediate results, making evaluation of changes in categories of FIB-4 complicated. Using continuous values of biomarkers measured over time may better classify patients at risk of incident MALO. Furthermore, beyond the individual value of a biomarker, it is unknown if the *rate* of change in a biomarker over time is additionally informative of the risk of MALO. Such information may help improve monitoring the progression of fibrosis in patients with non-cirrhotic MASLD. To this end, this study aimed to assess if longitudinal values and the rate of change in biomarkers over time are associated with a higher risk of MALO in patients with non-cirrhotic MASLD.

METHODS

Study design and population

This study pooled data from 4 cohorts of 1330 patients aged $\geq$18 years, with MASLD diagnosed at 3 Swedish university hospitals between December 18, 1974, and December 31, 2020.[13,14] Information on data collection has been previously reported in detail.[15] Briefly, patients were diagnosed with MASLD through liver biopsy, radiological measures such as controlled attenuation parameter, ultrasound, or other radiological examinations after ruling out other causes for steatosis, any concurrent liver disease, reported daily alcohol consumption of more than 30 g for men or 20 g for women, binge drinking, or previous liver transplantation.

The staging of fibrosis was scored on a 5-point scale (F0–F4) using either the Kleiner or METAVIR classification systems.[16,17] As not all patients underwent biopsy, the fibrosis stage was defined based on the liver stiffness measurement on VCTE if a biopsy was missing and VCTE was available. Fibrosis was then defined as "no/mild" if the biopsy showed a stage of F0–F1 or liver stiffness measurement of $<$10 kPa on VCTE when a biopsy was missing. "Moderate or advanced" fibrosis was defined as a biopsy stage of F2–F3 or a VCTE value between 10 and 15 kPa if a biopsy was missing. Patients with F4 on biopsy and those with a VCTE value $>$15 kPa on VCTE were excluded, as they presumably had cirrhosis. This left us 1260 patients with non-cirrhotic MASLD (Figure 1).

The index date was defined as the time of liver biopsy or of other diagnostic modalities if a biopsy was missing, and a baseline period was defined as within 1 month of the index date. Patients were followed up at prespecified time points (1, 2, 5, 10, and 20 years after the index date), with detailed clinical and biochemical assessments extracted from patient charts at those times.

Baseline characteristics

Information at baseline and during follow-up were collected from multiple sources. Granular data from

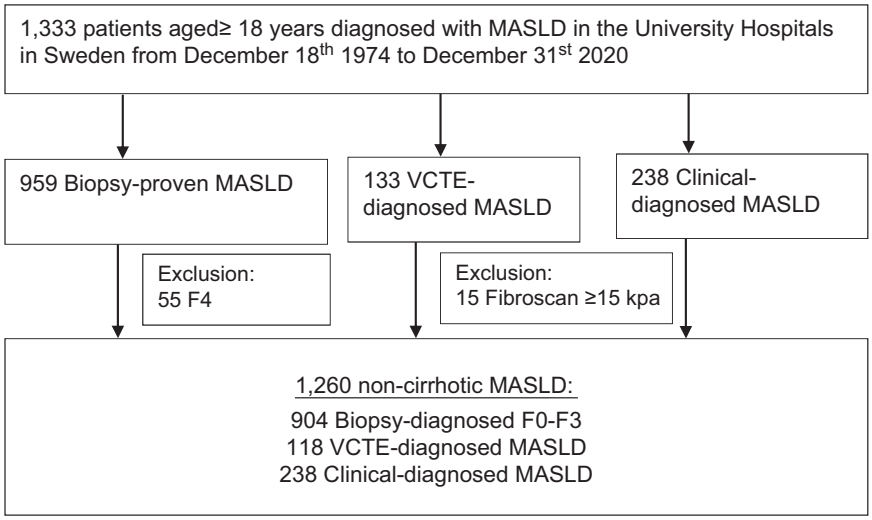

FIGURE 1 Flowchart of study population. Abbreviations: MASLD, metabolic dysfunction–associated steatotic liver disease; VCTE, vibration-controlled transient elastography.

patient's charts included clinical and biochemical parameters (eg, blood pressure, drug prescription data, and body weight). To complement this, data on recorded diagnoses based on International Classification of Diseases (ICD) codes from the National Patient Register (NPR) and data on dispensed drugs from the Prescribed Drug Register were used (definitions in Supplemental Table S2, http://links.lww.com/HEP/I604). The NPR started in 1964 and has positive predictive values of ICD-based diagnoses of around 85%–95% depending on the diagnosis.[18] Diagnoses related to cirrhosis and MASLD have been formally validated and found to have positive predictive values of more than 90%.[19,20] The Prescribed Drug Register was initiated in July 2005 and includes information on dispensed drugs from Swedish pharmacies.

A number of comorbidities were defined based on these data sources. Type 2 diabetes was defined as a registered diagnosis in the charts or NPR, fasting glucose ≥ 7 mmol/L, or having any antidiabetic medication recorded in charts or the Prescribed Drug Register. Hypertension was defined as a registered diagnosis, a resting blood pressure of $\geq 140/90$ mm Hg, or any prescribed antihypertensive medications. Hyperlipidemia was defined as a registered diagnosis, prescribed treatment with statins or other lipid-lowering medications, or a fasting total cholesterol value of ≥ 5.18 mmol/L. Height and weight were measured by medical staff, and body mass index (BMI, kg/m^2) was calculated.

