Long Term Outcomes and Predictive Scores for Hepatocellular Carcinoma and HBsAg Seroclearance after HBeAg Seroclearance

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Abbreviations:

HBV, hepatitis B virus

CHB, chronic hepatitis B

HBeAg, hepatitis B e-antigen

ESC, e-antigen seroclearance

HCC, hepatocellular carcinoma

AFP, alpha-fetoprotein

LLOD, lower limit of detection

ROC, receiver operating characteristics

AUROC, area under receiver operating characteristics

LOOCV, leave-one-out cross validation

ULN, upper limit of normal

AASLD, American Association for the Study of Liver Diseases

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Abstract:

The significance of hepatitis B e-antigen seroclearance (ESC) in the long term is not well defined. The current study aimed to determine the clinical outcomes, the factors and predictive scores for hepatocellular carcinoma (HCC) and hepatitis B surface antigen (HBsAg) seroclearance of a large cohort of patients undergoing ESC. Patients with documented ESC were followed up 3-6 monthly. Baseline characteristics and longitudinal laboratory results were recorded. Predictive scores for HCC (HCC-ESC) and HBsAg seroclearance (HBsAg-ESC) were derived from multivariate Cox regression models. A total of 723 patients underwent ESC with a median ESC age and follow-up of 36.0 and 18.3 years respectively. Only 3.5% and 3.0% had persistently normal ALT and HBV DNA <2logs IU/mL respectively after ESC. For patients with 100%, 100-90%, 90-50%, 50-10%, 10-0%, and 0% normal ALT after HBeAg seroclearance, the rate of HCC was 4.3%, 2.2%, 3.6%, 3.9%, 17.3%, and 37.2% at 20 years after ESC respectively ($p<0.001$). At 20 years after ESC, the cumulative incidence of HCC and HBsAg seroclearance was 7.9% and 13.5% respectively, with an overall survival of 91.5%. ESC age, male sex, cirrhosis, hypoalbuminemia, viral load, and ALT were significant factors for HCC, whereas ESC age, male sex, viral load, and antiviral therapy were significant factors for HBsAg seroclearance. The AUROC for HCC-ESC and HBsAg-ESC scores to predict HCC and HBsAg seroclearance at 20 years after ESC was 0.92 and 0.74 respectively. **Conclusions:** Male gender, older age at ESC, ALT, and higher level of HBV DNA were associated with higher rates of HCC after ESC. HCC-ESC and HBsAg-ESC predictive scores can determine the likelihood of developing HCC and achieving HBsAg seroclearance.

With an estimated 257 to 284 million people infected with the hepatitis B virus (HBV), chronic hepatitis B (CHB) continues to be a significant health burden in many parts of the world, where infection remains at an endemic rate (1, 2). There are typically several different phases in the natural history of CHB infection (3, 4). The hepatitis B e-antigen (HBeAg)-positive chronic HBV infection is characterized by a positive HBeAg, very high viral load, and with minimal to no hepatitic activity (5, 6). In the HBeAg-positive CHB phase, the patients are still HBeAg positive, but with fluctuating viral load and liver enzymes indicating underlying hepatic inflammation (7). Eventually, patients will undergo HBeAg seroclearance (ESC) to enter the HBeAg-negative chronic HBV infection phase, where the viral load is modest to low with minimal to no clinical evidence of hepatitis (8). In the HBeAg-negative CHB phase, patients may have fluctuating moderate-to-high HBV DNA levels and fluctuating or persistently elevated ALT levels. The hepatitis B surface antigen (HBsAg)-negative phase is characterized by loss of HBsAg with or without the development of anti-HBs.

The important milestone of ESC has traditionally been viewed as a treatment endpoint and a measurement of treatment success for HBeAg positive subjects (9). It is still regarded by major regional treatment guidelines as a parameter for stopping antiviral therapy. However, a significant proportion of patients who undergo ESC may progress to the HBeAg negative CHB phase, with high fluctuating viral load and evidence of active hepatitis (10, 11). Furthermore, a significant proportion of patients will develop liver cirrhosis and hepatocellular carcinoma (HCC) after ESC even with low HBV DNA levels and minimally raised ALT levels while remaining HBeAg negative (12).

Therefore, despite being an important milestone in the natural history of CHB infection, the significant and clinical events after ESC need to be defined more accurately. The current study aimed to determine the clinical outcomes of a large cohort of patients who had undergone ESC, and to determine factors associated with hepatitis flares, persistent viremia, development of HCC, and mortality after ESC. We also aimed to derive predictive scores for the probability of HCC and HBsAg seroclearance using this large cohort based study.

Patients & Methods:

As part of the Hong Kong Chronic Liver Disease study, all records of patients seen at the Liver and Hepatitis Clinics at Queen Mary Hospital, Hong Kong, from January to December 2004 were reviewed (13). Of the 6,430 patients, 6,106 had evidence of chronic liver disease, of which 5,460 had CHB. Those that had significant alcoholic intake and hepatitis C infection or other chronic liver diseases were excluded. In 2007, patients that were still actively followed up were reviewed for evidence of ESC during their follow-up period for recruitment into the current study. Of the initial 5,460 CHB patients, 871 were lost to follow up or referred out upon requests from patients because of geographical reasons. Another 39 patients passed away, of which 5 were HBeAg-positive at presentation (and remained positive at the time of death), and 34 were HBeAg-negative at presentation. A total of 4,550 patients were actively followed up in 2007, of which 3,204 were already HBeAg-negative at the time of first presentation (without documented time of ESC). In the remaining 1,346 patients who were HBeAg-positive at the time of presentation, 623 patients remained

HBeAg-positive at the time of study recruitment. The remaining 723 HBeAg-positive patients underwent ESC during the follow-up period and were included in the current study. The flow of patients is shown in figure 1.

For the dataset, basic demographic parameters, and longitudinal laboratory results including routine liver biochemistry and HBV serology were recorded. Clinical outcomes including hepatitis flares, the development of HCC, HBsAg seroclearance, and liver-related mortality were also recorded.

Patient monitoring

Patients with CHB were followed up regularly at a 3-6 monthly interval, with routine liver biochemistry, HBV serology, and alpha-fetoprotein (AFP). Patients were also advised to have regular ultrasound surveillance at 6 monthly intervals. Cirrhosis was defined clinically on ultrasonographical features including small-sized liver, nodular surface, and splenomegaly, with or without the manifestations of portal hypertension.

Viral load

HBV DNA levels were measured using the Cobas Taqman assays (Roche Diagnostics, Branchburg, NJ), with a lower limit of detection (LLOD) of 12 IU/mL initially and 20 IU/mL subsequently as provided by the manufacturer in the later version of the test, with the latter adopted for statistical analysis. Measurements of HBV DNA were not routinely funded, and performed at the discretion of the attending physicians. For this study, only HBV DNA measurements performed in treatment-naïve patients or prior to commencing antiviral therapy were

included. On-treatment HBV DNA measurements were not included for analysis, and were censored at the time of starting antiviral therapy.

Core promoter mutation analysis and genotyping

The core promoter mutation and not precore mutation has been shown previously to be associated with adverse clinical significant outcomes (14, 15). Therefore, only the core promoter mutation and genotype was determined on available samples at 3-24 months after ESC. The method of HBV core promoter detection was described in our previous study (16). The presence of core promoter mutations was denoted by the presence of the A1762T-G1764A double mutations. Genotyping was performed by phylogenetic comparison of the sequences from these amplicons with HBV reference sequences using the NCBI HBV genotyping tools (17).

Antiviral therapy

As the antiviral medications were largely self-funded prior to 2009, the decision for treatment and the type of antiviral agent used was at the discretion of the patients. The mainstay of therapy was oral nucleos(t)ide analogs, with the use of interferon being mostly at the time prior to the availability of oral antiviral therapy. As a result, only 2 patients received pegylated interferon. The recommendation to treat was based on available treatment guidelines at the time(18, 19), and later from the major regional guidelines (20), and also from available clinical trials during the study period. Patients were continued on antiviral therapy after ESC, and cessation of therapy was at the patient's own discretion. If the patients do decide to stop therapy, it will be on the

recommendation of at least 1 year of consolidative therapy after ESC together with persistently undetectable viral load and normal ALT.

