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SYSTEMATIC REVIEWS AND META-ANALYSIS

Clinical Consequences of Hepatitis B Surface Antigen Loss in Chronic Hepatitis B Infection: A Systematic Literature Review and Meta-Analysis

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BACKGROUND AND AIMS: Functional cure, which requires sustained hepatitis B surface antigen (HBsAg) loss after treatment cessation, is currently the optimal treatment endpoint for chronic hepatitis B virus infection. We performed a systematic literature review (SLR) and meta-analyses to assess the association between HBsAg loss and long-term clinical outcomes. **METHODS:** We performed a SLR of scientific literature published in Medline and Embase reporting the incidence of cirrhosis, hepatic decompensation (HD), hepatocellular carcinoma (HCC), liver-related mortality (LRM), and all-cause mortality (ACM) in relation to HBsAg status. Bayesian hierarchical commensurate prior meta-analyses synthesized evidence on the association between HBsAg loss and each outcome. **RESULTS:** Thirty-eight studies, comprising 50,354 patients with 350,734 patient-years of follow-up, were included in the meta-analyses, reporting on cirrhosis (n = 12), HD (n = 12), HCC (n = 36), LRM (n = 12), and ACM (n = 16). Pooled incidence rate ratios (IRRs; vs HBsAg persistence) and respective credible intervals (CrIs) were 0.28 (0.060–1.070) for cirrhosis, 0.13 (0.013–0.38) for HD, 0.27 (0.11–0.53) for HCC, 0.17 (0.028–0.61) for LRM, and 0.64 (0.24–1.17) for ACM. Single-predictor-adjusted IRRs remained consistent with those from the primary analyses for all outcomes except cirrhosis and LRM. Outcome incidence rates were modified by selected study, patient and infection characteristics, but trended in the same direction of reduced risk after loss. **CONCLUSION:** Overall, HBsAg loss was associated with a reduced risk of most clinically relevant outcomes. While the magnitude of the effect differed across subgroups, the direction of the association remained similar. Our results validate the need to develop new strategies to achieve HBsAg loss.

Keywords: HBsAg Loss; Biomarker; Chronic HBV Infection; Clinical Outcomes; Meta-Analysis

Introduction

Chronic hepatitis B virus (HBV) infection, which affected an estimated 292 million people worldwide in 2019[1] and led to an estimated 820,000 deaths,[2] is expected to remain a major global issue for the next 40–50 years.[3] Despite the existence of effective antiviral therapy, including potent nucleos(t)ides analogues (NAs), chronic HBV infection remains among the leading causes of liver-related mortality (LRM) and morbidity. Untreated, it can lead to life-threatening complications such as cirrhosis and hepatocellular carcinoma (HCC), which alone account for 96% of viral hepatitis-related deaths.[4] Notably, more than half of all HCC, a leading cause of cancer-related mortality, is associated with chronic HBV infection.[5]

Anti-HBV therapies seek to prevent liver-related complications and reduce mortality. Entecavir, tenofovir disoproxil fumarate, and tenofovir alafenamide, recommended by major international guidelines[6–8] along with peginterferon-α-2a (peg-IFN) as first-line anti-HBV therapies, efficiently induce and maintain strong viral suppression. Complete viral suppression, while associated with decreased risk of liver-related complications, does not eliminate HCC risk. Recent research suggests an incremental HCC risk reduction with viral suppression when the latter is accompanied by hepatitis B surface antigen (HBsAg) loss.[9] Produced in vast amounts, HBsAg plays a fundamental role in HBV chronicity. While its impact on HBV-specific T cells has not yet been unequivocally established, HBsAg seems to act chiefly as a decoy for hepatitis B surface

Abbreviations used in this paper: **ACM, all-cause mortality; ALT, alanine aminotransferase; CrI, credible interval; EQ, equivalent; GLMM, generalized linear mixed model; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCP, hierarchical commensurate prior; HCV, hepatitis C virus; HD, hepatic decompensation; HIV, human immunodeficiency virus; IR, incidence rate; IRD, incidence rate difference; IRR, incidence rate ratio; LRM, liver-related mortality; NA, nucleos(t)ide analogue; NOS, Newcastle-Ottawa Scale; peg-IFN, peginterferon-α-2a; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RR, relative risk; SLR, systematic literature review; WPRO, Western Pacific Region.**

Most current article

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antibodies (anti-HBs), leading to failure to clear the infection.[10,11] HBsAg loss is currently seldom achieved either spontaneously or induced by treatment with NAs or peg-IFN[12]; where observed, HBsAg loss is seemingly stable with low risk of reactivation,[13] indicating the potential for finite treatment. In fact, functional cure, which requires sustained HBsAg loss after treatment cessation, is now often regarded as the optimal HBV treatment endpoint, with several treatments in development aimed at eliciting it.[14] Such therapies may facilitate a treat-all strategy, as established for HIV and hepatitis C virus (HCV) infections, meaning large patient populations at risk of HCC but not currently indicated for treatment could potentially benefit (eg, those in chronic HBV infection phases of the disease, ie, without active liver inflammation or aminotransferase elevation).[15]

Previous studies assessing the association between HBsAg loss and long-term clinical outcomes have been contradictory at times and their interpretation impaired by small sample size, insufficiently long follow-up duration, and/or low numbers of HBsAg loss events. These factors are compounded by different HBsAg loss definitions and a lack of comparator groups. Recently, Anderson et al attempted to overcome some of these problems by performing a meta-analysis to synthesize the findings from 28 studies examining HBsAg loss impact.[16] Associations between HBsAg loss and reduced risk of HCC, liver decompensation, and liver transplantation and/or death were reported.[16] The present study seeks to build on Anderson's approach by employing Bayesian statistics to facilitate the inclusion of single-arm studies (specifically excluded from Anderson's study), with findings presented in context.

Methods

Systematic literature review

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) reporting guidelines were followed.[17]

Search strategy. Global English language literature published in Medline and Embase between 1995 and 12 February 2019 was included. Search strategies are provided in Supplementary Table 1. Reference lists of existing systematic reviews and meta-analyses were also reviewed to identify relevant missing studies.

