



**ANTIVIRAL THERAPY FOR PREVENTION OF
HEPATOCELLULAR CARCINOMA AND MORTALITY IN
CHRONIC HEPATITIS B: SYSTEMATIC REVIEW AND META-
ANALYSIS**

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2013-003265
Article Type:	Research
Date Submitted by the Author:	21-May-2013
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Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Evidence based practice
Keywords:	Adult gastroenterology < GASTROENTEROLOGY, Hepatobiliary disease < GASTROENTEROLOGY, Hepatobiliary tumours < ONCOLOGY

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10 **CHRONIC HEPATITIS B: SYSTEMATIC REVIEW AND META-**
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12 **ANALYSIS**
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19 **Short title:** Antiviral therapy for hepatitis B
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52
53
54 **Abstract word count: 298**

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57 **Total word count: 5790**
58
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ABSTRACT

Background: The effect of antiviral therapy on clinical outcomes in chronic hepatitis B (HBV) is not established.

Objectives: To assess the effect of antiviral treatment (interferon and/or nucleoside analogues) versus placebo or no intervention on prevention of hepatocellular carcinoma (HCC) and mortality in chronic hepatitis B.

Design: Random effects pair-wise meta-analysis of randomised trials and observational studies.

Data sources: Electronic and manual searches were combined.

Study selection: Randomised controlled trials (RCTs) were included in the primary analyses. Observational studies were included in sensitivity analyses.

Data extraction: Two independent reviewers extracted data and evaluated bias control. The primary outcome measures were HCC incidence and mortality.

Data synthesis: We included eight RCTs, eight prospective cohort studies and 19 case-control studies with a total of 3433 patients allocated to antiviral therapy and 4625 controls. The maximum duration of follow up was 23 years. Randomised trials found no effect of antiviral therapy on HCC or mortality. Cohort studies found that antiviral therapy increased the risk of HCC (risk ratio 1.43; 95% confidence interval 1.06 to 1.95) whereas case control studies found a decreased risk of HCC in the intervention group (risk ratio 0.69; 95% confidence interval 0.54 to 0.88). There was a clear difference between the results of RCTs and observational studies (test for subgroup differences $P < 0.001$).

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4 Antiviral therapy did not affect mortality in cohort studies, but reduced mortality in case
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6 control studies (relative risk 0.71; 95% confidence interval 0.54 to 0.93; test for subgroup
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8 differences, P=0.406).
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11 **Conclusions:** The effect of antiviral therapy on clinical outcomes in HBV remains to be
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13 established. Although there was a positive effect in the sensitivity analyses, the strength of
14
15 the evidence does not allow for extrapolation to clinical practice as research design plays
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17 an essential role in the overall assessment.
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21 **Trial registration number:** Prospero number CRD42013003881
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ARTICLE SUMMARY

Article focus

- The effect of antiviral treatment for chronic hepatitis B has been assessed using surrogate markers.
- An evaluation of the effect on hepatocellular carcinoma and mortality is missing.

Key messages

- Research design plays an essential role on hepatocellular carcinoma incidence estimates. As prospective cohorts and case-control series show opposing results, reports from such trials should be interpreted with caution.
- Sensitivity analyses show a positive effect of treatment on mortality

Strengths and limitations of this study

- A large number of observational studies were included allowing for detailed sensitivity analyses with tests for subgroup differences
- Only 8 randomised controlled trials were included
- The effect of modern nucleos(t)ides could not be assessed as newer trials does not include placebo treated or untreated patients in the control groups

INTRODUCTION

Worldwide, two billion people have been infected with hepatitis B. Chronic hepatitis B (HBV) may lead to hepatocellular carcinoma (HCC), cirrhosis and liver failure and each year, about 600 000 people die due to hepatitis¹⁻³. Globally, HCC is the fifth most common cause of cancer deaths in men, and the sixth in women⁴⁻⁶. Vaccine programs have decreased the incidence of HBV^{7 8}, but mortality from HBV related HCC and cirrhosis is increasing due to the high prevalence of chronically infected patients^{9 10}. The aim of antiviral treatment is to prevent progression to these clinical outcome measures¹¹⁻¹³. Recommended treatments include interferon and nucleos(t)ide analogues (NA)^{14 15}. A viral response may reduce the risk of HCC¹², but the results of clinical studies and meta-analyses on antiviral therapy are not consistent¹⁶⁻²⁴. One meta-analysis²⁵ found that antiviral therapy decreased liver-related mortality whereas a cohort series found decreased overall mortality in patients with a viral response to interferon²⁶. On the other hand, RCTs have failed to show an effect on HCC or mortality^{27 28}. We therefore conducted a systematic review of the evidence on antiviral treatment for prevention of HCC and mortality in patients with HBV.

METHODS

Scope

This systematic review evaluates the effects of antiviral therapy versus placebo or no intervention on prevention of HCC and mortality in patients with HBV. The review is based on a registered written protocol (Prospero number CRD42013003881) according to the methods specified in the Cochrane Handbook for Reviews on Interventions²⁹ and the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies³⁰. For a more detailed description of the methods, please see the MOOSE checklist (appendix 1).

Data sources

Eligible trials were identified through electronic and manual searches. Electronic searches were performed in MEDLINE (1966-2012), EMBASE (1928-2012), and Web of Science (1900-2012). Literature searches included keywords for HCC, chronic hepatitis B, and antiviral treatment. Manual searches included scanning of reference lists in relevant papers and conference proceedings and the International Clinical Trials Registry Platform.

Study selection

Our primary analyses included RCTs (primary analyses) on antiviral interventions (interferon and/or NA) versus placebo or no intervention for patients with HBV who had not previously received antiviral therapy (treatment naïve). Due to the expected prognosis and the duration of follow up necessary to evaluate intervention effects on clinical outcome measures in HBV, observational studies were included in sensitivity analyses. The primary outcome measures were HCC diagnosed using recommended criteria^{31 32} and all-cause

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4 mortality. To avoid prevalent cases of HCC the outcomes were assessed after at least 12
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6 months of follow up. The secondary outcome measure was HCC related mortality.
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9 10 **Data extraction and quality assessment**

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12 Two authors extracted data in an independent manner. When data were not available in
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14 the published reports, additional information was retrieved through correspondence with
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16 the primary investigators.
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19 The Cochrane Collaboration's Tool for Assessing Risk of Bias was used to evaluate bias
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21 control in RCTs. The assessment included the randomisation methods (allocation
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23 sequence generation and allocation concealment), blinding (of participants, personnel and
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25 investigators), the completeness of outcome data, reporting of data and other biases³³. All
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27 observational studies were classed as having a high risk of bias. Based on the MOOSE
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29 guidelines, the assessment of potential sources of bias within observational studies
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31 included documentation of how data were classified and coded (multiple raters, blinding
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33 and interrater reliability), assessment of confounding (comparability of cases and controls
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35 in studies where appropriate) and blinding of quality assessors, stratification or regression
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37 on possible predictors of study results.
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43 **Data synthesis and analysis**

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45 Statistics were performed using Stata Version 12 (Statacorp, College Station, TX, USA)
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47 and Trial Sequential Analysis (CTU, Copenhagen, Denmark). Meta-analyses were
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49 performed with results expressed as risk ratios, 95% CI and I^2 as a marker of
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51 heterogeneity. For meta-analyses showing a statistically significant effect the number
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53 needed to treat was calculated based on the risk difference. Initial sensitivity analyses
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4 included repeating all meta-analyses using both random and fixed effect models. The
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6 results of these analyses were only reported if the conclusions differed. Regression
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8 analyses were performed to assess for publication bias and other small study effects
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10 (Egger's test). Sequential analyses were performed for meta-analyses showing an
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12 intervention effect after adjusting for the risk of bias associated with cumulative testing³⁴.
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14 The sequential analysis was performed using a random-effects model, alpha (5%), power
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16 (80%) and the incidence rates and the intervention effects identified in the meta-analyses.
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18 Pre-planned sensitivity analyses were performed with inclusion of observational studies.
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20 These analyses were performed stratified by study design (RCT, prospective cohort or
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22 case-control study) and with fixed-effect inverse variance models that compared the
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24 results of subgroups. The result of the subgroup comparisons was expressed as P values
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26 (test for subgroup differences). Additional sensitivity analyses were performed to evaluate
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28 the influence of bias control (limiting the analysis to trials with adequate randomisation),
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30 the type of antiviral therapy (comparing interferon, NA or both), and the effect HCC
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32 screening (comparing the results of trials with or without screening). Finally, subgroup
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34 analyses including only patients with cirrhosis were performed.
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RESULTS

Literature searches and study inclusion

The electronic and manual searches identified 27 474 potentially relevant records (figure 1). After excluding duplicates and studies that did not fulfil our inclusion criteria, 36 references referring to eight RCTs, eight prospective cohort studies and 19 case control studies were included^{26-28 35-67}.

Characteristics of included RCTs and observational studies

The RCTs were conducted in Europe (n=4), Asia (n=2) and Africa (n=2). The duration of follow up ranged from one to 11 years. One trial performed HCC screening. Six trials assessed interferon and two trials NA (table 1). The proportion of men ranged from 70 to 100% and the mean age from 33 to 44 years. The proportion of patients with cirrhosis at inclusion ranged from zero to 66% (table 2). The proportion of patients with a virological response ranged from seven to 58% in the treatment group and from one to 22% of controls. A biochemical response was achieved for 14 to 66% of patients in the treatment and one to 20% of controls. The randomisation methods were described as adequate in three trials (table 3).

The prospective cohorts and case control studies were conducted in Europe (n=12), Asia (n=13), North America (n=1) and South America (n=1). The duration of follow up ranged from two to 23 years. HCC screening was performed in all prospective cohort studies and in 13 of the case control studies. Eighteen studies assessed interferon, seven assessed NA and two combined therapy with interferon and NA (table 1). The proportion of men ranged from 53 to 95% and the mean age from 27 to 65 years. The proportion of patients

with cirrhosis ranged from zero to 100% (table 2). In the prospective cohorts, the proportion of patients with a virological response in the treatment and control groups was 23 to 69% and zero to 23%, respectively. A biochemical response was achieved for 23 to 69% of patients in the treatment groups and 31% in the control group (only reported in one study). In the case control series, the proportion of patients with a virological response in the treatment and control group ranged from 7 to 78% and two to 100%, respectively. A biochemical response in the two groups was 27 to 68% and four to 51%, respectively.

Prevention of HCC

HCC was diagnosed in 22 of 840 patients in the treatment group versus 19 of 447 controls (relative risk 0.58; 95% confidence interval 0.32-1.07; $I^2=0\%$). There was no evidence of small study effects (Egger's test, $P=0.269$) and no difference between subgroups of trials assessing interferon or NA (test for subgroup differences $P=0.854$). The overall result was confirmed in sensitivity analyses including RCTs with a low risk of bias and trials with HCC screening.