This study was approved by the Institutional Review Board/Ethics Committee of The University of Hong Kong/Western Cluster of Hospital Authority, Hong Kong. As this was a review of clinical data without intervention, the need for informed consent was waived. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Statistical analysis

All statistical analyses were performed using SPSS and R version 3.2.3 (R Foundation for Statistical Computing) statistical software. Mann-Whitney test was used for comparing two continuous variables with skewed distribution. Kruskal-Wallis test was used for comparison between three or more variables. The cumulative incidence of liver-related events was analyzed using the Kaplan-Meier method, with log-rank testing between different variables. A P value of <0.05 was considered statistically significant.

Predictive scores for the two outcomes (HCC and HBsAg seroclearance) were formulated as a weighted sum of the significant independent variables. The weights were assigned based on the corresponding estimated coefficients derived from multivariable Cox regression models after being divided by the smallest coefficient and rounded to the nearest integer. The predictive accuracy of the two scores for predicting the development of the corresponding outcomes at 5, 10 and 20 years was assessed by estimating a time-dependent receiver

operating characteristics curve (ROC) by the nearest neighbor estimation method. The area under ROC (AUROC) was then calculated with the 95% confidence interval (95% CI) obtained by sampling the original group of patients for 1000 bootstrap samples. To assess the performance of the two scores in new data, they were assessed by the leave-one-out cross-validation (LOOCV). In brief, one patient was dropped from the cohort each time to re-determine the weights for calculating the scores for that particular patient, and the process was repeated for other patients. Cut-off values for predicting HCC and HBsAg seroclearance at 5, 10 and 20 years were derived by maximizing the Youden index (sensitivity – [1 – specificity]) from the time-dependent ROC analysis. Predictive accuracy using the optimal cut-off value at each time point was assessed by the sensitivity, specificity, predictive values and likelihood ratios. Similarly, the 95% CIs were derived by 1000 bootstrap samples, and the cut-off values were assessed by the LOOCV (supplementary table 1).

Results

A total of 723 patients were included (figure 1). At the time of initial consultation, the median age was 32 years (range, 18-83), with a median age of ESC of 36 years (range, 19-85). The median follow-up length was 18.3 years (range, 2.8-32.9), totaling 13,827 patient-years. The patient characteristics and laboratory values at the time of ESC are summarized in table 1.

During the entire follow-up period, 296 (40.9%) patients remained treatment-naïve, while the remaining 427 (59.1%) received antiviral therapy. Fifty (7%) patients received antiviral therapy prior to ESC (but were not on therapy at the

time of ESC), of which 41 had interferon-based therapy, and 10 patients received lamivudine for a limited duration. Of the 41 patients who received interferon therapy prior to ESC, only 1 patient received pegylated interferon, and 7 patients achieved ESC within 1 year after stopping therapy.

One hundred and sixty nine (23%) were on therapy at the time of ESC, of which the majority was receiving lamivudine. After ESC, 231 (32%) required commencement of antiviral therapy, of which some had already received therapy prior to HBeAg seroclearance. There were different time-points for the resumption of therapy, and some patients may have received more than one type of antiviral therapy. Therefore the cumulative number of treated patients at different time points might be higher than the absolute number of patients receiving antiviral therapy. A summary of antiviral therapy in this cohort is shown in table 2.

Serum ALT levels

The longitudinal profiles of all patients were analyzed, with a total pooled sample of 32,222 results. The upper limit of normal (ULN) ALT was defined as according to the American Association for the Study of Liver Diseases (AASLD) recommendations of 30 and 19 U/L for males and females respectively (21). Only 3.5% had completely normal ALT after ESC, and 10.1% had normal ALT at ≥90% of the follow-up period after ESC. Conversely, 10.1% had persistently elevated ALT after ESC, with 19.4% having abnormal ALT for ≥90% of the follow-up period (supplementary figure 1A).

The patterns of ALT after ESC were divided into four groups, with flares being defined as 3x ULN (absolute value: 90 IU/L for male; 57 IU/L for female). This cut-off was chosen because the normal ranges provided by the hospital laboratory was higher than the AASLD ULN. We regarded those with 2x ULN from the hospital laboratory as flares, which approximately equals to 3x ULN using the AASLD limits. The groups are defined as follows: 1 = persistently normal ALT, 2 = normal ALT with episodes of ALT up to <3x ULN, 3 = normal ALT with episodes of ALT ≥3x ULN, and 4 = persistently abnormal ALT. The majority of patients had either achieved normal ALT but with evidence of episodes of elevated ALT <3x ULN (group 2) and ≥3x ULN (group 3) (42.2% and 44.3% respectively, supplementary figure 1B). Overall, 73 (10.1%) of patients (group 4) did not achieve normal ALT after ESC.

Viral load

A total of 2,547 HBV DNA measurements were performed in 518 patients. Excluding those patients who were already on antiviral therapy at the time of ESC, 518/553 (94%) patients had HBV DNA measured after ESC, and 450 (81%) had at least two measurements. For those with two or more measurements, only 149 (33%), 69 (15%), and 13 (3%) had HBV DNA consistently <4 logs, <3 logs, and <2 logs IU/mL respectively. In a pooled analysis determining the trend of viral load over time, there was no significant difference in the median viral load at 1, 2, 3, 4, 5, 5-10, 10-15, 15-20, 20-25, and >25 years after ESC (3.67, 3.62, 3.52, 3.49, 3.50, 3.47, 3.37, 3.43, 3.21, 3.62, and 3.08 logs IU/mL respectively, p=0.116, supplementary figure 2).

Hepatocellular carcinoma

The cumulative incidence of HCC was 0.1%, 2.2%, 4.6%, 7.9% and 8.6% after 1, 5, 10, 20, and 30 years respectively after ESC, with a total of 44 cases. For patients who underwent ESC before age 30, 30-40, and over 40 years of age, the cumulative rate of HCC a was 1.2%, 1.6% and 11.7% respectively at 10 years post ESC, and 1.2%, 4.9%, and 20% respectively at 20 years post ESC ($p< 0.001$) (fig 2A). The median age of HCC development was 55 years.

To exclude the potential younger age bias for patients undergoing ESC at a younger age who might not have been followed up for a sufficiently long period, all patients age 50 years or older without liver-related complications at the time of last follow up were compared to those who developed HCC. A total of 413 non-complicated patients with a median age of 56 (range, 50-92) showed a significantly lower age of ESC compared to patients who developed HCC (39 vs 46 years respectively, $p<0.001$).

Using the previous ALT group stratification, there was a significantly higher rate of HCC for those who have persistently elevated ALT (group 4) (fig 2B). At 20 years post ESC, the cumulative rate of HCC was 37.2% for ALT group 4, compared to 4.3%, 3.4%, and 6.3% for ALT groups 1, 2, and 3 respectively ($p<0.001$). For those with 100%, 100-90%, 90-50%, 50-10%, 10-0%, and 0% normal ALT after ESC, the rate of HCC was 4.3%, 2.2%, 3.6%, 3.9%, 17.3%, and 37.2% at 20 years after ESC respectively, $p<0.001$, fig 2C). A significantly higher rate of HCC was observed in patients with higher HBV DNA. At 20 years post ESC, the rate of HCC was 11.1%, 1.2%, 0%, and 0% for those with HBV DNA >4, <4, <3,

and <2 logs IU/mL respectively (p=0.006) (fig 2D). There was a trend towards higher rate of HCC at 20 years after ESC for those with core promoter mutation compared to those without (10.4% vs 3.7% respectively, =0.051), and higher rates observed in genotype C compared to B (9.0% vs 1.4% respectively, p=0.048).

After multivariate analysis, age, male sex, cirrhosis, hypoalbuminemia, HBV DNA and ALT flares or persistently abnormal ALT remained significant factors for HCC development (table 3). The HCC-ESC score to predict the HCC risk for up to 20 years after ESC was calculated using age (years) + 20 * sex (male=1; female=0) + 29 * cirrhosis (presence=1; absence=0) + 5 * DNA (log IU/mL) + 31 * ALT group (flares or persistently abnormal ALT=1; otherwise=0) + 23 * hypoalbuminemia (<39 g/L, presence=1; absence=0), and with risk shown in fig 3A. With an optimal cut-off of 129, 121, and 114, the AUROC for predicting development of HCC at 5, 10 and 20 years after ESC was 0.95, 0.91, and 0.92 respectively (table 4).