Study selection and quality assessment. The titles and abstracts of identified references were entered into EndNote X4 and duplicates removed. The first 30% of titles and abstracts were systematically screened by 2 independent researchers for eligibility (Table). As the level of concordance achieved was <95%, an additional 10% were screened in duplicate, resulting in >95% concordance. A single researcher screened the remaining references. The full text of the first 10% of articles retained in the preceding step was reviewed independently by the same researchers, resulting in >95% concordance. The Newcastle-Ottawa Scale (NOS) was used to critically appraise eligible studies (Supplementary Table 2).

Data extraction and transformation. Data were extracted into a standardized form by one researcher and reviewed by another. Data calculations and transformations were independently reviewed (Supplementary Methods). For studies that did not report a summary demographic measure (ie, age and sex) separately for each group, the overall summary demographic measure was used for both. Rates (events per patient-year) were utilized as the measurement scale for all outcomes. For the HBsAg loss group, the preferred follow-up start period, if reported, was from seroclearance; otherwise, time from index was included. A sensitivity analysis, in which HBsAg loss follow-up time was halved, was conducted; 50% corresponded to the average time after seroclearance, as a proportion of the total follow-up time, derived from studies reporting both total follow-up duration and that following seroclearance.[18–22]

Meta-analysis methods

Primary analysis. Meta-analyses were performed separately on 5 clinical outcomes: developing cirrhosis, experiencing hepatic decompensation (HD), developing HCC, LRM, and all-cause mortality (ACM) (Supplementary Methods). Incidence rate ratios (IRRs) and incidence rate differences were calculated with the HBsAg-persistent group as the reference. Bayesian hierarchical commensurate prior (HCP) models were used to synthesize data from both two-arm and single-arm studies by assuming different rate parameters for the 2 study types. Outcomes were modeled with a Poisson distribution with normally distributed random effects terms to account for overdispersion. Single-arm study HBsAg loss group rate estimates served as informative prior rate parameters of the HBsAg loss group in two-arm studies. Exact likelihood specifications precluded the need for continuity corrections for studies with zero events. Bayesian equivalent (EQ) analyses, where all studies were treated as equivalent, were also performed.

All Bayesian analyses (Supplementary Methods) were conducted using WinBUGS 1.4.3. Median point estimates and corresponding 95% credible intervals (CrIs) were derived from the posterior distributions of the Monte Carlo simulations.

Due to the relatively few studies for some outcomes and several observations having no events, informative gamma priors were used for the inverse of the heterogeneity parameters. The parameter values for the gamma distributions were elicited from GSK hepatology experts (DT and SK). Non-informative priors were used in a sensitivity analysis.

Secondary analysis. Results from the primary analyses were compared with the results from frequentist meta-analyses models and generalized linear mixed models (GLMMs), employing both fixed effects and random effects models (Supplementary Methods). For both frequentist and GLMM analyses, 2 separate sets of analyses, one using only two-arm studies and another all studies, were conducted. Sensitivity analyses omitting one study at a time were conducted to assess the stability in the relative rate estimates.

Heterogeneity assessment. Frequentist standard and GLMM meta-regression models were each used to measure and identify possible sources of heterogeneity. Heterogeneity was evaluated using Q (significance level of 0.10) and I^2 statistics (I^2>50% was considered significant). The convention of Higgins et al was used to characterize the heterogeneity, with I^2

Table. Systematic Literature Review Eligibility Criteria

Inclusion criteria	Morais et al	Anderson et al[16]
Study population	Patients with chronic HBV infection	Patients with chronic HBV infection
Exposure	HBsAg loss/seroclearance and/or seroconversion to anti-HBsAg positive	HBsAg loss/seroclearance
Comparator	Persistent HBsAg positivity Single-arm studies were also included	Persistent HBsAg positivity
Outcomes (at least one of)	Cirrhosis, hepatic decompensation, hepatocellular carcinoma, liver-related mortality, all-cause mortality, liver transplant, viral reactivation (latter 2 excluded during meta-analysis)	Hepatic decompensation, hepatocellular carcinoma, liver transplant, death
Study design	Observational studies or control group of RCTs	-
Language	English	English
Other	Meta-analysis or other systematic literature reviews (for inspection of list of references only) Abstract available	≥50 patients Data on HBsAg at baseline and during follow-up
Exclusion criteria		
Study population	Patients with acute HBV infection Studies in selected populations (eg, children, coinfected with HIV/HCV/HDV)–excluded during meta-analysis	Studies in HBV reactivation, liver transplant recipients, patients with liver decompensation or hepatocellular carcinoma prior to HBsAg loss, entire patient cohort infected with HCV, HIV, or HDV
Exposure	HBsAg seroclearance after liver transplantation or in specific populations (eg, patients undergoing chemotherapy)	-
Comparator	-	No persistent HBsAg group
Study design/type of studies	Animal studies, phase I/II trials, case series, case reports, narrative review articles, editorials, conference abstracts	Case-control studies
Follow-up	No data on follow-up time–excluded during meta-analysis	<2 y mean/median follow-up
Other	No quantitative data Insufficient detail in the methods	Data from duplicated population

HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; RCT, randomized controlled trial.

values of 0%, 25%, 50%, and 75% representing no, low, moderate, and high heterogeneity, respectively.[23]

Meta-regressions to assess confounding and examining subgroup effects. For outcomes where significant heterogeneity was identified, subgroup and single-predictor frequentist meta-regression analyses were conducted on the variables listed in Supplementary Table 2, provided there were at least 10 studies for each variable. Subsequently, single-predictor meta-regression models utilizing the primary analysis Bayesian framework were conducted, provided the variable was identified via the Wald test for the regression coefficient as significantly affecting the outcome rate in the GLMM or frequentist meta-analysis model.

Results

Systematic literature review results and study characteristics

Title and abstract were screened for 3184 unique references of the 5889 total identified; the full text was reviewed for 275 and 54 were included in the review (Supplementary Figure 1).[9,13,18–22,24–70] Exclusion reasons included: lack of study objective data (n = 162), acute HBV infection in the HBsAg loss group (n = 27), and unavailable full text (n = 19).

