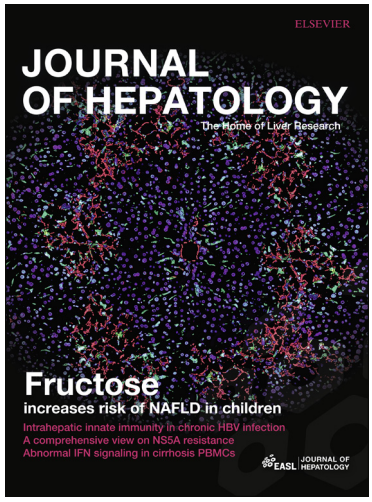

Impact of age and gender on risk of hepatocellular carcinoma after hepatitis B surface antigen seroclearance

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Impact of age and gender on risk of hepatocellular carcinoma after hepatitis B surface antigen seroclearance

Running title: HBsAg loss and HCC

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Keywords (not in the title): antiviral therapy; HBsAg seroclearance; HBsAg seroreversion; sustained response.

Abbreviations: ALT = alanine aminotransferase, anti-HBs = antibody to hepatitis B surface antigen, CDARS = Clinical Data Analysis and Reporting System, CHB = chronic hepatitis B, CI = confidence intervals, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HDV = hepatitis D virus, HR = hazard ratio, NA = nucleos(t)ide analogues, TDF = tenofovir disoproxil fumarate.

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Authorship Statement

Terry Yip, Yee-Kit Tse, Kelvin Lam and Grace Wong had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors were responsible for the study concept and design. Terry Yip, Yee-Kit Tse, Kelvin Lam and Grace Wong were responsible for the acquisition and analysis of data. All authors were responsible for the interpretation of data, the drafting, and critical revision of the manuscript for important intellectual content.

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Statement of Interests

Henry Chan is a consultant for AbbVie, Bristol-Myers Squibb, Gilead, Janssen and Roche, has received honorarium for lecture for Abbvie, Bristol-Myers Squibb, Echosens, Gilead, Glaxo-Smith-Kline, Merck, Novartis and Roche, and has received an unrestricted grant from Roche for hepatitis B research.

Vincent Wong has served as an advisory committee member for Abbvie, Roche, Novartis, Gilead and Otsuka. He has also served as a speaker for Abbvie, Bristol-Myers Squibb, Roche, Novartis, Abbott Diagnostics and Echosens.

Grace Wong has served as an advisory committee member for Gilead Sciences. She has also served as a speaker for Abbott, Abbvie, Bristol-Myers Squibb, Echosens, Furui, Gilead, Janssen, and Roche.

The other authors declare that they have no competing interests.

ABSTRACT

Background and Aims

Previous studies suggested spontaneous seroclearance of hepatitis B surface antigen (HBsAg) after 50 years old was still associated with an increased risk of hepatocellular carcinoma (HCC). This study aimed to evaluate the risk of HCC after HBsAg seroclearance and the impact of gender on HCC.

Methods

All chronic hepatitis B patients under medical care in Hospital Authority, Hong Kong who have cleared HBsAg from January 2000 to August 2016 were identified. The age of HBsAg seroclearance, gender, and subsequent development of HCC were captured and analyzed.

Results

4,568 patients with HBsAg seroclearance were identified; 793 (17.4%) were treated by nucleos(t)ide analogues and 60 (1.3%) had received interferon treatment. At a median (interquartile range) follow-up of 3.4 (1.5–5.0) years, 54 patients developed HCC; cumulative incidence of HCC at 1, 3 and 5 years were 0.9%, 1.3% and 1.5%, respectively. Age above 50 years (adjusted hazard ratio 4.31, 95% confidence interval 1.72 – 10.84; *P*=0.002) and male gender (2.47, 1.24 – 4.91; *P*=0.01) were two independent risk factors of HCC. Female patients aged ≤50 years (n=545) had zero risk of HCC in 5 years. Male patients aged ≤50 years (n=769), female patients aged >50 years (n=1,149) and male patients aged >50 years (n=2,105) had a 5-year cumulative incidence of HCC 0.7%, 1.0% and 2.5%, respectively. Similar findings were observed in patients with spontaneous and antiviral treatment-induced HBsAg seroclearance.

Conclusions

Female patients aged 50 years or below have zero risk of HCC after HBsAg seroclearance, whereas female patients aged above 50 years and all male patients are still at risk of HCC.

INTRODUCTION

Chronic hepatitis B (CHB) is the leading cause of hepatocellular carcinoma (HCC) worldwide [1]. Hepatitis B surface antigen (HBsAg) seroclearance is a surrogate of ultimate immune control of hepatitis B virus (HBV). The annual incidence of spontaneous HBsAg seroclearance varies from 0.12% to 2.38% in Asian cohorts and from 0.54% to 1.98% in Western cohorts [2]. The rate and durability of HBsAg seroclearance induced by nucleos(t)ide analogues (NA) is recently found to be comparable to those developed spontaneously [3]. Patients who achieve this endpoint often have a much favorable clinical course and very low risk of hepatocellular carcinoma (HCC) [4-6].

Despite a favorable clinical course, the risk of HCC still exists in patients achieved HBsAg seroclearance. Among all the well-established risk factors, age of HBsAg seroclearance, co-infection with hepatitis C and/or D virus, and presence of cirrhosis are the most important ones [5, 7, 8]. In particular, age above 50 years at the time of HBsAg seroclearance has been considered as a clinically important cutoff in previous studies, and shown to be independently associated with the development of HCC after HBsAg seroclearance [9, 10]. However, due to the limited sample size on female patients, there has been little data concerning the impact of gender, a well-defined risk factor for HCC development in chronic hepatitis B (CHB) patients [1, 11], on top of age on the risk of HCC after HBsAg seroclearance. In this large cohort study, we aimed to determine the impact of age and gender on the HCC risk in CHB patients who achieved HBsAg seroclearance and the potential interaction between these two factors.

