Statins associate with better clinical outcomes in chronic hepatitis B patients with HBsAg seroclearance

Short title: statins use after HBsAg-seroclearance

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Abstract

Introduction: We aimed to describe long-term clinical outcomes in chronic hepatitis B (CHB) patients after HBsAg seroclearance, and identify factors that modify disease outcomes.

Methods: CHB patients with HBsAg seroclearance occurring between 1986 and 2017 were recruited. Primary outcome was cirrhosis/hepatocellular carcinoma (HCC), and secondary outcomes were hepatic decompensation, liver-related death/transplantation, and all-cause mortality. Multivariable Cox model included demographics, prior antivirals, comorbidities, drugs (statins, metformin, proton-pump inhibitors, non-selective beta-blockers), and laboratory parameters (platelet, liver function test, prothrombin time, alpha-fetoprotein [AFP], anti-HBs). Statin users were propensity score matched (PSM) with non-users (1:2 ratio) for survival analysis of all outcomes.

Results: Of 913 patients with HBsAg seroclearance (male:613[67.1%]; median age:53.4 years [range:18.5–87.0]), 129 (14.1%) were statin users. During median follow-up of 7.7 years (up to 29.1 years), 64/833 (7.7%) developed cirrhosis, 25/905 (2.8%) developed HCC, 3/913 (0.3%) underwent transplantation, and 76/913 (8.3%) died. Statins associated with lower cirrhosis/HCC risk (adjusted hazard ratio [aHR]:0.44;95% CI:0.20–0.96; aHR for every one-year increase in use:0.85;95% CI:0.75–0.97). Statin users had no hepatic decompensation or liver-related death/transplantation (vs 18/778 [2.3%] and 18/784 [2.3%] cases in statin non-users, respectively). Statins also associated with lower all-cause mortality risk (aHR:0.21;95% CI:0.08–0.53). PSM yields consistent results for beneficial effects of statins (log-rank $p<0.05$ for all outcomes). Other factors for cirrhosis/HCC included increasing age (aHR:1.06), diabetes (aHR:2.03), higher creatinine (aHR:1.008), GGT>50U/L (aHR:3.25) and AFP>9ng/ml (aHR:10.14).

Conclusion: Patients with HBsAg seroclearance have favorable long-term survival. However, liver-related adverse outcomes still develop, necessitating further investigations on beneficial effects of statins.

Introduction

Chronic hepatitis B (CHB) infection affects 292 million people worldwide.[1] It can lead to hepatitis flare, cirrhosis,[2] hepatocellular carcinoma (HCC),[3-5] hepatic decompensation, and mortality. Even in quiescent phase, hepatitis B virus (HBV) reactivation triggered by immunosuppressive use could result in life-threatening liver failure.[6]

Hepatitis B surface antigen (HBsAg) seroclearance is a clinical event with an annual incidence of 1.2%,[7] occuring either spontaneously or during treatment with nucleos(t)ide analogues (NAs) or interferon.[8-10] Time-dependent decline in liver stiffness was demonstrated after HBsAg seroclearance. It is an important milestone for CHB infection and currently regarded as functional cure of CHB infection, as it is associated with better prognosis (lower cirrhosis and HCC risk) compared with HBsAg-positive patients.[11-13]

However, HBsAg seroclearance does not imply elimination of adverse outcomes. Studies investigating risk factors for adverse outcomes after HBsAg seroclearance are sparse. While anti-HBs positivity is a protective factor against HBV reactivation in occult HBV carriers receiving rituximab,[14] its prognostic significance after HBsAg seroclearance is currently unknown. In addition, modifiable risk factors including pharmacological agents and cardiometabolic risk factors, were under-reported. Identification of factors for adverse outcomes after HBsAg seroclearance and drugs that modify disease course helps risk stratification and streamline management in ters of follow-up and HCC surveillance interval.

In this context, we aimed to conduct a large cohort study to 1)describe clinical course of CHB patients after a prolonged period of HBsAg seroclearance, and 2)identify factors for adverse events, in particular the role of statins on modifying disease course.

Methods

Study design and patient recruitment

This was a retrospective cohort study with longitudinal follow-up of consecutive CHB patients (HBsAg-positivity ≥6 months) who achieved HBsAg seroclearance. CHB patients aged ≥18 years, who followed up at Hepatology Clinic of Queen Mary Hospital between 27 October 1986 and 30 June 2017, with documented HBsAg seroclearance, defined as HBsAg negativity on repeat testing (Abbott Laboratories, Chicago, IL) for ≥6 months and during subsequent follow-ups, were identified. Exclusion criteria were non-Asians, chronic hepatitis C infection, autoimmune hepatitis, primary biliary cholangitis and Wilson's disease.

Ethics statement

The study protocol was approved by Institutional Review Board, University of Hong Kong and West Cluster of Hospital Authority, Hong Kong (UW 19-818).

Outcomes of interest

The primary outcome was cirrhosis (definition to be provided in subsequent section) and/or HCC development. Patients were observed from HBsAg seroclearance to development of cirrhosis or HCC (whichever occurred first), or censored at last clinic follow-up, liver transplantation or death.

The secondary outcomes were 1)hepatic decompensation, 2)composite outcome of liver-related death or liver transplantation, and 3)all-cause mortality. Hepatic decompensation was defined by occurrence of ascites, variceal bleeding, hepatic encephalopathy, or hepatorenal syndrome. Liver-related death was defined as death

related to adverse liver outcomes including spontaneous bacterial peritonitis (SBP), variceal bleeding, hepatic encephalopathy, hepatorenal syndrome and HCC.

