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ORIGINAL ARTICLE

Effect of Concurrent Metabolic Dysfunction-Associated Steatotic Liver Disease on Serial Non-invasive Fibrosis Markers in Chronic Hepatitis B

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Abstract

Background & Aims Concurrent hepatic steatosis has diverse effects on chronic hepatitis B (CHB), however the combined effects of metabolic dysfunction-associated steatotic liver disease (MASLD) and CHB on liver fibrosis progression remains unclear. The primary aim of this study was to utilize serial fibrosis measurements to compare the dynamic change in fibrosis in CHB patients with/without concurrent MASLD. The secondary aim was to investigate factors associated with steatosis development and regression in CHB patients.

Methods This was a retrospective cohort study of all non-cirrhotic CHB patients identified from 1/1/2011 to 31/12/2016. Hepatic steatosis was diagnosed by ultrasound. Fibrosis markers included liver stiffness (LSM) by transient elastography, APRI and FIB-4. General linear mixed effects modelling was used to fit polynomial and linear estimates.

Results Of 810 CHB patients ($n=2{,}373$ LSM measurements; median age 44.4y; 48% male; 24% HBeAg positive), 14% had concurrent MASLD. LSM was higher at baseline but decreased in MASLD patients over time, while LSM remained stable in non-MASLD patients, such that all patients had similar LSM beyond 4–5 years. MASLD patients had lower APRI compared to non-MASLD patients, which was predominately due to a higher platelet count and higher ALT over time. There was substantial discordance between LSM, APRI and FIB-4. Baseline BMI was the only factor that predicted steatosis development and regression.

Conclusions We found no evidence of an association between concurrent MASLD and fibrosis progression amongst CHB patients without baseline advanced liver disease. APRI and FIB-4 may have reduced accuracy in MASLD patients.

Keywords NAFLD · MAFLD · Non-alcoholic · Fatty liver · Kinetics · Liver stiffness · LSM · Elastography · Steatosis · CHB · HBV

Abbreviations

CHB	chronic hepatitis B
HBV	hepatitis B virus
HCC	hepatocelular carcinoma
LSM	liver stiffness measurement
AST	asparate aminotransferase
APRI	AST platelet ratio index
FIB-4	Fibrosis-4 index
MASLD	metabolic dysfunction-associated steatotic liver disease
MAFLD	metabolic dysfunction-associated fatty liver disease
BMI	body mass index
ALT	alanine aminotransferase
T2DM	type 2 diabetes mellitus
HR	hazard ratio

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Introduction

Chronic hepatitis B (CHB) is a persistent liver infection with the hepatitis B virus (HBV) that affects approximately 250 million people globally [1]. CHB-related morbidity and mortality are due to the development of liver failure from cirrhosis, as well as the accelerated carcinogenesis that leads to hepatocellular carcinoma (HCC) development. Although CHB is not yet curable, ongoing treatment with modern nucleos(t)ide analogue antiviral therapies is effective in suppressing viral replication and reducing liver inflammation and fibrosis [2, 3]. However, fibrosis continues to progress in a proportion of patients, so regular monitoring and strategies to mitigate fibrosis progression are crucial. The need for regular monitoring has led to the progressive acceptance of non-invasive fibrosis markers in place of the gold standard of liver biopsy [4, 5]. These include ultrasound-based techniques such as liver stiffness measurement (LSM) by transient elastography, as well as biomarker-derived scores such as the aspartate aminotransferase (AST) platelet ratio index (APRI) and Fibrosis-4 index (FIB-4) [6, 7].

As viral suppression is achieved with nucleos(t)ide analogue therapy, more attention needs to be paid to non-viral risk factors, in particular to the increasing global incidence of obesity, metabolic syndrome and metabolic dysfunction-associated steatotic liver disease (MASLD) as potential co-factors for the development of CHB-associated adverse events [8, 9]. There are diverse effects of concurrent hepatic steatosis on CHB which are subject to much interest and ongoing research [10]. Concurrent hepatic steatosis appears to suppress HBV viral load and increases the rate of HBsAg seroconversion [11–14]. Despite this, liver steatosis is correlated with liver fibrosis [15], and persistent severe steatosis appears to accelerate fibrosis progression [14], but the effects of milder degrees of steatosis remains unclear. Further, there is increasing evidence that both liver steatosis and metabolic dysfunction increase the risk of HCC development in CHB [16–19]. Thus, the new MASLD framework (previously known as metabolic dysfunction-associated fatty liver disease or MAFLD) is useful as it incorporates the addition of metabolic factors to steatotic liver disease as part of its definition [9, 20, 21].

The primary aim of this study was therefore to model and compare the dynamics of liver fibrosis and viral response between non-cirrhotic CHB patients with or without concurrent MASLD. The secondary aim was to investigate factors associated with steatosis development and regression in CHB patients over time.

Methods

Study Design

This was a retrospective longitudinal cohort study conducted at Eastern Health, a large teaching health service in Australia comprising three acute hospitals. All non-cirrhotic CHB patients were categorized into two groups at baseline: MASLD vs. non-MASLD, and followed up over time. This allowed the analysis of serial laboratory and elastography measurements to rigorously monitor the progression of non-invasive fibrosis markers over time, accounting for baseline covariates. Ultrasound examination was used to determine steatosis development and regression over time. The study was approved and a requirement for informed consent was waived by the Eastern Health Human Research Ethics Committee (approval number CQ22-001).

