Reduced hepatic steatosis is associated with higher risk of hepatocellular carcinoma in chronic hepatitis B infection

Short title: Liver fat tied with less HCC in CHB

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Total word count of manuscript: 3961

Total number of figures and tables: 4 figures and 2 tables

Abstract

Background: Concomitant chronic hepatitis B infection (CHB) and non-alcoholic fatty liver disease (NAFLD) is common, but the implications of NAFLD on clinical outcomes of CHB, including hepatocellular carcinoma (HCC), are not well-investigated.

Methods: CHB patients were recruited for transient elastography assessment for liver stiffness (LS), and controlled attenuation parameter (CAP), a non-invasive quantification of hepatic steatosis, and were prospectively followed up for development of HCC. Steatosis and severe steatosis were diagnosed by CAP ≥248 dB/m and ≥280 dB/m respectively, and advanced fibrosis/ cirrhosis was diagnosed by LS ≥9 kPa. The independent effect of hepatic steatosis on HCC was examined via propensity score matching (PSM) of LS and other significant clinical variables.

Results: Forty-eight patients developed HCC among 2403 CHB patients (55.6% male, median age 55.6 years, 57.1% antiviral-treated, median ALT 26 U/L) during a median follow-up of 46.4 months. Multivariate Cox regression analysis showed age (HR 1.063), male (HR 2.032), Albumin-Bilirubin score (HR 2.393) and CAP (HR 0.993) were associated with HCC development. The cumulative probability of HCC was 2.88%, 1.56% and 0.71%, respectively for patients with no steatosis, mild-to-moderate steatosis, and severe steatosis, respectively (p=0.01). The risk of HCC increased from 1.56% to 8.89% in patients without severe steatosis if advanced fibrosis/cirrhosis were present (p<0.001). PSM yielded 957 pairs of CHB patients and hepatic steatosis was independently associated with HCC (HR 0.41).

Conclusion: Reduced hepatic steatosis was significantly associated with a higher risk of incident HCC in CHB infection. Routine CAP and LS measurements are important for risk stratification.

(Word count: 250)

Keywords: HBV, hepatocellular carcinoma, NAFLD, liver stiffness, controlled attenuation parameter

List of abbreviations:
CHB: chronic hepatitis B, NAFLD: non-alcoholic fatty liver disease, HCC: hepatocellular carcinoma, HCV: hepatitis C virus, HBV: hepatitis B virus, HBsAg: hepatitis B surface antigen, NA: nucleos(t)ide analogue, CAP: controlled attenuation parameter, HbA1c: glycated hemoglobin, FG: fasting glucose, TG: triglyceride, LDL: low density lipoprotein, HDL: high density lipoprotein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, HBeAg: hepatitis B e antigen, LS: liver stiffness, F0/F1: no/ minimal fibrosis, F3: advanced fibrosis, F4: cirrhosis, dB/m: decibel/ meter, IQR: interquartile

range, HR: hazard ratio, CI: confidence interval, NASH: non-alcoholic steatohepatitis, HBcAg: hepatitis B core antigen

Introduction

Chronic hepatitis B (CHB) infection and non-alcoholic fatty liver disease are two common chronic liver conditions which affect 3.9% and 29.6% of the global population, respectively.[1, 2] Each condition is capable of causing significant liver-related morbidities and mortality from development of cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC).[2-4] CHB patients with co-existing non-alcoholic fatty liver disease (NAFLD) are frequently encountered in clinical practice, with prevalence rates ranging from 14% to more than 50%.[5, 6] Although hepatitis C virus (HCV) can be steatogenic via insulin resistance and viral protein-induced lipid accumulation,[7, 8] hepatitis B virus (HBV) is not known to cause hepatic steatosis mechanistically. In fact, it has been suggested that HBV confers protective effect on hepatic steatosis, or vice versa, as reflected by the lower incidence of NAFLD in CHB patients compared to non-CHB persons,[9] the negative association between HBV viral load and hepatic steatosis,[10, 11] the earlier age of achieving hepatitis B surface antigen (HBsAg) seroclearance,[12 13] and the milder histological inflammation and fibrosis in CHB patients with steatosis compared to those without steatosis.[5] It remains controversial regarding the effects of hepatic steatosis on the natural course of CHB. While a cross-sectional study reported no effects of hepatic steatosis on hepatic fibrosis,[6] other studies showed that hepatic steatosis is associated with severe fibrosis and progression of fibrosis.[14, 15] The impact of concomitant hepatic steatosis on the clinical outcome of CHB, in particular HCC, especially in the current era of increasing nucleos(t)ide analogue (NA) treatment coverage, is largely unknown.

The non-invasive assessment of liver fibrosis via transient elastography is now recognized as standard-of-care in the management and monitoring of CHB.[16] Transient elastography is an ultrasound-based technique, which allows diagnosis of severe hepatic fibrosis or cirrhosis in a non-invasive manner.[17, 18] Current versions of transient elastography additionally quantify the amount of liver steatosis via controlled attenuation parameter (CAP) measurements, with well-defined cut-offs applied for different degrees of steatosis.[19] In a recent meta-analysis that included 16 studies and 2346 patients with histology-controlled CAP measurement, CAP can effectively recognize significant steatosis in patients with viral hepatitis.[20] Utilizing this easily accessible way of steatosis quantification, we designed a prospective study to assess the effect of concomitant hepatic steatosis on the risk of HCC in a large cohort of CHB patients.