METHODS

Study design and data source

We performed a retrospective cohort study using data from the Clinical Data Analysis and

Reporting System (CDARS) of the Hospital Authority (HA), Hong Kong. CDARS facilitates the retrieval of clinical data captured from different operational systems for analysis and reporting and provides good quality information to support retrospective clinical and management decisions by integrating the clinical data resided in Data Warehouse [12]. All subjects with HBsAg checked positive between January 1, 2000 and August 31, 2016 were identified. Patients aged below 18 years and those who had incomplete demographic data; undergone liver transplantation; HCC before HBsAg seroclearance; and viral hepatitis C and/or D co-infection based on viral and/or serological markers were excluded. To avoid inadvertent labeling of acute hepatitis B as chronic hepatitis B with HBsAg seroclearance, we excluded patients with positive immunoglobulin M to hepatitis B core antigen unless it was more than 6 months apart from a positive HBsAg result, and patients converting from positive HBsAg result to a negative HBsAg result within 6 months unless there were two positive HBsAg results more than 6 months apart before the negative HBsAg result. Patients were followed from the date of first documented HBsAg seroclearance to death, diagnosis of HCC, last follow-up date (31 October, 2016), or 5 years of follow-up, whichever came first. The study protocol was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee.

Data collection

Data were retrieved from the CDARS in November 2016. Baseline date was defined as of the date of the first HBsAg seroclearance. Demographic data including gender and date of birth were captured. At baseline, liver and renal biochemistries, hematological and virologic parameters were collected. Thereafter, serial liver and renal biochemistries as well as HBV viral markers (HBsAg, anti-HBs, HBV DNA) were collected until the last follow-up. Other relevant diagnoses, procedures, concomitant drugs, and laboratory parameters were retrieved

and studied.

NA-treated patients were defined as those prescribed and dispensed one or more NA for CHB (*i.e.* lamivudine, adefovir dipivoxil, entecavir, telbivudine, or tenofovir disoproxil fumarate) for any duration prior to HBsAg seroclearance. Interferon exposure was also checked by the dispensing record of interferon (IFN) alpha-2a/2b or peginterferon (PEG-IFN) alpha-2a/2b/lambda-1a. These medications were identified by HA's internal drug codes (Supplementary Table 1).

Definition of HBsAg seroclearance, HCC, and cirrhosis

HBsAg seroclearance was defined as loss of HBsAg detectability for once or more. HCC was identified by ICD-9-CM diagnosis codes for HCC (155.0 - hepatocellular carcinoma and 155.2 - carcinoma of liver). Cirrhosis was identified by low platelet count (<150 $x10^9$/L) at baseline, and the ICD-9-CM diagnosis codes for cirrhosis and its related complications prior to baseline including portal hypertension, varices, variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, and hepatorenal syndrome (Supplementary Table 2).

Statistical analyses

Data were analyzed using Statistical Product and Service Solutions (SPSS) version 22.0 (SPSS, Inc., Chicago, Illinois) and R software (3.3.2; R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were expressed in mean ± standard deviation or median (interquartile-range [IQR]), as appropriate, while categorical variables were presented as number (percentage). Qualitative and quantitative differences between two subgroups were analyzed by chi-square test for categorical parameters and Student t test or Mann-Whitney's

test for continuous parameters, as appropriate. Overall, qualitative and quantitative differences between three or more subgroups were analyzed by chi-square test for categorical parameters and one-way ANOVA or Kruskal-Wallis test for continuous parameters, as appropriate. Post-hoc analysis was performed upon overall statistically significant difference in subgroups. Kaplan-Meier's method was used to estimate the cumulative incidence of HCC with 95% confidence interval (CI); log-rank test was used to compare the cumulative probabilities of different subgroups. On multivariable analyses, adjusted hazard ratios (HR) with 95% CIs of HCC incidence were estimated with Cox proportional hazards regression, respectively. The accuracy of the CU-HCC score in predicting HCC development for patients after HBsAg seroclearance was assessed by the area under the receiver operating characteristic curve (AUROC) with 95% CI [13, 14]. Sensitivity analysis was performed to evaluate the impact of NA treatment and clearance of HBsAg after 60 years old. All statistical tests were two-sided. Statistical significance was taken as $P<0.05$. P values in post-hoc analysis were adjusted by Bonferroni correction.

RESULTS

We identified 73,493 subjects who had positive HBsAg result for at least once; 68,925 were excluded according to the inclusion and exclusion criteria (Figure 1). Finally, 4,568 CHB patients with HBsAg seroclearance were included for analysis (Table 1). Their mean age was 56.7 ± 13.8 years, and 2,874 (62.9%) patients were male. Most patients had compensated liver function and normal alanine aminotransferase (ALT) level at the time of HBsAg seroclearance; their mean serum albumin was 41.4 ± 5.9 g/L, total bilirubin was 15.5 ± 29.3 μmol/L, and median (IQR) ALT was 21.0 (15.0 – 33.0) IU/L. NA was prescribed to 793 (17.4%) patients, whereas 60 (1.3%) patients had received (PEG)-IFN therapy prior HBsAg seroclearance.

Cumulative incidence of HCC

At a median (IQR) follow-up of 3.4 (1.5 – 5.0) years, 54 (2.9%) patients developed HCC. The cumulative incidence rates of HCC at 1, 3 and 5 years after HBsAg seroclearance were 0.9% (95% CI: 0.6% to 1.2%), 1.3% (1.0% to 1.7%) and 1.5% (1.1% to 1.9%), respectively in the entire cohort (Figure 2A).

Impact of age and gender on HCC risk

Age ≤50 versus >50 years

Patients in the subgroup older than 50 years old (n=3,254) were more likely to be male, had lower platelet count, lower serum albumin and higher serum ALT, and less likely to receive NA treatment when compared to the counterpart of age 50 or below at the time of HBsAg seroclearance (Table 1). Five patients with HBsAg seroclearance at 50 years old or younger developed HCC; whereas 49 patients with HBsAg seroclearance older than 50 years old developed HCC. The cumulative incidence rates of HCC at 1, 3 and 5 years after HBsAg seroclearance were 0.3% (95% CI: 0.1% to 0.8%), 0.4% (0.2% to 1.0%) and 0.4% (0.2% to 1.0%), respectively in the ≤50 years old subgroup; the corresponding rates were 1.1% (0.8% to 1.5%), 1.7% (1.2% to 2.2%) and 2.0% (1.5% to 2.7%) in the >50 years old subgroup (Figure 2B). Patients who had HBsAg seroclearance at age >50 years old had significantly higher risk of HCC than those at age ≤50 years old (log-rank test, P<0.001). Patients who had HBsAg seroclearance at age above 60 years old had significantly higher risk of HCC than those at age ≤60 years old (log-rank test, P=0.006) (Supplementary Figure 2A).