Biochemical variables

Routine biochemical variables within 1 month of the MASLD diagnosis were collected from patient charts at baseline and at repeated time points when available: alanine aminotransferase (ALT, μkat/L), aspartate aminotransferase (AST, U/L), platelet count (10^9/L), albumin (g/L), bilirubin (mg/dL), international normalized ratio, creatinine (mg/dL), fasting total cholesterol (mmol/L) and triglycerides (mmol/L), LDL (mmol/L), and HDL (mmol/L). The Model for End-Stage Liver Disease score was calculated based on the formula[21]: Model for End-Stage Liver Disease $= 9.57 \times \ln$ (creatinine) + $3.78 \times \ln$ (total bilirubin) + $11.2 \times \ln$ (international normalized ratio) + 6.43. The FIB-4 score was calculated according to the published formula: [age × AST]/[platelets × ALT$^{1/2}$] and categorized as low risk for advanced fibrosis (< 1.30), intermediate (1.30–2.67), and high (> 2.67).[11]

Outcomes

Outcomes were defined by the NPR, the Swedish Cancer Register, and the Causes of Death Register (CDR) between 1974 and 2020. Malignancies are documented in the Swedish Cancer Register which contains around 96% of all diagnosed cancers in Sweden.[22] The CDR contains data on causes of death for all Swedish citizens. It is mandatory for Swedish physicians to report the cause of death and any diagnosis that might have contributed to death to the CDR.[23] Both primary and contributing diagnoses were used to identify outcomes in the NPR and CDR.

MALOs was a composite outcome, defined as having any diagnosis related to cirrhosis as defined by ICD codes in the aforementioned registers. These included compensated or decompensated cirrhosis, chronic or unspecified hepatic failure, liver transplantation, HCC, a Model for End-Stage Liver Disease score ≥ 15 by patient chart review, or liver-related death from the CDR

(definitions in Supplemental Table S1, http://links.lww.com/HEP/I604). The validity for cirrhosis and HCC diagnoses from the NPR in patients with known liver disease have positive predictive values ranging from 91% to 96%.[19]

Statistical analysis

Baseline characteristics were expressed as median and IQR or frequency and percentage where applicable. Patients were followed from baseline until the date of diagnosis of MALO or were censored at the date of emigration, diagnosis of any other liver disease than MASLD, death, or end of the study period (December 31, 2020), whichever occurred first. The multi-step variable selection strategy was used to decide which biomarkers and clinical factors should be included in the longitudinal analysis. Univariable Cox regression models were first fitted for each of the prespecified variables that might be associated with MALO based on clinical judgment and previous literature.[24–26] These variables included age at index date, sex, ALT, AST, platelet count, triglycerides, total cholesterol, BMI, type 2 diabetes, hypertension, hyperlipidemia, estimated glomerular filtration rate, and fibrosis stage (by biopsy or VCTE). Platelet count was modeled in the unit of every 10 Giga/L to allow for clinically meaningful interpretation. Variables with $p < 0.1$ were included in an elastic net regression model, and those that had non-zero coefficients in the model (meaning that the variables are likely associated with the outcome) were selected into the final Cox regression model.[27] HRs and 95% CIs were estimated based on 2 models: (1) crude HR from the univariable models and (2) adjusted HR from the multivariable model with selected variables. The final model also incorporated sex and BMI, given the clinical relevance for MASLD, regardless of their significance level.

The trajectories of selected biomarkers over time before MALO were modeled using a linear mixed-effects model. The values of biomarkers were log-transformed if normality was violated graphically. Random intercepts and slopes for time were introduced to account for variance between and within individuals' measurements over time. Since a nonlinear trajectory of biomarkers over time was observed, a random effect for time was modeled using natural cubic splines.

The joint model approach links the predicted trajectory of biomarkers, as obtained from a linear mixed-effects model, with a Cox regression model. This method, hence, uses data from all possible measurements of a biomarker over time, increasing statistical power. This allows for the estimation of how changes in biomarkers that are measured repeatedly over time are associated with the risk of an event.[28]. This approach is likely superior to a time-varying Cox approach or landmark analysis because such methods imply strong assumptions about the path of the time-varying exposures, which may be unrealistic for biomarker evolution.[28,29] Since we were interested in the longitudinal value (ie, the current value at the time of measurement) and the slope (ie, the speed of reaching the current value at the time of measurement) of the biomarkers in relation to the outcomes, we explored such relationships using 2 parameterizations. First, we estimated the association between a patient's current value of biomarkers at time *t* and the risk of MALO at the same time point. Second, we estimated the association between the slope, which reflected the speed and direction of the biomarker's trajectories at time *t*, and the risk of MALO following this. Since some biomarkers were naturally log-transferred and the coefficients in the Cox regression models represent the log hazard, doubling in biomarkers levels associated with the HR was calculated for ease of interpretation. HR adjusted for age and sex, HR adjusted for age, sex, BMI, type 2 diabetes, and hyperglycemia, and HR additionally adjusted for fibrosis stage were reported.

The individualized probability of MALO was predicted based on a set of repeated measurements of FIB-4 and baseline covariates from the fully adjusted joint model in a dynamic matter. This means that the individual's probability of the event was updated every time a new measurement of FIB-4 was performed. This graph consists of 2 parts: the predicted trajectory is plotted on the left side and the corresponding predicted 10-year event-free probabilities with 95% CI for that patient on the right side. We here used FIB-4 for illustration as it is one of the most adopted biomarkers in the field and showed a strong correlation with incident MALO in our analyses.

The R-package JMBayes was used to fit the joint models and subsequent dynamic prediction.[28] Multiple imputation with chained equations was used for missing data. Variables that exhibited $>30\%$ missingness were removed from the analysis altogether. The analyses were performed in STATA/MP 17 (StataCorp LP) and R version 4.1.1 (R Foundation).