Sensitivity analyses including prospective cohort studies and case control studies were performed. In the cohort studies, HCC was diagnosed in 51 of 689 patients in the treatment group and 174 of 2283. In the case control studies the numbers were 99 of 1904 and 201 of 1895 patients, respectively. A meta-analysis that combined RCTs and observational studies found no effect of antiviral therapy on HCC (relative risk 0.88; 95% confidence interval 0.73 to 1.05; $I^2=63\%$). There was no evidence of small study effects (Egger's test $P=0.730$). Subgroup analyses showed a clear difference between the RCTs, prospective cohorts and case control studies (test for subgroup differences $P<0.001$) (figure 2). The prospective cohort studies found that antiviral therapy increased the risk of

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4 HCC (relative risk 1.44; 95% confidence interval 1.06 to 1.95) whereas the case control
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6 studies found that antiviral therapy reduced the risk of HCC (relative risk 0.69; 95%
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8 confidence interval 0.54 to 0.88). Due to the high heterogeneity, a post-hoc meta-
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10 regression analysis was performed. We evaluated study and patient characteristics not
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12 accounted for in the sensitivity analyses, which may have influenced the result. No
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14 modifiers were found when adjusting for the following variables: proportion of men
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16 (coefficient -0.074; P=0.08) mean age of treated patients at inclusion (coefficient 0.020;
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18 P=0.94), mean age of untreated patients at inclusion (coefficient 0.121; P=0.65),
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20 proportion with cirrhosis at inclusion (coefficient -0.001; P=0.76), and region of trial
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22 (coefficient -0.394; P=0.55).
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31 Sensitivity analyses were performed to evaluate the risk of HCC among patients with
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33 cirrhosis. In the RCTs, one of 20 patients in the treatment group and two of 12 controls
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35 developed HCC (relative risk 0.75; 95% confidence interval 0.10 to 5.77). In the
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37 prospective cohort studies 32 of 184 versus 142 of 482 patients developed HCC whereas
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39 the numbers were 63 of 680 versus 161 of 955, respectively for case control studies.
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41 Overall, antiviral therapy reduced the risk of HCC when including data from RCTs and
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43 observational studies (relative risk 0.74; 95% confidence interval 0.57 to 0.96; $I^2=0\%$;
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45 number needed to treat 28 patients) (fig 3). The results of RCTs and observational studies
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47 were similar (test for subgroup differences P=0.159). There was no evidence of small
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49 study effects (Egger's test P=0.890). In trial sequential analysis, the monitoring and alpha-
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51 spending boundary did not cross suggesting that the result was not robust to adjustment
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53 for multiple testing.
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Mortality

In the RCTs, there was no difference in mortality between the treatment and control group (21 of 840 versus 9 of 447 patients; relative risk 1.24; 95% confidence interval 0.58 to 2.66; $I^2=0\%$). No evidence of small study effects (Egger's test $P=0.783$) and no difference between trials stratified by treatment (test for subgroup differences $P=0.668$) or HCC screening ($P=0.828$). In the observational studies, the number of patients in the treatment and control groups who died was 51 of 689 versus 247 of 2283 for prospective cohort studies and 71 of 1904 versus 92 of 1895 in the cohort studies. When combining RCTs and observational studies, random effects meta-analysis showed that antiviral treatment decreased mortality (relative risk 0.76; 95% confidence interval 0.62 to 0.95; $I^2=14\%$; number needed to treat 77; Egger's test $P=0.487$) (fig 4). There was no difference between RCTs and observational studies (test for subgroup differences $P=0.406$). In the trial sequential analysis, the monitoring boundary crossed the alpha-spending boundary in 2004 suggesting that the meta-analysis was robust to adjustments for multiple testing.

Only observational studies reported mortality in patients with cirrhosis. The number of patients who died in the intervention and control groups was 36 of 864 versus 141 of 1 477 (relative risk 0.61; 95% confidence interval 0.44 to 0.86; $I^2=9\%$; number needed to treat 16 patients). There were no small study effects (Egger's test $P=0.533$) and no difference between prospective cohort and case control studies (test for subgroup differences $P=0.292$).

HCC related mortality

Antiviral therapy had no effect on HCC related mortality (3 of 840 versus 2 of 447; relative risk 0.50; 95% confidence interval 0.10 to 2.44; $I^2=0\%$, $n=2$ RCT). Including data from

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4 observational studies had little influence on the overall result (41 of 3433 versus 146 of
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6 4625; relative risk 0.83; 95% confidence interval 0.5 to 1.20; $I^2=0\%$; Egger's test $P=0.248$).

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9 There was no difference between subgroups of trials stratified by design (test for subgroup
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11 differences $P=0.481$).

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DISCUSSION

This systematic review found that the evidence of the effect of antiviral therapy on clinical outcomes is weak. RCTs found no benefit of treatment on HCC, mortality or HCC related mortality in HBV. The total number of patients and duration of follow up may be too small to determine clinical effects. The inclusion of observational studies did not strengthen the overall findings because there was clear evidence of bias suggesting that the study design was closely related to the estimated treatment effects. The prospective cohort studies found that antiviral therapy increased the risk of HCC and had no effect on mortality in HBV. The case control studies found that antiviral therapy reduced both HCC and mortality. These findings suggest that detection and ascertainment bias as well as confounding by indication had a considerable influence on the overall result, which may explain why previous meta-analyses have disagreed in their assessment of the benefit of antiviral therapy^{16-18 20 21 23 24}.

The main limitation of our review is the limited number of RCTs. Only one of the included trials had prevention of HCC as a primary outcome measure²⁷ and none were designed to evaluate the effect on mortality or HCC related mortality. The current recommendation to treat patients with HBV is primarily based on surrogate outcomes. At present the evidence supporting the use of virological markers as surrogate outcomes is weak. The fact that some studies have found a correlation between a virological response and improved liver histology does not necessarily validate their use as surrogate outcomes. Previous evidence shows that interventions supported by surrogate markers may in fact have no benefit or even harmful effects on clinical outcome measures⁶⁸. Still, our findings are not sufficiently convincing and do not allow for changes in clinical practice.

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4 Another limitation of the current review is our failure to extract data for analyses of
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6 treatment responders versus non-responders. However, only six cases of HCC were
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8 reportedly diagnosed in patients with biochemical or viral treatment response. This
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10 suggests that treatment response does not lead to elimination of the HCC risk, but
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12 probably decreases HCC incidence compared to non- or partial-responders. This would be
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14 in line with previous findings^{19 25}. The majority of included trials in the current review
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16 assessed first generation NA and interferon, as reflected in low response rates. It was
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18 however not within the scope of the review to investigate modern antiviral treatments, as
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20 we included untreated control groups. Newer treatments will likely result in more patients
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22 achieving sustained suppression of HBV-DNA. It is therefore possible that the current
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24 review underestimates a potential treatment effect. It would also have been of interest had
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26 we been able to adjust for other common risk factors for HCC such as non-alcoholic
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28 steatohepatitis, alcoholic liver disease and coinfection with hepatitis C, hepatitis D and
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30 human immunodeficiency virus. Although these data were extracted, there was not
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32 enough data to allow for analyses.
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38 There are several potential explanations for the discrepancies between RCTs and
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40 observational studies⁶⁹. The fact that only prospective cohort studies found an increased
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42 risk of HCC among patients receiving antiviral therapy opposes speculations that the
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44 treatment affected HCC development. The findings are more likely to reflect baseline
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46 differences in the viral load, genotype and degree of liver disease. The degree of
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48 monitoring in the treatment and control group is also likely to differ and may lead to
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50 detection bias. The importance of detection bias is further supported by the subgroup
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52 differences observed according to HCC screening. The case control studies are likely to
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4 have an even higher risk of bias, as confounding by indication and ascertainment bias is
5 likely to exist in retrospective studies. Reporting bias should also be considered³³.
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9 The subgroup differences with regards to type of intervention suggests a possible anti-
10 carcinogenic effect of interferon, as seen in HCV⁷⁰. We additionally found a decrease in
11 both HCC incidence and overall mortality in sensitivity analyses of patients with cirrhosis.
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13 This could support the case for continued treatment of patients with cirrhosis.
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18 We found a beneficial effect of interferon and/or NA on mortality in HBV when including
19 RCTs and observational studies in chronic HBV patients. The assessment of mortality is
20 robust to bias⁷¹. Accordingly, our subgroup analysis showed no clear relation between the
21 results and the study design. HCC mortality is more prone to bias. Whether antiviral
22 treatment for HBV decreases mortality except from HCC is unknown.
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31 In conclusion, antiviral treatment for HBV has no proven effect on the clinical outcomes
32 HCC and mortality. Bias has a paramount impact on treatment effect estimates in
33 observational studies and we recommend a critical approach to conclusions drawn in such
34 studies. Future trials on antiviral treatment for HBV should be designed to show an effect
35 on clinical endpoints rather than surrogate markers.
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47 **Contributors:** MT, LLG and AK conceived the idea and design. MT and EKD collected
48 and assembled data. MT, LLG and AK analysed and interpreted the data. MT and LLG
49 drafted the manuscript. LLG and AK revised the manuscript for important intellectual
50 content. All authors discussed and approved of the final version of the manuscript. MT is
51 guarantor.
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4 **Competing interests:** All authors have completed the ICMJE uniform disclosure form at
5 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author)
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7
8 and declare: no support from any organisation for the submitted work; no financial
9
10 relationships with any organisations that might have an interest in the submitted work in
11
12 the previous three years; no other relationships or activities that could appear to have
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14 influenced the submitted work.
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17
18 **Ethical approval:** Not required.
19

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21 **Data sharing:** Dataset available from the corresponding author.
22
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24
25 **Licence statement:** Corresponding Author has the right to grant on behalf of all authors
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FIGURE LEGENDS

Figure 1. Study flow diagram.

Figure 2. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on hepatocellular carcinoma in patients with chronic hepatitis B, subgroups according to trial design.

Figure 3. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on hepatocellular carcinoma in patients with chronic hepatitis B and cirrhosis, subgroups according to trial design.

Figure 4. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on mortality in patients with chronic hepatitis B, subgroups according to trial design.