A further 13 studies were excluded from the meta-analyses: 8[29,31,40,41,47,58,65,67] did not report on the outcomes of interest, the sequence of events was unclear in 2,[56,60] and 3[27,28,68] were conducted in selected populations (ie, children, HIV infection, and HCV infection). Three additional studies were excluded from the quantitative analyses due to the absence of data on follow-up duration.[36,55,57]

Thirty-eight studies, comprising 50,345 patients (16,777 HBsAg loss and 33,568 HBsAg-persistent) with 350,734 patient-years of follow-up (85,446 and 265,288, respectively), were finally included in the meta-analyses: cirrhosis (n = 12), HD (n = 12), HCC (n = 36), LRM (n = 12), and ACM (n = 16).[9,13,18–22,24–26,30,32–35,37–39,42–46,48–54,59,61–64,66,69,70] Supplementary Table 3 summarizes the NOS results. All studies (n = 38) were published between 1998 and 2019

(Supplementary Table 4), most were conducted in Asia (n = 24) and approximately half (n = 20) were retrospective in design. Fourteen included untreated patients, 11 included treated patients, and another 11 included a mix. Of the 10 studies including IFN-treated patients, IFN was the only treatment received in 2. The baseline characteristics of patients included in each study are summarized in Supplementary Table 5. Briefly, baseline mean age was ≤50 years in most studies (28/38), with a male predominance across all but one study[63]; most studies included a mix of hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients (20/38), with 12 studies including HBeAg-negative patients only. Fifteen studies[20,26,30,33,35,38,39,43–45,51,52,61,62,64] lacked a definition of HBsAg loss, while it varied in the remaining studies (Supplementary Table 8).

Meta-analysis results

Primary analysis—hierarchical commensurate prior Bayesian model. *Pooled outcome incidence rate ratios.* The HBsAg loss group was at reduced risk of HD (median [95% CrI] IRR 0.13 [0.013, 0.38]), HCC (0.27 [0.11, 0.53]), and LRM (0.17 [0.028, 0.61]) compared with the HBsAg-persistent group. While IRRs for cirrhosis (0.28 [0.060, 1.070]) and ACM (0.64 [0.24, 1.17]) trended in the same direction, the 95% CrI crossed one (Figure 1). EQ models yielded similar estimates across all outcomes (Figure 1); point estimates from GLMMs were more heterogeneous but contained within the 95% CrIs of the HCP models. Incidence rate differences are presented in Supplementary Figure 2. Outcome incidence rates are presented in Figure 2 and Supplementary Table 6.

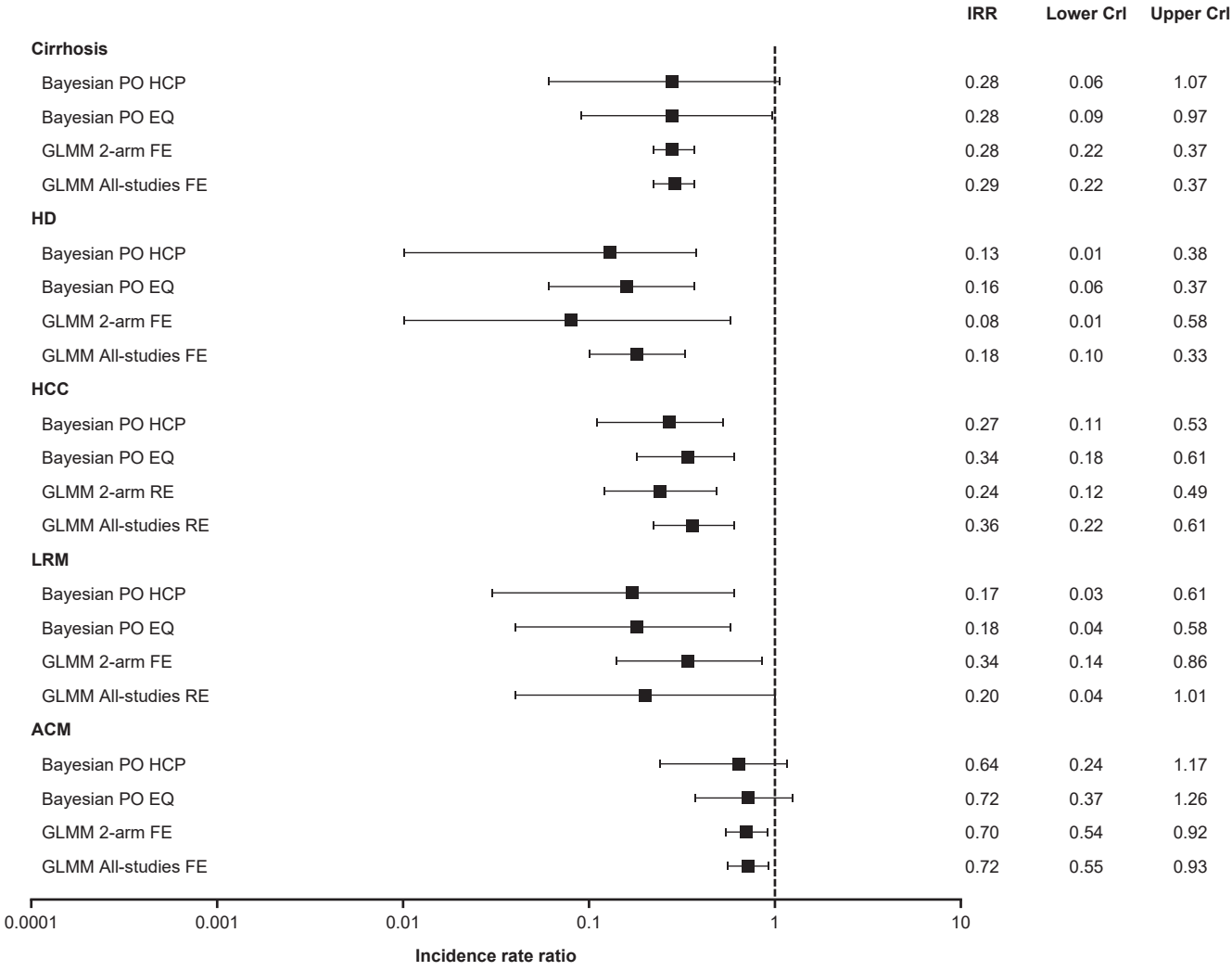

Figure 1. Pooled incidence rate ratio by outcome and analytical model. ACM, all-cause mortality; CrI, credible interval; EQ, equivalent; FE, fixed effects; GLMM, generalized linear mixed model; HCC, hepatocellular carcinoma; HCP, hierarchical commensurate prior; HD, hepatic decompensation; IRR, incidence rate ratio; LRM, liver-related mortality; PO, Poisson; RE, random effects.