Laboratory monitoring, diagnosis of cirrhosis and surveillance for hepatocellular carcinoma

Clinical characteristics included age at HBsAg seroclearance, sex, spontaneous or on-treatment HBsAg seroclearance status, diabetes mellitus (DM), other comorbidities including cardiovascular diseases (defined by the presence of ischemic heart disease, stroke, use of aspirin and clopidogrel), smoking, alcoholism, and concomitant medications (statins,[15] metformin,[16] proton-pump inhibitors,[17] and non-selective beta-blockers [NSSBs][18]). Chronic obstructive pulmonary disease (COPD) and alcohol-related diseases (gastrointestinal, hepatic, cardiac and neurological) were used as proxies for heavy smoking and alcoholism as smoking and drinking history were not documented in some patients. Available statins included simvastatin, atorvastatin and rosuvastatin, while NSSBs included propranolol, nadolol and carvedilol. Drug exposure was defined as ≥30-day use after HBsAg seroclearance.

Laboratory parameters included complete blood count, liver function test (albumin, bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], gamma-glutamyl transferase [GGT]), creatinine, prothrombin time (PT), alpha-fetoprotein (AFP) and anti-HBs positivity (lower limit of detection:10mIU/mL). As 240 (26.3%) of the cohort did not undergo ultrasonography (USG) and none had data of controlled attenuation parameter from transient elastography at or before HBsAg seroclearance, raised GGT >50 U/L was used as a surrogate marker of hepatic steatosis.

Follow-up, surveillance of hepatocellular carcinoma and diagnosis of adverse outcomes

Patients were followed up every 6-12 months with regular blood tests. They were advised for USG of liver at 6-monthly intervals. HCC was diagnosed by histology or imaging (triphasic computed tomography [CT] scan or magnetic resonance imaging [MRI]).

Cirrhosis was diagnosed by (1)imaging (USG/CT/MRI showing small liver, irregular liver outline, or features of portal hypertension–splenomegaly/varices/ascites), (2)transient elastography (liver stiffness [LS] >12 kilopascals [kPa] with normal ALT, or >13.4kPa if ALT 1-5 x upper limit of normal[19]), (3)liver biopsy, or (4)clinical features (thrombocytopenia, coagulopathy, ascites, SBP, hepatic hydrothorax, gastroesophageal varices, hepatic encephalopathy). LS was measured by Fibroscan (Echosens®, Paris, France) using M and XL probe for patients with body mass index (BMI)<30kg/m^2 and ≥30kg/m^2, respectively. Cirrhotic patients were offered endoscopy to screen for gastroesophageal varices.

Statistical analysis

All statistical analyses were performed using R version 3.2.3 (R Foundation for Statistical Computing) statistical software. Continuous variables were expressed as median and interquartile range (IQR). Mann-Whitney U-test was used to compare continuous variables of two groups. Chi-square test or Fisher's exact test when appropriate, was applied for comparing categorical variables. Wilcoxon signed rank test was used to compare the median values of liver function parameters at baseline and five years after HBsAg seroclearance among statin users.

Multivariable Cox proportional hazards model was used to derive adjusted hazard ratio (aHR) of primary and secondary outcomes. To deal with missing data, multiple imputation was used to construct 50 complete datasets by imputing missing variables.[20] All variables were included into multivariable analysis due to negative confounding.[21] For example, statin users tend to have more cardiometabolic risk factors/diseases which are risk factors for cirrhosis progression. Without adjustment for cardiometabolic risk factors/diseases (i.e. negative confounding factor), potential beneficial effect of statins may be biased towards null or even opposite direction.

Subgroup analysis for the primary outcome was performed according to anti-HBs positivity and duration of HBsAg seroclearance (<5 vs ≥5 years).

Sensitivity analysis was performed by propensity score matching (PSM) to control for confounding. PS was estimated by multivariable logistic regression based on aforementioned covariates.[21] In the current study, statin users were matched to non-statin users in a 1:2 ratio without replacement using a greedy distance-based matching algorithm with the logit of the PS within 0.1 standard deviation. Balance of covariates between the two groups was assessed by absolute standardized difference (ASD), with value of <0.20 indicating good balance. This was achieved for statin users and non-users (data not shown).

As a statistically significant association was found between outcomes and statins, duration-response relationship was elicited to strengthen causality. Duration of statin use was categorized into three groups: i)<180days, ii)≥180 to <3years, and iii)≥3years.

For survival analysis, Kaplan-Meier method was used to analyze adverse outcomes with log-rank test. As Kaplan-Meier method did not control for confounding, we used PS matched cohort to ensure balance in characteristics between statin users and non-users.

A two-sided p-value of <0.05 was used to define statistical significance.

Results

Patient Characteristics

A total of 913 patients with HBsAg seroclearance between 1986 and 2017 were identified**.** Patient recruitment process is depicted in **Supplementary figure 1**. Clinical characteristics and laboratory parameters are shown in **Table 1**. There were 613 (67.1%) males, and median age at HBsAg seroclearance was 53.4years (IQR:45.2–60.9;range:18.5–87.0). Among them, 818 (89.6%) were treatment naïve, while 95 (10.4%) had prior antiviral treatment (interferon:3, lamivudine:29, adefovir:2, telbivudine:3, entecavir:53, tenofovir:5); 513 (56.2%) developed anti-HBs at a median duration of 1.0year (IQR:0–3.0).

