Serious adverse events after cessation of nucleos(t)ide analogues in individuals with chronic hepatitis B: A systematic review and meta-analysis
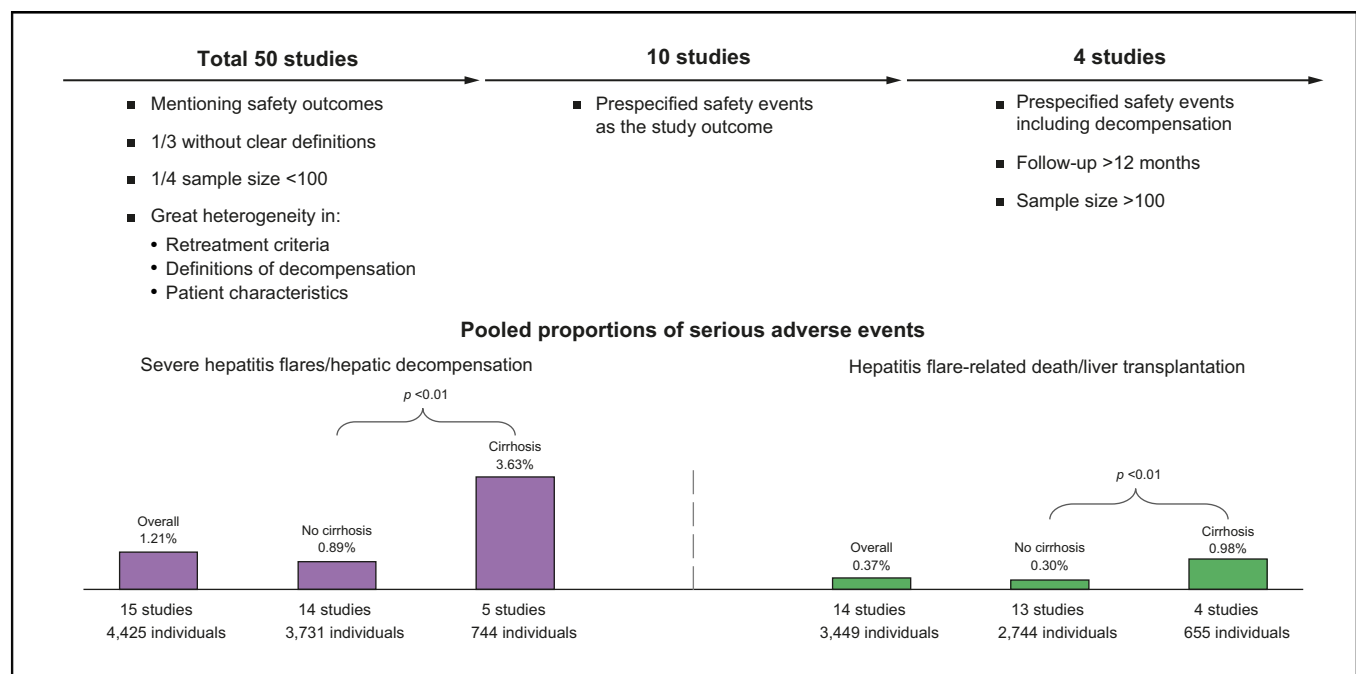
Authors

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Graphical abstract

Highlights

- Current literature on the safety of stopping nucleos(t)ide analogues in HBV infection is limited with important caveats.

- Serious safety events were not often reported in small studies with short follow-up and/or unclear outcome definition.

- Severe withdrawal flares or hepatic decompensation occurred in about 1% of overall nucleos(t)ide analogue stoppers.

- Approximately 0.3–0.4% of people require liver transplantation or die after stopping nucleos(t)ide analogues.

- Individuals with cirrhosis were significantly more vulnerable to serious adverse events following treatment withdrawal.

Impact and implications

Current literature regarding the safety concerns surrounding NUC cessation for individuals with chronic hepatitis B is limited and heterogeneous in designs and characteristics, and thus should be interpreted with great caution. Based on currently available data, the proportion of patients that develop severe hepatitis flares or hepatic decompensation was estimated at 1.21% and that of flare-related death or liver transplantation at 0.37%. Our findings are important for individuals receiving nucleos(t)ide analogues for hepatitis B virus infection because we not only pooled currently available data to estimate the risk of serious clinical adverse events following treatment cessation but also uncovered critical limitations of existing literature regarding the safety of finite therapy.

Serious adverse events after cessation of nucleos(t)ide analogues in individuals with chronic hepatitis B: A systematic review and meta-analysis

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Background & Aims: The risk of serious clinical outcomes following cessation of nucleos(t)ide analogues (NUCs) in individuals with chronic hepatitis B remains poorly characterized. This systematic review and meta-analysis aimed to evaluate current literature on this issue.
Methods: We searched PubMed, Embase, and Web of Science for NUC stop studies that noted clinical outcomes published between January 1, 2006 and August 18, 2022. We performed meta-research analyses to examine the relationships of reported outcomes with study designs and characteristics and also pooled studies with non-overlapping populations to provide risk estimates for the proportions of (1) severe hepatitis flares or hepatic decompensation or (2) hepatitis flare-related death or liver transplantation.
Results: The meta-research analysis included 50 studies of highly heterogeneous designs and characteristics. We found that reporting of safety outcomes varied widely according to outcome definition, follow-up duration, and sample size. Only ten studies prespecified safety events as the study outcome, and only four had an outcome definition to include hepatic insufficiency, a follow-up duration >12 months, and a sample size >100 patients. We further pooled 15 studies with 4,525 individuals and estimated that severe hepatitis flares or decompensation would occur in 1.21% (95% CI 0.70–2.08%), with significant heterogeneity (I^2 = 54%, p <0.01), while hepatitis flare-related death or liver transplantation would occur in 0.37% (95% CI 0.20–0.67%), without significant heterogeneity (I^2 = 0.00%, p = 1.00).
Conclusions: Current literature on the risk of serious clinical outcomes following NUC cessation is very limited and highly heterogeneous. Pooled analyses of available data found approximately 1% of patients who stopped NUCs developed severe flares or hepatic decompensation.
Impact and implications: Current literature regarding the safety concerns surrounding NUC cessation for individuals with chronic hepatitis B is limited and heterogeneous in designs and characteristics, and thus should be interpreted with great caution. Based on currently available data, the proportion of patients that develop severe hepatitis flares or hepatic decompensation was estimated at 1.21% and that of flare-related death or liver transplantation at 0.37%. Our findings are important for individuals receiving nucleos(t)ide analogues for hepatitis B virus infection because we not only pooled currently available data to estimate the risk of serious clinical adverse events following treatment cessation but also uncovered critical limitations of existing literature regarding the safety of finite therapy.

