Received: 20 June 2021 | Revised: 20 October 2021 | Accepted: 24 October 2021

DOI: 10.1002/hep.32231

ORIGINAL ARTICLE

Incidence and prediction of HBsAg seroclearance in a prospective multi-ethnic HBeAg-negative chronic hepatitis B cohort

Norah A. Terrault[1] | Abdus S. Wahed[2] | Jordan J. Feld[3] | Stewart L. Cooper[4] | Mark G. Ghany[5] | Mauricio Lisker-Melman[6] | Robert Perrillo[7] | Richard K. Sterling[8] | Mandana Khalili[9] | Raymond T. Chung[10] | Philip Rosenthal[11] | Robert J. Fontana[12] | Arif Sarowar[3] | Daryl T. Y. Lau[13] | Junyao Wang[2] | Anna S. Lok[14] | Harry L. A. Janssen[3]

[1]Gastrointestinal and Liver Diseases Division, Keck Medicine of University of Southern California, Los Angeles, California, USA

[2]Department of Biostatistics and Epidemiology Data Center, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[3]Toronto Center for Liver Disease, University of Toronto, Toronto, Ontario, Canada

[4]San Francisco Center for Liver Disease, California Pacific Medical & Research Institute, San Francisco, California, USA

[5]Liver Diseases Branch, National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA

[6]Washington University School of Medicine and John Cochran VA Medical Center, St. Louis, Missouri, USA

[7]Baylor University Medical Center, Dallas, Texas, USA

[8]Section of Hepatology, Virginia Commonwealth University, Richmond, Virginia, USA

[9]Department of Medicine, University of California San Francisco, San Francisco, California, USA

[10]Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

[11]Department of Pediatrics, University of California San Francisco, San Francisco, California, USA

[12]Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA

[13]Liver Center, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard University, Boston, Massachusetts, USA

[14]Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, Michigan, USA

Correspondence
Norah Terrault, Gastrointestinal and Liver Diseases Division, Keck Medicine of University of Southern California, 1520 San Pablo St, Suite 1000, Los Angeles, CA 90033, USA.
Email: terrault@usc.edu

Funding information
The Hepatitis B Research Network (HBRN) was funded as a cooperative agreement between the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the following investigators: Anna Suk-Fong Lok, M.D. (U01-DK082863), Steven H. Belle, Ph.D., M.Sc.Hyg. (U01-DK082864), Adrian M. Di Bisceglie, M.D. (U01-DK082871), William M. Lee, M.D. (U01-DK082872),

Abstract

Background and Aims: Achieving HBsAg loss is an important landmark in the natural history of chronic hepatitis B (CHB). A more personalized approach to prediction of HBsAg loss is relevant in counseling patients. This study sought to develop and validate a prediction model for HBsAg loss based on quantitative HBsAg levels (qHBsAg) and other baseline characteristics.

Methods: The Hepatitis B Research Network (HBRN) is a prospective cohort including 1240 untreated HBeAg-negative patients (1150 adults, 90 children) with median follow-up of 5.5 years. Incidence rates of HBsAg loss and hepatitis B surface antibody (anti-HBs) acquisition were determined, and a predictor

Abbreviations: ALT, alanine aminotransferase; anti-HBe, hepatitis B e antibody; anti-HBs, hepatitis B surface antibody; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; BCP, basal core promoter; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; HBRN, Hepatitis B Research Network; HBRN SQuARe, sex, ΔquantHBsAg, age, race; IQR, interquartile range; PC, precore; PY, person-years; qHBsAg, quantitative HBsAg levels; ULN, upper limit of the normal.

© 2021 American Association for the Study of Liver Diseases.

Harry L. A. Janssen, M.D., Ph.D. (U01-DK082874), Daryl T-Y Lau, M.D., M.P.H. (U01-DK082919), Richard K. Sterling, M.D., M.Sc. (U01-DK082923), and Mandana Khalili, M.D. (U01-DK082944); an interagency agreement with NIDDK: Lilia M. Ganova-Raeva, Ph.D. (A-DK-3002-001) and support from the intramural program, NIDDK, NIH: Marc G. Ghany, M.D. Additional funding to support this study was provided to NIH Public Health Service Research (M01-RR00040), Richard K. Sterling, M.D., M.Sc. (UL1TR000058; NCATS [National Center for Advancing Translational Sciences], NIH), Norah A. Terrault, M.D., M.P.H. (CTSA UL1TR000004), and Anna Suk-Fong Lok (CTSA UL1RR024986 and U54TR001959.) Additional support was provided by Gilead Sciences, Inc., and Roche Molecular Systems through a CRADA through the NIDDK

score of HBsAg loss using readily available variables was developed and externally validated.

Results: Crude incidence rates of HBsAg loss and anti-HBs acquisition were 1.6 and 1.1 per 100 person-years (PY); 67 achieved sustained HBsAg loss for an incidence rate of 1.2 per 100 PY. Increased HBsAg loss was significantly associated with older age, non-Asian race, HBV phenotype (inactive CHB vs. others), HBV genotype A, lower HBV-DNA levels, and lower and greater change in qHBsAg. The HBRN-SQuARe (sex,ΔquantHBsAg, age, race) score predicted HBsAg loss over time with area under the receiver operating characteristic curve (AUROC) (95% CIs) at 1 and 3 years of 0.99 (95% CI: 0.987–1.00) and 0.95 (95% CI 0.91–1.00), respectively. In validation in another cohort of 1253 HBeAg-negative patients with median follow-up of 3.1 years, HBRN SQuARe predicted HBsAg loss at 1 and 3 years with AUROC values of 0.99 (0.98–1.00) and 0.88 (0.77–0.99), respectively.

Conclusion: HBsAg loss in predominantly untreated patients with HBeAg-negative CHB can be accurately predicted over a 3-year horizon using a simple validated score (HBRN SQuARe). This prognostication tool can be used to support patient care and counseling.

INTRODUCTION

Functional cure of chronic hepatitis B (CHB) is defined as a sustained loss of HBsAg and HBV DNA from serum, with or without hepatitis B surface antibody (anti-HBs) seroconversion.[1] The importance of achieving functional cure is emphasized by the lower rates of liver complications, including HCC, in patients who have lost HBsAg versus those with sustained HBV-DNA suppression but persistence of HBsAg.[2] Patients who achieve a functional cure remain at risk for spontaneous or immunosuppressive therapy–induced HBV reactivation.[3] Sterilizing cure, with eradication of integrated HBV DNA and covalently closed circular DNA (cccDNA), is the more desirable objective, but largely beyond reach with currently approved therapies.[1] Thus, functional cure is generally regarded as the optimal outcome for patients with CHB and is the primary endpoint for many of the therapies under development.

Recent systematic reviews highlighted the relative rarity of HBsAg loss among persons with CHB, estimating the pooled annual incidence of spontaneous HBsAg loss at approximately 1% per year.[4,5] Comparison of treated cohorts suggests peg-interferon alone or in combination with nucleos(t)ide analogue therapy may enhance HBsAg loss to 3%–8% at end of study,[6–8] but overall the rates of HBsAg loss are low. These data emphasize both the need for better therapies to enhance rates of HBsAg loss as well as the need for prognostic models to better estimate the trajectory of HBsAg loss. The factor most frequently associated with loss of HBsAg is quantitative levels of HBsAg, but the specific threshold and factors influencing the rate of HBsAg loss are not well established.[9]

Much of what is known about the natural history of CHB has emerged from Asian or European cohorts, with much more limited data from North American patients with CHB. While CHB in North America is primarily found among the immigrant populations[10–12] existing data on CHB outcomes from other countries may not be applicable. Differing epidemiologic and environmental factors, liver disease comorbidities, access to treatment, and monitoring may influence the natural history of CHB and likelihood of achieving HBsAg loss, thereby stimulating the need to study these key questions in a representative North American population with CHB.

As HBeAg loss precedes HBsAg loss, patients with HBeAg-negative CHB are the prime group to evaluate predictors of HBsAg loss or functional cure. The Hepatitis B Research Network (HBRN), a North American, prospective, multi-ethnic, multicenter cohort of children and adults, provided an opportunity to determine the incidence of key events in the natural history of HBeAg-negative CHB and to develop and validate a prognostic score for HBsAg loss over a several-year time span.

PATIENTS AND METHODS

Study population

The HBRN is a collaborative network including 28 clinical sites (21 adult, 7 pediatric) from diverse regions in the United States and Canada that prospectively followed

participants with CHB from January 2011 to December 2019, funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NCT01263587).[13,14] At enrollment, all participants were HBsAg-positive and not currently receiving treatment. Those with hepatic decompensation, liver cancer, solid organ or bone marrow transplantation, human immunodeficiency virus infection, or active HCV or HDV coinfections were excluded. Participants with less than 24 weeks of follow-up from baseline were also excluded. Treatment after enrollment occurred as per standard of care at individual sites or through an HBRN clinical trial. Follow-up visits and laboratory testing were completed at week 12 after enrollment and then every 24 weeks thereafter in adults, and at 24 weeks and 48 weeks and annually thereafter in children. For this analysis, all HBsAg-positive adult and pediatric participants whose first available HBeAg test showed a negative result, regardless of the presence of hepatitis B e antibody (anti-HBe), were included. Historical data on HBeAg status (before enrollment) was not captured. HBRN phenotypes were defined as previously published.[15]

The HBRN Steering Committee approved all study protocols. Institutional review board or ethics committee approval was obtained from all clinical sites. All participants or their parents/guardians provided written informed consent.