Materials and Methods

Study design and patient population

This is a prospective study involving Asian CHB patients from the Hepatology Clinic, Queen Mary Hospital, Hong Kong. Patients with CHB, defined as persistent seropositivity for HBsAg for ≥ 6 months, (aged ≥18, treatment-naïve or on NA) were consecutively recruited for transient elastography assessment between January 2015 and January 2019. We excluded patients with prior history of HCC, concomitant HCV or human immunodeficiency virus infection, primary biliary cholangitis, Wilson's disease, autoimmune hepatitis, significant alcohol intake (≥30 gram per day for male, or ≥20 gram per day for female), on steatogenic medications (see below), prior liver transplantation, and those who already developed HBsAg seroclearance. The cross-sectional findings of the first recruited 1606 patients, demonstrating an association between fibrosis and steatosis in CHB, had been previously described.[14] In this present study, a total of 2403 subjects were recruited and the patient disposition is shown in Figure 1. The present study was approved by the Institutional Review Board/ Ethics Committee of the University of Hong Kong and the Hong Kong West Cluster of Hospital Authority. All study subjects provided written informed consent prior to any study-related procedures.

Clinical evaluation and laboratory assessment

Detailed history including demographic details, alcohol consumption, concomitant medications including steatogenic drugs (systemic corticosteroids, amiodarone, tamoxifen, valproic acid, and methotrexate) were taken. To identify metabolic risk factors, anthropometric measurement including the body weight, body height, body mass index (BMI), waist circumference, hip circumference, blood pressure measurement was carried out at the time of performing transient elastography. In addition, serum glycated hemoglobin (HbA1c), fasting glucose (FG), cholesterol and triglyceride (TG) was checked. Diabetes mellitus was defined as FG ≥7.0 mmol/L, HbA1c ≥6.5%, or the use of anti-diabetic medications. Dyslipidaemia was defined as total cholesterol ≥5.2 mmol/L, low-density lipoprotein (LDL) cholesterol ≥3.4 mmol/L, high-density lipoprotein (HDL) cholesterol ≤1.3 mmol/L for female or ≤1.0 mmol/L for male, TG ≥1.7 mmol/L, or the use of lipid-lowering therapy. Serum liver biochemistry

including alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelet count, and alpha fetoprotein was measured at each visit at 3-6 months interval, together with viral markers, including serum HBsAg, hepatitis B e-antigen (HBeAg), and serum HBV DNA (lower limit of detection 20 IU/mL or 1.3 log IU/mL). Residual liver function was assessed by Albumin-Bilirubin Grade (ALBI) using the formula as follows:

$$ALBI = (\log_{10}\text{bilirubin} \times 0.66) + (\text{albumin} \times -0.085)$$

ALBI scores of ≤-2.60, >-2.60 to ≤-1.39 and >-1.39 represent ALBI grades of 1, 2 and 3, respectively.[21]

The NA entecavir and tenofovir disoproxil fumarate were the mainstay of antiviral therapy in our locality. Patients were started on subsidized NA if they developed raised ALT (different ALT cut-offs were used owing to variations in laboratory policies. For tenofovir-treated patients, the ALT cut-off was defined as 60 U/L for males or 38 U/L for females[22]; for patients treated with other NAs, the ALT cut-off was defined as 50 U/L at that time[23]) together with high HBV viral load (defined as HBV DNA >20,000 IU/mL for HBeAg-positive patients or >2,000 IU/mL for HBeAg-negative patients), any level of detectable serum HBV DNA in the presence of cirrhosis, or the diagnosis of HCC.

Radiological assessment

Regular six-monthly ultrasonography of the upper abdomen was advised to all subjects. For patients with abnormal ultrasound findings showing suspicion of liver nodule, contrast-enhanced imaging with either computerized tomography or magnetic resonance imaging was arranged. HCC was diagnosed by the typical features of arterial phase hyper-enhancement and porto-venous washout of contrast, with or without histological proof.[24, 25]

Transient elastography

Fibroscan (Echosens®, Paris, France) was used to perform transient elastography assessment for recruited subjects. The M probe was used for patients with BMI <30 kg/m^2, while the XL probe was used for patients with BMI ≥30kg/m^2. Two certified operators with prior formal training from Echosens® and at least 500 transient elastography procedures performed the transient elastography. Liver stiffness (LS)

was expressed as the median value of ≥10 successful acquisition (kilopascal, kPa). No significant fibrosis (F0/F1) was defined as LS <6 kPa. Advanced liver fibrosis (F3) was defined as LS >9 kPa (>12 kPa for elevated ALT) and cirrhosis (F4) was defined as LS >12 kPa (≥13.5 kPa for elevated ALT). This classification was in accordance with the European Association for Study of Liver, Asociación Latinoamericana para el Estudio del Hígado clinical guidelines.[26] Patients with ALT more than 5 times the upper limit of normal will be excluded from transient elastography analysis as these elevated ALT levels may falsely increase the liver stiffness value. CAP was determined and expressed in decibel per meter (dB/m) with a linear range of 100-400 dB/m. CAP was only considered valid with an interquartile range of <40 dB/m.[27] Steatosis was categorized as mild (CAP 248-267 dB/m), moderate (CAP 268-279 dB/m) and severe (≥ 280 dB/m) according to the CAP values.[19]

