Nested case–control study: hepatocellular carcinoma risk after hepatitis B surface antigen seroclearance

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SUMMARY

Background
Hepatocellular carcinoma (HCC) risk after resolving chronic hepatitis B virus (HBV) infection is unclear.

Aim
To compare HCC risk between Alaska Native (AN) patients with and without hepatitis B surface antigen (HBsAg) seroclearance.

Methods
We selected persons with (case-patients) and without (control-patients) HBsAg seroclearance from a cohort of 1346 chronically HBV-infected AN patients followed during 1982 2013. We attempted to match two control-patients/case-patient on sex, HBV genotype, and age. Person-years of follow-up for case-patients began on the date of HBsAg resolution and for control-patients began on the date equivalent to the cohort entry date plus the years of HBsAg duration for their corresponding case-patient. We compared HCC risk using a Cox proportional hazards model.

Results
The 238 case-patients (4 with HCC) and 435 control-patients (9 with HCC) were similar in age [P-value (P) = 0.30], sex (P = 0.53) and HBV genotype (P = 0.99). Case-patients had longer person-years of follow-up than control-patients (11.7 vs. 10.1 years; P = 0.04). The HCC rate/100 000 persons was similar between case- (132) and control-patients (178; P = 0.65). The adjusted hazard ratio comparing case- and control-patients was similar for HCC [0.7; 95% confidence interval (CI): 0.2–2.4], increased for each 1-year increment for age (1.1; CI: 1.0–1.1; P < 0.01), and was greater if the initial HBeAg was positive (3.5; CI: 1.1–11.0; P = 0.03).

Conclusions
Hepatitis B surface antigen seroclearance was not associated with reduced HCC risk; the HCC risk estimates are limited by wide 95% confidence intervals. Persons meeting HCC surveillance indications prior to HBsAg seroclearance could benefit from continued surveillance after seroclearance.

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INTRODUCTION

Approximately, 248 million persons worldwide have chronic hepatitis B virus (HBV) infection.[1] Persons with chronic HBV infection are at increased risk for cirrhosis and hepatocellular carcinoma (HCC).[2] Depending on genotype, 0.5–2% of HBV-infected persons will clear hepatitis B surface antigen (HBsAg) annually.[3–5] The American Association for the Study of Liver Diseases HCC practice guidelines state that HCC surveillance is cost-effective in populations, where the HCC incidence exceeds 0.2% per year. No recommendations for HCC surveillance exist for persons with spontaneous HBsAg seroclearance because of conflicting data on HCC risk in this population.[6]

Previous studies evaluating HCC risk after HBsAg seroclearance have reached diverging conclusions.[7–10] Reasons for the variation in reported HCC risk include differences in the predominant circulating genotype (HCC risk varies with genotype),[11] study design (clinic-based vs. population-based cohort), and lack of uniform follow-up after HBsAg seroclearance (since time of seroclearance is unknown for persons in some studies). In addition, those previous studies included the years of follow-up prior to HBsAg clearance when calculating the HCC incidence among persons who resolve HBsAg. Recent evidence indicates that the eventual risk for developing HCC might be substantially influenced by factors early in the course of disease such as the HBV DNA level at the time of diagnosis.[10, 12] Therefore, restricting the HCC risk analysis to the time period after HBsAg seroclearance can provide a more precise estimate of the effect of HBsAg seroclearance on the subsequent risk for HCC.

During 1982 1987, 53 000 AN persons representing 84% of the Alaska Native (AN) population in Alaska were tested for HBsAg as part of a statewide HBV vaccination campaign.[13] All persons testing positive for HBsAg during/after the vaccination campaign were provided healthcare through the Alaska Tribal Health System (ATHS) and offered HCC surveillance regardless of age or risk factors.[14] Previous studies of this cohort of AN HBV carriers have documented an HBsAg seroclearance rate of 0.5–0.7% per year.[5, 15] We conducted a nested case–control study among this cohort of HBV-infected AN persons to compare the risk for developing HCC after HBsAg seroclearance with the risk for HCC among persons who did not clear HBsAg. We specifically aimed to evaluate the HCC risk only during the time period after HBsAg seroclearance for case-patients or the equivalent time period of time for control-patients.

PATIENTS AND METHODS

Study population

There were approximately 143 000 AN persons in Alaska in 2013.[16] All AN persons with chronic HBV, defined as having two positive HBsAg results >6 months apart, have been entered into an HBV clinical registry maintained by the ATHS. The clinical registry helps track when patients are due for routine screening exams and records clinical outcomes. Persons newly diagnosed with chronic HBV infection were continuously added to the clinical registry. In addition, the names of newly diagnosed patients with HBV infection were cross-referenced with the Alaska Area Specimen Bank, which contains >266 000 biological specimen from persons who participated in research studies dating back to 1961[17]; if serum specimen from these persons were located, it was tested for HBsAg to more precisely estimate the date of infection.