The median follow-up duration of the whole cohort was 7.7years (IQR:4.5–13.2; up to 29.1). The median follow-up duration in statin users and statin non-users was 7.9 years (IQR: 4.5–12.0) and 7.6 years (IQR: 4.5–13.2), respectively. Before HBsAg seroclearance, 79 (8.7%) had cirrhosis (eight decompensated cases) and eight (0.9%) had HCC. After patients with baseline cirrhosis were excluded, 64 of 833 (7.7%) developed cirrhosis after HBsAg seroclearance at a median of 6.6years (IQR:2.0–11.7) (**figure 1a**). After those with prior HCC were excluded, 25 of 905 (2.8%) developed HCC at a median of 6.6years (IQR:2.8–9.8;range:0.6–21.0) (**figure 1b**). Eighteen of 64 (28.1%) with cirrhosis development progressed to develop hepatic decompensation (median:6.2years;IQR:3.2–11.5). Three (0.3%) underwent liver transplantation, and 76 (8.3%) patients died (liver-related deaths:17; non-liver-related deaths:59) (**figures 1c and 1d**).

Before HBsAg seroclearance, six had ascites (two had SBP), 13 had gastroesophageal varices (two had bleeding), and two had hepatic encephalopathy. After HBsAg seroclearance, 12 of 907 (1.3%) without baseline ascites developed ascites (median:6.5years;IQR:2.7–12.9), 15 of 900 (1.7%) developed gastroesophageal varices (7 [46.7%] had bleeding) at a median of 4.4years (IQR:2.3–8.8), and 3 of 911 (0.3%) developed hepatic encephalopathy (median:3.5years;IQR:2.2–4.1).

There were 129 (14.1%) patients used statins (median duration:2.8 years;IQR:1.0–5.0), 36 (3.9%) used NSSBs (median:1.8 years;IQR:0.2–5.2), 66 (7.2%) used metformin (median:2.6 years;IQR:0.8–5.4), and 109 (11.9%) used PPIs (median:0.3 years;IQR:0.04–2.1). Statin users were older (61.2 vs 52.1 years;p<0.001) with a higher proportion having DM (47.7% vs 20.6%;p<0.001), cardiovascular diseases (53.5% vs 4.8%;p<0.001), and being smokers (4.7% vs 0.9%;p=0.005**)** (**Table 1**). Statin users had a higher median level of baseline creatinine (85 vs 81umol/L;p=0.005), ALT (23 vs 20U/L;p=0.002), AST (23 vs 22U/L;p=0.032), ALP (65 vs 62U/L;p=0.024), and GGT level (27 vs 22U/L;p<0.001). A higher proportion of statin users received NSSBs (7.8% vs 3.3%;p=0.016), metformin (31.0% vs 3.3%;p<0.001), and PPIs (37.2% vs 7.8%;p<0.001).

Factors associated with cirrhosis/hepatocellular carcinoma after HBsAg seroclearance (primary outcome)

Table 2 shows independent risk factors for cirrhosis/HCC when patients without baseline cirrhosis or prior HCC at HBsAg seroclearance were analyzed (n=833). These include increasing age (aHR:1.06;95% CI:1.03–1.09), DM (aHR:2.03;95% CI:1.17–3.51), higher creatinine level (aHR:1.008;95% CI:1.005–1.012),

GGT>50U/L (aHR:3.25;95% CI:1.77–5.97), and AFP>9ng/ml (aHR:10.14;95% CI:3.33–30.94). The aHR was 2.07 (95% CI:1.15–3.72;p=0.015) by comparing age ≥50 vs <50years.

Statins were associated with lower cirrhosis/HCC risk (aHR:0.44;95% CI:0.20–0.96) (**Table 2**). PSM yields consistent result (aHR:0.30;95% CI:0.10–0.86) (**Table 3**). For every one-year increase in statin use, aHR of cirrhosis/HCC was 0.85 (95% CI:0.75–0.97;p=0.018). Compared with statin use for <180days, aHR was 1.36 (95% CI:0.53–3.47;p=0.517) for statin use ≥180days but <3years, and 0.36 (95% CI:0.14–0.93;p=0.034) for statin use ≥3years.

Effects of statins on secondary outcomes after HBsAg seroclearance

Table 3 shows effects of statins on hepatic decompensation, liver-related mortality/liver transplantation, and all-cause mortality. There were no cases of hepatic decompensation or liver-related death/liver transplantation in statins users compared with 18 of 778 (2.3%) and 18 of 784 (2.3%) cases in statin non-users, respectively.

Statins were associated with lower risk all-cause mortality risk (aHR:0.21;95% CI:0.08–0.53). PSM yields consistent result (aHR:0.33;95% CI:0.13–0.87) (**Table 3**). For every one-year increase in statin use, aHR of death was 0.80 (95% CI:0.69–0.92;p=0.002). Compared with statin use for <180days, aHR was 0.31 (95% CI:0.08–1.16;p=0.082) for statin use ≥180days but <3years, and 0.26 (95% CI:0.08–0.84;p=0.025) for statin use ≥3years.

Risk factors associated with secondary outcomes after HBsAg seroclearance

Supplementary Table 1 shows independent factors for hepatic decompensation. These included increasing age (aHR:1.13;95% CI:1.07–1.19), male sex (aHR:5.47;95% CI:1.14–26.30), baseline cirrhosis (aHR:13.21;95% CI:4.82–36.25), thrombocytopenia (aHR:2.79;95% CI:1.05–7.42), higher creatinine level (aHR:1.014;95% CI:1.002–1.026), bilirubin>17umol/L (aHR:6.91;95% CI:2.44–19.53), ALP>110U/L (aHR:5.23;95% CI:1.60–17.05), GGT>50U/L (aHR:4.87;95% CI:1.84–12.88), and metformin (aHR:8.25;95% CI:2.62–25.95). The aHR was 16.34 (95% CI: 2.08–128.19;p=0.008) by comparing age ≥50 vs <50years.