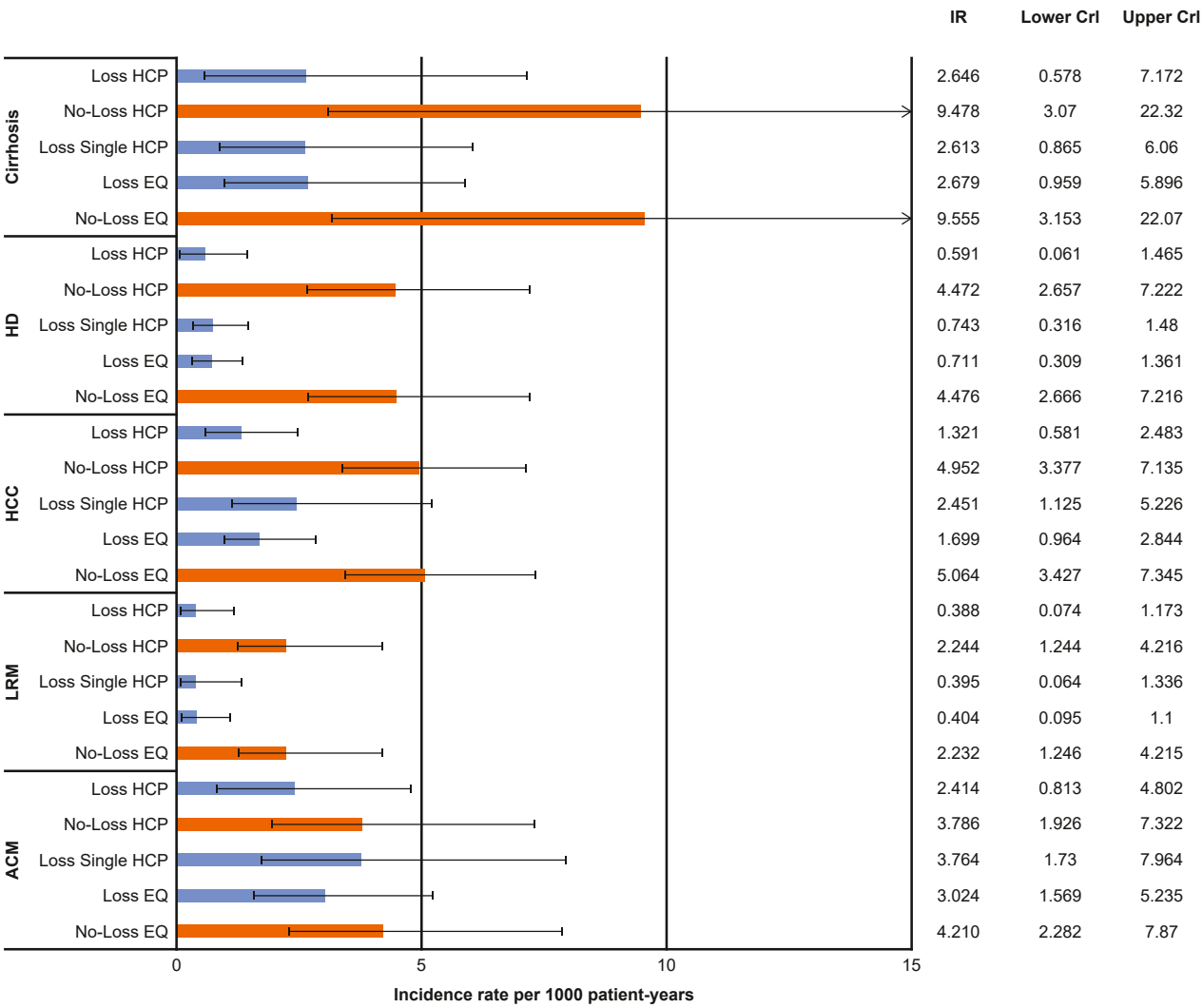

Figure 2. Pooled outcome incidence rates by HBsAg group and Bayesian analytical model. ACM, all-cause mortality; CrI, credible interval; EQ, equivalent; HCC, hepatocellular carcinoma; HCP, hierarchical commensurate prior; HD, hepatic decompensation; IR, incidence rate; LRM, liver-related mortality.

Assessment of heterogeneity. Incidence rate heterogeneity was high across studies (I^2>75%) in both the loss and no loss groups for all outcomes except HD (25% < I^2<50%). Heterogeneity remained moderate to high (I^2>50%) in most subgroups and significant (Q statistic *P* value <.10) in meta-regression models that adjusted for each covariate separately.

Bayesian meta-regression analyses to assess confounding. Single-predictor-adjusted IRRs were consistent with those from the primary analyses (Figure 3). For cirrhosis, however, most adjustments (except coinfection and percentage HBeAg-positive) resulted in a significant reduction in the cirrhosis risk associated with HBsAg loss. Adjusting for baseline percentage HBeAg-positive attenuated the benefit of HBsAg loss for LRM.

Bayesian meta-regression models to examine subgroup effects. Cirrhosis rates were not modified by setting (interaction parameters [95% CrI], 0.54 [−1.96, 2.98]), age (continuous, 0.90 [−0.59, 2.39]; <40 years vs ≥ 40 years, 0.96 [−1.82, 3.88]), or sex (<65% vs ≥ 65% male, 1.54 [−1.49, 4.76]). Rates were, however, modified by follow-up, with IRR decreasing by approximately 41% for a 1-year increase in duration (Figure 4).

Rates of HD were modified by sex and alanine aminotransferase (ALT) (Figure 4). A 1% absolute increase in percentage male resulted in a ∼25% decrease in IRR, while a 1-unit increase in mean baseline ALT levels resulted in a ∼100% decrease in IRR.

For HCC, risk reduction associated with HBsAg loss was most pronounced in treated (IRR 0.06 [95% CrI 0.01, 0.22]) vs untreated studies (IRR 0.50 [95% CrI 0.25, 0.97]); most studies in the treated group comprised NA-treated patients (n = 8/10). Rates were also modified by baseline HBV DNA levels; a 1-log unit increase resulted in a decrease in IRR by ∼42% (Figure 4).