Patient Selection

All patients referred to the viral hepatitis clinic were screened. We included all adult patients aged ≥ 18 years with CHB (defined as HBsAg positive with HbsAb negative) with an index abdominal ultrasound between January 2011 and December 2016. Exclusion criteria were: diagnosis of cirrhosis, significant alcohol intake (> 210 g/week for males, > 140 g/week for females) and hepatitis C, hepatitis D and/or HIV co-infection. Liver steatosis was diagnosed on ultrasound (diffusely increased echogenicity). MASLD was diagnosed according to new criteria [9]: presence of liver steatosis with the addition of one of the following criteria: (1) presence of type 2 diabetes mellitus, (2) overweight as defined by body mass index (BMI) > 25 kg/m^2, (3) presence of hypertension, (4) presence of hypercholesterolemia or (5) presence of hypertriglyceridemia.

Baseline Variables

The following baseline characteristics were collected: patient demographics, BMI, comorbidities, LSM via transient elastography (Fibroscan), liver ultrasound, HBV serology, platelet count, liver biochemistry, HBV viral load and HBV treatment status.

Outcomes and Follow-Up

The following outcomes were tracked over time: LSM, APRI, FIB-4, BMI, alanine aminotransferase (ALT), AST, platelet count, HBV DNA and liver ultrasound. Serial outcomes were retrieved from electronic medical records. We excluded serial measurements that occurred within 3 months to remove unnecessary data fluctuations that may have

occurred from hospital admissions. APRI and FIB-4 were calculated if all constituent biomarkers (ALT, AST, platelet count) were measured within 3 months of each other.

Transient elastography was performed by a certified operator, using the M probe for patients with skin to capsule distance < 2.5 cm or an XL probe for patients with skin to capsule distance > 2.5 cm. Patients were fasted for > 2 h prior to transient elastography. The median of at least 10 successful measurements was recorded as the LSM. The IQR-median ratio and success rate were used to assess the reliability of each scan. Timing and indication for repeat transient elastography were decided by the treating clinician, which typically occurs once every 1 to 3 years at our institution.

Development of steatosis was defined as the first follow-up ultrasound that identified liver steatosis in patients without steatosis at baseline. Regression of steatosis was defined as the first follow-up ultrasound that identified absence of liver steatosis in patients with steatosis at baseline.

Patients were followed up until August 2020 or until loss to follow-up. Prior and new commencement of anti-viral therapy was documented during follow-up. Commencement of antivirals during follow-up was at the discretion of the treating clinician, broadly following consensus guidelines [22].

Statistical Analysis

Baseline continuous variables were compared with the Mann-Whitney *U* test or a *t*-test if they were non-parametric or parametric respectively. Baseline categorical variables were compared using the chi-squared test.

General linear mixed effects regression with was used to model change in fibrosis markers over time. A random intercept was used to account for patient-level clustering. All covariates including time were considered to be fixed effects. Polynomial time covariates were used to model the non-linear relationship with time, where the optimal degree of the polynomial was determined by Akaike's information criterion. Linear terms were used for all other baseline covariates, which included: age, sex, BMI, HBeAg status, pre-existing antiviral therapy, commencement of antiviral therapy at baseline, concurrent diabetes mellitus, hypertension and hyperlipidaemia. An interaction term between MASLD and time was used to assess for differences in rate of change. Both the fitted polynomial models as well as basic linear models for each outcome were plotted for comparison. A log transformation was applied to non-parametric variables to correct for skewness, therefore non-parametric outcomes were expressed as percentage change instead of absolute change. Due to the finding of differences in trajectories of APRI and FIB-4, we additionally modelled the dynamics of platelet count and AST to determine if any specific constituent was responsible for the difference. Additionally, as sensitivity analysis, the general linear mixed models were re-evaluated by replacing the variable MASLD with liver steatosis. This was to assess whether differences in dynamics over time might be due to steatosis in general as opposed to MASLD specifically.

Cox proportional hazards regression was used to model steatosis development (in the non-steatotic group) and regression (in the steatotic group). In each group, a multivariable model including all predictors was used to adjust for baseline covariates. Patients entered the risk group upon study entry and were censored at last follow-up.

A two-sided $P < 0.05$ was used to indicate statistical significance. All analysis was performed in Stata/IC 16.1 (StataCorp LP, Texas, USA, 2020).

Results

Study Cohort

A total of 810 CHB patients were included, comprising 112 (14%) patients with concurrent MASLD (see Table 1), with analysis of 2,373 LSM measurements, 4,127 APRI/FIB-4 measurements and 9,930 HBV DNA measurements. On univariable analysis, MASLD and non-MASLD patients were of similar age (median 45.5 vs. 43.8 years, $P = 0.25$), however MASLD patients were more likely to be male (64% vs. 45%, $P < 0.001$), had higher BMI (median 27.0 vs. 22.7 kg/m^2, $P < 0.001$) and were more likely to have diabetes, hypertension and high cholesterol (see Table 1). MASLD patients had higher ALT, LSM and platelet count, but similar AST and bilirubin levels. MASLD patients had a numerically lower baseline HBV viral load (2.58 vs. 2.91 $\log_{10}$ IU/L, $P = 0.06$) and similar rates of HBeAg positivity (19% vs. 25%, $P = 0.23$).