Definitions and outcomes

The primary outcome for this analysis was HBsAg loss. HBsAg loss was categorized as transient or sustained, with the latter defined as having persistently negative results for 6 months or more. Additional outcomes included anti-HBs seroconversion, HBeAg seroreversion, alanine aminotransferase (ALT) flares, and new onset of cirrhosis. If there were missing data between two timepoints, we assumed that the outcome had not changed; in other words, the outcome at the first timepoint was carried forward to the second timepoint with an outcome.

Anti-HBs seroconversion was categorized as transient and sustained, with the latter defined as having persistently detectable anti-HBs positivity for 6 months or more. HBeAg seroreversion was defined as transient or sustained, with the latter defined by detectable HBeAg measured at least 6 months apart without any intervening negative tests. ALT flares were defined as ALT level ≥ 10 times the upper limit of the normal range (ULN). The ULN for adult men and women were 30 U/L and 20 U/L,[15] respectively, and for children were 33 U/L for male and female infants <1 year old; 25 U/L for male and female children 1 year to <13 years old; and 25 U/L for males and 22 U/L for females between 13 years and <18 years old.[16] New onset of cirrhosis was based on clinical, radiologic (splenomegaly or a nodular liver), laboratory (platelet count < 120,000 mm^3) and histologic data, and a cirrhosis diagnosis within 6 months of cohort entry was considered to be present at baseline. All outcomes were reviewed and confirmed by an adjudication committee.[17]

Laboratory testing

HBsAg and HBeAg quantitation, and qualitative anti-HBs and HBV-DNA level were measured centrally at an HBRN-funded virology laboratory (University of Washington, Seattle, WA, USA). Additional qualitative anti-HBs was performed at the University of Texas Southwestern (Dallas, TX, USA). Local laboratory results were used to supplement missing values. HBV DNA was tested using a real-time PCR assay (ROCHE COBAS Ampliprep/COBAS Taqman HBV Test, v2.0) with lower limit of detection of 10 IU/ml. Quantitative HBsAg and HBeAg levels were measured by Elecsys HBsAg II Quant and Elecsys HBeAg II Quant assay (Roche Molecular Systems, Inc.) with lower limits of detection of 0.05 IU/ml and 0.3 IU/ml, respectively. Central testing of qualitative anti-HBs used the Food and Drug Administration–approved EIA assay (ETI-AB-AUK PLUS, DiaSorin, Italy) with values ≥10 mIU/mL being considered positive. Qualitative assays for HBsAg, anti-HBs, HBeAg, and anti-HBe were performed locally using commercially available ELISA assays. Genotyping of HBV was performed by the Centers for Disease Control and Prevention using mass spectrometry.[18] Precore (PC) and basal core promoter (BCP) variants were determined by Sanger sequencing.[19] All other laboratory results were obtained locally. Hyperlipidemia and diabetes were self-reported clinical diagnoses or use of diagnosis-specific medications. Aspartate aminotransferase (AST)–to-platelet ratio index (APRI) was calculated as (AST/upper limit of normal)/platelet count (expressed as platelets × 10^9/l) × 100.[20]

Statistical analyses

The baseline categorical variables of participants were summarized using frequencies and percentages, and continuous variables summarized with medians and interquartile ranges. Duration of follow-up for each individual was determined by the time between baseline and the last follow-up visit. Rates of clinical outcomes were calculated and expressed per 100 person-years (PY). The CIs for PY rates were calculated using Poisson regression (log-linear regression for rates with log follow-up time as offset).

Poisson regression with log of the follow-up time as offset was used to assess the association between baseline characteristics and incidence rates of HBsAg

loss, with results expressed as rate ratios and corresponding 95% CIs or adjusted rate ratios when adjusted for other covariates in the model. The Poisson regression was preferred to Cox regression, as follow-ups were not as frequent. Each variable was evaluated in univariable and then multivariable Poisson regression models. Because genotype and race were highly correlated, multivariable models only included race. The receipt of ≥24 weeks of antiviral therapy during follow-up was evaluated as a time-varying covariate. Sensitivity analysis was conducted with censoring of participants at the initiation of treatment. A similar approach was used for the analysis of anti-HBs seroconversion rates.

Prediction model development and validation

A parametric Weibull regression model with time to HBsAg loss as the outcome was fit using age, sex, race, quantitative HBsAg levels (qHBsAg), qHBsAg change in a year (maximum of three measurements within 48 weeks), and their interaction. A parametric regression model over the semi-parametric Cox proportional hazard model was chosen considering the small number of events (HBsAg loss) over time. The variables selected were prespecified with a focus on qHBsAg and its change over a period of 1 year as predictive markers in addition to age, sex, and race (surrogate for genotype), which are other known predictors of HBsAg loss. The prespecification of covariates was done to prevent model overfitting due to small number of events and to mostly evaluate the predictive ability of qHBsAg and its change. For the same reasons, no model calibration was considered. The model including qHBsAg change per year as a predictor (hereafter referred to as HBRN SQuARe [Sex, ΔQuantitave HBsAg, Age, Race]) required participants to have more than 1 year of follow-up with at least two measurements of qHBsAg during the first year. For each participant, the qHBsAg change in the first year was calculated as the slope of a simple linear regression model of qHBsAg on time during the first year, meaning that if a participant had only two measurements during this period, the resulting change could be calculated by dividing their difference by the time between the two measurements. For this model formulation, "time origin" to calculate time to HBsAg loss was taken to be the time of last qHBsAg measurement within the first year. Age was treated as a categorical variable to provide predictions for specific age groups. Additional models evaluated HBV genotype (B/C vs. others) in lieu of race and a single baseline HBsAg. For models that did not include qHBsAg change, the time origin was the baseline visit.

Missing covariate data were treated as missing completely at random, whereas the censoring was assumed independent of observed data. This implied that the likelihood-based estimation in models such as that in the Poisson and Weibull regression was consistent. The goodness of fit for the models was investigated using the time-dependent area under the receiver operating characteristics curve (AUROC) values as well as the sensitivity and specificity values. We specifically evaluated the AUROCs at years 1, 2, and 3 as the time origin. AUROC values from various models were compared using Wald-type tests. An additional sensitivity analysis excluded the 131 participants who had any prior history of HBV treatment. Finally, the HBRN-SQuARe score was externally validated on an HBeAg-negative cohort of 1253 patients (51 of whom lost HBsAg) from the Toronto Center for Liver Disease with similar inclusion criteria to the HBRN cohort.

The HBRN-SQuARe model was applied to the validation cohort to calculate the predicted probability of HBsAg loss at years 1, 2, and 3. Model performance was assessed using the time-dependent AUROC, similar to the development process.

All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 3.5.3 (R Core Team, 2013). All tests reported were two-sided, and *p*-values less than 0.05 were considered statistically significant. All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

Cohort characteristics

Of the 2458 HBsAg-positive participants at entry, 1408 met the initial eligibility criteria for this study. Following exclusion of those HBeAg-negative participants with less than 24 weeks of follow-up from baseline, a total of 1240 were eligible for the longitudinal analytic sample (1150 adults and 90 children) (Figure S1). All participants had at least two and at maximum 18 follow-up visits, with most participants (79.4%) having five or more follow-up visits. The median duration of follow-up was 5.5 years, and 17.1% of participants had 8 years or more of follow-up. Of the 1057 participants who reached 1 year of follow-up, 7.9% had initiated HBV therapy within the prior 24 weeks, and at 5 years of follow-up, 21.1% (125 of 588) had initiated antiviral therapy.

The baseline characteristics of the study cohort are summarized in Table 1. The mean age of children was 12 years and of adults was 43 years. Children and young adults (<30 years of age) represented 17.7% of the participants, whereas 11.9% were older than age 60. Most (69.9%) of the participants were of Asian ethnicity with near equal representation of males and females. With respect to virological and laboratory characteristics, the median HBV DNA was 3.2 $\log_{10}$ IU/ml; 96.6% were anti-HBe-positive (96.4% of these were consistently positive); and 34.2% had normal ALT levels. With respect to the phase of HBV infection, 24.7%

TABLE 1 Baseline characteristics of eligible adult and children with HBeAg-negative CHB

Baseline characteristics	*n*, Value
Age (years)	*n* = 1240
<18	90 (7.3%)
18 to <30	130 (10.5%)
30 to <50	619 (49.9%)
50 to <60	254 (20.5%)
≥60	147 (11.9%)
Female	610 (49.2%)
Race	*n* = 1236
White	154 (12.5%)
Black	185 (15.0%)
Asian	864 (69.9%)
Other/mixed	33 (2.7%)
Phenotype[a]	*n* = 1176
HBeAg-negative CHB	290 (24.7%)
Inactive carrier	366 (31.1%)
Indeterminant	520 (44.2%)
IND B	465 (89.4%)
IND C	55 (10.6%)
HBV genotype	*n* = 1096
A	204 (18.6%)
B	441 (40.2%)
C	301 (27.5%)
D	109 (9.9%)
Other (E, F, and multiple)	41 (3.8%)
qHBsAg (log_{10} IU/ml)	*n* = 1110
Median (25th:75th)	3.2 (2.5:3.8)
<2	159 (14.3%)
2–3	299 (26.9%)
>3	652 (58.7%)
Anti-HBe	*n* = 1036
Positive	1001 (96.6%)
HBV DNA (log_{10} IU/ml)	*n* = 1237
Median (25th:75th)	3.2 (2.4:4.3)
<3	549 (44.4%)
3 to <4	329 (26.6%)
4 to <5	187 (15.1%)
≥5	172 (13.9%)
ALT × ULN	*n* = 1216
Median (25th to 75th)	1.3 (0.9:1.8)
≤1 ULN	416 (34.2%)
>1 to ≤2 ULN	558 (45.9%)
>2 ULN	242 (19.9%)
Platelets ($\times 10^3/mm^3$)	*n* = 1044
Median (25th:75th)	222.0 (182.5:262.0)
APRI (AST-platelet-ratio index)	*n* = 1035
Median (25th:75th)	0.3 (0.2:0.4)
≤0.50	831 (80.3%)
>0.50–1.50	173 (16.7%)
>1.50	31 (3.0%)
BCP/PC mutation (A1762T, G1764A, G1896A)	*n* = 628
Wild type	178 (28.3%)
BCP only	152 (24.2%)
PC only	174 (27.7%)
BCP and PC	124 (19.7%)
Prior HBV treatment	*n* = 131 (10.6%)
Median (25th:75th), year-to-entry	5.0 (1.3, 9.0)
Interferon	29 (17.9%)
Entecavir	23 (14.2%)
Telbivudine	6 (3.7%)
Lamivudine	48 (29.6%)
Adefovir	13 (8.0%)
Peg-interferon	19 (11.7%)
Tenofovir	10 (6.2%)
Unknown	14 (8.6%)