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Introduction

Hepatitis B virus (HBV) infection remains a global health problem, with 296 million infected people worldwide.[1] Chronic hepatitis B (CHB) is the leading cause of hepatocellular carcinoma and liver-related mortality,[2–5] but the risk can be substantially reduced by long-term (often indefinite) nucleos(t)ide analogue (NUC) therapy.[6–9]

The optimal duration of NUC treatment is currently unknown and poorly defined. Generally, hepatitis B surface antigen (HBsAg) seroclearance is considered durable and consequently the most sought-after treatment endpoint, but it rarely occurs on continuous NUC therapy (0.15-0.33% per year).[10–12] Meanwhile, long-term or indefinite duration of treatment can be costly or unaffordable, leading to efforts to define strategies for more finite NUC therapy. Additionally, recent data also suggested a higher HBsAg seroclearance rate after NUC cessation compared to continuous NUC treatment.[12–14] Nevertheless, NUC cessation may lead to serious clinical events such as severe hepatitis flares that may progress to liver failure and possibly death.[15–19]

Risk estimation for serious clinical outcomes following NUC cessation has been difficult because most events have been reported as sporadic cases,[15] based on which quantitative analysis has been challenging.[20] In order to fill in the knowledge gap, a critical appraisal of existing literature is necessary and a pooled analysis based on all available data is indispensable. Therefore, we conducted this systematic review and meta-analysis to appraise published studies that reported safety outcomes of NUC cessation and then estimated the proportions of patients who developed serious clinical events including severe hepatitis flares, hepatic decompensation, liver-related mortality, and liver transplantation. In this analysis, we focused on NUC cessation following entecavir (ETV) or tenofovir disoproxil fumarate (TDF) therapy because older generations of NUCs are no longer in routine clinical use.[21–23]

Materials and methods

Search strategy and study selection
This study was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Table S1).[24] We searched PubMed, Embase, and Web of Science without language restriction for relevant full-text studies published between January 1, 2006, and August 18, 2022. The search strategy was developed in collaboration with a medical librarian (JAB) and involved the use of keywords related to CHB, NUC cessation, withdrawal, stoppage, and discontinuation (details in Table S2). Two authors (CHT and THC) independently screened the literature and extracted data using a case report form developed for this study. Discrepancies were resolved via consensus and/or discussion with two senior investigators (MHN and YCH).

Eligible studies are full-length articles that reported virological, biochemical, and/or clinical outcomes of adults (≥18 years) with CHB who received NUCs for at least 1 year before cessation. Studies were excluded if the sample size was <30 patients, if patients had viral coinfection (e.g., with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus), if they included unique populations (e.g., patients who received organ transplants or had end-stage renal disease or rheumatic diseases), if they included patients who had achieved HBsAg seroclearance during the antiviral therapy, if patients receiving treatments with experimental drugs or interferon were included, if patients testing positive for hepatitis B e-antigen (HBeAg) at the time of treatment cessation were included, or if <50% of the study population received ETV or TDF.

Data extraction
Study data, including baseline age, sex, pretreatment HBeAg status, the proportion of individuals with cirrhosis, the types of NUC used, treatment duration, consolidation duration, end-of-treatment HBsAg levels, follow-up duration, and criteria for NUC cessation and retreatment, were extracted into a spreadsheet. The safety outcomes of interest included (1) severe hepatitis flares or hepatic decompensation as stated by each study, and (2) hepatitis flare-related death or liver transplantation.

We first conducted a meta-research analysis to characterize all included articles and evaluate the relationships between study characteristics and outcomes.[25,26] Then we pooled aggregate data from selected studies without overlapping populations for risk estimation. The criteria for studies to be selected into data pooling were (1) studies without overlapping populations (for studies with substantial overlapping populations, we selected the one that was largest in sample size or most comprehensive in information), (2) studies not exclusively composed of special populations such as those with low HBsAg levels or prior severe acute exacerbation, and (3) studies with a mean or median follow-up duration >12 months. A minimum duration of follow-up was necessary for pooling the event data because most studies reported occurrence of serious clinical events as proportions rather than incidences. A time frame of 12 months was appropriate in that most flare-related clinical events occurred within 1–2 years after NUC cessation.[17,18,27]

Statistical analysis
A random-effects model and the inverse variance method were adopted to pool the reported proportions of (1) severe hepatitis flares or hepatic decompensation as respectively defined in each study, and (2) hepatitis flare-related death or liver transplantation. Studies that reported zero outcomes were adjusted for in the analysis through continuity correction.[28] We evaluated between-study heterogeneity by using I^2 statistics. Comparison between the studies with and those without exclusion of individuals with cirrhosis was carried out using the Wald test. All tests are two-sided, and statistical significance was defined as a p value <0.05. Egger's funnel plot was used to assess publication bias. All statistical analysis was performed using R (version 4.1.3).

Risk-of-bias assessment
Each study's risk of bias was assessed with the modified Newcastle–Ottawa scale, which classified the quality of reporting safety issues as high (7–9 points), fair (4–6 points), or low (<4 points) (Table S3).[29]

Results
We identified 6,297 articles through the initial electronic database search (Table S2). After removing duplicates and reviewing the titles and abstracts, we retrieved 160 potentially relevant articles for full-text evaluation. Ultimately, we identified 50 eligible studies which reported the events of severe hepatitis flares, hepatic decompensation, hepatitis flare-related death, or liver transplantation (Fig. 1).[12–14,17–19,27,30–72] One of the studies involved two distinct cohorts, of which one consisted exclusively of individuals with prior severe acute exacerbation and the other consisted of patients who had never experienced such an event.[31] Therefore, 50 studies with 51 cohorts were included in the meta-research analysis. Results of the risk-of-bias assessment of the included studies are provided in Table S4.