HBsAg seroclearance

A total of 63 patients achieved HBsAg seroclearance, of which 46 had evidence of HBsAg seroconversion with the development of anti-HBs. The cumulative rate of HBsAg seroclearance was 1.1%, 3.3%, 13.5% and 23.4% at 5, 10, 20, and 30 years after ESC. There was no significant difference in the rate of HBsAg seroclearance between those who underwent ESC at age before 30, 30-40, and over 40 years (p=0.431). There was a significantly higher rate of HBsAg seroclearance for those who did not require oral nucleos(t)ide analog therapy

compared to those that received oral therapy (21.3% vs 4.3% respectively at 20 years post ESC, p<0.001). This may highlight the fact that those that do not require antiviral therapy may intrinsically have a better disease profile with more favorable virological and biochemical characteristics.

There was a significantly lower rate of HBsAg seroclearance for those who have persistently elevated ALT without achieving normal ALT (fig 4A). At 20 years post ESC, the cumulative rate of HBsAg seroclearance was 1.8% for ALT group 4, compared to 79.4%, 15.5%, and 9.8% for ALT groups 1, 2, and 3 respectively (p<0.001). By stratifying the proportion of normal ALT after ESC, there was a stepwise increase in HBsAg seroclearance with increasing percentage of normal ALT (fig 4B). For those with persistently normal ALT, 90 to <100%, 50 to <90%, 10 to <50%, >0 to <10%, and persistently abnormal ALT, the HBsAg seroclearance rate was 79.4%, 25.9%, 17.8%, 7.5%, 0%, and 1.8% respectively at 20 years after ESC (p<0.001, fig 4B). A higher rate of HBsAg seroclearance at 20 years post ESC was observed for those with core promoter mutation compared to those without (20.2% vs 8.2% respectively, p=0.048), with no difference observed between genotype B and C.

After multivariate analysis, age, male gender, viral load, and history of antiviral therapy remained significant factors associated with HBsAg seroclearance (table 3). The HBsAg-ESC score to predict HBsAg seroclearance up to 20 years was calculated using age (years) + 15 * sex (male=1; female=0) - 6 * DNA (log IU/mL) - 40 * history of treatment (presence=1; absence=0), with the chance shown in fig 3B). Using optimal cut-offs of 19, 17, and 12 to predict HBsAg seroclearance at

5, 10, and 20 years after ESC were associated with an AUROC of 0.88, 0.84, and 0.74 respectively (table 4).

Survival

The 5, 10, 20, and 30 year overall survival after ESC was 99.2%, 96.6%, 91.5%, and 88.3% respectively. Overall, 23 patients died of liver-related events (19 HCC and 4 decompensation), and 5 patients underwent liver transplantation for HCC. The cumulative liver-related mortality (including liver transplantation) at 5, 10, 20, and 30 years after ESC was 0.7%, 2.7%, 4.9%, and 6.5% respectively. Those that underwent ESC at age over 40 years had higher liver-related events compared to those that seroconverted at 30-40 years and below 30 years (15.7% vs 1.7% vs 0.6% respectively at 20 years after ESC, p<0.001, fig 4C).

Higher rates of liver-related mortality/liver transplantation were observed for ALT group 4 compared to 1, 2, and 3 (24.4% vs 4.3%, 3.1%, and 2.6% at 20 years post ESC respectively, P<0.001, figure 4D) and for those who had persistently elevated ALT compared to those who had >0 to <10%, 10 to <50%, 50 to <90%, 90 to <100%, and persistently normal ALT (25.6% vs 7.1% vs 2.6% vs 1.7% vs 2.2% vs 4.2% respectively, p<0.001). Significantly higher liver-related events were seen in those with cirrhosis compared to non-cirrhotics (24.6% vs 1.5% respectively, p<0.001). No difference was observed between patients treated with oral nucleos(t)ide analogs compared to those who did not receive oral antiviral therapy (p=0.904).

Discussion

The current study followed up a large cohort of 723 CHB patients from the time of ESC over a long period of up to 30 years, totaling 13,827 patient-years, with analysis of over 32,000 longitudinal blood results. Although the majority (88%) achieved normalization of ALT (with or without antiviral treatment), only a minority (5.3%) maintained persistently normal ALT after ESC. The viral load was likewise only low (<4 logs IU/mL) in 34%, with the majority having a higher viral load at some points after ESC. The overall proportion receiving antiviral therapy was 59.1%, with 31.8% commencing antiviral therapy after ESC. These results suggests that even after ESC, the overwhelming majority of patients will still have significant viremia and elevated transaminases necessitating antiviral therapy. The rate of HCC development is modest (8.6% at 30 years after ESC). Higher rates of HCC were observed in patients who were older at the time of ESC, had persistent elevated ALT and higher HBV DNA post ESC, or had underlying established cirrhosis. The overall rate of HCC should have been higher if there is no widespread use of long-term antiviral therapy in CHB patients. This highlights the importance of viral suppression with normalization of ALT.

Those with persistently normal ALT after ESC had a much more favorable outcome, including a low rate of HCC development, high rate of HBsAg seroclearance, and low liver-related mortality (2.9%, 70.3%, and 4.2% respectively at 20 years after ESC). In fact, those patients achieving normal ALT at some points after ESC (groups 1, 2 and 3) consistently had a more favorable outcome with significantly lower rates of HCC and liver-related mortality and higher rate of HBsAg seroclearance compared to those patients that never achieved normalization of ALT (group 4). The importance of ALT has been

established previously in another study of 3,233 patients showing that higher levels of ALT were at a greater risk of developing complications in Asian CHB patients (22).

Previous studies have also demonstrated the increased risks of developing HCC associated with higher levels of HBV DNA, although these studies were based on viral load at a single time point in community-based patients not on antiviral therapy (23, 24). The current study included patients who required antiviral treatment before and/or after ESC, and non-treated patient, within a tertiary care setting. In the current study, those with HBV DNA consistently less than 4 logs IU/mL had a lower rate of HCC development compared to those with levels above this threshold. The level of HBV DNA appeared to be stable over time from early after ESC to over 25 years. However, this may not be strictly representative given that 55.2% were on antiviral therapy at the time of ESC or commenced afterwards, and their viral load were censored at the time of antiviral therapy. Nevertheless, the study provides a longitudinal picture of viral load without treatment after ESC and their association with HCC development and HBsAg seroclearance.

After multivariate analysis, older age at ESC, male sex, higher HBV DNA, cirrhosis, hypoalbuminemia, and persistently abnormal ALT/flares were significant independent predictors for developing HCC. For HBsAg seroclearance, older age at ESC, male sex, lower HBV DNA levels and the absence of antiviral therapy were significant predictors. The development of the HCC-ESC and HBsAg-ESC predictive scores allows long-term risk stratification for patients for

up to 20 years, identifying patients with both favorable (HBsAg seroclearance) and unfavorable (HCC) profiles. For predicting HCC risk, the score can achieve a very high sensitivity and specificity values throughout 5 to 20 years. The high NPV, combined with an AUROC of ≥0.91 for up to 20 years after ESC provides reassurance for those within the optimal cut-off values, where the risk is minimal. Apart from the ESC age and gender, the remaining components of the HCC-ESC model are dynamic, and can be calculated accordingly during the follow up after ESC. Hence patients may not remain in the low risk category during their follow-up period if they develop cirrhosis, significant viremia, or hepatitis.

In addition to the new HCC-ESC score, the present study is the first to derive the HBsAg-ESC predictive score, which has very high sensitivity (100%) and specificity (76%) at year 5, and maintaining good accuracy even up to year 20. Both scores were validated by the stringent leave-one-out statistical analyses, with a high sensitivity and specificity of predictions at 5, 10, and 20 years. This is an important finding to allow clinicians and patients to have ideas on their chances of HBsAg seroclearance, the best achievable endpoint of treatment to date. It has been shown that cessation of treatment in patients on antiviral therapy after achieving HBsAg seroclearance was associated with negligible rate of viral rebound (25).