[a]According to HBRN phenotype definitions.[15] Inactive carrier is defined by HBeAg-negative, normal ALT, and HBV DNA ≤ 10,000 IU/ml; IND-B by HBeAg-negative, elevated ALT, and HBV DNA ≤ 10,000 IU/ml; and IND-C by HBeAg-negative, normal ALT, and HBV DNA > 10,000 IU/ml.

Abbreviations: Anti-HBe, hepatitis B e antibody; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; BCP, basal core promoter; IND, indeterminant; PC, precore; ULN, upper limit of normal.

met the HBRN criteria for immune-active CHB; 31.1% were inactive carriers; and 44% were an indeterminant phenotype.[15,16] Genotypes B (40.2%) and C (27.5%) were most prevalent, followed by genotype A (18.6%); 71.7% of those tested (*n* = 628) had evidence of PC/BCP variants. The median HBsAg level at baseline was 3.2 log_{10} IU/ml (interquartile range [IQR] 2.8–3.8). Only 3% of the population had evidence of advanced fibrosis or cirrhosis at entry with APRI scores > 1.5.

Incidence of clinical outcomes

Clinical outcomes, including key serologic events, are summarized in Table 2. Among the 1240 HBeAg-negative participants, 92 ever became HBsAg-negative, for a crude incidence rate of 1.6 per 100 PY, of whom 71 met the criteria for sustained HBsAg loss for an incidence rate of 1.2 per 100 PY. The time course for HBsAg loss among those who ever lost HBsAg is shown in Figure 1. Baseline characteristics of participants with and without sustained HBsAg loss are given in Table S1.

TABLE 2 Summary of clinical outcomes

Outcomes[a]	Post baseline			
	Number of events	Number of events within 24 weeks of baseline	Median follow-up time in weeks (min, max)	Incidence (per 100 PY, 95% CI)[b]
Ever HBsAg negative[a]	92	0	211 (25, 384)	1.6 (1.3, 2.0)
Sustained HBsAg negative[c]	71	0	194 (25, 345)	1.2 (1.0, 1.6)
Ever HBeAg seroreversion	25	10	26 (0.4, 362)	0.4 (0.3, 0.6)
Sustained HBeAg seroreversion[c]	8	4	25 (20, 360)	0.1 (0.1, 0.3)
Ever anti-HBs positive[d]	63	1	193 (23, 399)	1.4 (1.1, 1.8)
Sustained anti-HBs positive[c,d]	33	0	148 (40, 338)	0.7 (0.5, 1.0)
Initiation of any HBV treatment[e]	291	63	92 (0.1, 376)	5.9 (5.2, 6.6)
New onset of ALT flare[f]	44	8	52 (1, 313)	0.8 (0.6, 1.0)
New onset of cirrhosis	23	10	29 (1, 167)	0.4 (0.3, 0.6)

[a]Includes transient and sustained HBsAg loss.

[b]Incidence estimate and 95% CI were obtained from Poisson regression.

[c]Defined as confirmed first HBsAg negative or HBeAg positive or anti-HBs positive (per the outcome of interest) measurement ≥24 weeks apart without any HBsAg positive or HBeAg negative or anti-HBs positive measurement in between.

[d]Among the 1240 HBeAg-negative participants, 926 (74.7%) had negative anti-HBs at study baseline and are eligible for anti-HBs analysis.

[e]Only nucleo(s)tide analogue therapies were given. None were treated with peg-interferon.

[f]ALT flare is defined as ≥10 × ULN.

A total of 926 (74.7%) were tested and negative for anti-HBs at study baseline and eligible for an evaluation on anti-HBs development. The remainder were not tested within the window used to define baseline. Of the 926 with testing at baseline, 594 (64.1%) had at least one anti-HBs test in follow-up (median number of tests = 1, IQR 0–5). Sixty-three ever became anti-HBs-positive, for a crude incidence rate of 1.4 per 100 PY, and was sustained in 33, for an incidence rate of 0.7 per 100 PY. The time course for anti-HBs seroconversion among those who ever lost HBsAg is shown in Figure S2. Of the 92 participants with HBsAg loss, 24 (26.1%) participants had anti-HBs detected at the same (n = 19) or an earlier time point (n = 5), 25 (27.1%) following HBsAg loss (11 within 1 year and 14 beyond 1 year of HBsAg loss), and the remainder were without anti-HBs (n = 37) or testing (n = 6) at last follow-up. In a time-to-event analysis restricted to patients with anti-HBs testing at least annually after HBsAg loss, the estimated median time between HBsAg loss and anti-HBs detection was approximately 1.5 years (95% CI 0.7–2.2).

Cirrhosis developed in 23 participants for an incidence rate of 0.4 per 100 PY. As 10 occurred within the first 24 weeks of cohort entry (and therefore likely present at baseline), excluding these resulted in an incidence rate of *de novo* cirrhosis of 0.2 per 100 PY (95% CI 0.1–0.4). No participant with HBsAg loss progressed to cirrhosis. ALT flares, defined as a serum ALT ≥ 10 times the ULN, were observed in 44 participants, for an incidence rate of 0.8 per 100 PY. Only 1 participant had an ALT flare before HBsAg loss (11 months before). Baseline characteristics associated with ALT flares were HBeAg-negative CHB and indeterminant phenotypes (vs. inactive CHB) (p < 0.001), higher HBV-DNA levels (p < 0.001), and lower platelet count (p = 0.04). Ever experiencing HBeAg seroreversion was observed in 25 participants, for an incidence rate of 0.4 per 100 PY, and was sustained in only 6 (0.1 per 100 PY); 8 (32%) were anti-HBe-negative at baseline. Seroreversion was not associated with receipt of immunosuppressive therapy. A total of 291 HBeAg-negative participants started treatment over the duration of follow-up (5.9 per 100 PY).

Baseline and time-varying factors associated with HBsAg loss and seroconversion

The crude incidence rate of ever HBsAg loss varied by baseline characteristics (Table S2 and Figure 2). In Poisson regression analysis, the incidence rate ratios for HBsAg loss were significantly associated with increasing age, non-Asian race, baseline HBV phenotype (inactive carrier vs. others), HBV genotype, and lower quantitative HBsAg and HBV DNA (Table 3). Those older than 60 years had an almost 80% higher rate of HBsAg loss compared with those younger than 30 years (p = 0.001). Participants of White and Black race had a 37% higher rate of HBsAg loss compared with those of Asian race (p = 0.03). Participants with an inactive CHB phenotype had the highest rate of HBsAg loss compared to participants with other HBV phenotypes (p < 0.001). Rate ratios of HBsAg loss were significantly lower in those with high qHBsAg and high HBV-DNA levels with the rate declining by 75% for each $\log_{10}$ increase in qHBsAg level (p < 0.001) and

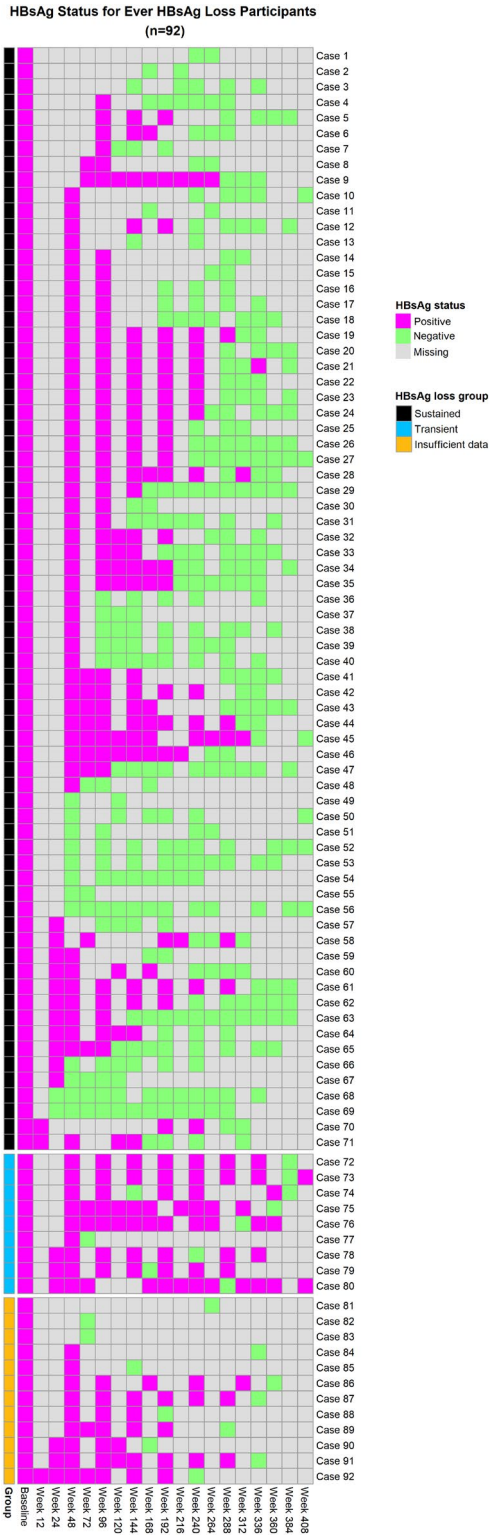

FIGURE 1 Evolution of HBsAg status among participants with HBsAg loss during study years. Each participant's follow-up is shown as a row, with the time of follow-up on the x-axis. Each time interval is represented by a box: Pink boxes indicate HBsAg is positive; light green boxes indicate HBsAg is negative; and gray boxes indicate missing test results. Furthermore, the participants are grouped into three broad categories indicated by the colored bar on the far left: black bar, those with sustained HBsAg loss; blue bar, transient HBsAg loss; and orange bar, insufficient follow-up. Most (67 of 92) participants had sustained HBsAg loss.