LRM was the singular most modifiable outcome. Rates differed by setting (population-based, IRR 0.62 [95% CrI 0.11, 3.08] vs clinical, IRR 0.03 [95% CrI 0.00, 0.22]) and by

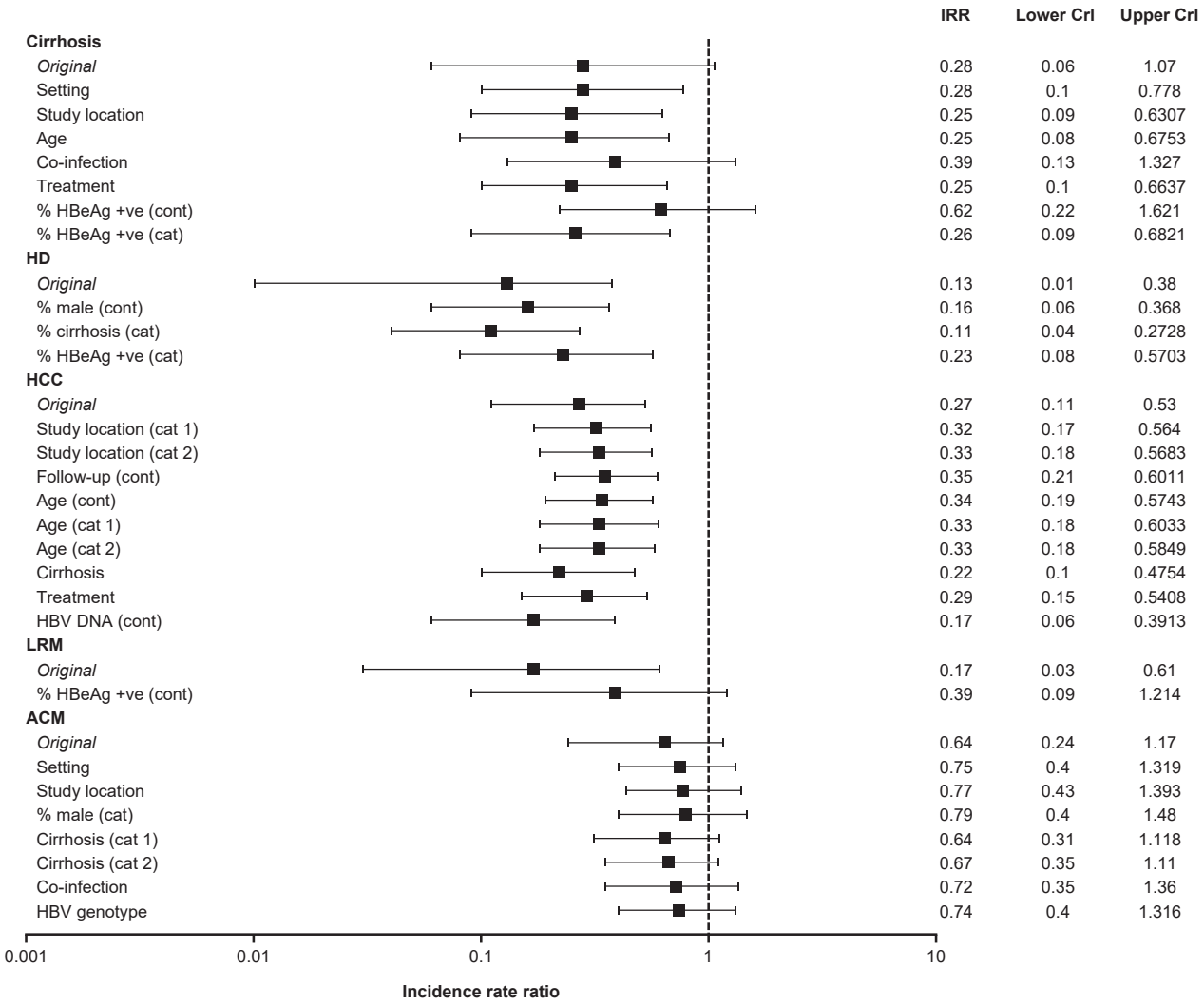

Figure 3. Bayesian meta-regression analyses to assess confounding. ACM, all-cause mortality; cat, categorical; cont, continuous; CrI, credible interval; DNA, deoxyribonucleic acid; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HD, hepatic decompensation; IRR, incidence rate ratio; LRM, liver-related mortality.

study design (retrospective, IRR 0.47 [95% CrI 0.09, 1.86] vs prospective, IRR 0.01 [95% CrI 0.00, 0.17]). Furthermore, IRRs decreased with a 1-year increase in follow-up duration (~43%), a 1% absolute increase in percentage male (~22%), and a 1% absolute increase in baseline percentage cirrhosis (~18%) (Figure 5).

For ACM, risk reduction associated with HBsAg loss was lower in studies conducted outside the World Health Organization (WHO) Western Pacific Region (WPRO; IRR 0.28 [95% CrI 0.03, 0.87]) vs within (IRR 1.57 [95% CrI 0.67, 4.32]). Furthermore, for a 1% absolute increase in percentage baseline cirrhosis and 1% absolute increase in percentage male, the IRRs each decreased by 6% (Figure 5).

Sensitivity analyses. Study findings were insensitive to the use of informative vs non-informative heterogeneity priors (Supplementary Figures 3 and 4) and to shortening follow-up time by 50% (Supplementary Figure 4 and Supplementary Table 7). While this was also true for the omission of single studies (Supplementary Figure 5), the removal of Gounder et al[38] had the greatest impact on point estimates and accompanying 95% CrIs in several instances. This was believed to be more reflective of difficulties in fitting GLMMs to the data set rather than to the study removal itself.

Discussion

We report on a systematic literature review and meta-analysis that informs further on the association between HBsAg loss and long-term clinical outcomes. In contrast to Anderson et al,[16] we leveraged a Bayesian analytical framework to facilitate the inclusion of single-arm studies. This, plus differing eligibility criteria (Table), meant that only 10 studies[9,18,19,30,43,48,50,52,54,66] included in Anderson et al were also included in our meta-analysis. Despite these differences in study design and analytical approach, we report remarkably similar findings as outlined below.