Ethical consideration

The study was approved by the Regional Ethical Review Board of Stockholm (dnr 2018/880-31).

RESULTS

Baseline characteristics of patients

A total of 1260 patients with non-cirrhotic MASLD were included (median age: 52 y; 59% male), of which 904 (71.7%) had biopsy-proven MASLD, 118 (9.4%) had VCTE-estimated fibrosis, and 238 (18.9%) had a clinical

TABLE 1 Basic characteristics of the study population (n = 1260)

Parameter	Complete data	Median (IQR)/N (%)
Age, y	1260	52 (39–60)
Male	1260	748 (59)
Follow-up time, y	1260	12.2 (5.7–23.9)
Calendar periods	1260	
1974–1980		39 (3.1)
1981–1990		361 (28.7)
1991–2000		182 (14.4)
2001–2010		284 (22.5)
2011–2019		394 (32.3)
Fibrosis stage by biopsy	904	
F0		222 (24.6)
F1		372 (41.2)
F2		210 (23.3)
F3		100 (11.1)
Transient elastography	118	
CAP (dB/m)	42	328 (287–355)
LSM (kPa)	118	6.4 (5.1–7.9)
IQR, LSM	95	1.1 (0.7–1.6)
Success rate (%)	86	100 (83–100)
Fibrosis stage by VCTE or biopsy	1022	
No or mild (F0–F1 on biopsy or VCTE < 10 kPa)		698 (68.3)
Moderate or advanced (F2–F3 on biopsy or VCTE 10–15 kPa)		324 (31.7)
Biochemical variables		
ALT, U/L	1225	68 (45–104)
AST, U/L	1205	41 (30–58)
Platelets count, 10^9/L	1039	241 (198–287)
Albumin, g/L	1073	41 (39–44)
Bilirubin, mg/dL	1153	0.59 (0.47–0.82)
INR	1009	1 (1–1)
Creatinine, mg/dL	998	0.93 (0.79–1.04)
eGFR, mL/min/1.73 m^2	998	96 (88–105)
BMI, kg/m^2	1028	29 (26–32)
Triglycerides, mmol/L	654	1.9 (1.3–2.7)
Total cholesterol, mmol/L	703	5.7 (4.8–6.5)
LDL, mmol/L	202	3.2 (2.2–3.0)
HDL, mmol/L	236	1.1 (0.9–1.3)
Fasting glucose, mmol/L	731	5.6 (5.0–6.7)
Key comorbidities		
Type 2 diabetes	1260	319 (25.3)
Hypertension	1260	833 (66.1)
Hyperlipidemia	1260	258 (20.5)
Scoring systems		
MELD score	843	6 (6–8)
FIB-4 score	1016	0.97 (0.69–1.51)
Low (< 1.30)		696 (68.5)
Intermediate (1.30 to < 2.67)		248 (24.4)
High (≥ 2.67)		72 (7.1)

Abbreviations: ALT, alanine aminotransferease; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4 score; INR, international normalized ratio; LSM, liver stiffness measurement; MELD, Model for End-Stage Liver Disease; VCTE, vibration-controlled transient elastography.

diagnosis of MASLD lacking biopsy and VCTE. Hence, there were 1022 patients with information on fibrosis stage based on biopsy or VCTE, of which 698 (68.3%) were classified as having no or mild fibrosis (F0–F1) and 324 (31.7%) as moderate or advanced fibrosis (F2–F3). Baseline characteristics are shown in Table 1. There were 25.3% of patients with type 2 diabetes, 66.1% with hypertension, and 20.5% with hyperlipidemia at baseline. In addition, the median (IQR) FIB-4 value was 0.97 (0.69–1.51), and 68.5% of patients were classified as at low risk for fibrosis based on FIB-4 (ie, < 1.3).

MALO and baseline risk factors and biomarkers

During a median follow-up of 12.2 years (IQR: 5.7–23.9, corresponding to 18,657 person-years), 111 (8.8%, incidence rate: 5.9/1000 person-years) patients developed MALO. The individual liver outcome is presented in Supplemental Table S4, http://links.lww.com/HEP/I604. The baseline factors predictive of MALO, based on univariable Cox regression, are reported in Table 2. For example, for 1 year increase in age, the hazard of MALO increased by 5% (95% CI: 1.03–1.07). A nonlinear relationship between continuous FIB-4 and incident MALO was observed, as the HR for continuous FIB-4 was 1.01 (95% CI: 0.99–1.02), whereas intermediate (2.89, 95% CI: 1.90–4.41) or high FIB-4 category (6.74, 95% CI: 4.03–11.3) was associated with an increased risk in the outcome, compared to the low FIB-4 category. Multivariable and elastic net regression showed that baseline factors, including age at index date, type 2 diabetes, hyperlipidemia, fibrosis stage, AST, platelets count, and FIB-4, were associated with incident MALO.