Case- and control-patients

We selected case- and control-patients from among HBV registry patients who were followed during 1982 2013 and consented to participate in study (Figure 1).[5, 13, 15] Case-patients were defined as persons with HBsAg seroclearance and control-patients as persons without HBsAg clearance. We attempted to match two control-patients for each case-patient on sex, HBV genotype and age group at cohort entry (control age group ±2.5 years if case aged <30 years, ±7.5 years if case aged 30–50 years and ±10 years if case aged ≥50 years). For a cohort person to be eligible for selection as a control-patient, the documented HBsAg duration must have been at least as long as their corresponding case-patient's HBsAg duration. Because this study used data from an observational clinical cohort, rather than a prospective cohort designed specifically to study HCC in HBV-infected persons, the presence of other risk factors for HCC, such as hepatitis C virus (HCV)-coinfection, diabetes mellitus, family history of HCC or non-alchoholic steatohepatitis,[18–21] were not comprehensively documented for study participants not developing HCC. Therefore, we were unable to exclude HBV-infected patients with other risk factors for HCC from analysis.

Laboratory testing

All AN persons with chronic HBV infection in the clinical registry were reminded semiannually by mail to go to their clinical provider for a blood draw. The sera were
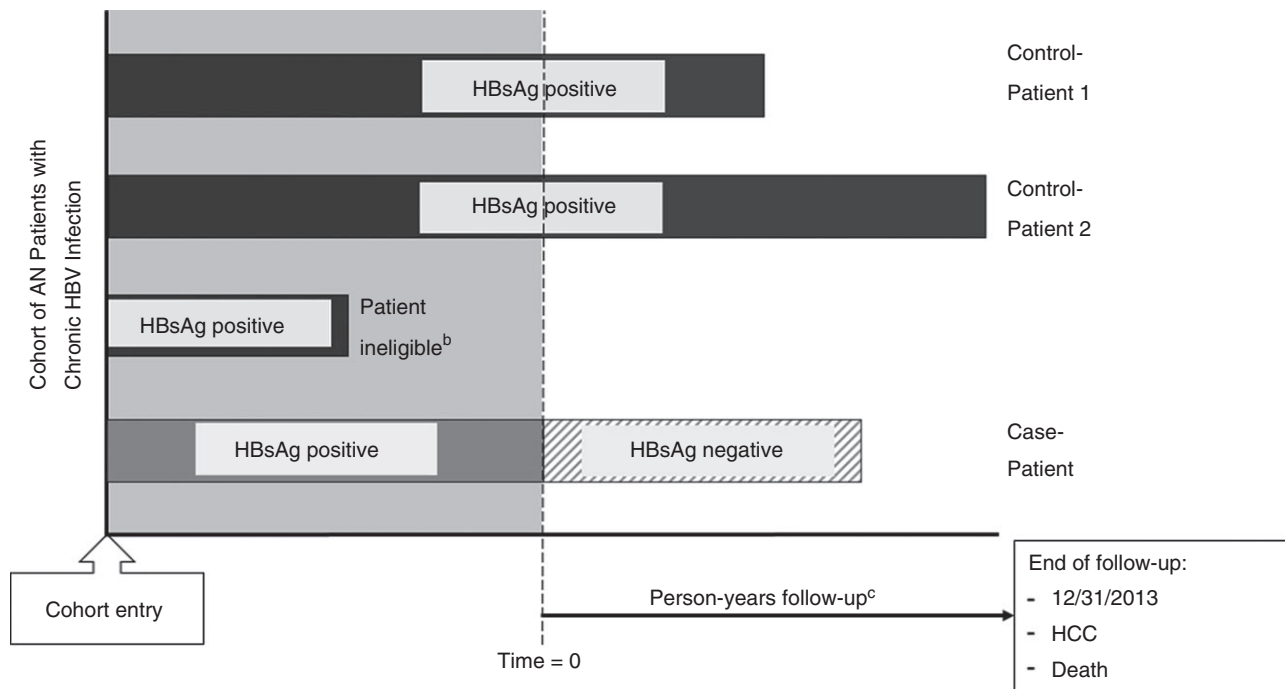
Figure 1 | Selection of case- and control-patients.[a] AN, Alaska Native; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma. [a]Case-patients (persons with HBsAg seroclearance) and control-patients (persons without HBsAg seroclearance) were selected from a cohort of 1346 chronically HBV-infected AN persons; matched two control-patients for each case-patient on sex, HBV genotype, and age group at cohort entry (control age group ±2.5 years if case aged <30 years, ±7.5 years if case aged 30–50 years, and ±10 years if case aged ≥50 years). [b]Cohort patients without HBsAg seroclearance who were not followed for at least as long as corresponding case-patient's HBsAg duration were ineligible for selection as control-patients. [c]Person years of follow-up for calculating HCC incidence began on case-patient's HBsAg seroclearance date (time = 0) or the corresponding number of years after cohort entry for control-patients; years of follow-up shaded in grey were excluded for calculating HCC incidence.

