Master of Public Health

Master de Santé Publique

Natural history of chronic hepatitis B virus infection according to DNA and ALT levels: A systematic review and meta-analysis

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Class and year of the Master:
Master of Public Health 2022-2023

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ACKNOWLEDGEMENT

I would first like to thank my professional advisor, Dr Yusuke Shimakawa, for giving me the privilege and the opportunity to dedicate my practicum to this interesting project and for his guidance, support, and for sharing his vision towards a healthier world. I extend my gratitude to Dr Arthur Rakover, Dr Yu Ri Im, Zakary Warsop, Dr Rukmini Jagdish and Dr Emma Chen for and their contributions during references screening, research papers review, data verification and insightful discussion throughout this project.

Additionally, I would like to express my special gratitude to my supervisor Prof. Mary Beth Terry for her brilliant feedback and timely guidance during the process of my practicum and thesis writing.

I thank Professor Philippa Easterbrook (WHO) and Professor Roger Chou (Oregon Health and Science University, USA) as well, for supporting this project with their extensive expertise on the subject matter, through valuable discussion and advice.

Importantly, I would also like to thank all my colleagues of “Unité de Recherche et d’Expertise Epidémiologie des maladies Emergentes” at Institut Pasteur for welcoming me and providing me with the environment and all the useful resources needed to complete this project. Finally, I would like to extend my sincere appreciation and gratitude to my parents, sister and grandmother for their everlasting care and love throughout my life, none of my work would have been possible without them.

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List of abbreviations

Abbreviation or Term	Definition/Explanation
HBV	Hepatitis B virus
CHB	Chronic Hepatitis B
VL	Viral load
ALT	Alanine transaminase
EASL	European Association for the Study of the Liver
AASLD	American Association for the Study of Liver Diseases
APASL	Asian Pacific Association for the Study of the Liver
APASL	Asian Pacific Association for the Study of the Liver
WHO	World Health Organization
HBeAg	Hepatitis B e antigen
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
HCV	Hepatitis C virus
HDV	Hepatitis D virus
DNA	Deoxyribonucleic Acid
ULN	Upper limit of normal
FU	Follow-up
PYFU	Person-years of follow-up

Abstract

Background

In 2015, the World Health Organization (WHO) launched the first hepatitis B treatment guidelines, alongside the first global viral hepatitis elimination targets. Currently, decisions around antiviral treatment aim to target individuals at high risk of disease progression. However, with emerging evidence, it is important to re-evaluate treatment eligibility. Thus, this systematic review and meta-analysis aims to review and synthetize evidence on clinical outcomes in untreated, non-cirrhotic HBV patients, stratified by HBV DNA and alanine transaminase (ALT) levels.

Methods

We searched PubMed, Embase, Web of Science, and the Cochrane Library for studies published up to Feb 2023. Two reviewers independently screened titles and abstracts and extracted data from full-text articles. Outcomes included hepatocellular carcinoma (HCC), cirrhosis and liver-related mortality. The pooled incidence rates of each outcome were stratified by viral load (<2,000, 2,000-20,000, 20,000-200,000, and ≥200,000 IU/mL) or ALT levels (1x, 1-2x, ≥2x ULN). We performed a meta-analysis using a generalized mixed model.

Results

Of 13,124 identified and screened studies, we identified 45 eligible studies. In low viral load strata - HBV DNA <200 IU/mL and <2,000 IU/mL, the pooled HCC incidence (per 100 person-years) was low: 0.131 (95% CI: 0.097-0.177) and 0.176 (0.117-0.265), respectively. For viral load above 2,000 IU/mL, there was a dose-response relationship: 0.312 (0.245-0.396), 0.874 (0.735-1.040), and 0.941 (0.664-1.335) when HBV DNA levels were 2,000-20,000, ≥20,000-200,000, and ≥200,000 IU/mL, respectively. A similar relationship was observed for the incidence of cirrhosis and liver-related mortality. There were only two studies in children and no clear association between viral load and clinical outcomes was found.

Conclusion

This represents the most comprehensive systematic review and meta-analysis to date of chronic HBV infection natural history. Cirrhosis and HCC incidence rates were low in individuals with HBV DNA<2000 IU/ml. There was paucity of data in the pediatric population.

Abstract in French

Contexte

En 2015, l'OMS publie ses premières directives pour le traitement de l'hépatite B et lance des objectifs d'éradication globale. Ces actions requièrent une expansion importante de la thérapie antivirale, ciblant surtout les sujets à haut risque d'aggravation de la maladie. Cette méta-analyse vise à évaluer cette incidence d'aggravation et à synthétiser les preuves des résultats cliniques chez les patients non-cirrhotiques atteints d'HBV, en fonction des niveaux d'ADN du VHB et d'ALT.

Méthodes

Nous avons recherché PubMed, Embase, Web of Science et Cochrane Library des études publiées jusqu'en février 2023 Deux examinateurs ont revu les titres et résumés de manière indépendante et ont extrait les données des articles complets. Les résultats incluaient le carcinome hépatocellulaire (CHC), la cirrhose et la mortalité liée au foie. Les taux d'incidence cumulés de chaque résultat étaient stratifiés par charge virale (<2 000, 2 000-20 000, 20 000-200 000, et ≥200 000 IU/mL) ou niveaux d'ALT (1x, 1-2x, ≥2x ULN). Une méta-analyse utilisant un modèle mixte généralisé a été réalisée.

Résultats

Sur 13 124 études identifiées et examinées, 45 ont été incluses. Dans les strates de charge virale faible - ADN du VHB <200 IU/mL et <2 000 IU/mL, l'incidence cumulée de CHC (par 100 années-personnes) était faible: 0,131 (IC 95%: 0,097-0,177) et 0,194 (0,121-0,310) respectivement. Pour une charge virale supérieure à 2 000 IU/mL, une relation dose-réponse était observée. Une relation similaire était observée pour l'incidence de la cirrhose et de la mortalité hèpatique. Aucune association claire n'a été trouvée dans 2 études pédiatriques.

Conclusion

Cette revue est la plus exhaustive à ce jour sur l'évolution naturelle de l'infection chronique par le VHB. Les taux d'incidence de la cirrhose et du CHC étaient faibles chez les individus avec un ADN du VHB <2000 IU/ml. Il y avait une pénurie de données dans la population pédiatrique.

1. Introduction

1.1 Background information

Hepatitis B virus (HBV) represents an important public health concern worldwide. In 2019, approximately 296 million people were chronically infected and over 820,000 individuals died globally due to related complications such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) (1,2). Approximately one in four individuals with chronic hepatitis B virus (HBV) infection are at risk of premature death from related complications, despite the availability of effective tools and technologies, such as testing, vaccines, and antiviral therapies (3). Antiviral therapies lower HBV viral load (VL) and can reduce the risk of developing cirrhosis and hepatocellular carcinoma (4). However, not all the individuals with chronic HBV are eligible for antiviral therapy as it is targeted towards those at high risk of liver-related complications (4).

In recent years, there have been several efforts to establish guidelines to classify individuals with chronic HBV at high risk of liver disease progression who are eligible to receive treatment (4). This classification often requires multiple tests including alanine transaminase (ALT), HBV DNA level, and assessment of the degree of liver fibrosis (either via a non-invasive marker or a liver biopsy) (5,6). Guidelines covering these recommendations have been developed by organizations such as the European Association for the Study of the Liver (EASL) (7), the American Association for the Study of Liver Diseases (AASLD) (5), the Asian Pacific Association for the Study of the Liver (APASL) (6) and the World Health Organization (WHO) (4). These guidelines are fairly similar in their treatment indications, yet, they often differ on the HBV DNA level and ALT threshold designated to indicate antiviral treatment, particularly in non-cirrhotic individuals (8).

In 2015, the WHO launched its first guidelines for the prevention, care and treatment of persons with chronic HBV (4). These guidelines represented an attempt to simplify treatment decisions and used HBV DNA, ALT and fibrosis assessment to establish individuals at risk of disease progression, particularly for resource-limited countries. Specifically, in the case of non-cirrhotic patients, WHO guidelines recommended antiviral therapy for patients with persistently abnormal ALT (defined as three ALT determinations above the upper limit of normal, during a 12-month period) and HBV DNA > 20 000 IU/mL (4). This recommendation was made based on moderate quality of evidence, and it did not distinguish for hepatitis B e antigen (HBeAg) status. Additionally, one of its main limitations was lack of available data in resource-limited settings (8).

1.2 Rationale

Since 2015, new evidence has emerged on the eligibility for chronic HBV infection treatment and on the characteristics of the individuals who might benefit the most from receiving therapy. Thus, it is important to synthesize the evidence, including studies that have been recently published, in order to identify individuals at higher risk of liver complications who might benefit from treatment. Therefore, this study aims to review and synthetize available literature on the incidence rate of liver complications in individuals with chronic HBV infection and without cirrhosis, according to HBV DNA and ALT levels.

1.3 Review question

What is the incidence rate of clinical outcomes without treatment in HBV-infected people without cirrhosis, stratified according to the HBV DNA levels (<2000, 2000-20,000, 20,000-200,000, and ≥200,000 IU/mL) and ALT levels (<1x, 1-2x, ≥2x upper limit of normal (ULN))?

1.4 Objectives

- To review and summarize available evidence on the incidence rate of liver complications (HCC, cirrhosis and liver-related deaths) in HBV-infected individuals without cirrhosis and without treatment.

- To estimate the incidence rate of liver complications in HBV-infected individuals without cirrhosis, when not receiving treatment. Stratified by HBV DNA levels and ALT levels.

2. Methods

This study used a systematic literature review and meta-analysis as methods to identify relevant studies and synthesize the incidence rates of liver complications (HCC, cirrhosis, and liver-related deaths) in HBV-infected individuals without cirrhosis. This systematic review was reported following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (9).

2.1 Protocol and registration

For all the methods used for this systematic review, a prespecified protocol was developed and followed. This protocol was registered in PROSPERO, the international prospective register of systematic reviews.

2.2 PICO question and eligibility criteria

Table 1 describes the PICO framework elements that were considered to include studies in the review.

Table 1. PICO framework for inclusion of studies

Population	People of any age with CHB[*1] without cirrhosis and without concomitant anti-HBV therapy who had both HBV DNA quantification and ALT measurement at baseline[*2]
Intervention	No treatment (natural history) [*3] Studies that included participants who initiated antiviral therapy during the follow-up were also included
Comparison	Not applicable
Outcome	Incidence rate of clinical outcomes[*4] reported by HBV DNA levels (<2000, 2000-20,000, 20,000-200,000, and ≥200,000 IU/mL)[*5] and ALT levels (<1x, 1-2x, ≥2x ULN)[*6]

[*1] Definition of chronic HBV infection (CHB)

CHB is defined as a positive HBsAg serology test on two occasions at least 6 months apart (10). However, because new HBV infections in adults are rare in highly endemic countries where the majority of HBsAg-positive people acquired the infection perinatally or during childhood, HBsAg positivity on only one occasion in adults living in highly prevalent countries was assumed to reflect CHB (11).

[*2] Evaluation at baseline

Some longitudinal studies of people with CHB may define subgroups based on HBV DNA levels and ALT levels evaluated at a single time point, but others may define them based on several measurements taken during several visits. We considered both types of studies in this systematic review and performed subgroup analysis based on this parameter (single time point *versus* multiple time points).

[*3] Natural history of CHB

Our primary focus was on longitudinal studies that assess the natural history of CHB in all participants, ideally without any participants initiating anti-HBV therapy during follow-up. However, most contemporary studies provide antiviral therapy to those who become eligible

during follow-up and retrospectively exclude or censor those who initiated antiviral therapy. This practice may have introduced bias, because participants who become eligible for anti-HBV therapy during follow-up and started treatment are more likely to develop cirrhosis or HCC than those who never meet eligibility criteria during the follow-up. For instance, in a cohort stratified by gender, HBeAg status, and baseline HBV DNA levels, the REAL-B registry database found that the incidence rates of HCC were higher when participants who initiated antiviral therapy during follow-up were included in the analysis, compared to when they were excluded (12). This suggests that studies that exclude participants who start antivirals during follow-up may underestimate the risk of HCC. To ensure a comprehensive analysis of the natural history, we also included a study that included participants who started antiviral therapy during follow-up.

[*4] Outcomes

We considered HCC, cirrhosis and liver-related mortality.

[*5] HBV DNA levels

All HBV DNA levels will be reported in IU/mL; values given as copies/mL were converted to IU/mL after dividing by a factor of 5 (10 000 copies/mL = 2000 IU/mL; 100 000 copies/mL = 20 000 IU/mL; 1 million copies/mL = 200 000 IU/mL) (13).

[*6] Upper limit of normal (ULN) for ALT levels

The ULN of ALT varies between the guidelines. We considered the ULN of whatever the original study used according to the different guidelines. Table 2 describes different possible cut-off values for the upper limit of normal (ULN) of ALT according to different guidelines.

Table 2. Upper limit of normal (ULN) of ALT (IU/L) according to the different guidelines

Guidelines	Men	Women
AASLD, 2018 (5)	35	25
EASL, 2017 (7)	40	40
APASL, 2015 (6)	40	40
WHO, 2015 (4)	30	19

Articles published in any language were considered and the following criteria were considered for inclusion.

