Original Article

Advanced Liver Fibrosis Predicts Liver Outcomes in Biopsy-proven Metabolic Dysfunction-associated Steatotic Liver Disease: A U.S.-based Single-center Retrospective Cohort Study

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Abstract

Background and Aims: Data regarding risk factors and long-term outcomes of U.S. patients with biopsy-proven metabolic dysfunction-associated steatotic liver disease (MASLD) are limited. This study aimed to investigate the role of clinical and histologic risk factors on long-term outcomes in patients with MASLD. ***Methods:*** A retrospective cohort study of 451 adults with biopsy-proven MASLD was conducted at a U.S. academic hospital from 2012 to 2020. An experienced pathologist evaluated the index liver biopsy. Patients with a prior liver transplant or alternative etiologies of chronic liver disease were excluded. The duration of the risk exposure was determined from the date of the index liver biopsy to an outcome event or the last follow-up examination. Outcome events of interest included incident liver-related events, liver decompensation, and all-cause mortality. ***Results:*** In the final cohort of 406 patients followed for a median of 3.7 years (interquartile range: 4.8 years), 35 patients died, 41 developed hepatic decompensation, and 70 experienced a liver-related event. Among histologic risk factors, stage 3 (adjusted Hazard ratio (aHR) 2.68, 95% confidence interval (CI) 1.18–6.11) and stage 4 (aHR 6.96, 95% CI 3.55–13.64) fibrosis were associated with incident liver-related events compared to stage 0–1 fibrosis. Stage 4 (aHR 8.46, 95% CI 3.26–21.99) fibrosis alone was associated with incident liver decompensation events compared to stage 0–1 fibrosis. Among clinical risk factors, hypertension (aHR 2.58, 95% CI 1.05–6.34) was associated with incident liver decompensation. ***Conclusions:*** In a U.S. single-center cohort of patients with biopsy-proven MASLD, advanced fibrosis was the primary risk factor for incident liver decompensation and liver-related events.

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is the most common cause of chronic liver disease and a leading cause of liver transplantation in the U.S.[1–3] The prevalence of MASLD is projected to increase to 100.9 million affected individuals in the U.S. by 2030, growing in parallel with the epidemic of metabolic syndrome, including obesity and type 2 diabetes.[4] MASLD also contributes to a substantial national economic burden, with $103 billion spent annually on direct medical costs in the U.S.[5]

Liver biopsy is considered the gold standard for diagnosing and grading the severity of MASLD, which ranges from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH), with or without fibrosis.[6,7] The fibrosis stage is one of the most important prognostic factors in patients with MASLD—an increasing fibrosis stage is associated with a higher risk of overall mortality and liver-related outcomes such as hepatocellular carcinoma and hepatic decompensation.[8–11]

Understanding risk factors with prognostic implications can help stratify patients who may benefit from early specialty referral and MASH-directed therapy. Most previous studies investigating clinical outcomes in patients with biopsy-confirmed MASLD have been based on international populations, either through pooled data from multiple countries or single-country studies outside the U.S.[9,12–15] Data on long-term outcomes in U.S.-based populations with biopsy-confirmed MASLD remain limited. MASLD is a complex and heterogeneous condition that can vary geographically due to differences in ethnicity, diet, and metabolic comorbidities.[16,17] Therefore, the goal of this study was to investigate the role of clinical and histologic risk factors on the long-term prognosis of U.S. patients with biopsy-proven MASLD.

Methods

Study design and data source

We conducted a retrospective cohort study of patients

with biopsy-confirmed MASLD at Yale-New Haven Hospital (YNHH), a large U.S. academic medical center. YNHH uses an EPIC electronic medical record system which was initiated in early 2010, with full adoption across hospital services by 2012. EPIC contains comprehensive data on demographics, medical diagnoses, social and family history, medications, and laboratory results. Our study utilized the YNHH pathology database, which contains documentation of all liver biopsy specimens, pathology reports, and pathology slides. The study was approved by the Institutional Review Board of Yale University. The reporting of this study followed the STROBE guidelines.