MALO and longitudinal biomarkers

In total, 678 (53.8%) patients had > 1 follow-up visit where FIB-4 could be calculated. We included 2411

TABLE 2 HRs and 95% CIs of major adverse liver outcomes in relation to baseline parameters

	HR	*p*	aHR[a]	*p*
Age, y	**1.05 (1.03–1.07)**	**< 0.001**	**1.04 (1.02–1.06)**	0.001
Male	**0.68 (0.46–1.00)**	**0.05**	1.12 (0.69–1.80)	0.651
ALT, U/L	**1.00 (1.00–1.01)**	**0.011**		
AST, U/L	**1.01 (1.01–1.01)**	**0.001**	**1.01 (1.00–1.01)**	0.005
Platelets count, 10 Giga/L	**0.94 (0.92–0.97)**	**< 0.001**	**0.96 (0.92–0.99)**	0.023
Triglycerides, mmol/L	1.00 (0.87–1.16)	0.957	—	
Total cholesterol, mmol/L	1.02 (0.81–1.12)	0.765	—	
BMI, kg/m^2	1.01 (0.97–1.05)	0.688	0.99 (0.93–1.04)	0.624
Type 2 diabetes	**2.78 (1.87–4.15)**	**< 0.001**	**1.97 (1.23–4.93)**	0.005
Hyperlipidemia	**2.25 (1.45–3.48)**	**< 0.001**	**1.95 (1.17–3.26)**	0.011
Hypertension	**1.84 (1.17–2.89)**	**0.008**	—	
eGFR, mL/min/1.73 m^2	**0.98 (0.97–0.99)**	**0.001**	—	
Bilirubin, mg/dL	1.21 (0.85–1.74)	0.280	—	
Albumin, g/L	**0.93 (0.89–0.98)**	**0.004**	—	
Biopsy or VCTE-estimated fibrosis				
No or mild	Reference		Reference	
Moderate or advanced	**4.57 (2.97–7.05)**	< 0.001	**3.10 (1.95–4.93)**	< 0.001
FIB-4 (continuous)	1.01 (0.99–1.02)	0.407	0.98 (0.89–1.07)	0.616
FIB-4 (categorized)				
Low (< 1.30)	Reference			
Intermediate (1.30 to < 2.67)	**2.89 (1.90–4.41)**	< 0.001		
High (≥ 2.67)	**6.74 (4.03–11.3)**	< 0.001		

[a]Adjusted for age, sex, AST, platelets, BMI, type 2 diabetes, hyperlipidemia, fibrosis stage, and continuous FIB-4.
Abbreviations: aHR, adjusted hazard ratio; ALT, alanine aminotransferease; AST, aspartate aminotransferase; BMI, body mass index; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4 score; INR, international normalized ratio; VCTE, vibration-controlled transient elastography.

instances where repeated biomarkers were collected from these patients over a median follow-up of 5 years (IQR: 1–11). In this cohort, 33.4% of MALO events occurred in patients with low FIB-4 at baseline. The median time from the low FIB-4 category to the high FIB-4 category was 10 years (IQR: 3–19). 85.7% of those with MALO events had intermediate or high FIB-4 at any time during follow-up.

Individual measurements (log-transferred FIB-4, platelets, and log-transferred AST) and individual biomarker trajectories, as well as the population average trajectories, are presented in Figure 2. Higher values in the log-transferred FIB-4 trajectory over time among patients who developed MALO compared to those who did not were observed (Figure 2A). Similar patterns were observed for log-transferred AST and, to some extent, platelets (Figures 2B, C).

The standardized coefficients of the joint model are presented in Table 3, suggesting a strong association between the most recent measurement of FIB-4, AST, and platelets and incident MALO. The joint models showed that a doubling in the FIB-4 level at any time point was associated with a 3.29-fold (95% CI: 2.31–4.79) increased risk of MALO after adjustment for age and sex. After adjustment for age, sex, BMI, type 2 diabetes, and hyperlipidemia, the HRs were slightly lower, 2.81 (95% CI: 2.08–3.84). This estimate was further reduced to 2.60 (95% CI: 1.89–3.50) after additionally adjusting for the fibrosis stage at baseline. The growth rate, that is, the slope of FIB-4 trajectory, was however not associated with incident MALO after adjusting for baseline covariates, including fibrosis stage (adjusted hazard ratio [aHR]: 1.04; 95% CI: 0.67–1.61). Similar patterns were observed for AST and platelets, in that doubling the U/L in the current value of AST (aHR: 2.69; 95% CI: 2.57–3.05) and every 10 Giga/L decrease in the current value of platelets (aHR: 0.93, 95% CI: 0.90–0.97) were associated with a higher risk of MALO disease after full adjustment, while the slopes of both biomarkers were not.

Dynamic prediction of MALO in individual patients

Figure 3 illustrates how FIB-4 measurements taken over time can improve the prediction of the 10-year probability of MALO, depicted for 2 female patients with fibrosis stage 1. Patient B had comorbid type 2 diabetes and hyperlipidemia, while patient A did not.

Generally, FIB-4 levels tend to increase over time; as FIB-4 values increased, the probability of MALO also