by 65% for each $\log_{10}$ increase in HBV-DNA level ($p < 0.001$). Treatment for a period longer than 24 weeks as a time-varying covariate was associated with a 66% lower rate of HBsAg loss compared with not starting treatment ($p = 0.02$).

The relationships between HBsAg loss and age, race, HBV phenotype, qHBsAg, and HBV DNA were robust and remained significant in adjusted models (Table 3). Male sex, which was not associated with HBsAg loss in unadjusted analysis, became statistically significant after controlling for age, race, and treatment. Conversely, AST and APRI were no longer significantly associated with HBsAg loss after controlling for age, race, sex, and treatment. HBsAg loss was not significantly associated with HBV genotype, presence of BCP/PC mutation, serum ALT, and platelet count in adjusted models.

The crude incidence rate of ever developing anti-HBs varied by baseline characteristics is provided in Table S3. In Poisson regression analysis, the incidence rate ratios for anti-HBs acquisition were significantly higher among those older than 60 years, with low baseline qHBsAg and low HBV-DNA level (Table S4).

Prediction of HBsAg loss

Two models were evaluated using age, sex, race, and either a single qHBsAg value or rate of qHBsAg change (minimum of two and maximum of three measurements) over a year. The median (IQR) time between qHBsAg measurements was 48.9 (46.6, 51.7) weeks. Based on 1077 HBeAg-negative participants, a model using age, sex, race, and a single qHBsAg value at baseline was developed to predict HBsAg loss over time (Table 4, model 1). The AUROC for predicting loss of HBsAg at 1 year was 0.95 (95% CI 0.91–0.99), at 2 years was 0.94 (95% CI 0.91–0.97), and at 3 years was 0.94 (95% CI 0.92–0.97). The second model with the rate of qHBsAg change (minimum of two and maximum of three measurements) over a year as well as the baseline age, sex, race, and baseline qHBsAg was based on a total of 739 individuals, with 56 achieving HBsAg loss (Table 4, model 2). The AUROC for predicting loss of HBsAg at 1 year was 0.99 (95% CI 0.987–1.00), at 2 years was 0.96 (95% CI 0.89–1.00), and at 3 years was 0.95 (95% CI 0.91–1.00) and was significantly better than model 1 in the first year ($p = 0.01$). We refer to this as the HBRN-SQuARe (Sex, ΔQuantitave HBsAg, Age, Race) model. The receiver operating characteristic (ROC) curves for models 1 and 2 for years 1-3 are given in Figure 3. Inclusion of an interaction term for baseline qHBsAg and change in HBsAg did not improve model prediction. Graphical depiction of the predicted probability of HBsAg loss in representative patient groups is shown in Figure 4.

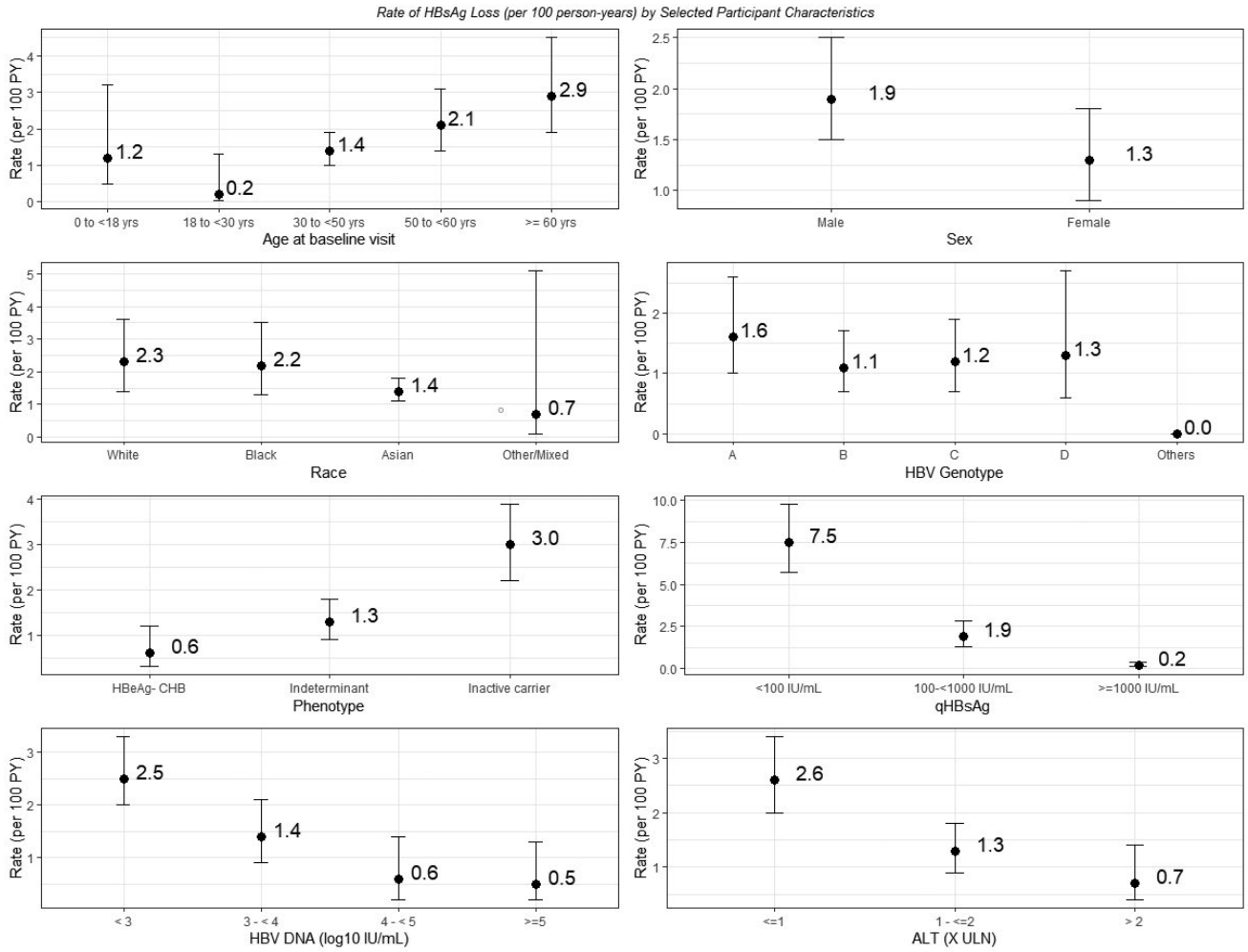

FIGURE 2 Crude incidence rate (per 100 person-years [PY]) for HBsAg loss by selected baseline characteristics. Participants with HBsAg loss differed by baseline characteristics. Crude incidence rates by key baseline factors are shown: age, sex, race, HBV genotype, chronic HBV (CHB) phenotype, alanine aminotransferase (ALT), HBV DNA, and HBsAg quantitation

Other models evaluating prediction of HBsAg loss evaluated genotype instead of race and genotype plus race in the model, but were not superior to the HBRN-SQuARe model (model 2) (Table S5). HBRN SQuARe also yielded very similar results after excluding 131 participants who had any prior history of HBV treatment, except that race was no longer a significant predictor (results not shown). Finally, the model was rerun by replacing the continuous qHBsAg with baseline HBsAg categories (<100, 100 to <1000, and >1000) with very similar Akaike information criterion statistic compared with the HBRN-SQuARe model. A web application for the HBRN-SQuARe model is provided at https://abduswahed.shinyapps.io/HBRN-SQuARe/.

External validation of HBRN-SQuARe model

For external validation of the predictive model, HBRN SQuARe models 1 and 2 were validated retrospectively in a separate single center (Toronto Center for Liver Disease). Baseline characteristics of the validation cohort (Table S7) included 71% (*n* = 893) Asian, 45% (*n* = 558) female, with young adults (<30 years of age but no children) comprising only 6% of the cohort, in contrast to the HBRN that included children and 18% of participants were under age 30. Furthermore, the proportion of patients in the Toronto cohort over the age of 60 was 28% versus 18% of the HBRN cohort. Baseline qHBsAg, proportion female, and proportion Asian were similar. The mean follow-up duration for patients was 3.1 (SD: 1.9) years, with 10% of patients having 6 years or more of follow up. A total of 69 (5.5%) patients achieved HBsAg loss for an incidence rate of 1.8 per 100 PY, very similar to our cohort.