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27.323 records identified through database searching

151 additional records identified through other sources

27.474 records screened

26.990 records excluded because they were either duplicates or clearly irrelevant

484 full-text articles assessed for eligibility

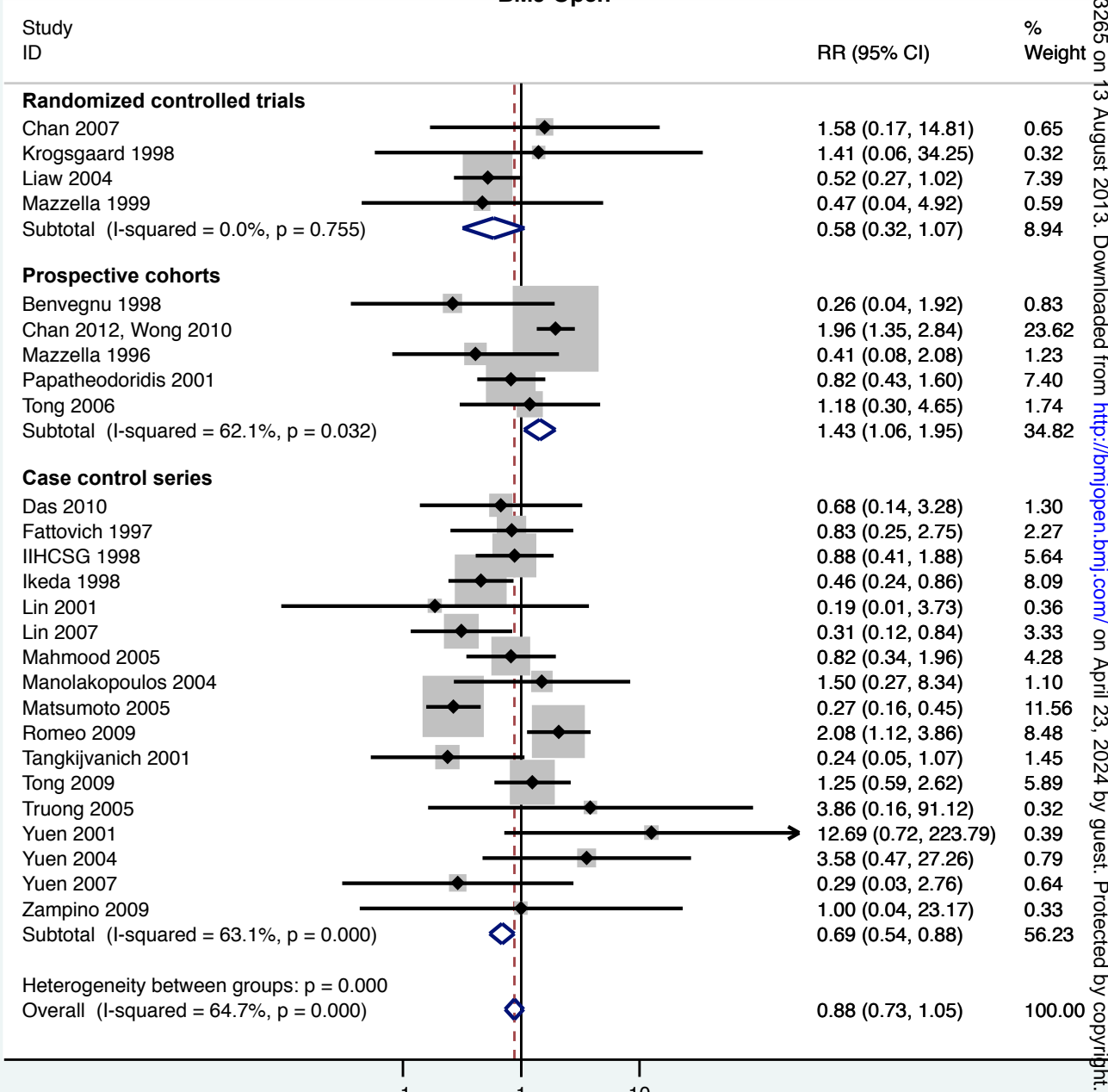
448 full-text articles excluded, with reasons:
Turned out not to be prospective or case-cohort (85).
Did not have an untreated control group (47).
Did not have a treated control group (54).
Did not report HCC incidence or mortality (88).
Did not report outcome reported for treatment groups (48).
A more recent report was available (65).
Outcome assessment was derived from central registries (40).
Had a follow up period of less than 12 month (19).

36 articles referring to 35 studies included in qualitative synthesis

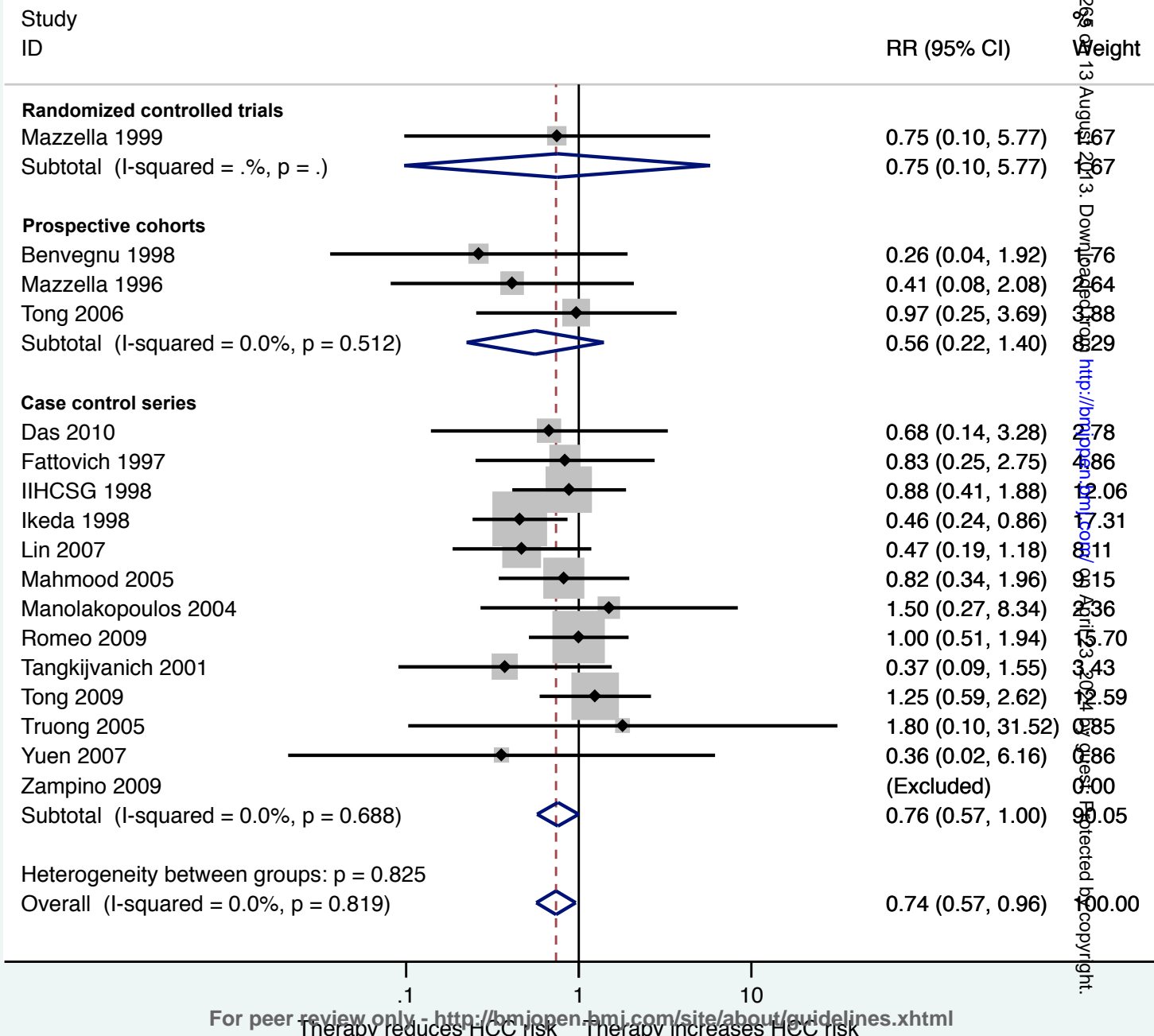
36 articles referring to 35 studies included in quantitative synthesis (meta-analysis)