Dynamics of Fibrosis Markers

Liver Stiffness

At entry, MASLD patients had a 15% higher LSM compared to non-MASLD patients (95% CI 7–23%, $P < 0.001$). LSM decreased over time in all patients, but the drop was faster in MASLD patients such that both groups reached similar LSM levels after 2–3 years (see Fig. 1A). On average, LSM was 15% higher in males (95% CI 11–19%, $P < 0.001$), 10% higher in patients who were commenced on antiviral therapy on entry (95% CI 3–18%, $P = 0.003$) and 3% higher for every $\log_{10}$ IU/L of baseline HBV DNA (95% CI 2–4%, $P < 0.001$). There was no significant association

Table 1 Baseline characteristics of study cohort ($n=810$)

Characteristic	Non-MASLD ($n=698$)	MASLD ($n=112$)	P
Age, years, median (IQR)	43.8 (35.1–53.3)	45.5 (37.0–55.2)	0.25
Male, *n* (%)	317 (45)	71 (64)	< 0.001
Liver steatosis, *n* (%)	73 (10)	112 (100)	< 0.001
BMI, kg/m^2, median (IQR)	22.7 (20.4–24.9)	27.0 (25.5–28.9)	< 0.001
BMI, categorical, *n* (%)			
Normal	529 (76)	17 (15)	< 0.001
Overweight	147 (21)	78 (70)	< 0.001
Obese	22 (3)	17 (15)	< 0.001
HBeAg positive, *n* (%)	171 (25)	21 (19)	0.23
LSM, kPa, median (IQR) ($n=790$)	4.8 (3.9–5.9)	5.6 (4.7–6.8)	< 0.001
LSM, categorical, *n* (%) ($n=790$)			
<6.0 kPa	518 (76)	65 (58)	< 0.001
6.0–9.0 kPa	133 (20)	38 (34)	< 0.001
>9.0 kPa	27 (4)	9 (8)	0.09
Prior antiviral exposure, *n* (%)			
Lamivudine	35 (5)	1 (1)	0.048
Interferon	21 (3)	3 (3)	> 0.99
Adefovir	15 (2)	0 (0)	0.25
Baseline antiviral therapy, *n* (%)			
Entecavir monotherapy	31 (4)	6 (5)	0.63
TDF monotherapy	34 (5)	1 (1)	0.07
Other	13 (2)	0 (0)	0.23
Total	78 (11)	7 (6)	0.14
Commenced antiviral at entry, *n* (%)	55 (8)	8 (7)	> 0.99
Concomitant medical history, *n* (%)			
Diabetes mellitus	23 (3)	24 (21)	< 0.001
Hypertension	49 (7)	24 (21)	< 0.001
Dyslipidaemia	43 (6)	30 (27)	< 0.001
Coronary artery disease	4 (1)	2 (2)	0.20
Pathology, median (IQR)			
High ALT > 19 IU/L (females) or > 30 IU/L (males), *n* (%)	352 (50)	79 (71)	< 0.001
ALT, IU/L	25 (18–35)	31 (25–42)	< 0.001
AST, IU/L ($n=770$)	23 (20–30)	24 (20–30)	0.31
Platelets, 10^9/L	207 (178–244)	217 (193–259)	0.002
Bilirubin, µmol/L	9 (6–12)	9 (7–11)	0.36
Albumin, g/L	41 (38–43)	41 (39–44)	0.028
HBV DNA, $\log_{10}$ IU/L	2.91 (1.53–4.43)	2.58 (1.49–3.75)	0.06

with age, BMI, HBeAg status, antiviral therapy, diabetes, hypertension or hyperlipidaemia. The dynamics of LSM did not appear to correlate with BMI changes over time, where BMI remained higher in MASLD patients over time compared to non-MASLD patients (see Fig. 2A).

When considering steatosis alone, the estimated dynamics of LSM overtime appeared similar to the analysis comparing MASLD with non-MASLD (see Fig. 1B). As sensitivity analysis, the linear mixed model was refitted after combining all metabolic factors into one variable (being overweight, type 2 diabetes mellitus (T2DM), hypertension, hyperlipidaemia). An interaction term of this composite metabolic factor with time was also included. In this model, liver steatosis but not the composite metabolic variable affected LSM over time: presence of steatosis caused an estimated decrease in LSM by 9.4% after 8 years (95% CI 1.3 to 16.8%, $P=0.024$), however presence of metabolic risk factors did not affect LSM (estimated change of +0.8% after 8 years, 95% CI -6.1% to +8.4%, $P=0.82$).

APRI

There was no significant difference in baseline APRI in MASLD patients (7% lower, 95% CI -6 to 18%, $P=0.29$). APRI decreased over time in all patients (see Fig. 1C). On average, APRI was 3% higher for every 10 years of age (95% CI 0.4–5%, $P=0.022$), 17% higher in males (95% CI 11–24%, $P<0.001$), 3% higher for every $\log_{10}$ IU/L of baseline HBV DNA (95% CI 2–5%, $P<0.001$), 31% higher for every natural log increase in LSM (95% CI 20–44%, $P<0.001$) and 7% lower for every 10 kg/m^2 BMI (95% CI 2–11%, $P<0.001$). There was no significant association with HBeAg status, antiviral therapy, diabetes, hypertension or hyperlipidaemia. Repeating the analysis by comparing patients with and without liver steatosis showed similar results (see Fig. 1D).

On analysis of the individual components of APRI, AST was similar in MASLD and non-MASLD patients, and decreased over time in all patients (see Fig. 3A). Platelet count was higher in MASLD patients, and increased over time in all patients (see Fig. 3C). The analysis comparing patients with or without liver steatosis showed similar findings (see Fig. 3B and D).