Inclusion criteria:

- Studies presenting longitudinal data on people of any age with CHB[*1] without cirrhosis and without concomitant anti-HBV therapy,[*2] who had both HBV DNA quantification and ALT measurement at baseline,[*3] and reporting the number of events and person-years-at-risk for clinical outcomes[*4] in a group of participants stratified by both HBV

DNA levels (<2000, 2000-20,000, 20,000-200,000, and ≥200,000 IU/mL)[*5] and ALT levels (<1x, 1-2x, ≥2x ULN)[*6]

- Clinical outcomes should be reported for a group of participants who were free of cirrhosis at baseline, had no history of anti-HBV therapy before the start of the study, did not receive any such therapy during the study, and were followed up for at least 1 year, with an interval not exceeding 2 years.
- The clinical outcomes should be stratified according to the predefined thresholds for HBV DNA levels and ALT levels. It should be noted that some studies may report subgroups defined by broader categories, for example, HBV DNA levels ≥20,000 IU/mL & ALT levels ≥1x.
- Not all longitudinal studies reporting the number of events for a specific outcome present the person-years-at-risk for that specific clinical outcome. We still included these studies if they reported: i) incidence rate for the outcome; ii) number of participants at the start of follow-up and mean/median duration of follow-up specific for the outcome; or iii) a life table specific for the outcome with the number at risk at each interval.
- Both original articles and conference proceedings were be considered if they reported enough information to meet the inclusion criteria.

Exclusion criteria:

- Studies focused on people with a primary condition other than hepatitis B (e.g., malignancy, autoimmune disease, hemodialysis).
- Studies focused on people co-infected with HIV, HCV, or HDV.
- Studies focused on people who have already lost HBsAg.
- Studies focused on people who have received anti-HBV therapy before recruitment.
- Studies focused on pregnant women.

2.3 Search strategy and Information sources

The databases searched included: PubMed, Embase, Web of Science, and the Cochrane Library. The exact search strategies are adapted to each database and is detailed in **Appendix 1.** The search terms employed covered hepatitis B infection AND parameters used to stratify participants (i.e. ALT, HBV DNA) AND clinical outcomes (i.e. progression of fibrosis, cirrhosis, HCC, death) AND type of research. Additionally, it was restricted to papers published from January of 2000 up to February of 2023.

We also performed a manual search through the references of included studies, as well as through those of relevant systematic reviews identified through the literature search, to identify any further eligible studies.

2.4 Selection process

The search was conducted in the selected databases using the reported search strategy and the results were transferred to a .ris file. Duplicates were identified and removed using a references manager. Subsequently, references were screened by titles and abstracts by two reviewers to assess their eligibility. The decision to include studies were made after screening was complete, and dissimilarities were solved by a third reviewer. Reasons for exclusion were labeled and recorded. Corresponding author for all potentially eligible studies with missing information were contacted by e-mail or phone call.

2.5 Data extraction and description of the database

Data from selected studies were extracted using a pre-piloted data extraction form. All relevant data was extracted including: (i) reference's general characteristic, (ii) General information about the study design and recruitment (iii) Characteristics of study participants (number of participants at baseline, inclusion and exclusion criteria for participants, number of male/female, mean age, number with positive HBeAg, HBV genotype, fibrosis stage, number of participants coinfected with hepatitis C virus, hepatitis D virus and human immunodeficiency virus (HIV), (iv) Baseline HBV DNA and ALT data, (v) measured outcome and outcome's definition (HCC, cirrhosis and liver-related mortality), (vi) method to estimate the time of follow-up, (vii) number of events during the follow-up, and (viii) follow up expressed either in person-years, mean follow-up or median follow-up.

One reviewer extracted the data independently and once data extraction was completed, an independent reviewer verified the data for accuracy. After completing data extraction, articles with potentially overlapping populations were identified by checking the recruitment period, recruitment place and enrolment criteria. Afterwards, articles reporting results for the same research project were identified and references containing less information or reporting results for a subsample were excluded.

2.6 Quality assessment

The quality of included studies was rated using the Newcastle–Ottawa Scale (14) for observational studies (see Appendix 2).

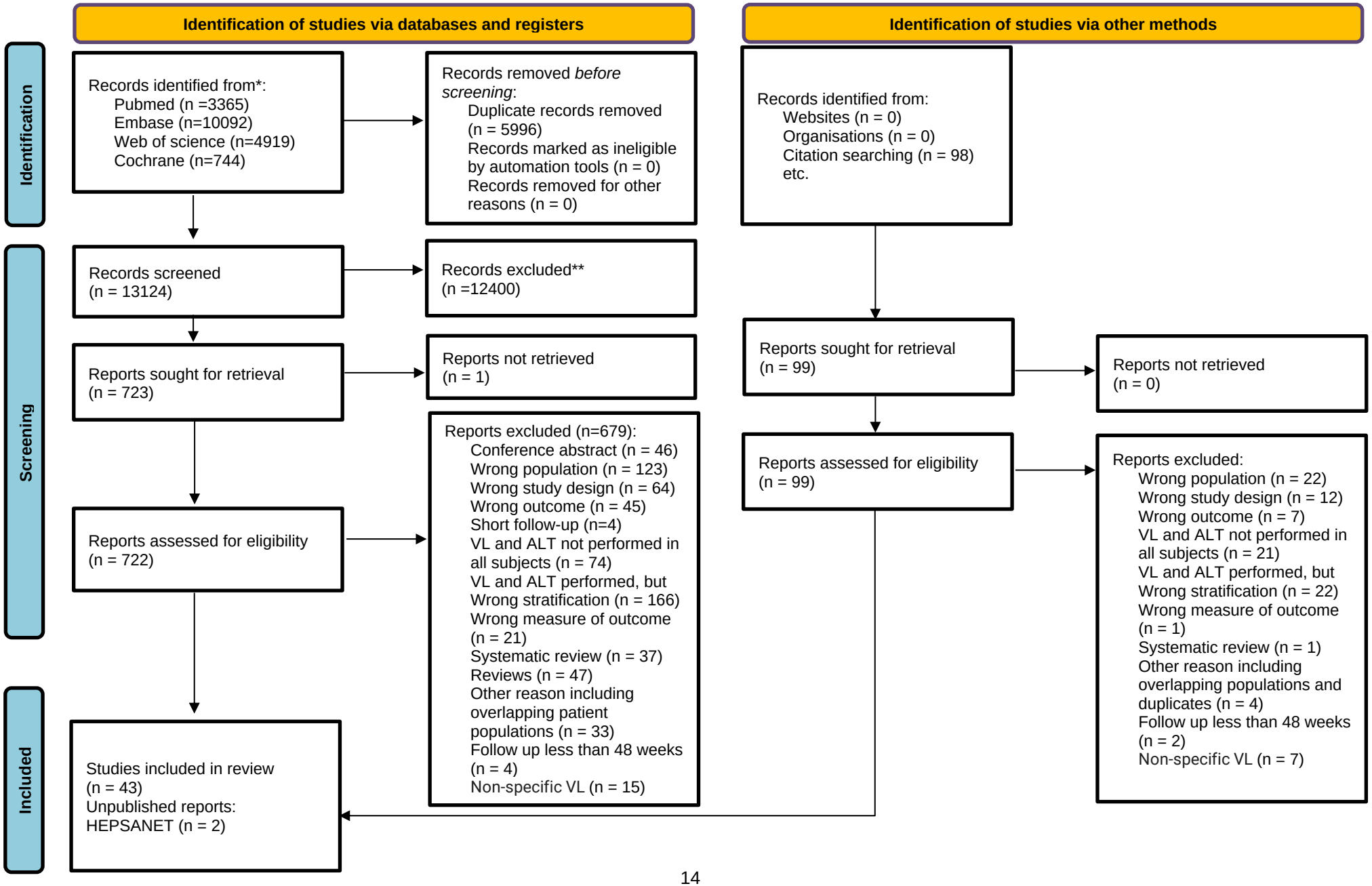
2.7 Data synthesis and statistical analysis

We tabulated extracted data to summarize the characteristics of included studies. We also performed a random-effects meta-analysis using a generalized linear mixed model (GLMM) with the logit link by the “metarate” command in R 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). We choose random-effects model over fixed-effects model as a high level of heterogeneity is expected among the included studies. We employed I^2 to assess the statistical heterogeneity. We performed subgroup analyses based on: (i) HBV DNA and ALT pre-defined groups; (ii) adults and children’s cohorts; and (iii) single and multiple time HBV DNA and ALT assessments.

Results

We initially identified a total of 13,124 references through our electronic database search. After a thorough screening process, 43 studies were included in our meta-analysis; the studies were published from 2002 to 2023 and included 43 studies conducted in adults and 2 studies conducted in children/adolescents. Additionally, to address the lack of data on CHB in sub-Saharan Africa, unpublished longitudinal data from the region was identified through the Hepatitis B in Africa collaborative Network (HEPSANET) (15). As a result, we integrated aggregated data from two cohort studies: the Ethiopian cohort (Johannessen A, Desalegn H et al.) and the Gambian cohort (Ndow G, Lemoine M et al.). Figure 2 shows the literature search flowchart.

Figure 2. Flow chart of study inclusion and exclusion



3.1 General characteristics of included studies

The majority of the included studies were from the Western Pacific region (64.4%, 29/45), followed by Europe (11.1%, 5/45), Americas (6.7%, 3/45), Eastern Mediterranean (6.7%, 3/45), and Africa (6.7%, 3/45). Most of the studies focused on adults (86.7%, 39/45), while 12.5% (10/45) focused on adolescents/children and 2.5% (2/45) included both. Over a half of the studies were conducted before 2015 (31.1%), around 64.4% (29/45) after 2015 and two included references remain unpublished. Table 3 presents the general characteristics of selected studies including WHO region, year of publication, study population, study design, fibrosis assessment and follow-up duration.

Table 3. General characteristics of included studies

Variables		Number (Total n=45)	%
WHO regions			
	WPR	29	64.4%
	EMR	2	4.4%
	EUR	5	11.1%
	AFR	3	6.7%
	AMR	3	6.7%
	Combination	3	6.7%
Year of publication			
	Before 2015	14	31.1%
	After 2015 (including 2015)	29	64.4%
	Unpublished	2	4.4%
Study population			
	Adults	39	86.7%
	Children (<18 years)	2	4.4%
	Mixed	4	8.9%
Study design			
	Prospective cohort study	22	48.9%
	Retrospective cohort study	23	51.1%
Fibrosis assessment			
	Not reported	34	75.6%
	F0-1	1	2.2%
	F0-2	1	2.2%
	F0-3	9	20.0%
Duration of follow-up			
	< 5 years	9	20.0%
	≥ 5 years	36	80.0%

Across all 45 included references, 22 (48,9%) prospective studies, and 23 retrospective studies (51,1%) were included. Table 4 presents the main characteristics of participants in included studies.

Table 4. Summary of included studies

Author, year	Country	Study design (prospective, retrospective)	Total number of participants at baseline	Male sex (n/N, %)	Age category	Mean age (±SD) or median (range or IQR) in years	HBeAg-positive (n/N, %)	HBV genotype: A, B, C, D, E, F, G, H, I, J (n/N, %)	Fibrosis stage	HCV (n/N, %)	HDV (n/N, %)	HIV (n/N, %)	Alcohol (n/N, %)
Ahn, 2014 (16)	Korea	Retrospective	309	55,0%	Adults	Mean 46.8 SD 10.2	0,0%	N/R	N/R	N/R	N/R	N/R	N/R
Akbulut, 2014 (17)	Turkey	Retrospective	150	64,7%	Children	Mean 14.97 SD 2.92	60,7%	N/R	F0-3	0,0%	0,0%	N/R	N/R
Bonacci, 2018 (18)	Spain	Prospective	287	N/R	Adults	N/R	0,0%	N/R	F0-2	0,0%	0,0%	0,0%	0,0%
Brouwer, 2016 (19)	Multinational	Prospective	292	55,5%	Adults	Mean 43.2 SD 13.3	0,0%	A 16%; B 14%; C 11%; D 37%; Other 5%	Unclear distribution	0,0%	0,0%	0,0%	N/R
Chang, 2017 (20)	Korea	Retrospective	484	50%	Adults	N/R	100,00%	C 484	F0-3	0,00%	0,00%	Not reported	Not reported
Chen, 2012 (21)	Taiwan	Retrospective	62	N/R	Both	Range 17–57	0,0%	B 47; C 15	N/R	0,0%	0,0%	0,0%	N/R
Chen, 2006 (22)	Taiwan	Prospective	3653	61,9%	Adults	N/R	15,5%	N/R	N/R	0,0%	N/R	N/R	N/R
Chen, 2010 (23)	Taiwan	Prospective	1932	58%	Adults	Mean 47.4, SD 10	0,00%	N/R	N/R	0,00%	Not reported	Not reported	0.00%
Cho, 2014 (24)	Korea	Retrospective	1014	60,0%	Adults	Mean 51.7 SD10.2	0,0%	N/R	N/R	0,0%	NR	0,0%	N/R
Choi, 2019 (25)	Korea	Retrospective	5414	N/R	Adults	N/R	0,0%	N/R	N/R	0,0%	N/R	0,0%	N/R
Farzi, 2014 (26)	Iran	Prospective	399	74,4%	Adults	N/R	0,0%	N/R	N/R	0,0%	0,0%	0,0%	0,0%
Hsu, 2021 (27)	Taiwan	Prospective	81	80,2%	Adults	Median 43, (IQR 37-51)	25.93%	N/R	F0-3	0.00%	0.00%	N/R	N/R
Hsu, 2013 (28)	Taiwan	Prospective	95	63%	Children	Mean 5.8 (Range 0.5–18.4)	42,11%	B 62; C 25	N/R	0,00%	0,00%	0,00%	Not reported
Huang, 2021 (29)	Multinational	Prospective	3366	68,7%	Adults	Mean 45.60 SD 10.83	15,2%	N/R	N/R	0,0%	0,0%	0,0%	N/R
Iloeje, 2007 (30)	Taiwan	Prospective	3931	59,3%	Adults	N/R	14,4%	N/R	N/R	0,0%	N/R	N/R	11,9%
Jeon, 2021 (31)	Korea	Retrospective	125	61,6%	Adults	Median 38.6 (IQR 28.1–50.7)	100,0%	N/R	Unclear distribution	0.00%	N/R	N/R	0.00%
Johannessen, unpublished	Ethiopia	Prospective	1303	59,1%	Adults	Median 31 (range 18-72)	12,1%	N/R	F0-3	2,6%	0,9%	0.00%	3,5%
Kim, 2018 (32)	Korea	Retrospective	4535	N/R	Adults	N/R	100,0%	N/R	N/R	0,0%	0,0%	0,0%	N/R
Kim, 2020 (33)	Korea	Retrospective	6949	55,9%	Adults	Mean 45 SD12	29,9%	N/R	N/R	0,0%	0,0%	0,0%	N/R
Koc, 2022 (34)	Belgium & Netherlands	Retrospective	404	N/R	Adults	N/R	0,0%	N/R	N/R	0,0%	0,0%	0,0%	0,0%
Kumada, 2022 (35)	Japan	Retrospective	526	51,9%	Adults	Median 53 (IQR 43–64)	0,0%	A 20; B 60; C 296; D 3	F0-3	0.00%	0.00%	0.00%	26,0%
Kusakabe, 2011 (36)	Japan	Prospective	479	45,9%	Adults	N/R	3,5%	N/R	N/R	0.00%	N/R	N/R	0.00%
Lee, 2020 (37)	Korea	Prospective	747	54,5%	Adults	Mean 56.4 SD 11.8	16,9%	N/R	F0-3	0,0%	0,0%	0,0%	