Study population and selection criteria

The source cohort was identified by reviewing and retrieving original biopsy slides from the YNHH pathology database for patients with a biopsy diagnosis keyword of "steatosis" or "steatohepatitis" between January 2012 and December 2020. An experienced gastrointestinal pathologist (D.J.), who was unaware of the patient's clinical and laboratory data, reviewed the obtained biopsy slides to assess if the original report findings were consistent with a histologic diagnosis of MASLD. Each patient's clinical chart was then thoroughly reviewed for imaging, serologic workup, and clinical documentation to verify a diagnosis of clinical MASLD without alternative etiology. Patients with reported weekly alcohol consumption exceeding 140 g for women and 210 grams for men at the time of the index biopsy and during follow-up were categorized as having alcohol-associated fatty liver disease and were excluded. Additional exclusion criteria included a prior history of liver transplant or alternative etiologies of chronic liver disease, such as viral hepatitis, autoimmune or cholestatic liver disorders, and genetic or metabolic liver disorders. All included patients were aged ≥18 years at the time of the index biopsy and had at least one adequate liver biopsy for scoring.

Histopathology evaluation

All index liver biopsy slides for eligible study patients were reviewed and scored again by our gastrointestinal pathologist (D.J.). The index liver biopsy was defined as the patient's first liver biopsy if they had undergone multiple biopsies. A minimum of 5% hepatocyte steatosis was required on the index biopsy for a MASLD diagnosis. Liver biopsies were scored for various histologic features based on NASH Clinical Research Network criteria, including fibrosis stage (0–4), steatosis (0–3), lobular inflammation (0–3), portal inflammation (0–3), ballooning (0–2), and Mallory bodies (0–2).[18] The NAFLD activity score was the sum of scores for steatosis, lobular inflammation, and ballooning.

Baseline characteristics and laboratory data

Baseline demographics, anthropometrics, medical comorbidities, and medication data were extracted from patient charts at the time of the index liver biopsy. Diagnoses of medical comorbidities were identified using ICD-9 and ICD-10 codes. A former smoker was defined as an adult who had smoked at least 100 cigarettes in their lifetime but had quit smoking at least 28 days prior to the index biopsy. BMI was calculated as weight (in kg) divided by height (in meters^2). Medications were grouped into classes, specifically statins, metformin, thiazolidinediones, GLP-1 agonists, DPP-4 inhibitors, and vitamin E. Laboratory results within a one-month period closest to the index liver biopsy were considered baseline values. These included routine liver function tests (alanine transaminase, aspartate transaminase, alkaline phosphatase, total bilirubin, direct bilirubin, and albumin), complete blood count, fasting lipids, and hemoglobin A1C.

Outcome events

The primary outcome events of interest were any liver-related event, defined as liver decompensation, hepatopulmonary syndrome, development of varices, hepatocellular carcinoma, or liver-related death; liver decompensation, defined as ascites, variceal hemorrhage, hepatic encephalopathy, spontaneous bacterial peritonitis, or hepatorenal syndrome; and all-cause mortality. Individual outcome events and their dates were obtained from the patient charts based on ICD-9 and ICD-10 codes. Events occurring within 90 days of the index biopsy were excluded.

Exposure and follow-up

Risk exposure was determined from the date of the index liver biopsy to the earliest occurrence of an outcome event or the last follow-up examination. All-cause mortality was determined using the date of death recorded in the patient chart.

Statistical analysis

Analyses were performed using SAS software (version 9.4, SAS Institute, Cary, NC). For descriptive analyses, we reported medians and interquartile ranges for continuous variables and frequencies and percentages for categorical variables unless otherwise specified. Kaplan-Meier analysis was used to estimate cumulative survival probabilities for patients in each fibrosis stage, with comparisons among groups performed using the Log-rank test. Given that all-cause mortality is a competing risk for liver-related events and liver decompensation, cumulative incidence functions were plotted for liver-related events and decompensation, and comparisons among fibrosis groups were made using Fine and Grey's test. We performed cause-specific multivariable Cox regression analysis to estimate adjusted hazard ratios (aHR) and 95% confidence intervals (CI) for risk factors associated with time-to-event outcomes. Variables were chosen for inclusion in the multivariable model based on univariable analysis findings, frontline clinical experience, and review of published data. *p*-values less than 0.05 were considered statistically significant.