Data were pooled to generate risk estimates from 15 selected studies with 4,525 patients overall.[17,19,27,31,32,47,48,50,54,56,59,62,]
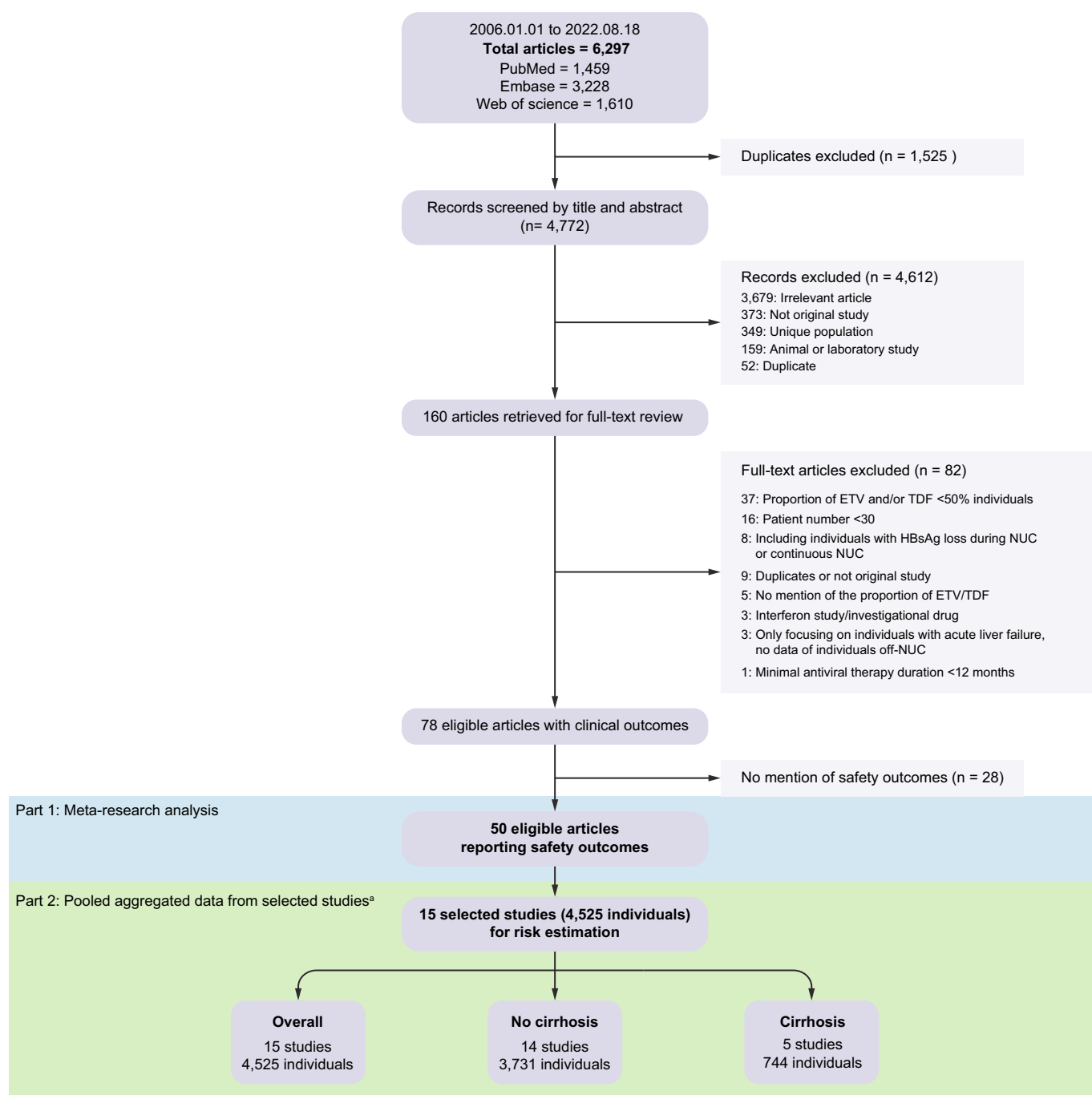
Fig. 1. Flow chart of study selection. The first part of analysis (part 1) is to characterize all the included articles and evaluate the relationships between study designs/characteristics and outcomes in a meta-research analysis. The second part (part 2) is to pool data from selected studies without overlapping populations for risk estimation. a: criteria of study selection for pooling aggregate data: (1) no overlapping populations, (2) a mean or median follow-up duration >12 months, (3) not for special populations such as studies exclusively composed of individuals with prior severe acute exacerbation or low serum HBsAg level. CHB, chronic hepatitis B; ETV, entecavir; HBsAg, hepatitis B surface antigen; NUC, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate.

[64,66,69] The analysis was further stratified by cirrhosis status: the non-cirrhotic subgroup analysis from 14 studies with 3,731 patients,[17,19,27,31,32,47,48,50,56,59,62,64,66,69] and the cirrhotic subgroup analysis from five studies with 744 patients.[17,19,27,56,62] The funnel plot did not detect a significant publication bias among the 15 studies (Fig. S1).

Meta-research analysis

Study characteristics

Major characteristics of the included studies were summarized in Table 1 and additional details were provided in Tables S5–S7. The vast majority (74.00%) of these studies were conducted in Asia, while two large international studies included cohorts from

Table 1. Characteristics of the 50 studies reporting safety outcomes.

	Number of studies (%)
Safety outcomes as the prespecified events:	
Yes	10 (20%)
No	40 (80%)
Region	
Asia	37 (74.00%)
Western countries	8 (16.00%)
Mixed Asia and the West	5 (10.00%)
Study design:	
Clinical cohort study - prospective[a]	35 (70.00%)
Clinical cohort study - retrospective	13 (26.00%)
Electronic health record study	2 (4%)
Criteria to define severe hepatitis flares or decompensation[b]	
Hyperbilirubinemia + coagulopathy, or clinical symptoms	12 (26.09%)
Hyperbilirubinemia or coagulopathy	20 (43.48%)
No clear definition	14 (30.43%)
Principle of stopping NUC:	
International guideline/Local regulation/Others/No mention	35 (70.00%)/9 (18.00%)/5 (10.00%)/1 (2.00%)
Principle of resuming NUC:	
VR/CR/CR with a withholding period/Others/No mention	4 (8.00%)/6 (12.00%)/29 (58.00%)/2 (4.00%)/9 (18.00%)
Cirrhosis status	
Pure non-cirrhosis/Mixed/Pure cirrhosis/No mention	32 (62%)/16 (32%)/1 (2%)/1 (2%)
Patient number:	
>501/500-101/≤ 100	9 (18%)/28 (56%)/13 (26%)
Follow-up duration (months)[c,d]	
≥24/between 12-24/≤12	24 (48.98%)/14 (28.57%)/11 (22.45%)
Pretreatment HBeAg status:	
Only HBeAg(-)/Mixed with HBeAg(+) and (-)/Only HBeAg(+)	23 (46.00%)/21 (42.00%)/6 (12.00%)
Studies focusing on special patient populations:	
Lower HBsAg (<100 or 200 log IU/ml)	3 (6.00%)[33,51,52]
Prior severe acute exacerbation	1 (2.00%)[31]

