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Dynamics of liver stiffness in chronic hepatitis B patients with concurrent metabolic-associated fatty liver disease

To the Editor:

We read with interest the article by Mak *et al.*[1] and commend their work exploring the diverse relationship between hepatic steatosis and chronic hepatitis B (CHB). Through prospective follow-up of 330 patients with normal alanine aminotransferase (ALT) and low viraemia, Mak *et al.* showed persistent severe steatosis (controlled attenuation parameter ≥280 dB/m) was associated with progression of fibrosis category after 3 years. As modern nucleos(t)ide analogues are able to achieve effective long-term viral/biochemical suppression and attenuate fibrosis development,[2] the focus of CHB management has shifted to address non-viral risk factors such as concurrent steatosis – with approximately 30% prevalence amongst patients with CHB[3] – and metabolic dysfunction-associated fatty liver disease (MAFLD).[4] Although Mak *et al.* provided useful insight into the effects of severe steatosis on virologically quiescent CHB, the broader relationship between these two conditions remains elusive. We aimed to provide additional insight by modelling serial liver stiffness measurements (LSM) over time and comparing their trajectories amongst patients with or without concurrent MAFLD.

We retrospectively identified all non-cirrhotic patients with CHB at a tertiary Australian centre. Patients were followed up from first review between 01/01/2010–31/12/2016 until 31/08/2020 or loss-to-follow-up. Steatosis was diagnosed radiologically (diffusely increased echogenicity on ultrasound) and MAFLD was diagnosed using new criteria.[4] Transient elastography was performed by a certified operator in accordance with best clinical practice for quality and probe selection.[5] The median of ≥10 successful measurements was recorded. Multivariable general linear mixed-effects regression (random intercept at the patient level) was used to model LSM, BMI, ALT and viral load using optimal order polynomial time covariates (by Akaike's information criterion) and time interaction terms. Each model was controlled for baseline age, sex, LSM, ALT, BMI, viral load, antiviral status, prior antiviral exposure and metabolic risk factors. Analysis was performed in Stata/IC 16.1 (StataCorp LP, USA, 2020).

Of 660 patients (median follow-up 6.0 years; IQR 4.1–8.3), 172 (26%) had concurrent MAFLD. Data comprised 1,997 LSM, 10,647 ALT and 8,223 DNA measurements. Patients with MAFLD were of similar age (median 45.5 *vs.* 43.0 years, $p = 0.09$), but were more commonly male (65% *vs.* 44%, $p < 0.001$). There was no significant difference (MAFLD *vs.* non-MAFLD) in the proportion who were HBeAg positive (19% *vs.* 25%, $p = 0.21$), on antiviral therapy at baseline (7% *vs.* 12%, $p = 0.18$) or commenced on therapy during follow-up (25% *vs.* 29%, $p = 0.49$). Most (61%) were never on antiviral therapy. Interestingly, we found that although average LSM was 15% higher initially (95% CI 7–24%, $p < 0.001$) amongst patients with concurrent MAFLD, this improved over time such that there was no significant difference after 2–3 years (Fig. 1). Although LSM is known to be elevated in obesity, the trajectory of LSM in patients with MAFLD did not appear to correlate with BMI, but instead mirrored the trajectory of HBV DNA and ALT, reflective of the underlying virological and biochemical response. On average, DNA was 35% lower in patients with MAFLD (95% CI 14–51%, $p = 0.003$), and this difference remained over time. ALT decreased in all patients over time but was 13% higher in patients with MAFLD on entry (95% CI 4–23%, $p = 0.003$), and remained higher throughout follow-up. Of note, a similar degree of improvement in LSM was not observed in patients without MAFLD, despite no difference in the rate of change in ALT or viral load between MAFLD and non-MAFLD patients.

Perhaps the most striking finding of these data is the lack of any apparent worsening of LSM on average over time – in fact, LSM *improved* in patients with MAFLD, with similar findings when analysing only untreated patients (data not shown). However, our cohort was substantially different to the cohort by Mak *et al.* as we included all patients with CHB, the majority of whom had elevated HBV DNA and ALT throughout follow-up. This perhaps suggests viraemia and/or raised ALT has a more pronounced influence on LSM, and that the effect of steatosis is unmasked only after viral and biochemical suppression. Another

Received 22 December 2020; received in revised form 31 January 2021; accepted 9 February 2021; available online 20 February 2021
https://doi.org/10.1016/j.jhep.2021.02.013