Validation of models 1 and 2 was performed by assessing the performance of predicting HBsAg loss at years 1, 2, and 3 by estimating the AUROC. AUROC estimates with 95% CI for model 1 at years 1, 2, and 3 were 0.70 (0.54–0.86), 0.75 (0.65–0.85), and 0.77

TABLE 3 Association between patient characteristics and incidence of first HBsAg loss expressed as rate ratios[a]

	n	Unadjusted rate ratio (95% CI)	*p* value	*n*	Adjusted rate ratio (95% CI)[b]	*p* value
Baseline characteristics						
Age, years (ref ≥60)	1240		0.01			
<18		0.41 (0.14, 1.16)				
18 to <30		0.06 (0.01, 0.45)				
30 to <50		0.47 (0.28, 0.79)				
50 to <60		0.71 (0.40, 1.24)				
Female sex	1240	0.67 (0.45, 1.02)	0.06			
Asian (ref: non-Asian)[c]	1203	0.63 (0.41, 0.95)	0.03			
Phenotype (ref: inactive carrier)	1176		<0.001	1143		<0.001
HBeAg(-) CHB		0.22 (0.11, 0.44)			0.24 (0.12, 0.49)	
Indeterminant		0.43 (0.27, 0.66)			0.44 (0.28, 0.69)	
Non-A genotype[d]	1055	0.72 (0.41, 1.27)	0.26	1055	0.77 (0.43, 1.35)	0.36
BCP/PC mutation (ref: wild type)	628		0.07	609		0.11
BCP only		0.41 (0.15, 1.11)			0.42 (0.15, 1.16)	
PC only		0.27 (0.09, 0.81)			0.29 (0.10, 0.87)	
BCP and PC		0.72 (0.30, 1.76)			0.63 (0.23, 1.74)	
Time-varying variables						
HBV treatment (≥24 weeks in past 36 weeks)	1240	0.34 (0.14, 0.84)	0.02			
qHBsAg per 1-$\log_{10}$ IU/ml increase	1219	0.25 (0.18, 0.35)	0.001	1182	0.24 (0.18, 0.32)	<0.001
HBV DNA per 1-$\log_{10}$ IU/ml increase	1240	0.35 (0.28, 0.43)	<0.001	1203	0.22 (0.16, 0.30)	<0.001
ALT per 1 × ULN increase	1234	0.56 (0.31, 1.02)	0.06	1197	0.63 (0.36, 1.11)	0.11
Platelets per 10^3/mm^3 increase	1185	1.002 (0.998, 1.01)	0.03	1150	1.003 (0.999, 1.01)	0.11

[a]The ratio of the rate at which participants experienced HBsAg loss, where a lower rate suggests a longer time to HBsAg-negative. Thus, a value <1 indicates longer time to HBsAg loss.

[b]Each estimate is from a unique model adjusting for age group, sex, race, and treatment with one exception: Genotype is not adjusted for race. For the adjusted model, age was regrouped to combine all <30 in one group, because of lack of HBsAg loss in lower age groups (Table S1).

[c]Does not include "other/mixed" race (*n* = 33).

[d]Does not include "other (E, F, and multiple): genotype (*n* = 41).

TABLE 4 Model for predicting log time to HBsAg loss based on qHBsAg level, age, race, and sex

	Model 1		Model 2 (HBRN SQuARe)	
Parameter	**Estimate (SEM)**	***p* value**	**Estimate (SEM)**	***p* value**
Intercept	1.11 (0.17)	<0.0001	1.94 (0.24)	<0.0001
Age (years) (ref: ≥60 years)				
<30 years	1.11 (0.58)	0.05	0.65 (0.44)	0.14
30 to <50	−0.03 (0.16)	0.86	−0.18 (0.17)	0.29
50 to <60	−0.08 (0.17)	0.64	−0.47 (0.17)	0.01
Sex (female vs. male)	0.41 (0.14)	0.004	0.33 (0.13)	0.004
Race (Asian vs. others)	0.65 (0.14)	<0.0001	0.12 (0.15)	<0.0001
qHBsAg (log 10 IU/ml) baseline	0.55 (0.06)	<0.0001	0.37 (0.07)	<0.0001
Rate of change in qHBsAg (log 10 IU/ml/year)			1.26 (0.18)	<0.0001
Interaction (qHBsAg*rate of change in qHBsAg)			−0.25 (0.08)	<0.0001
Akaike information criterion	462.19		258.71	

Note: Model 1: *n* = 1077, events = 80 and model 2: *n* = 739, events = 56. Estimates refer to mean change in log time to HBsAg loss with respect to one-unit change in continuous variable, and the mean difference in log time to HBsAg loss between comparison and reference groups. Thus, a positive coefficient indicates longer time to HBsAg loss, or reduced likelihood of HBsAg loss.

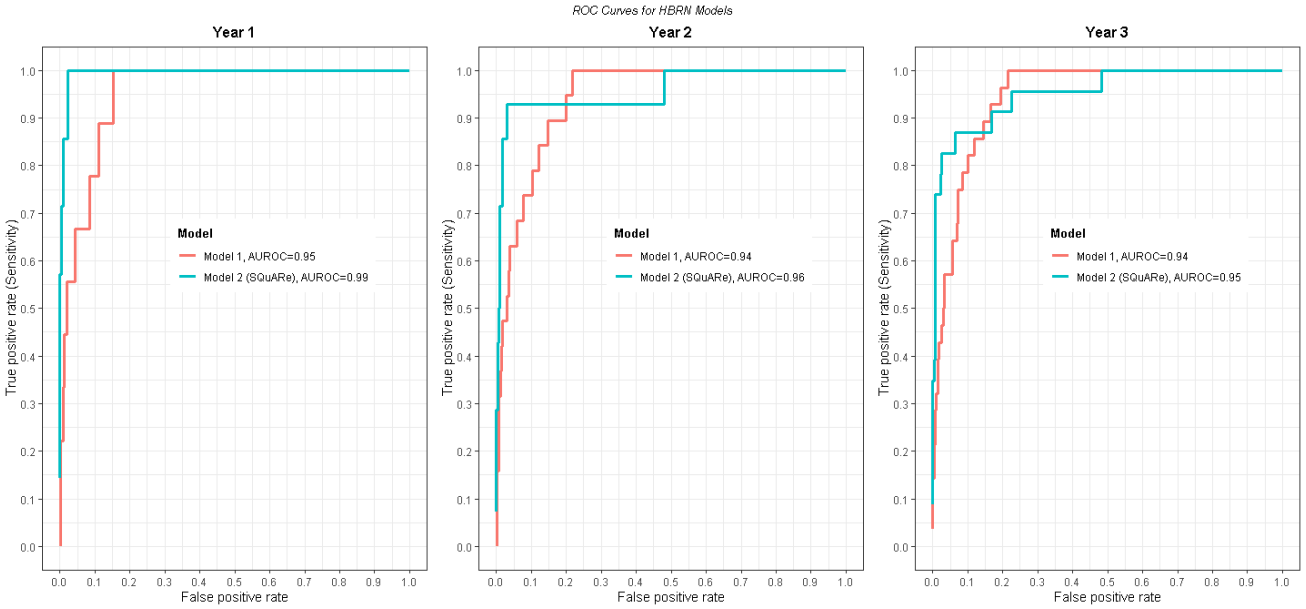

FIGURE 3 Area under the receiver operating characteristic curves (AUROCs) for predicting HBsAg loss using models 1 and 2 (HBRN SQuARe). The SQuARe model (model 2 = sex,ΔquantHBsAg, age, race) can estimate predicted probability of HBsAg loss over time (https://abduswahed.shinyapps.io/RShiny/)