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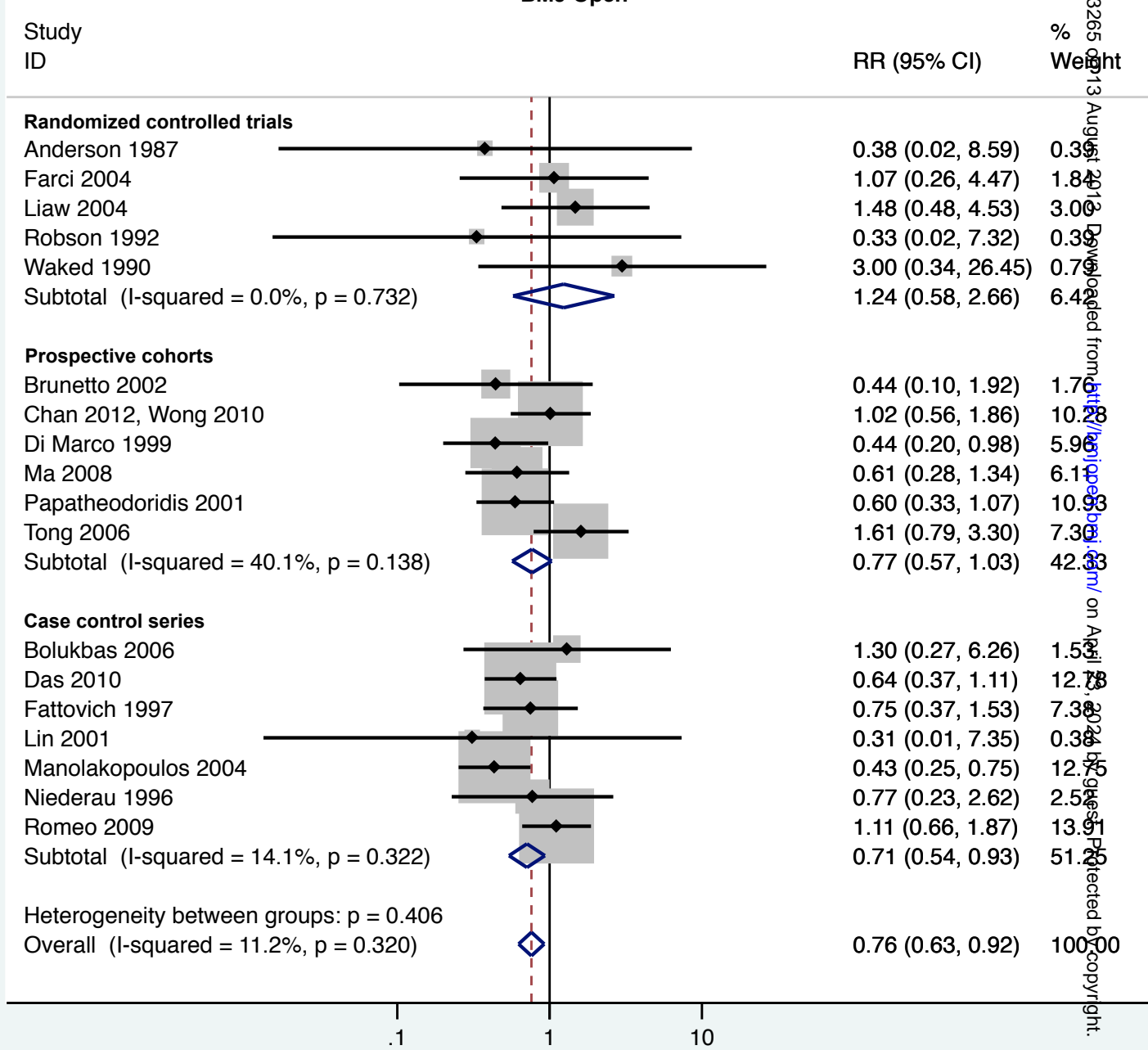
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Table 1. Characteristics of Trials						
Randomized controlled trials						
Study, year (reference)	Country of origin	Intervention (dose)	Number of patients	Follow up (mean/median year)	HCC screening (yes/no)	Outcomes reported
Anderson 1987 (35)	England	IFN (2.5-7.5 MU/m ² /d)	I: 14 C: 16	I: 1.0 C: 1.0	No	Overall mortality
Chan 2007 (39)	China	Lamivudine (100mg/d)	I: 89 C: 47	I: 2.5 C: 2.5	No	HCC incidence
Farci 2004 (43)	Italy	IFN (3-9 MU/x3w)	I: 28 C: 10	I: 10.8 C: 10.8	No	Overall mortality
Krogsgaard 1998 (28)	Europe	IFN (1.5-18 MU/x3w)	I: 210 C: 98	I: 1.3 C: 1.3	No	HCC incidence and -mortality
Liaw 2004 (27)	Asia	Lamivudine (100mg/d)	I: 436 C: 215	I: 2.7 C: 2.7	Yes	HCC incidence Overall mortality
Mazzella 1999 (53)	Italy	IFN (648MU total)	I: 33 C: 31	I: 7.2 C: 6.6	No	HCC incidence and -mortality
Robson 1992 (56)	South Africa	IFN (10MU/x3w)	I: 10 C: 10	I: 1.4 C: 1.4	No	Overall and HCC mortality
Waked 1990 (62)	Egypt	IFN (5MU/m ² /x3w - 5MU/m ² /d)	I: 20 C: 20	I: 1.3 C: 1.3	No	Overall and HCC mortality
Prospective cohorts						
Study, year (reference)	Country of origin	Intervention (dose)	Number of patients	Follow up (mean/median year)	HCC screening (yes/no)	Outcomes reported
Benvegna 1998 (36)	Italy	IFN (5-10 MU/x3w)	I: 13 C: 24	I: 6.0 C: 6.0	Yes	HCC incidence Overall mortality
Brunetto 2002 (38)	Italy	IFN (9MU/x3w)	I: 103 C: 61	I: 6.0 C: 6.0	Yes	Overall mortality
Chan 2012 (40) Wong 2010 (63)	China	Nucleos(t)ides IFN (NS)	I: 158 C: 1271	I: 10.1 C: 10.1	Yes	HCC incidence, overall and HCC mortality
Di Marco 1999 (42)	Italy	IFN (NS)	I: 109 C: 193	I: 7.8 C: 7.8	Yes	Overall mortality
Ma 2008 (49)	China	Nucleos(t)ides (NS)	I: 41 C: 176	I: 2.9 C: 2.9	Yes	Overall mortality
Mazzella 1996 (52)	Italy	IFN (10MU/x3w)	I: 34 C: 28	I: 4.1 C: 4.0	Yes	HCC incidence
Papatheodoridis 2001 (55)	Greece	IFN (3MU/x3w)	I: 209 C: 152	I: 6.0 C: 6.1	Yes	HCC incidence, overall and HCC mortality
Tong 2006 (59)	USA	IFN (NS)	I: 22 C: 378	I: 7.0 C: 7.0	Yes	HCC incidence, overall and HCC mortality
Case control series						
Study, year (reference)	Country of origin	Intervention (dose)	Number of patients	Follow up (mean/median year)	HCC screening (yes/no)	Outcomes reported
Bolukbas 2006 (37)	Turkey	Lamivudine (100mg/d)	I: 23 C: 15	I: 1.5 C: 2.0	Yes	Overall and HCC mortality
Das 2010 (41)	India	Lamivudine Adefovir (NS)	I: 151 C: 102	I: 4.0 C: 3.8	Yes	HCC incidence, overall and HCC mortality
Fattovich 1997 (44)	Europe	IFN (36MU to	I: 40	I: 6.2	No	HCC incidence,

		>300MU total)	C: 50	C: 6.2		overall and HCC mortality
IHCSCG 1998 (45)	Italy, Argentina	IFN (9MU/w)	I: 49 C: 97	I: 5.8 C: 6.9	Yes	HCC incidence
Ikeda 1998 (46)	Japan	IFN (6MU/x2w)	I: 94 C: 219	I: 6.8 C: 7.0	Yes	HCC incidence
Lin 2001 (47)	China	IFN (5MU/x3w)	I: 30 C: 28	I: 2.7 C: 2.6	No	HCC incidence, overall and HCC mortality
Lin 2007 (48)	China	IFN (6-9 MU/m ² /x3w)	I: 233 C: 233	I: 6.8 C: 6.1	Yes	HCC incidence and -mortality
Mahmood 2005 (50)	Japan	IFN (6MU/d)	I: 23 C: 68	I: 7.0 C: 7.0	Yes	HCC incidence
Manolakopoulos 2004 (26)	Greece	Lamivudine (100mg/d)	I: 30 C: 30	I: 1.5 C: 1.8	Yes	HCC incidence, overall and HCC mortality
Matsumoto 2005 (51)	Japan	Lamivudine (100mg/d)	I: 508 C: 231	I: 2.7 C: 5.3	No	HCC incidence
Niederrau 1996 (54)	Germany	IFN (2-5 MU/x3w)	I: 103 C: 53	I: 4.2 C: 3.2	No	Overall mortality
Romeo 2009 (57)	Italy	Lamivudine (NS) IFN (6-9 MU)	I: 102 C: 135	I: 22.4 C: 16.5	Yes	HCC incidence, overall and HCC mortality
Tangkijvanich 2001 (58)	Thailand	IFN (3-6 MU/x3w)	I: 67 C: 72	I: 4.9 C: 4.9	Yes	HCC incidence
Tong 2009 (60)	USA	Lamivudine (NS)	I: 27 C: 101	I: 5.3 C: 5.3	Yes	HCC incidence and -mortality
Truong 2005 (61)	Japan	IFN (174-687 MU total)	I: 27 C: 35	I: 7.0 C: 6.2	Yes	HCC incidence and -mortality
Yuen 2001 (64)	China	IFN (2.5-10 MU/m ² /x3w)	I: 208 C: 203	I: 8.9 C: 9.0	Yes	HCC incidence and -mortality
Yuen 2004 (65)	China	IFN (NS)	I: 6 C: 86	I: 10.5 C: 10.5	No	HCC incidence
Yuen 2007 (66)	China	Lamivudine (100mg/d)	I: 142 C: 124	I: 7.5 C: 9.0	Yes	HCC incidence
Zampino 2009 (67)	Italy	IFN (5MU/m ² /x3w)	I: 41 C: 13	I: 23 C: 23	No	HCC incidence
/x3w = thrice weekly; /d = daily; C = Control; CC = Case Control Series; HCC = hepatocellular carcinoma; I = Intervention; IFN = Interferon; MU = Million Units; NS = Not Stated; PC = Prospective Cohort; RCT = Randomized Controlled Trial.						

Table 2. Patient Characteristics in Included Trials

Randomized controlled trials						
Study, year (reference)	Median/ Mean Age (years)	Proportion of men (%)	Proportion with cirrhosis (%)	Proportion with elevated ALT (%)	Proportion positive for HBeAg (%)	HBeAg sero- converters (n,%)
Anderson 1987 (35)	I: 36 C: 35	100	20	77	100	I: 2; 14% C: 0; 0%
Chan 2007 (39)	I: 39 C: 39	84	16	77	5	NS
Farci 2004 (43)	I: 35 C: 38	83	66	100	2	I: NA C: 1; 100%
Krogsgaard 1998 (28)	I: 36 C: 36	81	19	100	100	NS
Liaw 2004 (27)	I: 43 C: 44	85	33	78	58	NS
Mazzella 1999 (53)	I: 36 C: 41	78	0	100	100	I: 30; 91% C: 19; 61%
Robson 1992 (56)	I: 33 C: 31	70	NS	100	100	I: 5; 50% C: 1; 10%
Waked 1990 (62)	I: 35 C: 35	78	40	100	100	I: 13; 81% C: 5; 33%
Prospective cohorts						
Study, year (reference)	Median/ Mean Age (I/C; years)	Proportion of men (%)	Proportion with cirrhosis (%)	Proportion with elevated ALT (%)	Proportion positive for HBeAg (%)	HBeAg sero- converters (n,%)
Benvegnu 1998 (36)	I: 57 C: 60	65	100	NS	NS	NS
Brunetto 2002 (38)	I: 40 C: 40	80	38	NS	0	NA
Chan 2012 (40) Wong 2010 (63)	NS	67	32	87	NS	NS
Di Marco 1999 (42)	I: 33 C: 35	71	29	100	29	I: 35; 32% C: 29; 15%
Ma 2008 (49)	I: 54 C: 54	72	100	NS	24	NS
Mazzella 1996 (52)	I: 48 C: 49	73	100	NS	NS	NS
Papatheodoridis 2001 (55)	I: 47 C: 49	83	31	100	0	NA
Tong 2006 (59)	I: 48 C: 48	71	35	NS	49	NS
Case control series						
Study, year (reference)	Median/ Mean Age (I/C; years)	Proportion of men (%)	Proportion with cirrhosis (%)	Proportion with elevated ALT (%)	Proportion positive for HBeAg (%)	HBeAg sero- converters (n,%)
Bolukbas 2006 (37)	I: 45 C: 46	82	100	NS	0	NA
Das 2010 (41)	I: 42 C: 46	91	100	NS	45	I: 12; 13% C: 3; 10%

1	Fattovich 1997 (44)	I: 47 C: 45	87	100	100	100	I: 27; 68% C: 30; 60%	
2								
3	IIHCSG 1998 (45)	I: 54 C: 54	64	100	NS	NS	NS	
4								
5	Ikeda 1998 (46)	I: 41 C: 44	79	100	NS	52	NS	
6								
7	Lin 2001 (47)	I: 39 C: 41	95	10	100	0	NA	
8								
9	Lin 2007 (48)	I: 32 C: 31	94	9	NS	100	I: 115; 49% C: 86; 37%	
10								
11	Mahmood 2005 (50)	I: 49 C: 49	69	100	NS	36	NS	
12								
13	Manolakopoulos 2004 (26)	I: 65 C: 63	80	100	100	0	NA	
14								
15	Matsumoto 2005 (51)	I: 42 C: 41	73	18	NS	55	NS	
16								
17	Niederrau 1996 (54)	I: 40 C: 41	78	28	100	100	I: 53; 51% C: 7; 13%	
18								
19	Romeo 2009 (57)	NS	77	35	NS	27	NS	
20								
21	Tangkijvanich 2001 (58)	I: 37 C: 40	72	20	NS	100	I: 24; 36% C: 7; 10%	
22								
23	Tong 2009 (60)	I: 46 C: 46	86	100	14	53	NS	
24								
25	Truong 2005 (61)	I: 33 C: 37	53	2	100	60	I: 9; 53% C: 11; 55%	
26								
27	Yuen 2001 (64)	I: 27 C: 28	64	NS	32	100	I: 96; 46% C: 93; 46%	
28								
29	Yuen 2004 (65)	I: 43 C: 43	71	NS	NS	23	NS	
30								
31	Yuen 2007 (66)	I: 34 C: 33	74	0	48	100	NS	
32								
33	Zampino 2009 (67)	NS	67	0	NS	54	I: 16; 62% C: NS	
34								
35	I = Intervention; C = Control; NA = Not Applicable; NS = Not Stated; ALT = Alanine amino transferase							

Table 3. Risk of Bias Summary

Study, year (reference)	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting
Anderson 1987 (35)	?	?	+	+	?	?
Chan 2007 (39)	+	+	+	+	?	?
Farci 2004 (43)	+	+	+	+	?	?
Krogsgaard 1998 (28)	?	?	+	+	–	?
Liaw 2004 (27)	+	+	+	+	+	+
Mazzella 1999 (53)	?	?	+	+	+	?
Robson 1992 (56)	?	?	+	+	?	?
Waked 1990 (62)	?	?	+	+	?	?