sent to the Alaska Native Medical Center in Anchorage, Alaska for testing. Since 1982, sera have been tested for HBsAg and alpha-foetoprotein (AFP) semiannually, and for hepatitis B e antigen (HBeAg) and antibody to HBe (anti-HBe) annually. Beginning in 2001, sera were also tested for liver function tests semiannually, including aspartate and alanine aminotransferase (AST and ALT, respectively) levels, and to obtain a baseline HBV DNA level and HBV genotype. The HBV DNA level was repeated every 6–12 months for persons with a baseline HBV DNA level >2000 IU/mL, a family history of HCC, or if aminotransferase levels were elevated. We tested for HBeAg, anti-HBe, antibody to HBsAg (anti-HBs), antibody to hepatitis B core antigen (anti-HBc), and HBV DNA using commercially available assays as previously described.[5, 22] Complete blood count, which includes a platelet count, was not routinely requested because of specimen instability associated with the time required to

transport specimen from certain rural Alaskan villages to the Alaska Native Medical Center for testing.

Identification of persons with HCC

Most AN persons with HCC were initially detected by the ATHS HCC surveillance programme. As many AN persons live in small rural Alaskan communities that are inaccessible by road and without ultrasound capability, the ATHS has offered HCC surveillance to all AN persons with chronic HBV infection by semiannual AFP measurements. The AFP threshold for referring for liver imaging was >25 ng/mL during 1982–1992 and was reduced to >15 ng/mL beginning in 1993, and >10 ng/mL after 2000. Persons with an elevated AFP, a family history of HCC, or cirrhosis were also offered diagnostic liver imaging by ultrasound or computed tomography. All persons with radiologic findings concerning for HCC were offered further evaluation/treatment at the Alaska

Native Medical Center; histological confirmation of HCC was available for persons who received a biopsy/surgical resection of their tumour. Persons who declined biopsy/resection of their liver lesion were diagnosed with HCC based on their clinical presentation, including an elevated AFP level, and compatible findings on radiographic imaging. We likely captured all HCC cases in the study population because all patients received care at the Alaska Native Medical Center. To ensure no study patients were diagnosed/treated for HCC at another hospital in Alaska, we cross-referenced the names of study patients with the Alaska Native Tumor Registry, a National Cancer Institute Surveillance, Epidemiology and End Results Programme registry in operation since 1969.[23]

Statistical analysis

Demographic and clinical characteristics between case- and control-patients were compared by using the Wilcoxon rank-sum test for ordered variables, and chi-squared or Fisher's exact test for categorical variables. Median values are reported with 25th and 75th percentiles (Q_1–Q_3). Person-years of follow-up for case-patients began on the date of HBsAg resolution (Figure 1). The equivalent time zero to mark the start of control-patient's person-years of follow-up began on the date equivalent to the control-patient's cohort entry date plus the years of HBsAg duration for their corresponding case-patient. We estimated the date of HBsAg resolution as the mid-point between the last HBsAg-positive and the first HBsAg-negative test. Person-years of follow-up ended for case- and control-patients on the date of HCC diagnosis, death or end of study period. Case-patients without at least one matching control-patient were excluded from analysis. Patients who are simultaneously positive for HBeAg and anti-HBe are considered HBeAg-positive for analysis. The initial HBeAg test was defined as the first test done after cohort entry and the final HBeAg test was defined as the last test done before end of follow-up. The duration of HBeAg positivity was defined as the difference between the first and last positive HBeAg test results.

We calculated the unadjusted HCC rate/100 000 person-years by dividing the total number of HCC tumours in case- and control-patients by their respective person-years of follow-up after time zero. We compared the HCC rate between case- and control-patients by using a Cox proportional hazards model that adjusted for exact age and initial HBeAg status (the first recorded HBeAg level after cohort entry).

Analysis was conducted with STATA 10 (College Station, TX, USA). $P < 0.05$ were considered statistically significant, and all tests were two-sided.

Human subjects review

This study was approved by the Institutional Review Boards of the Alaska Area and the Centres for Disease Control and Prevention. It also received review and approval by the Alaska Native Tribal Health Consortium.

RESULTS

Study cohort characteristics

A total of 1346 chronically HBV-infected AN persons were enrolled in the study cohort during 1982–2013. Among cohort persons, 58% (782) were male, the median age at cohort entry was 23 years (minimum–maximum years: 0–87), 35% (476/1343) and 4% (53/1343) had a positive HBeAg result on the initial and final tests, respectively, 19% (254) had HBsAg seroclearance, 4% (51) developed HCC, and 34% (460) died (all causes; proportion liver-related unknown).