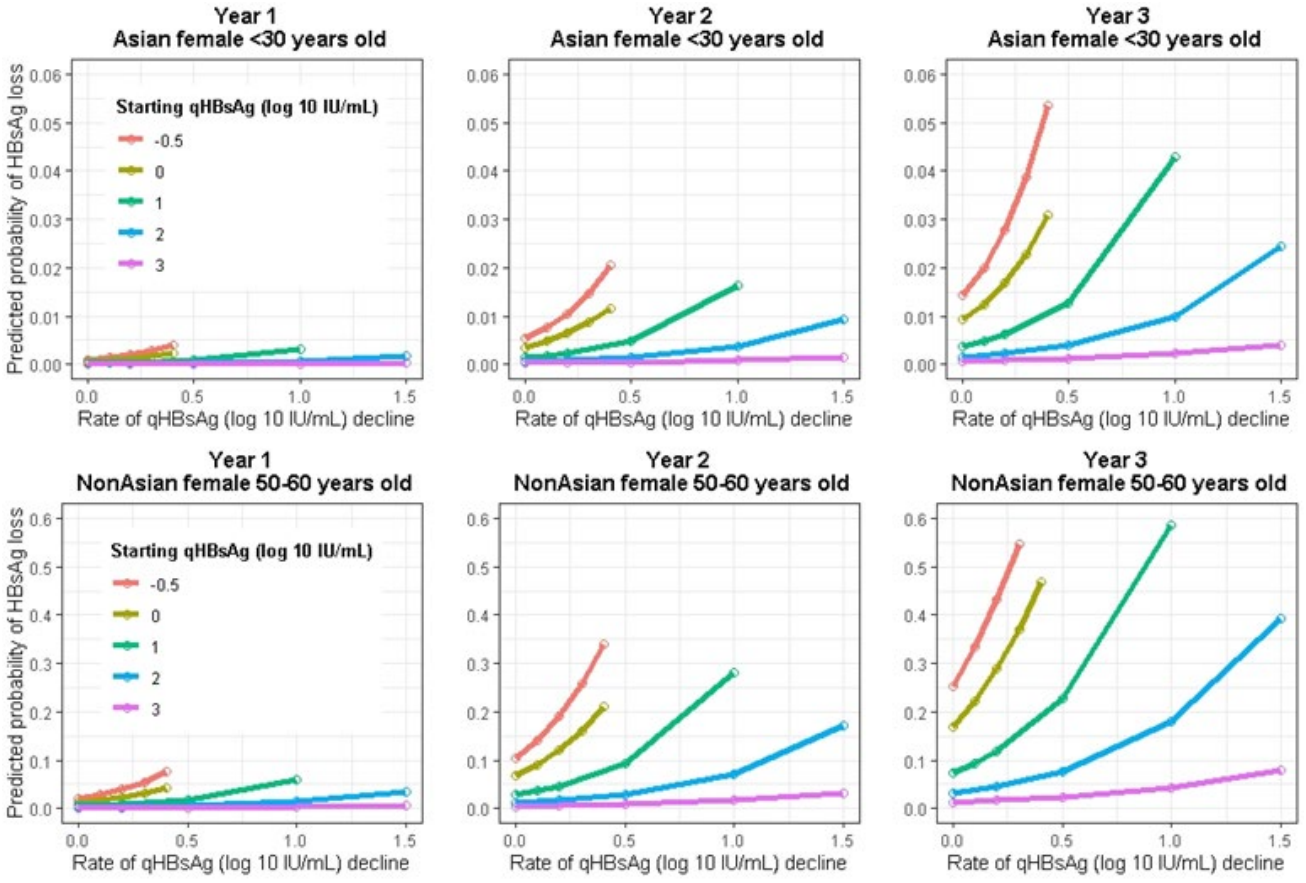

FIGURE 4 Examples of predicting probability of HBsAg loss using HBRN-SQuARe model. Predicted probability of HBsAg loss as a function of baseline quantitative HBsAg (qHBsAg) levels and change over a 1-year period for selected participants of varying race and age. Upper row for a <30-year-old Asian female participant and the lower panel for a non-Asian female in her 50s

(0.69–0.85), respectively. AUROC for model 2 (HBRN SQuARe) estimates with 95% CI at years 1, 2, and 3 were 0.99 (0.98–1.00), 0.90 (0.80–1.00), and 0.88 (0.77–0.99), respectively. A total of 728 individuals with 22 (3%) achieving HBsAg loss met the criteria for inclusion in this latter model. The ROC curves for the validation models are shown in Figure S3.

DISCUSSION

HBsAg loss is an infrequent but critically important event in the natural history of chronic HBV infection. In this large, multi-ethnic North American cohort with median follow-up of 5.5 years (nearly 20% with 8 years or more), we found that HBsAg loss occurred at a rate of 1.6 per 100 PY, similar to what has been reported in other cohorts and systematic reviews.[4,5] However, the rates of HBsAg loss differed markedly by baseline characteristics, ranging from <0.5 per 100 PY to over 3 per 100 PY. Using sex, age, race, and quantitative HBsAg, the HBRN-SQuARe model was developed and internally and externally validated to accurately predict HBsAg loss over a 3-year period. The information provided by the HBRN SQuARe score can be used to convey likelihood of HBsAg loss with a 3-year horizon for untreated HBeAg-negative patients with CHB. Identification of patients who are more or less likely to spontaneously clear HBsAg allows for prognostication, which is clearly important for patients, but also may help prioritize individuals for therapies. For example, those who are unlikely to clear HBsAg spontaneously may be more inclined to opt for new therapies, whereas patients predicted to have higher spontaneous clearance rates may be informed that need for additional therapies to enhance HBsAg loss is less urgent.

Understanding the predictors of HBsAg loss is important for many reasons. Long-term follow-up data consistently confirm the importance of HBsAg loss, with markedly lower rates of progressive liver disease and HCC, particularly when HBsAg loss occurs in patients younger than 50 years without cirrhosis.[21,22] The clear clinical benefits and durability of HBsAg loss have made it the preferred therapeutic endpoint for current HBV therapies in development. Yet, given the heterogeneity in patients with CHB, models that can help to stratify likelihood of HBsAg loss in the absence of treatment may be especially helpful in the design of randomized trials, to ensure balance across treatment arms and potentially to allow for improved matching of patients with specific therapeutic approaches that are more likely to be effective based on a given clinical profile.

We found the strongest predictors of HBsAg loss were older age, non-Asian ethnicity, inactive disease, and low HBsAg and HBV-DNA levels. The association of very low or undetectable levels of HBsAg and HBV DNA with HBsAg loss suggests that clearance may occur when declining cccDNA levels and/or transcriptional activity fall below a threshold, not necessarily invoking a specific viral or immunological event that triggers clearance. Importantly, we were not able to distinguish HBsAg derived from cccDNA from HBsAg that is transcribed from HBV DNA integrated into the host genome. Tools to distinguish the source of HBsAg would be very helpful, particularly if the prognostic implications of the HBsAg from integrated versus cccDNA differ. Understanding the processes by which HBsAg clearance occurs could have important implications for the development of treatments that lead to functional cure.

Few studies have examined the durability of spontaneous HBsAg loss and seroreversion. We found that HBsAg loss was durable in most participants (73%), with anti-HBs developing concurrent with or in the subsequent year in approximately half the participants. As anti-HBs testing was less frequent than HBsAg testing in the cohort, the timing of anti-HBs acquisition is less precise than the trajectory of HBsAg loss and seroreversion. However, similar rates of durable spontaneous HBsAg loss were reported in other large cohort studies.[23–25] HBsAg seroreversion was rare in our study with an incidence of 0.4% per year, which was lower than the 4.1% reported in the Hong Kong cohort.[25] Differences may reflect differences in the baseline characteristics of the population, with our study more limited to those that entered the cohort having already achieved HBeAg negativity. Similar to other studies, we observed a variable interval between HBsAg loss and anti-HBs seroconversion. Thus, the current definition of HBV functional cure does not mandate detection of anti-HBs.

Clinical events among the cohort with HBeAg-negative CHB were infrequent, as previously highlighted in the outcomes among all adult HBRN participants and in the pediatric cohort.[17,26] ALT flares defined by ALT values greater than 10 times normal occurred in 44 patients at a median of 52 weeks of follow-up, for a rate of 0.8% per year, a rate lower than reported in the entire HBRN cohort (incidence = 1.51 per 100 PY) but reflective of differences in ALT flare rates between HBeAg-positive versus HBeAg-negative CHB. Treatment initiation occurred at a rate of 5.9% in this cohort and was associated with a lower rate of HBsAg loss compared with those who remained untreated. It is likely that treatment was a surrogate for active disease rather than truly negatively influencing the likelihood of HBsAg clearance. Treatment decisions were made by individual health care providers (not protocolized) and final models adjusted for treatment initiation as a time-varying covariate. In *post hoc* analysis, factors associated with treatment initiation were elevated ALT and HBV-DNA levels (data not shown), supporting our hypothesis that treatment initiation was a surrogate for

active CHB. Finally, progression to cirrhosis, as determined by an adjudication committee, occurred in 23 patients (rate of 0.4% per year), but nearly half (10 of 23) were diagnosed within first 24 weeks, suggesting that it was likely present at baseline, and none of the participants with HBsAg loss progressed to cirrhosis. This low rate of progression to cirrhosis aligns with our previously reported low rate of clinically relevant outcomes in the HBRN cohort and likely reflects the benefits of active management of chronic HBV infection.[17]

This study has some limitations. HBsAg loss was an infrequent event, limiting the number of covariates that could be included in the prediction models. However, the cohort represents a large multi-ethnic population from North America with long-term and comprehensive prospective follow-up and the risk prediction score developed from our cohort was externally validated with good accuracy. HBV genotype representation is dominated by genotype B and C, although genotypes A–G are also represented. This limited our ability to perform genotype-specific models for HBsAg loss. Regarding our prediction models, internal cross-validation was not possible due to the modest number of HBsAg loss events, but external validation in another North American cohort strengthens this work. As expected, the accuracy of HBRN SQuARe in predicting HBsAg in the validation cohort was less than in the original cohort, possibly related to the difference in the percentage of patients at the extremes of age, with the Toronto cohort having a smaller proportion under age 30 (6% vs. 18% in HBRN). Nonetheless, the AUROC value of 0.88 at 3 years for HBRN SQuARe in the externally validated cohort indicates good to excellent discrimination. We found that use of serial qHBsAg data through week 48 performed better than a single baseline qHBsAg value. Therefore, the preferred final model was HBRN SQuARe, based on serial values over one year rather than a single qHBsAg value.

In conclusion, we found that HBsAg seroclearance occurred infrequently in a large cohort of predominantly untreated North American patients with HBeAg-negative CHB. HBsAg loss was associated with older age, non-Asian ethnicity, and inactive CHB, and can be predicted over 3 years using a simple validated HBRN-SQuARe score model. This information is important to disease prognostication and prioritization of future therapeutic approaches in CHB.