Lee, 2020 (38)	Korea	Retrospective	946	45,3%	Adults	Median 36.8 (IQR 27.6-45.7)	100,0%	N/R	F0-1	0,0%	0,0%	0,0%	0,0%
Lee, 2019 (39)	Korea	Retrospective	773	N/R	Adults	N/R	N/R	N/R	N/R	0,0%	0,0%	0,0%	N/R
Liu, 2016 (40)	Taiwan	Prospective	1529	N/R	Adults	N/R	0,0%	B 775; B 337	N/R	0,0%	N/R	N/R	N/R
Liu, 2021 (12)	Multiple	Retrospective	8526	N/R	Adults	Mean 44.01 SD10.85	15,4%	N/R	Unclear distribution	0,0%	0,0%	0,0%	N/R
Lok, 2021 (41)	USA & Canada	Prospective	1418	48,5%	Adults	Median 41.1 years	16,2%	A 219; B 523; C 434; D 87; E 33	F0-3	0,0%	0,0%	0,0%	7,1%
Nakazawa, 2011 (42)	Japan	Prospective	104	54,8%	Adults	Median 49 SD 11 range (22–74)	0,0%	A 4; B 24; C 74; Other 2	N/R	0,0%	N/R	N/R	0,0%
Ndow, Unpuplished	The Gambia	Prospective	857	61,8%	Adults	Median 35 (IQR 31-43)	100,0%	A 40; E 267	F0-3	0,9%	0,9%	2,8%	6,4%
Ormeci, 2016 (43)	Turkey	Prospective	120	48,3%	Both	Mean 42.8 SD 11.32	0,0%	N/R	N/R	0,0%	0,0%	0,0%	0,0%
Raptopoulou-Gigi, 2002 (44)	Greece	Retrospective	307	62,9%	Adults	Mean 45.65 SD 11.37	0,0%	N/R	N/R	0,0%	N/R	0,0%	N/R
Sali, 2016 (45)	Iran	Prospective	420	62,1%	Adults	N/R	0,0%	N/R	N/R	N/R	N/R	N/R	N/R
Seong, 2022 (46)	Korea	Retrospective	651	62,1%	Adults	Median 36 (28-45)	100,0%	N/R	F0-3	0,0%	N/R	0,0%	N/R
Shimakawa, 2016 (47)	The Gambia	Prospective	405	50,4%	Both	Median 10.8 (IQR 4.6-21.8)	44,8%	A 5; E 97	N/R	N/R	N/R	N/R	N/R
Sinn, 2019 (48)	Korea	Retrospective	3624	N/R	Adults	Mean 48.0 SD11.9	25,7%	N/R	N/R	0,0%	NR	0,0%	N/R
Suzuki, 2021 (49)	Japan	Retrospective	462	57,1%	Adults	N/R	N/R	N/R	Unclear distribution	0,0%	N/R	0,0%	N/R
Tseng, 2012 (50)	Taiwan	Retrospective	390	67,7%	Adults	N/R	0,0%	N/R	N/R	0,0%	0,0%	0,0%	N/R
Tseng, 2012 (51)	Taiwan	Retrospective	2668	61,2%	Adults	N/R	19,5%	B 80.7%; C 19.3%	N/R	0,0%	0,0%	0,0%	N/R
Tseng, 2022 (52)	Taiwan & Japan	Retrospective	2150	61,0%	Adults	Mean 42,4 SD 10,1	0,0%	B 1700; C 331	N/R	0,0%	0,0%	0,0%	N/R
Tohme, 2013 (53)	USA	Prospective	414	43,2%	Adults	N/R	0,0%	A 57; B 9; C 23; D 209; F 71	N/R	0,0%	N/R	0,0%	N/R
Tong, 2013 (54)	USA	Prospective	146	46,6%	Adults	Mean 40.6 SD 12.3	100,0%	A 5; B 43; C 30; D 3; H 1; B+C 1	N/R	0,0%	N/R	0,0%	N/R
Tseng, 2013 (55)	Taiwan	Retrospective	2165	N/R	Adults	N/R	0,0%	B 87%; C 13%	N/R	0,0%	0,0%	0,0%	0,0%
Tseng, 2021 (56)	Taiwan	Retrospective	1673	56,2%	Adults	N/R	0,0%	B 1324; C 248; Other 101	N/R	0,0%	0,0%	0,0%	N/R
Yasunaka, 2016 (57)	Japan	Prospective	657	50,4%	Both	Median 49, (range11-88)	26,1%	B 20; C 178	N/R	0,0%	N/R	0,0%	N/R

3.2 Risk of bias

26 studies were rated poor-quality, four fair-quality and 15 as good-quality. Regarding the representativeness of the study participants, the majority of the reports (n=40, 88.9%) selected truly representative exposed cohorts or somewhat representative cohorts that identified participants to carry HBsAg as clinically indicated for suspected liver disease or known to carry HBsAg through general population testing or clinically indicated and have been followed by specialist services. All the included studies clearly defined how participants were categorized based on HBV DNA levels and ALT levels. Additionally, most of the reports (n=40, 97.8%) verified that the outcome of interest was not present at the start of the study. With respect to the outcome score, the majority of the included studies (n=28, 62.2%) did not described how the assessment of the outcome was performed. Yet, the follow-up was ≥ 5 years in 80.0% of the included studies. Finally, most of the assessed reports lacked a clear statement for the adequacy of follow up (n=38, 84.4%). Table 5 reports the quality assessment for the included references.

Table 5. Risk of bias assessment according to the New Castle Ottawa scale.

Author & Year	1)Representativeness of the exposed cohort (number of stars)	2) Ascertainment of exposure (number of stars)	4) Demonstration that outcome of interest was not present at start of study	Selection score	1) Assessment of outcome	2) Was follow-up long enough for outcomes to occur	3) Adequacy of followup of cohorts	Outcome score	Interpretation
Ahn 2014 (16)	★	★	★	3	★			1	Poor quality
Akbulut 2014 (17)		★	★	2	★	★		2	Fair quality
Bonacci 2018 (18)	★	★	★	3		★		1	Poor quality
Brouwer 2016 (19)	★	★	★	3		★	★	2	Good quality
Chang 2017 (20)	★	★	★	3				0	Poor quality
Chen 2012 (21)		★	★	2		★		1	Poor quality
Chen 2006 (22)	★	★	★	3	★	★		2	Good quality
Chien 2016 (24)	★	★	★	3		★	★	2	Good quality
Cho 2014 (24)	★	★	★	3				0	Poor quality
Choi 2019 (25)	★	★	★	3	★	★		2	Good quality
Farzi 2014 (26)	★	★	★	3		★		1	Poor quality
Hsu 2013 (28)	★	★	★			★	★	2	Good quality
Hsu 2021 (27)		★	★	2	★		★	2	Fair quality

Huang 2021 (29)	★	★	★	3		★		1	Poor quality
Iloeje 2007 (30)	★	★	★	3	★	★		2	Good quality
Jeon 2021(31)		★	★	2		★		1	Poor quality
Johannessen Unpublished	★	★	★	3		★	★	2	Good quality
Kim 2018 (32)	★	★		2	★	★		2	Fair quality
Kim 2020 (33)	★	★	★	3	★	★		2	Good quality
Koc 2022 (34)		★	★	2		★		1	Poor quality
Kumada 2022 (35)	★	★	★	3		★		1	Poor quality
Kusakabe 2011 (36)	★		★	2	★	★		2	Fair quality
Lee 2019 (39)	★	★	★	3		★		1	Poor quality
Lee 2020 (37)	★	★	★	3		★		1	Poor quality
Lee 2020 (38)	★	★	★	3		★		1	Poor quality
Liu 2016 (40)	★	★	★	3	★	★		2	Good quality
Liu 2021 (12)	★	★	★	3		★		1	Poor quality
Lok 2021 (41)	★	★	★	3		★		1	Poor quality
Nakazawa 2011 (42)	★	★	★	3	★	★		2	Good quality
Ndow Unpublished	★	★	★	3	★	★	★	3	Good quality
Ormeci 2016 (43)	★	★	★	3				0	Poor quality
Raptopoulou-Gigi 2002 (44)	★	★	★	3		★		1	Poor quality
Sali 2016 (45)	★	★		2				0	Poor quality
Seong 2022 (46)	★	★	★	3	★	★		2	Good quality
Shimakawa 2016 (47)	★	★	★	3	★	★	★	3	Good quality
Sinn 2019 (48)	★	★	★	3	★			1	Poor quality
Suzuki 2021 (49)	★	★	★	3				0	Poor quality
Tohme 2013 (53)	★	★		2		★		1	Poor quality
Tong 2013 (54)	★	★	★	3		★		1	Poor quality
Tseng 2012 (50)	★	★	★	3		★		1	Poor quality
Tseng 2012 (51)	★	★	★	3		★		1	Poor quality
Tseng 2013 (55)	★	★	★	3		★		1	Poor quality
Tseng 2021 (56)	★	★	★	3	★	★		2	Good quality
Tseng 2022 (52)	★	★	★	3	★	★		2	Good quality
Yasunaka 2016 (57)	★	★		2				0	Poor quality