Male versus female

Male patients (n=2,874) were older, had lower platelet count, slightly higher serum albumin,

higher total bilirubin and higher serum ALT, and more likely to receive NA when compared to the female patients (Table 1). Ten female and 44 male patients developed HCC after HBsAg seroclearance. The cumulative incidence rates of HCC at 1, 3 and 5 years after HBsAg seroclearance were 0.5% (95% CI: 0.3% to 1.1%), 0.7% (0.3% to 1.2%) and 0.7% (0.3% to 1.2%), respectively in female subgroup; the corresponding rates were 1.1% (0.7% to 1.5%), 1.6% (1.2% to 2.2%) and 1.9% (1.4% to 2.6%) in male subgroup (Figure 2C). Male patients had a significantly higher risk of developing HCC than female patients after HBsAg seroclearance (log-rank test, P=0.005).

Interaction between age and gender

Four subgroups defined with age and gender showed different risks of HCC (log-rank test, P<0.001), and the risk is the highest in male patients with HBsAg seroclearance older than 50 years old. The corresponding cumulative incidence rates of HCC at 1, 3 and 5 years after HBsAg seroclearance were 1.3% (95% CI: 0.9% to 1.9%), 2.0% (1.4% to 2.8%) and 2.5% (1.8% to 3.5%), respectively. Female patients with HBsAg seroclearance at 50 years old or younger had the lowest and zero risk of HCC up to 5 years. The other two subgroups, female patients with HBsAg seroclearance older than 50 years old and male patients with HBsAg seroclearance at 50 years old or younger had intermediate and comparable risks of HCC. The cumulative incidence rates of HCC at 1, 3 and 5 years after HBsAg seroclearance were 0.8% (0.4% to 1.6%), 1.0% (0.5% to 1.8%) and 1.0% (0.5% to 1.8%), respectively in female >50 subgroup; the corresponding rates were 0.5% (0.2% to 1.4%), 0.7% (0.3% to 1.6%) and 0.7% (0.3% to 1.6%) in male ≤50 years old subgroup (Figure 2D). On multivariable analysis, both age >50 years old and male gender were independent risk factors of HCC development in patients with HBsAg seroclearance; the adjusted HRs (95% CI) were 4.31 (1.72 – 10.84; P=0.002) and 2.47 (1.24 – 4.91; P=0.01), respectively.

Compared to patients who developed HCC despite HBsAg seroclearance after the age of 50, the 5 male patients with HCC after HBsAg seroclearance at age 50 years or younger tend to have lower platelet count, higher total bilirubin, lower serum albumin and higher serum ALT; four had liver cirrhosis at baseline under the definition (Table 2).

Performance of the CU-HCC score

Eight hundred and thirty-nine (18.4%) patients were identified with cirrhosis among 4,568 patients with HBsAg seroclearance. CU-HCC score achieved an AUROC (95% CI) of 0.73 (0.67 – 0.80). Under the recommended dual cutoffs of 5 and 20, 3,335 patients (73.0%), 626 patients (13.7%) and 607 patients (13.3%) were classified into the low risk, intermediate risk and high risk groups, respectively. In the corresponding risk groups, 21 (0.6%), 12 (1.9%) and 21 (3.5%) developed HCC over 5 years after HBsAg seroclearance; the 5-year cumulative incidence rates were 0.9, 2.3% and 4.1%, respectively (log-rank test, $P<0.001$) (Supplementary Figure 2C). CU-HCC score achieved 61% (95% CI 47% – 74%) sensitivity and 87% (86% – 88%) specificity with corresponding negative predictive value of 99% (99% – 100%) and positive predictive value of 3% (2% – 5%) under the dual cutoffs.

Sensitivity analysis - spontaneous versus NA-induced HBsAg seroclearance

Similar impact of age and gender on HCC risk was observed in the subgroup of patients with spontaneous HBsAg seroclearance (n=3,775) (Figure 2E). In the subgroup of patients with NA-induced HBsAg seroclearance (n=793), HCC developed only among male patients of age >50 years at the time of HBsAg seroclearance; whereas all female patients and male patients of age ≤50 years at the time of HBsAg seroclearance did not develop HCC up to 5 years (Figure 2F).

Sensitivity analysis - interaction between 60 years of age and gender

Different risks of HCC were consistently observed in four subgroups defined with 60 years of age and gender (log-rank test, *P*=0.001). Female patients with HBsAg seroclearance at 60 years old or younger had minimal risk of HCC up to 5 years, with a 5-year cumulative incidence rate of 0.1% (0% to 0.7%). The risk was the highest in male patients who cleared HBsAg after 60 years old; the cumulative incidence rates of HCC at 1, 3 and 5 years after HBsAg seroclearance were 1.2% (95% CI: 0.7% to 2.1%), 1.9% (1.2% to 3.0%) and 2.4% (1.5% to 3.9%), respectively. The other two subgroups, female patients with HBsAg seroclearance after 60 years old and male patients with HBsAg seroclearance at 60 years old or younger had intermediate and comparable risks of HCC. The cumulative incidence rates of HCC at 1, 3 and 5 years after HBsAg seroclearance were 1.3% (0.6% to 2.5%), 1.6% (0.8% to 3.1%) and 1.6% (0.8% to 3.1%), respectively in female >60 subgroup; the corresponding rates were 0.9% (0.6% to 1.5%), 1.4% (0.9% to 2.2%) and 1.6% (1.1% to 2.5%) in male ≤60 years old subgroup (Supplementary Figure 2B). On multivariable analysis, both age >60 years old and male gender were independent risk factors of HCC development in patients with HBsAg seroclearance; the adjusted HRs (95% CI) were 2.05 (1.20 – 3.51; *P*=0.009) and 2.56 (1.29 – 5.08; *P*=0.007), respectively.

DISCUSSION

This is one of the largest cohorts of CHB patients with HBsAg seroclearance aiming to examine the effects of gender on top of age and their interaction on the risk of HCC after HBsAg seroclearance. Our findings showed that CHB patients who achieve HBsAg seroclearance were associated with low yet definite risk of HCC, with a 5-year incidence of 1.5%. We further established male gender as an independent risk factor of HCC in patients

with HBsAg seroclearance on top of the well-established risk factor of age above 50 years. We demonstrated that female patients with seroclearance at 50 years or younger had zero risk of HCC up to 5 years. Male patients with seroclearance at 50 years or younger and female patients with seroclearance older than 50 years had mild and comparable risks of HCC, i.e. 5-year cumulative incidences of 0.7% and 1.0%, when compared to that of male patients with seroclearance older than 50 years, showing a 5-year incidence of 2.5%. The persistent risk of developing HCC in male patients with HBsAg seroclearance at 50 years or younger is most likely related to the presence of cirrhosis as reflected by the diagnosis codes, as well as the lower platelet count, higher total bilirubin, and lower serum albumin in the patients developed HCC when compared to other age and gender subgroups.