ACKNOWLEDGMENT

In addition to the authors, the HBRN wishes to acknowledge the contributions of the following: ***Harvard Consortium*****:** Jianghe Niu, Ph.D., Asad Javaid, M.B.B.S., Bilal Nasir, M.B.B.S., Ammu Susheela, M.B.B.S., Imad Nasser, M.D. (Beth Israel Deaconess Medical Center, Boston, MA, USA), Arley Donovan, Nifasha Rusibamayila, and Cara Foley (Massachusetts General Hospital, Boston, MA, USA). ***Minnesota Alliance for Research in Chronic Hepatitis B*****:** Alisha C. Stahler, Linda Stadheim, R.N. (Mayo Clinic Rochester, Rochester, MN, USA), John Lake, M.D., Philip Lacher (University of Minnesota, Minneapolis, MN, USA), and Shannon M. Riggs, L.P.N., A.S. (Department of Pediatrics, University of Minnesota Masonic Children's Hospital, Minneapolis, MN, USA). ***Midwest Hepatitis B Consortium*****:** Kathryn Rushing, R.N., Rosemary A. Nagy, R.D.N., L.D., M.B.A., Jacki Cerkoski, R.N., M.S.N. (St. Louis University School of Medicine, St. Louis, MO, USA), Debra DeMarco Shaw, R.N., B.S.N., Lisa Kessels, R.N., and Michael K. Klebert, Ph.D., R.N., A.N.P.-B.C. (Washington University School of Medicine, St. Louis, MO, USA). ***University of Toronto Consortium*****:** Seham Noureldin, Ph.D., Danie La, R.N., Lucie Liu, M.Sc., C.C.R.P., Diana Kaznowski, R.N., Jiayun Chen, Fengfei Huang, Doinita Vladutu, Orlando Cerocchi (Toronto General Hospital, Toronto, ON, Canada), and Athena Hau, B.Sc. (Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada). ***HBV CRN North Texas Consortium*****:** Debra Rowan, L.V.N. (Division of Digestive and Liver Diseases, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA), Sheila Bass (University of Texas Southwestern, Dallas, TX, USA), Barbara Lilly, B.S. (Baylor University Medical Center, Dallas, TX, USA), Laurie A. Rodgers–Augustyniak, R.N., Shirley Montanye, R.N. (Department of Pediatrics, UTSW, Dallas, TX, USA). ***Los Angeles Hepatitis B Consortium*****:** Samuel French, M.D., and Velma Peacock, R.N. (David Geffen School of Medicine, UCLA, Los Angeles, CA, USA). ***San Francisco Hepatitis B Research Group Consortium*****:** Marion Peters, M.D., Ashley Shobe, M.S., Rayshawnda Davis, Romuald Kuras, Claudia Ayala, M.S., Ivy Lau, B.S. (University of California-San Francisco, San Francisco, CA, USA), Veronika Podolskaya, B.S., N.C.P.T., Anna von Bakonyi, L.V.N., C.C.R.C., Nata DeVole, R.N. (California Pacific Medical Center Research Institute, San Francisco, CA, USA), Natasha Feier, M.S., Joel Feier, B.S., and Camille Langlois, M.S. (Department of Pediatrics, UCSF, San Francisco, CA, USA). ***Michigan Hawaii Consortium*****:** Barbara McKenna, M.D., Karen Choi, M.D., Kelly Oberhelman, P.A.C., Sravanthi Kaza, Bpharm, Isabel Moran (University of Michigan, Ann Arbor, MI, USA), Leslie Huddleston, N.P., and Richmond Wong (The Queen's Medical Center, University of Hawaii, Honolulu, HI, USA). ***Chapel Hill, NC Consortium*****:** A. Sidney Barritt, M.D., Tiffany Marsh, B.A., Vikki Metheny, A.N.P., and Danielle Cardona, P.A-C. (University of North Carolina at Chapel Hill, Chapel Hill, NC, USA). ***Virginia Commonwealth University Medical Center*****:** Paula G. Smith, R.N., B.S.N., and Charlotte Hofmann, R.N. (Virginia Commonwealth University Health System, Richmond, VA, USA). ***PNW/Alaska Clinical Center Consortium*****:** Alycia Wolfstone, R.N., M.N. (University

of Washington Medical Center, Seattle, WA, USA), Jody Mooney, Lupita Cardona-Gonzalez (Virginia Mason Medical Center, Seattle, WA, USA), and Kara L. Cooper (Center for Clinical and Translational Research, Seattle Children's Institute, Seattle, WA, USA). ***Johns Hopkins University*****:** Hongxia Li, M.B.B.S., M.S., Douglas Mogul, M.D., Robert Anders, M.D., Ph.D., Hejab Imteyaz, Peter Lee, M.D., Kiyoko Oshima, M.D., Kim Kafka, R.N., and Naureen Islam, B.S. **(**Department of Pediatrics, Johns Hopkins Medical Institutions, Baltimore, MD, USA). ***Liver Diseases Branch, NIDDK, NIH*****:** Nancy Fryzek, R.N., B.S.N., Elenita Rivera, B.S.N., Nevitt Morris, Vanessa Haynes-Williams, Amy Huang, R.N., Catherine Nadal, R.N., M.S, and Jaha Norman-Wheeler, R.N., B.A. (National Institutes of Health, Bethesda, MD, USA). ***Liver Disease Research Branch, NIDDK, NIH:*** Averell H. Sherker, M.D., Rebecca J. Torrance, R.N,. M.S., and Sherry R. Hall, M.S. (National Institutes of Health, Bethesda, MD, USA). ***Immunology Center*****:** Mary E. Valiga, R.N., Keith Torrey, B.S., Danielle Levine, B.S., James Keith, B.S., Michael Betts, Ph.D. (University of Pennsylvania, Philadelphia, PA, USA), and Luis J. Montaner, D.V.M., D.Phil. (Wistar Institute, Philadelphia, PA, USA). ***Data Coordinating Center*****:** Frani Averbach, M.P.H., Tamara Haller, Regina Hardison, M.S., Stephanie Kelley, M.S., Christina M. Lalama, M.S., Sharon Lawlor, M.B.A., Hsing-Hua S. Lin, M.S., Ph.D., Manuel Lombardero, M.S., Andrew Pelesko, B.S., Donna Stoliker, Melissa Weiner, M.P.H., Ella Zadorozny, M.S., and Qian Zhao, Ph.D. (Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA).

The HBRN: *Minnesota Alliance for Research in Chronic Hepatitis B Consortium***:** Lewis R. Roberts, M.B., Ch.B., Ph.D. (Mayo Clinic Rochester, Rochester, MN, USA), Mohamed A. Hassan, M.D. (University of Minnesota, Minneapolis, MN, USA), and Sarah Jane Schwarzenberg, M.D. (Department of Pediatrics, University of Minnesota Masonic Children's Hospital, Minneapolis, MN, USA). ***Midwest Hepatitis B Consortium*****:** Adrian M. Di Bisceglie, M.D., (St. Louis University School of Medicine, St. Louis, MO, USA) and Jeffrey Teckman, M.D. (Department of Pediatrics, Cardinal Glennon Children's Medical Center, St. Louis University, St. Louis, MO, USA). ***University of Toronto Consortium*****:** David K. Wong, M.D. (Toronto General Hospital, Toronto, ON, Canada), Joshua Juan, M.D. (Toronto General Hospital, Toronto, ON, Canada), Colina Yim, N.P., M.N. (Toronto General Hospital, Toronto, ON, Canada), Keyur Patel, M.D. (Toronto General Hospital, Toronto, ON, Canada), and Simon C. Ling, M.B.Ch.B. (Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, ON, USA). ***HBV CRN North Texas Consortium:*** William M. Lee, M.D. (Division of Digestive and Liver Diseases, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA), Son Do, M.D. (University of Texas Southwestern, Dallas, TX, USA), and Norberto Rodriguez-Baez, M.D. (Department of Pediatrics, University of Texas Southwestern, Dallas, TX, USA). ***Los Angeles Hepatitis B Consortium*****:** Steven-Huy B. Han, M.D. (David Geffen School of Medicine, UCLA, Los Angeles, CA, USA) and Tram T. Tran, M.D. (Cedars Sinai Medical Center, Los Angeles, CA, USA). ***Michigan Hawaii Consortium*****:** Naoky Tsai, M.D. (The Queen's Medical Center, University of Hawaii, Honolulu, HI, USA) and Barak Younoszai, D.O. (The Queen's Medical Center, University of Hawaii, Honolulu, HI, USA). ***Chapel Hill, NC Consortium*****:** Michael W. Fried, M.D. (University of North Carolina at Chapel Hill, Chapel Hill, NC, USA), Andrew Muir, M.D. (Duke University Medical Center, Durham, NC, USA), Donna Evon, Ph.D. (University of North Carolina at Chapel Hill, Chapel Hill, NC, USA), and Jama M. Darling, M.D. (University of North Carolina at Chapel Hill, NC, USA). ***PNW/Alaska Clinical Center Consortium*****:** Robert C. Carithers, M.D. (University of Washington Medical Center, Seattle WA, USA), Margaret Shuhart, M.D. (Harborview Medical Center, Seattle WA, USA), Kris V. Kowdley, M.D. (Virginia Mason Medical Center, Seattle WA, USA), Chia C. Wang, M.D. (Virginia Mason Medical Center, Seattle WA, USA), and Karen F. Murray, M.D. (Department of Pediatrics, University of Washington, Seattle, WA, USA). ***Virginia Commonwealth University Medical Center*****:** Velimir A. Luketic, M.D. (Virginia Commonwealth University Health System, Richmond, VA, USA). ***Johns Hopkins University*****:** Kathleen B. Schwarz, M.D. (Department of Pediatrics, Johns Hopkins Medical Institutions, Baltimore, MD, USA**)**. ***Liver Diseases Branch, NIDDK*****:** T. Jake Liang, M.D. (National Institutes of Health, Bethesda, MD, USA). ***Liver Disease Research Branch, NIDDK:*** Jay H. Hoofnagle, M.D. (National Institutes of Health, Bethesda, MD, USA) and Edward Doo, M.D. (National Institutes of Health, Bethesda, MD, USA). ***Immunology Center*****:** Kyong-Mi Chang, M.D. (University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA) and Jang-June Park, Ph.D. (University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA). ***Data Coordinating Center*****:** Steven H. Belle, Ph.D., M.Sc.Hyg. (Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA), Wendy C. King, Ph.D. (Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA). ***Central Pathology*****:** David Kleiner, M.D., Ph.D. (Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, USA).