There are several limitations of the current study. Firstly, this is an observational cohort study and not a randomized controlled study. Therefore untreated patients may be biased to have more favorable virological and biochemical profile compared to those requiring antiviral therapy. This may account for the fact that there was no difference in HCC development between treated and non-

treated patients, and the higher rate of HBsAg seroclearance observed in non-treated patients. There was also a paucity of patients treated with pegylated interferon. Secondly, viral load was not performed at regular intervals, although the majority did have at least one measurement performed. Thirdly, the HBV genotype and core promoter mutation were not routinely performed and were not available for all patients. The precore mutation was also not performed, although previous studies have shown that it was not significantly associated with HCC or cirrhosis (14, 15, 26). Fourthly, the definition of cirrhosis was defined on ultrasound findings, which may not have identified patients with cirrhosis but without overt features on imaging. Finally, the HCC-ESC and HBsAg-ESC scores were derived in a single cohort. External validation should be performed by future studies, with collaboration from different centers to confirm the results of the current study.

In conclusion, persistent viremia and elevated ALT remained common after ESC. Male gender, older age at ESC, failure to normalize ALT, and higher levels of HBV DNA were associated with higher rates of HCC after ESC. The use of the HCC-ESC and HBsAg-ESC scores should provide clinicians with new tools to offer more guided management for CHB patients.

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Figure legends:

Figure 1. Patient flow and inclusion

Figure 2. Cumulative rate of hepatocellular carcinoma according to (A) age of HBeAg seroclearance, (B) ALT profile, (C) proportional of normal ALT, and (D) HBV DNA levels

Figure 3. Chance of (A) HCC and (B) HBsAg seroclearance at 5, 10, and 20 years after HBsAg seroclearance

Figure 4. Rate of HBsAg seroclearance according to (A) ALT profile and (B) proportion of normal ALT. Rate of liver-related mortality/transplantation according to (C) age of HBeAg seroclearance, and (D) ALT profile

'Table 1. Patient demographics and laboratory parameters

Parameter	Values
Cohort number (n)	723
Male sex, n(%)	438 (60.6%)
Age at baseline (years)	32 (18-83)
Age at HBeAg seroclearance (years)	36 (19-85)
Age at last follow-up (years)	53 (22-92)
Length of follow-up (years)	18.3 (2.8-32.9)
At time of HBeAg seroclearance	
Bilirubin (umol/L)	11 (1-360)
Alkaline phosphatase (U/L)	70 (27-172)
Gamma-glutamyl transferase (U/L)	25 (4-330)
Alanine aminotransferase (U/L)	32 (5-825)
Aspartate aminotransferase (U/L)	29 (2-570)
Albumin (g/L)	44 (26-60)

Continuous variables are expressed as median values (range).

Table 2. Antiviral therapy use at different phases of chronic hepatitis B

Treatment time-point and type of therapy	Value
Before HBeAg seroclearance *	
Total number of patients	50
Interferon-based therapy**	41
Lamivudine	10
On-treatment during HBeAg seroclearance***	
Total number of patients	169
Lamivudine	150
Interferon-based therapy	19
Entecavir	3
Adefovir	13
After HBeAg seroclearance****	
Total number of patients	231
Entecavir	172
Lamivudine	51
Telbivudine	10
Adefovir	14
Tenofovir	20

* Antiviral therapy given before but not at the time of HBeAg seroclearance

** 7 patients achieved HBeAg seroclearance within 1 year of stopping interferon

*** Antiviral therapy given before and during the time of HBeAg seroclearance

**** Antiviral therapy given after HBeAg seroclearance

Table 3. Multivariate analysis of factors associated with HCC and HBsAg seroclearance

Parameter	Adjusted HR	95% CI	P value
Development of HCC			
Age at HBeAg seroclearance	1.06	1.03 – 1.10	<0.001
Male sex	3.39	1.22 – 9.41	0.019
Cirrhosis	6.07	2.55 – 14.43	<0.001
Hypoalbuminemia	4.10	1.53 – 10.96	0.005
HBV DNA (log)	1.33	1.01 – 1.73	0.040
Normal with flares or persistently abnormal ALT	6.77	1.98 – 23.20	0.002
HBsAg seroclearance			
Age at HBeAg seroclearance	1.05	1.01 – 1.09	0.010
Male sex	2.03	1.02 1.02 – 4.03	0.0453
HBV DNA (log)	0.75	1.03 0.61 – 0.92	0.006
History of antiviral therapy	0.15	1.04 0.04 – 0.51	0.003

Table 4. Performance of risk scores for HCC and HBsAg seroclearance up to 20 years after HBeAg seroclearance

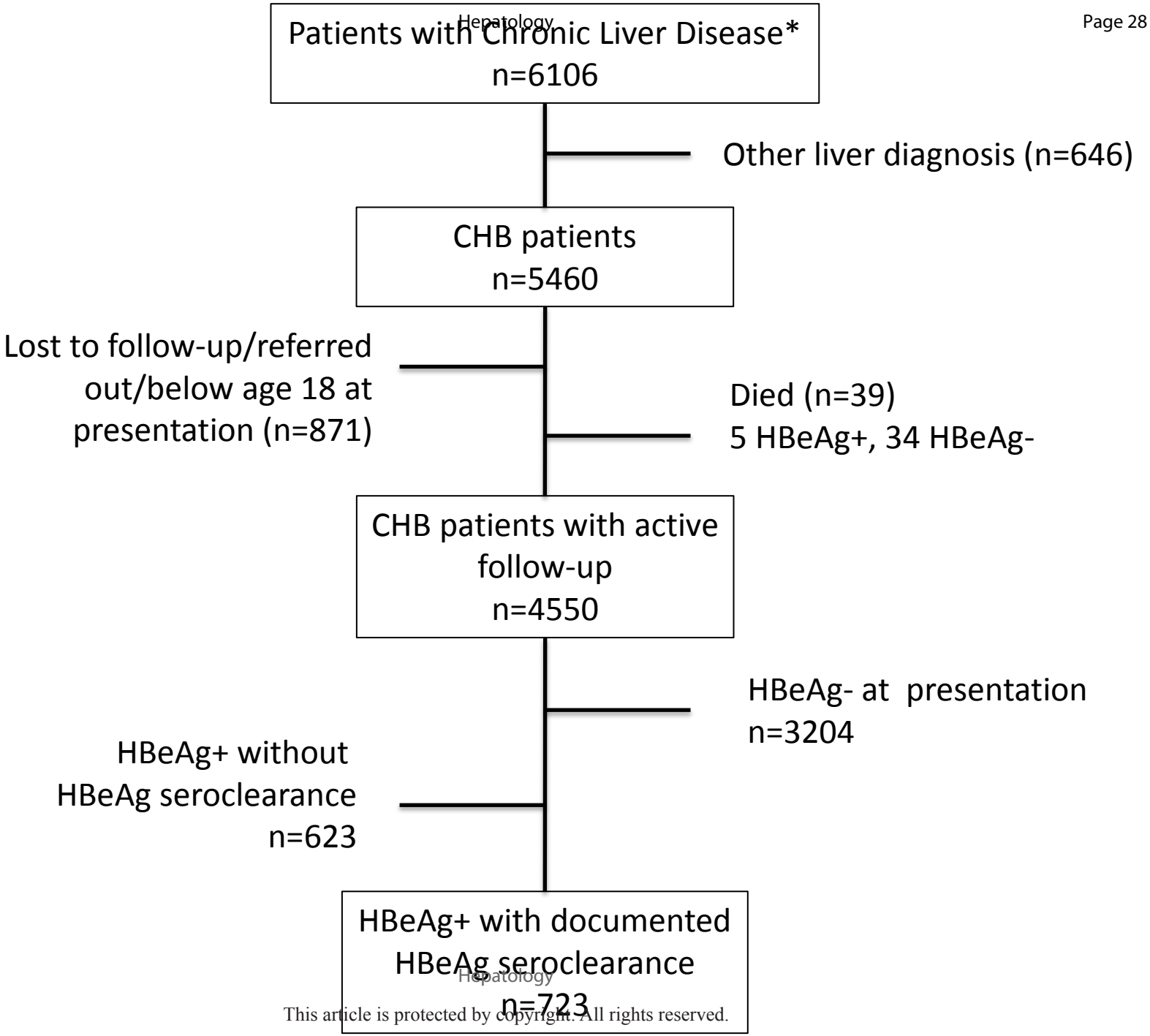
	5 years	10 years	20 years
HCC *			
Optimal cut-off	129	121	114
Sensitivity (%)	98.5 (96.6-100)	87.2 (72.6-99.8)	90.7 (78.7-99.7)
Specificity (%)	86.5 (83.1-90.5)	81.8 (67.0-88.8)	79.7 (68.1-90.3)
PPV (%)	12.6 (10.5-17.0)	16.5 (11.0-24.4)	26.6 (19.6-42.3)
NPV (%)	99.97 (99.9-100)	99.4 (98.7-99.99)	99.1 (98.1-99.96)
PLR	7.28 (5.90-10.30)	4.78 (3.00-7.82)	4.47 (3.01-9.04)
NLR	0.02 (0-0.04)	0.16 (0-0.32)	0.12 (0.01-0.24)
AUROC	0.95 (0.94-0.97)	0.91 (0.84-0.96)	0.92 (0.88-0.96)
HBsAg seroclearance**			
Optimal cut-off	19	17	12
Sensitivity (%)	100 (99.5-100)	83.0 (65.9-100)	72.0 (65.6-97.1)
Specificity (%)	76.1 (72.0-90.3)	74.3 (43.2-83.5)	68.0 (38.4-73.5)
PPV (%)	3.2 (2.7-7.5)	7.4 (4.1-11.2)	24.0 (17.4-29.4)
NPV (%)	100 (99.9-100)	99.4 (98.9-100)	94.5 (93.3-99.1)
PLR	4.18 (3.57-10.30)	3.30 (1.75-5.09)	2.25 (1.50-2.97)
NLR	0 (0.07)	0.23 (0-0.44)	0.41 (0.07-0.51)
AUROC	0.88 (0.82-0.96)	0.84 (0.75-0.92)	0.74 (0.65-0.81)