Statistical analysis

Continuous variables were expressed as median (interquartile range, IQR), and categorical variables were expressed as proportions. Follow-up time was censored at the date of HCC diagnosis, all-cause mortality, or end of follow-up (December 31 2019). Statistical comparisons for continuous variables were carried out using Mann-Whitney U test or Kruskal-Wallis test as appropriate, while Chi-square test and Fisher's exact test were used to compare categorical variables. Correlation between two continuous variables was analyzed by Spearmen's correlation coefficient.

A Cox proportional hazards regression model was established to determine whether clinical factors were independently associated with HCC development. Variables with a P <0.1 in univariate analyses were entered into multivariate analysis performed by Cox regression, with hazard ratio (HR) and 95% confidence interval (CI) calculated. Kaplan-Meier survival analysis was used to compare the probability of HCC between patients with different risk factors, with differences tested for significance by using the log-rank test. Cox regression analysis was performed to evaluate the probability of HCC development. We additionally performed sensitivity analyses with logistic regression model on the effect of hepatic steatosis on HCC by categorizing the degree of hepatic steatosis and performing a number of sub-group analyses (treatment status, treatment duration and fibrosis status).

We further use propensity score (PS) matching to evaluate the independent effect of hepatic steatosis (≥248 dB/m) on the risk of HCC. Missing data were assumed to be missing at random. They were replaced with substituted values by multiple imputation with chained equations to create 20 complete data sets after the first 10 iterations. The imputed variables, in descending order of missingness, were HBV DNA (8.0%), platelet (0.9%), AST (0.5%), albumin (0.4%), bilirubin (0.4%), gender (0.2%). Imputed values were constrained within plausible ranges. Patients with and without hepatic steatosis were matched in a 1:1 ratio with caliber of 0.2. The matching variables were age, gender, LSM, platelet, HBV DNA, albumin, bilirubin, AST, NA.

A two-tailed P value of <0.05 was considered statistically significant. Multiple imputation and PS matching was performed using R software (version 4.0.4). All other statistical analysis was performed using Statistical package for Social Sciences version 20.0 (SPSS Inc, Chicago, IL, USA).

Results

Baseline characteristics

Among the 2403 recruited CHB patients (median age 55.6, 55.3% male), the follow-up duration after baseline assessment was 46.4 (interquartile range IQR: 24.7 – 51.1) months. Serum HBeAg was positive in 230 (9.6%) patients, and 1 patient achieved HBsAg seroclearance during follow-up. More than half of recruited patients were on NA treatment (57.1%) (**Table 1**). Majority (96.1%) patients had a low ALBI grade. The proportion of patients with no hepatic steatosis, mild-to-moderate steatosis, and severe steatosis was 1247 (51.9%), 450 (18.7%) and 706 (29.4%), respectively. The proportion of patients with advanced fibrosis/ cirrhosis was 371 (15.4%). More than one-quarter and half of the patients had diabetes mellitus and dyslipidaemia, respectively (**Table 1**).

HCC development

A total of 48 patients developed HCC during a median interval of 21.7 (IQR: 7.5 – 52.8) months from baseline. Majority of patients with HCC development were NA-treated (91.7%), HBeAg-negative (91.5%) and were male (75%). None of them achieved HBsAg seroclearance by the end of follow up. The median liver stiffness at recruitment was 9.3 (IQR 7.5 – 16.3) kPa, with 47.9% of them having at

least advanced fibrosis and 29.2% having cirrhosis by transient elastography criteria. Eleven patients (22.9%) had imaging features of cirrhosis on ultrasonography (including small nodular liver, splenomegaly, presence of ascites etc). The median CAP was 216 (IQR 197 – 248) dB/m, and majority of them did not have hepatic steatosis (75%). The details of histological evaluation for 22 HCC patients with surgical resection/ liver transplantation were shown in **Supplementary table 1**. Five out of 22 (22.7%) were found to have hepatic steatosis on histology around the time of HCC diagnosis, and 4/5 (80%) were detected at baseline CAP assessment with baseline CAP values being 212, 255, 277, 359 and 375 dB/m. Advanced fibrosis/ cirrhosis was found on histology in 10 patients around the time of HCC diagnosis, and 8/10 (80%) were detected at baseline liver stiffness measurement.