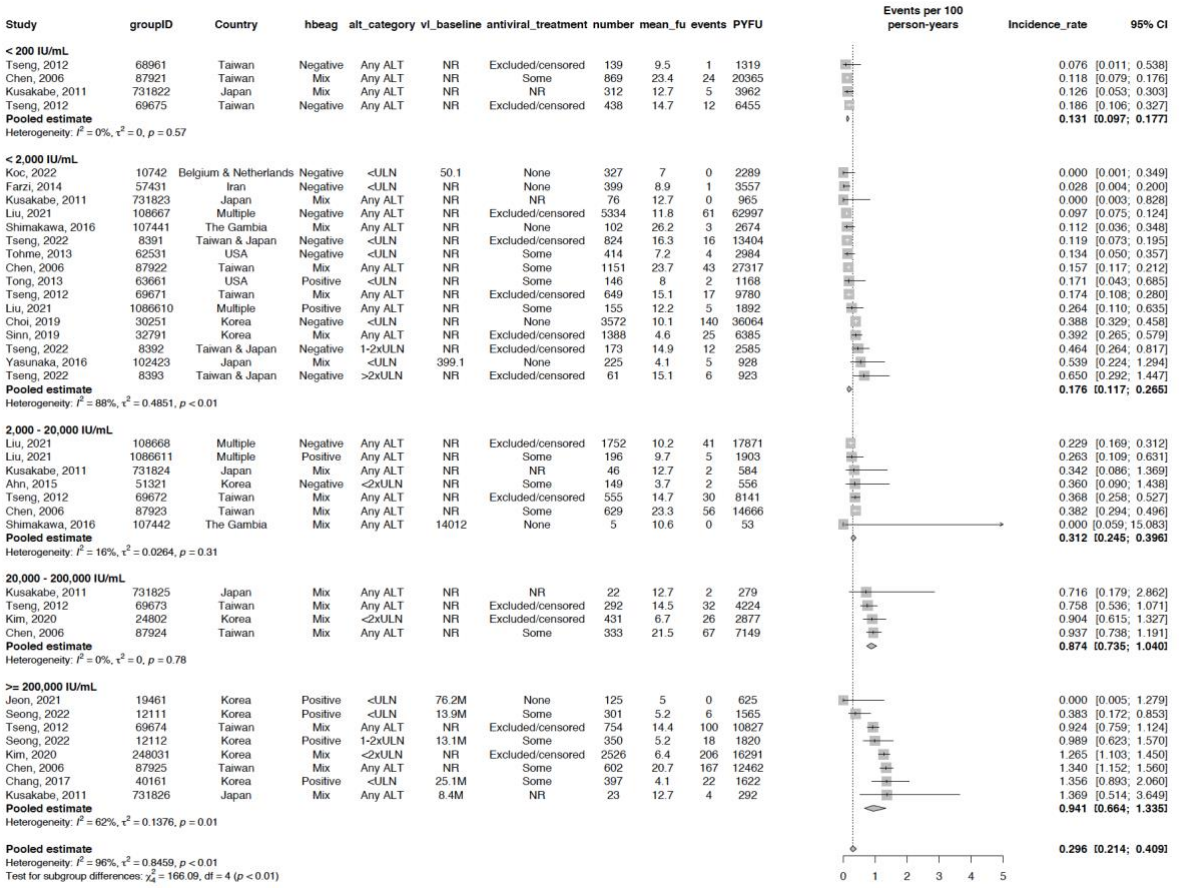
3.3 Meta-analysis

3.3.1 HCC in adults with a single HBV DNA assessment

22 studies provided a total of 39 distinct within-study groups, 4, 16, 7, 4, 8 for the viral load strata of <200 IU/mL, <2,000 IU/mL, 20,000-200,000 IU/mL, and ≥200,000 IU/mL, respectively. There was a dose-response relationship between HBV DNA levels at baseline and the incidence rates of HCC (per 100 person-years): 0.131 (95% CI: 0.097-0.177, I^2 = 0%) in the <200 IU/mL stratum; 0.176 (0.117-0.265, I^2 = 88%) in the <2,000 stratum; 0.312 (0.245-0.396, I^2 = 16%) in the 2,000-20,000 stratum; 0.874 (0.735-1.040, I^2 = 0%) in the 20,000-200,000 stratum; and 0.941 (0.664-1.335, I^2 = 62%) in the ≥200,000 stratum (p for heterogeneity between subgroups < 0.01) (Figure 1).

The pooled incidence rates of the first three strata (<200, <2,000, and 2,000-20,000 IU/mL) were all relatively low, at less than 0.35 per 100 person-years. The 95% confidence intervals for these rates overlapped. The mean duration of follow-up was higher than 5 years for most of the studies included in all strata (<200, <2,000, 2,000-20,000, 20,000-200,000, and >200,000 IU/mL) (Figure 1).

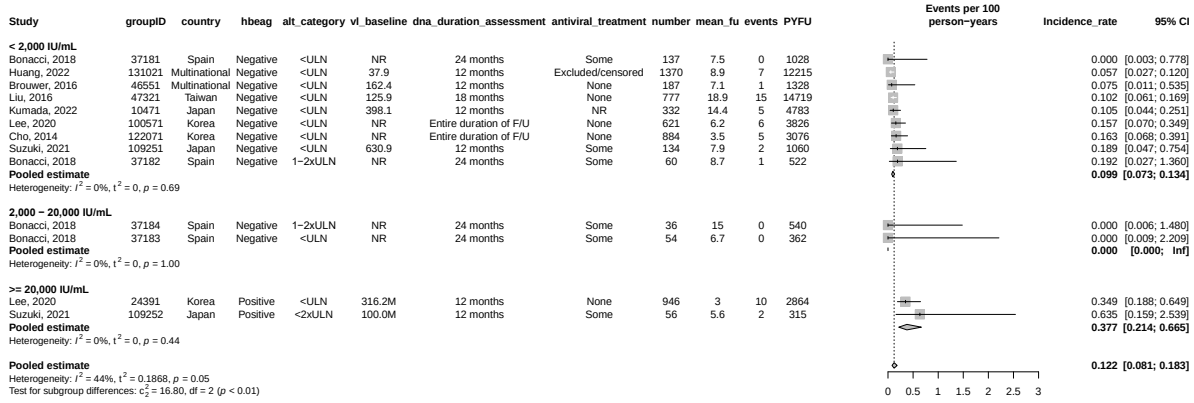
Figure 1. Forest plot of pooled HCC incidence rate in adults, according to a single HBV DNA assessment



3.3.2 HCC in adults with multiple HBV DNA assessment

8 studies provided 9, 2 and 2 distinct within-study groups for the viral load strata of <2,000 IU/mL, 2,000-20,000 IU/mL, > 20,000 IU/mL, respectively. The pooled incidence rate of HCC (per 100 person-years) was very low in persistently below 2,000 IU/mL stratum (0.099, 95% CI: 0.073-0.134, I2 = 0%) (Figure 2). Additionally, there were only two within-study groups for the viral load stratum between 2,000 and 20,000 IU/mL, there were no events reported, yet the number of individuals in this stratum was relatively low.

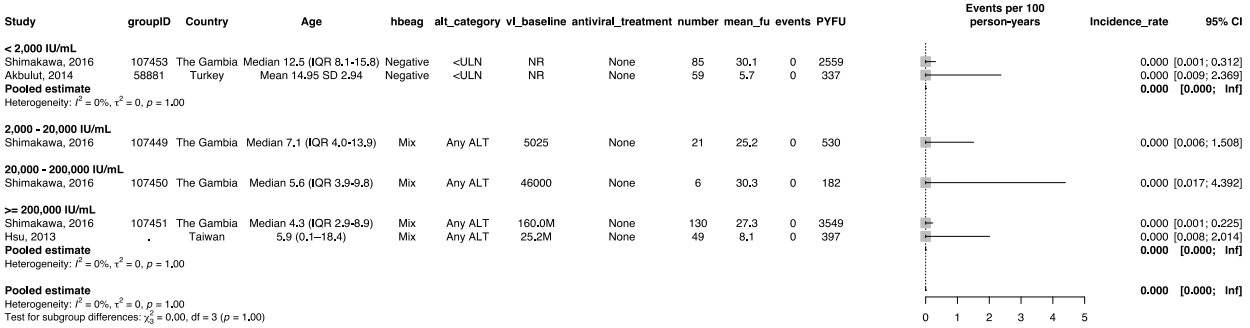
Figure 2. Forest plot of pooled HCC incidence rate in adults, according to multiple HBV DNA assessments



3.3.3 HCC in children with a single HBV DNA assessment

There were six groups from three studies reporting on HCC in children. There were no cases of HCC in any of these groups. The baseline median age was less than 10 years in four groups (4.3, 5.9, 5.8, 7.1 years) and >12 years in two groups (12.5 and 14.9 years) (Figure 3). The mean duration of follow-up was longer than 5 years in most of the studies and only 2 studies had a follow up shorter than 5 years. We did not identify any study reporting multiple VL assessments in children.

Figure 3. Forest plot of pooled HCC incidence rate in children, according to a single HBV DNA assessment

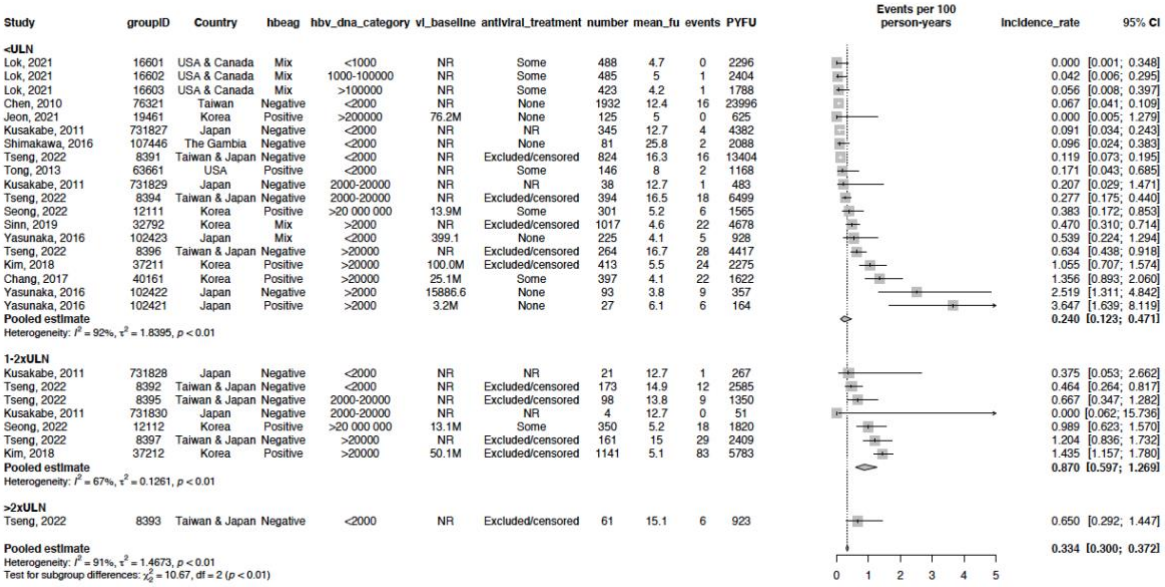


3.3.4 HCC in adults with a single ALT assessment

14 studies provided 27, distinct within-study groups for the ALT strata of <ULN, 1-2ULN and, >2ULN. The pooled incidence rate of HCC (per 100 person-years) in the < ULN ALT stratum was 0.240 (95% CI: 0.123-0.471), but there was significant heterogeneity across the studies within this stratum (I^2 = 92%). We also identified a wide variation in HBV DNA levels at baseline. There were four within-study groups

that reported an incidence rate of more than 1.0 per 100 person-years; all of these studies recruited participants with HBV DNA levels of >2,000 or >20,000 IU/mL. The pooled incidence rate in the strata with 1-2x ULN was statistically significantly higher than the normal ALT stratum: 0.240 (95% CI: 0.123-0.471, I^2 = 92%) in <1x ULN, 0.870 (0. 597-1.269, I^2 = 67%) in 1-2x ULN. However, we identified only 1 reference for the in >2x ULN stratum. This study reported an incidence rate of 0.650 (0.292-1.447) (Figure 4).

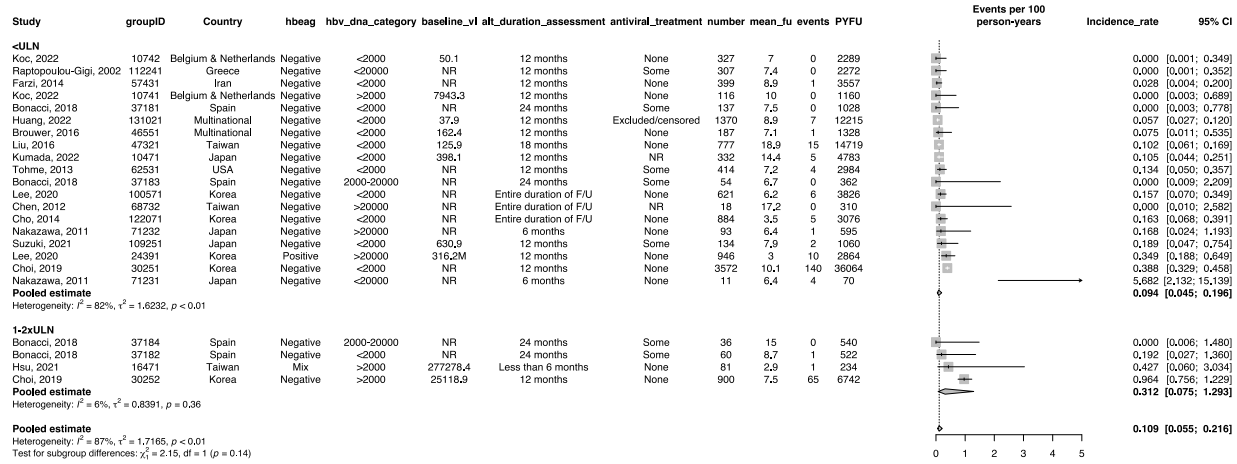
Figure 4. Forest plot of pooled HCC incidence rate in adults, according to a single ALT assessment



3.3.5 HCC in adults with multiple ALT assessments

In individuals with persistently normal ALT levels, the incidence rates of HCC were consistently low in all included within-study groups, at less than 0.4 per 100 person-years, with the exception of one stud (42). This study was led by Nakazawa in 2011 and had only 11 participants in this ALT category, 4 individuals developed HCC after a mean follow-up duration of 6.4 years (42). The overall pooled incidence rate in the groups of individuals with persistently normal ALT levels was low: 0.094 (95% CI: 0.045-0.196, I^2 = 82%). This rate was slightly higher in a group with persistently elevated ALT between 1-2x ULN (0.312, 0.075-1.293, I^2 = 6%) (Figure 5).

Figure 5. Forest plot of pooled HCC incidence rate in adults, according to multiple ALT assessment



3.3.6 HCC in children with single ALT assessment

We identified only two references reporting results on children with normal ALT levels (<ULN). There was no case of HCC in any of these groups. The median duration of follow-up was more than 5 years in both studies (Table 6).

Table 6. Studies reporting HCC in children, according to a single ALT assessment.