* HCC risk = age (years) + 20 * sex (male = 1; female = 0) + 29 * cirrhosis (presence =1; absence = 0) + 5 * DNA (in log IU/mL) + 31 * ALT group (flares or

persistently abnormal ALT = 1; otherwise = 0) + 23 * hypoalbuminemia (presence =1; absence = 0)

** HBsAg seroclearance = age (years) + 15 * sex (male = 1; female = 0) - 6 * DNA (in log IU/mL) - 40 * history of treatment (presence =1; absence = 0)

AUROC = area under receiver operating characteristic; NLR = negative likelihood ratio; NPV = negative predictive value; PLR = positive likelihood ratio; PPV = positive predictive value



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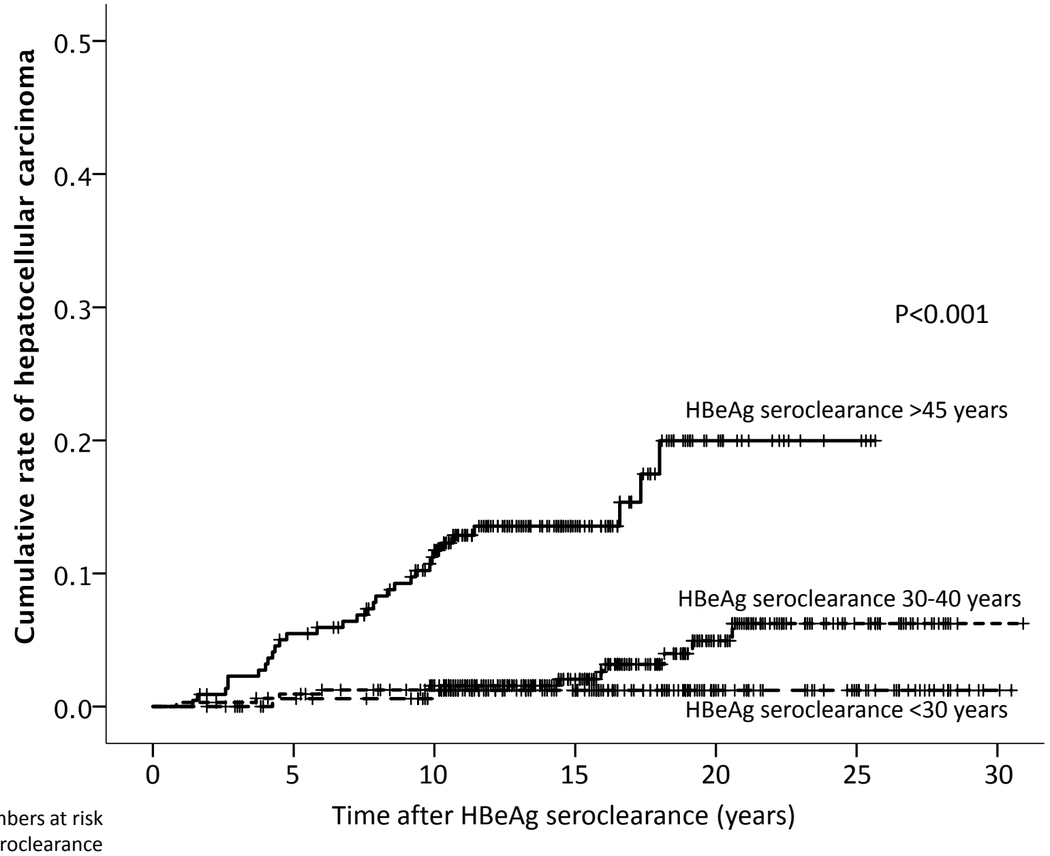
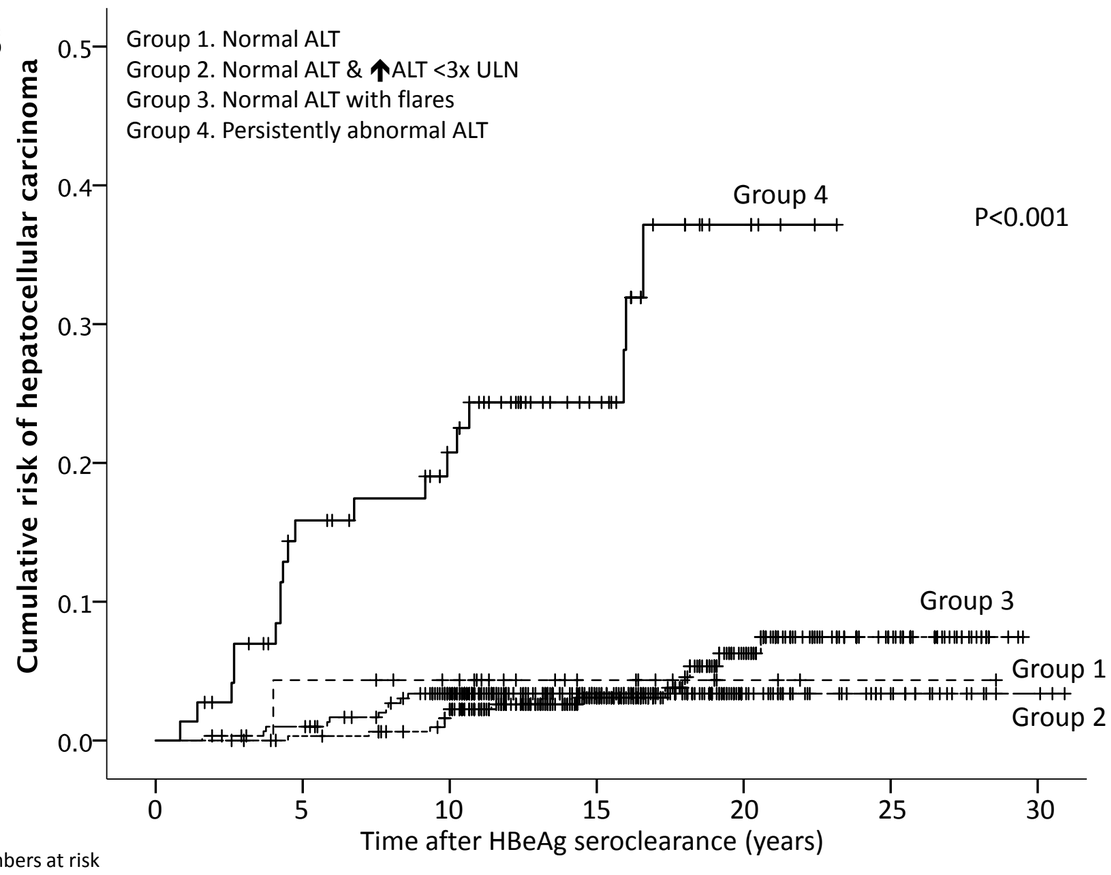