Table 4 shows independent factors for liver-related death/liver transplantation. These included increasing age (aHR:1.10;95% CI:1.05–1.16), male sex (aHR:7.45;95% CI:2.08–26.63), baseline cirrhosis (aHR:13.58;95% CI:4.77–38.64), thrombocytopenia (aHR:3.46;95% CI:1.29–9.30), higher creatinine level (aHR:1.007;95% CI:1.005–1.010), longer PT (aHR:1.26;95% CI:1.09–1.45), NSSBs (aHR:3.11;95% CI:1.18–8.18), metformin (aHR:5.93;95% CI:1.64–21.37) and PPIs (aHR:5.38;95% CI: 2.07–13.97). The aHR was 2.60 (95% CI:0.71–9.54;p=0.150) by comparing age ≥50 vs <50years.

Supplementary Table 2 shows independent factors for all-cause mortality. These included increasing age (aHR:1.10;95% CI:1.07–1.13), antiviral treatment (aHR:2.69;95% CI:1.08–6.70), baseline cirrhosis (aHR:4.03;95% CI:2.13–7.61), cardiovascular diseases (aHR:2.13;95% CI:1.12–4.06), higher creatinine level (aHR:1.004;95% CI:1.001–1.008), ALP>110U/L (aHR:3.57;95% CI:1.21–10.52). The aHR was 4.80 (95% CI:2.29–10.07;p<0.001) by comparing age ≥50 vs <50years.

Serial changes in liver function parameters among statin users

Supplementary Table 3 shows the serial changes in liver function parameters at baseline and five years after HBsAg seroclearance among statin users. There was statistically significant difference in the median values of albumin (43 vs 44), AST (23 vs 25), ALP (65 vs 69) and INR (1.0 vs 1.0), but no statistically significant difference in the median values of bilirubin, ALT, and GGT.

Subgroup analysis

Among patients with positive baseline anti-HBs, aHR of cirrhosis/HCC with statin use was 0.96 (95% CI:0.33–2.83). Among patients with negative baseline anti-HBs, aHR was 0.29 (95% CI:0.07–1.12) (**Supplementary Table 4**).

Among patients with duration of HBsAg seroclearance of <5years, aHR of cirrhosis/HCC with statin use was 0.17 (95% CI:0.04–0.84). Among patients with duration of HBsAg seroclearance of ≥5years, aHR was 0.45 (95% CI:0.15–1.34) (**Supplementary Table 4**).

Survival analysis

Cirrhosis and/or HCC

For whole cohort, overall 5-year, 10-year, 15-year and 20-year probability of cirrhosis/HCC-free survival was 95.7% (95% CI:94.2–97.1%), 90.4% (95% CI:87.9–93.0%), 86.7% (95% CI: 83.2–90.3%), and 83.2% (95% CI:78.5–88.1%), respectively. In PS matched cohort, statin users had better cirrhosis/HCC-free survival (log rank p=0.020) (**figure 2a**)

Hepatic decompensation

Overall 5-year, 10-year, 15-year and 20-year probability of hepatic decompensation-free survival was 99.0% (95% CI:98.3–99.7%), 98.0% (95% CI:96.9–99.1%), 96.8% (95% CI:95.0–98.5%), and 94.7% (95% CI:91.2–98.2%), respectively. Among statin users, there no events of hepatic decompensation. In PS matched cohort, statin users had better decompensation-free survival (log rank p=0.020) (**figure 2b**).

Liver-related death and/or liver transplantation

Overall 5-year, 10-year, 15-year and 20-year probability of liver-related death/transplantation-free survival was 99.0% (95% CI:98.3–99.7%), 97.9% (95% CI:96.8–99.1%), 97.0% (95% CI:95.3–98.8%), and 94.1% (95% CI:89.8–98.5%), respectively. Among statin users, there were no events of liver-related death or transplantation. In PS matched cohort, statin users had better transplantation-free survival (log rank p=0.030) (**figure 2c**).

All-cause mortality

Overall 5-year, 10-year, 15-year and 20-year survival probability was 97.0% (95% CI:95.9–98.2%), 93.5% (95% CI:91.6–95.5%), 89.0% (95% CI: 85.9–92.1%), and 76.0% (95% CI:69.3–83.3%), respectively. In PS matched cohort, statin users had better survival (log rank p=0.010) (**figure 2d**).

Discussion

We described long-term clinical outcomes after HBsAg seroclearance and identified risk factors for adverse outcomes. Importantly, statin use is shown first time in literature to reduce adverse outcomes after HBsAg seroclearance, including cirrhosis/HCC, hepatic decompensation, liver-related mortality/transplantation, and all-cause mortality.

To our knowledge, this is one of the largest cohorts with prolonged follow-up (up to 21 years) after HBsAg seroclearance. Previous studies had relatively smaller sample size (ranging from 49 to 422),[12, 22, 23] and most had limited follow-up duration (mean:1.6-1.9years).[12, 23] While various risk factors for adverse outcomes were identified including well-known ones like older age, male sex and cirrhosis, importance of cardiometabolic risk factors/diseases should be highlighted. DM was associated with higher cirrhosis/HCC risk (aHR:2.03), and raised GGT (a surrogate marker of hepatic steatosis) was associated with higher risk of cirrhosis/HCC (aHR:3.25) and hepatic decompensation (aHR:4.87). Cardiovascular diseases were associated with higher all-cause mortality risk (aHR:2.13). Cardiometabolic risk factors/diseases like DM and metabolic syndrome are known risk factors for hepatic steatosis and hence cirrhosis progression.[24-26]

Prior antiviral treatment was a risk factor for death (aHR:2.69), which may signify underlying advanced fibrosis/cirrhosis not detected clinically/biochemically at baseline. Nevertheless, there was no statistically significant difference in the outcomes of cirrhosis/HCC and liver-related mortality/transplantation between NA-induced and spontaneous HBsAg seroclearance. This may be related to the smaller

sample size and number of events leading to underpower. While Chen et al[22] could not demonstrate a difference in adverse outcomes between NA-induced and spontaneous HBsAg seroclearance, their cohort had few adverse outcomes (only one death and subsequent cirrhosis after HBsAg seroclearance was not reported).