Results

Demographics and baseline laboratory studies

A flow diagram of the study selection is shown in Figure 1. A total of 451 patients with biopsy-proven MASLD at YNHH were identified during the study period. After applying inclusion and exclusion criteria, the final study cohort consisted of 406 patients (Table 1). The cohort had a median age of 54 years (interquartile range [IQR] 20.4) and a median BMI of 33 (IQR 11). Patients were predominantly female (58.2%), White (76.3%), and never smokers (57.4%). The most common medical comorbidities included obesity (69%), hypertension (60.3%), and type 2 diabetes mellitus (49.8%). Laboratory results showed median serum ALT levels (48 mg/dL, IQR 51 mg/dL) greater than AST (42 mg/dL, IQR 34 mg/dL), with elevated hemoglobin A1C (6.3%, IQR 2.2%). Median platelet count (221.5 × 10^3 platelets/μL, IQR 97.5 × 10^3 platelets/μL), international normalized ratio (1.0, IQR 0.2), and albumin levels (4.3 g/dL, IQR 0.6 g/dL) were within normal ranges.

Index liver biopsy characteristics

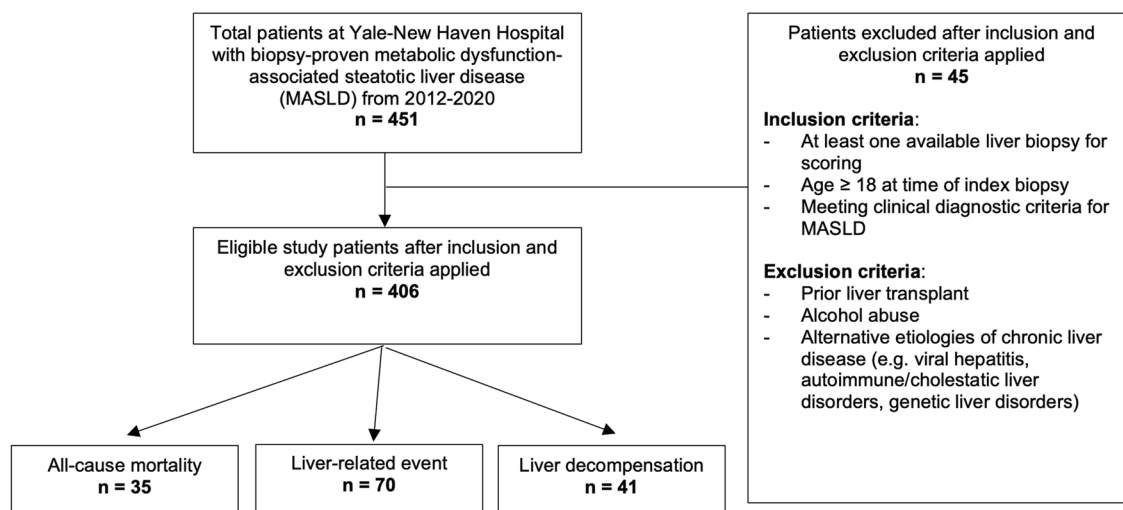The largest proportion of the study cohort had an index

Fig. 1. The flow of patients through the study.

Table 1. Summary of baseline characteristics of the patient cohort with biopsy-proven metabolic dysfunction-associated steatotic liver disease (MASLD)

	Study cohort (n = 406)
Demographics	
Age, median (IQR)	54 (20.4)
Male sex, n (%)	170 (41.8%)
Race, n (%)	
White	310 (76.3%)
Black	20 (4.9%)
Asian	9 (2.2%)
Unknown	68 (16.7%)
Ethnicity, n (%)	
Hispanic	101 (24.8%)
Non-Hispanic	298 (73.3%)
Unknown	8 (2%)
Smoking	
Current	40 (9.8%)
Former	131 (32.3%)
Never	233 (57.4%)
Unknown	3 (0.7%)
BMI, median (IQR)	33 (11)
Medical comorbidities	
Hypertension, n (%)	245 (60.3%)
Overweight, n (%)	95 (23.4%)
Obese, n (%)	280 (69%)
Type 2 diabetes mellitus, n (%)	202 (49.8%)
Coronary artery disease, n (%)	36 (8.8%)
Chronic kidney disease, n (%)	30 (7.4%)
Congestive heart failure, n (%)	15 (3.69%)

(continued)