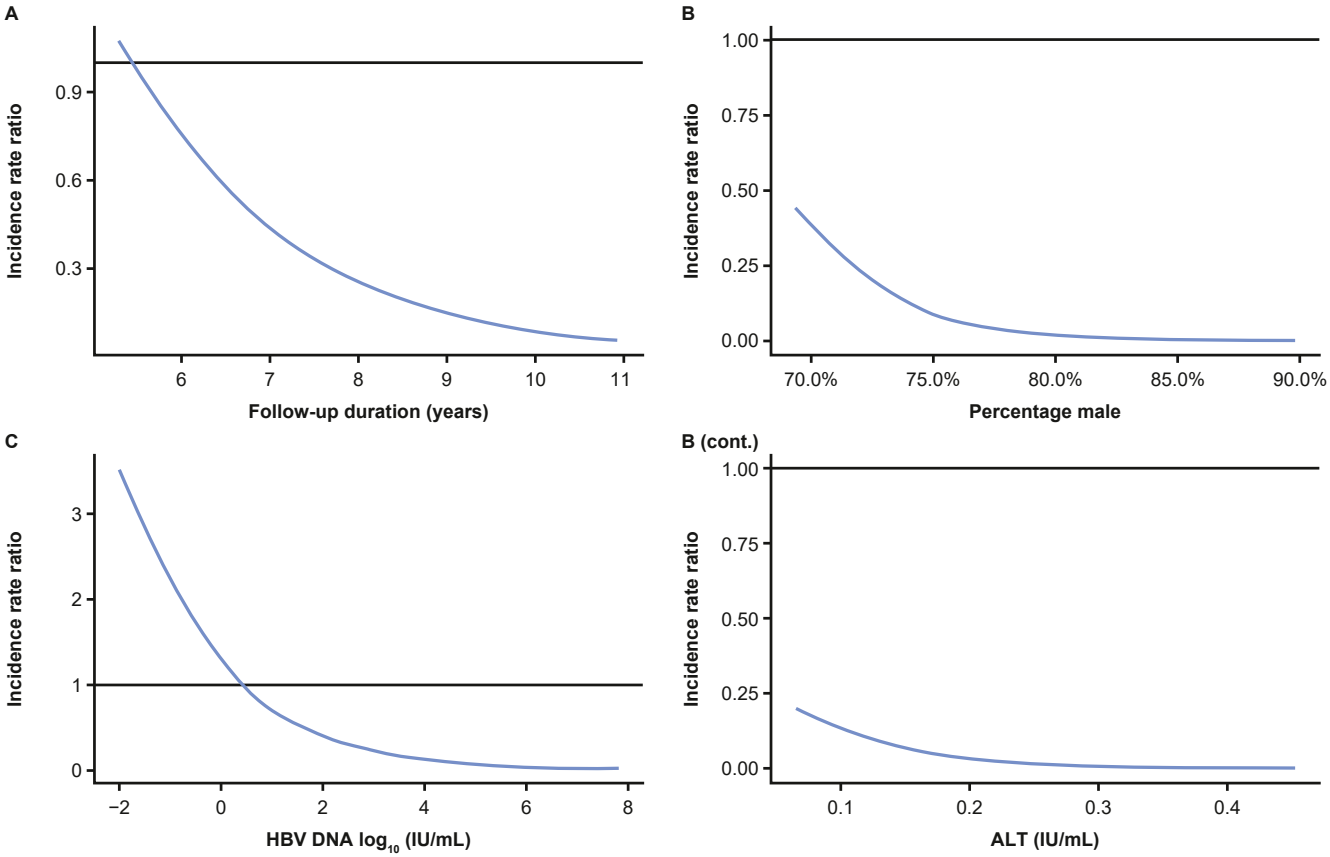

Figure 4. Bayesian meta-regression models—subgroup analyses (significant continuous variables). (A) Cirrhosis; (B) Hepatic decompensation; (C) Hepatocellular carcinoma. ALT, alanine aminotransferase; HBV, hepatitis B virus.

Liver cirrhosis

While the association between HBsAg loss and cirrhosis did not achieve statistical significance in our primary (HCP) analysis, both Bayesian EQ and fixed effects GLMM approaches found a statistically significant risk reduction (Figure 1). This can be partly explained by how study uncertainty is managed in each analysis, its effect on variance, and resulting 95% CrIs: HCP Bayesian analyses consider within- and between-study uncertainty, which increases variance and 95% CrI width. Uncontrolled confounding may have also contributed. HBsAg loss significantly reduced cirrhosis risk after a single adjustment for setting, study location, baseline age, treatment status, and percentage HBeAg-positive. Exposure group imbalance meant that the HBsAg loss group was more likely to have been recruited from clinical settings, to be older or of Asian ethnicity, to be HBeAg-negative or be treated (Supplementary Tables 4 and 5), all of which may reflect an increased baseline risk of developing cirrhosis compared with the HBsAg-persistent group.[71]

Longer follow-up duration was associated with a greater reduction in cirrhosis risk after HBsAg loss. Ascertainment may explain this: cirrhosis identified soon after HBsAg loss may reflect depletion of as yet undiagnosed disease at the point of loss rather than true post-loss incident cases. The significant reduction observed when the analysis was adjusted for clinical setting may support this if patients are managed more effectively in this setting.

Hepatocellular carcinoma and hepatic decompensation

Like Anderson et al,[16] we found a significant reduction in the risk of HCC and HD after HBsAg loss.

Hepatocellular carcinoma. Higher levels of baseline HBV DNA and receiving treatment were each associated with a greater reduction in HCC risk after HBsAg loss. High HBV DNA level and active viral replication have been identified as independent risk factors for HCC.[72] Extensive HBV DNA integration in the host genome and hepatocyte expansion, possibly preceding hepatocarcinogenesis, occur early in the disease's natural history, opening a pathway for HCC development independent of liver fibrosis progression.[6,72] Accordingly, the observed greater reduction in HCC risk with higher HBV DNA levels, a possible marker of early disease, suggests that the timing of HBsAg loss may be important. Studies with higher mean HBV DNA levels were comparable in terms of age and ALT levels to those