While the clearance of covalently closed circular DNA in CHB patients is almost impossible with current NA treatment [15], HBsAg seroclearance is regarded as a surrogate of ultimate immune control of HBV and a realistic antiviral treatment endpoint, as it leads to reduced risk of HCC [16-18]. However, the risk of HCC persists in patients achieved HBsAg seroclearance. One of the possible reasons is the integration of the viral DNA into host genome, leading to insertional mutagenesis and genomic instability [19]. The reactivation of the intrahepatic replicative activity of covalently closed circular DNA after HBsAg seroclearance to produce HBV pregenomic RNA for HBV replication acts as another reason for disease progression. On the other hand, the presence of cirrhosis is still a major risk factor for HCC ever after HBsAg seroclearance [5, 8]. NA-induced HBsAg seroclearance may have abolished the HCC risk in all female and young male patients; however a small risk still persist in male patients above 50 years old at the time of HBsAg seroclearance.

HCC surveillance is considered to be cost-effective when the annual risk of HCC exceeds 0.2% in CHB patient according to American Association for the Study of Liver Diseases (AASLD) guideline [20]. Our findings suggest that HCC surveillance can be omitted from female patients with HBsAg seroclearance at 50 years or younger, whereas it remains cost-effective over time in male patients with seroclearance older than 50 years old, who had an annual HCC incidence of 0.5%. These are in line with the recommendation of current guidelines on HCC surveillance. For CHB patients, AASLD guideline recommends that Asian males over age 40 and females over age 50 to receive HCC surveillance, while the AASLD, Asian Pacific Association for the Study of the Liver (APASL) and European Association for the Study of the Liver (EASL) guidelines recommend patients with cirrhosis to receive HCC surveillance [20-22]. Therefore, HCC surveillance may also be required in male patients who cleared HBsAg at 50 years or younger unless cirrhosis has been confidently excluded. In contrast, our findings show that a higher threshold of age for HCC surveillance may be considered for female who have lost HBsAg. The risk of HCC in female patients who cleared HBsAg at 60 years old or younger was so low that HCC surveillance may not be cost-effective, whereas HCC surveillance may be cost-effective in female patients with HBsAg seroclearance after 60 years old, whose annual HCC incidence was 0.32%. As our study could not clearly differentiate cirrhotic patients from non-cirrhotic patients, it is possible for patients without liver cirrhosis to have a much lower risk of HCC after HBsAg seroclearance and thus HCC surveillance may not be cost-effective.

CU-HCC score was originally derived to predict the risk of HCC for CHB patients using patients' age, HBV DNA level, albumin level, bilirubin level, and presence of cirrhosis. It has been validated in different cohorts of CHB patients, with or without receiving antiviral therapy, on the prediction of HCC development in 5 years [13, 14]. Our findings show that

CU-HCC score is still predictive on the development of HCC for patients who cleared HBsAg with an AUROC of 0.73. An apparently lower AUROC when compared to other validation cohorts of CHB patients may be a result of inaccuracy in the identification of cirrhosis, difference in disease course and lower incidence of HCC after HBsAg seroclearance. Adjustment on current HCC risk scores may be required to better capture the patients after HBsAg seroclearance on their risk of HCC development.

Our study has the strength of a large sample size and the provision of clinically important data on the impact of gender on top of age on the risk of HCC after HBsAg seroclearance. We also adopted stringent exclusion criteria to minimize bias. Data from real-life cohorts represent a spectrum of patients wider than those in randomized controlled trials, in which patients at multiple co-morbidities are often excluded, and are applicable to routine clinical practice. However, our study has a few limitations. First, we could not avoid missing data and irregular intervals of laboratory measurement as in other retrospective studies, though these biases can partly be compensated by our large cohort size. Second, we were not able to correctly identify cirrhotic patients in this analysis. In real-life clinical practice, physicians may use different criteria to diagnose liver cirrhosis, which may affect their coding of liver cirrhosis in the computer system. Liver biopsy is not a routine clinical practice, and liver stiffness measurement is not widely available across public hospitals in Hong Kong. We fully acknowledge that some of our patients who developed HCC after HBsAg seroclearance had underlying liver cirrhosis, but it can partly be reflected by their age of clearing HBsAg and male gender. Third, we did not have information on the HBV genotype of the patients. Previous studies have shown that the majority of CHB patients in Hong Kong have either genotype B or C HBV, and genotype C HBV is associated with a higher risk of HCC [23, 24]. Fourth, we did not have information on the menstruation status. The risk of HCC is known to

be lower in pre-menopausal females and rises after menopause [25]. A study in Hong Kong mentioned the median age of peri-menopausal female was 48 years, ranging from 40 to 60 years [26]. Therefore, the analysis on the risk of HCC for female patients with age over 50 and 60 years can partly reflect the impact of menopause on the risk of HCC development after clearance of HBsAg.

In conclusion, the results of this study have laid down some guidance on HCC surveillance for CHB patients achieved HBsAg seroclearance. Risk of HCC may still persist in CHB patients who achieved HBsAg seroclearance. HCC surveillance may not be necessary for female patients with HBsAg seroclearance at 50 years or younger, but may still be cost-effective for other patients.