FIB-4

At entry, FIB-4 was 19% lower in MASLD patients compared to non-MASLD patients (95% CI 9 to 28%, $P<0.001$). FIB-4 increased over time in all patients (see Fig. 1E). On average, FIB-4 was 25% higher for every natural log increase in baseline LSM (95% CI 14–37%, $P<0.001$), 29% higher in diabetic patients (95% CI

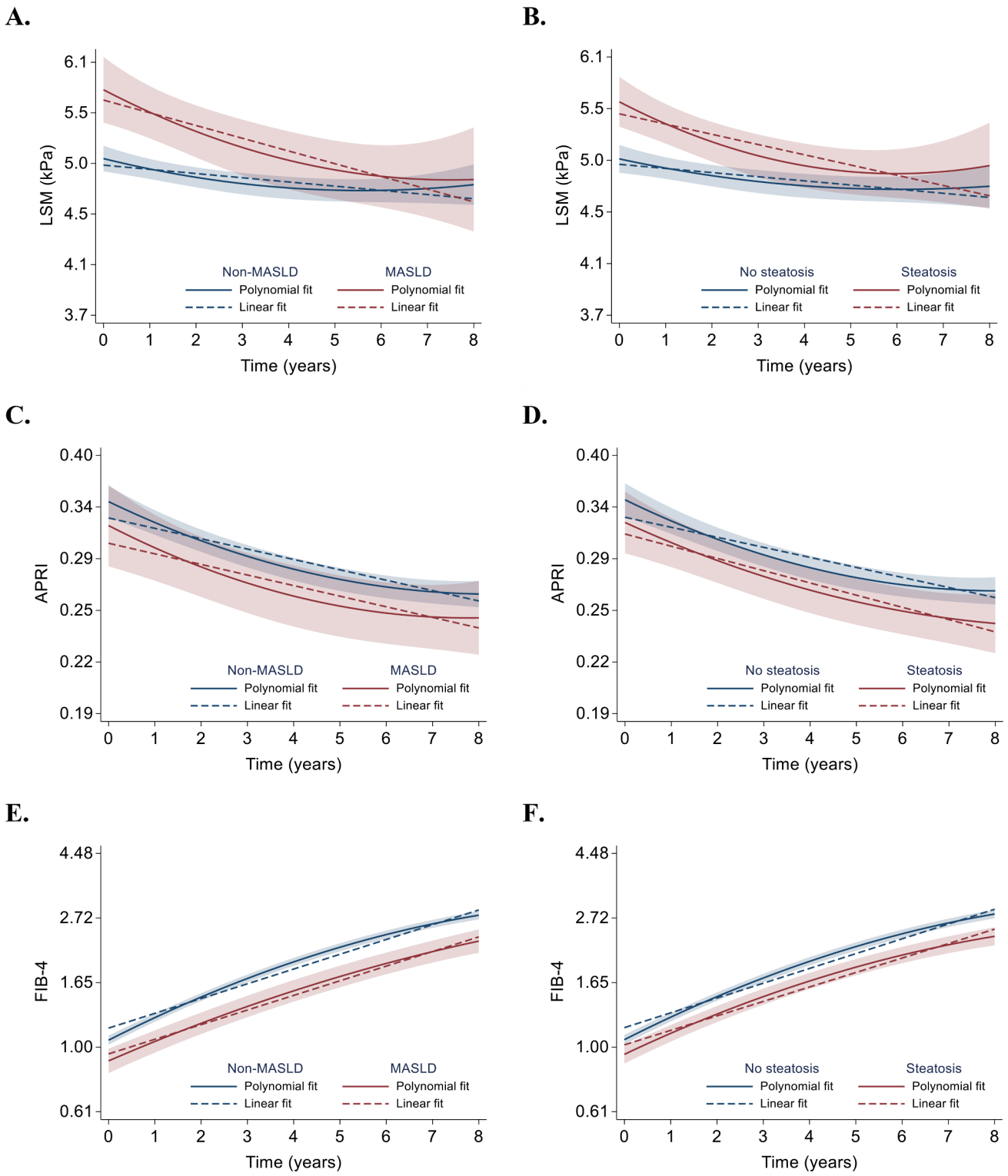

Fig. 1 Results of general linear mixed effects regression of fibrosis measurements over time in patients with chronic hepatitis B: (**A**) liver stiffness (LSM) stratified by MASLD status; (**B**) LSM stratified by steatosis; (**C**) APRI stratified by MASLD status; (**D**) APRI stratified by steatosis; (**E**) FIB-4 stratified by MASLD status; (**F**) FIB-4 stratified by steatosis