+ Low risk of bias. – High risk of bias. ? Unknown risk of bias.

peer review only

MOOSE Checklist

ANTIVIRAL THERAPY FOR PREVENTION OF HEPATOCELLULAR CARCINOMA AND MORTALITY IN CHRONIC HEPATITIS B: SYSTEMATIC REVIEW AND META-ANALYSIS

Corresponding Author:
Maja Thiele, M.D.

Abbreviations:
HBV: Chronic Hepatitis B
HCC: Hepatocellular carcinoma
IFN: Interferons
NA: Nucleos(t)ide analogues
RR: Relative Risk

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Patients with HBV have an increased risk of HCC and death. It is debated whether antiviral treatment decreases incidence of HCC and mortality.
√	Hypothesis statement	Antiviral treatment decreases the incidence of HCC and mortality in HBV.
√	Description of study outcomes	Primary outcomes: HCC and all-cause mortality. Secondary outcomes: HCC related mortality.
√	Type of exposure or intervention used	Exposure: Chronic hepatitis B. HBV was defined as sustained hepatitis B surface antigen (HBsAg) positivity for more than six months. Interventions: IFN or NA without restrictions on duration or dose of therapy. Control: No treatment or placebo
√	Type of study designs used	We included case control studies, prospective cohort studies and randomized trials..
√	Study population	Patients with HBV were included.
Reporting of search strategy should include		
√	Qualifications of searches	The searches were performed in several electronic databases and combined with manual searches.
√	Search strategy, including time period included in the synthesis and keywords	No restriction on time period. Keywords for HCC were: HCC, hepatoma, hepatocell*, liver cancer, liver neoplasm*, liver cell carcinoma. Keywords for HBV were: HBV, CHB, hepatitis B, B

		hepatitis, dane particle, *HBs*, *HBe*, chronic hepatitis B, chronic B hepatitis. Keywords for antiviral therapy were: IFN, interferon*, Placebo*, Lamivud*, Telbivud*, Emtricitab*, Entecavir, Adefovir, Tenofovir, Medical therapy, antiviral*, Drug Therapy, nucleoside*, nucleotide*
√	Databases and registries searched	PubMed, EMBASE, Science Citation Index Expanded, Cochrane Central Register of Controlled Trials
√	Search software used, name and version, including special features	EndNote was used to manage retrieved citations.
√	Use of hand searching	Included articles and relevant reviews were manually searched for additional eligible articles.
√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in the flow chart. A list of excluded articles is available upon request.
√	Method of addressing articles published in languages other than English	All articles eligible for inclusion were published in English.
√	Method of handling abstracts and unpublished studies	All trials and studies eligible for inclusion were published as full paper articles.
√	Description of any contact with authors	When necessary authors of eligible trials were contacted in order to provide additional information.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Inclusion and exclusion criteria are described in the methods section.
√	Rationale for the selection and coding of data	Data on patient characteristics at inclusion and during follow up were selected based on the prognostic factors in HBV and known risk factors for HCC. Extracted data included: country of origin, duration of follow up, interventions (type, length of treatment and treatment doses), proportion of men; proportion of patients with histological or clinical cirrhosis; severity of underlying liver disease; proportion of hepatitis envelope antigen (HBeAg) positive patients; proportion of patients with a virological response (loss of hepatitis B virus DNA (HBV DNA)), serological response (HBeAg- and HBsAg-seroconversions) or biochemical response (normalisation of liver function tests including

		aspartate aminotransferase and alanine aminotransferase); and whether HCC screening was performed.
√	Assessment of confounding	Sensitivity analyses are described in the methods section.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Bias assessment are described in short in the methods section. In details: The allocation sequence generation was classed as adequate if based on computer generated random numbers, a table of random numbers or similar. The allocations concealment was classed as adequate if patients were randomised through a central independent unit, serially numbered, opaque sealed envelopes or similar. Due to the objective nature of the primary outcome measures assessed, the importance of blinding was limited. Blinding was primarily extracted for the assessment of the secondary outcome measure and was classed as adequate if patients, personnel or investigators were blinded. Outcome data were classed as complete if there were no missing data, if reasons for missing data were unlikely to be related to true outcome, if missing data were balanced in numbers between intervention groups and if the proportion of missing data was too small to induce clinically relevant impact on the intervention effect estimate. Reporting of data was classed as adequate if all expected clinically relevant outcomes were reported. Other bias included whether a sample size calculation had been performed and whether the sample size was met.
√	Assessment of heterogeneity	Assessment of heterogeneity is described in the methods section. I^2 values below 30% were considered unimportant. Values between 30% and 50% represented a moderate risk of heterogeneity, values between 50% and 75% a substantial risk of heterogeneity and values between 75% and 100% represented considerable heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	The statistics used are described in the methods section.
√	Provision of appropriate tables and graphics	We have provided the following: Figure 1: Trial Flow Diagram;; Figure 2-4: Forest plots for subgroups and treatment effect on HCC incidence and mortality; Table 1: Characteristics of Included Studies; Table 2: Patient Characteristics in Included Studies; Table

		3: Risk of Bias Summary
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figure 2-4
√	Table giving descriptive information for each study included	Table 1-3
√	Results of sensitivity testing	Sensitivity and subgroup analyses are described under the results section.
√	Indication of statistical uncertainty of findings	Results are presented as RR with 95% confidence intervals and I ² values. Sensitivity analyses using a fixed effects model have been done for all analyses. For results showing a statistical significant effect Eggers test of bias and sequential analyses have been performed to test for small study effects and multiple testing.
Reporting of discussion should include		
√	Quantitative assessment of bias	Quantitative assessment of bias are described in the discussion section.
√	Justification for exclusion	Reasons for exclusion of observational studies in the primary analyses are described in the discussion.
√	Assessment of quality of included studies	Quality assessment are described in both the results and the discussion sections.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	Alternative explanations for observed results have been described in the discussion section
√	Generalization of the conclusions	Generalizations have been described in the discussion section
√	Guidelines for future research	Guidelines for future research have been described in the discussion section
√	Disclosure of funding source	Disclosures and author contributions are reported.



**ANTIVIRAL THERAPY FOR PREVENTION OF
HEPATOCELLULAR CARCINOMA AND MORTALITY IN
CHRONIC HEPATITIS B: SYSTEMATIC REVIEW AND META-
ANALYSIS**

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2013-003265.R1
Article Type:	Research
Date Submitted by the Author:	08-Jul-2013
Complete List of Authors:	Thiele, Maja; Odense University Hospital, Department of Gastroenterology and Hepatology Gluud, Lise; Copenhagen University Hospital Gentofte, Department of Medicine Dahl, Emilie; Copenhagen University, Faculty of Health Sciences Krag, Aleksander; Odense University Hospital, Department of Gastroenterology and Hepatology
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Evidence based practice, Pharmacology and therapeutics
Keywords:	Adult gastroenterology < GASTROENTEROLOGY, Hepatobiliary disease < GASTROENTEROLOGY, Hepatobiliary tumours < ONCOLOGY

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4 **ANTIVIRAL THERAPY FOR PREVENTION OF**
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7 **HEPATOCELLULAR CARCINOMA AND MORTALITY IN**
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10 **CHRONIC HEPATITIS B: SYSTEMATIC REVIEW AND META-**
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12 **ANALYSIS**
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19 **Short title:** Antiviral therapy for hepatitis B
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54 **Abstract word count: 298**
55

56
57 **Total word count: 5955**
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59
60

ABSTRACT

Background: The effect of antiviral therapy on clinical outcomes in chronic hepatitis B (HBV) is not established.

Objectives: To assess the effect of antiviral treatment (interferon and/or nucleoside analogues) versus placebo or no intervention on prevention of hepatocellular carcinoma (HCC) and mortality in chronic hepatitis B.

Design: Random effects pair-wise meta-analysis of randomised trials and observational studies.

Data sources: Electronic and manual searches were combined.

Study selection: Randomised controlled trials (RCTs) were included in the primary analyses. Observational studies were included in sensitivity analyses.

Data extraction: Two independent reviewers extracted data and evaluated bias control. The primary outcome measures were HCC incidence and mortality.

Data synthesis: We included eight RCTs, eight prospective cohort studies and 19 case-control studies with a total of 3433 patients allocated to antiviral therapy and 4625 controls. The maximum duration of follow up was 23 years. Randomised trials found no effect of antiviral therapy on HCC or mortality. Cohort studies found that antiviral therapy increased the risk of HCC (risk ratio 1.43; 95% confidence interval 1.06 to 1.95) whereas case control studies found a decreased risk of HCC in the intervention group (risk ratio 0.69; 95% confidence interval 0.54 to 0.88). There was a clear difference between the results of RCTs and observational studies (test for subgroup differences $P < 0.001$).

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4 Antiviral therapy did not affect mortality in cohort studies, but reduced mortality in case
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6 control studies (relative risk 0.71; 95% confidence interval 0.54 to 0.93; test for subgroup
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8 differences, P=0.406).
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10
11 **Conclusions:** The effect of antiviral therapy on clinical outcomes in HBV remains to be
12
13 established. Although there was a positive effect in the sensitivity analyses, the strength of
14
15 the evidence does not allow for extrapolation to clinical practice as research design plays
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17 an essential role in the overall assessment.
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21 **Trial registration number:** Prospero number CRD42013003881
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ARTICLE SUMMARY

Article focus

- The effect of antiviral treatment for chronic hepatitis B has been assessed using surrogate markers.
- An evaluation of the effect on hepatocellular carcinoma and mortality is missing.

Key messages

- Research design plays an essential role on hepatocellular carcinoma incidence estimates. As prospective cohorts and case-control series show opposing results, reports from such trials should be interpreted with caution.
- Sensitivity analyses show a positive effect of treatment on mortality.