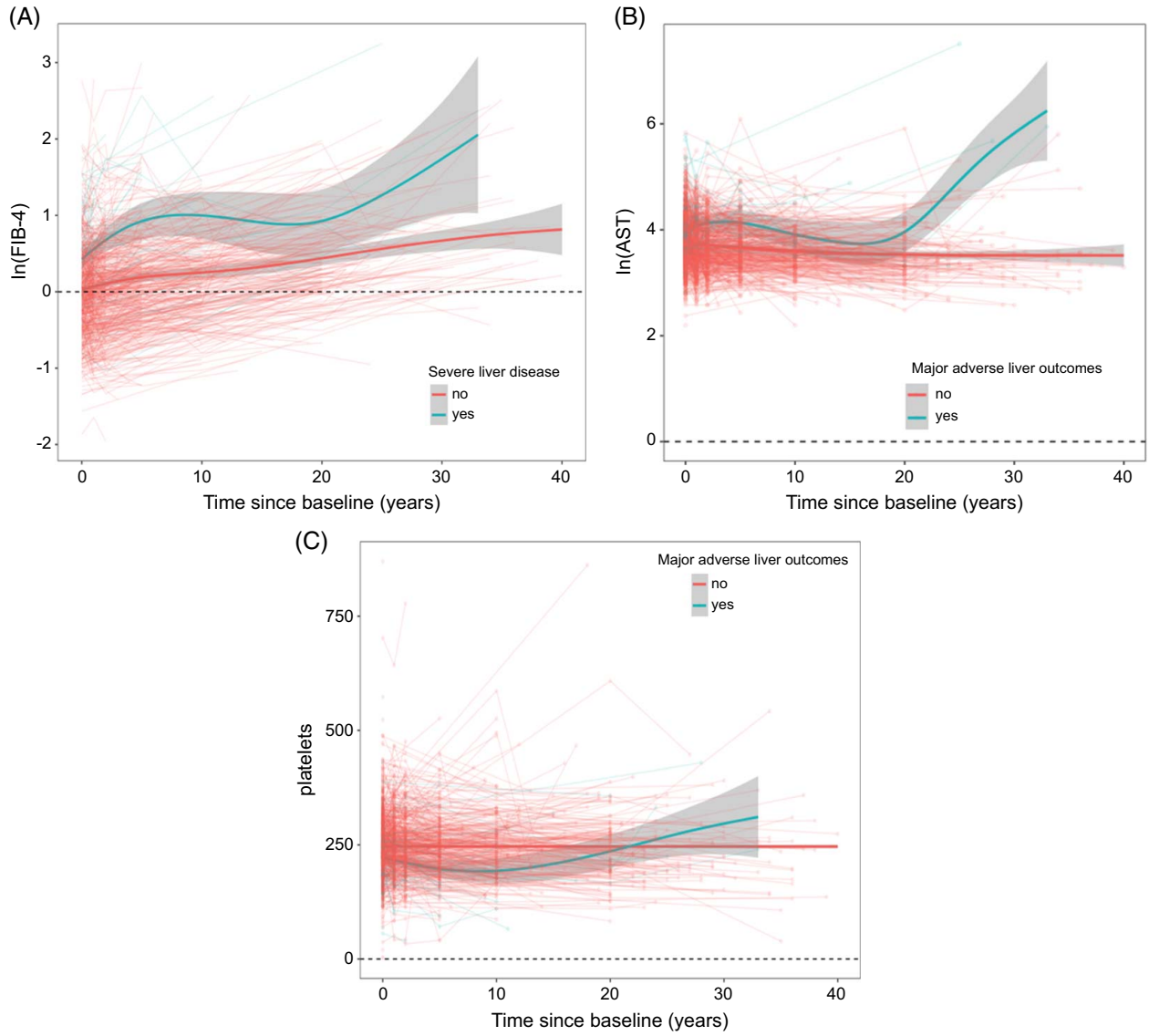

FIGURE 2 Individual biomarkers (A: ln [FIB-4], B: ln [AST], and C: platelets) measurements over time (dots), individual biomarkers trajectories (shaded lines),and the population average biomarkers trajectory (solid lines) before the development of MALO. Trajectories are color-coded by MALO status (red: free from MALO and blue: incident MALO). Abbreviations: AST, aspartate aminotransferase; FIB-4, fibrosis-4 score; MALO, major adverse liver outcome.

increased. Patient A had a relatively high FIB-4 repeatedly from baseline until year 5. The FIB-4 value then increased rapidly at year 10, leading to a higher probability of the outcome. In contrast, patient B had a lower FIB-4 at baseline but had comorbid metabolic risk factors, resulting in a relatively comparable 10-year probability to patient A. Patient B had a stable FIB-4 over time, and hence, the probability remained stable as more measurements were added.

DISCUSSION

In this large cohort study in patients with MASLD with highly granular data collected over time, biomarkers such as FIB-4, AST, and platelets count, when measured longitudinally, were significantly associated with an increased risk of MALOs. However, the *rate* of change in these biomarker values did not appear to further help in predicting the risk of incident MALO. To our knowledge, this is one of the largest studies of its kind to investigate such associations over longer periods of time in patients with primarily biopsy-proven MASLD. Our findings suggest that in addition to baseline measurements, monitoring biomarkers value over time could aid in identifying high-risk patients. The longitudinal value of biomarkers may be indicative of fibrosis severity in these patients, whereas how fast the biomarkers evolved may not be clinically relevant to a patient's prognosis.

TABLE 3 The association between longitudinal biomarkers and major adverse liver outcomes