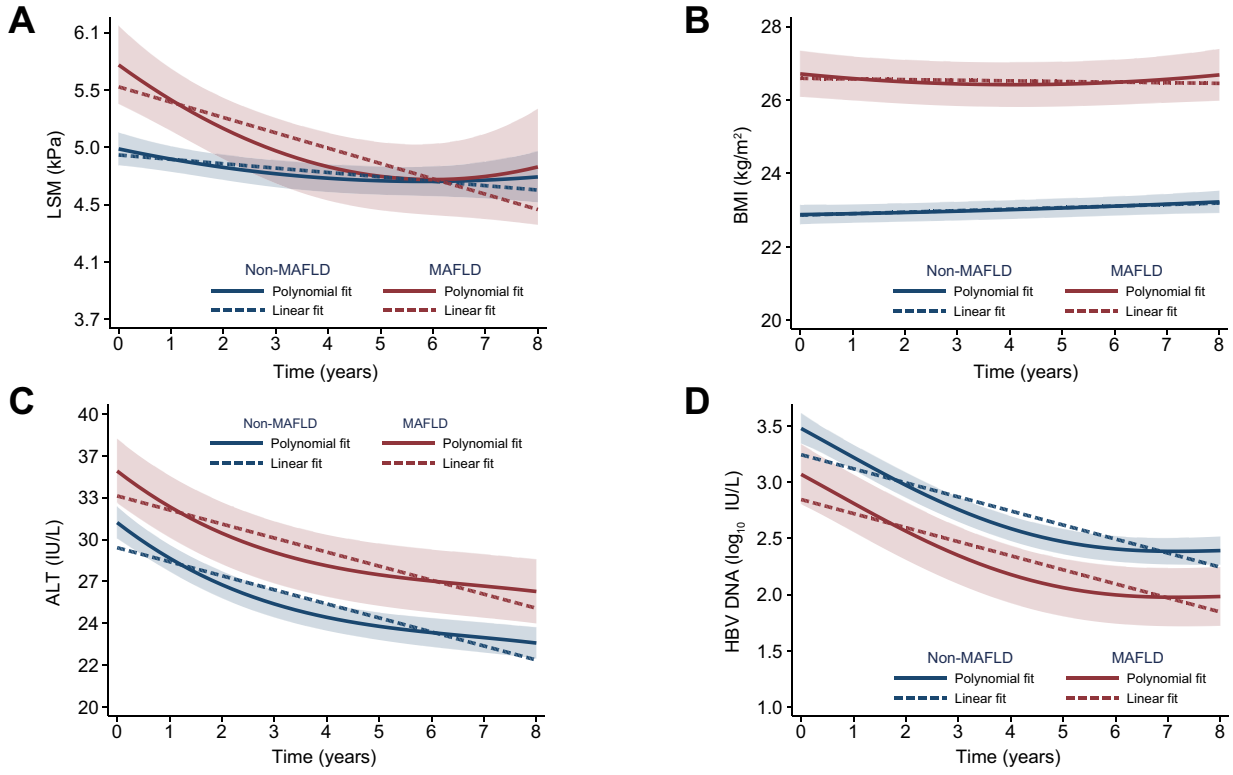

Fig. 1. Estimated trends in liver stiffness (LSM), BMI, ALT and HBV DNA over time using general linear mixed effects regression in patients with chronic hepatitis B, stratified by presence of concurrent MAFLD (n = 660). Both optimal order polynomial fit with 95% confidence bands as well as linear fit for comparison are shown. (A) LSM; (B) BMI; (C) ALT; (D) HBV DNA. (This figure appears in color on the web.)

explanation could be the influence of improved lifestyle changes not captured in our data; however, BMI did not substantially change over time. Although we were limited by the lack of controlled attenuation parameter measurements to quantify steatosis degree, ultrasound is less sensitive for steatosis so the patients in our cohort were likely to have moderate-to-severe steatosis. Thus, our results are markedly different from Mak *et al.* who showed the rate of fibrosis progression appeared highest in patients with persistent severe steatosis (41%) and new onset severe steatosis (35%). However, data for milder degrees of steatosis were not provided. It remains speculative whether the negative effects of steatosis on liver fibrosis outweigh the apparent protective effects, since concurrent steatosis attenuates viraemia,[6,7] accelerates HBsAg seroclearance,[1] and possibly even improves the rate of response to antiviral therapy.[8] Additional prospective studies are required to further clarify the unique relationship between steatosis and CHB.

Financial support
This study was not directly funded and there are no other financial disclosures to declare.

Conflicts of interest
The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
DC: conceptualization, data collection, statistical analysis, data interpretation, manuscript drafting. DCC: data collection, data interpretation. JL: data collection, data interpretation, revision of manuscript critically for important intellectual content. RS: conceptualization, data interpretation, revision of manuscript critically for important intellectual content. SB: conceptualization, data interpretation, revision of manuscript critically for important intellectual content.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.02.013.