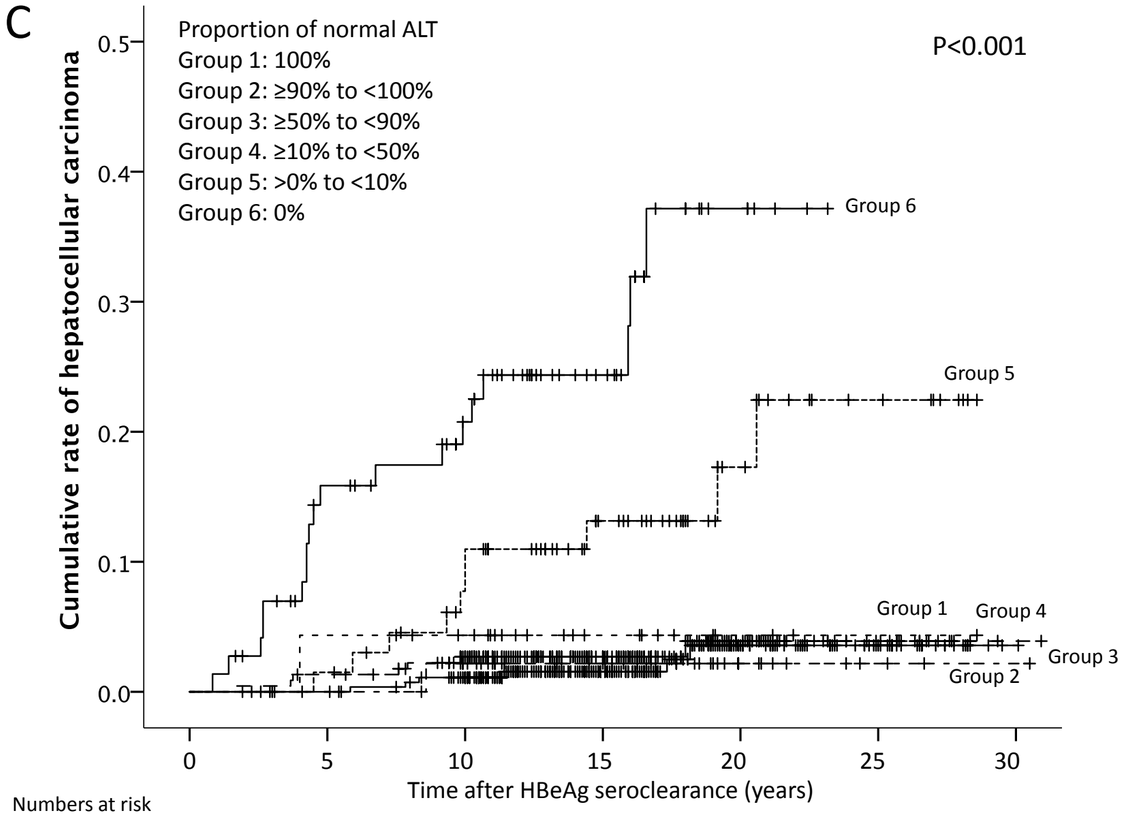
Numbers at risk Age of HBeAg seroclearance							
>45 years	221	206	172	66	18	4	
30-40 years	324	318	299	193	80	26	1
<30 years	178	169	158	106	46	26	2

B



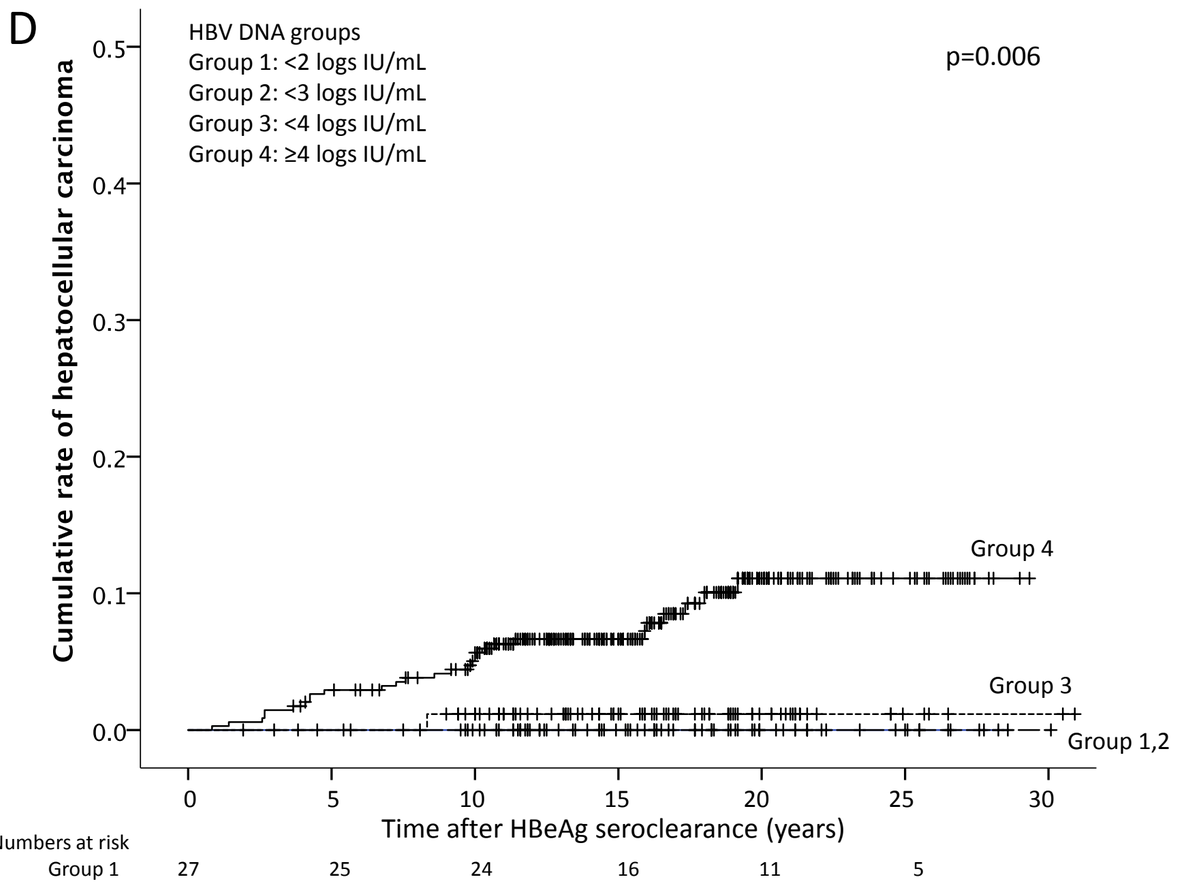
Numbers at risk							
Group 1	25	22	19	9	3	1	
Group 2	305	298	263	140	46	19	3
Group 3	320	317	303	193	89	36	
Group 4	73	56	45	24	6		