Risk factors for HCC development

The differences in baseline characteristics and laboratory parameters between patients developing HCC and those who did not develop HCC were shown in **Supplementary table 2**. Patients with HCC were older, with higher proportion of NA use and male, and with the following baseline parameters including lower platelet count, lower albumin, higher bilirubin, higher AST, higher ALBI score, lower proportion of detectable serum HBV DNA, higher liver stiffness, and lower CAP compared to the latter group of patients. Multivariate Cox regression analysis showed that increased age (HR 1.063, 95%CI 1.034 – 1.093), male gender (HR 2.032, 95%CI 1.015 – 4.066), higher ALBI score (HR 2.393, 95%CI 1.134 – 5.05), and reduced CAP (HR 0.994, 95%CI 0.989 – 0.999) were independent risk factors for HCC development (**Table 2**). These implied that a reduction of CAP by 10 dB/m increased the risk of HCC by 6%.

In view of the finding that a lower CAP was independently associated with HCC development, stratified analysis was performed based on the absence of steatosis, presence of mild-to-moderate steatosis, or severe steatosis. The cumulative 48-month probability of HCC was 2.88%, 1.56% and 0.71%, respectively (log rank: p=0.01) (**Figure 2**).

Patients were divided into 4 groups based on the presence of severe steatosis and advanced fibrosis/cirrhosis: group 1: no advanced fibrosis/cirrhosis + severe steatosis, group 2: advanced fibrosis/cirrhosis + severe steatosis, group 3: no advanced fibrosis/cirrhosis + no severe steatosis, group

4: advanced fibrosis/cirrhosis + no severe steatosis. The risk of HCC was highest in patients in group 4, followed by group 2, group 3 and lowest in group 1 (8.89%, 2.05%, 1.56% and 0.35%, respectively, log rank p<0.001). (**Figure 3**)

Sensitivity analysis for the whole cohort

Sensitivity analysis was performed to assess the effect of different degrees of hepatic steatosis (as categorical variables) and fibrosis on the risk of HCC development. Multivariate analyses on steatosis showed that any degree of steatosis was independently associated with lower risk of HCC (**Supplementary figure 1**). When subgroup analysis was performed for patients without advanced fibrosis/cirrhosis, CAP remained to be inversely associated with risk of HCC (OR 0.991, 95%CI 0.983 – 0.999). In contrast, when subgroup analysis was performed in patients with advanced fibrosis/cirrhosis, CAP becomes an insignificant variable (**Supplementary table 3**).

Subgroup analysis for NA-treated patients

Since only 4 treatment naïve patients (0.39%) developed HCC, the frequency was too low to evaluate for statistical significance. Subgroup analysis of NA-treated patients was therefore performed. For NA-treated patients, after excluding 23 patients started on NA after baseline assessment, a total of 44 patients (3.3%) developed HCC. Majority of patients had undetectable serum HBV DNA (85.6%). Univariate analysis showed that older age, lower platelet count, lower albumin, higher bilirubin, higher AST, higher ALBI score, lower CAP and higher liver stiffness were associated with HCC development (**Supplementary table 4**). Multivariate Cox regression analysis showed that increased age (HR 1.059, 95%CI 1.029 – 1.09), increased ALBI score (HR 2.91, 95%CI 1.425 – 5.942) and reduced CAP (HR 0.993, 95%CI 0.987 – 0.999) were independent risk factors for HCC development (**Supplementary table 5**). These implied that a reduction of CAP by 10 dB/m increased the risk of HCC by 7%.

We additionally performed stratified analysis of NA-treated patients based on the absence of steatosis, presence of mild-to-moderate steatosis, or severe steatosis. The cumulative probability of HCC was 4.45%, 3% and 1.07%, respectively (log rank: p=0.025) (**Supplementary figure 2**).

Sensitivity analysis for NA-treated patients

Sensitivity analysis was performed to assess the effect of different degrees of hepatic steatosis (as categorical variables) and fibrosis on the risk of HCC development in NA-treated patients. Multivariate analyses showed that any degree of steatosis was independently associated with lower risk of HCC (**Supplementary figure 3 & Supplementary table 6**). Further subgroup analysis was performed based on the presence of advanced fibrosis/ cirrhosis, choice of NA and duration of NA. A reduced CAP was independently associated with HCC in patients without advanced fibrosis/ cirrhosis (OR 0.987, 95%CI 0.978 – 0.996) and in those receiving ≥3 years of NA (OR 0.992, 95%CI 0.985 – 1) (**Supplementary table 6**).

Since both CAP was independent variable for HCC development, patients were divided into 4 groups in a similar manner as stated in the previous section. The risk of HCC was highest in patients in group 4 (i.e. advanced fibrosis/cirrhosis + no severe steatosis), followed by group 2, group 3 and lowest in group 1 (11.2%, 3.13%, 2.52% and 0.36%, respectively, log rank p<0.001) (**Figure 4**).

Propensity score matching

957 pairs of CHB patients were identified after matching for age, gender, LSM, platelet, HBV DNA, albumin, bilirubin, AST, NA. After PS matching, the absolute standardized difference of all matching variables were <0.1, which indicates good balance (**Supplementary table 7**). The HR of HCC with the presence of hepatic steatosis was 0.41 (95% CI 0.21-0.83).