Characteristics of case- and control-patients

We identified 435 matched control-patients, who remained HBsAg-positive throughout the follow-up period, for 238 case-patients, who had HBsAg seroclearance (41 case-patients had only one matching control-patient); we excluded from analysis 16 case-patients without any matching control-patients (Table 1). There were no significant differences between case- and control-patients with respect to the matching criteria of age, sex, and HBV genotype (Table 1). Case-patients were followed up for a median of 28.9 years (Q_1–Q_3: 24.4–30.2 years) prior to HBsAg clearance and matched to control-patients who were followed up for at least a median of 28.9 years (Q_1–Q_3: 20.7–30.3 years). Case-patients were followed up for a median of 11.7 years (Q_1–Q_3: 6.5–18.3 years) after HBsAg clearance; the equivalent median years of follow-up for control-patients were 10.1 years (Q_1–Q_3: 4.8–17.9 years). Case-compared with control-patients were less likely to have an initial positive HBeAg result (22% vs. 37%; $P < 0.01$) and less likely to have received anti-viral therapy for HBV infection (1% vs. 7%; $P < 0.01$). The two case-patients who received anti-viral therapy were treated with lamivudine for immune-active HBV infection, which possibly facilitated HBsAg seroclearance; an additional five case-patients were placed on anti-viral

Table 1 | Demographical and clinical characteristics of case-patients (persons with HBsAg seroclearance) and matched control-patients (persons without HBsAg seroclearance) selected from a cohort of HBV-infected Alaska native persons followed during 1982–2013*

Characteristics	Study participants			Study participants with HCC		
	Case-patients	Control-patients	P-value†	Case-patients	Control-patients	P-value†
Total, N	238	435		4	9	
Male sex, N (%)	152 (64)	267 (61)	0.53	2 (50)	4 (44)	1.0
Age (years) at cohort entry,‡ Median (Q₁–Q₃)§	28.8 (15.9–42.2)	27.2 (14.9–38.8)	0.30	52.7 (40.2–59.6)	42.1 (32.0–48.1)	0.28
Person-years follow-up, median (Q₁–Q₃)						
From cohort entry¶	28.9 (24.4–30.2)	28.9 (20.7–30.3)	0.40	12.1 (9.2–15.9)	19.2 (13.2–25.1)	0.28
After HBsAg seroclearance**	11.7 (6.5-18.3)	10.1 (4.8-17.9)	**0.04**	7.3 (3.4-9.3)	6.7 (2.3-13.0)	0.88
+HBeAg, N (%)††,‡‡						
Initial test	51 (22)	159 (37)	**<0.01**	1 (25)	5 (56)	0.56
Final test	1 (0.4)	10 (2.3)	0.11	0	2 (22)	1.0
Any test	71 (30)	200 (46)	**<0.01**	3 (75)	6 (67)	1.0
Duration +HBeAg, Median years (Q₁–Q₃)§§	1.0 (0.0–4.6)	3.6 (0.1–27.4)	**<0.01**	0.0 (0.0–0.1)	13.0 (7.2–14.4)	0.07
Anti-HBs positive, N (%)‡‡,¶¶,***						
Final test	216 (91)	55 (14)	**<0.01**	2 (50)	0 (0)	0.11
Any test	226 (95)	72 (18)	**<0.01**	4 (100)	0 (0)	**<0.01**
HBV genotype, N (%)***						
A	22 (12)	40 (11)	0.99	2 (50)	0	0.10
B	2 (1)	4 (1)		0	0	
C	8 (4)	16 (4)		0	4 (44)	
D	119 (64)	236 (66)		0	1 (11)	
F	36 (19)	64 (18)		2 (50)	4 (44)	
Unknown	51	75		0	0	

therapy after HBsAg seroclearance ahead of planned immunosuppressive therapy. Case- and control-patients were similar in terms of the percentage that died during follow-up (28% vs. 33%) and the percentage with HCV coinfection (4% vs. 3%). Among the patients selected for the nested case–control study, three had HIV coinfection (one control-patient, one case-patient before HBsAg seroclearance, and one case-patient after HBsAg seroclearance), and 13 developed HCC (four case-patients and nine control-patients). An additional two cohort patients developed HCC prior to HBsAg seroclearance; these patients were not included as case-patients because person-years of follow-up for this analysis began after HBsAg seroclearance. There were no significant differences between case- and control-patients developing HCC in terms of the percentage that died (100% vs. 78%), had HCV coinfection (25% vs. 11%), had cirrhosis at time of HCC diagnosis (25% vs. 63%), or a family history of HCC (0% vs. 50%). The specific characteristics of the 13 case- and control-patients who developed HCC are detailed in Table 2.