CR, clinical relapse; EHR, electronic health record; HBeAg, hepatitis B e-antigen; NUC, nucleos(t)ide analogue; VR, virological relapse.
[a] Prospective in combination with retrospective study was classified as prospective study.
[b] From 46 studies reporting the events of severe hepatitis flares or decompensation.
[c] Mean or median.
[d] From 49 studies with available follow-up duration.

both Asia and Western countries that overlapped considerably with other published studies.[18,63]

The characteristics of these studies including study sample size, follow-up duration, proportion of individuals with cirrhosis, definition of safety outcomes, pretreatment HBeAg status and criteria for NUC cessation and retreatment were all highly heterogeneous. Regarding study design, most studies were primarily designed to analyze virological and/or biochemical relapses, and only ten studies prespecified safety outcomes with a clear definition.[17–19,27,30–34,69] Among these ten studies, only four included manifestations of hepatic insufficiency (such as jaundice, coagulopathy, or related clinical complications), instead of a certain serum alanine aminotransferase (ALT) level alone, in the outcome definition and had a sample size of >100 patients and a follow-up duration of >12 months.[17,18,27,69] The other six studies either relied on serum ALT levels alone to define the safety outcomes,[19,31,33,34] had a small (N = 46) sample size,[32] had a short (≤6 months) duration of follow-up,[30,34] or was exclusively composed of a special patient population (those with HBsAg <200 IU/ml[33]) (Table S8). The criteria for antiviral retreatment were also remarkably heterogeneous. In the absence of hepatic decompensation that indicated immediate retreatment, patients could resume treatment because of virological relapse alone (4 of 50 studies, 8.00%), biochemical relapse alone or accompanied by virological relapse (6 of 50 studies, 12.00%), biochemical relapse that did not subside after an observation period (29 of 50 studies, 58.00%), or local regulations of the participating institutions (2

of 50 studies, 4.00%). The criteria of retreatment were not mentioned at all in 9 (18.00%) studies.

A total of 46 of 50 studies reported data on severe flares or hepatic decompensation, but heterogeneously defined them as hyperbilirubinemia (without or without concurrent coagulopathy), ascites, encephalopathy, and/or variceal bleeding, in addition to abrupt elevation of serum ALT.[12–14,17–19,27,30–32,34–59,61–67,69,70,72] The definition of hyperbilirubinemia was also heterogeneous and could be a level of serum bilirubin ≥2 mg/dl, >3 mg/dl, and >2.5x the upper limit of normal. Similarly, coagulopathy was heterogeneously defined by prolongation of prothrombin time (PT) >3 seconds or an international normalized ratio >1.5 (Table S7). Of note, 14 (30.43%) of the 46 articles did not provide specific definitions for the said clinical events.[34,42,49,54–57,59,61,62,64–67]

In the analysis of the relationships between the reported safety outcomes and the characteristics of the studies, we excluded four cohorts that were comprised exclusively of special patient populations (3 cohorts of individuals with low HBsAg levels and one cohort of only individuals with prior severe acute exacerbation,[31,33,51,52]) and included 44 cohorts reporting severe hepatitis flares or hepatic decompensation,[12–14,17–19,27,30–32,34–50,53–59,61–67,69,70,72] and 45 reporting hepatitis flare-related death or liver transplantation.[12–14,17,18,27,30–32,34–50,54–72]