REFERENCES

[1] Chan SL, Wong VW, Qin S, Chan HL. Infection and Cancer: The Case of Hepatitis B. J Clin Oncol. 2016;34:83-90.
[2] Chu CM, Liaw YF. Hepatitis B surface antigen seroclearance during chronic HBV infection. Antivir Ther. 2010;15:133-43.
[3] Yip TC, Wong VW, Tse YK, Chan HL, Wong GL. Hepatitis B surface antigen seroclearance in a cohort of 154,740 patients with chronic hepatitis B: a 15-year follow-up study. Hepatol Int. 2017;11:S75.
[4] Ahn SH, Park YN, Park JY, Chang HY, Lee JM, Shin JE, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol. 2005;42:188-94.
[5] Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Batrla-Utermann R, et al. Spontaneous seroclearance of hepatitis B seromarkers and subsequent risk of hepatocellular carcinoma. Gut. 2014;63:1648-57.
[6] Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology. 2010;51:1531-7.
[7] Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology. 2002;123:1084-9.
[8] Kim GA, Lim YS, An J, Lee D, Shim JH, Kim KM, et al. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. Gut. 2014;63:1325-32.
[9] Kim GA, Lee HC, Kim MJ, Ha Y, Park EJ, An J, et al. Incidence of hepatocellular carcinoma after HBsAg seroclearance in chronic hepatitis B patients: a need for surveillance. J Hepatol. 2015;62:1092-9.
[10] Liu F, Wang XW, Chen L, Hu P, Ren H, Hu HD. 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.
[11] Yang HI, Yuen MF, Chan HL, Han KH, Chen PJ, Kim DY, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol. 2011;12:568-74.
[12] Cheng MC, Tong YH, Kwok TC, Cheng IT, Chung AP, Leung JK, et al. Development Journey of Clinical Data Analysis and Reporting System (CDARS) in Hospital Authority of Hong Kong. MEDINFO. 2010:1468.
[13] Wong GL, Chan HL, Chan HY, Tse PC, Tse YK, Mak CW, et al. Accuracy of risk scores for patients with chronic hepatitis B receiving entecavir treatment. Gastroenterology. 2013;144:933-44.
[14] Wong VW, Chan SL, Mo F, Chan TC, Loong HH, Wong GL, et al. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. J Clin Oncol. 2010;28:1660-5.
[15] Trepo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet. 2014;384:2053-63.
[16] European Association For The Study Of The L. EASL Clinical Practice Guidelines: Management of chronic hepatitis B virus infection. J Hepatol. 2012;57:167-85.
[17] Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10:1-98.
[18] Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH, et al. AASLD guidelines for treatment of chronic hepatitis B. Hepatology. 2016;63:261-83.
[19] Levrero M, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. J

Hepatol. 2016;64:S84-101.
[20] Bruix J, Sherman M, American Association for the Study of Liver D. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53:1020-2.
[21] European Association For The Study Of The L, European Organisation For R, Treatment Of C. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56:908-43.
[22] Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int. 2010;4:439-74.
[23] Chan HL, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, et al. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut. 2004;53:1494-8.
[24] Wong GL, Chan HL, Yiu KK, Lai JW, Chan VK, Cheung KK, et al. Meta-analysis: The association of hepatitis B virus genotypes and hepatocellular carcinoma. Aliment Pharmacol Ther. 2013;37:517-26.
[25] McGlynn KA, Sahasrabuddhe VV, Campbell PT, Graubard BI, Chen J, Schwartz LM, et al. Reproductive factors, exogenous hormone use and risk of hepatocellular carcinoma among US women: results from the Liver Cancer Pooling Project. Br J Cancer. 2015;112:1266-72.
[26] Li S, Ho SC, Sham A. Relationship between menopause status, attitude toward menopause, and quality of life in Chinese midlife women in Hong Kong. Menopause. 2016;23:67-73.

Table 1. Clinical characteristic at the time of hepatitis B surface antigen (HBsAg) seroclearance.

Clinical Characteristic	**All patients N=4,568**	**Age ≤50 N=1,314**	**Age >50 N=3,254**	***P* values[#]**	**Female N=1,694**	**Male N=2,874**	***P* values[*]**
Age (years)	56.7 ± 13.8	40.0 ± 7.6	63.5 ± 9.4	<0.001	55.8 ± 14.9	57.3 ± 13.2	0.001
Male sex (n, %)	2,874 (62.9)	769 (58.5)	2105 (64.7)	<0.001	-	-	-
Platelet (x10^9/L)	210.5 ± 78.5	222.0 ± 74.2	206.1 ± 79.6	<0.001	217.7 ± 78.4	206.2 ± 78.3	<0.001
Missing (%)	11.9	15.8	10.4		11.5	12.2	
Albumin (g/L)	41.4 ± 5.9	43.0 ± 4.9	40.9 ± 6.1	<0.001	41.2 ± 5.5	41.6 ± 6.1	0.029
Missing (%)	1.8	4.9	0.6		4.0	0.5	
Total bilirubin (μmol/L)	15.5 ± 29.3	14.6 ± 30.2	15.9 ± 28.9	0.213	12.3 ± 13.6	17.4 ± 35.1	<0.001
Missing (%)	1.8	4.9	0.5		3.9	0.5	
Alanine aminotransferase (IU/L)	21.0 (15.0-33.0)	20.0 (13.0-31.0)	22.0 (15.0-34.0)	0.001	18.0 (13.0-26.0)	24.0 (16.0-36.0)	<0.001
Missing (%)	1.7	4.8	0.5		3.8	0.5	
Follow-up duration (years)	3.4 (1.5-5.0)	4.9 (2.4-5.0)	3.0 (1.2-5.0)	<0.001	3.4 (1.5-5.0)	3.4 (1.5-5.0)	0.535
Antiviral therapy (n, %)							
NA prior HBsAg seroclearance	793 (17.4)	272 (20.7)	521 (16.0)	<0.001	232 (13.7)	561 (19.5)	<0.001
(PEG)-IFN prior HBsAg	60 (1.3)	34 (2.6)	26 (0.8)	<0.001	16 (0.9)	44 (1.5)	0.093

P values compared patients ≤50 years old with patients >50 years old.
* *P* values compared female patients with male patients.
Alanine aminotransferase and duration of exposure were expressed in median (interquartile range), whereas other continuous variables were expressed in mean ± standard deviation. Percentages were based on non-missing data.
NA = nucleos(t)ide analogue; (PEG)-IFN = (peg)interferon.

Table 2. Clinical characteristic of patients who developed hepatocellular carcinoma (HCC) after hepatitis B surface antigen (HBsAg) seroclearance.