A platelet count necessary to calculate an aspartate aminotransferase-to-platelet ratio index (APRI), a non-invasive marker for liver fibrosis, was available for 131 (55%) case-patients and 258 (59%) control-patients.[24] Among case-patients with an APRI, 88% had an index <0.5, 9% had an index 0.5–1.5, and 2% had an index >1.5. Among control-patients with an APRI, 81% had an index <0.5, 14% had an index 0.5–1.5, and 5% had an index >1.5. A FIB-4 index, another non-invasive liver fibrosis marker calculated using platelets, alanine aminotransferase, aspartate aminotransferase and patient age, was available for 131 (55%) case-patients and 256 (59%) control-patients.[25] Among case-patients with a FIB-4 index, 69% had an index <1.45, 24% had an index 1.45–3.25 and 7% had an index >3.25. Among control-patients with a FIB-4 index, 71% had an index <1.45, 19% had an index 1.45–3.25 and 10% had an index >3.25. There was no difference between case- and control-patients in the percentage of patients with an APRI >1.5 vs. ≤1.5 (P = 0.19) or in the percentage with a Fib4 score >3.25 vs. a score ≤3.25 (P = 0.34).

Table 1 | (Continued)

Characteristics	Study participants			Study participants with HCC		
	Case-patients	Control-patients	*P*-value†	Case-patients	Control-patients	*P*-value†
Died during follow-up, N (%)	66 (28)	145 (33)	0.13	4 (100)	7 (78)	1.0
HCV RNA positive, N (%)	10 (4)	11 (3)	0.23	1 (25)	1 (11)	1.0
Received HBV treatment, N (%)	2 (1)†††	32 (7)	**<0.01**	0 (0)	5 (56)	0.11
Cirrhosis, N (%)‡‡‡	–	–		1 (25)	5 (63)§§§	0.55
Family history of HCC, N (%)	–	–		0 (0)	4 (50)§§§	0.21

–, not available; +HBeAg, serology positive for hepatitis B e antigen serum; +HBsAg, serology positive for hepatitis B surface antigen; anti-HBs, antibody to HBsAg; HBV, hepatitis B virus; Q1-Q3, interquartile range; N, number.

* Attempted to match 2 control-patients per case-patient on sex, HBV genotype, age group at cohort entry (control-patient age group ±2.5 years if case-patient aged <30 years, ±7.5 years if case-patient aged 30–50 years, and ±10 years if case-patient aged ≥50 years), and with HBsAg duration ≥corresponding case-patients' HBsAg duration; 41 case-patients only had 1 matching control-patient and 16 case-patients had were excluded because no matching control-patients were identified.

† Medians compared by rank-sum test and proportions by chi-squared test or Fisher's exact test; p-values <0.05 indicated in **bold.**

‡ Cohort entry defined as 1 January 1982 if positive for before that time or date of first +HBsAg result if after 1 January 1982.

§ Q1, 25th percentile; Q3, 75th percentile.

¶ Person-years of follow-up calculated from cohort entry until death, HCC, or 31 December 2013.

** Person-years of follow-up for case-patients began on the date of HBsAg resolution and for control-patients began on the date equivalent to the cohort entry date plus the years of HBsAg duration for their corresponding case-patient; follow-up ended at death, HCC, or 31 December 2013.

†† HBeAg status tested for 237 case-patients (4 had <2 HBeAg test results recorded) and 435 control-patients (1 had <2 HBeAg test results recorded); all 13 patients with HCC had ≥2 HBeAg test results recorded.

‡‡ Initial test refers to result of first test done after cohort entry, final test refers to result of last test done before end of follow-up, and any test refers to result of any test done from cohort entry until end of follow-up.

§§ Calculated as the time between initial and final +HBeAg result among the 71 case-patients and 200 control-patients with an initial +HBeAg result.

¶¶ Anti-HBs titer >0 mIU/mL.

*** Per cent calculated among case- and control-patients with a known HBV genotype.

††† 5 (2%) additional patients received treatment after HBsAg seroclearance to prevent reactivation of infection when undergoing immunosuppressive therapy.

‡‡‡ Status at the time of HCC diagnosis; status not comprehensively known for case- and control-patients not developing HCC.

§§§ Presence/absence of cirrhosis at the time of HCC diagnosis and family history of HCC not recorded for 1 of 9 control-patient.

HCC rate

The HCC rate/100 000 persons was similar between case-patients with HBsAg seroclearance [132; 95% confidence interval (CI): 36–338] and control-patients without HBsAg seroclearance (178; CI: 81–338). The risk for HCC did not differ significantly between case- and control-patients in the multivariable Cox proportional hazards model [adjusted hazard ratio (aHR): 0.7; CI: 0.2–2.4]. The risk for HCC was associated with greater age at cohort entry (aHR for each 1-year increment: 1.1; CI: 1.0–1.1; $P < 0.01$) and having a positive initial HBeAg result compared with a negative result (aHR: 3.5; CI: 1.1–11.0).