CONFLICT OF INTEREST

Dr. Janssen consults and received grants from Gilead, GlaxoSmithKline, Roche, Janssen, and Vir. He consults for Aligos, Antios, Arbutus, Eiger, Merck, and Viroclinics. Dr. Terrault consults for EXIGO, Saol Therapeutics and Moderna. She

received grants from Gilead, Roche/Genentech, and GlaxoSmithKline. Dr. Wahed consults for Merck. Dr. Feld consults for Arbutus, Antios, Enanta, Eiger, Gilead, GlaxoSmithKline, and Janssen. Dr. Lisker-Melman is on the speakers' bureau for AbbVie and Gilead. Dr. Sterling is on the data security monitoring board for AskBio and Pfizer. He received grants from Roche, Abbott, Gilead, and AbbVie. Dr. Khalili consults and received grants from Gilead. She received grants from Intercept. Dr. Chung received grants from AbbVie, Gilead, Bristol-Myers Squibb, Merck, Roche, Boehringer Ingelheim, GlaxoSmithKline, and Janssen. Dr. Rosenthal consults and received grants from AbbVie, Albireo, Gilead, and Mirum. He consults for Audentes, Biomarin, Discerna, Encoded, and Ambys. He received grants from Arrowhead and Merck. Dr. Fontana received grants from Gilead and AbbVie. Dr. Lau consults for Abbott and advises Gilead. Dr. Lok advises and received grants from TARGET. She advises Arbutus, ClearB, Enanta, Enochian, GNI, and Janssen. She received grants from Gilead.

AUTHOR CONTRIBUTIONS

Study design, data acquisition and interpretation, manuscript editing and critical review: J. J. Feld, S. L. Cooper, M. G. Ghany, M. Lisker-Melman, R. Perrillo, R. K. Sterling, M. Khalili, R. T. Chung, P. Rosenthal, R. J. Fontana, D. T. Y. Lau, A. S. Lok, H. L. A. Janssen. Study design, data acquisition and interpretation, manuscript draft, editing and critical review: N. A. Terrault. Analysis, data interpretation, manuscript editing and critical review: A. S. Wahed. Data acquisition and interpretation, and critical review: A. Sarowar. Analysis, data interpretation and critical review: J. Wang.

ORCID

Norah A. Terrault https://orcid.org/0000-0003-4143-1950
Richard K. Sterling https://orcid.org/0000-0002-8637-2475
Mandana Khalili https://orcid.org/0000-0001-9178-9139
Daryl T. Y. Lau https://orcid.org/0000-0003-4139-1987

REFERENCES

1. Cornberg M, Lok A-F, Terrault NA, Zoulim F, Berg T, Brunetto MR, et al. EASL-AASLD HBV Treatment Endpoints Conference. J Hepatol. 2019;2020:539–57.
2. Yip T-F, Wong G-H, Chan H-Y, Tse Y-K, Lam K-Y, Lui G-Y, et al. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. J Hepatol. 2019;70:361–70.
3. Su YC, Lin PC, Yu HC, Wu CC. Hepatitis B virus reactivation in patients with resolved hepatitis B virus infection receiving chemotherapy or immunosuppressive therapy. Eur J Gastroenterol Hepatol. 2018;30:925–9.
4. Zhou K, Contag C, Whitaker E, Terrault N. Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analyses. Lancet Gastroenterol Hepatol. 2019;4:227–38.
5. Yeo YH, Ho HJ, Yang HI, Tseng T-C, Hosaka T, Trinh HN, et al. Factors associated with rates of HBsAg seroclearance in adults with chronic HBV infection: a systematic review and meta-analysis. Gastroenterology. 2019;156:635–46 e9.
6. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. Gastroenterology. 2008;135:459–67.
7. Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. Gastroenterology. 2009;136:2169–79 e1–4.
8. Bourlière M, Rabiega P, Ganne-Carrie N, Serfaty L, Marcellin P, Barthe Y, et al. Effect on HBs antigen clearance of addition of pegylated interferon alfa-2a to nucleos(t)ide analogue therapy versus nucleos(t)ide analogue therapy alone in patients with HBe antigen-negative chronic hepatitis B and sustained undetectable plasma hepatitis B virus DNA: a randomised, controlled, open-label trial. Lancet Gastroenterol Hepatol. 2017;2:177–88.
9. Liaw YF. Clinical utility of HBV surface antigen quantification in HBV e antigen-negative chronic HBV infection. Nat Rev Gastroenterol Hepatol. 2019;16:631–41.
10. Rein DB, Lesesne SB, O'Fallon A, Weinbaum CM. Prevalence of hepatitis B surface antigen among refugees entering the United States between 2006 and 2008. Hepatology. 2010;51:431–4.
11. Mitchell T, Armstrong GL, Hu DJ, Wasley A, Painter JA. The increasing burden of imported chronic hepatitis B-United States, 1974–2008. PLoS One. 2011;6:e27717.
12. Coffin CS, Ramji A, Cooper CL, Miles D, Doucette KE, Wong P, et al. Epidemiologic and clinical features of chronic hepatitis B virus infection in 8 Canadian provinces: a descriptive study by the Canadian HBV Network. CMAJ Open. 2019;7:E610–7.
13. Ghany MG, Perrillo R, Li R, Belle SH, Janssen HLA, Terrault NA, et al. Characteristics of adults in the hepatitis B research network in North America reflect their country of origin and hepatitis B virus genotype. Clin Gastroenterol Hepatol. 2015;13:183–92.
14. Schwarz KB, Cloonan YK, Ling SC, Murray KF, Rodriguez-Baez N, Schwarzenberg SJ, et al. Children with chronic hepatitis B in the United States and Canada. J Pediatr. 2015;167:1287–94 e2.
15. Di Bisceglie AM, Lombardero M, Teckman J, Roberts L, Janssen HLA, Belle SH, et al. Determination of hepatitis B phenotype using biochemical and serological markers. J Viral Hepat. 2017;24:320–9.
16. Schwarz KB, Lombardero M, Di Bisceglie AM, Murray KF, Rosenthal P, Ling SC, et al. Phenotypes of chronic hepatitis B in children from a large North American cohort. J Pediatr Gastroenterol Nutr. 2019;69:588–94.
17. Lok AS, Perrillo R, Lalama CM, Fried MW, Belle SH, Ghany MG, et al. Low incidence of adverse outcomes in adults with chronic hepatitis B virus infection in the era of antiviral therapy. Hepatology. 2021;73:2124–40.
18. Ganova-Raeva L, Ramachandran S, Honisch C, Forbi JC, Zhai X, Khudyakov Y. Robust hepatitis B virus genotyping by mass spectrometry. J Clin Microbiol. 2010;48:4161–8.
19. Lau DTY, Ganova-Raeva L, Wang J, Mogul D, Chung RT, Lisker-Melman M, et al. Precore and basal core promoter hepatitis B virus (HBV) variants are present from a young age and differ across HBV genotypes. Hepatology. 2021;73:1637–51.
20. Xiao G, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. Hepatology. 2015;61:292–302.

21. Liu J, Yang H-I, Lee M-H, Jen C-L, Batrla-Utermann R, Lu S-N, et al. Serum levels of hepatitis B surface antigen and DNA can predict inactive carriers with low risk of disease progression. Hepatology. 2016;64:381–9.
22. Liu J, Yang H-I, Lee M-H, Lu S-N, Jen C-L, Batrla-Utermann R, et al. Spontaneous seroclearance of hepatitis B seromarkers and subsequent risk of hepatocellular carcinoma. Gut. 2014;63:1648–57.
23. Lok AS, Zoulim F, Dusheiko G, Chan HLY, Buti M, Ghany MG, et al. Durability of hepatitis B surface antigen loss with nucleotide analogue and peginterferon therapy in patients with chronic hepatitis B. Hepatol Commun. 2020;4:8–20.
24. Alawad AS, Auh S, Suarez D, Ghany MG. Durability of spontaneous and treatment-related loss of hepatitis B s antigen. Clin Gastroenterol Hepatol. 2020;18:700–9 e3.
25. Yip T-F, Wong G-H, Wong V-S, Tse Y-K, Lui G-Y, Lam K-Y, et al. Durability of hepatitis B surface antigen seroclearance in untreated and nucleos(t)ide analogue-treated patients. J Hepatol. 2018;68:63–72.
26. Ling SC, Lin HS, Murray KF, Rosenthal P, Mogul D, Rodriguez-Baez N, et al. Chronic hepatitis is common and often untreated among children with hepatitis B infection in the United States and Canada. J Pediatr. 2021;237:24–33.e12.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Terrault NA, Wahed AS, Feld JJ, Cooper SL, Ghany MG, Lisker-Melman M, et al. Incidence and prediction of HBsAg seroclearance in a prospective multi-ethnic HBeAg-negative chronic hepatitis B cohort. Hepatology. 2022;75:709–723. https://doi.org/10.1002/hep.32231