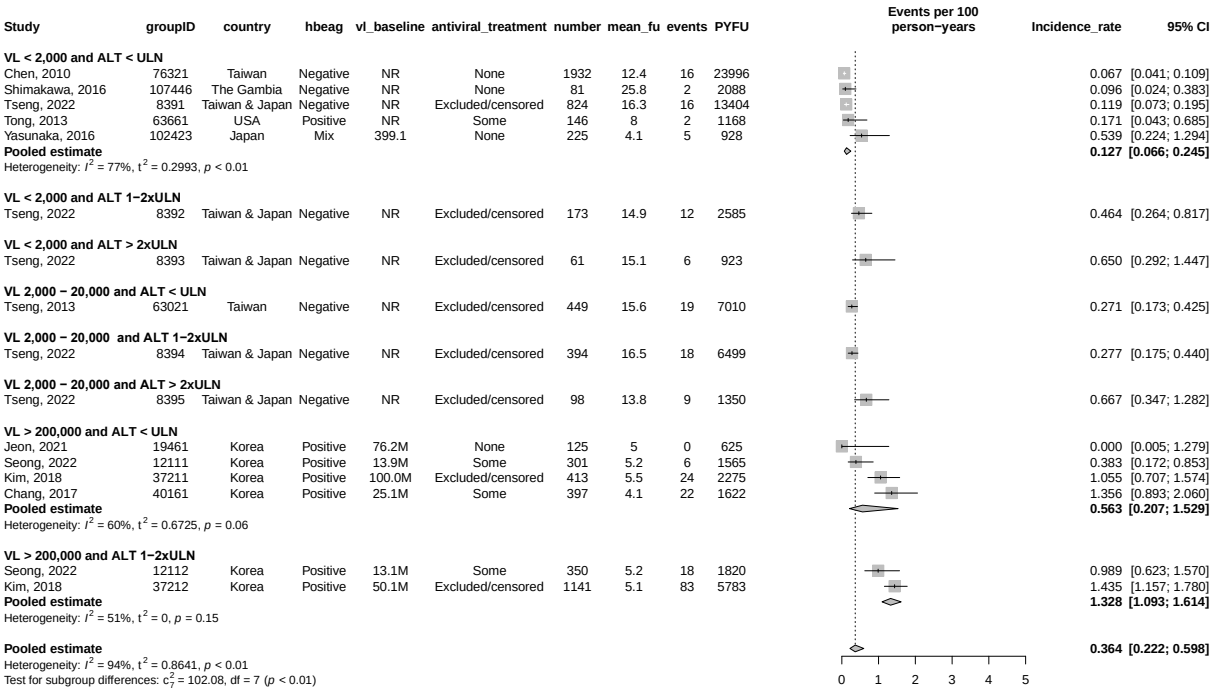
Study	Country	Mean age (±SD) or median (range or IQR) in years	HBeAg	VL category	Antiviral treatment	Number of participants	Mean or median of FU	Events	PYFU	Incidence rate (100 person-years)
< ULN										
Akbulut, 2014 (17)	Turkey	Mean 14.9 SD 2.9	Negative	<2000 IU/mL	None	59	5.7	0	337	0.000 (95% CI: 0.009-2.369)
Shimakawa, 2016 (47)	Gambia	Median 12.5 (IQR 8.1-15.8)	Negative	<2000 IU/mL	None	85	30.1	0	2559	0.000 (95% CI: 0.001-0.312)

3.3.7 HCC in adults with a single HBV DNA and ALT assessment

There was only one study with available data in most of the viral load and ALT strata, especially for the strata of VL 2,000-20,000 IU/mL stratified also by ALT levels. In the strata with VL <2,000 IU/mL, there was a dose-response relationship between the baseline ALT levels and the incidence rates of HCC per 100 person-years: 0.127 (95% CI: 0.066-0.245, I^2 = 77%) in the normal ALT stratum, 0.464 (0.264-0.817, only one study) in the 1-2x ULN stratum, and 0.650 (0.292-1.447, only one study) in the >2x ULN stratum.

In the strata with VL 2,000-20,000 IU/mL, there was only one study for each ALT stratum. The incidence rates of HCC per 100 person-years were 0.271 (95% CI: 0.175-0.440) in the normal ALT stratum, 0.277 (0.175-0.440) in the 1-2x ULN stratum, and 0.667 (0.347-1.282) in the >2x ULN stratum (Figure 6).

Figure 6. Forest plot of pooled HCC incidence rate in adults characterized according to a single HBV DNA and ALT assessment.

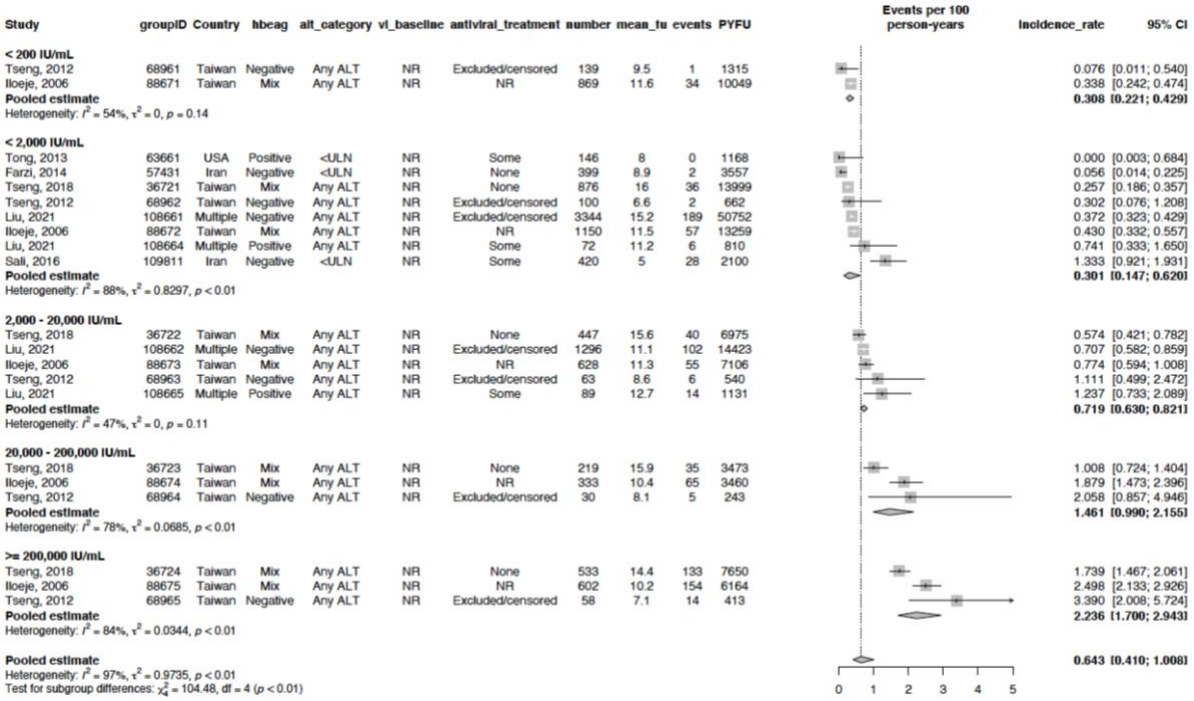


3.3.8 Cirrhosis in adults with a single HBV DNA assessment

7 studies provided 2, 8, 5, 3 and 3 distinct within-study groups for the viral load strata of <200, <2,000, 2,000-20,000, 20,000-200,000, and >200,000 IU/mL, respectively. The pooled incidence rates of cirrhosis (per 100 person-years) were similar between the <200 IU/mL stratum (0.308, 95% CI: 0.221-0.429, I^2 = 54%) and <2,000 IU/mL stratum (0.301, 0.147-0.620, I^2 = 88%) with overlapping 95% CIs. Then, there was a dose-response relationship between HBV DNA levels at baseline and the incidence rates of cirrhosis: 0.301 (95% CI: 0.147-0.620, I^2 = 88%), 0.719 (0.630-0.821, I^2 = 47%), 1.461 (0.990-2.155, I^2 = 78%), and 2.236 (1.700-2.943, I^2 = 84%), in the strata of <2,000, 2,000-20,000, 20,000-200,000, and ≥200,000 IU/mL, respectively (p for test for subgroup differences <0.01) (Figure 7). Additionally, the lower boundary of the 95% CI of the pooled incidence rate in the stratum of 2,000-20,000 IU/mL was higher than the upper boundary of those estimated in the strata of <200 IU/mL or <2,000 IU/mL. The mean duration of follow-up was larger than 5 years in all

included studies in the strata of <200, <2,000, 2,000-20,000, 20,000-200,000, and >200,000 IU/mL.

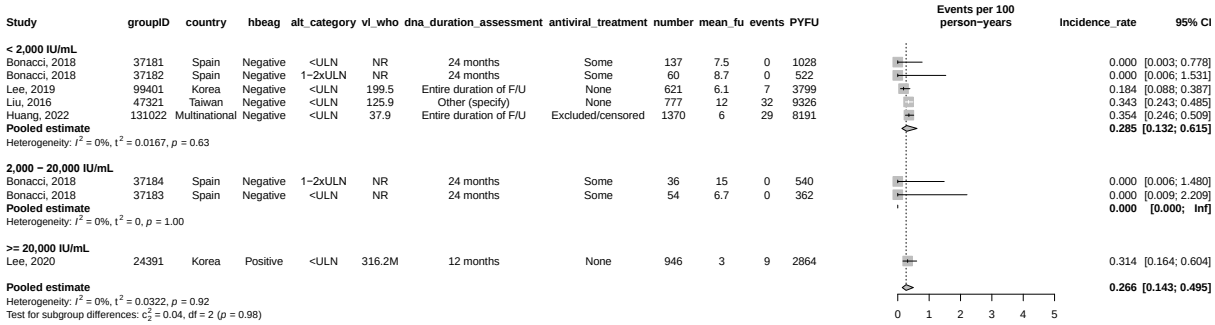
Figure 7. Forest plot of pooled cirrhosis incidence rates in adults, according to a single HBV DNA assessment



3.3.9 Cirrhosis in adults with multiple HBV DNA assessments

5 studies provided 5, 2, 1 distinct within-study groups for the viral load strata of <2,000, 2,000-20,000, and >200,000 IU/mL, respectively. The pooled incidence rate of cirrhosis (per 100 person-years) was low in persistently below 2,000 IU/mL stratum (0.285, 95% CI: 0.132-0.615, I^2 = 0%). There were only two within-study groups for the viral load stratum between 2,000 and 20,000 IU/mL, and there were no events. The mean duration of follow-up lasted more than 5 years in all the groups identified in the strata of <2,000 and 2,000-20,000 IU/mL (Figure 8).

Figure 8. Forest plot of pooled cirrhosis incidence rates in adults, according to multiple HBV DNA assessment



3.3.10 Cirrhosis in children with single HBV DNA assessment

There were only two studies assessing cirrhosis in children. There was no case of cirrhosis in these studies. Mean age was 5.9 years and 14.9 years, respectively, and mean follow-up was 8.0 years and 5.7 years, respectively (Table 7). We did not identify any study reporting cirrhosis as outcome and assessing VL multiple times in children.

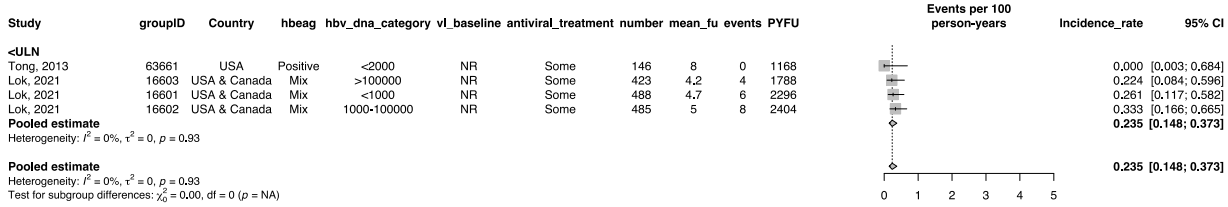
Table 7. Studies reporting cirrhosis in children, according to a single HBV DNA assessment.

Study	Country	Mean age (±SD) or median (range or IQR) in years	HBeAg	VL category	Antiviral treatment	Number of participants	Mean or median of FU	Events	PYFU	Incidence rate (100 person-years)
< ULN										
Akbulut, 2014 (17)	Turkey	Mean 14.9 SD 2.9	Negative	<2000 IU/mL	None	59	5.7	0	337	0.000 (95% CI: 0.009-2.369)
Hsu, 2013	Taiwan	Mean 5.9 (0.1-18.4)	Mix	>=200000 IU/mL	None	49	8.1	0	397	0.000 (95% CI: 0.008-2.014)

3.3.11 Cirrhosis in adults with single ALT assessment

There were only four within-study groups from two studies with normal ALT. The pooled incidence rate of cirrhosis (per 100 person-years) was 0.235 (95% CI: 0.148-0.373, I^2 = 0%) in individuals with normal ALT levels at baseline (Figure 9). Both included studies were conducted in North America.

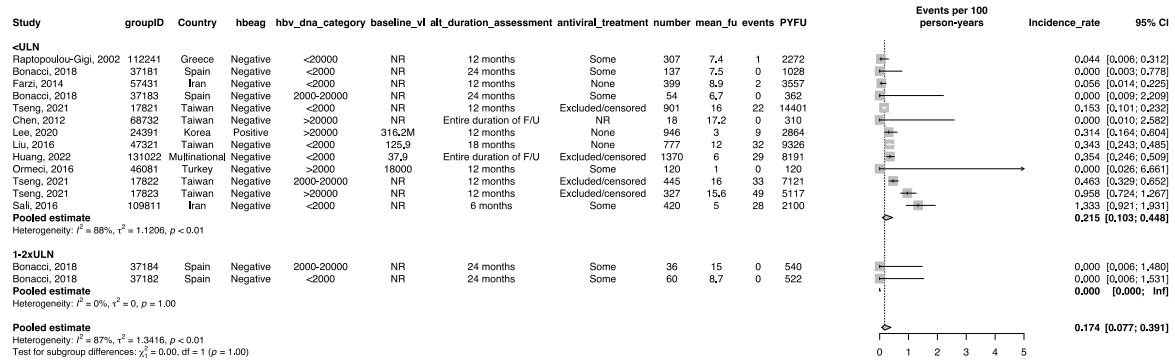
Figure 9. Forest plot of pooled cirrhosis incidence rate in adults, according to a single ALT assessment.