Biomarkers	Coefficient[a]	HR[a]	*p*	Coefficient[b]	HR[b]	*p*	Coefficient[c]	HR[c]	*p*
ln (FIB-4)									
Longitudinal value of ln (FIB-4)	1.72 (1.21–2.26)	3.29 (2.31–4.79)	< 0.001	1.49 (1.06–1.94)	2.81 (2.08–3.84)	< 0.001	1.37 (0.92–1.81)	2.60 (1.89–3.50)	< 0.001
Slope and longitudinal value of ln (FIB-4)									
ln (FIB-4)—slope	0.12 (−0.39 to 0.67)	1.09 (0.76–1.59)	0.655	0.05 (−0.06 to 0.69)	1.04 (0.96–1.61)	0.897	0.05 (−0.57 to 0.69)	1.04 (0.67–1.61)	0.919
ln (FIB-4)—value	1.54 (1.06–2.84)	2.91 (2.08–7.16)	< 0.001	1.51 (0.88–2.08)	2.85 (1.94–4.23)	< 0.001	1.47 (0.95–2.00)	2.77 (1.93–4.00)	< 0.001
ln (AST), U/L									
Longitudinal value of ln (AST)	0.002 (0.001–0.003)	1.00 (1.00–1.01)	< 0.001	1.24 (1.24–1.25)	2.36 (2.36–2.38)	< 0.001	1.43 (1.36–1.61)	2.69 (2.57–3.05)	< 0.001
Slope and longitudinal value of ln (AST)									
ln (AST)—slope	−0.01 (−0.02 to −0.01)	0.99 (0.98–1.00)	0.059	−0.01 (−0.01 to −0.00)	0.99 (0.99–1.00)	0.06	−0.01 (−0.01 to 0.01)	0.99 (0.00–1.01)	0.085
ln (AST)—value	0.02 (0.02–0.03)	1.01 (1.01–1.02)	< 0.001	0.02 (0.02–0.03)	1.01 (1.01–1.02)	< 0.001	0.02 (0.02–0.03)	1.01 (1.01–1.02)	< 0.001
Platelets count, 10 Giga/L									
Longitudinal value of platelets	−0.08 (−0.12 to −0.04)	0.92 (0.89–0.96)	< 0.001	−0.07 (−0.12 to −0.03)	0.93 (0.87–0.97)	< 0.001	−0.07 (−0.11 to −0.03)	0.93 (0.90–0.97)	< 0.001
Slope and longitudinal value of platelets									
Platelets—slope	−0.05 (−0.11 to 0.02)	0.95 (0.89–1.02)	0.157	−0.05 (−0.12 to 0.03)	0.95 (0.88–1.03)	0.223	−0.09 (−0.18 to 0.02)	0.91 (0.83–1.02)	0.178
Platelets—value	−0.06 (−0.11 to −0.01)	0.94 (0.89–0.99)	< 0.001	−0.05 (−0.11 to 0.02)	0.95 (0.89–1.02)	0.195	−0.02 (−0.10 to 0.05)	0.98 (0.90-1.05)	0.625

[a]Model 1 adjusted for age and sex.
[b]Model 2 adjusted for age, sex, body mass index, type 2 diabetes, and hyperlipidemia.
[c]Model 3 adjusted for age, sex, body mass index, type 2 diabetes, hyperlipidemia, and fibrosis stage.
Abbreviations: AST, aspartate aminotransferase; FIB-4, fibrosis-4 score.