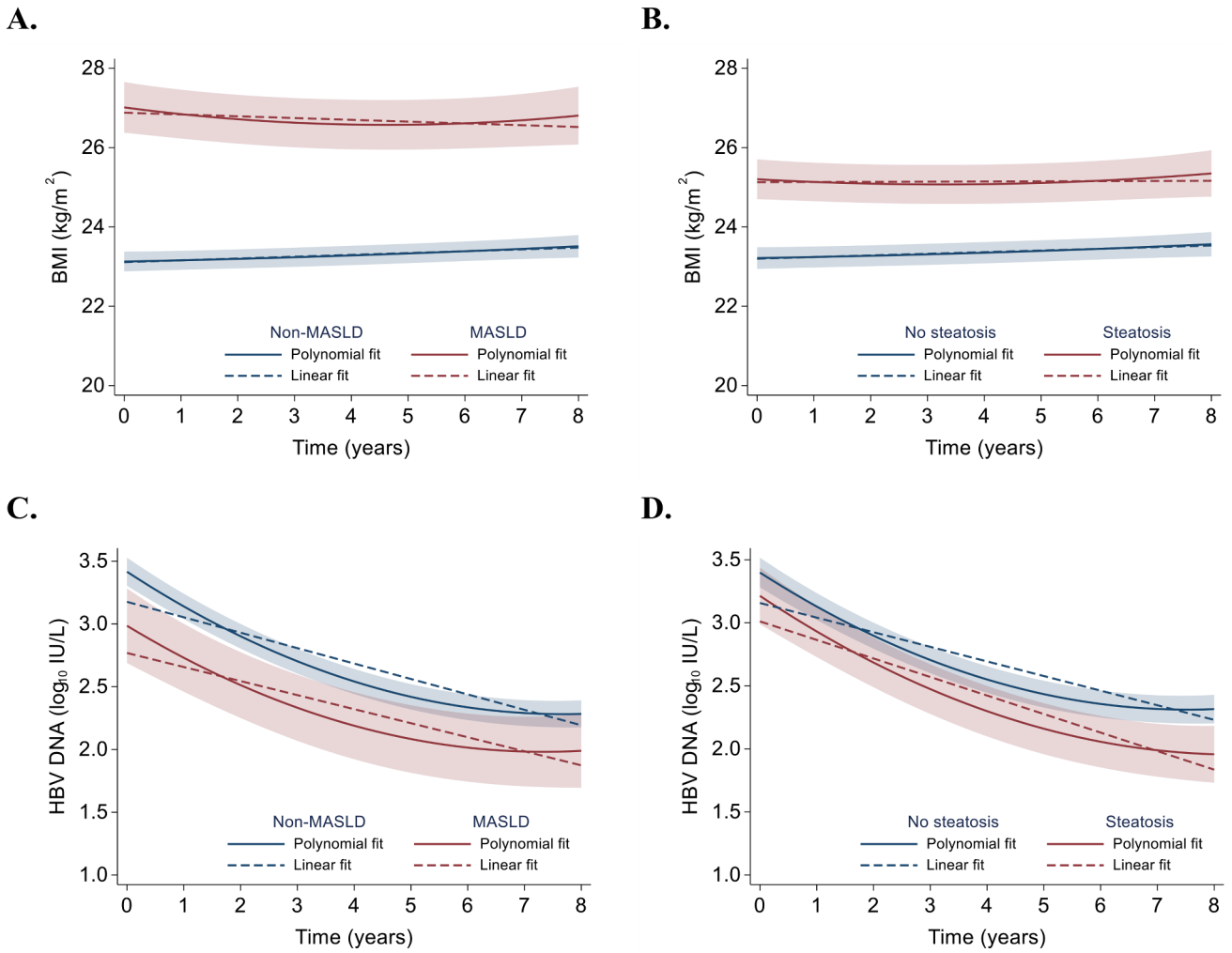

Fig. 2 Results of general linear mixed effects regression of BMI and HBV DNA over time in patients with chronic hepatitis B: (**A**) BMI stratified by MASLD status; (**B**) BMI stratified by steatosis; (**C**) HBV DNA stratified by MASLD status; (**D**) HBV DNA stratified by steatosis

14–46%, $P<0.001$), 15% higher in hypertensive patients (95% CI 3–27%, $P=0.009$), 9% lower for each 10 kg/m^2 increase in BMI (95% CI 5–13%, $P<0.001$) and 11% lower in HBeAg positive patients (95% CI 4–17%, $P=0.003$). There was no association with antiviral therapies, sex, baseline viral load or hyperlipidaemia. The analysis comparing patients with or without liver steatosis showed similar findings (see Fig. 1F).

Dynamics of the Host and Viral Response

ALT

At entry, MASLD patients had a 16% higher ALT than non-MASLD patients (95% CI 6–28%, $P=0.002$). ALT decreased in all patients over time (see Fig. 3E). There was no significant difference in rate of change in ALT over time between MASLD and non-MASLD patients. On average, ALT was 27% higher in males (95% CI 21–35%, $P<0.001$), 25% higher for every natural log increase in LSM (95% CI 15–36%, $P<0.001$), 16% higher in patients with hyperlipidaemia (95% CI 5–28%, $P=0.004$), and 7% lower for every 10 years of age (95% CI 5–9%, $P<0.001$). There was no significant association with antiviral therapy, BMI, HBeAg status, diabetes or hypertension. The analysis comparing patients with or without liver steatosis showed similar findings (see Fig. 3F).

HBV DNA

At entry, viral load (log10 IU/L) was 35% lower in MASLD patients (95% CI 10–53%, $P=0.009$). On average, viral load decreased in all patients over time (see Fig. 2C). On average over the entire follow-up, viral load was 296% higher in HBeAg positive patients (95% CI 217–394%, $P<0.001$), 78% lower in patients on baseline antiviral therapy (95% CI