3.3.12 Cirrhosis in adults with multiple ALT assessments

In the group of individuals with persistently normal ALT levels, the pooled incidence rate of cirrhosis (per 100 person-years) was 0.215 (95% CI: 0.103-0.448, I^2 = 88%) (Figure 10). There were only two within-study groups from one study in the stratum with persistently abnormal ALT levels (1-2x ULN).

Figure 10. Forest plot of pooled cirrhosis incidence rate in adults, according to multiple ALT assessment.



3.3.13 Cirrhosis in children with a single ALT assessment

There was only one group in children having normal ALT levels. There were no cases of cirrhosis in this group. We did not identify any study reporting cirrhosis as outcome and assessing VL multiple times in children (Table 8).

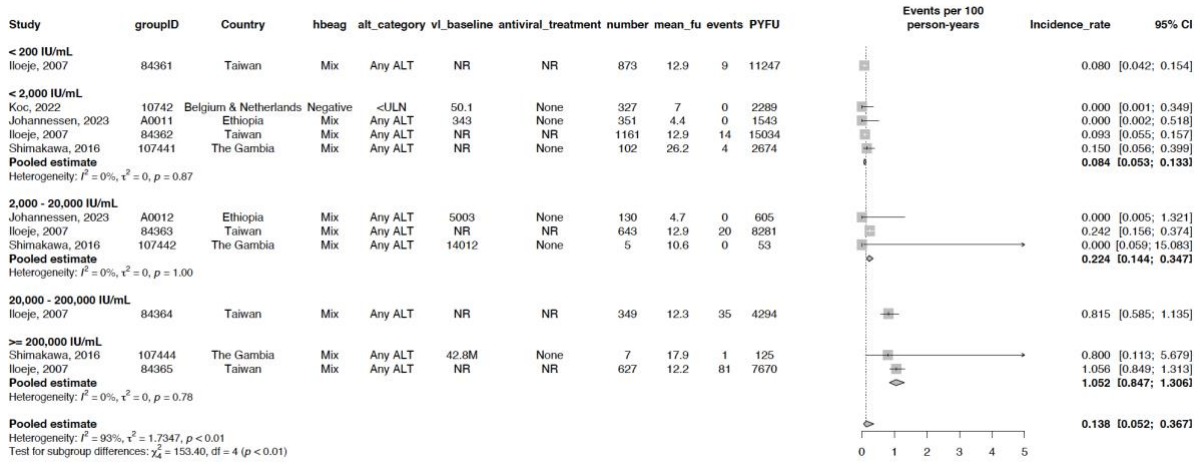
Table 8. Studies reporting cirrhosis in children, according to a single ALT assessment.

Study	Country	Mean age (±SD) or median (range or IQR) in years	HBeAg	VL category	Antiviral treatment	Number of participants	Mean or median of FU	Events	PYFU	Incidence rate (100 person-years)
< ULN										
Akbulut, 2014 (17)	Turkey	Mean 14.95 SD 2.94	Negative	<2000 IU/mL	None	59	5.7	0	337	0.000 (95% CI : 0.009-2.369)

3.3.14 Liver related deaths in adults with single HBV DNA assessment

Studies provided 1, 4, 3, 1 ,2 distinct within-study groups for the viral load strata of <200, <2,000, 2,000-20,000, 20,000-200,000, and ≥200,000 IU/mL. The pooled liver-related mortality rates (per 100 person-years) were similarly very low in the <200 IU/mL stratum (0.080, 95% CI: 0.042-0.154, only one study) and the <2,000 IU/mL stratum (0.084, 0.053-0.133, I^2 = 0%). But, there was a dose-response relationship between HBV DNA levels at baseline and the liver-related mortality rates: 0.084 (95% CI: 0.053-0.133, I^2 = 0%), 0.224 (0.144-0.347, I^2 = 0%), 0.815 (0.585-1.135, only one study), and 1.056 (0.849-1.313, only one study), in the strata of <2,000, 2,000-20,000, 20,000-200,000, and ≥200,000 IU/mL, respectively, although the number of studies in high viral load strata was limited.

Figure 11. Forest plot of pooled liver-related deaths rate in adults, according to single HBV DNA assessment.



3.3.15 Liver related deaths in adults with multiple HBV DNA assessment

There were only two studies (both in the viral load stratum of persistently below 2,000 IU/mL). The liver-related mortality rate (per 100 person-years) was very low in both studies and all participants had ALT values bellow the ULN at baseline evaluation. Additionally, the mean duration of follow-up was more than 5 years in both references (Table 9).

Table 9. Studies reporting liver related deaths in adults, according to multiple HBV DNA assessments.

Study	Country	HBeAg	ALT category	Antiviral treatment	Number of participants	Mean FU	Events	PYFU	Incidence rate (100 person-years)
< 2000 IU/mL									
Kumada, 2022	Japan	Negative	<ULN	None	332	14.5	3	4820	0.062 (95% CI : 0.020-0.193)

Brouwer, 2016	Multinational	Negative	<ULN	Not reported	187	7.1	1	1328	0.075 (95% CI : 0.011-0.535)

3.3.16 Liver related deaths in children with a single HBV DNA assessment

There was one single study reporting on liver related deaths according to VL group. There were no cases of liver-related deaths (Table 10). There was no study reporting on liver related deaths in children according to multiple VL assessments.

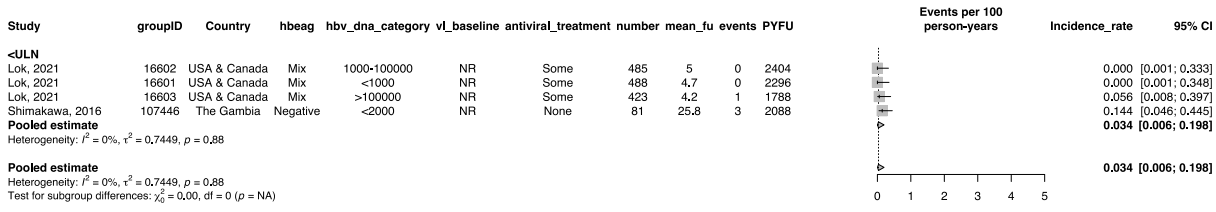
Table 10. Studies reporting liver related deaths in children with a single HBV DNA assessment.

Study	Country	HBeAg	ALT category	Antiviral treatment	Number of participants	Mean FU	Events	PYFU	Incidence rate (100 person-years)
< 2000 IU/mL									
Shimakawa, 2016 (47)	Gambia	Negative	<ULN	None	85	30.1	0	2559	0.000 (95% CI : 0.001-0.312)
2000-20000 IU/mL									
Shimakawa, 2016 (47)	Gambia	Mix	Any ULN	None	21	25.2	0	530	0.000 (95% CI : 0.006-1.508)
20000-200000 IU/mL									
Shimakawa, 2016 (47)	Gambia	Mix	Any ULN	None	6	30.3	0	182	0.000 (95% CI : 0.017-4.392)
>=200000 IU/mL									
Shimakawa, 2016 (47)	Gambia	Mix	Any ULN	None	130	27.3	0	3549	0.000 (95% CI : 0.001-0.225)

3.3.17 Liver related deaths in adults with a single ALT assessment

There were only four within-study groups from two studies in a stratum of normal ALT levels at baseline. The pooled liver-related mortality rate (per 100 person-years) was very low (0.034, 0.006-0.198, I^2 = 0%). The mean duration of follow-up was longer than 4 years in all studies (Figure 12).

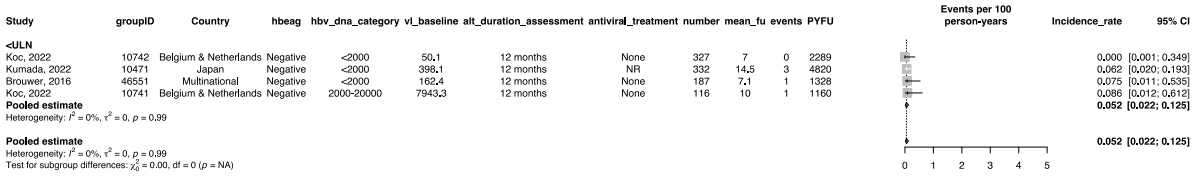
Figure 12. Forest plot for liver-related deaths in adults with a single ALT assessment



3.3.18 Liver deaths in adults with multiple ALT assessments

There were four studies in a stratum of persistently normal ALT levels. The pooled liver-related mortality rate (per 100 person-years) was very low (0.054, 0.022-0.125, I^2 = 0%). The median (range) duration of follow-up was 8.55 (7-14.5) years.

Figure 13. Forest plot for liver-related deaths in adults with multiple ALT assessments



3.3.19 Liver deaths in children with single ALT assessments

There was only one study in a stratum of normal ALT (<ULN) levels at baseline. There was no case of liver-related death.

Table 11. Studies reporting liver related deaths in children with a single HBV DNA assessment.

Study	Country	Mean age (±SD) or median (range or IQR) in years	HBeAg	VL category	Antiviral treatment	Number of participants	Mean or median of FU	Events	PYFU	Incidence rate (100 person-years)
< ULN										
Shimakawa, 2016 (47)	The Gambia	Median 10.8 (IQR 5.4-14.9)	Negative	<2000 IU/mL	None	85	30.1	0	2559	0.000 (95% CI : 0.001-0.312)

4. Discussion

In this systematic review and meta-analysis, we identified a total of 13,124 articles through our electronic database search. After a thorough screening process, 45 studies were included in our meta-analysis. In addition, to address the lack of data on CHB in sub-Saharan Africa, the WHO reached out to the Hepatitis B in Africa collaborative Network (HEPSANET) to identify unpublished longitudinal data from the region. As a result, we integrated aggregated data from two cohort studies: the Ethiopian cohort (Johannessen A, Desalegn H et al.) and the Gambian cohort (Ndow G, Lemoine M et al.). Most of the included studies were from the Western Pacific region (64.4%, 29/45), followed by Europe (11.1%, 5/45). Most of the studies included only adults (86.7%, 39/45) and more than a half (64.4%, 29/45) were published after 2015.

For adults, a substantial number of estimates were available for different viral load strata, especially for important clinical outcomes, such as HCC and cirrhosis. Similarly to previous literature (58) that describes HBV DNA levels among the main risk factors for HCC, our results found higher HCC incidence rates for the groups with higher viral load levels. The pooled incidence rates of HCC in adults (per 100 person-years) were similarly low between the stratum with less than 200 IU/mL of HBV DNA (0.131, 95% CI: 0.097-0.177, I2 = 0%), the stratum with less than 2,000 IU/mL (0.176, 95% CI: 0.117-0.265, I2 = 88%), and the stratum with 2,000-20,000 IU/mL (0.312, 95% CI: 0.245-0.396, I^2 = 16%), with overlapping 95% CIs. However, for the viral load strata above 2,000 IU/ml, there was a clear dose-response relationship between HBV DNA levels at baseline and the incidence rates of HCC. The incidence rates of HCC were 0.312 (95% CI: 0.245-0.396, I^2 = 16%), 0.874 (0.735-1.040, I^2 = 0%), and 0.941 (0.664-1.335, I^2 = 62%), for strata with 2,000-20,000, 20,000-200,000, and ≥200,000 IU/mL, respectively ($p < 0.01$). The meta-analysis of multiple viral load assessments was more limited as there were fewer studies in the high viral load strata with repeated viral load measurements. However, in the group of individuals with persistently low viremia of <2,000 IU/mL, the pooled incidence rate of HCC was relatively low (0.099, 95% CI: 0.073-0.134, I^2 = 0%).

Importantly, in a group of individuals with persistently normal (<ULN) ALT levels (Figure 5), the incidence rates of HCC per 100 person-years were consistently low, with the exception of one outlier study with a small sample size (n=11). The pooled incidence rate in this stratum was 0.094 (95% CI: 0.045-0.196, I^2 = 82%).

Similarly, the pooled incidence rates of cirrhosis (per 100 person-years) were similar between the <200 IU/mL stratum (0.308, 95% CI: 0.221-0.429, I^2 = 54%) and <2,000 IU/mL stratum (0.301, 0.147-0.620, I^2 = 88%). But, there was a clear dose-response relationship between HBV DNA levels at baseline and the incidence rates of cirrhosis: 0.301 (95% CI: 0.147-0.620, I^2 = 88%), 0.719 (0.630-0.821, I^2 = 47%), 1.461 (0.990-2.155, I^2 = 78%), and 2.236 (1.739-2.236, I^2 = 84%), for strata with <2,000, 2,000-20,000, 20,000-200,000, and ≥200,000 IU/mL, respectively (p for test for subgroup differences <0.01). In individuals with persistently low viremia of <2,000 IU/mL we also found a relatively low incidence rate of cirrhosis (0.285, 95% CI: 0.132-0.615, I^2 = 0%).