Our study is the first to demonstrate statins were protective against hepatic-related adverse outcomes after HBsAg seroclearance. It is noteworthy that statins users were generally older, and a higher proportion of were smokers and had cardiometabolic diseases, which are also risk factors for cirrhosis. This provides further proof that statin use is associated with better clinical outcomes despite the higher associated intrinsic risk in statin users after multivariable analyses. This protective effect is likely mediated via both cardiovascular disease prevention and reduction of cirrhosis. A meta-analysis shows statins associate with lower risk of cirrhosis progression, decompensation and mortality among cirrhotic patients.[15] Statins mitigate oxidative stress by suppressing activation of inflammatory cells, improve endothelial function by enhancing nitric oxide synthesis, and increase number of endothelial progenitor cells. Subgroup analysis does not show protective effect of statins on cirrhosis/HCC among patients with positive anti-HBs (HR:0.96;p=0.945). On the other hand, there was a borderline significance among patients with negative anti-HBs (HR:0.29;p=0.073), which is likely due to underpower from subgroup analysis. This marked difference in the statin effect as regards the presence of anti-HBs warrants further investigation. Interestingly, although there was statistically significant difference in the median values of albumin, AST, ALP and INR among statin users at five years after HBsAg seroclearance, the clinical significance of this is not prominent given the small numerical differences. One of the possible reasons is the generally

good clinical outcome of patients with HBsAg seroclearance who used statins. While a beneficial effect of statins has been demonstrated on prevention of cirrhosis/HCC and hepatic decompensation (categorical outcome), the biochemical improvement of liver function parameters (numerical outcome) may not be distinctly demonstrated.

As for liver-related death/transplantation, NSSBs (aHR:3.11), metformin (aHR:5.93), and PPIs (aHR:5.38) were risk factors. Poorer glycemic control mandates metformin use, explaining its higher risk of hepatic decompensation (aHR:8.25) and liver-related death (aHR:5.93). Indeed, metformin improves survival of diabetic patients with cirrhosis.[27] Studies of diabetic patients with HBsAg seroclearance adjusting for serial HbA1c measurements and other anti-diabetic medications are required to investigate role of metformin in liver outcomes. A meta-analysis showed PPIs associated with higher risk of adverse liver outcomes and mortality which may be due to gut dysbiosis.[17] NSSB use may signify more severe liver disease (and hence liver-related mortality) in our current study, although recent study showed patients with decompensated cirrhosis had higher mortality if cardiac output was <5L/min.[28]

A few limitations of this study should be acknowledged. First, a significant proportion of patients did not undergo USG, transient elastography and liver biopsy at HBsAg seroclearance, precluding a more homogeneous definition of cirrhosis and hepatic steatosis. Data on BMI at the time of HBsAg seroclearance was not available Second, data on HBV genotype, a risk factor for adverse hepatic outcomes, were unavailable. Third, due to incomplete documentation of smoking and alcohol intake, COPD and alcohol-related diseases were chosen as surrogate markers to reflect more severe disease spectrum. Fourth, generalizability of study results should be confirmed by

multi-center studies involving Caucasians, as different genotypes predominate in Asian and non-Asian countries.[29]

Our study has several important clinical implications. Identification of factors for adverse outcomes after HBsAg seroclearance helps streamline management in a cost-effective manner based on risk stratification. Particular attention should be paid to cardiometabolic risk factors apart from known factors like older age and cirrhosis. Importance of monitoring of hepatic steatosis (via controlled attenuation parameter measurement) should be emphasized, as resolution of severe steatosis is associated with fibrosis regression in quiescent CHB patients.[25] Initiation of statins may be considered in patients with HBsAg seroclearance at high risk of adverse clinical outcomes, especially those with borderline indications for cardiovascular disease prevention.

Conclusion

There is overall good long-term prognosis after HBsAg seroclearance, although some patients still develop adverse clinical outcomes. In addition to known risk factors like older age, male sex and cirrhosis, cardiometabolic risk factors are important for prognostication. Statins are associated with improved prognosis after HBsAg seroclearance.

Data availability

Data will not be shared due to confidentiality.

Animal research (Ethics)

This study did not involve animal research.

Consent to participate (Ethics)

This is a retrospective cohort study and has been approved by Institutional Review Board, University of Hong Kong and West Cluster of Hospital Authority, Hong Kong.

Consent to publish (Ethics)

Participate identity was anonymized in this study.

Clinical Trials Registration

This is not a clinical trial.

Authors contributions

Drs. Ka-Shing Cheung and Wai-Kay Seto were involved with study concept and design; acquisition of data; analysis and interpretation of data; drafting of manuscript; **Dr. Lok Ka Lam** was involved with acquisition of data. **Dr. Lung-Yi Mak and James Fung** were involved with analysis and interpretation of data; and critical revision of the manuscript for important intellectual content. **Prof Man-Fung Yuen** were involved with the study concept and design; analysis and interpretation of data; drafting of manuscript; critical revision of the manuscript for important intellectual content; and study supervision. The corresponding author had full access to all data, and was fully responsible for the data integrity and statistical analysis. All authors revised the manuscript and approved the final version of this article.