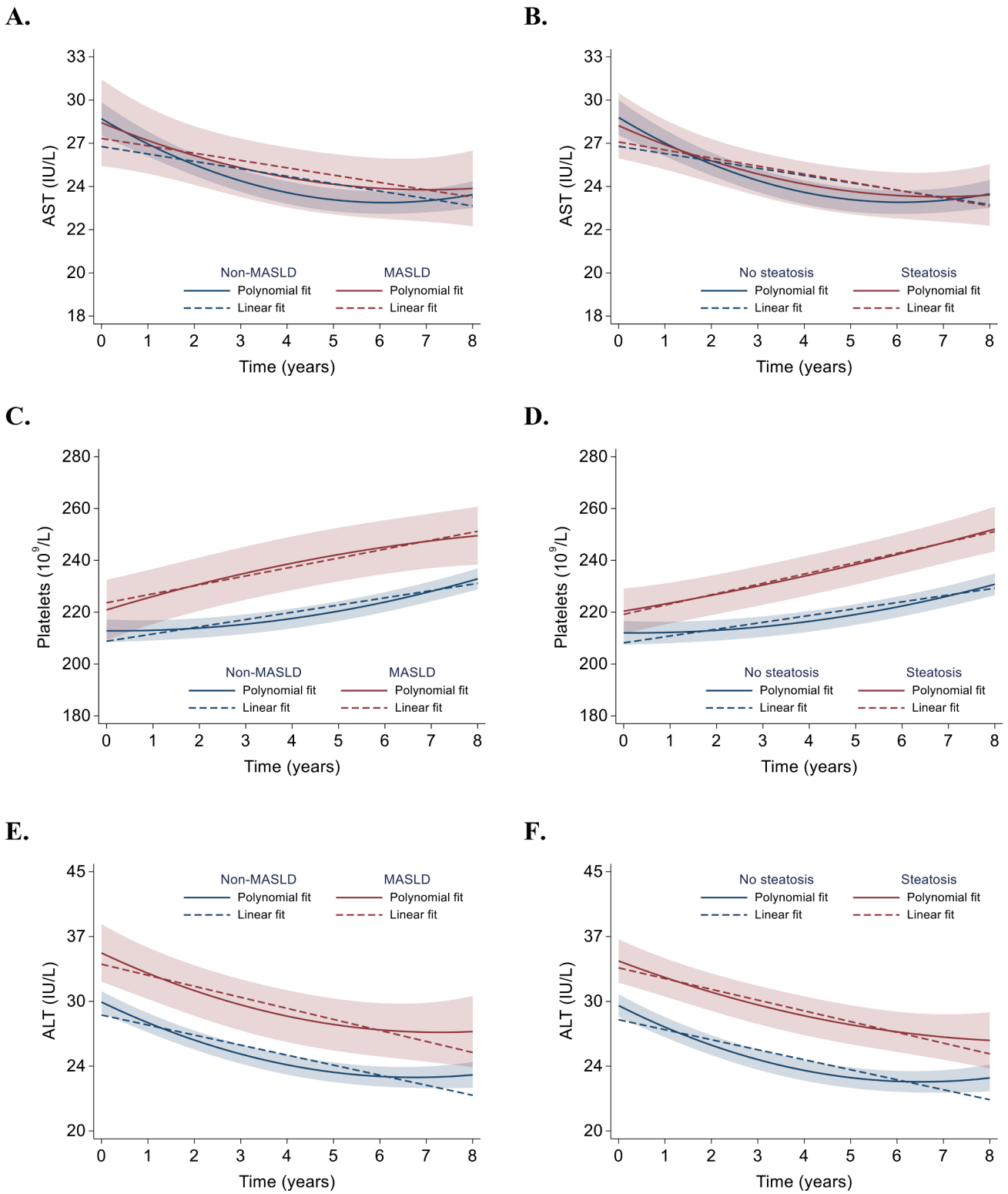

Fig. 3 Results of general linear mixed effects regression of APRI/FIB-4 constituent markers over time in patients with chronic hepatitis B: (**A**) AST stratified by MASLD status; (**B**) AST stratified by steatosis; (**C**) platelet count stratified by MASLD status; (**D**) platelet count stratified by steatosis; (**E**) ALT stratified by MASLD status; (**F**) ALT stratified by steatosis

70–84%, $P<0.001$), 71% lower in patients who commenced antiviral therapy at entry (95% CI 60–79%, $P<0.001$) and 36% lower in patients who had prior antiviral exposure (95% CI 9–55%, $P=0.012$). Viral load was not associated with age, sex, liver stiffness, BMI and individual metabolic risk factors. The analysis comparing patients with or without liver steatosis showed a slightly smaller separation in the curves (see Fig. 2D).

Dynamics of Liver Steatosis

Development of Steatosis

Of 624 non-steatotic CHB patients with a median observation time of 63.5 months, 182 (29%) developed steatosis corresponding to a yearly incidence rate of 5.5%. Factors associated with steatosis development on univariable analysis included: older age, male sex, hypertension, being overweight and HBeAg negative status (see Table 2). In a multivariable model including all predictors, only BMI ≥ 25 kg/m^2 (adjusted HR 1.57, 95% CI 1.14–2.18, $P=0.006$) was independently associated with steatosis development. Steatosis development occurred in non-overweight patients with a yearly incidence rate of 4.5% (95% CI 3.7–5.5%), and in overweight patients with a yearly incidence rate of 8.5% (95% CI 6.7–10.7%) (see Fig. 4).

Regression of Steatosis

Of 185 steatotic CHB patients with median observation time of 69.8 months, 30 (16%) had steatosis regression corresponding to a yearly incidence rate of 3.0% per year (see Table 2). The only factor associated with steatosis regression on univariable analysis was normal BMI < 25 kg/m^2 (HR 3.48, 95% CI 1.55–7.83, $P=0.003$). Normal BMI was the only significant predictor of steatosis regression on multivariable modelling (adjusted HR 3.43, 95% CI 1.48–7.92, $P=0.004$). Steatosis regression occurred in non-overweight patients with a yearly incidence rate of 5.1% (95% CI 3.4–7.8%), and in overweight patients with a yearly incidence rate of 1.5% (95% CI 0.7–2.9%) (see Fig. 4).

On univariable analysis, having non-metabolic associated steatotic liver disease (cryptogenic SLD) was associated with a higher rate of steatosis regression (HR 2.37 95% CI 1.15 to 4.89, $P=0.019$) compared to MASLD patients.

Table 2 Regression analysis for steatosis development in CHB patients without baseline steatosis ($n=625$), and steatosis regression in CHB patients with baseline steatosis ($n=185$)