Table 1. *(continued)*

	Study cohort (n = 406)
Obstructive sleep apnea, n (%)	94 (23.1%)
Medications	
Statin, n (%)	168 (41.4%)
Metformin, n (%)	39 (9.6%)
Thiazolidinedione, n (%)	5 (1.2%)
GLP-1 agonist, n (%)	21 (5.2%)
DPP-4 inhibitor, n (%)	8 (2%)
Vitamin E, n (%)	8 (2%)
Laboratory data	
Alanine transaminase (ALT), median (IQR)	48 mg/dL (51 mg/dL)
Aspartate transaminase (AST), median (IQR)	42 mg/dL (34 mg/dL)
Alkaline phosphatase, median (IQR)	84 mg/dL (43 mg/dL)
Albumin, median (IQR)	4.3 g/dL (0.6g/dL)
Total bilirubin, median (IQR)	0.5 mg/dL (0.4 mg/dL)
Direct bilirubin, median (IQR)	0.2 mg/dL (0.1 mg/dL)
Hemoglobin A1C, median (IQR)	6.3% (2.2%)
International normalized ratio (INR), median (IQR)	1.0 (0.2)
Platelets, median (IQR)	221.5×10^3/ μL (97.5×10^3/ μL)
Triglycerides, median (IQR)	138 mg/dL (99 mg/dL)
Low-density lipoprotein (LDL) cholesterol, median (IQR)	95 mg/dL (44 mg/dL)
High-density lipoprotein (HDL) cholesterol, median (IQR)	43mg/dL (20 mg/dL)
Triglycerides, median (IQR)	138 mg/dL (99mg/dL)
Total cholesterol, median (IQR)	171 mg/dL (51mg/dL)

IQR, interquartile range; BMI, body mass index.

liver biopsy suggestive of stage 0–1 fibrosis (44.3%), with the remaining distribution including stage 2 (19.2%), stage 3 (14.8%), and stage 4 (21.7%) fibrosis. The median NAFLD activity score was 4 (IQR 2). A detailed description of the index liver biopsy characteristics is provided in Table 2.

Follow-up and outcomes

The median follow-up duration for the 406 patients was 3.7 years (IQR 4.8 years), as shown in Table 3. During this period, 35 patients died (8.6%), 70 developed a liver-related event (17.2%), and 41 experienced liver decompensation (10%). We sought to understand the clinical and histologic risk factors associated with the development of any liver-related event, liver decompensation, and all-cause mortality. Univariable-unadjusted hazard ratios for clinical and histologic risk factors associated with clinical outcome events are reported in Supplementary Tables 1–3.

A Kaplan-Meier survival curve for all-cause mortality was generated (Fig. 2) and compared among fibrosis stage groups, showing that patients with stage 0–1 fibrosis had significantly better survival than those in the other three stages (p <0.0001). Cumulative incidence curves of any liver-related event (Fig. 3) and liver decompensation (Fig. 4) also demonstrated that patients with stage 3 and 4 fibrosis had higher cumulative event rates over time compared to those with stages 0–2 (p < 0.0001).

In multivariable analysis (Table 4), patients with stage 3 (aHR 2.68, 95% CI 1.18–6.11) and stage 4 (aHR 6.96, 95% CI 3.55–13.64) liver fibrosis had significantly higher rates of liver-related events compared to those with stage 0–1 fibrosis, in a stepwise fashion. Stage 4 fibrosis alone (aHR 8.46, 95% CI 3.26–21.99) was associated with an increased rate of liver decompensation compared to stage 0–1 fibrosis. Among clinical risk factors, hypertension (aHR 2.58, 95% CI 1.05–6.34) was associated with a higher risk of liver decompensation. Former smoking (aHR 2.60, 95% CI 1.18–5.70) was significantly associated with higher all-cause mortality compared to non-smokers. No other clinical risk factors or histologic features were significantly associated with the outcome events.