Conflicts of Interest

WK Seto is an advisory board member of AbbVie and Gilead Sciences, and received speaker fees from AbbVie, Gilead Sciences and Mylan. J Fung received research funding from Novartis. MF Yuen is advisory board member and received speaker's fees from AbbVie, Janssen, Biocartis NV, Bristol Myers Squibb, Fujirebio Incorporation, Gilead Sciences, Merck Sharp and Dohme, Sysmex Corporation. MF Yuen does consulting for Aligos Pharmaceuticals, Assembly Biosciences, Arbutus Biopharma, Dicerna Pharmaceuticals, Clear-B Therapeutics, GlaxoSmithKline, Immunocore, Springbank Pharmaceuticals and also received research funding from Bristol Myers Squibb and Gilead Sciences. LY Mak is advisory board member of Gilead Sciences. The remaining authors have no conflicts of interest.

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References

1. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol 2018;3:383-403.
2. Chen YC, Chu CM, Liaw YF. Age-specific prognosis following spontaneous hepatitis B e antigen seroconversion in chronic hepatitis B. Hepatology 2010;51:435-44.
3. Cheung KS, Seto WK, Wong DK, et al. Wisteria floribunda agglutinin-positive human Mac-2 binding protein predicts liver cancer development in chronic hepatitis B patients under antiviral treatment. Oncotarget 2017;8:47507-47517.
4. Seto WK, Lau EH, Wu JT, et al. Effects of nucleoside analogue prescription for hepatitis B on the incidence of liver cancer in Hong Kong: a territory-wide ecological study. Aliment Pharmacol Ther 2017;45:501-509.
5. Cheung KS, Mak LY, Liu SH, et al. Entecavir vs Tenofovir in Hepatocellular Carcinoma Prevention in Chronic Hepatitis B Infection: A Systematic Review and Meta-Analysis. Clin Transl Gastroenterol 2020;11:e00236.
6. Cheung KS, Seto WK, Lai CL, et al. Prevention and management of hepatitis B virus reactivation in cancer patients. Hepatol Int 2016;10:407-14.
7. Zhou K, Contag C, Whitaker E, et al. Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic

review and pooled meta-analyses. Lancet Gastroenterol Hepatol 2019;4:227-238.
8. Lai CL, Shouval D, Lok AS, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011-20.
9. Yuen MF, Seto WK, Chow DH, et al. Long-term lamivudine therapy reduces the risk of long-term complications of chronic hepatitis B infection even in patients without advanced disease. Antivir Ther 2007;12:1295-303.
10. Fattovich G, Giustina G, Sanchez-Tapias J, et al. Delayed clearance of serum HBsAg in compensated cirrhosis B: relation to interferon alpha therapy and disease prognosis. European Concerted Action on Viral Hepatitis (EUROHEP). Am J Gastroenterol 1998;93:896-900.
11. Yuen MF, Wong DK, Fung J, et al. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology 2008;135:1192-9.
12. Ahn SH, Park YN, Park JY, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol 2005;42:188-94.
13. Liu F, Wang XW, Chen L, et al. Systematic review with meta-analysis: development of hepatocellular carcinoma in chronic hepatitis B patients with hepatitis B surface antigen seroclearance. Aliment Pharmacol Ther 2016;43:1253-61.
14. Seto WK, Wong DK, Chan TS, et al. Association of Hepatitis B Core-Related Antigen With Hepatitis B Virus Reactivation in Occult Viral Carriers Undergoing High-Risk Immunosuppressive Therapy. Am J Gastroenterol 2016;111:1788-1795.
15. Kim RG, Loomba R, Prokop LJ, et al. Statin Use and Risk of Cirrhosis and Related Complications in Patients With Chronic Liver Diseases: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2017;15:1521-1530.e8.
16. Cunha V, Cotrim HP, Rocha R, et al. Metformin in the prevention of hepatocellular carcinoma in diabetic patients: A systematic review. Ann Hepatol 2020;19:232-237.
17. Wang J, Wu Y, Bi Q, et al. Adverse outcomes of proton pump inhibitors in chronic liver disease: a systematic review and meta-analysis. Hepatol Int 2020.
18. Kockerling D, Nathwani R, Forlano R, et al. Current and future pharmacological therapies for managing cirrhosis and its complications. World J Gastroenterol 2019;25:888-908.
19. Seto WK, Fung J, Cheung KS, et al. Body-mass index is associated with fibrosis regression during long-term nucleoside analogue therapy in chronic hepatitis B. Alimentary Pharmacology & Therapeutics 2016;44:1071-1079.
20. White IR, Royston P. Imputing missing covariate values for the Cox model. Stat Med 2009;28:1982-98.
21. Cheung KS, Leung WK, Seto WK. Application of Big Data analysis in gastrointestinal research. World J Gastroenterol 2019;25:2990-3008.
22. Chen YC, Jeng WJ, Chien RN, et al. Clinical outcomes after spontaneous and nucleos(t)ide analogue-treated HBsAg seroclearance in chronic HBV infection. Aliment Pharmacol Ther 2016;43:1311-8.

23. Huo TI, Wu JC, Lee PC, et al. Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. Hepatology 1998;28:231-6.
24. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol 2020;73:202-209.
25. Mak LY, Wan-Hin Hui R, Fung J, et al. Diverse effects of hepatic steatosis on fibrosis progression and functional cure in virologically quiescent chronic hepatitis B. J Hepatol 2020.
26. Mak LY, Seto WK, Hui RW, et al. Fibrosis evolution in chronic hepatitis B e antigen-negative patients across a 10-year interval. J Viral Hepat 2019;26:818-827.
27. Zhang X, Harmsen WS, Mettler TA, et al. Continuation of metformin use after a diagnosis of cirrhosis significantly improves survival of patients with diabetes. Hepatology 2014;60:2008-16.
28. Alvarado Tapias E, Ardevol A, Garcia-Guix M, et al. Short-term hemodynamic effects of β-blockers influence survival of patients with decompensated cirrhosis. J Hepatol 2020.
29. Norder H, Hammas B, Lee SD, et al. Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. J Gen Virol 1993;74 (Pt 7):1341-8.