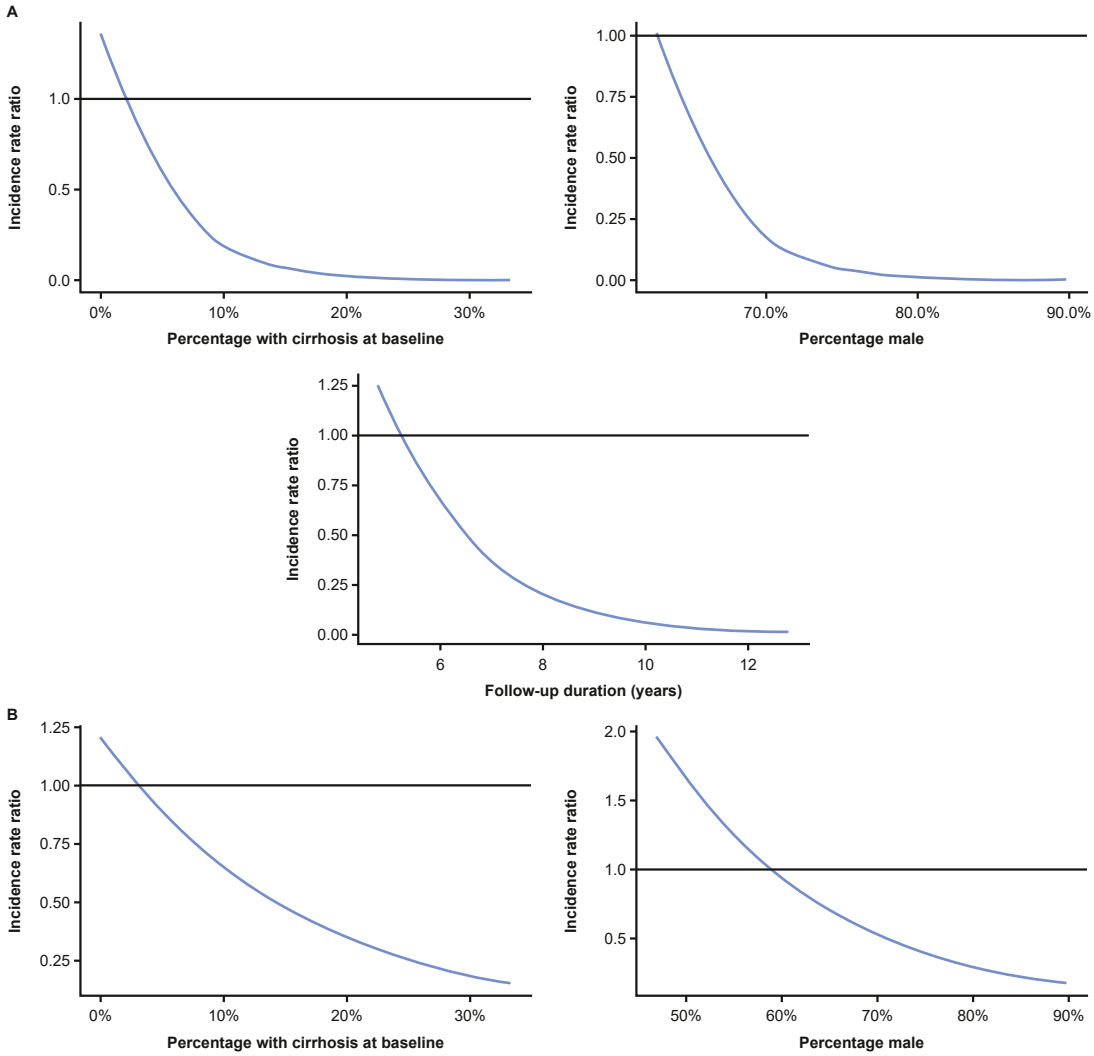

Figure 5. Bayesian meta-regression models—subgroup analyses (significant continuous variables) cont. (A) Liver-related mortality; (B) All-cause mortality.

reporting lower mean HBV DNA levels but had higher HBsAg levels, more HBeAg-positivity, more cirrhosis, and less treatment. Whereas some of these characteristics suggest the predominance of less active disease, as seen in the HBeAg-positive chronic infection phase,[6–8] the imbalance for cirrhosis is unexpected. These differences, however, were not tested for statistical significance, and the aggregated nature of the data limits the scope for additional analyses to inform further on this finding.

We found a significant post-loss reduction in HCC in both treated and untreated cohorts; however, like Anderson et al,[16] the magnitude of the reduction was far greater in treated patients. A recent Hong Kong study reported that HBsAg loss had a greater impact on HCC risk than NA-induced viral suppression alone.[9] Similarly, in their meta-analysis, Song et al found a significantly lower HCC incidence after treatment-induced vs spontaneous loss, reinforcing the value of early HBsAg loss.[73] Compared with those with untreated patients, studies with treated patients were similar in regard to many baseline characteristics, but the higher HBV DNA levels and greater cirrhosis prevalence may have preceded and precipitated treatment. Confounding by indication may also play a role: it is not clear (and beyond the scope of this study) whether the observed risk reduction reflects the greater impact of HBsAg loss in a population at higher risk of HCC or an effect of HBsAg loss beyond that of NA-induced viral suppression.

Hepatic decompensation. Higher baseline ALT levels and higher proportion of males were each significantly associated with a greater reduction in HD risk. Studies with higher ALT reported higher cirrhosis prevalence—the single most important risk factor for HD—hence this finding may reflect a higher intervention impact on a high-risk group.

A similar underlying mechanism may explain the more nuanced risk reduction of HD after HBsAg loss associated with male predominance, also seen in relation to the risk of LRM and ACM. Males with chronic HBV infection have been highlighted as being at higher risk of developing liver disease, its complications, and of dying compared with

females.[74,75] Notably, included studies with more males also reported a higher prevalence of baseline cirrhosis.

In their recent study, Yip et al[76] identified, in an Asian cohort, a lower cumulative HCC incidence in patients with HBsAg loss compared to those with inactive chronic HBV infection. When analyzing the annual decline in HCC rate after HBsAg loss, however, the authors found a significant annual reduction in the incidence of HD (−0.23% [95% CI −0.40% to −0.06%]) but not in that of HCC (−0.04% [95% CI −0.13% to 0.04%]). Moreover, cirrhosis and older age were each associated with an increased risk of HCC after HBsAg loss. Phenomena preceding hepatocarcinogenesis such as HBV DNA integration in the host genome and hepatocyte expansion as well as other pro-oncogenic events—all non-modifiable by HBsAg loss after onset—may have already taken place at the time of HBsAg loss in patients with no clinical manifestations of HCC. Together, these findings reaffirm the need to continue HCC surveillance even after HBsAg loss and suggest timing of HBsAg loss may play an important role in the subsequent risk of HCC.