Strengths and limitations of this study

- A large number of observational studies were included allowing for detailed sensitivity analyses with tests for subgroup differences.
- Only 8 randomised controlled trials were included.
- The effect of modern nucleos(t)ides could not be assessed as newer trials does not include placebo treated or untreated patients in the control groups.

INTRODUCTION

Worldwide, two billion people have been infected with hepatitis B. Chronic hepatitis B (HBV) may lead to hepatocellular carcinoma (HCC), cirrhosis and liver failure and each year, about 600 000 people die due to hepatitis¹⁻³. Globally, HCC is the fifth most common cause of cancer deaths in men, and the sixth in women⁴⁻⁶. Vaccine programs have decreased the incidence of HBV^{7 8}, but mortality from HBV related HCC and cirrhosis is increasing due to the high prevalence of chronically infected patients^{9 10}. The aim of antiviral treatment is to prevent progression to these clinical outcome measures¹¹⁻¹³. Recommended treatments include interferon and nucleos(t)ide analogues (NA)^{14 15}. A viral response may reduce the risk of HCC¹², but the results of clinical studies and meta-analyses on antiviral therapy are not consistent¹⁶⁻²⁴. One meta-analysis²⁵ found that antiviral therapy decreased liver-related mortality whereas a cohort series found decreased overall mortality in patients with a viral response to interferon²⁶. On the other hand, RCTs have failed to show an effect on HCC or mortality^{27 28}. We therefore conducted a systematic review of the evidence on antiviral treatment for prevention of HCC and mortality in patients with HBV.

METHODS

Scope

This systematic review evaluates the effects of antiviral therapy versus placebo or no intervention on prevention of HCC and mortality in patients with HBV. The review is based on a registered written protocol (Prospero number CRD42013003881) according to the methods specified in the Cochrane Handbook for Reviews on Interventions²⁹ and the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies³⁰. For a more detailed description of the methods, please see the MOOSE checklist (appendix 1).

Data sources

Eligible trials were identified through electronic and manual searches. Electronic searches were performed in MEDLINE (1966-2012), EMBASE (1928-2012), and Web of Science (1900-2012). Literature searches included keywords for HCC, chronic hepatitis B, and antiviral treatment. Manual searches included scanning of reference lists in relevant papers and conference proceedings and the International Clinical Trials Registry Platform.

Study selection

Our primary analyses included RCTs (primary analyses) on antiviral interventions (interferon and/or NA) versus placebo or no intervention for patients with HBV who had not previously received antiviral therapy (treatment naïve). Due to the expected prognosis and the duration of follow up necessary to evaluate intervention effects on clinical outcome measures in HBV, observational studies were included in sensitivity analyses. The primary outcome measures were HCC diagnosed using recommended criteria^{31 32} and all-cause

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4 mortality. To avoid prevalent cases of HCC the outcomes were assessed after at least 12
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6 months of follow up. Some studies did not perform screening ultrasonography and would
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8 therefore not detect small HCC present at inclusion. Twelve months was therefore
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10 chosen as a limit. The secondary outcome measure was HCC related mortality.

14 **Data extraction and quality assessment**

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17 Two authors extracted data independently. When data were not available in the published
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19 reports, additional information was retrieved through correspondence with the primary
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21 investigators.

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24 The Cochrane Collaboration's Tool for Assessing Risk of Bias was used to evaluate bias
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26 control in RCTs. The assessment included the randomisation methods (allocation
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28 sequence generation and allocation concealment), blinding (of participants, personnel and
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30 investigators), the completeness of outcome data, reporting of data and other biases³³. All
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32 observational studies were classed as having a high risk of bias. Based on the MOOSE
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34 guidelines, the assessment of potential sources of bias within observational studies
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36 included documentation of how data were classified and coded (multiple raters, blinding
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38 and interrater reliability), assessment of confounding (comparability of cases and controls
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40 in studies where appropriate) and blinding of quality assessors, stratification or regression
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42 on possible predictors of study results.
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48 **Data synthesis and analysis**

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50 Statistics were performed using Stata Version 12 (Statacorp, College Station, TX, USA)
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52 and Trial Sequential Analysis (CTU, Copenhagen, Denmark). Meta-analyses were
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54 performed with results expressed as risk ratios, 95% CI and I^2 as a marker of
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4 heterogeneity. For meta-analyses showing a statistically significant effect the number
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6 needed to treat was calculated based on the risk difference. Initial sensitivity analyses
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8 included repeating all meta-analyses using both random and fixed effect models. The
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10 results of these analyses were only reported if the conclusions differed. Regression
11
12 analyses were performed to assess for publication bias and other small study effects
13
14 (Egger's test). Sequential analyses were performed for meta-analyses showing an
15
16 intervention effect after adjusting for the risk of bias associated with cumulative testing³⁴.
17
18 The sequential analysis was performed using a random-effects model, alpha (5%), power
19
20 (80%) and the incidence rates and the intervention effects identified in the meta-analyses.
21
22 Pre-planned sensitivity analyses were performed with inclusion of observational studies.
23
24 These analyses were performed stratified by study design (RCT, prospective cohort or
25
26 case-control study) and with fixed-effect inverse variance models that compared the
27
28 results of subgroups. The result of the subgroup comparisons was expressed as P values
29
30 (test for subgroup differences). Additional sensitivity analyses were performed to evaluate
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32 the influence of bias control (limiting the analysis to trials with adequate randomisation),
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34 the type of antiviral therapy (comparing interferon, NA or both), and the effect HCC
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36 screening (comparing the results of trials with or without screening). Finally, subgroup
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38 analyses including only patients with cirrhosis were performed.
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RESULTS

Literature searches and study inclusion

The electronic and manual searches identified 27 474 potentially relevant records (figure 1). After excluding duplicates and studies that did not fulfil our inclusion criteria, 36 references referring to eight RCTs, eight prospective cohort studies and 19 case control studies were included^{26-28 35-67}.

Characteristics of included RCTs and observational studies

The RCTs were conducted in Europe (n=4), Asia (n=2) and Africa (n=2). The duration of follow up ranged from one to 11 years. One trial performed HCC screening. Six trials assessed interferon and two trials NA (table 1). A total of 840 patients received antiviral therapy and 447 patients received placebo or no intervention. The proportion of men ranged from 70 to 100% and the mean age from 33 to 44 years. The proportion of patients with cirrhosis at inclusion ranged from zero to 66% (table 2). The proportion of patients with a virological response ranged from seven to 58% in the treatment group and from one to 22% of controls. A biochemical response was achieved for 14 to 66% of patients in the treatment and one to 20% of controls. The randomisation methods were described as adequate in three trials (table 3).

The prospective cohorts and case control studies were conducted in Europe (n=12), Asia (n=13), North America (n=1) and South America (n=1). The duration of follow up ranged from two to 23 years. HCC screening was performed in all prospective cohort studies and in 13 of the case control studies. Eighteen studies assessed interferon, seven assessed NA and two combined therapy with interferon and NA (table 1). A total of 2593 patients

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4 received antiviral therapy and 4178 patients received no intervention. The proportion of
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6 men ranged from 53 to 95% and the mean age from 27 to 65 years. The proportion of
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8 patients with cirrhosis ranged from zero to 100% (table 2). In the prospective cohorts, the
9
10 proportion of patients with a virological response in the treatment and control groups was
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12 23 to 69% and zero to 23%, respectively. A biochemical response was achieved for 23 to
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14 69% of patients in the treatment groups and 31% in the control group (only reported in one
15
16 study). In the case control series, the proportion of patients with a virological response in
17
18 the treatment and control group ranged from 7 to 78% and two to 100%, respectively. A
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20 biochemical response in the two groups was 27 to 68% and four to 51%, respectively.
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25 **Prevention of HCC**

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28 HCC was diagnosed in 22 of 768 patients in the treatment group versus 19 of 391 controls
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30 (relative risk 0.58; 95% confidence interval 0.32-1.07; $I^2=0\%$). There was no evidence of
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32 small study effects (Egger's test, $P=0.269$) and no difference between subgroups of trials
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34 assessing interferon or NA (test for subgroup differences $P=0.854$). The overall result was
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36 confirmed in sensitivity analyses including RCTs with a low risk of bias and trials with HCC
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38 screening.
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42 Sensitivity analyses including prospective cohort studies and case control studies were
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44 performed. In the cohort studies, HCC was diagnosed in 51 of 436 patients in the
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46 treatment group and 174 of 1853. In the case control studies the numbers were 99 of 1778
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48 and 201 of 1827 patients, respectively. A meta-analysis that combined RCTs and
49
50 observational studies found no effect of antiviral therapy on HCC (relative risk 0.88; 95%
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52 confidence interval 0.73 to 1.05; $I^2=63\%$). There was no evidence of small study effects
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54 (Egger's test $P=0.730$). Subgroup analyses showed a clear difference between the RCTs,
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4 prospective cohorts and case control studies (test for subgroup differences $P < 0.001$)
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6 (figure 2). The prospective cohort studies found that antiviral therapy increased the risk of
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8 HCC (relative risk 1.44; 95% confidence interval 1.06 to 1.95) whereas the case control
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10 studies found that antiviral therapy reduced the risk of HCC (relative risk 0.69; 95%
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12 confidence interval 0.54 to 0.88). Due to the high heterogeneity, a post-hoc meta-
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14 regression analysis was performed. We evaluated study and patient characteristics not
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16 accounted for in the sensitivity analyses, which may have influenced the result. No
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18 modifiers were found when adjusting for the following variables: proportion of men
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20 (coefficient -0.074; $P = 0.08$) mean age of treated patients at inclusion (coefficient 0.020;
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22 $P = 0.94$), mean age of untreated patients at inclusion (coefficient 0.121; $P = 0.65$),
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24 proportion with cirrhosis at inclusion (coefficient -0.001; $P = 0.76$), and region of trial
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26 (coefficient -0.394; $P = 0.55$).
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32 To further evaluate the influence of bias on overall results, we performed additional
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34 subgroup analysis in which trials were stratified for HCC screening. The analysis found
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36 eight trials that did not perform HCC screening (relative risk 0.40; 95% confidence interval
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38 0.26 to 0.63) and 18 trials that did perform HCC screening (relative risk 1.03; 95%
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40 confidence interval 0.84 to 1.25). The results of subgroups were clearly different (test for
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42 subgroup differences $P < 0.001$).
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46 Sensitivity analyses were performed to evaluate the risk of HCC among patients with
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48 cirrhosis. In the RCTs, one of 20 patients in the treatment group and two of 12 controls
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50 developed HCC (relative risk 0.75; 95% confidence interval 0.10 to 5.77). In the
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52 prospective cohort studies 32 of 184 versus 142 of 482 patients developed HCC whereas
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54 the numbers were 63 of 680 versus 161 of 955, respectively for case control studies.
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4 Overall, antiviral therapy reduced the risk of HCC when including data from RCTs and
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6 observational studies (relative risk 0.74; 95% confidence interval 0.57 to 0.96; $I^2=0\%$;
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8 number needed to treat 28 patients) (fig 3). The results of RCTs and observational studies
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10 were similar (test for subgroup differences $P=0.159$). There was no evidence of small
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12 study effects (Egger's test $P=0.890$). In trial sequential analysis, the monitoring and alpha-
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14 spending boundary did not cross suggesting that the result was not robust to adjustment
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16 for multiple testing.
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20 21 **Mortality**