Analysis of severe hepatitis flares or hepatic decompensation
Fig. S2 presents proportions of the events according to presence of cirrhosis, number of patients, and length of follow-up. The reported proportions ranged from 0 to 4.27% with a scattered
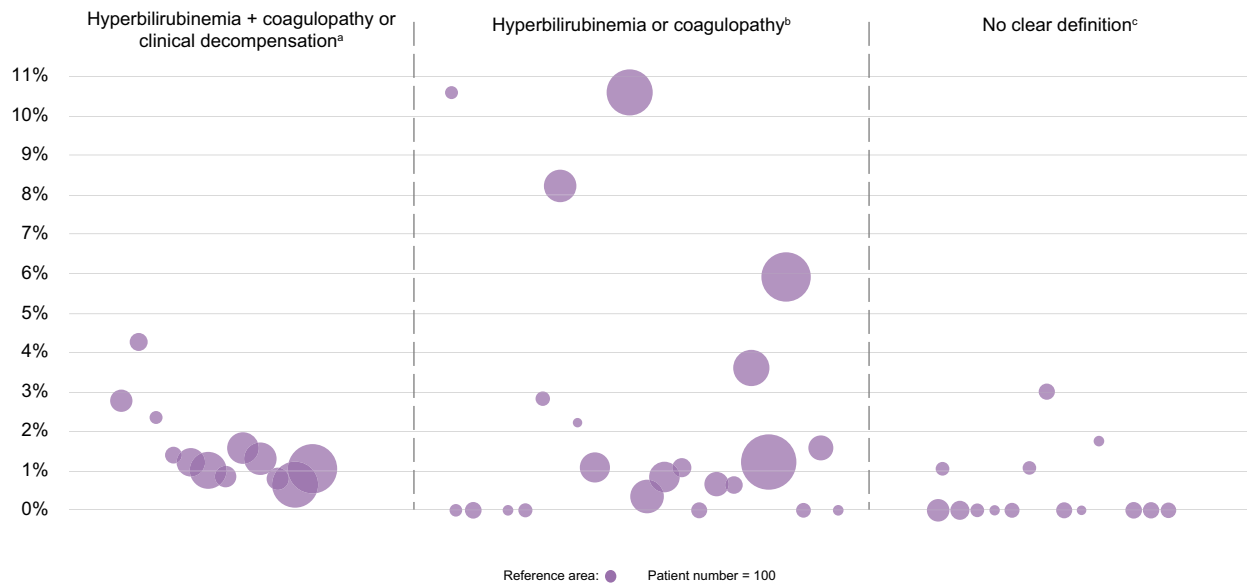
Fig. 2. Reported proportions of individuals with severe hepatitis flare or hepatic decompensation according to definitions of the events. Each circle indicates a study and its area represents the sample size of the study. The proportions ranged from 0.65% to 4.27% in studies that defined the events by clinical complications of liver failure or manifestations of both hyperbilirubinemia and coagulopathy (left panel), from 0 to 10.60% among studies where either hyperbilirubinemia or coagulopathy was sufficient to define the event (middle panel). Notably, 14 of the 46 studies (30.43%) did not provide clear definitions of the events (right panel). These 14 studies were remarkably smaller in sample size (median number of 113 patients) and were more likely (10 of 14 studies, 71.43%) to report zero events. a: references,[12–14,19,27,35–37,44,45,47,53] b: references,[17–19,27,30–32,38–41,43,44,46–48,50,58,63,69,70,72] c: references.[34,42,49,54–57,59,61,62,64–67]
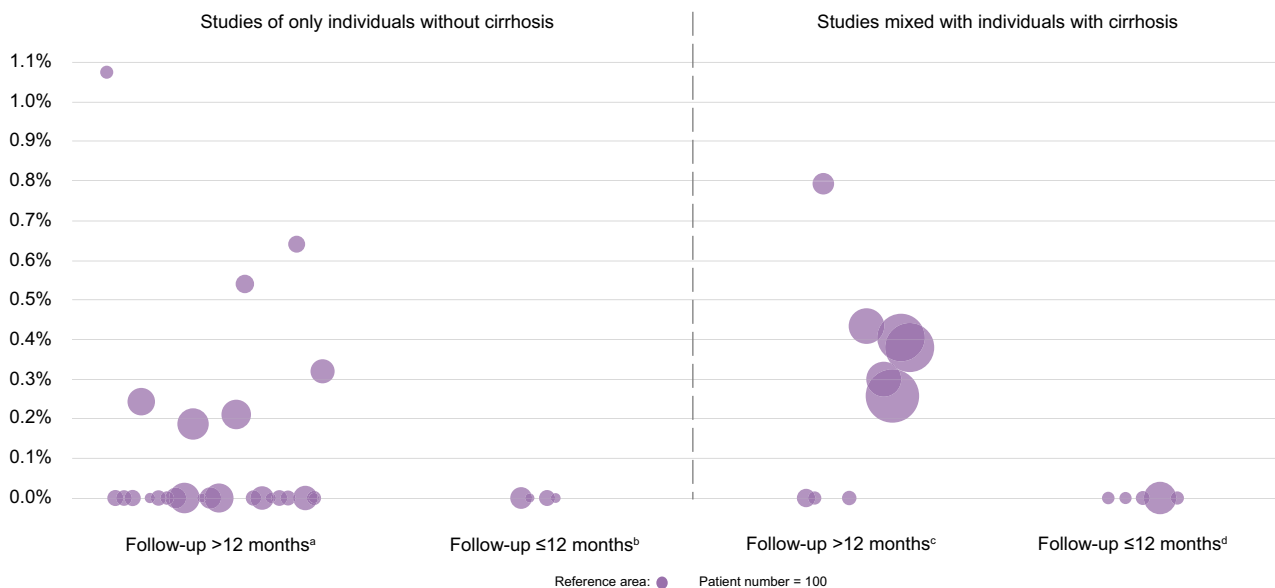
Fig. 3. Reported occurrences of hepatitis flare-related death or liver transplantation according to inclusion of individuals with cirrhosis, follow-up duration, and sample size. Each circle indicates a study and its area represents the sample size of the study. Whether the studies were exclusively composed of individuals without cirrhosis (left panel) or mixed with individuals with cirrhosis (right panel), there were no cases of flare-related death or liver transplantation if the mean or median duration of follow-up did not exceed 12 months. Furthermore, no events were recorded in 30 of 36 (83.33%) studies with a sample size of fewer than 500 individuals. a: references,[13,14,31,32,35,37–43,45–48,50,57,59–61,64,66,69,70,72] b: references,[34,65,67,68] c: references,[12,17,18,27,36,49,56,62,71] d: references.[30,44,55,58,63]

Table 2. Summary of the 15 studies selected for risk estimation and two multinational studies containing patient populations overlapping with other published studies.

First author, year	Patient number	Cirrhosis[g] (%)	Off-NUC criteria	Retreatment criteria	Severe hepatitis flares or decompensation[a] N (%)	Hepatitis flare-related death, liver transplantation N (%)
Studies selected for risk estimation (without overlapping populations)						
Liem,[32] 2019	45	0%	EASL 2017[c]	Per trial protocol[d]	1 (2.22%)	0%
Wong,[19] 2020	1,076	8.27%	n.a.	n.a.	Bilirubin: >2x ULN: 114 (10.60%); >5x ULN: 19 (1.77%); Decompensation: 7 (0.65%)	n.a.
Lai,[31] 2021 Non-prior SAE group	156	0%	Taiwan reimbursement[c]	Taiwan reimbursement[d]	1 (0.64%)	1 (0.64%)
Hsu[17 b], 2022	665	14.29%	Taiwan reimbursement[c]	Taiwan reimbursement[d]	24 (3.61%)	2 (0.30%)
Liu,[27] 2022	1,234	40.11%	APASL 2012[c]	Taiwan reimbursement[d]	Bilirubin ≥2 mg/dl: 73 (5.92%); Decompensation: 13 (1.05%)	5 (0.41%)
Papatheodoridis,[48] 2018	57	0%	Similar to EASL 2017[c]	Greece regulation[d]	0 (0%)	0 (0%)
Su,[50] 2018	100	0%	APASL 2012[c]	Taiwan reimbursement[d]	0 (0%)	0 (0%)
Ma,[47] 2019	535	0%	APASL 2012[c]	Taiwan reimbursement[d]	Bilirubin >2 mg/dl: 44 (8.22%); >3 mg/dl: 10 (1.87%); APASL definition: 7 (1.31%)	1(0.19%)
Sohn,[62] 2014	95	46.32%	APASL 2008[c]	HBV DNA >60 IU/ml	0 (0%)[f]	0 (0%)
Fong,[54] 2015	54	n.a.	HBeAg seroconversion with undetectable DNA ≥1 month	n.a.	0 (0%)[f]	0 (0%)
Jung,[56] 2016	113	18.58%	APASL 2012[c]	HBV DNA >2,000 IU/ml	0 (0%)[f]	0 (0%)
Wang,[64] 2016	93	0%	Taiwan reimbursement or APASL 2012[c]	HBV DNA >2,000 IU/ml and ALT >2x ULN	1(1.08%)[f]	1(1.08%)
Manolakopoulos,[59] 2021	57	0%	Similar to EASL 2017[c]	Greek regulations[d]	1 (1.75%)[f]	0 (0%)
Xia,[66] 2021	135	0%	Similar to APASL 2012[c]	HBV DNA >2,000 IU/ml and ALT >2x ULN	0 (0%)[f]	0 (0%)
Hall,[69] 2022	110	0%	Similar to APASL 2012[c]	Per protocol[d]	0 (0%)	0 (0%)
Multinational studies containing populations substantially overlapped in other published studies						
RETRACT-B study Hirode,[18] 2022	1,552	11.86%	Per different institutions[e]	Per different institutions[e]	19 (1.22%)	4 (0.26%)
CREATE study Sonneveld,[63] 2022	572	n.a.	Per different institutions	Per different institutions	2 (0.35%)	0 (0%)

ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; EASL, European Association for the Study of the Liver; ETV, entecavir; HBV, hepatitis B virus; n.a., not available; NUC, nucleo(s)tide analogue; SAE, severe acute exacerbation (defined by ALT >5x ULN and bilirubin >2 mg/dl or prolonged prothrombin time >3 seconds before antiviral agents); TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.
[a] Defined by each study. For study without its own definition, we extracted the data for individuals with bilirubin ≥2 mg/dl or clinical presentation of jaundice, ascites, encephalopathy, or variceal bleeding if available. Details in Table S7.
[b] Data from the subgroup of patients exclusively receiving ETV or TDF.
[c] Details in Table S9.
[d] Details in Table S6.
[e] Details in Table S10.
[f] No clear definition of severe hepatitis flares or decompensation.
[g] The definition of cirrhosis or advanced fibrosis in Table S12.

distribution in the 31 studies that had a sample size <300.[13,14,30–32,34–36,38,39,42,44,46,48–50,53–59,61,62,64–67,69,72] Moreover, 17 of these 31 (54.83%) studies did not record any event (Fig. S3). By contrast, the proportions of patients who developed severe flares or hepatic decompensation ranged from 0.36% to 3.61% in studies with a sample size >300, none of which reported zero events. Fig. 2 illustrated the relationships of outcome definitions and sample sizes with reporting of the outcomes. Studies without clear definitions of serious clinical outcomes had considerably smaller sample sizes (median number of 113 patients) and were more likely (10 of 14 studies, 71.43%) to report zero events.[34,42,49,54–57,59,61,62,64–67] The proportions of individuals with severe hepatitis flares or decompensation ranged from 0-10.60% when the outcome was defined by either hyperbilirubinemia or coagulopathy.[17–19,27,30–32,38–41,43,44,46–48,50,58,63,69,70,72] and 0.65-4.27% if the definition required both hyperbilirubinemia and coagulopathy unless clinical complications of liver failure were present.[12–14,19,27,35–37,44,45,47,53]

*Analysis of hepatitis flare-related death or liver transplantation*
Fig. 3 presents the reported proportions of patients who experienced hepatitis flare-related death or liver transplantation according to follow-up duration, inclusion of individuals with cirrhosis, and number of enrolled patients. Most of the studies (30 of 37 studies, 81.08%) with a sample size <500 reported zero events,[13,14,30–32,34–44,46,48–50,54–62,64–70,72] and two of the five

studies with a sample size of 500–1,000 also reported zero events (Fig. S4).[45,63] The proportions with hepatitis flare-related death or liver transplantation in the largest four studies from different institutions were 0.26% (RETRACT-B, international cohort study, 1,552 patients), 0.48% (single-center prospective cohort study, 1,309 patients), 0.30% (single-center retrospective electronic health record database study, 665 patients), and 0.19% (single-center prospective cohort study, 535 patients) respectively.[17,18,47,71]