Clinical Characteristic	**All HCC N=54**	**Male, age ≤50 N=5**	**Female, age >50 N=10**	**Male, age >50 N=39**	***P* values[#]**
Age (years)	63.3 ± 11.0	46.4 ± 3.9	70.2 ± 8.9	63.7 ± 9.9	-
Male sex (n, %)	44 (81.5)	-	-	-	-
Platelet (x10^9/L)	191.0 ± 106.5	146.6 ± 88.4	259.1 ± 166.1	178.3 ± 80.3	0.062
Missing (%)	5.6	0	0	7.7	
Albumin (g/L)	37.7 ± 7.4	34.5 ± 9.0	34.5 ± 7.1	39.0 ± 7.1	0.138
Missing (%)	0	0	0	0	
Total bilirubin (μmol/L)	40.8 ± 78.6	74.1 ± 121.4	43.4 ± 69.9	35.8 ± 75.8	0.596
Missing (%)	0	0	0	0	
Alanine aminotransferase (IU/L)	35.0 (19.0-47.3)	39.0 (19.5-50.3)	37.5 (20.3-48.0)	35.0 (19.0-48.0)	1.000
Missing (%)	0	0	0	0	
Follow-up duration (months)	3.7 (0.6-20.1)	4.0 (1.8-12.9)	0.9 (0.6-5.9)	6.7 (0.6-25.9)	0.485
Antiviral therapy (n, %)					
NA prior HBsAg seroclearance	5 (9.3)	0 (0)	0 (0)	5 (12.8)	0.347
(PEG)-IFN prior HBsAg	0 (0)	0 (0)	0 (0)	0 (0)	-

P values compared male patients of age ≤50, female patients of age >50 and male patients of age >50.
Alanine aminotransferase and duration of exposure were expressed in median (interquartile range), whereas other continuous variables were expressed in mean ± standard deviation. Percentages were based on non-missing data.
NA = nucleos(t)ide analogue; (PEG)-IFN = (peg)interferon.

FIGURE LEGENDS

Figure 1. Patient flowchart. HBsAg = hepatitis B surface antigen; CHB= chronic hepatitis B; HCV = hepatitis C virus; HDV = hepatitis D virus; NA = nucleos(t)ide analogue.

Figure 2. Kaplan–Meier analysis of HCC in A. entire cohort; B. age ≤50 versus >50 years; C. male versus female; D. four subgroups based on age and gender; E. four subgroups in patients with spontaneous HBsAg seroclearance; and F. four subgroups in patients with nucleos(t)ide analogue-induced HBsAg seroclearance. HBsAg = hepatitis B surface antigen; HCC = hepatocellular carcinoma.

Figure 1.

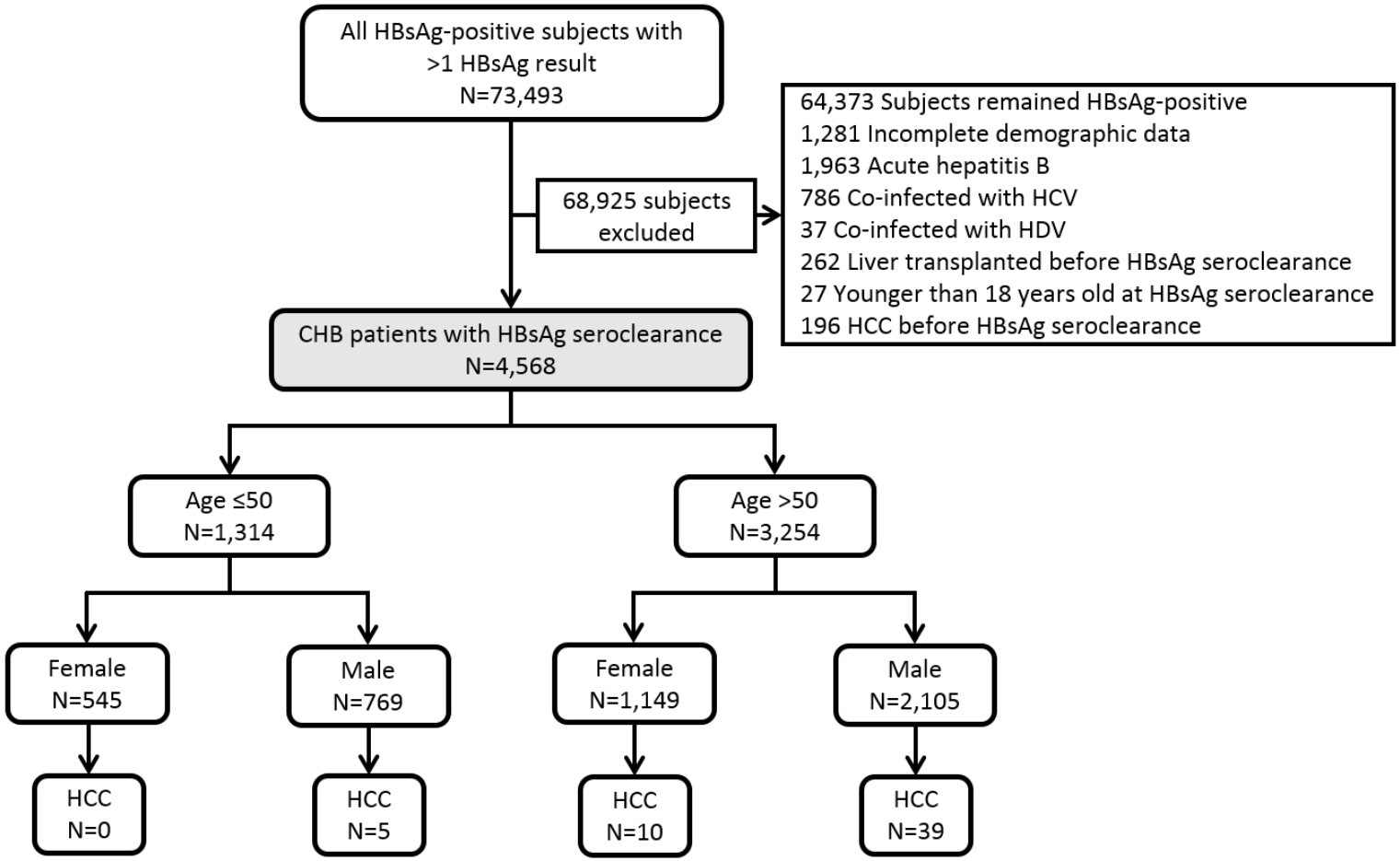
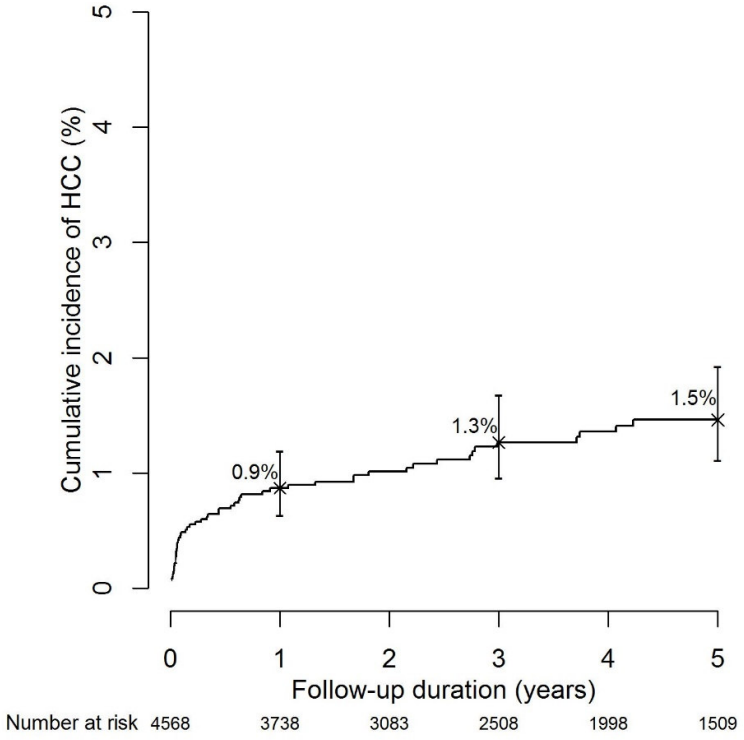