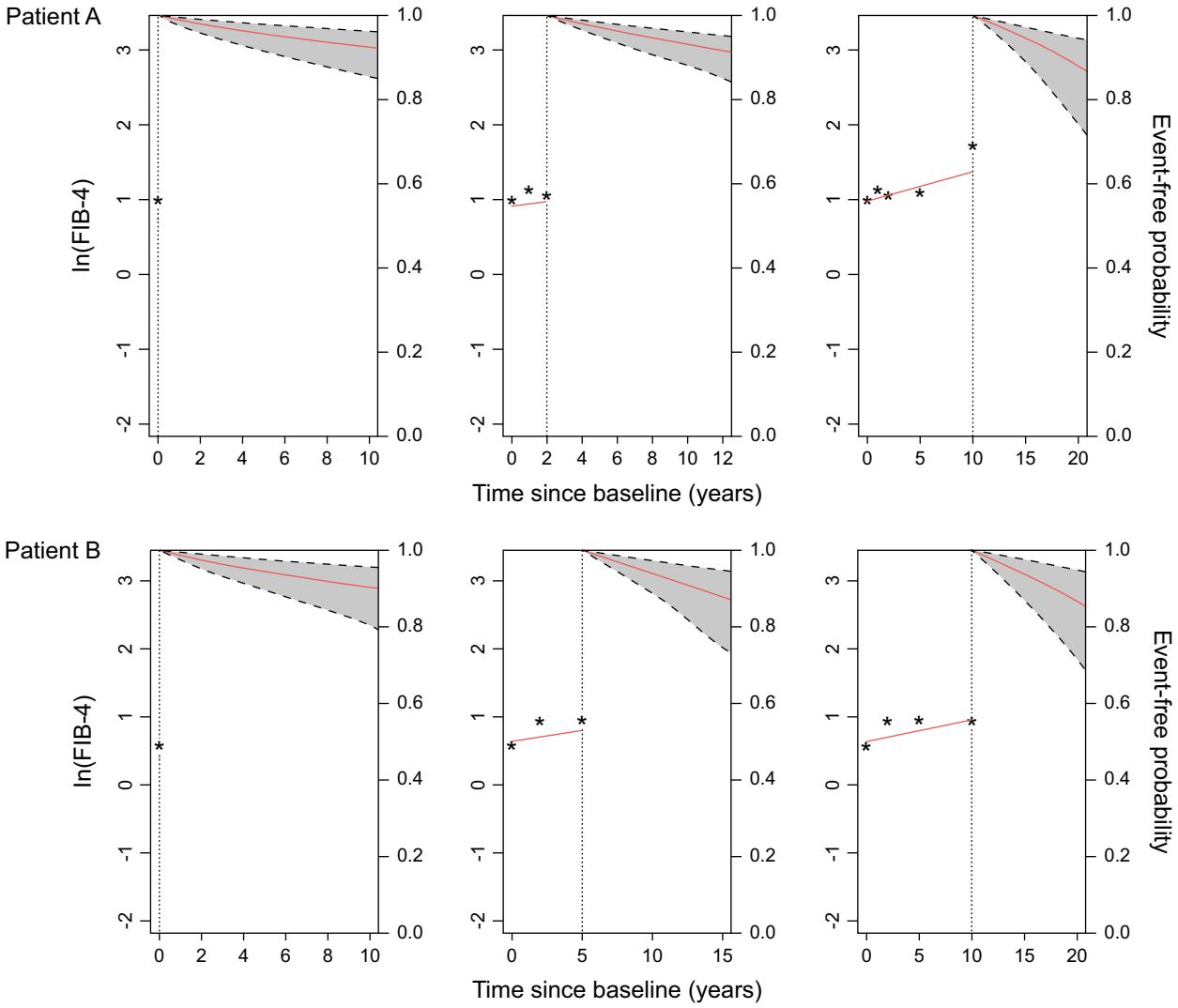

FIGURE 3 Dynamic prediction of MALOs using FIB-4 values in a single patient. The plots on the left-hand side represent a measurement of FIB-4, and the line represents the predicted trajectory of FIB-4 over time. The plots on the right-hand side update the probability of MALO every time a new FIB-4 measurement is available. We present 2 patients here with different clinical profiles: patient A is a female, 63 years old, with fibrosis stage of 1, without type 2 diabetes and hyperlipidemia, and has a body mass index of 28; patient B is a female, 55 years old, with fibrosis stage of 1, with type 2 diabetes and hyperlipidemia, and had a body mass index of 33. Abbreviations: FIB-4, fibrosis-4 score; MALO, major adverse liver outcome.

These results are partly in line with several recent studies that investigated the impact of changes in the FIB-4 category on long-term liver outcomes.[11,12,30] According to a previous population-based study in Sweden involving 40,729 individuals from the general population with repeated FIB-4 measurements over 5 years, a transition from a low- or intermediate-risk group to a high-risk group was associated with a greater risk of MALO (aHR of 7.99 and 8.64, respectively). Similarly, a cohort study of 202,319 veterans with MASLD from the United States showed that the risk of liver-related outcomes was higher for all combinations of transitions, even for those regressed from intermediate- to low-risk group (aHR: 2.75) compared to those who remained in low-risk groups at both time points (baseline and within 3 years). However, these studies reported population average levels and did not consider the dependence of repeated measurements within individuals. We modeled the individual trajectory of biomarkers over multiple time points, minimizing the possibility of false negatives regarding the test values. Despite the different methodologies between studies, these findings demonstrate that serial biomarker measurements may be useful to improve the identification of high-risk patients with MASLD.

Importantly, this study revealed that the longitudinal value of biomarkers is independently associated with liver outcomes regardless of baseline fibrosis stage in patients in secondary care. This signals the potential role of biomarkers, such as FIB-4, that could be used not only as a "gatekeeper" for ruling out advanced fibrosis as part of the triage process in primary care,[5] but also for monitoring patients without the need

for repeated biopsies or imaging in secondary management. This is supported by the previous findings from the United Kingdom, which demonstrated a significant correlation between the change in fibrosis stage and the change in FIB-4 between 2 paired biopsies.[31] However, this correlation was only modest (Spearman correlation coefficient = 0.24, $p = 0.03$). In fact, the progressors who advanced to F3–F4 were more likely to have a higher FIB-4 at any time point than non-progressors who remained F0–F2, even though the degree of change in FIB-4 may not have been different. This suggests that although an increase in FIB-4 value may indicate some degree of fibrosis progression, it is not necessarily a strong indicator of advanced fibrosis. Instead, patients with higher FIB-4 scores at follow-up are more likely to have progressed to advanced fibrosis, regardless of the speed of change in their FIB-4 score over time. We repeated the analysis in patients with FIB-4 values < 1.3 since such patients are recommended to be monitored in primary care only. These results were similar to the main analysis in that the slope of FIB-4 change did not further add to the prediction of MALO in addition to the latest FIB-4 value. This highlights the importance of considering the absolute value of FIB-4 in monitoring fibrosis progression as an additional aspect of the current clinical practice guidelines for using FIB-4 in the surveillance of patients with MASLD.

The study has some notable strengths. The long follow-up time allowed for capturing events that have a long natural history. We used high-quality national registers to identify MALO which have high positive predictive values, resulting in a low risk for misclassification bias of the outcome. The use of individual biomarker trajectories allowed for predicting MALO based on the patient's clinical profile. Furthermore, as seen in the individual dynamic prediction, those who had comorbidity but low FIB-4 value had a similar probability of developing MALO as those who had high FIB-4. This means that apart from monitoring absolute FIB-4, patients with MASLD may benefit from management that is tailored to their specific clinical profile. Limitations should be noted. First, patients were selected solely from university hospitals, which may introduce selection bias, potentially resulting in higher estimates than those from patients with MASLD seen only in primary care. Second, patients who had repeated measurements did not differ in terms of clinical profile or baseline biomarkers value compared to those with only baseline measurement, except for those with only 1 measurement tended to be older and have a lower BMI. Third, these data do not support evidence for how often repeated biomarkers should be sampled. Finally, there may be residual confounding, such as genetic predisposition, that were not adjusted for in the analysis.

In conclusion, our study provides evidence that longitudinal values of biomarkers for liver fibrosis are associated with future risk of MALOs in patients with MASLD; however, the rate of change in these biomarkers did not seem to provide additional information to risk prediction. Our findings highlight the importance of continuously monitoring these biomarkers over time. The absolute value of the biomarker over time may be sufficient for clinicians to evaluate the patient's disease severity, and there may not be a need to scrutinize the patient's previous biomarker history. Further studies are warranted to confirm our study findings.

AUTHOR CONTRIBUTIONS

Study concept and design: Ying Shang, Hannes Hagström, Xiao Zhang, and Tongtong Wang. Acquisition of data: Camilla Akbari, Patrik Nasr, Johan Vessby, Maja Dodd, Stergios Kechagias, Fredrik Rorsman, Per Stål, Mattias Ekstedt, and Hannes Hagström. Statistical analysis: Ying Shang. Analysis and interpretation of data: All. Drafting of the manuscript: Ying Shang. Critical revision: All. Guarantor of article: Hannes Hagström. All authors approved the final version of the article, including the authorship list.