Our results are consistent with the findings from the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer–Hepatitis B Virus (REVEAL-HBV) cohort, which reports increase in the incidence of both HCC and cirrhosis in proportion to the HBV DNA level, from

<60 IU/mL (undetectable) to ≥ 200,000 IU/mL (22). These associations remained statistically significant in this cohort even after adjustment for age, sex, smoking, alcohol drinking, and HBeAg status (22,59). Such a dose-response relationship was also observed for liver-related mortality rates: 0.084 (95% CI: 0.053-0.133, I^2 = 0%), 0.224 (0.144-0.347, I^2 = 0%), 0.815 (0.585-1.135, only one study), and 1.056 (0.849-1.313, only one study), in the stratum of <2,000, 2,000-20,000, 20,000-200,000, and ≥200,000 IU/mL, respectively.

Our findings are also consistent with a prior review of the literature (60) on the natural history of chronic HBV infection. That study reports high levels of HBV at enrolment and during follow-up as one of the best predictors for progression of clinical outcomes, including HCC and cirrhosis. However, this review limited its search to PubMed and only considered articles written in English up to June of 2007. A subsequent systematic review and meta-analysis was conducted by Raffetti *et al*. in 2016 (61), who also identified an increase in HCC incidence rates with respect to HBV DNA levels >2,000 IU /ml. Nevertheless, this study did not consider other important clinical outcomes and did not conduct further stratification based on viral load or ALT levels.

In adults, compared to the normal ALT level stratum, the pooled incidence rates of HCC (per 100 person-years) were slightly higher in the strata with ALT 1-2x ULN and ALT >2x ULN: 0.259 (95% CI: 0.125-0.537, I^2 = 92%), 0.741 (0.425-1.292, I^2 = 92%), and 0.660 (0.398-1.095, I^2 = 0%), respectively (p for test for subgroup difference = 0.06). But there was high heterogeneity across the studies within the normal ALT level stratum and the stratum with ALT 1-2x ULN (I^2 = 92% for both estimates). These heterogeneities may be partially due to the difference in HBV DNA levels at baseline. For example, in the normal ALT stratum, there were four within-study groups that reported an incidence rate of more than 1.0 per 100 person-years; all of these groups recruited participants with HBV DNA levels greater than 2,000 IU/mL.

For children, we included fewer studies, and we did not observe any clear association between viral loads at baseline and clinical outcomes. In the limited number of studies included, both HCC and cirrhosis were rare events in this population, and some included studies did not document any occurrence of these outcomes despite a long follow-up in one study from The Gambia. Since chronic HBV infection is highly dynamic, and the large majority of children who were infected at birth or shortly after have a prolonged period of HBeAg-positive chronic HBV infection, a one-time assessment of viral load or ALT at baseline may be less predictive for

their natural history compared to adults. This highlights the importance of follow-up with the paediatric population, to better identify those who might benefit from receiving treatment, and not determining eligibility for treatment based on one single measurement of HBV DNA levels.

This study has several limitations. First, we only included studies that provided specific viral load strata (<2,000, 2,000-20,000, 20,000-200,000, and ≥200,000 IU/mL). However, most of these studies were conducted after wide availability of antiviral therapy as the standard of care. Although historical studies conducted before the availability of antiviral therapy may provide valuable insights on the natural history of CHB, there was no sensitive HBV DNA quantification as available nowdays. Due to the methodological challenge that this encompass, we had to include contemporary studies that were conducted in individuals who that might receive antiviral therapy if they became eligible for treatment during follow up. Both including participants who subsequently started antiviral therapy or excluding them from the analysis may result in an underestimation of the true incidence rates of clinical outcomes. Although we did not conduct a formal sensitivity analysis, we did not observe a clear tendency that studies excluding treated participants or including them provided lower incidence rates of clinical outcomes than historical studies without antiviral therapy.

This study was also limited due to the limited number of studies, we could not adequately adjust for potential sources of heterogeneity, such as the baseline distribution of fibrosis stage or the presence of comorbidities including alcohol and non-alcoholic fatty liver disease. With regards to co-infection with HCV, HIV, or HDV, many of the studies excluded participants with these infections. Thirdly, the majority of included studies were from the Western Pacific Region, particularly from mainland China and Taiwan, with a limited number of studies from South East Asia, Africa and the Eastern Mediterranean Region. Finally, many of the paediatric studies included both children (≤12 years) and adolescents (>12 years) and we were not able to stratify further by these age groups.

Despite these limitations, this study stands as the most extensive systematic review and meta-analysis currently available on the natural history of chronic HBV infection that quantifies the incidence rate of adverse clinical outcomes (cirrhosis, HCC, and liver related mortality) according to the viral load and ALT levels. Furthermore, it contributes to a better understanding of the natural history of the disease. This is particularly valuable to inform on the groups of patients at higher risk of disease progression, who might benefit the most from access to treatment.

5. Conclusion

This study represents the most comprehensive systematic review and meta-analysis to date of chronic HBV infection natural history. Cirrhosis and HCC incidence rates were low in individuals with HBV DNA <2000 IU/ml. The incidence rate of HCC was also relatively low for adults with persistently normal (<ULN) ALT levels. Additionally, we identified paucity of data in pediatric and adolescents' populations and in individuals with undetectable viral load levels. Thus, more research is needed in this specific area to further characterize the natural history of HBV.

Appendixes

Appendix 1. Search strategy

PubMed

#	Searches 01.01.2000 - 06.02.2023	Results
1	"hepatitis b virus"[MeSH Terms] OR "hepatitis-b"[MeSH Terms] OR "hepatitis b surface antigens"[MeSH Terms]	78,291
2	"hepatitis b"[Title/Abstract] OR "type b hepatitis"[Title/Abstract] OR "hepatitis type b"[Title/Abstract] OR "hbv"[Title/Abstract] OR "vhb"[Title/Abstract] OR "hep b"[Title/Abstract] OR "hbsag"[Title/Abstract] OR "aghbs"[Title/Abstract] OR "hbs ag"[Title/Abstract] OR "hbs antigen*"[Title/Abstract]	99,564
3	#1 OR #2	112,460
4	"alanine transaminase"[MeSH Terms]	33,348
5	"alanine transaminase*"[Title/Abstract] OR "alanine aminotransferase*"[Title/Abstract] OR "glutamic pyruvic transaminase*"[Title/Abstract] OR "glutamic pyruvate transaminase*"[Title/Abstract] OR "glutamate pyruvic transaminase*"[Title/Abstract] OR "glutamate pyruvate transaminase*"[Title/Abstract] OR "alt"[Title/Abstract] OR "alat"[Title/Abstract] OR "gpt"[Title/Abstract] OR "sgpt"[Title/Abstract] OR "liver function test*"[Title/Abstract] OR "lft"[Title/Abstract] OR "liver enzyme*"[Title/Abstract]	100,947
6	#4 OR #5	113,768
7	"viral load"[MeSH Terms] OR "viremia"[MeSH Terms] OR "dna, viral"[MeSH Terms]	132,711
8	"viral load*"[Title/Abstract] OR "virus load"[Title/Abstract] OR "viremi*"[Title/Abstract] OR "viraemi*"[Title/Abstract] OR "hbv dna"[Title/Abstract] OR "hepatitis b virus dna"[Title/Abstract]	70,056
9	#7 OR #8	167,833

10	"liver cirrhosis"[MeSH Terms] OR ("liver"[MeSH Terms] AND "fibrosis"[MeSH Terms]) OR ("liver"[MeSH Terms] AND "biopsy"[MeSH Terms]) OR "elasticity imaging techniques"[MeSH Terms] OR “carcinoma, hepatocellular”[MeSH Terms] OR "hepatic insufficiency"[MeSH Terms] OR “mortality”[MeSH Terms] OR “death”[MeSH Terms]	791,869
11	"cirrho*"[Title/Abstract] OR "liver fibrosis"[Title/Abstract] OR "hepatic fibrosis"[Title/Abstract] OR "advanced fibrosis"[Title/Abstract] OR "significant fibrosis"[Title/Abstract] OR "liver biops*"[Title/Abstract] OR "hepatic biops*"[Title/Abstract] OR "liver histolog*"[Title/Abstract] OR "hepatic histolog*"[Title/Abstract] OR "liver histopatholog*"[Title/Abstract] OR "hepatic histopatholog*"[Title/Abstract] OR "metavir"[Title/Abstract] OR "ishak"[Title/Abstract] OR "elastograph*"[Title/Abstract] OR "fibroscan"[Title/Abstract] OR "liver stiffness"[Title/Abstract] OR "liver ultrasound"[Title/Abstract] OR "liver ultrasonography"[Title/Abstract] OR "hepatic ultrasound"[Title/Abstract] OR "hepatic ultrasonography"[Title/Abstract] OR "abdominal ultrasound"[Title/Abstract] OR "abdominal ultrasonography"[Title/Abstract] OR "abdomen ultrasound"[Title/Abstract] OR "abdomen ultrasonography"[Title/Abstract] OR "ultrasound abdomen"[Title/Abstract] OR "ultrasonography abdomen"[Title/Abstract] OR "fibrosis-4"[Title/Abstract] OR "fib-4"[Title/Abstract] OR "fib4"[Title/Abstract] OR "apri"[Title/Abstract] OR "fibrotest*"[Title/Abstract] OR “liver carcinom*”[Title/Abstract] OR “hepatocellular	2,048,275

	carcinom*”[Title/Abstract] OR “liver cancer*”[Title/Abstract] OR “liver neoplasm*”[Title/Abstract] OR “liver tumor*”[Title/Abstract] OR “hepatic tumor*”[Title/Abstract] OR “liver tumour*”[Title/Abstract] OR “hepatic tumour*”[Title/Abstract] OR “hepatoma” [Title/Abstract] OR “hcc” [Title/Abstract] OR “liver failure”[Title/Abstract] OR “hepatic failure”[Title/Abstract] OR “liver decompensat*”[Title/Abstract] OR “hepatic decompensat*”[Title/Abstract] OR "end stage liver disease*"[Title/Abstract] OR “death*”[Title/Abstract] OR “mortality”[Title/Abstract] OR “active hepatitis” [Title/Abstract] OR "active chronic hepatitis"[Title/Abstract] OR "active CHB"[Title/Abstract] OR "active CH"[Title/Abstract] OR "hbeag negative hepatitis"[Title/Abstract] OR "hbeag negative chronic hepatitis"[Title/Abstract] OR "hbeag negative CHB"[Title/Abstract] OR "hbeag negative CH"[Title/Abstract] OR "hbeag (-) hepatitis"[Title/Abstract] OR "hbeag (-) chronic hepatitis"[Title/Abstract] OR "hbeag (-) CHB"[Title/Abstract] OR "hbeag (-) CH"[Title/Abstract] OR "hepatitis flare*"[Title/Abstract] OR "hepatic flare*"[Title/Abstract]	
12	#10 OR #11	2,384,016
13	"cohort studies"[MeSH Terms] OR "clinical studies as topic"[MeSH Terms] OR "survival analysis"[MeSH Terms]	2,948,170
14	"observational study"[Publication Type] OR "clinical study"[Publication Type] OR "comparative study"[Publication Type] OR "evaluation study"[Publication Type] OR "meta analysis"[Publication Type]	3,169,523
15	#13 OR #14	5,291,666

16	#3 AND (#6 OR #9) AND #12 AND #15	4,052
17	#16 NOT ("animals"[MeSH Terms] NOT "humans"[MeSH Terms])	4,032
18	#17 NOT ("letter"[Publication Type] OR "news"[Publication Type] OR "comment"[Publication Type] OR "editorial"[Publication Type] OR "congress"[Publication Type])	4,011
19	#18 AND 2000/01/01:3000/12/31[Date - Publication]	**3,365**

Embase

#	**Searches** **01.01.2000 – 06.02.2023**	**Results**
1	'hepatitis b virus'/exp OR 'hepatitis b'/exp OR 'hepatitis b antigen'/exp	186,584
2	'hepatitis b':ti,ab OR 'type b hepatitis':ti,ab OR 'hepatitis type b':ti,ab OR 'hbv':ti,ab OR 'vhb':ti,ab OR 'hep b':ti,ab OR 'hbsag':ti,ab OR 'aghbs':ti,ab OR 'hbs ag':ti,ab OR 'hbs antigen*':ti,ab	148,714
3	#1 OR #2	197,294
4	'alanine aminotransferase'/exp	154,150
5	'alanine transaminase*':ti,ab OR 'alanine aminotransferase*':ti,ab OR 'glutamic pyruvic transaminase*':ti,ab OR 'glutamic pyruvate transaminase*':ti,ab OR 'glutamate pyruvic transaminase*':ti,ab OR 'glutamate pyruvate transaminase*':ti,ab OR 'alt':ti,ab OR 'alat':ti,ab OR 'gpt':ti,ab OR 'sgpt':ti,ab OR 'liver function test*':ti,ab OR 'lft':ti,ab OR 'liver enzyme*':ti,ab	167,955
6	#4 OR #5	254,666
7	'virus load'/exp OR 'viremia'/exp OR 'virus dna'/exp	192,800
8	'load*':ti,ab OR 'virus load':ti,ab OR 'viremi*':ti,ab OR 'viraemi*':ti,ab OR 'hbv dna':ti,ab OR 'hepatitis b virus dna':ti,ab	659,396
9	#7 OR #8	766,107
10	'liver cirrhosis'/exp OR ('liver'/exp AND 'fibrosis'/exp) OR ('liver'/exp AND 'biopsy'/exp) OR	2,402,384