FIGURE LEGEND

Figure 1: Kaplan Meier plot of proportion of patients developing (a) cirrhosis; (b) HCC; (c) liver-related death or liver transplantation; (d) all-cause death of the whole study cohort after HBsAg seroclearance

Abbreviations: HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; PS, propensity score

Figure 2: Kaplan Meier plot of (a) cirrhosis and/or HCC-free survival; (b) hepatic decompensation-free survival; (c) liver-related death and/or liver transplantation-free survival; (d) survival after HBsAg seroclearance according to statin use among PS matched cohort

Abbreviations: HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; PS, propensity score

Table 1. Characteristics of patients with HBsAg seroclearance

	Whole cohort (n=913)	Statin users (n=129)	Statin non-users (n=784)	p-value
Age	53.4 (45.2 –60.9)	61.2 (54.2 – 67.6)	52.1 (44.0 – 59.5)	**<0.001**
Male (n, %)	613 (67.1%)	92 (71.3%)	521 (66.5%)	0.276
Anti-HBs positivity (n, %)	513 (56.2%)	80 (62.0%)	433 (55.2%)	0.150
Treatment naïve (n, %)	818 (89.6%)	114 (88.4%)	704 (90.0%)	0.624
Cirrhosis	80 (8.8%)	11 (8.5%)	69 (8.8%)	0.919
DM*	205/827 (24.8%)	61/128 (47.7%)	144/699 (20.6%)	**<0.001**
Alcoholism[#]	11 (1.2%)	2 (1.6%)	9 (1.1%)	0.660
Smoking[#]	13 (1.4%)	6 (4.7%)	7 (0.9%)	**0.005**
Cardiovascular diseases	107 (11.7%)	69 (53.5%)	38 (4.8%)	**<0.001**
Platelet ($x10^9$/L)	204 (171 – 241)	199 (169 – 226)	205 (171 – 243)	0.148
Creatinine (umol/L)*	82 (68 – 93)	85 (73 – 100)	81 (68 – 92)	**0.005**
Albumin (g/L)*	43 (41 – 45)	43 (41 – 45)	43 (41 – 46)	0.324
Bilirubin (umol/L)*	9 (6 – 12)	9 (6 – 12)	9 (6 – 12)	0.442
ALT (U/L)*	20 (15 – 28)	23 (16 – 33)	20 (15 – 27)	**0.002**
AST (U/L)*	22 (19 – 27)	23 (20 – 28)	22 (19 – 27)	**0.032**
ALP (U/L)*	62 (52 – 73)	65 (55 – 77)	62 (52 – 73)	**0.024**
GGT (U/L)*	22 (16 – 33)	27 (20 – 38)	22 (16 – 32)	**<0.001**
PT (seconds)*	11.1 (10.6 – 11.8)	11.1 (10.7 – 11.8)	11.1 (10.6 – 11.8)	0.983
AFP (ng/ml)*	2 (2 – 3)	2 (2 – 3)	2 (2 – 3)	0.520
NSSBs (n, %)	36 (3.9%)	10 (7.8%)	26 (3.3%)	**0.016**
Metformin (n, %)	66 (7.2%)	40 (31.0%)	26 (3.3%)	**<0.001**
PPIs (n, %)	109 (11.9%)	48 (37.2%)	61 (7.8%)	**<0.001**

p-value < 0.05 is bold

*missing data – DM: 86 (9.4%); creatinine: 138 (15.1%); platelet: 13 (1.4%); albumin: 4 (0.4%); bilirubin: 4 (0.4%); ALT: 5 (0.5%); AST: 7 (0.8%); ALP: 4 (0.4%); GGT: 17 (1.9%); PT: 82 (9.0%); AFP: 6 (0.7%)

[#]Chronic obstructive pulmonary disease (COPD) and alcohol-related diseases (gastrointestinal, hepatic, cardiac and neurological) were used as proxies for heavy smoking and alcoholism as the smoking and drinking history were not documented in some patients.

Abbreviations: anti-HBs, anti-hepatitis B surface antigen; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase;

ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; PT, prothrombin time; AFP, alpha fetoprotein; NSSBs, non-selective beta blockers; PPIs, proton pump inhibitors

Table 2. Risk factors for cirrhosis and/or hepatocellular carcinoma development after HBsAg seroclearance (cohort number= 833, events=71)