Discussion

In a large observational cohort of patients with biopsy-proven MASLD, advanced fibrosis was the primary histologic risk factor associated with the development of liver-related and liver decompensation events, demonstrating a stepwise increase in risk for liver-related events in stage 3 (aHR 2.68) and stage 4 (aHR 6.96) fibrosis. Furthermore, patients with stage 4 fibrosis experienced higher rates of liver decompensation events (aHR 8.46). Hypertension was predictive of

Table 2. Index liver biopsy characteristics

	Study cohort (n = 406)
Fibrosis, n (%)	
0–1	180 (44.3%)
2	78 (19.2%)
3	60 (14.8%)
4	88 (21.7%)
Steatosis, n (%)	
0–1	146 (35.9%)
2	162 (40.0%)
3	98 (24.1%)
Lobular inflammation, n (%)	
0	70 (17.2%)
1	176 (43.4%)
2	134 (33.0%)
3	26 (6.4%)
Portal inflammation, n (%)	
0	269 (66.3%)
1	115 (28.3%)
2	22 (5.4%)
Ballooning, n (%)	
0	203 (50%)
1	177 (43.6%)
2	26 (6.4%)
Mallory bodies, n (%)	
0	331 (81.5%)
1	68 (16.7%)
2	7 (1.7%)
Acidophils, n (%)	
0	386 (95.1%)
1	20 (4.9%)
NAFLD activity score, median (IQR)	4 (2)
Microvesicular fat, median (IQR)	0 (0)
Iron, median (IQR)	0 (0)

IQR, Interquartile range; NAFLD, non-alcoholic fatty liver disease.

Table 3. Summary of follow-up and outcomes for patient cohort

	Study cohort (n = 406)
Follow-up time, median (IQR)	3.7 years (4.8 years)
All-cause mortality, n (%)	35 (8.6%)
Liver decompensation, n (%)	41 (10%)
Hepatic encephalopathy	35
Spontaneous bacterial peritonitis	6
Variceal bleeding	7
Ascites	1
Liver-related event, n (%)	70 (17.2%)
Liver decompensation	41
Hepatocellular carcinoma	16
Variceal development	45
Hepatopulmonary syndrome	2

IQR, Interquartile range.

study with biopsy-proven MASLD found that the incidence of liver-related decompensation for stage 3 and 4 fibrosis was 18.7 times and 46.1 times that of patients with stages 0 to 2 fibrosis, respectively.[20] Unlike prior studies, we did not find a statistically significant association between advanced fibrosis and mortality, which may be attributable to the limited number of events and statistical power.

The association between advanced fibrosis and both liver decompensation and liver-related events is mechanistically related to portal hypertension.[21,22] Extensive liver fibrosis disrupts liver architecture and increases resistance to portal blood flow, ultimately leading to portal hypertension.[23] Patients with a hepatic venous pressure gradient above 5 mmHg are directly at risk for clinical events such as ascites, variceal bleeding, and hepatic encephalopathy.[24,25]

While current management of MASLD is based on weight loss (through diet, exercise, and/or medications such as GLP-1 receptor agonists) and strict control of metabolic comorbidities, current tools to prevent and treat MASH

liver decompensation.

Given the heterogeneous burden of MASLD across geographical regions, this study of a U.S. cohort provides further evidence confirming the primary importance of the histologic fibrosis stage as a predictor of clinical outcomes.[16,19] Our findings are consistent with those from prior studies involving cohorts of patients with biopsy-proven MASLD. A multicenter retrospective study of patients with biopsy-proven MASLD from the U.S., Europe, and Thailand found that patients with stage 3 and 4 fibrosis had 14.2 times and 51.5 times the risk of liver-related events, respectively, compared to stage 0 fibrosis.[9] Similarly, a prospective U.S. multicenter registry
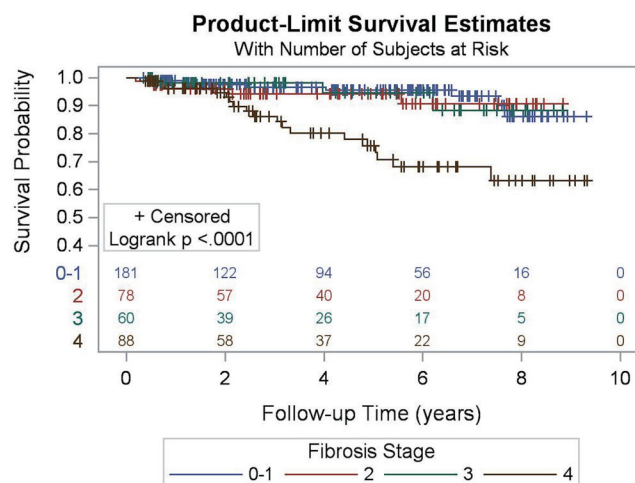