Risk estimation from selected studies without overlapping populations

Pooled proportions of safety outcomes
We pooled the proportions of adverse events from the 15 selected studies without overlapping populations,[17,19,27,31,32,47,48,50,54,56,59,62,64,66,69] and also presented data from two large international multicenter studies whose patient populations overlapped considerably with those of the other published studies (Table 2, details in Tables S5–S7).[18,63] As indicated in Fig. 4, the pooled proportions of patients in the overall, non-cirrhotic, and cirrhotic populations who experienced severe hepatitis flares or hepatic decompensation were 1.21% (95% CI 0.70-2.08%), 0.89% (95% CI 0.44-1.79%), and 3.63% (95% CI 1.77-7.29%), respectively, and the corresponding proportions for hepatitis flare-related death or liver transplantation were 0.37% (95% CI 0.20-0.67%), 0.30% (95% CI 0.12-0.73%) and 0.98% (95% CI 0.45-2.14%), respectively. As such,
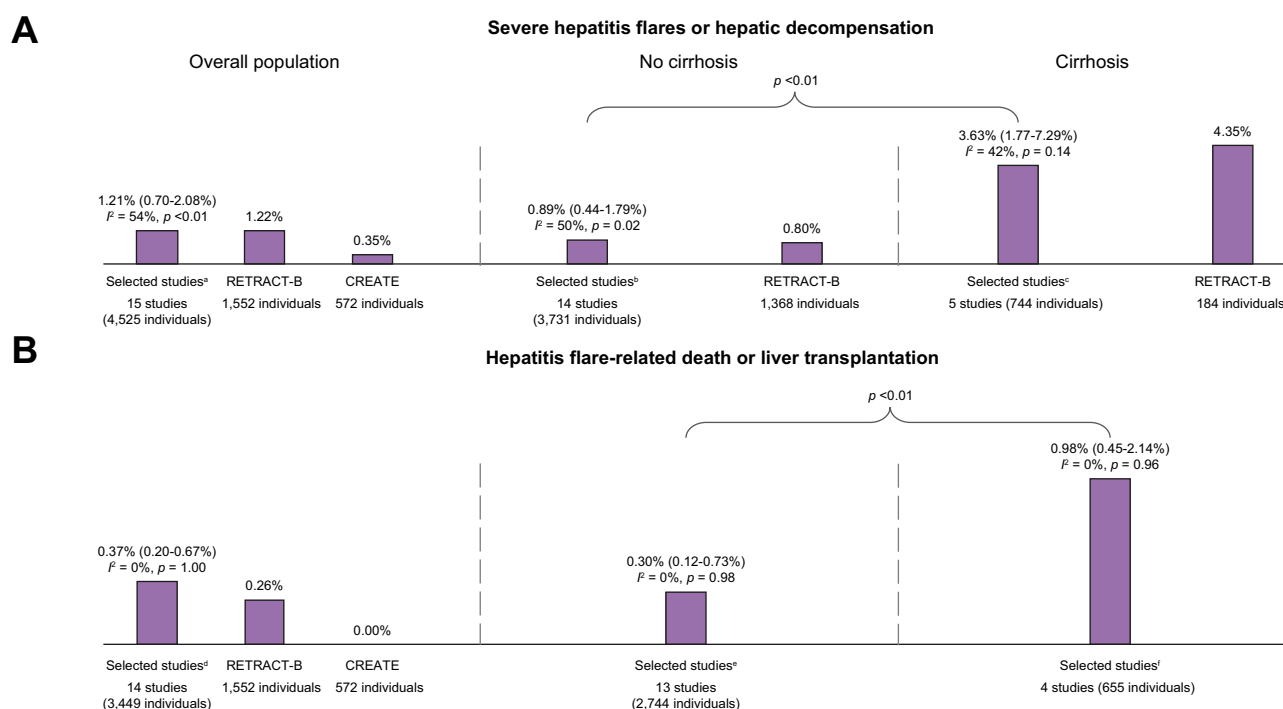
Fig. 4. Pooled estimates for the proportions of individuals suffering serious adverse clinical events following cessation of nucleos(t)ide analogues. Criteria of study selection for pooling aggregate data included (1) no overlapping populations, (2) a mean or median follow-up duration >12 months, (3) not for special populations such as studies exclusively composed of individuals with prior severe acute exacerbation or low serum HBsAg level. Two large multinational studies (RETRACT-B and CREATE) containing substantial overlapping populations in other published studies were also listed. (A) Estimated proportions of severe hepatitis flares or hepatic decompensation according to cirrhosis (upper panel). (B) Estimated proportions of hepatitis flare-related death or liver transplantation according to cirrhosis (lower panel). Estimated risks, generated by random-effects model and the inverse variance method, are expressed by proportions with 95% CIs. Heterogeneity among studies is indicated by the I^2 statistics. Comparison between the studies with and those without exclusion of individuals with cirrhosis was carried out using the Wald test. All tests are two-sided. a: references,[17,19,27,31,32,47,48,50,54,56,59,62,64,66] b: references,[17,19,27,31,32,47,48,50,56,59,62,64,66] c: references.[17,19,27,56,62] d: references,[17,27,31,32,47,48,50,54,56,59,62,64,66] e: references,[17,27,31,32,47,48,50,56,59,62,64,66] f: references.[17,27,56,62]

compared to those without cirrhosis, individuals with cirrhosis were significantly more likely to suffer both severe hepatitis flares or decompensation (p <0.01) and hepatitis flare-related death or liver transplantation (p <0.01). The heterogeneity among the studies pooled for severe flares or decompensation was significant (I^2 = 54%, p <0.01) but not in the pooled analysis for hepatitis flare-related death or liver transplantation (I^2 = 0%, p = 1.00) (details in Table S11 and Figs. S5–S7).

Discussion

In this systematic review and meta-analysis that focused on serious clinical events following NUC cessation in individuals with CHB, we not only pooled currently available data to estimate the risk but also examined the quality of current literature in reporting such events. Using a meta-research approach to appraise 50 studies, we noted a wide variation across studies in the proportions of patients who experienced serious clinical events, and more importantly we uncovered several pitfalls in the current literature, which could account for substantial heterogeneity among the studies and lead to inaccurate risk interpretation. We found most of the studies did not prespecify safety outcomes as a study endpoint and few were designed to adequately address this issue. Among the 46 studies that included severe hepatitis flares or hepatic decompensation in their reports, one-third (14 studies, 30.43%) did not provide clear details on what constituted the definitions and the remaining studies applied remarkably heterogeneous definitions for a serious clinical event. We further showed that reporting of these safety outcomes was influenced by the study sample size, follow-up duration, and the definition of study outcomes, with zero events more likely to be observed in studies with a smaller sample size, shorter follow-up, or unclear outcome definition. Of all 50 original studies analyzed, only four prespecified safety events with a clear definition that included manifestation of hepatic insufficiency (instead of a certain ALT level alone), had a follow-up duration >12 months, and had a sample size >100 patients.[17,18,27,69] Finally, we provided estimation of the risks by pooling aggregate data from 15 selected studies (4,525 patients) without overlapping populations and found overall 1.21% (95% CI 0.70–2.08%) of individuals with CHB who stopped NUC (predominantly ETV or TDF) would run into severe flares or hepatic decompensation; the risk was significantly higher in those with cirrhosis (3.63%; 95% CI 1.77-7.29%) than those without (0.89%; 95% CI 0.44-1.79%; p <0.01). The pooled estimate for the proportion of hepatitis flare-related mortality or liver transplantation was 0.37% (95% CI 0.20-0.67%), and the risk was again significantly higher in individuals with cirrhosis (p <0.01). Collectively, our findings expose the limitations of current literature on the safety of NUC withdrawal in individuals with CHB and highlight the need to interpret published data with great caution.

Our analysis revealed that studies of small sample size and/or short follow-up could risk spurious results that are biased towards a lower event rate and a false sense of safety. In fact, these small or short studies were designed primarily for an endpoint defined by laboratory measurement, such as resurgence of viremia or rise of serum ALT, rather than a more serious safety outcome. As outcome observation could be censored upon occurrence of the virological or biochemical endpoints, studies that did not prespecify clinical safety events as study outcomes

were presumably more likely to underreport such an event. Besides, the heterogeneity of retreatment criteria should also be considered when weighing available data to discuss the pros and cons of finite NUC therapy. When to resume the treatment following NUC cessation is currently unknown and fervently debated because early retreatment may miss the "good flares" whereas delayed retreatment may fail to abrogate preventable catastrophes.[73,74]

With regards to the reporting of severe hepatitis flares or decompensation after NUC cessation, various definitions and cut-off values for defining hyperbilirubinemia and coagulopathy were employed, ranging from either serum bilirubin level ≥2 mg/dl or prolongation of PT ≥3 seconds to both serum bilirubin level >3.5 mg/dl and PT prolongation >3 seconds. A stricter criterion would lead to a lower reported rate of such adverse events but also indicate that patients might be close to liver failure. For example, Liu *et al.* reported that 6.85% (5/73) of individuals with a serum bilirubin level ≥2 mg/dl died of or received liver transplantation for acute-on-chronic liver failure following NUC withdrawal, and the percentage of fatal consequences rose to 38.46% (5/13) if the denominator was confined to individuals with a bilirubin level ≥2 mg/dl and a prolongation of PT ≥3 seconds.[27] Conceivably, interpretation of the safety data was more difficult if the event was not prespecified or even defined.