HBV DNA and ALT Levels

The distribution (statistical spread of values) of HBV DNA levels among case-patients was compared with control-patients at time periods before and after HBsAg seroclearance (Table 3). At least one HBV DNA measurement was available for 97% of case-patients (median: two measurements; Q_1–Q_3: 1–4 measurements) and 82% of control-patients (median: three measurements; Q_1–Q_3: 1–6); the time periods for aggregating HBV DNA measurements were selected to optimise sample size for analysis. There was no difference in the distribution of the HBV DNA level between case- and control-patients ≥9 years prior to HBsAg seroclearance ($P = 0.39$) and

Table 2 | Characteristics of case-patients (persons with HBsAg seroclearance) and control-patients (persons without HBsAg seroclearance) who developed hepatocellular carcinoma (HCC)

Patient	Advanced age at HCC diagnosis*	HBV Genotype	Family history of HCC	Cirrhosis	HCV coinfection	Duration (years)			Peak HBV DNA level§
						Positive HBsAg	Negative HBsAg†	Positive HBeAg‡	
Case									
1	No	A	No	Yes	Past	3	9.5	0.1	0
2¶	Yes	F	No	No	Never	2.6	9.1	0	100
3	Yes	A	No	No	Never	18	1.3	0	132
4	Yes	F	No	No	Never	1.3	5.4	0	Unknown
Control									
1	No	F	Yes	Unknown	Never	29.9	N/A	16.2	3.7×10^7
2	No	F	No	No	Never	19.2	N/A	0	1109
3	Yes	C	Yes	Yes	Never	25.1	N/A	12.2	2.4×10^7
4	Yes	D	Unknown	Yes	Past	14.6	N/A	0	700
5	Yes	C	Unknown	Unknown	Never	19.7	N/A	7.2	144
6	Yes	C	Yes	Yes	Never	25.9	N/A	13.8	954
7	Yes	F	No	Yes	Never	13.2	N/A	14.4	Unknown
8	Yes	F	No	No	Never	1.5	N/A	0	Unknown
9	Yes	C	Yes	Yes	Never	6.2	N/A	0.1	Unknown

HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; N/A, not applicable.

No patients with HCC had autoimmune hepatitis.

* Male aged >40 years/female aged >50 years based on American Association for the Study of Liver Diseases HCC practice guidelines for persons who could benefit from HCC surveillance.

† Date of HBsAg resolution defined as the mid-point between the last positive HBsAg and the first negative HBsAg.

‡ Defined as the difference between the first and last positive HBeAg test results.

§ Testing cohort patients for HBV DNA level (IU/mL) started in 2001 and are unknown for persons who died before 2001.

¶ Case-patient tested positive on one of 24 HBsAg tests after initial HBsAg seroclearance.

<9 years prior to HBsAg seroclearance ($P = 0.12$). The HBV DNA level was lower among case-patients compared with control-patients <9 years after HBsAg seroclearance (median: 0 vs. 212 IU/mL; % with HBV DNA >0 IU/mL: 40% vs. 87%; % with HBV DNA >2000 IU/mL: 1% vs. 28%; $P < 0.01$) and ≥9 years after HBsAg seroclearance (median: 0 vs. 259 IU/mL; % with HBV DNA >0 IU/mL: 48% vs. 92%; % with HBV DNA >2000 IU/mL: 2% vs. 29%; $P < 0.01$). Among the 98 case-patients followed up for ≥9 years after HBsAg seroclearance, 95% (93) were anti-HBs positive on the last serum specimen tested before end of follow-up and 51% (47) had detectable HBV DNA. The five case-patients who were followed up for ≥9 years after HBsAg seroclearance and remained anti-HBs negative also had detectable HBV DNA on their final serum sample tested before end of follow-up.

We also compared the distribution of ALT levels among case-patients with control-patients at time periods before and after HBsAg seroclearance (Table 4). The ALT level was similar between case- and control-patients ≥9 years prior to HBsAg seroclearance ($P = 0.39$) and <9 years prior to HBsAg seroclearance ($P = 0.99$). The ALT level was lower among case-patients compared with control-patients <9 years after HBsAg seroclearance ($P < 0.01$) and ≥9 years after HBsAg seroclearance ($P = 0.03$).