Discussion

In the current study, the risk of incident HCC in 2403 CHB patients was significantly increased with decreasing amount of hepatic steatosis and increasing burden of fibrosis. Every 10 dB/m decrease in CAP was associated with 6% increase in HCC risk. The 4-year cumulative HCC risk was 2.88%, 1.56% and 0.71% for patients with no steatosis, mild-to-moderate steatosis, and severe steatosis, respectively. The other independent risk factors for HCC in this study included older age, male gender, and higher ALBI score, which are well-reported and consistent with the literature. This prospective study involved a large cohort of CHB patients, and has been adjusted for the underlying metabolic risk factors including

obesity, central obesity, hypertension and diabetes mellitus. The use of transient elastography to quantify hepatic steatosis and liver fibrosis aided the demonstration of the significant inverse relationship between steatosis and HCC development, which was synergistic with liver fibrosis.

Animal studies showed that viral antigen expression and HBV DNA levels were decreased in mice with concomitant NAFLD and HBV compared to HBV alone.[28, 29] For clinical studies, our group previously showed that HBV viral load was inversely associated with hepatic steatosis,[11] and suggests a possible inhibitory effect of hepatic steatosis on HBV viral replication. Similarly, in another paper, we found that hepatic steatosis was associated with lower quantitative HBsAg levels and higher chance of HBsAg seroclearance, although severe hepatic steatosis was associated with advanced fibrosis or fibrosis progression.[14, 30] In the current study, advanced fibrosis/ cirrhosis is found to synergistically agonize the risk of HCC in patients without severe steatosis (i.e. group 4 - see text in the Results section), which suggests that there is complex interaction between hepatic steatosis and fibrosis in CHB patients. The correlation between CAP and HBV DNA was -0.065 (p=0.002). In patients with CAP ≥248 dB/m (i.e. any degree of hepatic steatosis), the proportion of serum HBV DNA detectability was 540/1156 (46.1%) vs. 564/1247 (45.2%) for patients without hepatic steatosis; p=0.486. For the lower HBV DNA load in patients with HCC, it is likely to be due to the fact that 91.7% of them were on antiviral therapy compared to 56.4% for those without HCC (**Supplementary table 2**). For patients on NA, the proportion of HBV DNA detectability was 217/1372 (15.8%) vs 887/1031 (86%) for patients not on NA (p<0.001). Taken together, the very weak negative association between CAP and HBV DNA could not fully explain the apparent protective effect of hepatic steatosis on HCC. However, serum HBV DNA is just one of the many viral biomarkers that could be evaluated, and it remains unknown whether the presence of hepatic steatosis inhibits other HBV-related activities including upstream transcriptional activity and DNA integration into the host genome. It is possible that in NA-treated patients with suppressed reverse transcriptase activity, the residual viral replication (including transcription, translation and eventually production of oncogenic proteins) is inhibited by the presence of hepatic steatosis. The exact step of viral replication affected by hepatic steatosis is unknown. In a study involving histological assessment of patients having concomitant CHB and NAFLD, viral antigens staining for HBsAg and HBV core antigen (HBcAg) in hepatocytes were lower compared to patients with CHB alone.[31] Theoretically, translation for viral proteins or any upstream steps of viral lifecycle could be affected by hepatic steatosis, leading

to reduced production of oncogenic products as well as DNA integration (**Supplementary Figure 4**). This hypothesis would need further mechanistic studies to explore.

On the other hand, it is well known that patients with burnt-out non-alcoholic steatohepatitis (NASH) do not have excess hepatic fat any more.[32, 33] Viewing from this perspective, reduction in CAP might signify building up of liver fibrosis, i.e. 'burnt-out NASH' which provides another explanation for the negative association between hepatic fat and HCC development observed in the current study (**Supplementary Figure 4**). The relative contribution of HBV and NAFLD on HCC risk is not clear. It is well reported that in South-East Asia, i.e. HBV-endemic areas, perinatal infection or horizonal infection early in childhood are the main routes of HBV transmission.[34, 35 36] Therefore, the age of the patient at recruitment likely represents the duration of CHB. For NAFLD, the natural history is not entirely clear in Chinese and is presumed to parallel the onset of metabolic syndrome that usually sets in during adulthood[37] and the prevalence of which increases with age.[38] As the prevalence of metabolic syndrome among Chinese adolescents is as low as 2.4%,[39] it is reasonable to assume that the age of onset of NAFLD is during adulthood. In a study conducted by our group, the prevalence of NAFLD in those aged ≤25, 26-35, 36-45, 46-55, and >55 was 14.7%, 29%, 43.5%, 50.4% and 58%, respectively.[40] The rate of disease progression of NAFLD is slow, i.e. annual fibrosis progression rate was 0.07 stages for NAFL and 0.14 stages for NASH, translating into 1 stage per 14 years for NAFLD and 1 stage per 7 years for NASH.[41] Judging from these observations, HBV is likely the major contributor to the risk of HCC development in terms of cumulative damage to the liver.