The pooled risk estimates for severe hepatitis flares or decompensation (1.21%) and hepatitis flare-related death or liver transplantation (0.37%) from 15 selected studies with 4,525 individual patients were similar to those reported from an international consortium of 1,552 patients (RETRACT-B, 1.22% and 0.26%, respectively). The moderate heterogeneity among the studies pooled for severe hepatitis flares or decompensation (I^2 = 42-54%) could be partially explained by different definitions for such adverse outcomes. Interestingly, the heterogeneity was low among the studies for flare-related mortality or transplantation (I^2 = 0%), indicating that, regardless of various definitions of severe withdrawal flares or decompensation, protocols of follow-up, and criteria for antiviral retreatment, the estimated proportions of patients who would suffer the most serious outcomes were rather consistent among the studies.

Severe clinical adverse events were not reported or only narrated without quantitative analyses in previous systematic reviews with or without meta-analysis for the issue of NUC cessation in individuals with CHB. In fact, they mainly focused on the incidences of virological/biochemical relapse or seroclearance of HBsAg after NUC cessation.[15,16,75] For instance, Hall and colleagues identified 31 decompensation events (0.5%) after reviewing 36 studies in their recent meta-analysis and considered it safe to stop NUCs.[15] Nonetheless, neither was the definition for safety outcomes clarified nor were the study populations adequately characterized (*e.g.* by removing data from overlapping cohorts or a unique cohort with certain specific features).[20] In contrast, we attended to this safety issue, which should have constituted the major consideration for the practice of stopping NUC therapy, with a deeper look into the research design and the relationship of study characteristics with outcomes reported. On the basis of comprehensive search and critical appraisal of currently available literature, our analysis generated the best estimates so far to inform clinical practice. Moreover, our findings exposed several critical points that lacked consensus among investigators and should be included in the future research agenda: detailed criteria for stopping NUCs,

clearly defined patient outcomes as safety endpoints, protocols of off-NUC follow-up, and indications for antiviral retreatment.

This study has several limitations. First, we used proportions instead of incidence rate to evaluate the safety outcomes because few of the included studies provided time-to-event data. This approach could not precisely estimate the risk of adverse events because the follow-up durations of the studies varied. In order to mitigate this limitation, we pooled data for studies with duration of the median or mean follow-up of 12 months or longer. Second, because of the lack of individual patient-level data and high heterogeneity in study design and patient characteristics, some key factors such as age, ethnicity, criteria for NUC cessation, end-of-treatment serum HBsAg level, follow-up protocol, consolidation duration, and retreatment criteria, could not be fully examined for their potential influence on patient outcomes. Third, our risk estimates were generated for individuals with CHB at large and might not be applicable to special populations such as those with a prior experience of severe acute exacerbation, whose risks of serious clinical events were reportedly higher.[31] Fourth, significant liver fibrosis or even cirrhosis might be present in some individuals in the non-cirrhosis cohort because liver fibrosis was not graded and cirrhosis was not defined by histological exam in most of the studies. Also, it is unclear how cirrhosis regression during NUC treatment may affect the incidence and consequence of withdrawal flares. Lastly, the majority of studies came from referral centers with likely more intensive monitoring protocols and more adherent patients, so the data may not be generalizable to real-world community-based populations. More data on patients managed at community-based clinics are needed.

In summary, the overall safety data for NUC cessation is limited with great heterogeneity in study design and patient characteristics, indicating the need to interpret published data with great caution. The pooled estimates from available data were 1.21% for severe hepatitis flares or decompensation and 0.37% for hepatitis flare-related death or liver transplantation, with only moderate to no significant heterogeneity among studies and with a significantly higher risk in individuals with cirrhosis than those without. These findings can inform the discussion and decision about NUC-based treatment strategies for individuals with CHB.

Abbreviations

ALT, alanine transaminase; CHB, chronic hepatitis B; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NUC, nucleos(t)ide analogues; PT, prothrombin time; TDF, tenofovir disoproxil fumarate.

Conflict of interest

CHT: Speaker: Abbvie, Bristol-Myers Squibb, Gilead Sciences, Merck Sharp & Dohme, and Bayer. TYL: Research grant: Gilead Sciences, Merk Sharp & Dohme, and Roche. Consulting/advisory board/speaker: Gilead Sciences, Merk Sharp & Dohme, Roche, Abbvie, Bristol-Myers Squibb, Bayer, Janssen, and Eisai. MHN: Research grant: Pfizer, Glycotest, Enanta, Gilead, National Cancer Institute, Vir, BK Kee Foundation; Consulting/advisory board: Novartis, Spring Bank, Janssen, Gilead, Exact Sciences, Intercept, Eli Lilly, Bayer, Eisai, Laboratory of Advanced Medicine. YCH: Research grant: Gilead Sciences; Consulting/advisory board: Gilead Sciences; Speaker: Abbvie, Bristol-Myers Squibb, Gilead Sciences, Merck Sharp & Dohme, and Novartis. All other authors: Nothing to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept: CHT, YCH, MHN. Search key word determination: CHT, YCH, MHN, JAB. Literature search: CHT, THC. Data extraction: CHT, THC. Study quality assessment: CHT, THC. Data analysis: CHT, JLW. Manuscript writing: CHT, YCH, MHN, JLW. Data interpretation and critical review/revision of the manuscript: All authors.

Data availability statement

Data extracted and collected in this study and data dictionaries defining fields in the datasets will be available to access. Data access will be provided to investigators for academic research after a proposal has been approved by the study committee identified for this purpose. The investigator will sign a data access agreement on how to collaborate with consideration of potential overlaps between the proposal and ongoing efforts. Data will be available beginning with full publication of this Article. Proposals should be directed to holdenhsu@gmail.com, and the requested data will be transferred by email.

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Author names in bold designate shared co-first authorship

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