C

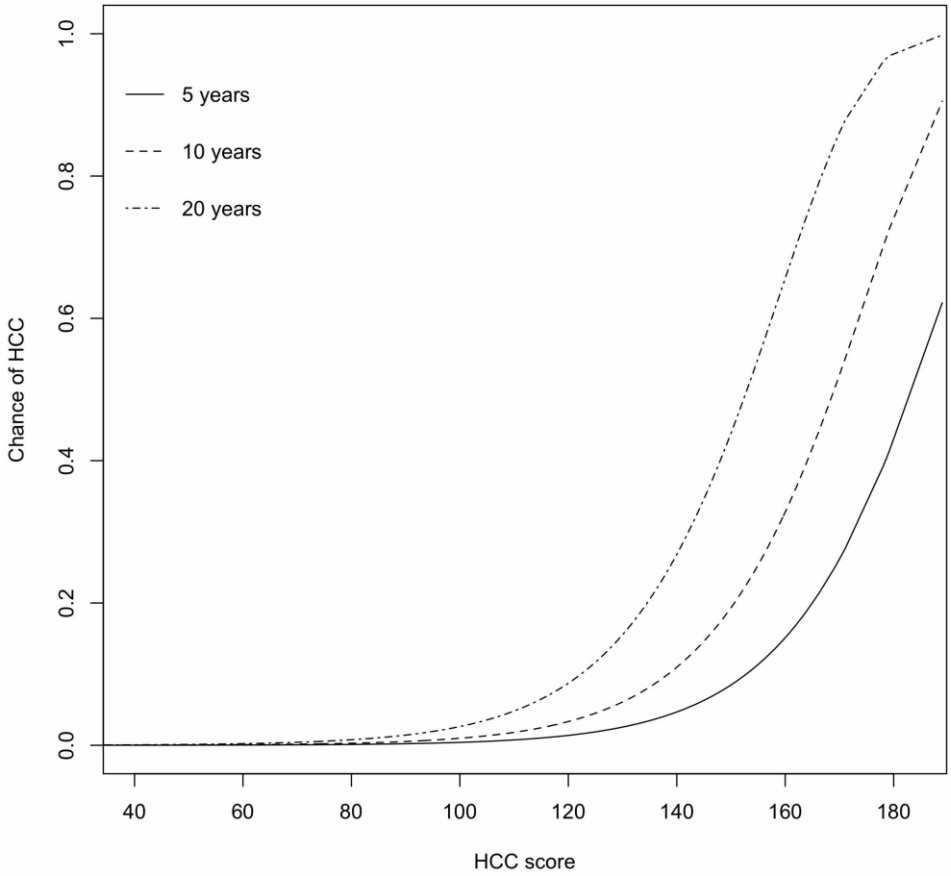


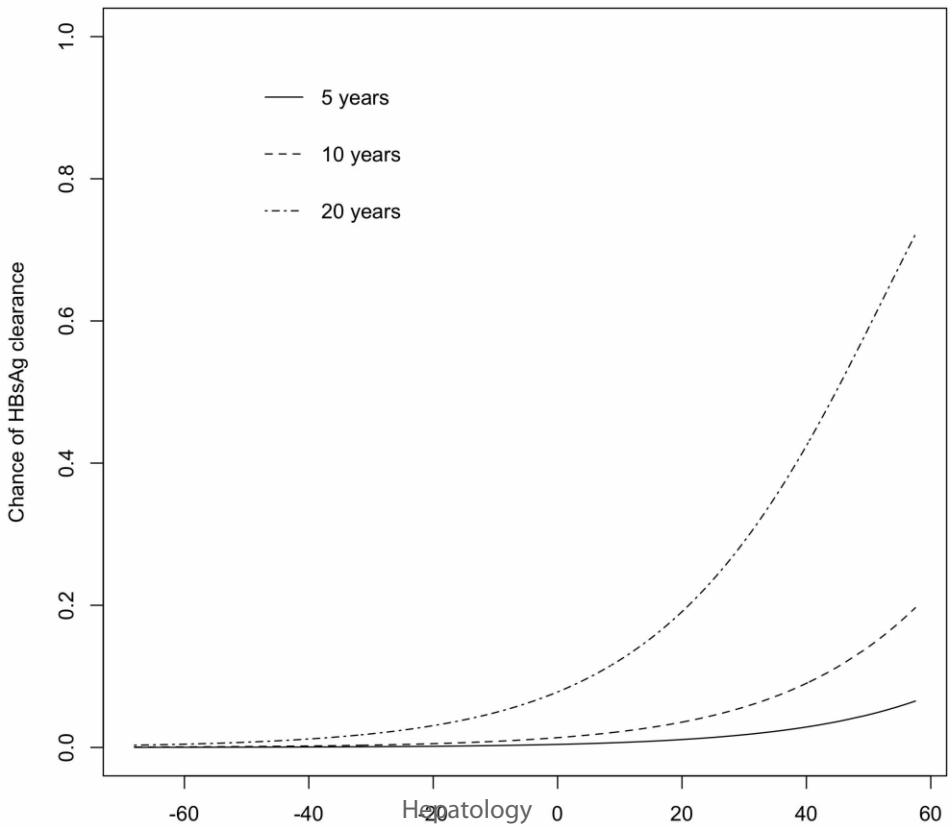
Numbers at risk							
Group 1	25	22	19	9	3	1	
Group 2	48	47	42	27	9	3	1
Group 3	283	279	263	146	56	25	1
Group 4	227	223	204	123	53	19	1
Group 5	67	66	56	37	17	8	
Group 6	73	56	45	24	6		

D



Numbers at risk							
Group 1	27	25	24	16	11	5	
Group 2	58	57	53	34	11	6	
Group 3	90	89	80	50	20	5	2
Group 4	343	330	307	178	67	23	





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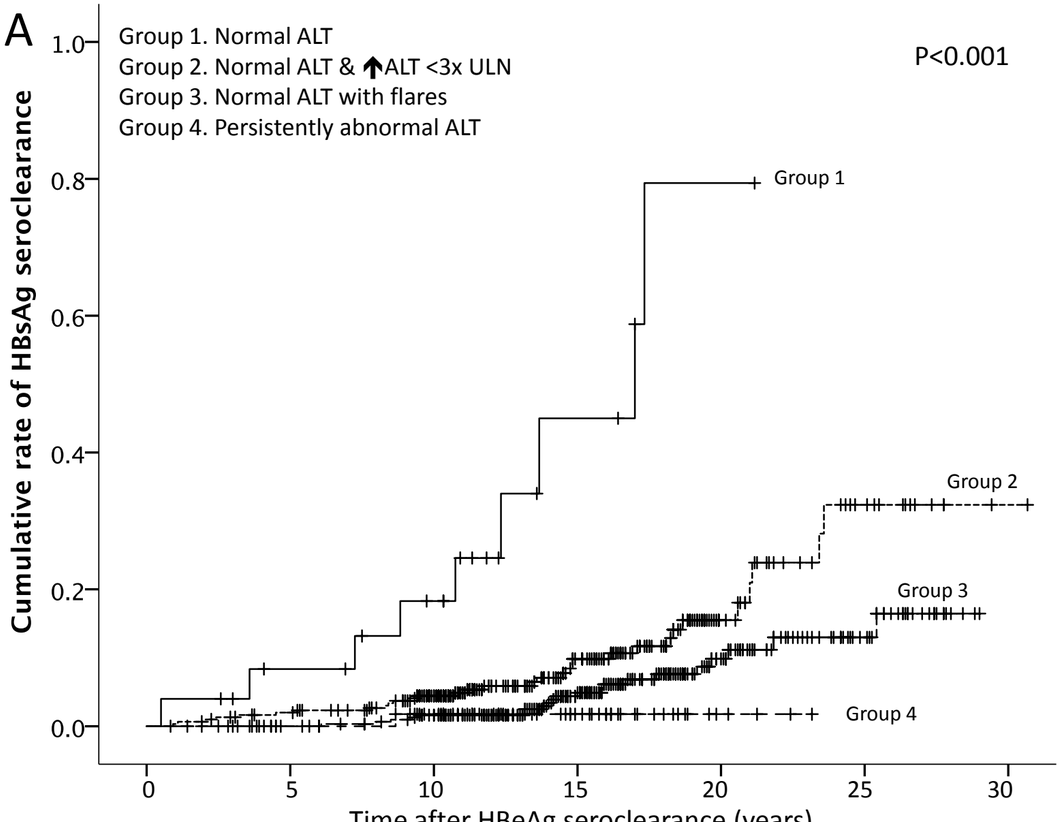
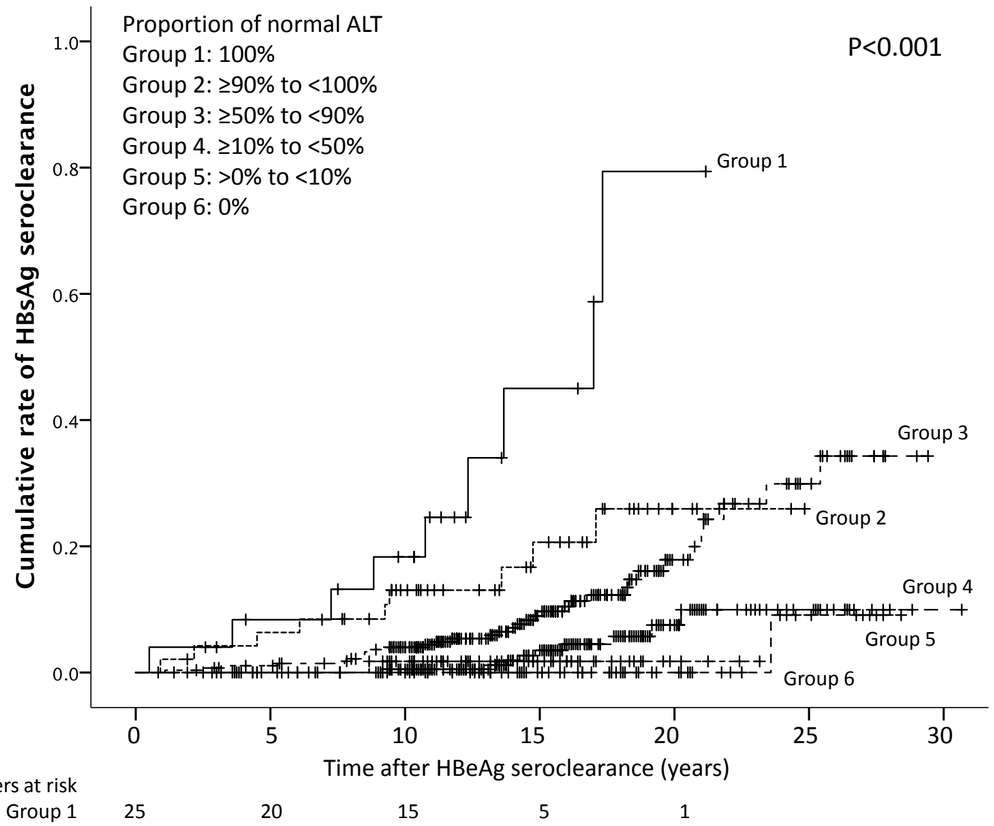