Variable	Univariable		Multivariable	
	HR (95% CI)	P	Adj. HR (95% CI)	P
Steatosis development				
Age, per year	1.02 (1.00–1.03)	0.005	1.01 (0.99–1.02)	0.33
Male sex	1.53 (1.14–2.05)	0.004	1.34 (0.97–1.85)	0.07
LSM, per $\log_e$(kPa)	1.33 (0.85–2.10)	0.21	1.15 (0.69–1.92)	0.60
HBeAg positive	0.69 (0.47–1.00)	0.049	0.86 (0.54–1.37)	0.53
HBV DNA, per $\log_{10}$(IU)	0.93 (0.86–1.00)	0.06	0.95 (0.86–1.04)	0.28
Previous antiviral treatment	1.01 (0.63–1.63)	0.95	1.11 (0.64–1.92)	0.70
Antiviral use at baseline	1.11 (0.73–1.69)	0.62	0.98 (0.58–1.63)	0.93
Antiviral initiation at baseline	0.82 (0.46–1.48)	0.51	0.57 (0.29–1.13)	0.11
Hypertension	2.03 (1.26–3.27)	0.003	1.43 (0.81–2.53)	0.22
T2DM	1.76 (0.90–3.44)	0.10	1.48 (0.72–3.02)	0.28
Hypercholesterolaemia	1.55 (0.92–2.64)	0.10	1.16 (0.66–2.03)	0.60
BMI ≥ 25 kg/m^2	1.94 (1.44–2.63)	< 0.001	1.57 (1.14–2.18)	0.006
BMI, per kg/m^2 †	1.10 (1.06–1.15)	< 0.001	-	-
Steatosis regression				
Age, per year	0.99 (0.96–1.03)	0.66	1.00 (0.96–1.04)	0.88
Male sex	1.18 (0.55–2.51)	0.68	1.26 (0.53–2.99)	0.59
LSM, per loge(kPa)	0.43 (0.15–1.25)	0.12	0.40 (0.13–1.22)	0.11
HBeAg positive	1.64 (0.75–3.59)	0.21	0.91 (0.31–2.68)	0.87
HBV DNA, per log10(IU)	1.13 (0.97–1.31)	0.12	1.12 (0.91–1.37)	0.29
Previous antiviral treatment	1.02 (0.14–7.50)	0.98	0.52 (0.05–5.05)	0.57
Antiviral use at baseline	1.29 (0.39–4.27)	0.67	2.73 (0.56–13.31)	0.21
Antiviral initiation at baseline	1.28 (0.39–4.23)	0.68	1.58 (0.43–5.85)	0.49
Hypertension	0.65 (0.20–2.13)	0.47	0.70 (0.19–2.54)	0.58
T2DM	1.29 (0.49–3.37)	0.60	2.10 (0.66–6.71)	0.21
Hypercholesterolaemia	0.90 (0.35–2.36)	0.84	1.06 (0.31–3.62)	0.92
BMI < 25 kg/m^2	3.48 (1.55–7.83)	0.003	3.43 (1.48–7.92)	0.004
BMI, per kg/m^2 ‡	0.80 (0.70–0.92)	0.001	-	-

†Sensitivity analysis demonstrates BMI remains a significant predictor in multivariable analysis when included as a continuous variable (adjusted HR 1.08 per kg/m^2, 95% CI 1.03–1.13, $P=0.001$)

‡Sensitivity analysis demonstrates BMI remains a significant predictor in multivariable analysis when included as a continuous variable (adjusted HR 0.81 per kg/m^2, 95% CI 0.70–0.93, $P=0.003$)

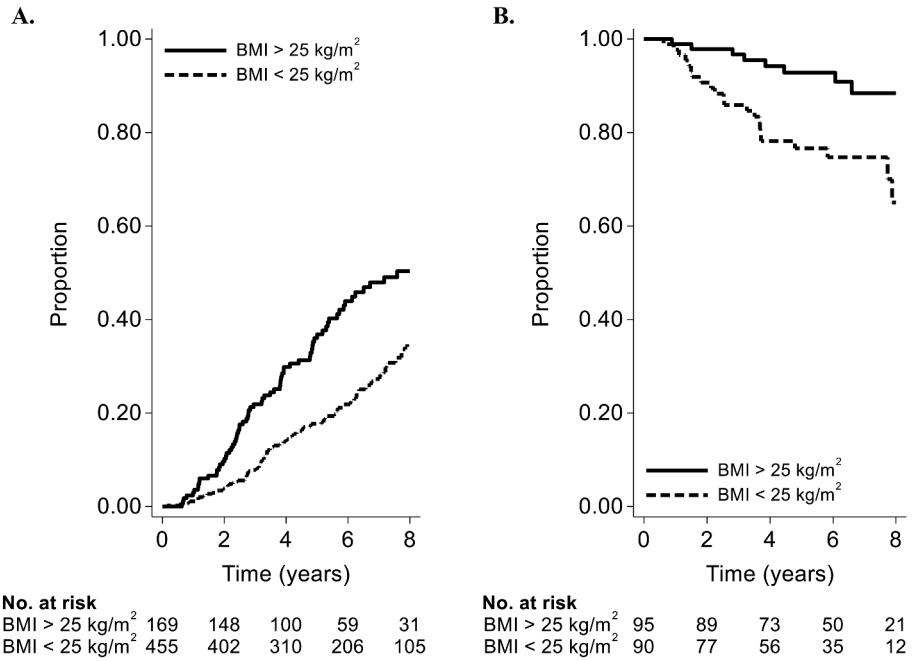

Fig. 4 Steatosis development and regression in patients with CHB stratified by overweight status. (**A**) Steatosis development in CHB patients without baseline steatosis ($n = 624$). (**B**) Steatosis regression in CHB patients with concurrent steatosis ($n = 185$)

Steatosis regression occurred in cryptogenic SLD patients with a yearly incidence rate of 4.8% (95% CI 3.0–7.7%), and in MASLD patients with a yearly incidence rate of 2.0% (95% CI 1.2–3.5%).

Discussion

Modern nucleos(t)ide antiviral therapies are able to achieve high rates of virological response and suppression of liver inflammation, and thus attenuate the rate of fibrosis progression in patients with CHB [2, 3]. Non-viral co-factors such as metabolic syndrome and MASLD are increasingly recognized to play crucial roles in the mediation of liver fibrosis and carcinogenesis. Although liver steatosis correlates with fibrosis cross-sectionally [15, 23], and persistent severe steatosis has been shown to correlate with fibrosis progression [14], the effect of milder degrees of steatosis on fibrosis progression remains unclear and contentious. Through a rigorous analysis of sequential fibrosis measurements, our study has demonstrated that concurrent MASLD does not appear to accelerate fibrosis progression in otherwise healthy, non-cirrhotic CHB patients, in the short to medium term.