CONCLUSIONS

Previous studies evaluating the risk for HCC associated with HBsAg loss have included the time during which persons were seropositive for HBsAg in calculating the HCC rate.[3, 7, 9, 10] Our study is unique because we attempted to isolate the effect of HBsAg seroclearance on subsequent risk for developing HCC. The results indicate that HBsAg seroclearance was not associated with reduced risk for HCC. Although the small number of persons who developed HCC limits the strength of our conclusion, our case- and control-patients were sampled from one of the largest and longest followed

Table 3 | Hepatitis B virus (HBV) DNA level relative to case-patient hepatitis B surface antigen (HBsAg) seroclearance date*

Years of follow-up relative to time zero	Case-patients				Control-patients				
		HBV DNA level (IU/mL)				HBV DNA level (IU/mL)			
			N (%)				N (%)		
	N	Median	>0	>2000	N	Median	>0	>2000	P-value†
≥9 years prior	15	416	15 (100)	7 (47)	11	289	9 (82)	4 (36)	0.39
<9 years prior	76	111	59 (78)	19 (22)	112	357	91 (81)	29 (26)	0.12
0 years	0				0				
<9 years after	164	0	66 (40)	2 (1)	217	212	188 (87)	61 (28)	**<0.01**
≥9 years after	98	0	47 (48)	2 (2)	184	259	169 (92)	53 (29)	**<0.01**

N, number of patients contributing an HBV DNA result during the specified follow-up time period.

* Case-patient's HBsAg seroclearance date (time zero to mark start of study follow-up) was defined as the mid-point between the last positive HBsAg and first negative HBsAg test result; the time zero for control-patients without HBsAg seroclearance was defined as the date of cohort entry plus the duration in years their corresponding case-patient had positive HBsAg.

† Wilcoxon rank-sum test with significant results indicated in **bold**; values for a patient averaged if the patient contributes >1 measurement during specified follow-up time period.

Table 4 | Alanine aminotransferase (ALT) level relative to case-patient hepatitis B surface antigen (HBsAg) seroclearance date*

Years of follow-up relative to time zero	Case-patients			Control-patients			
		ALT (U/L)			ALT (U/L)		
	N	Median	IQR	N	Median	IQR	P-value†
≥9 years prior	78	37	29–51	125	39	31–57	0.39
<9 years prior	212	30	23–45	372	32	24–43	0.99
0 years	0			0			
<9 years after	323	25	18–35	581	29	22–42	**<0.01**
≥9 years after	243	24	18–35	380	27	20–36	**0.03**

IQR, interquartile range; N, number of patients contributing an ALT result during the specified follow-up time period.

* Case-patient's HBsAg seroclearance date (time zero to mark start of study follow-up) was defined as the mid-point between the last positive HBsAg and first negative HBsAg test result; the time zero for control-patients without HBsAg seroclearance was defined as the date of cohort entry plus the duration in years their corresponding case-patient had positive HBsAg.

† Wilcoxon rank-sum test with significant results indicated in **bold**; values for a patient averaged if the patient contributes >1 measurement during specified follow-up time period.

population-based cohorts of persons with HBV infection in the world. As a result, this present study includes more persons with resolved HBsAg (including those who developed HCC) than similar previous studies that have evaluated the risk of HCC after resolving HBV infection.[3, 8, 9, 26] As it is unlikely a more precise estimate of HCC risk following HBsAg seroclearance can be obtained in the near future, it would be reasonable to offer HCC surveillance after HBsAg seroclearance for persons meeting AASLD practice guidelines criteria for surveillance prior to resolving HBV infection.[6]

Cirrhosis is an important risk factor for developing HCC among persons with chronic HBV infection. The presence of cirrhosis was not comprehensively known for our study participants who did not develop HCC in part because many patients lived in rural communities without ready access to liver biopsy capable facility. Therefore, we were unable to match case- and control-patients according to their cirrhosis status. For the subset of case- and control-patients with APRI and Fib4 available, we are reassured that there was no difference between the two groups in the proportion with advanced

liver fibrosis as measured by these non-invasive makers. Furthermore, knowing the cirrhosis status only for case-patients who developed HCC still provides insights into the risk for HCC. Unlike previous studies where the majority of patients who developed HCC after resolving chronic HBV infection had cirrhosis at the time of HBsAg seroclearance,[3, 9, 26] only one of the four case-patients with HCC in our study had cirrhosis. However, the other three case-patients without cirrhosis would have met AASLD age/sex criteria for continuing HCC surveillance after HBsAg seroclearance.[6]