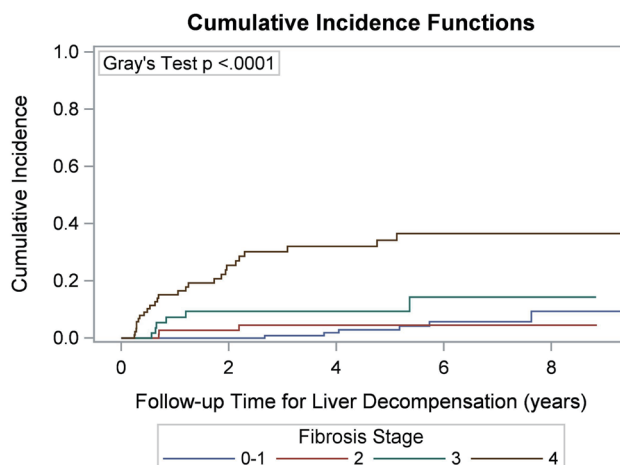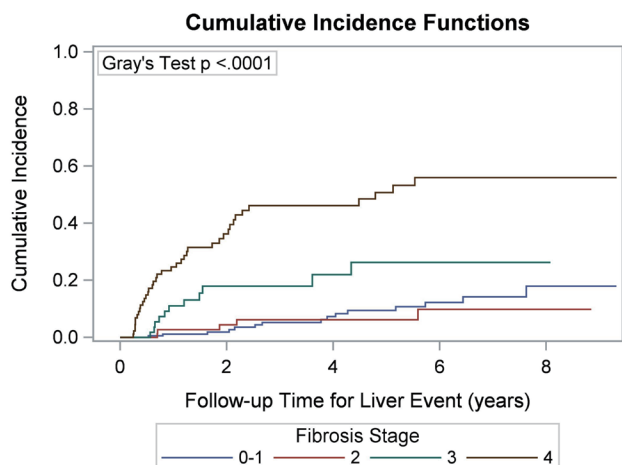**Fig. 2. Overall survival according to index liver fibrosis staging.**

Fig. 3. Cumulative incidence of a liver-related event according to initial index liver fibrosis staging.

Fig. 4. Cumulative incidence of liver decompensation according to initial index liver fibrosis staging.

Table 4. Multivariate-adjusted hazard ratios and 95% confidence intervals (CI) of selected covariates and outcome events

	Adjusted hazard ratio (aHR)	95% Confidence interval (CI) of aHR	*p*-value
All-cause mortality			
Fibrosis stage			
0–1	Reference	Reference	
2	1.06	0.35–3.23	0.92
3	0.75	0.19–2.93	0.68
4	2.35	0.93–5.94	0.07
Age	1.02	0.99–1.06	0.15
Body mass index (BMI)			
Underweight/Normal	Reference	Reference	
Overweight	0.76	0.21–2.75	0.68
Obese	0.55	0.17–1.77	0.32
Race			
White	Reference	Reference	
Asian	1.52	0.18–12.61	0.70
Black	1.05	0.14–8.10	0.97
Gender			
Male	Reference	Reference	
Female	0.98	0.48–2.01	0.95
Type 2 diabetes mellitus	1.89	0.84–4.27	0.12
Hypertension	1.34	0.56–3.23	0.51
Smoker			
Never smoker	Reference	Reference	
Former smoker	2.60	1.18–5.70	0.02
Current smoker	1.18	0.26–5.40	0.83
Liver-related event			
Fibrosis stage			
0–1	Reference	Reference	
2	0.78	0.28–2.20	0.65

(continued)