Concurrent liver steatosis attenuates HBV viraemia [11–13], accelerates HBsAg seroclearance [14], and may even improve the rate of response to antiviral therapy [24]—therefore the positive effects of steatosis and/or MASLD on HBV infection may be potential mechanisms for the lack of an effect on fibrosis progression found in this study. Additionally, it remains to be seen whether the degree of steatosis may have dynamic effects on fibrosis progression: in patients with mild steatosis, a lower viral activity may reduce the necro-inflammatory response in the liver and limit fibrogenesis in the early stages of follow-up, whereas in a patient with severe steatosis, the detrimental effects of steatosis on fibrogenesis may outweigh the protective effects of the relative viral suppression. However, the present study had no capacity to assess degree of liver steatosis, although the presence of steatosis on ultrasound suggests a steatosis percentage of at least 20–30% [4].

As expected, patients with MASLD had higher baseline liver stiffness in our study, yet somewhat surprisingly, liver stiffness appeared to become similar between groups after 4–5 years of follow-up, despite similar rates of baseline antiviral treatment. A mechanism not assessed in our study is the potential effect of clinician and patient factors that may have influenced metabolic risk profile over time, such as diet improvement or improved glycaemic, blood pressure and/or cholesterol control, although we showed that weight did not appear to substantially change over time. A second postulated mechanism may be a multiplicative effect of concurrent MASLD and viraemia on liver inflammation, such that untreated viraemic patients have a higher liver stiffness at baseline, which approaches similar liver stiffness levels over time after treatment. However, our study was not designed to answer this question. Although LSM and ALT are well-known to be correlated, interestingly ALT seemed to decrease by the same rate in all patients while LSM decreased more in the patients with MASLD compared to non-MASLD. The mechanism of this apparent phenomenon is unclear and should be further evaluated in future studies.

Interestingly, although both APRI and FIB-4 have been validated to be accurate non-invasive markers of liver fibrosis [6, 7], neither score mirrored the dynamics of liver stiffness over time in our study. In particular, FIB-4 appeared to have an inverse relationship with MASLD as compared to liver stiffness, where MASLD CHB patients had higher LSM yet lower FIB-4 compared to non-MASLD CHB patients. This appears to be related to the finding that MASLD patients had a higher platelet count and ALT level, which would result in a lower FIB-4 given their presence in the denominator of the FIB-4 formula. FIB-4 also increased over time given its dependence on age, while LSM and APRI generally decreased over time. It therefore remains unclear which non-invasive tool is more accurate and reliable in assessing liver fibrosis in patients with liver steatosis or MASLD.

This study has also shown that BMI appears to be the most important factor in liver steatosis development and regression. Although MASLD is considered to be a liver manifestation of the metabolic syndrome, it was BMI alone (and not diabetes or other metabolic factors) which seemed to influence the rate of steatosis change over time on multivariable analysis. This finding is not surprising given central obesity and visceral adiposity is suggested to be a greater risk factor for liver steatosis than general obesity [25].

The major limitation of this study is its retrospective nature, which inherently subjects it to potential bias due to misclassification and missing data. The study was conducted at a single centre which may limit its external validity. The study did not utilize liver biopsy as this was not routinely performed. Timing and frequency of repeat transient elastography were decided by the treating clinician, which may introduce bias where patients perceived to be at greater risk of fibrosis progression might have under gone more frequent elastographic examinations. Although we did include many confounding factors in the mixed effects modelling, we did not capture fasting duration, recent alcohol use or other liver factors at the time of LSM reading which may have influenced results. Although abdominal ultrasound has been shown to be reliable to assess for presence of steatosis, the use of non-invasive markers such as liver stiffness, APRI and FIB-4 are much less reliable. Further, although our study had a relatively long follow-up of up to 8 years, this is unlikely to be long enough to demonstrate fibrosis progression in otherwise healthy CHB patients. Although we did adjust for treatment status at baseline, we did not capture data regarding future treatment initiation, which may introduce a bias if one group (i.e. MASLD) were more likely to require treatment. However, we note that there were no baseline differences in prior, current or new treatment between MASLD and non-MASLD patients. Another limitation is the lack of ethnicity data which may influence outcomes. Further, some patients may be misclassified in terms of their overweight and MASLD status due to the lower threshold for being overweight in Asian females. The determination of liver steatosis by radiologists was subjective and is another limitation. Additional longer studies in external cohorts, as well as studies utilizing liver biopsy are required to confirm our findings, and to assess for an interaction with the degree of steatosis and the degree of fibrosis in determining fibrosis progression over time.

In conclusion, this study has shown that concurrent MASLD does not appear to contribute to the rate of fibrosis progression as measured by transient elastography in non-cirrhotic CHB patients. Non-invasive markers such as APRI and FIB-4 may be less reliable and accurate in estimating liver fibrosis in CHB patients with or without concurrent MASLD given their discrepancy with transient elastography. BMI appears to be the most important factor determining the rate of steatosis development and regression over time. A longer duration of follow-up may be required to detect the long-term effects of concurrent MASLD in CHB patients.

Author contributions Study conception and design were performed by DC, JSL, RS and SB. Data collection was performed by DC, ST and DCC. Statistical analysis and manuscript drafting was performed by DC. Manuscript revision for important intellectual content was performed by DCC, JSL, RS and SB. All authors read and approved the final version of the manuscript.

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Data availability The participants of this study did not give written consent for their data to be shared publicly, so due to the sensitive nature of the research supporting data is not available.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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