Liver-related and all-cause mortality

While Anderson et al found HBsAg loss reduced the risk of the composite endpoint liver transplant and/or death,[16] our study showed that HBsAg loss reduced the risk of LRM but not ACM. This is not surprising, since the effect of HBsAg loss on reducing mortality is likely mediated through its impact on the risk of liver-related complications of HD and HCC and may not impact on other causes of death. US data support this: a cohort of 4389 chronic HBV infection cases were at almost 16 times the risk of LRM compared with the general population (relative risk 15.91; 95% CI 15.81, 16.01), but the increased risk for ACM was more modest (relative risk 1.85; 95% CI 1.85, 1.86).[77] When adjusting for percentage HBeAg-positive, HBsAg loss was no longer associated with LRM. This may be driven by 2 studies,[25,32] excluded due to HBeAg missingness and reporting zero liver-related deaths in the HBsAg loss group. Full interpretation of the potential confounding effect of HBeAg-positivity is therefore not possible, since unadjusted and adjusted IRRs were drawn from different study populations.

Higher cirrhosis prevalence was significantly associated with a larger decrease in loss-related risk of both LRM and ACM. Cirrhosis, its complications, and HCC are leading causes of chronic hepatitis B-related death,[77] hence the present results suggest that, even in the presence of established liver disease, HBsAg loss may prevent negative downstream outcomes.

As with cirrhosis, longer follow-up time resulted in a greater reduction of LRM. Combined, these findings suggest long follow-up is required to adequately capture the impact of HBsAg loss on both liver disease and survival.

Study characteristics, including setting, location, and design, modified mortality rates. Populations recruited from clinical settings and those included in prospective studies presented with higher prevalence of biochemically active and histological liver disease. The greater impact of loss in reducing mortality in these populations reinforces the idea that high-risk groups seem to benefit the most in relative terms. Less clear is the increased risk of HBsAg loss-associated ACM in studies conducted in WHO WPRO countries (China, Japan, South Korea, and Taiwan). Only 3 studies contributed to the HBsAg-persistent group in the WHO WPRO subset, hence a full evaluation of study characteristics that might explain this finding is not possible.

In a cohort of Asian patients, Yip et al[69] identified age older than 50 years as an independent risk factor of HCC after HBsAg loss (adjusted hazard ratio 4.31 [95% CI 1.72 to 10.84]), with older age likely representing a longer duration of antigen exposure in this population. In our study, age was not identified either as a confounder or an effect modifier in any of the outcome analyses. This, however, may simply reflect the challenges of looking at the influence of age on the association between HBsAg loss and clinical outcomes using aggregated data, where only average age values were available, and results were not generally reported by age. The need for further research is highlighted here.

Limitations

Several limitations warrant discussion. First, relatively few studies reported events for both groups for many of the outcomes, and the inclusion of time prior to HBsAg loss (for studies which did not report on the follow-up duration after HBsAg loss) is suboptimal. Whereas the Bayesian approach allowed the incorporation of single-arm loss-only studies, the number of studies reporting outcomes for the HBsAg-persistent group remained low. Results were, nonetheless, consistent across analyses and not affected when halving follow-up. Second, the interpretation of the pooled effect estimates requires caution as heterogeneity remained moderate-to-high in most subgroup analyses and significant in meta-regression models. Third, the lack of patient-level data precluded the study of the definite effect of baseline characteristics on the association between HBsAg loss and study outcomes or to address confounding. Only aggregated data representing average values were available, and baseline measurements were not always reported separately for HBsAg-loss and HBsAg-persistent groups. Lastly, studies included in this analysis were observational in nature, thus bias inherent to the original study design cannot be precluded.

Conclusions

Here, we presented an alternative approach that included single-arm studies and considered multiple analytical methods. Our study reinforces the findings from Anderson et al in that, overall, and in specific subgroups, HBsAg loss appears to confer meaningful clinical benefits, including HCC, HD, and LRM risk reduction. HCC risk reduction was observed even among NA-treated populations. Although the relative contribution of HBsAg loss to

improved clinical outcomes is not conclusive, our findings align with recent reports suggesting a clinical benefit associated with HBsAg loss beyond that from NA-induced viral suppression.[9] Patients, presumably at an early phase of the infection, were found to benefit the most from HBsAg loss; in the advent of finite treatments aimed at eliciting functional cure, the importance of treatment timing is poised to become central to the discussion on how patients are managed, with further research required. Although remarkable, the reduction in cirrhosis risk did not achieve the conventional threshold for statistical significance. Longer studies with adequate adjustment for confounding are required to ascertain the effect of HBsAg loss on cirrhosis development. Notwithstanding the limitations here outlined, the present results validate the need to develop new strategies to achieve HBsAg loss.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2023.06.004.

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Eleonora Morais and Iain A. Gillespie equally contributed to Conceptualization. Eleonora Morais (supporting), Lauren Mason (lead), John Dever (supporting), Pam Martin (supporting), and Jing Voon Chen (supporting) contributed to Data curation. Eleonora Morais contributed to the Writing – original draft. Eleonora Morais, Lauren Mason, John Dever, Pam Martin, Jing Voon Chen, Leigh Felton, Stuart Kendrick, Dickens Theodore, and Iain A. Gillespie equally contributed to the Writing – review and editing. Eleonora Morais, Leigh Felton, Stuart Kendrick, Dickens Theodore, and Iain A. Gillespie equally contributed to Data interpretation. Lauren Mason, John Dever, Pam Martin, Jing Voon Chen equally contributed to Formal analysis.

Conflicts of Interest:
The authors disclose the following: Eleonora Morais, Leigh Felton, Stuart Kendrick, Dickens Theodore, and Iain A. Gillespie are employees of GSK and hold stocks/shares in the company. Lauren Mason is an employee of Pallas Health Research and Consultancy, which received funding from GSK to conduct the research study 212562. Pam Martin is an employee of Medical Decision Modeling Inc., which received funding from GSK to conduct study 213453. John Dever and Jing Voon Chen were employees of Medical Decision Modeling Inc. at the time study 213453 was conducted. Employees of Pallas Health Research and Medical Decision Modeling Inc. were not paid for manuscript development.

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
GSK makes available anonymized individual participant data and associated documents from interventional clinical studies which evaluate medicines upon approval of proposals submitted to www.clinicalstudydatarequest.com. To access data for other types of GSK sponsored research, for study documents without patient-level data, and for clinical studies not listed, please submit an enquiry via the website.

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