Figure 2A.



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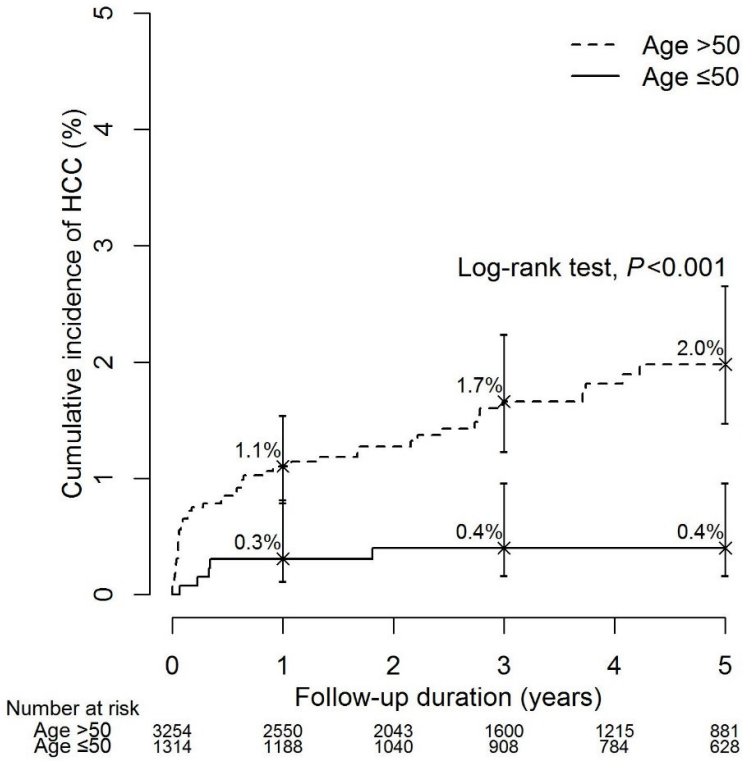

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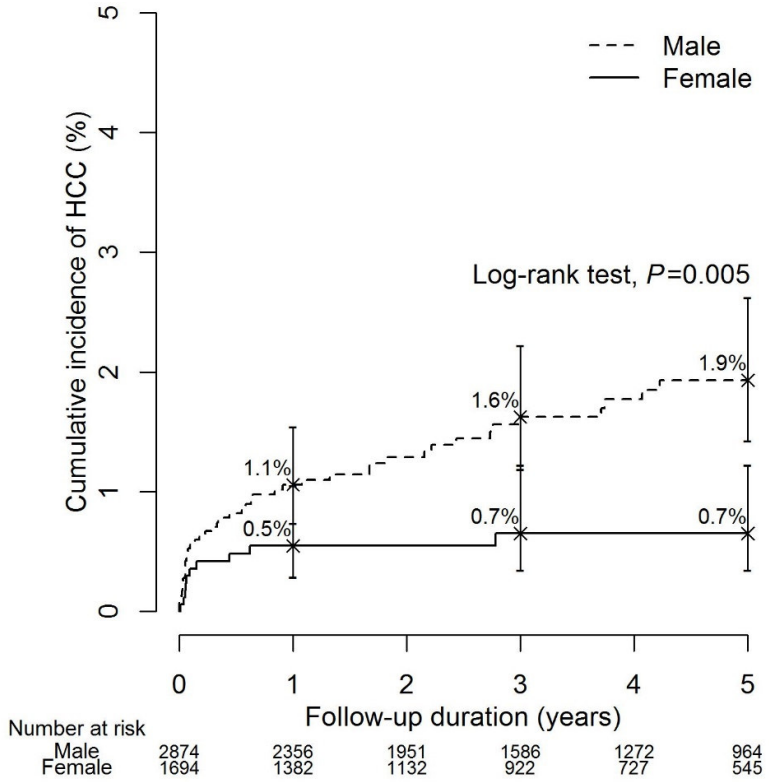

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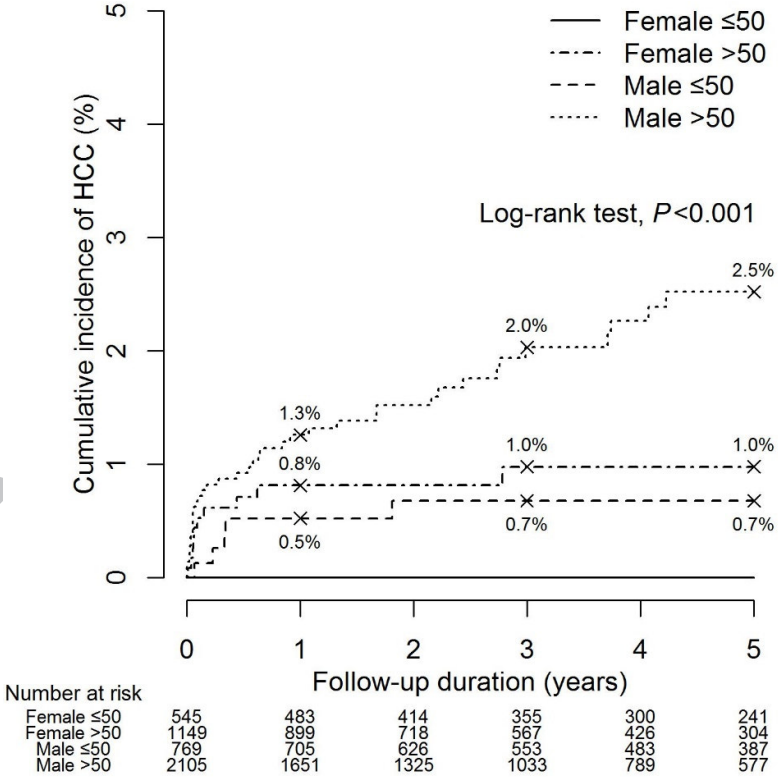