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CONFLICTS OF INTEREST

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REFERENCES

1. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): A systematic review. Hepatology. 2023;77:1335–47.
2. Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, et al. The prevalence and incidence of NAFLD worldwide: A systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2022;7:851–61.
3. Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology. 2019;69:2672–82.
4. Cheemerla S, Balakrishnan M. Global epidemiology of chronic liver disease. Clin Liver Dis (Hoboken). 2021;17:365–70.
5. European Association for the Study of the Liver (EASL). EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis—2021 update. J Hepatol. 2021;75:659–89.
6. Wong VW-S, Adams LA, de Lédinghen V, Wong GL-H Sookoian S. Noninvasive biomarkers in NAFLD and NASH—Current progress and future promise. Nat Rev Gastroenterol Hepatol. 2018;15:461–78.
7. Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: A meta-analysis. Hepatology. 2017;66:1486–501.
8. Boursier J, Hagström H, Ekstedt M, Moreau C, Bonacci M, Cure S, et al. Non-invasive tests accurately stratify patients with NAFLD based on their risk of liver-related events. J Hepatol. 2022;76:1013–20.
9. Hagström H, Nasr P, Ekstedt M, Stål P, Hultcrantz R, Kechagias S. Accuracy of noninvasive scoring systems in assessing risk of death and liver-related endpoints in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2019;17:1148–156.e4.
10. Mózes FE, Lee JA, Vali Y, Alzoubi O, Staufer K, Trauner M, et al. Performance of non-invasive tests and histology for the prediction of clinical outcomes in patients with non-alcoholic fatty liver disease: An individual participant data meta-analysis. Lancet Gastroenterol Hepatol. 2023;8:704–13.
11. Hagström H, Talbäck M, Andreasson A, Walldius G Hammar N. Repeated FIB-4 measurements can help identify individuals at risk of severe liver disease. J Hepatol. 2020;73:1023–9.
12. Cholankeril G, Kramer JR, Chu J, Yu X, Balakrishnan M, Li L, et al. Longitudinal changes in fibrosis markers are associated with risk of cirrhosis and hepatocellular carcinoma in non-alcoholic fatty liver disease. J Hepatol. 2023;78:493–500.
13. Hagström H, Nasr P, Ekstedt M, Kechagias S, Önnerhag K, Nilsson E, et al. Low to moderate lifetime alcohol consumption is associated with less advanced stages of fibrosis in non-alcoholic fatty liver disease. Scand J Gastroenterol. 2017;52:159–65.
14. Hagstrom H, Nasr P, Ekstedt M, Hammar U, Stal P, Hultcrantz R, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol. 2017;67:1265–73.
15. Akbari C, Dodd M, Stål P, Nasr P, Ekstedt M, Kechagias S, et al. Long-term major adverse liver outcomes in 1,260 patients with non-cirrhotic NAFLD. JHEP Rep. 2023;6:100915.
16. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313–21.
17. Bedossa P, Poynard T, The METAVIR Cooperative Study Group. An algorithm for the grading of activity in chronic hepatitis C. Hepatology. 1996;24:289–93.
18. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim J-L, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011;11:450.
19. Bengtsson B, Askling J, Ludvigsson JF, Hagström H. Validity of administrative codes associated with cirrhosis in Sweden. Scand J Gastroenterol. 2020;55:1205–10.
20. Åström H, Wester A, Hagström H. Administrative coding for non-alcoholic fatty liver disease is accurate in Swedish patients. Scand J Gastroenterol. 2023;58:931–6.
21. Kamath PS, Kim WR. The model for end-stage liver disease (MELD). Hepatology. 2007;45:797–805.
22. Barlow L, Westergren K, Holmberg L, Talbäck M. The completeness of the Swedish Cancer Register: A sample survey for year 1998. Acta Oncol. 2009;48:27–33.
23. Brooke HL, Talback M, Hornblad J, Johansson LA, Ludvigsson JF, Druid H, et al. The Swedish cause of death register. Eur J Epidemiol. 2017;32:765–73.
24. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Diabetologia. 2016;59:1121–40.
25. 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;64:73–84.
26. Stepanova M, Rafiq N, Makhlouf H, Agrawal R, Kaur I, Younoszai Z, et al. Predictors of all-cause mortality and liver-related mortality in patients with non-alcoholic fatty liver disease (NAFLD. Dig Dis Sci. 2013;58:3017–23.
27. Zou H, Hastie T. Regularization and variable selection via the elastic net. J R Stat Soc Series B Stat Methodol. 2005;67:301–20.
28. Rizopoulos D. Dynamic predictions and prospective accuracy in joint models for longitudinal and time-to-event data. Biometrics. 2011;67:819–29.
29. Baart SJ, van der Palen RLF, Putter H, Tsonaka R, Blom NA, Rizopoulos D, et al. Joint modeling of longitudinal markers and time-to-event outcomes: An application and tutorial in patients after surgical repair of transposition of the great arteries. Circ Cardiovasc Qual Outcomes. 2021;14:e007593.
30. Balkhed W, Åberg FO, Nasr P, Ekstedt M, Kechagias S. Repeated measurements of non-invasive fibrosis tests to monitor the progression of non-alcoholic fatty liver disease: A long-term follow-up study. Liver Int. 2022;42:1545–56.
31. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol. 2015;62:1148–55.

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