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23 In the RCTs, there was no difference in mortality between the treatment and control group
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25 (21 of 508 versus 9 of 271 patients; relative risk 1.24; 95% confidence interval 0.58 to
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27 2.66; $I^2=0\%$). No evidence of small study effects (Egger's test $P=0.783$) and no difference
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29 between trials stratified by treatment (test for subgroup differences $P=0.668$) or HCC
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31 screening ($P=0.828$). In the observational studies, the number of patients in the treatment
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33 and control groups who died was 51 of 655 versus 247 of 2231 for prospective cohort
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35 studies and 79 of 506 versus 92 of 413 in the cohort studies. When combining RCTs and
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37 observational studies, random effects meta-analysis showed that antiviral treatment
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39 decreased mortality (relative risk 0.76; 95% confidence interval 0.62 to 0.95; $I^2=14\%$;
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41 number needed to treat 77; Egger's test $P=0.487$) (fig 4). There was no difference
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43 between RCTs and observational studies (test for subgroup differences $P=0.406$). In the
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45 trial sequential analysis, the monitoring boundary crossed the alpha-spending boundary in
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47 2004 suggesting that the meta-analysis was robust to adjustments for multiple testing.
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53 Only observational studies reported mortality in patients with cirrhosis. The number of
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55 patients who died in the intervention and control groups was 36 of 298 versus 141 of 499
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4 (relative risk 0.61; 95% confidence interval 0.44 to 0.86; $I^2=9%$; number needed to treat 16
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6 patients). There were no small study effects (Egger's test $P=0.533$) and no difference
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8 between prospective cohort and case control studies (test for subgroup differences
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10 $P=0.292$).

14 **HCC related mortality**

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16 Antiviral therapy had no effect on HCC related mortality (3 of 282 versus 2 of 154; relative
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18 risk 0.50; 95% confidence interval 0.10 to 2.44; $I^2=0%$, $n=2$ RCT). Including data from
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20 observational studies had little influence on the overall result (38 of 1233 versus 144 of
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22 2632; relative risk 0.83; 95% confidence interval 0.5 to 1.20; $I^2=0%$; Egger's test $P=0.248$).
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25 There was no difference between subgroups of trials stratified by design (test for subgroup
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27 differences $P=0.481$).
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DISCUSSION

This systematic review found that the evidence of the effect of antiviral therapy on clinical outcomes in HBV is weak. RCTs found no benefit of treatment on HCC, mortality or HCC related mortality in HBV. The total number of patients and duration of follow up may be too small to determine clinical effects. The inclusion of observational studies did not strengthen the overall findings because there was clear evidence of bias suggesting that the study design was closely related to the estimated treatment effects. The prospective cohort studies found that antiviral therapy increased the risk of HCC and had no effect on mortality. The case control studies found that antiviral therapy reduced both HCC and mortality. These findings suggest that detection and ascertainment bias as well as confounding by indication had a considerable influence on the overall result, which may explain why previous meta-analyses have disagreed in their assessment of the benefit of antiviral therapy^{16-18 20 21 23 24}. The importance of detection bias was underlined in the subgroup analysis of HCC screening. No intervention effect was found in trials that performed systematic HCC screening.

The main limitation of our review is the limited number of RCTs. Only one of the included trials had prevention of HCC as a primary outcome measure²⁷ and none were designed to evaluate the effect on mortality or HCC related mortality. Tests to evaluate the robustness of the results (including Egger's test) were difficult to interpret.

The current recommendation to treat patients with HBV is primarily based on surrogate outcomes. At present the evidence supporting the use of virological markers as surrogate outcomes is weak. The fact that some studies have found a correlation between a

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4 virological response and improved liver histology does not necessarily validate their use as
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6 surrogate outcomes. Previous evidence shows that interventions supported by surrogate
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8 markers may in fact have no benefit or even harmful effects on clinical outcome
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10 measures⁶⁸. Still, our findings are not sufficiently convincing and do not allow for changes
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12 in clinical practice.
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16 Another limitation of the current review is our failure to extract data for analyses of
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18 treatment responders versus non-responders. However, only six cases of HCC were
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20 reportedly diagnosed in patients with biochemical or viral treatment response. This
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22 suggests that treatment response does not lead to elimination of the HCC risk, but
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24 probably decreases HCC incidence compared to non- or partial-responders. This would be
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26 in line with previous findings^{19 25}. The majority of included trials in the current review
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28 assessed first generation NA and interferon, as reflected in low response rates. It was
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30 however not within the scope of the review to investigate modern antiviral treatments, as
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32 we included untreated control groups. Newer treatments will likely result in more patients
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34 achieving sustained suppression of HBV-DNA. It is therefore possible that the current
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36 review underestimates a potential treatment effect. It would also have been of interest had
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38 we been able to adjust for other common risk factors for HCC such as non-alcoholic
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40 steatohepatitis, alcoholic liver disease and coinfection with hepatitis C, hepatitis D and
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42 human immunodeficiency virus. Although these data were extracted, there was not
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44 enough data to allow for analyses.
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50 There are several potential explanations for the discrepancies between RCTs and
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52 observational studies⁶⁹. The fact that only prospective cohort studies found an increased
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54 risk of HCC among patients receiving antiviral therapy opposes speculations that the
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4 treatment affected HCC development. The findings are more likely to reflect baseline
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6 differences in the viral load, genotype and degree of liver disease. The degree of
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8 monitoring in the treatment and control group is also likely to differ and may lead to
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10 detection bias. The importance of detection bias is further supported by the subgroup
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12 differences observed according to HCC screening. The case control studies are likely to
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14 have an even higher risk of bias, as confounding by indication and ascertainment bias is
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16 likely to exist in retrospective studies. Reporting bias should also be considered³³.
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21 The subgroup differences with regards to type of intervention suggests a possible anti-
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23 carcinogenic effect of interferon, as seen in HCV⁷⁰. We additionally found a decrease in
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25 both HCC incidence and overall mortality in sensitivity analyses of patients with cirrhosis.
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27 This could support the case for continued treatment of patients with cirrhosis.
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31 We found a beneficial effect of interferon and/or NA on mortality in HBV when including
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33 RCTs and observational studies in chronic HBV patients. The assessment of mortality is
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35 robust to bias⁷¹. Accordingly, our subgroup analysis showed no clear relation between the
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37 results and the study design. HCC mortality is more prone to bias. Whether antiviral
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39 treatment for HBV decreases mortality except from HCC is unknown.
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43 In conclusion, antiviral treatment for HBV has no proven effect on the clinical outcomes
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45 HCC and mortality. Bias has a paramount impact on treatment effect estimates in
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47 observational studies and we recommend a critical approach to conclusions drawn in such
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49 studies. Future trials on antiviral treatment for HBV should be designed to show an effect
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51 on clinical endpoints rather than surrogate markers.
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4 **Contributors:** MT, LLG and AK conceived the idea and design. MT and EKD collected
5 and assembled data. MT, LLG and AK analysed and interpreted the data. MT and LLG
6 drafted the manuscript. LLG, EKD and AK revised the manuscript for important intellectual
7 content. All authors discussed and approved of the final version of the manuscript. MT is
8 guarantor.
9

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15
16 **Competing interests:** All authors have completed the ICMJE uniform disclosure form at
17 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author)
18 and declare: no support from any organisation for the submitted work; no financial
19 relationships with any organisations that might have an interest in the submitted work in
20 the previous three years; no other relationships or activities that could appear to have
21 influenced the submitted work.
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30 **Ethical approval:** Not required.
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34 **Data sharing:** Dataset available from the corresponding author.
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36

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FIGURE LEGENDS

Figure 1. Study flow diagram.

Figure 2. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on hepatocellular carcinoma in patients with chronic hepatitis B, subgroups according to trial design.

Figure 3. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on hepatocellular carcinoma in patients with chronic hepatitis B and cirrhosis, subgroups according to trial design.

Figure 4. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on mortality in patients with chronic hepatitis B, subgroups according to trial design.

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8 **ANTIVIRAL THERAPY FOR PREVENTION OF**
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10 **HEPATOCELLULAR CARCINOMA AND MORTALITY IN**
11 **CHRONIC HEPATITIS B: SYSTEMATIC REVIEW AND META-**
12 **ANALYSIS**
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21 **Short title:** Antiviral therapy for hepatitis B
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50 **Abstract word count: 298**
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ABSTRACT

Background: The effect of antiviral therapy on clinical outcomes in chronic hepatitis B (HBV) is not established.

Objectives: To assess the effect of antiviral treatment (interferon and/or nucleo(t)side analogues) versus placebo or no intervention on prevention of hepatocellular carcinoma (HCC) and mortality in chronic hepatitis B.

Design: Random effects pair-wise meta-analysis of randomised trials and observational studies.

Data sources: Electronic and manual searches were combined.

Study selection: Randomised controlled trials (RCTs) were included in the primary analyses. Observational studies were included in sensitivity analyses.

Data extraction: Two independent reviewers extracted data and evaluated bias control. The primary outcome measures were HCC incidence and mortality.

Data synthesis: We included eight RCTs, eight prospective cohort studies and 19 case-control studies with a total of 3433 patients allocated to antiviral therapy and 4625 controls. The maximum duration of follow up was 23 years. Randomised trials found no effect of antiviral therapy on HCC or mortality. Cohort studies found that antiviral therapy increased the risk of HCC (risk ratio 1.43; 95% confidence interval 1.06 to 1.95) whereas case control studies found a decreased risk of HCC in the intervention group (risk ratio 0.69; 95% confidence interval 0.54 to 0.88). There was a clear difference between the results of RCTs and observational studies (test for subgroup differences $P < 0.001$).

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8 Antiviral therapy did not affect mortality in cohort studies, but reduced mortality in case
9 control studies (relative risk 0.71; 95% confidence interval 0.54 to 0.93; test for subgroup
10 differences, P=0.406).
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14 **Conclusions:** The effect of antiviral therapy on clinical outcomes in HBV remains to be
15 established. Although there was a positive effect in the sensitivity analyses, the strength of
16 the evidence does not allow for extrapolation to clinical practice as research design plays
17 an essential role in the overall assessment.
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23 **Trial registration number:** Prospero number CRD42013003881
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ARTICLE SUMMARY

Article focus

- The effect of antiviral treatment for chronic hepatitis B has been assessed using surrogate markers.
- An evaluation of the effect on hepatocellular carcinoma and mortality is missing.

Key messages

- Research design plays an essential role on hepatocellular carcinoma incidence estimates. As prospective cohorts and case-control series show opposing results, reports from such trials should be interpreted with caution.
- Sensitivity analyses show a positive effect of treatment on mortality.