Although liver biopsy was not performed for most patients, liver histology obtained at hepatic resection or liver transplantation around the time of HCC from 22 patients was studied, which showed that 5/22 (22.7%) and 10/22 (45.4%) patients had hepatic steatosis and advanced fibrosis/ cirrhosis, respectively, on liver histology. This highlighted that hepatic steatosis was retained in these patients even when HCC was formed. Moreover, the sensitivity of detection of hepatic steatosis and advanced fibrosis/ cirrhosis by transient elastography technique was 80% and 70%, respectively. It has been reported that liver stiffness could be influenced by many factors, including ALT,[42] CAP,[43] cholestasis,[44] hepatic congestion,[45, 46] and probe type.[47] We minimized the confounding effect of ALT by adopting a different liver stiffness cut-off as per recommendation of the EASL-ALEH guidelines and using appropriate

probes for patients with different BMI range (see Methods section). Moreover, the majority of patients in this study had BMI <30 and therefore M probe was used (**Table 1**). While one report (n=82) mentioned that CAP could be influenced by significant fibrosis in patients with NAFLD,[48] a bigger study involving 450 patients with NAFLD showed that probe type and steatosis did not affect LSM.[49] Although residual confounding between LSM and CAP could not be excluded, the independent effect of hepatic steatosis on HCC has been further elucidated by PSM analysis. After matching of age, gender, liver stiffness, platelet, HBV DNA, albumin, bilirubin, AST as continuous variables and antiviral treatment, hepatic steatosis was independent associated with reduced risk of HCC, with a hazard ratio of 0.41 (95% CI 0.21-0.83). This means that even with the same amount of liver fibrosis (as reflected by liver stiffness that is matched by PS) and other similar clinical parameters, the presence of hepatic steatosis is associated with almost 60% reduction in HCC risk.

There are two limitations of our study. Firstly, liver biopsies were not done for most patients to assess the histological steatosis, NASH activity and actual fibrosis stage. However, liver biopsy is an invasive procedure, and is not feasible to be performed for a large cohort of patients, vast majority being stable and asymptomatic, due to the associated risks of the procedure. Transient elastography demonstrates excellent performance in diagnosing advanced fibrosis and hepatic steatosis with high accuracy with references to histological findings,[50-52] which was similarly observed in the histology in 22 HCC patients around the time of HCC diagnosis. In addition, information on genotypes, known viral mutations (e.g. core promoter mutations)[53] and family history of HCC were not available in the current study.

In conclusion, our study found that decreasing quantity of hepatic steatosis, as measured by CAP, and increasing burden of liver fibrosis, as measured by liver stiffness, were significantly and independently associated with a higher risk of incident HCC among CHB patients. Our present study findings highlight the importance of routine liver stiffness and CAP measurements in the risk stratification and monitoring of CHB patients.

Declarations

Funding: This study was supported by the General Research Fund, Research Grants Council, The Government of the Hong Kong Special Administrative Region (ref no: 17125916); Innovative Research Fund of the State Key Laboratory for Liver Research, The University of Hong Kong (Ref no: SKLLR/IRF/2018/08); and the Outstanding Young Researcher Award, The University of Hong Kong.

Conflict of interest: J Fung has been a consultant for Gilead Sciences. MF Yuen received speaker fees and received research funding from Bristol-Myers Squibb and Gilead Sciences. WK Seto received speaker fees from Mylan and is an advisory board member, received speaker fees and research funding from Gilead Sciences. All other authors: none to declare.

Ethics approval: The present study was approved by the Institutional Review Board/ Ethics Committee of the University of Hong Kong and the Hong Kong West Cluster of Hospital Authority.

Consent for publication: Written informed consent was obtained from all study subjects prior to any study-related procedures.

Availability of data and material: data available within the article or its supplementary materials

Code availability: not applicable

Author contributions: The authors declare they have participated in the preparation of the manuscript and have seen and approved the final version. LY Mak was involved in data acquisition, data analysis and interpretation, and drafting of manuscript. Rex WH Hui and KS Cheung were involved in data acquisition and analysis. F Liu and DKH Wong were involved in data acquisition. BL was responsible for analysis of data. J Fung and MF Yuen was involved in critical revision of manuscript. WK Seto was involved in study concept and design, analysis and interpretation of data, critical revision of manuscript and overall study supervision.

Acknowledgement: The interim results of this study was presented in the Digital Liver Meeting 2020 as a poster (Hepatology 2020; volume 72, issue number 1 supplementary, P.658A; poster number 1084). The authors would like to thank Ms. Carmen Chan, Ms. Carol Chu and Mr. Justin Ma for the logistical arrangement of patients.