	Adjusted HR	95% CI	p-value
Age	1.06	1.03 – 1.09	**<0.001**
Male	1.48	0.85 – 2.58	0.170
Antiviral treatment	1.57	0.60 – 4.14	0.362
Anti-HBs positivity	0.73	0.44 – 1.21	0.220
DM	2.03	1.17 – 3.51	**0.011**
Alcoholism*	0.36	0.04 – 2.98	0.346
Smoking*	0.65	0.08 – 5.08	0.683
Cardiovascular diseases	1.40	0.70 – 2.78	0.339
Platelet <150x10^9/L	1.37	0.73 – 2.58	0.326
Creatinine (umol/L)	1.008	1.005 – 1.012	**<0.001**
Albumin <39 g/L	0.99	0.48 – 2.04	0.986
Bilirubin >17 umol/L	1.12	0.47 – 2.71	0.793
ALT >40 U/L	1.19	0.47 – 3.02	0.721
AST >40 U/L	1.69	0.53 – 5.39	0.371
ALP >110 U/L	1.31	0.40 – 4.31	0.661
GGT >50 U/L	3.25	1.77 – 5.97	**<0.001**
PT (seconds)	1.06	0.96 – 1.17	0.224
AFP > 9 ng/ml	10.14	3.33 – 30.94	**<0.001**
Statins	0.44	0.20 – 0.96	**0.038**
NSSBs	1.47	0.52 – 4.19	0.471
Metformin	1.88	0.82 – 4.30	0.133
PPIs	0.67	0.28 – 1.56	0.348

p-value < 0.05 is bold

*Chronic obstructive pulmonary disease (COPD) and alcohol-related diseases (gastrointestinal, hepatic, cardiac and neurological) were used as proxies for heavy smoking and alcoholism as the smoking and drinking history were not documented in some patients.

Abbreviations: HBsAg, hepatitis B surface antigen; HR, hazard ratio; 95% CI, 95% confidence interval; anti-HBs, anti-hepatitis B surface antigen; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; PT, prothrombin time; AFP, alpha fetoprotein; NSSBs, non-selective beta blockers; PPIs, proton pump inhibitors

Table 3. Effect of statins on primary and secondary outcomes after HBsAg seroclearance

Multivariable analysis	*aHR	95% CI	p-value	PS matching	HR	95% CI	p-value
Cirrhosis and/or hepatocellular carcinoma (cohort number=833, events=71)	0.44	0.20 – 0.96	**0.038**	Cirrhosis and/or hepatocellular carcinoma (cohort number=163, events=26)	0.30	0.10 – 0.86	**0.025**
Hepatic decompensation (cohort number=905, events=18)	No events in statin users	n.a. #	n.a. #	Hepatic decompensation (cohort number=198, events=8)	No events in statin users	n.a. #	n.a. #
Liver-related mortality and/or liver transplantation (cohort number=913, events=18)	No events in statin users	n.a. #	n.a. #	Liver-related mortality and/or liver transplantation (cohort number=208, events=8)	No events in statin users	n.a. #	n.a. #
All-cause mortality (cohort number=913, events=76)	0.21	0.08 – 0.53	**<0.001**	All-cause mortality (cohort number=207, events=29)	0.33	0.13 – 0.87	**0.026**

p-value <0.05 is bold

*adjusted for age, sex, antiviral treatment, anti-HBs positivity, DM, alcoholism, smoking, cardiovascular diseases, thrombocytopenia, creatinine, hypoalbuminemia, hyperbilirubinemia, ALT, AST, ALP, GGT, PT, AFP, NSSBs, metformin and PPIs

#could not be calculated as there were no events in patients with statin use

Abbreviations: HBsAg, hepatitis B surface antigen; aHR, adjusted hazard ratio; 95% CI, 95% confidence interval; anti-HBs, anti-hepatitis B surface antigen; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; PT, prothrombin time; AFP, alpha fetoprotein; NSSBs, non-selective beta blockers; PPIs, proton pump inhibitors

Table 4. Risk factors for liver-related mortality and/or liver transplantation after HBsAg seroclearance (cohort=913, events=18)

	Adjusted HR	95% CI	p-value
Age	1.10	1.05 – 1.16	**<0.001**
Male	7.45	2.08 – 26.63	**0.002**
Antiviral treatment	1.15	0.14 – 9.39	0.895
Anti-HBs positivity	0.54	0.21 – 1.41	0.211
Cirrhosis	13.58	4.77 – 38.64	**<0.001**
DM	0.96	0.37 – 2.51	0.936
Alcoholism*	2.78	0.34 – 22.98	0.344
Smoking*	n.a.[#]	n.a.[#]	n.a.[#]
Cardiovascular diseases	1.58	0.50 – 4.95	0.436
Platelet <150x10^9/L	3.46	1.29 – 9.30	**0.014**
Creatinine (umol/L)	1.007	1.005 – 1.010	**<0.001**
Albumin <39 g/L	0.74	0.28 – 1.97	0.550
Bilirubin >17 umol/L	0.32	0.10 – 1.00	0.051
ALT >40 U/L	n.a.[#]	n.a.[#]	n.a.[#]
AST >40 U/L	n.a.[#]	n.a.[#]	n.a.[#]
ALP >110 U/L	0.24	0.07 – 0.91	**0.036**
GGT >50 U/L	1.83	0.58 – 5.76	0.304
PT (seconds)	1.26	1.09 – 1.45	**0.001**
AFP >9 ng/ml	7.55	0.97 – 58.68	0.053
Statins	n.a.[#]	n.a.[#]	n.a.[#]
NSSBs	3.11	1.18 – 8.18	**0.022**
Metformin	5.93	1.64 – 21.37	**0.006**
PPIs	5.38	2.07 – 13.97	**<0.001**

p-value <0.05 is bold

*Chronic obstructive pulmonary disease (COPD) and alcohol-related diseases (gastrointestinal, hepatic, cardiac and neurological) were used as proxies for heavy smoking and alcoholism as the smoking and drinking history were not documented in some patients.

[#]results not available as there were no events in patients with smoking, ALT >40 U/L, AST >40 U/L, and statin use

Abbreviations: HBsAg, hepatitis B surface antigen; HR, hazard ratio; 95% CI, 95% confidence interval; anti-HBs, anti-hepatitis B surface antigen; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; PT, prothrombin time; AFP, alpha fetoprotein; NSSBs, non-selective beta blockers; PPIs, proton pump inhibitors