The reasons for why HBsAg seroclearance was not associated with reduced HCC rate are unknown but likely multifactorial. It is possible that factors early in the course of HBV infection, such as the HBV DNA level or degree of hepatic necroinflammation, might have had a greater influence on HCC risk.[10, 12, 27] We compared the distribution of HBV DNA level for the time periods before and after HBsAg seroclearance among case-patients and for the corresponding time periods among control-patients. The lack of difference in HCC rate between case- and control-patients corresponds with the similarity in HBV DNA levels among case-patients compared with control-patients before HBsAg clearance. These results support previous reports that the HBV DNA level before HBsAg seroclearance is an important predictor for developing HCC.[10, 12] It is likely for the reason that treatment with nucleos(t)ide analogues decreases both the risk for developing HCC and the risk of HCC recurrence after surgical resection.[28–31] One mechanism by which HBV infection causes HCC could be through the integration of HBV DNA into the host hepatocyte genome and as covalently closed circular (ccc) DNA in hepatocyte nuclei.[27, 32] The integrated viral DNA and cccDNA that result from HBV viremia persist after HBsAg seroclearance and might promote the development of HCC. Furthermore, a substantial proportion of case-patients in our study had a detectable HBV DNA level after HBsAg seroclearance. Thus, it is possible that ongoing low-level HBV DNA replication with continued integration into the host hepatocyte also contributes to the persistent HCC risk after HBsAg seroclearance.

In addition, the degree of HBV-associated hepatic inflammation, which can be assessed by measuring ALT levels, correlates with the risk for developing HCC.[27] The ALT level before HBsAg seroclearance was similar among case-patients compared with control-patients. The ALT level together with the HBV DNA level indicates that the majority of case- and control-patients were in the immune-inactive phase of HBV infection prior to

HBsAg seroclearance. Results from this present study confirm previous evaluations in this cohort demonstrating that most patients with chronic HBV infection are HBeAg-negative and remain in the immune-inactive phase after HBeAg clearance.[33, 34] The lack of difference in the degree of hepatic inflammation between case- and control-patients prior to HBsAg could also partly account for the lack of association between HBsAg sero-clearance and reduced HCC risk.

Our adjusted analysis indicates that the initial HBeAg status and increasing age at cohort entry were associated with HCC risk. The presence of HBeAg, indicating immune-active phase of disease, is associated with high HBV DNA levels and intermittent ALT elevations.[35] Therefore, HBeAg seropositivity could be associated with increased risk for HCC, because it is a surrogate marker for HBV DNA level and hepatic inflammation.[7, 36] Our adjusted analysis also confirmed results from previous studies indicating that increasing age in HBV-infected persons is a risk factor for HCC.[2, 37, 38] Although the exact date of HBV infection is unknown for patients in our study, it is likely that most patients acquired HBV infection in early childhood or at birth.[39] Thus, the age at cohort entry probably correlates with the duration of infection for most study patients. Since our control-patients were matched with case-patients on age and duration of follow-up, the results additionally suggest that increasing age might be a risk factor for HCC independent of duration of HBV infection.

This study has limitations. First, our HCC risk estimates had wide confidence intervals because few case- and control-patients developed HCC. Thus, we could have failed to detect a real reduction in HCC risk associated with HBsAg seroclearance because of insufficient statistical power. It is important to note, however, that both HBsAg seroclearance and development of HCC are rare events, and our study has more persons with HBsAg seroclearance and HCC than other similar studies.[3, 8–10, 26] Furthermore, it is important to note that the HCC incidence for a population cannot be calculated in a case–control study since the number of cases and controls are prespecified. The HCC rates we present allow for comparing the HCC risk between groups in this paper, but the absolute rates cannot be compared with the HCC incidence reported elsewhere. In addition, the presence of other HCC risk factors, such as HCV coinfection, family history of HCC, diabetes mellitus or fatty liver disease,[18–20] was not comprehensively known for our study participants not developing HCC. As a result, we could not adjust for several important HCC risk

factors in our model comparing the HCC rate between case- and control-patients. We did demonstrate, however, that case- and control-patients were similar in terms of certain key risk factors, such proportion with HCV coinfection, HBV DNA level, hepatic inflammation as measured by ALT levels, and liver fibrosis as measured by APRI and Fib4. Finally, our results based on the AN population might not be generalisable to other populations. The risk for HCC varies by HBV genotype[11]; AN persons infected with genotypes C and F have a higher incidence of HCC compared with persons infected with other genotypes.[22] Differences in the prevalence of HBV genotypes between those found in AN persons compared with other geographical regions of the world could affect the incidence of HCC observed between persons with and without HBsAg seroclearance.

The goals of HBV treatment are to reduce the risk of developing cirrhosis, liver decompensation and HCC. Therapy for HBV infection is indicated for patients in the immune-active phase but not for patients in the immune-inactive phase of HBV infection.[40] Most patients in our study were in the immune-inactive phase of infection and did not receive HBV therapy. However, study patients were still at high risk for developing HCC and HBsAg seroclearance did not reduce the HCC risk. Given the effectiveness of nucleos(t)ide analogues in reducing HCC risk for persons with elevated HBV DNA levels,[30] further research to better understand the factors early in the course of infection that predict future risk for developing HCC risk could help to identify a subset of immune-inactive patients who might benefit from early treatment.

AUTHORSHIP

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