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Tables

Table 1. Baseline characteristics of all patients (N=2403)

	Median/ frequency	Interquartile range
Age (years)	55.6	46.7 – 62.9
Gender (male)	1336 (55.6%)	-
Follow-up duration (months)	46.4	24.4 – 51.1
Body height (cm)	163	157 – 170
Body weight (kg)	64.7	56.2 – 73
Body mass index (kg/m^2)	24.0	21.7 – 26.9
Body mass index ≥25 kg/m^2 (yes)	945 (39.3%)	-
Body mass index ≥30 kg/m^2 (yes)	225 (9.4%)	-
Waist circumference (cm)	87	79 – 94
Hip circumference (cm)	96	92 – 101
Systolic blood pressure (mmHg)	133	121 – 147
Diastolic blood pressure (mmHg)	79	72 – 87
Presence of diabetes mellitus (yes)	657/2277 (28.9%)	-
Glycated hemoglobin (%)	5.7	5.3 – 6.4
Presence of dyslipidaemia (yes)	1275/2393 (53.3%)	-
Platelet count (x100/L)	208	165 – 248
Albumin (gram/L)	45	43 – 47
Bilirubin (umol/L)	10	7 – 13
Alanine aminotransferase (U/L)	26	19 – 36
Aspartate aminotransferase (U/L)	26	21 – 32
ALBI score*	-3.18	-3.34 to -3.02
ALBI grade		
1	2301/2394	96.1%
2	91/2394	3.8%
3	2/2394	0.1%
HBV DNA positivity (>20 IU/mL) (Yes)	1104/2403 (45.9%)	
HBeAg positivity (yes)	230 (9.6%)	-
On nucleos(t)ide analogue therapy (yes)	1372 (57.1%)	-
Controlled attenuation parameter (dB/m)	246	206 – 290
Proportion of severe steatosis	706 (29.4%)	-
Liver stiffness (kPa)	5.6	4.0 – 7.8
Proportion of advanced fibrosis/ cirrhosis	371 (15.4%)	-

ALBI: Albumin-Bilirubin, HBeAg: hepatitis B e antigen, HBV: hepatitis B virus
Serum HBV DNA lower limit of detection 20 IU/mL (1.3 log IU/mL)
*data missing in 9 patients

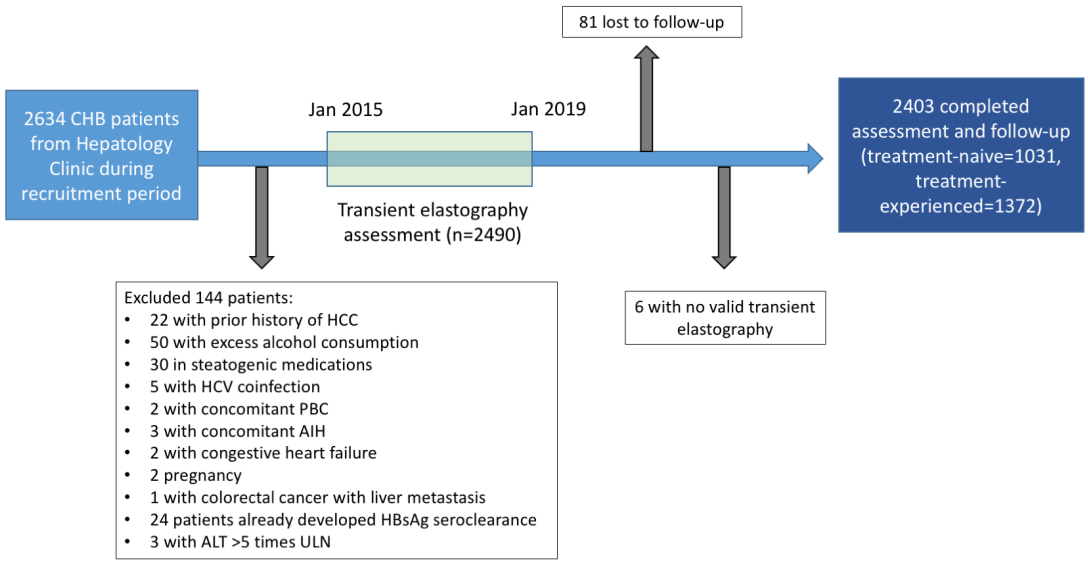
Table 2. Multivariate Cox regression analysis of risk factors for HCC development in all patients

	Hazard ratio	95% confidence interval	P value
Age (per year)	1.063	1.034 – 1.093	<0.001
Male gender (yes)	2.032	1.015 – 4.066	0.045
Platelet count (per 1 x 10^9/L)	0.996	0.991 – 1.002	0.159
Aspartate aminotransferase (per U/L)	1.007	0.998 – 1.017	0.132
ALBI score (per 1 score)	2.393	1.134 – 5.05	0.022
Serum HBV DNA (per log IU/mL)	0.86	0.516 – 1.433	0.562
Nucleos(t)ide analogue (yes)	3.659	0.827 – 16.187	0.087
Controlled attenuation parameter (per dB/m)	0.994	0.989 – 0.999	0.035
Liver stiffness (per kPa)	1.018	0.989 – 1.048	0.217
ALBI: Albumin-Bilirubin, HBV: hepatitis B virus			

Associated univariate analysis presented in Supplementary Table 2.
(Albumin and bilirubin were incorporated in ALBI score)