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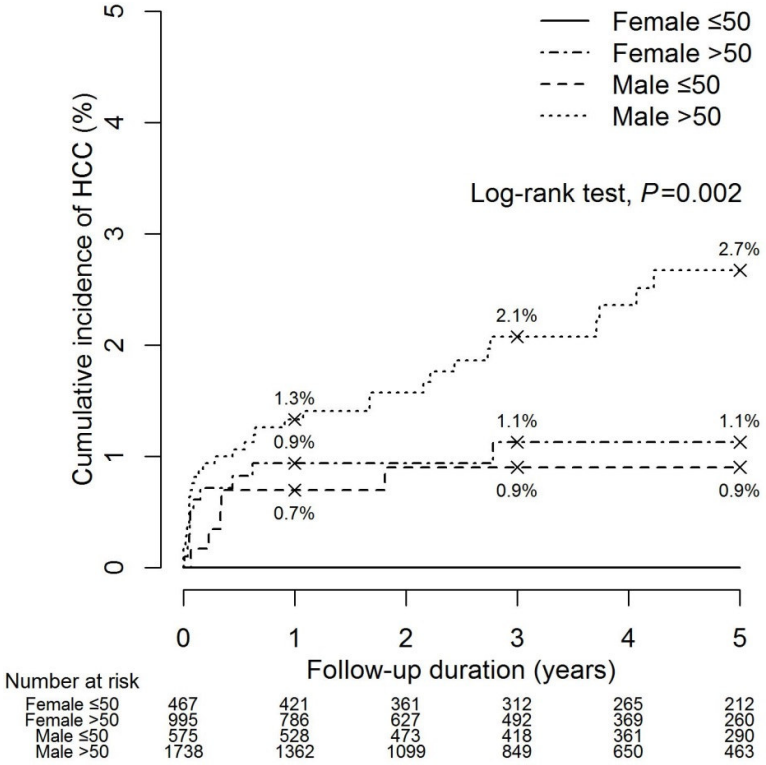

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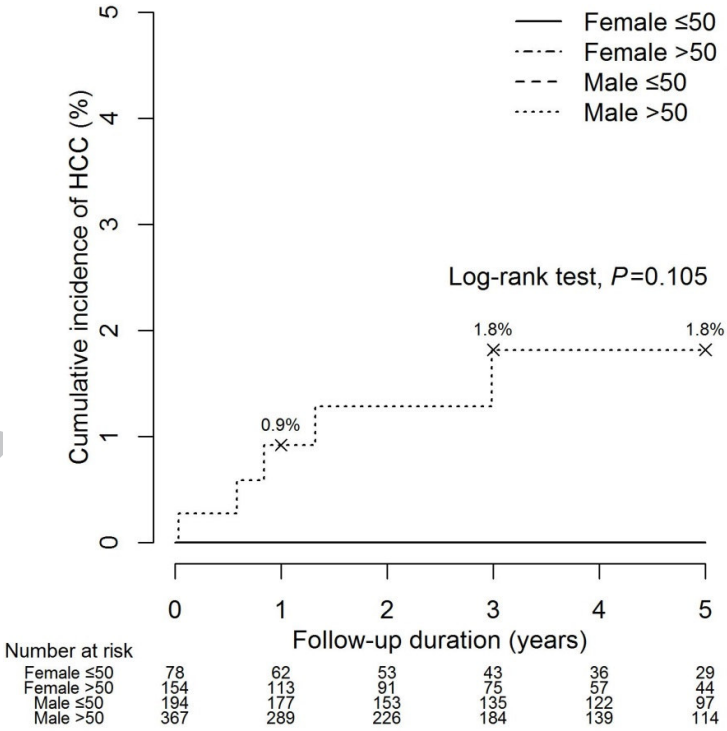

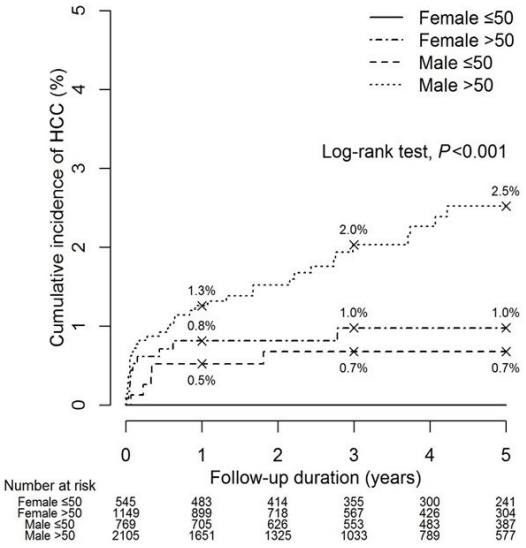

- 4,568 patients with HBsAg seroclearance were identified.
- At a median follow-up of 3.4 years, 54 patients developed HCC.
- Cumulative incidence of HCC at 1, 3 and 5 years were 0.9%, 1.3% and 1.5%, respectively.
- Age above 50 years (adjusted hazard ratio 4.31, 95% confidence interval 1.72 – 10.84; *P*=0.002) and male gender (2.47, 1.24 – 4.91; *P*=0.01) were two independent risk factors of HCC.
- Female patients aged ≤50 years (n=545) had zero risk of HCC in 5 years.
- Male patients aged ≤50 years (n=769), female patients aged >50 years (n=1,149) and male patients aged >50 years (n=2,105) had a 5-year cumulative incidence of HCC 0.7%, 1.0% and 2.5%, respectively.
- Similar findings were observed in patients with spontaneous and antiviral treatment-induced HBsAg seroclearance.

Highlights

- In patients with seroclearance of hepatitis B surface antigen (HBsAg), age above 50 years and male gender were two independent risk factors of hepatocellular carcinoma (HCC).
- Female patients aged 50 years or below had zero risk of HCC in 5 years.
- Female patients aged above 50 years and all male patients are still at risk of HCC.