	'elastography'/exp OR 'liver cell carcinoma'/exp OR 'liver failure'/exp OR 'mortality'/exp OR 'death'/exp	
11	'cirrho*':ti,ab OR 'liver fibrosis':ti,ab OR 'hepatic fibrosis':ti,ab OR 'advanced fibrosis':ti,ab OR 'significant fibrosis':ti,ab OR 'liver biops*':ti,ab OR 'hepatic biops*':ti,ab OR 'liver histolog*':ti,ab OR 'hepatic histolog*':ti,ab OR 'liver histopatholog*':ti,ab OR 'hepatic histopatholog*':ti,ab OR 'metavir':ti,ab OR 'ishak':ti,ab OR 'elastograph*':ti,ab OR 'fibroscan':ti,ab OR 'liver stiffness':ti,ab OR 'liver ultrasound':ti,ab OR 'liver ultrasonography':ti,ab OR 'hepatic ultrasound':ti,ab OR 'hepatic ultrasonography':ti,ab OR 'abdominal ultrasound':ti,ab OR 'abdominal ultrasonography':ti,ab OR 'abdomen ultrasound':ti,ab OR 'abdomen ultrasonography':ti,ab OR 'ultrasound abdomen':ti,ab OR 'ultrasonography abdomen':ti,ab OR 'fibrosis-4':ti,ab OR 'fib-4':ti,ab OR 'fib4':ti,ab OR 'apri':ti,ab OR 'fibrotest*':ti,ab OR 'liver carcinom*':ti,ab OR 'hepatocellular carcinom*':ti,ab OR 'liver cancer*':ti,ab OR 'liver neoplasm*':ti,ab OR 'liver tumor*':ti,ab OR 'hepatic tumor*':ti,ab OR 'liver tumour*':ti,ab OR 'hepatic tumour*':ti,ab OR 'hepatoma':ti,ab OR 'hcc':ti,ab OR 'liver failure':ti,ab OR 'hepatic failure':ti,ab OR 'liver decompensat*':ti,ab OR 'hepatic decompensat*':ti,ab OR 'end stage liver disease*':ti,ab OR 'death*':ti,ab OR 'mortality':ti,ab OR 'active hepatitis':ti,ab OR 'active chronic hepatitis':ti,ab OR 'active chb':ti,ab OR 'active ch':ti,ab OR 'hbeag negative hepatitis':ti,ab OR 'hbeag negative chronic hepatitis':ti,ab OR 'hbeag negative chb':ti,ab OR 'hbeag negative ch':ti,ab OR 'hbeag (-) hepatitis':ti,ab OR 'hbeag (-) chronic hepatitis':ti,ab OR 'hbeag (-) chb':ti,ab OR 'hbeag (-) ch':ti,ab OR 'hepatitis flare*':ti,ab OR 'hepatic flare*':ti,ab	2,931,434
12	#10 OR #11	2,692,517

13	'cohort analysis'/exp OR 'clinical study'/exp OR 'survival analysis'/exp	12,010,669
14	#3 AND (#6 OR #9) AND #12 AND #13	16,604
15	#14 AND ('article':it OR 'review':it)	11,259
16	#15 AND [humans]/lim	11,174
17	#16 AND [2000-2023]/py	**10,092**

Web of science

#	**Searches - 06.02.2023**	**Results**
1	TS=("hepatitis b" OR "hepatitis b virus" OR "hepatitis b" OR "hepatitis b antigen" OR "type b hepatitis" OR "hepatitis type b" OR "hbv" OR "vhb" OR "hep b" OR "hbsag" OR "aghbs" OR "hbs ag*" OR "hbs antigen*")	117 201
2	TS=("alanine aminotransferase" OR "alanine transaminase*" OR "alanine aminotransferase*" OR "glutamic pyruvic transaminase*" OR "glutamic pyruvate transaminase*" OR "glutamate pyruvic transaminase*" OR "glutamate pyruvate transaminase*" OR " alt" OR "alat" OR "gpt" OR "sgpt" OR "liver function test*" OR "lft" OR "liver enzyme*")	101 159
3	TS= ("load*" OR "virus load" OR "viremi*" OR "viraemi*" OR "virus dna" OR "hbv dna" OR "hepatitis b virus dna")	1 683 883
4	TS=("liver cirrho*" OR "cirrho*" OR "liver fibrosis" OR "hepatic fibrosis" OR "advanced fibrosis" OR "significant fibrosis"OR "liver biops*" OR "hepatic biops*" OR "liver histolog*" OR "hepatic histolog*" OR "liver histopatholog*" OR "hepatic histopatholog*" OR "metavir" OR "ishak" OR "elastograph*" OR "fibroscan" OR "liver stiffness" OR "liver ultrasound" OR "liver ultrasonography" OR "hepatic ultrasound" OR "hepatic ultrasonography" OR "abdominal ultrasound" OR "abdominal ultrasonography" OR "abdomen ultrasound" OR "abdomen ultrasonography" OR "ultrasound abdomen" OR "ultrasonography abdomen" OR "fibrosis-4" OR "fib-4"	2 531 918

	OR "fib4" OR "apri" OR "fibrotest*" OR "liver carcinom*" OR "hepatocellular carcinom*" OR "liver cancer*" OR "liver neoplasm*" OR "liver tumor*" OR "hepatic tumor*" OR "liver tumour*" OR "hepatic tumour*" OR "hepatoma" OR "hcc" OR "liver failure" OR "hepatic failure" OR "liver decompensat*" OR "hepatic decompensat*" OR "end stage liver disease*" OR "death*" OR "mortality" OR "active hepatitis" OR "active chronic hepatitis" OR "active chb" OR "active ch" OR "hbeag negative hepatitis" OR "hbeag negative chronic hepatitis" OR "hbeag negative chb" OR "hbeag negative ch" OR "hbeag (-) hepatitis" OR "hbeag (-) chronic hepatitis" OR "hbeag (-) chb" OR "hbeag (-) ch" OR "hepatitis flare*" OR "hepatic flare*")	
5	TS=("cohort" OR "longitud*" OR "observational stud*" OR "survival analysis" OR "follow*" OR "random*" OR "blind*" OR "placebo*" OR "RCT " OR "meta analysis")	8 622 303
6	#1 AND (#2 OR #3) AND #4 AND #5	**4919**

Cochrane

#	**Searches** **01.01.2000 - 06.02.2023**	**Results**
1	MeSH descriptor: [Hepatitis B] explode all trees	3,171
2	MeSH descriptor: [Hepatitis B virus] explode all trees	936
3	MeSH descriptor: [Hepatitis B Antigens] explode all trees	1,229
4	("hepatitis b" OR "type b hepatitis" OR "hepatitis type b" OR "hbv" OR "vhb" OR "hep b" OR "hbsag" OR "aghbs" OR "hbs ag" OR "hbs antigen*"):ti,ab,kw	10,765
5	#1 OR #2 OR #3 OR #4	10,765
6	MeSH descriptor: [Alanine Transaminase] explode all trees	1,796
7	("alanine transaminase*" OR "alanine aminotransferase*" OR "glutamic pyruvic transaminase*" OR "glutamic pyruvate transaminase*"	23,309

	OR "glutamate pyruvic transaminase*" OR "glutamate pyruvate transaminase*" OR "alt" OR "alat" OR "gpt" OR "sgpt" OR "liver function test*" OR "lft" OR "liver enzyme*"):ti,ab,kw	
8	#6 OR #7	23,309
9	MeSH descriptor: [Viral Load] explode all trees	2,927
10	MeSH descriptor: [Viremia] explode all trees	439
11	MeSH descriptor: [DNA Viruses] explode all trees	3,466
12	("viral load*" OR "virus load" OR "viremi*" OR "viraemi*" OR "hbv dna" OR "hepatitis b virus dna"):ti,ab,kw	10,201
13	#9 OR #10 OR #11 OR #12	13,062
14	MeSH descriptor: [Fibrosis] explode all trees	7,118
15	MeSH descriptor: [Liver Cirrhosis] explode all trees	3,474
16	MeSH descriptor: [Biopsy] explode all trees	6,805
17	MeSH descriptor: [Liver Neoplasms] explode all trees	3,835
18	MeSH descriptor: [Hepatic Insufficiency] explode all trees	1,078
19	MeSH descriptor: [Death] explode all trees	2,965
20	MeSH descriptor: [Mortality] explode all trees	16,600
21	("liver cirrhosis" OR "cirrhosis" OR "cirrhosis hepatis" OR "fibrosis stage" OR "advanced fibrosis" OR "significant fibrosis" OR "liver biopsy" OR "hepatic biopsy" OR "liver histolog*" OR "hepatic histolog*" OR "liver histopatholog*" OR "hepatic histopatholog*" OR "metavir" OR "ishak" OR "elastograph*" OR "fibroscan" OR "liver stiffness" OR "liver ultrasound" OR "liver ultrasonography" OR "hepatic ultrasound" OR "hepatic ultrasonography" OR "abdominal ultrasound" OR "abdominal ultrasonography" OR "abdomen ultrasound" OR "abdomen ultrasonography" OR "ultrasound abdomen" OR "ultrasonography abdomen" OR "fibrosis-4" OR "fib-4" OR "fib4" OR "apri" OR "fibrotest*" OR "liver carcinom*" OR "hepatocellular carcinom*" OR "liver cancer*" OR "liver neoplasm*" OR "liver tumor*" OR	173,236

	"hepatic tumor*" OR "liver tumour*" OR "hepatic tumour*" OR "hepatoma" OR "hcc" OR "liver failure" OR "hepatic failure" OR "liver decompensat*" OR "hepatic decompensat*" OR "end stage liver disease*" OR "death*" OR "mortality" OR "active hepatitis" OR "active chronic hepatitis" OR "active chb" OR "active ch" OR "hbeag negative hepatitis" OR "hbeag negative chronic hepatitis" OR "hbeag negative chb" OR "hbeag negative ch" OR "hepatitis flare*" OR "hepatic flare*"):ti,ab,kw	
22	#14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21	187,057
23	MeSH descriptor: [Cohort Studies] explode all trees	181,114
24	MeSH descriptor: [Clinical Studies as Topic] explode all trees	82,353
25	MeSH descriptor: [Survival Analysis] explode all trees	25,893
26	("cohort" OR "longitud*" OR "observational stud*" OR "survival analysis" OR "follow*" OR "random*" OR "blind*" OR "placebo*" OR "RCT" OR "meta analysis"):ti,ab,kw	855,586
27	#23 OR #24 OR #25 OR #26	939,521
28	#5 AND (#8 OR #13) AND #22 AND #27	823
29	#28 [Publication year from 2000 to 2023]	**744**

Appendix 2. Newcastle-Ottawa Quality Assessment Form for Cohort Studies of Q1

Note: A study can be given a maximum of one star for each numbered item within the Selection and Outcome categories. Comparability category is not applicable for Q1.

Selection

1) Representativeness of the exposed cohort

a) Truly representative ***(one star)***

- Participants identified to carry HBsAg through general population testing (i.e. routine testing throughout the entire population without attempting to identify high-risk behaviours or characteristics)[16]

b) Somewhat representative ***(one star)***

- Participants identified to carry HBsAg as clinically indicated for suspected liver disease
- Participants known to carry HBsAg through general population testing or clinically indicated and have been followed by specialist services

c) Selected group

- Participants identified to carry HBsAg through focused testing of high-risk groups (e.g., men who have sex with men (MSM), people who inject drugs (PWID), people in prisons and other closed settings, sex workers, transgender people, etc)[16]

d) No description of the derivation of the cohort

2) Selection of the non-exposed cohort

Not applicable since Q1 is primarily descriptive.

3) Ascertainment of exposure

a) Clearly defined how participants were categorized based on HBV DNA levels and ALT levels ***(one star)***

b) No description

c) Other

4) Demonstration that outcome of interest was not present at start of study

a) Yes ***(one star)***

- All participants screened for the presence of cirrhosis and HCC

b) No

Comparability

Not applicable since Q1 is primarily descriptive.

Outcome

1) Assessment of outcome

a) Independent blind assessment ***(one star)***

- Examiner of clinical outcomes (e.g., research physician) was blinded to the baseline HBV DNA and ALT levels

b) Record linkage ***(one star)***

- E.g., HCC event was ascertained by cancer registry, death was ascertained by death registry.

c) Self report

d) No description

e) Other

2) Was follow-up long enough for outcomes to occur

a) Yes ***(one star)***

- ≥5 years

b) No

- <5 years

3) Adequacy of follow-up of cohorts

a) Complete follow up ***(one star)***

- All subject accounted for and lost to follow-up reported clearly as zero

b) Subjects lost to follow up unlikely to introduce bias ***(one star)***

- Follow-up rate ≥80% or description of those lost suggested no different from those followed

c) Follow-up rate <80% and no description of those lost

d) No statement

- If not reporting any lost to follow-up, and also not mentioning clearly that "there were no cases lost to follow-up", then we should assume that lost to follow-up was not well reported and this should not be given a star.

<u>Thresholds for converting the Newcastle-Ottawa scales to AHRQ standards (good, fair, and poor):</u>

Good quality: 3 stars in selection domain AND 2 or 3 stars in outcome domain

Fair quality: 2 stars in selection domain AND 2 or 3 stars in outcome domain

Poor quality: 0 or 1 star in selection domain OR 0 or 1 starsin outcome domain

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