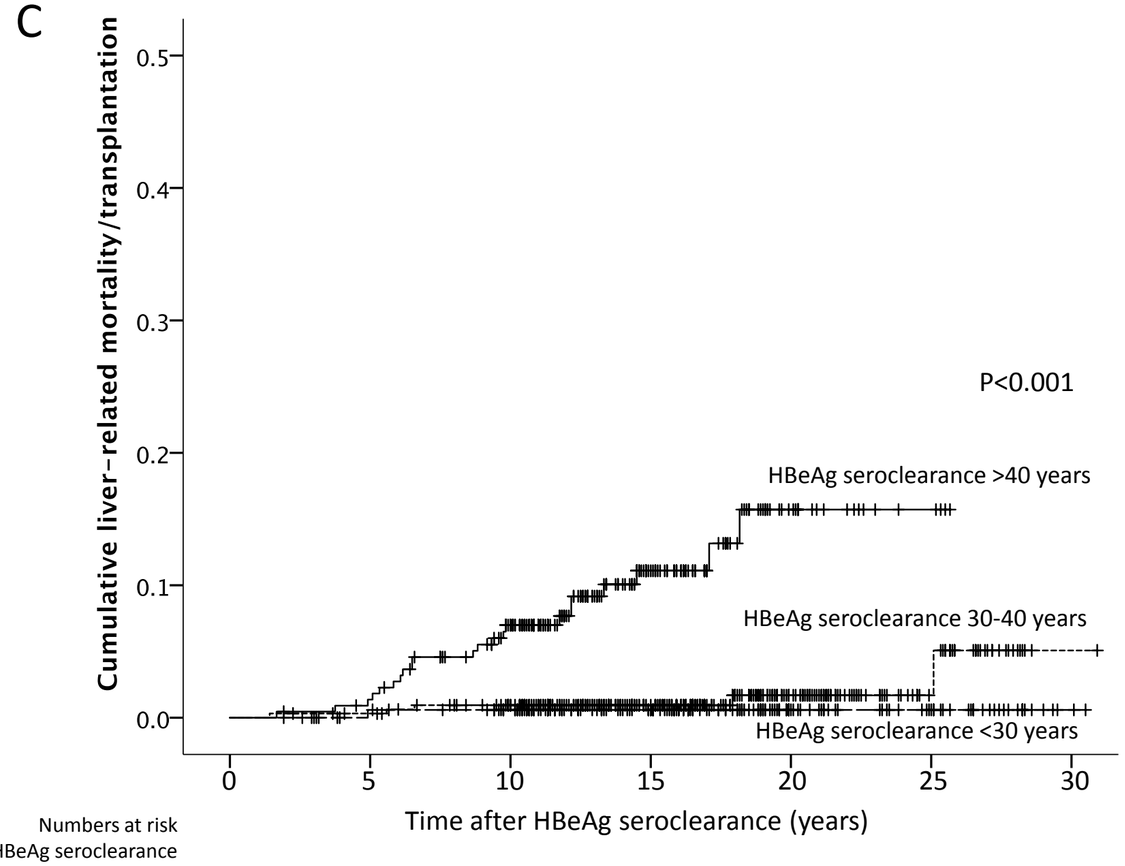
Numbers at risk							
Group 1	25	20	15	5	1		
Group 2	305	293	245	125	35	12	1
Group 3	320	317	297	180	74	28	
Group 4	73	59	50	24	4		

B



Numbers at risk							
Group 1	25	20	15	5	1		
Group 2	48	44	34	20	5		
Group 3	283	276	247	130	41	17	
Group 4	227	224	200	116	44	16	1
Group 5	67	66	56	37	17	8	
Group 6	73	59	50	24	4		

C



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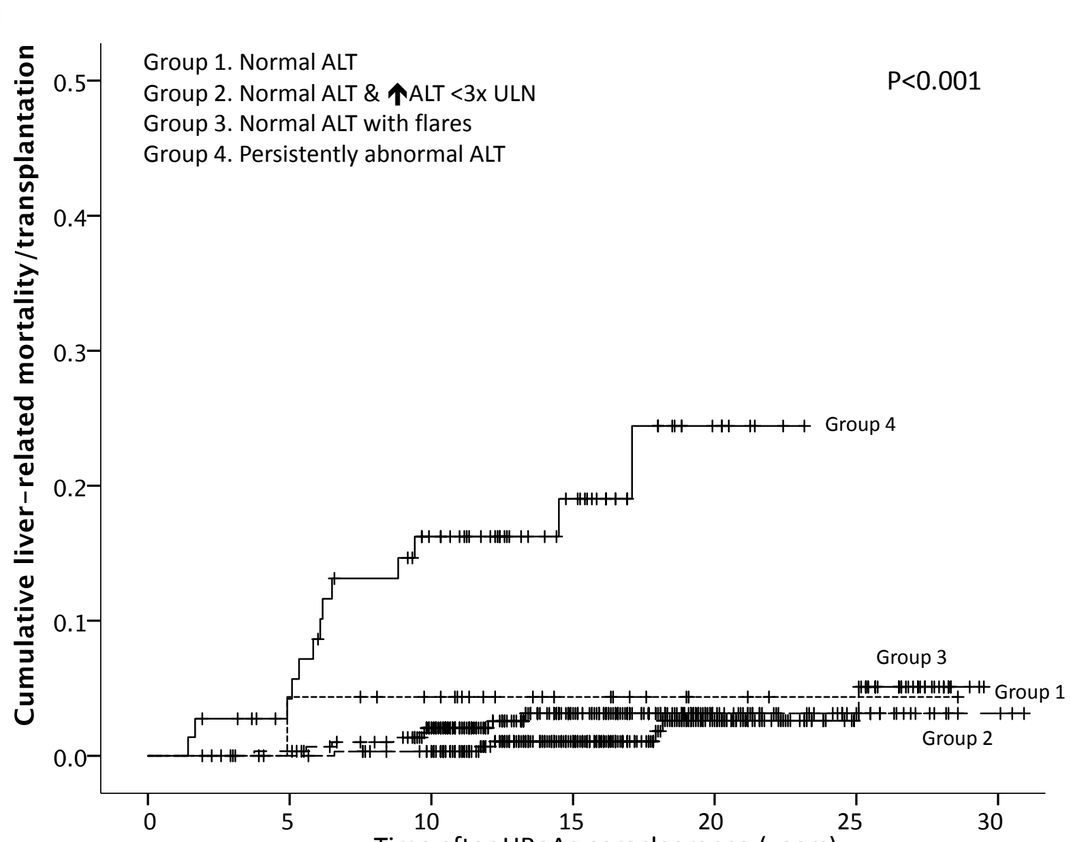

Numbers at risk							
Group 1	25	22	19	9	3	1	
Group 2	305	300	267	140	46	20	3
Group 3	320	318	307	198	91	39	
Group 4	73	65	50	28	7		