Table 4. *(continued)*

	Adjusted hazard ratio (aHR)	95% Confidence interval (CI) of aHR	*p*-value
3	2.68	1.18–6.11	0.02
4	6.96	3.55–13.64	<0.01
Age	1.02	1.00–1.04	0.06
BMI			
Underweight/Normal	Reference	Reference	
Overweight	2.44	0.79–7.51	0.12
Obese	1.06	0.36–3.10	0.92
Race			
White	Reference	Reference	
Asian	0.56	0.07–4.32	0.58
Black	1.76	0.52–5.96	0.36
Gender			
Male	Reference	Reference	
Female	0.66	0.40–1.08	0.10
Type 2 diabetes mellitus	1.18	0.68–2.04	0.56
Hypertension	1.18	0.64–2.16	0.59
Smoker			
Never smoker	Reference	Reference	
Former smoker	1.26	0.75–2.15	0.74
Current smoker	0.61	0.21–1.76	0.36
Liver decompensation			
Fibrosis stage			
0–1	Reference	Reference	
2	1.03	0.25–4.16	0.97
3	2.90	0.90–9.28	0.07
4	8.46	3.26–21.99	<0.01
Age	1.02	0.99–1.05	0.12
BMI			
Underweight/Normal	Reference	Reference	
Overweight	7.24	0.90–58.45	0.06
Obese	2.10	0.27–16.40	0.48
Race			
White	Reference	Reference	
Asian	0.73	0.09–5.94	0.77
Black	2.27	0.50–10.36	0.29
Gender			
Male	Reference	Reference	
Female	0.92	0.48–1.76	0.80
Type 2 diabetes mellitus	1.15	0.56–2.35	0.71
Hypertension	2.58	1.05–6.34	0.04
Smoker			
Never smoker	Reference	Reference	
Former smoker	1.62	0.82–3.22	0.17
Current smoker	0.62	0.14–2.77	0.53

fibrosis remain limited.[26] Novel investigational medications addressing MASH and MASH fibrosis are currently under evaluation in clinical trials, offering hope for improving clinical outcomes.[27,28] Due to the strong association between advanced fibrosis and liver-related outcomes, patients with liver stage 3 fibrosis or greater should be prioritized for MASH-directed therapy. Our study further supports clinical care pathways that prioritize risk stratification to identify patients with MASH fibrosis who may represent priority candidates for weight loss interventions and MASH pharmacotherapy.[29,30]

A key strength of our study was the restriction of the study population to patients with biopsy-proven MASLD, rather than those diagnosed based on noninvasive tests alone. Additionally, a single experienced pathologist reviewed and staged all liver biopsies using a validated scoring system (NASH Clinical Research Network system). To our knowledge, this study represents the largest U.S. single-center cohort study evaluating clinical outcomes in patients with biopsy-confirmed MASLD to date. While there is growing interest in non-invasive testing to assess liver fibrosis, these tests are limited by variability, inadequate accuracy, and potential error factors.[31] Our study provides further justification for using liver biopsy as the gold-standard diagnostic modality to grade fibrosis, given its prognostic value for liver outcomes. Our study had several important limitations. With only 21.7% of the cohort having cirrhosis at baseline, our median follow-up time of 3.7 years may have been insufficient to observe an adequate number of clinical events to determine differences between individual fibrosis stages, particularly regarding liver decompensation and hepatocellular carcinoma. Due to the limited number of outcome events, we were restricted in the number of covariates that could be included in the multivariable model to control for confounders. Most of our patients were White, so these results may not be generalizable to non-White patients, although 24.8% were identified as Hispanic. These demographics are similar to other U.S. studies, including a large U.S.-based multicenter study[20] and an MASLD cohort within the U.S. National Health and Nutrition Examination Survey.[32] Lastly, while our study carefully excluded patients with alcohol use disorder at the time of the index liver biopsy, the retrospective nature of our study could not account for interval increases in alcohol intake over time or alternative sources of hepatic injury during the follow-up period, which could contribute to the development of outcome events.

Conclusions

Our study showed that advanced fibrosis in a large U.S. single-center cohort of patients with biopsy-proven MASLD is associated with hepatic decompensation and liver-related events. Patients with advanced fibrosis should be identified using risk stratification and staging tools and prioritized for weight loss interventions and future MASH-directed pharmacotherapy.

Funding

None to declare.

Conflict of interest

JKL has received research contracts (to Yale University) from Gilead, Intercept, Inventiva, Novo Nordisk, Pfizer, and Viking; JKL has been an Executive Associate Editor of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests re-

lated to this publication.

Author contributions

Study conception and design (RL, DJ, JKL), data collection (RL, DJ, EA), All authors were involved in the analysis and interpretation of the results, drafting and revision of key manuscript, review of the results, approval of the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Ethical statement

The study was approved by the Institutional Review Board of Yale University (IRB # 2000029192). The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. The individual consent for this retrospective analysis was waived.

Data sharing statement

Data, analytic methods, and study materials may be made available to researchers upon request.

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