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Myofibroblast YAP/TAZ is dispensable for liver fibrosis in mice

To the Editor:

Severe fibrosis often leads to significant mortality as a result of liver failure and cirrhosis. Recent evidence arising in the *Journal of Hepatology etc.* shed light on the role of the mechanosensing Hippo/YAP/TAZ pathway during hepatic fibrosis.[1–5] Those studies suggested that the YAP/TAZ pathway was activated in various mouse models of liver fibrosis and in human fibrotic liver. However, conflicting data exist for both positive and negative regulation of the YAP/TAZ pathway in liver fibrogenesis. The study by Liu *et al.* elegantly demonstrated that YAP inhibition with verteporfin exacerbated liver fibrogenesis.[1] In contrast, several recent studies suggested that inhibition of YAP/TAZ signaling attenuated liver fibrosis, which highlighted YAP/TAZ as a critical driver of hepatic fibrosis.[2–5] Such discrepancy has sparked debate about the function of YAP/TAZ in liver fibrogenesis. In particular, the role of YAP/TAZ in myofibroblasts, a key cell type driving extracellular matrix production during fibrosis, is unclear. Herein, we independently addressed this issue using a newly generated *Postn-CreERT2* mouse line[6] to lineage trace activated myofibroblasts *in vivo* and genetically target Yap/Taz specifically in myofibroblasts within the fibrotic liver.

Periostin is a secreted matricellular protein which has been reported as marker of the myofibroblasts expressed exclusively in area of tissue injury.[6,7] To characterize whether *Postn-CreERT2* targets endogenous myofibroblasts in the liver, we crossed the *Postn-CreERT2* mice with the mT/mG reporter line, which switches from membrane-targeted Tomato expression to membrane-targeted GFP expression upon Cre-mediated recombination (Fig. 1A). We subjected Postn-CreERT2; mT/mG mice to a well-established model of carbon tetrachloride (CCl_4)-induced liver fibrosis followed by tamoxifen induction (Fig. 1B). Postn-CreERT2; mT/mG mice showed abundant expression of periostin protein in the injured liver as well as Postn-CreERT2-dependent expression of GFP (Fig. 1C). At the histological level, periostin lineage-traced GFP^+ cells were predominantly expressed in the portal area with the characteristic septal pattern of liver fibrosis (Fig. 1D). Approximately 95% of the GFP-positive cells were αSMA positive, while nearly 92% were collagen-positive and ~85% were desmin-positive but only few were hepatocyte marker HNF4α or endothelial marker CD31 reactive (Fig. 1D-E). Similarly, in bile duct-ligation (BDL, Fig. 1F) and ischemia-reperfusion injury (IRI, Fig. 1G) induced fibrosis models, Postn-CreERT2-induced GFP expression strongly overlapped with αSMA. Thus, *Postn-CreERT2* transgenic mice exclusively mark myofibroblasts in the fibrotic liver and Postn-expressing myofibroblasts should be considered as a primary target for anti-fibrotic therapies.

To test the contribution of YAP/TAZ in the regulation of liver fibrosis, we generated mice lacking YAP/TAZ in Postn-expressing myofibroblasts by crossing mice carrying floxed alleles of both Yap and Taz ($Yap^{fl/fl}$; $Taz^{fl/fl}$) with *Postn-CreERT2* animals (Fig. 1H-I). Consistent with previous reports, we found that in CCl_4-treated mice, YAP/TAZ was predominantly localized in the nucleus of αSMA-positive myofibroblasts but rarely present in the hepatocyte nucleus (Fig.1J). Myofibroblast-specific, tamoxifen-inducible YAP/TAZ deficient mice (Pn-Yap/Taz-KO) treated with CCl_4 showed strong recombination of both floxed alleles, resulting in loss of YAP and TAZ proteins specifically in the liver myofibroblasts (90.8% in floxed littermates *vs.* 2.5% in KO co-localization with $αSMA^+$ cell nucleus, Fig.1J). Western blot and RT-qPCR analyses confirmed the loss of YAP and TAZ in myofibroblasts isolated from Pn-Yap/Taz-KO (Fig.1K, N). We next examined the consequences of myofibroblast Yap/Taz knockout in liver fibrogenesis by histopathological H&E, Sirius red and Masson's trichrome staining. After 6 weeks of CCl_4 injections, we did not detect a difference in fibrosis formation between the Pn-Yap/Taz-KO and floxed littermates (Fig. 1L-M). Accordingly, the gross morphology, fibrous portal expansion, necrosis, inflammatory cell infiltration as well as extracellular matrix and collagen deposition were all unaltered in CCl_4-treated Pn-Yap/Taz-KO mice (Fig.1L-M). To exclude that our data may be specific to the

Received 25 January 2021; received in revised form 22 February 2021; accepted 23 February 2021; available online 03 March 2021
https://doi.org/10.1016/j.jhep.2021.02.026