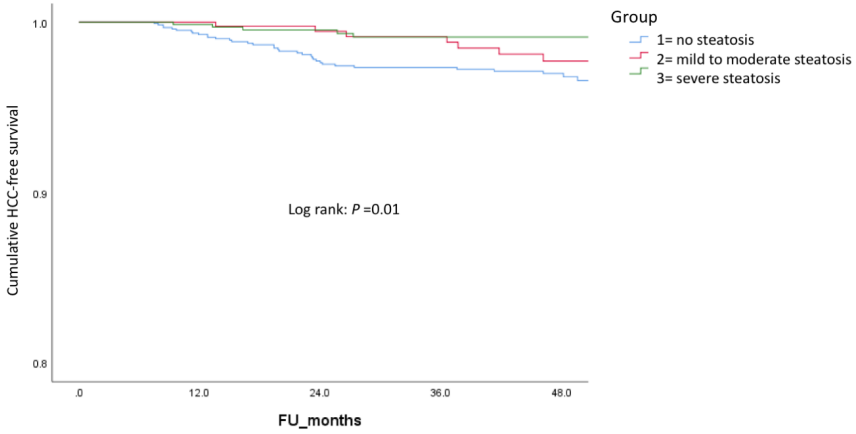
Figures legends

Figure 1. Patient disposition



AIH: autoimmune hepatitis, ALT: alanine aminotransferase, CHB: chronic hepatitis B, HBsAg: hepatitis B surface antigen, HCC: hepatocellular carcinoma, HCV: hepatitis C virus, PBC: primary biliary cholangitis, ULN: upper limit of normal

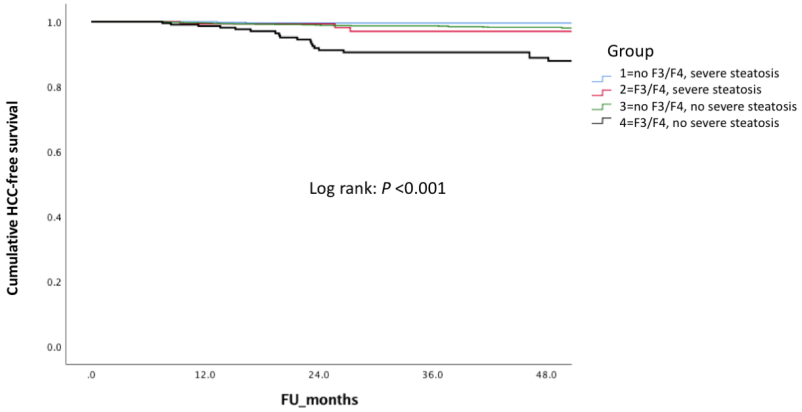
Figure 2. Cumulative HCC-free survival stratified by the severity of hepatic steatosis in all patients



Number at risk	0 months	12 months	24 months	36 months	48 months	Cumulative probability of HCC
Group 1	1248	1217	1019	952	519	2.88%
Group 2	450	445	328	299	161	1.56%
Group 3	705	700	490	417	222	0.71%

HCC: hepatocellular carcinoma

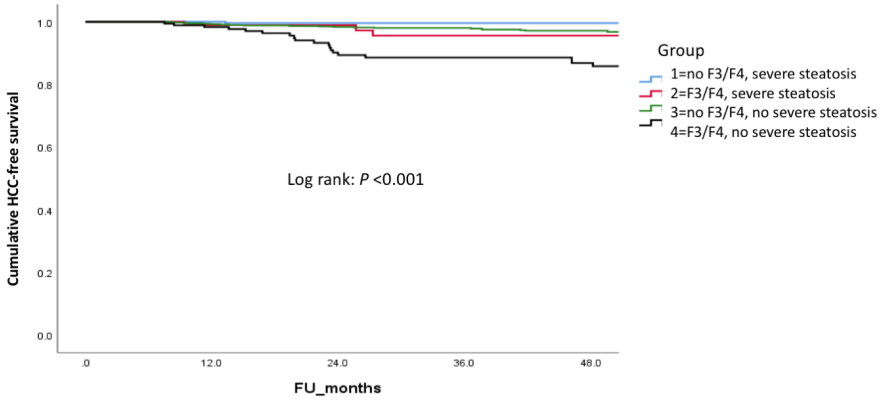
Figure 3. Cumulative HCC-free survival stratified by the severity of hepatic steatosis and liver fibrosis in all patients



Number at risk	0 months	12 months	24 months	36 months	48 months	Cumulative probability of HCC
Group 1	560	555	401	338	169	0.35%
Group 2	146	143	87	77	51	2.05%
Group 3	1472	1445	1208	1130	585	1.56%
Group 4	225	217	139	121	94	8.89%

F3/F4: advanced fibrosis/cirrhosis, HCC: hepatocellular carcinoma

Figure 4. Cumulative HCC-free survival stratified by the severity of hepatic steatosis and liver fibrosis in NA-treated patients



Number at risk	0 months	12 months	24 months	36 months	48 months	Cumulative probability of HCC
Group 1	277	269	187	163	91	0.36%
Group 2	96	92	59	55	41	3.13%
Group 3	797	774	623	576	310	2.52%
Group 4	179	173	115	106	85	11.2%

F3/F4: advanced fibrosis/cirrhosis, HCC: hepatocellular carcinoma