Strengths and limitations of this study

- A large number of observational studies were included allowing for detailed sensitivity analyses with tests for subgroup differences.
- Only 8 randomised controlled trials were included.
- The effect of modern nucleos(t)ides could not be assessed as newer trials does not include placebo treated or untreated patients in the control groups.

INTRODUCTION

Worldwide, two billion people have been infected with hepatitis B. Chronic hepatitis B (HBV) may lead to hepatocellular carcinoma (HCC), cirrhosis and liver failure and each year, about 600 000 people die due to hepatitis¹⁻³. Globally, HCC is the fifth most common cause of cancer deaths in men, and the sixth in women⁴⁻⁶. Vaccine programs have decreased the incidence of HBV^{7 8}, but mortality from HBV related HCC and cirrhosis is increasing due to the high prevalence of chronically infected patients^{9 10}. The aim of antiviral treatment is to prevent progression to these clinical outcome measures¹¹⁻¹³. Recommended treatments include interferon and nucleos(t)ide analogues (NA)^{14 15}. A viral response may reduce the risk of HCC¹², but the results of clinical studies and meta-analyses on antiviral therapy are not consistent¹⁶⁻²⁴. One meta-analysis²⁵ found that antiviral therapy decreased liver-related mortality whereas a cohort series found decreased overall mortality in patients with a viral response to interferon²⁶. On the other hand, RCTs have failed to show an effect on HCC or mortality^{27 28}. We therefore conducted a systematic review of the evidence on antiviral treatment for prevention of HCC and mortality in patients with HBV.

METHODS

Scope

This systematic review evaluates the effects of antiviral therapy versus placebo or no intervention on prevention of HCC and mortality in patients with HBV. The review is based on a registered written protocol (Prospero number CRD42013003881) according to the methods specified in the Cochrane Handbook for Reviews on Interventions²⁹ and the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies³⁰. For a more detailed description of the methods, please see the MOOSE checklist (appendix 1).

Data sources

Eligible trials were identified through electronic and manual searches. Electronic searches were performed in MEDLINE (1966-2012), EMBASE (1928-2012), and Web of Science (1900-2012). Literature searches included keywords for HCC, chronic hepatitis B, and antiviral treatment. Manual searches included scanning of reference lists in relevant papers and conference proceedings and the International Clinical Trials Registry Platform.

Study selection

Our primary analyses included RCTs (primary analyses) on antiviral interventions (interferon and/or NA) versus placebo or no intervention for patients with HBV who had not previously received antiviral therapy (treatment naïve). Due to the expected prognosis and the duration of follow up necessary to evaluate intervention effects on clinical outcome measures in HBV, observational studies were included in sensitivity analyses. The primary outcome measures were HCC diagnosed using recommended criteria^{31 32} and all-cause

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8 mortality. To avoid prevalent cases of HCC the outcomes were assessed after at least 12
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10 months of follow up. Some studies did not perform screening ultrasonography and would
11 therefore not detect small HCC present at inclusion. Twelve months was therefore
12 chosen as a limit. The secondary outcome measure was HCC related mortality.
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16 17 **Data extraction and quality assessment**

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19 Two authors extracted data ~~in an~~ independently manner. When data were not available in
20 the published reports, additional information was retrieved through correspondence with
21 the primary investigators.
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25 The Cochrane Collaboration's Tool for Assessing Risk of Bias was used to evaluate bias
26 control in RCTs. The assessment included the randomisation methods (allocation
27 sequence generation and allocation concealment), blinding (of participants, personnel and
28 investigators), the completeness of outcome data, reporting of data and other biases³³. All
29 observational studies were classed as having a high risk of bias. Based on the MOOSE
30 guidelines, the assessment of potential sources of bias within observational studies
31 included documentation of how data were classified and coded (multiple raters, blinding
32 and interrater reliability), assessment of confounding (comparability of cases and controls
33 in studies where appropriate) and blinding of quality assessors, stratification or regression
34 on possible predictors of study results.
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45 46 **Data synthesis and analysis**

47 Statistics were performed using Stata Version 12 (Statacorp, College Station, TX, USA)
48 and Trial Sequential Analysis (CTU, Copenhagen, Denmark). Meta-analyses were
49 performed with results expressed as risk ratios, 95% CI and I^2 as a marker of
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8 heterogeneity. For meta-analyses showing a statistically significant effect the number
9 needed to treat was calculated based on the risk difference. Initial sensitivity analyses
10 included repeating all meta-analyses using both random and fixed effect models. The
11 results of these analyses were only reported if the conclusions differed. Regression
12 analyses were performed to assess for publication bias and other small study effects
13 (Egger's test). Sequential analyses were performed for meta-analyses showing an
14 intervention effect after adjusting for the risk of bias associated with cumulative testing³⁴.
15 The sequential analysis was performed using a random-effects model, alpha (5%), power
16 (80%) and the incidence rates and the intervention effects identified in the meta-analyses.
17 Pre-planned sensitivity analyses were performed with inclusion of observational studies.
18 These analyses were performed stratified by study design (RCT, prospective cohort or
19 case-control study) and with fixed-effect inverse variance models that compared the
20 results of subgroups. The result of the subgroup comparisons was expressed as P values
21 (test for subgroup differences). Additional sensitivity analyses were performed to evaluate
22 the influence of bias control (limiting the analysis to trials with adequate randomisation),
23 the type of antiviral therapy (comparing interferon, NA or both), and the effect HCC
24 screening (comparing the results of trials with or without screening). Finally, subgroup
25 analyses including only patients with cirrhosis were performed.
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RESULTS

Literature searches and study inclusion

The electronic and manual searches identified 27 474 potentially relevant records (figure 1). After excluding duplicates and studies that did not fulfil our inclusion criteria, 36 references referring to eight RCTs, eight prospective cohort studies and 19 case control studies were included^{26-28 35-67}.

Characteristics of included RCTs and observational studies

The RCTs were conducted in Europe (n=4), Asia (n=2) and Africa (n=2). The duration of follow up ranged from one to 11 years. One trial performed HCC screening. Six trials assessed interferon and two trials NA (table 1). [A total of 840 patients received antiviral therapy and 447 patients received placebo or no intervention.](#) The proportion of men ranged from 70 to 100% and the mean age from 33 to 44 years. The proportion of patients with cirrhosis at inclusion ranged from zero to 66% (table 2). The proportion of patients with a virological response ranged from seven to 58% in the treatment group and from one to 22% of controls. A biochemical response was achieved for 14 to 66% of patients in the treatment and one to 20% of controls. The randomisation methods were described as adequate in three trials (table 3).

The prospective cohorts and case control studies were conducted in Europe (n=12), Asia (n=13), North America (n=1) and South America (n=1). The duration of follow up ranged from two to 23 years. HCC screening was performed in all prospective cohort studies and in 13 of the case control studies. Eighteen studies assessed interferon, seven assessed NA and two combined therapy with interferon and NA (table 1). [A total of 2593 patients](#)

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8 [received antiviral therapy and 4178 patients received no intervention](#). The proportion of
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10 men ranged from 53 to 95% and the mean age from 27 to 65 years. The proportion of
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12 patients with cirrhosis ranged from zero to 100% (table 2). In the prospective cohorts, the
13
14 proportion of patients with a virological response in the treatment and control groups was
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16 23 to 69% and zero to 23%, respectively. A biochemical response was achieved for 23 to
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18 69% of patients in the treatment groups and 31% in the control group (only reported in one
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20 study). In the case control series, the proportion of patients with a virological response in
21
22 the treatment and control group ranged from 7 to 78% and two to 100%, respectively. A
23
24 biochemical response in the two groups was 27 to 68% and four to 51%, respectively.

25 26 **Prevention of HCC**

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28 HCC was diagnosed in 22 of [840-768](#) patients in the treatment group versus 19 of [447-391](#)
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30 controls (relative risk 0.58; 95% confidence interval 0.32-1.07; $I^2=0\%$). There was no
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32 evidence of small study effects (Egger's test, $P=0.269$) and no difference between
33
34 subgroups of trials assessing interferon or NA (test for subgroup differences $P=0.854$).
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36 The overall result was confirmed in sensitivity analyses including RCTs with a low risk of
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38 bias and trials with HCC screening.

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40 Sensitivity analyses including prospective cohort studies and case control studies were
41
42 performed. In the cohort studies, HCC was diagnosed in 51 of [689-436](#) patients in the
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44 treatment group and 174 of [2283-1853](#). In the case control studies the numbers were 99 of
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46 [4904-1778](#) and 201 of [4895-1827](#) patients, respectively. A meta-analysis that combined
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48 RCTs and observational studies found no effect of antiviral therapy on HCC (relative risk
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50 0.88; 95% confidence interval 0.73 to 1.05; $I^2=63\%$). There was no evidence of small study
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52 effects (Egger's test $P=0.730$). Subgroup analyses showed a clear difference between the
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8 RCTs, prospective cohorts and case control studies (test for subgroup differences
9 $P < 0.001$) (figure 2). The prospective cohort studies found that antiviral therapy increased
10 the risk of HCC (relative risk 1.44; 95% confidence interval 1.06 to 1.95) whereas the case
11 control studies found that antiviral therapy reduced the risk of HCC (relative risk 0.69; 95%
12 confidence interval 0.54 to 0.88). Due to the high heterogeneity, a post-hoc meta-
13 regression analysis was performed. We evaluated study and patient characteristics not
14 accounted for in the sensitivity analyses, which may have influenced the result. No
15 modifiers were found when adjusting for the following variables: proportion of men
16 (coefficient -0.074; $P = 0.08$) mean age of treated patients at inclusion (coefficient 0.020;
17 $P = 0.94$), mean age of untreated patients at inclusion (coefficient 0.121; $P = 0.65$),
18 proportion with cirrhosis at inclusion (coefficient -0.001; $P = 0.76$), and region of trial
19 (coefficient -0.394; $P = 0.55$).

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23 To further evaluate the influence of bias on overall results, we performed additional
24 subgroup analysis in which trials were stratified for HCC screening. The analysis found
25 eight trials that did not perform HCC screening (relative risk 0.40; 95% confidence interval
26 0.26 to 0.63) and 18 trials that did perform HCC screening (relative risk 1.03; 95%
27 confidence interval 0.84 to 1.25). The results of subgroups were clearly different (test for
28 subgroup differences $P < 0.001$).

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44 Sensitivity analyses were performed to evaluate the risk of HCC among patients with
45 cirrhosis. In the RCTs, one of 20 patients in the treatment group and two of 12 controls
46 developed HCC (relative risk 0.75; 95% confidence interval 0.10 to 5.77). In the
47 prospective cohort studies 32 of 184 versus 142 of 482 patients developed HCC whereas
48 the numbers were 63 of 680 versus 161 of 955, respectively for case control studies.
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8 Overall, antiviral therapy reduced the risk of HCC when including data from RCTs and
9 observational studies (relative risk 0.74; 95% confidence interval 0.57 to 0.96; $I^2=0\%$;
10 number needed to treat 28 patients) (fig 3). The results of RCTs and observational studies
11 were similar (test for subgroup differences $P=0.159$). There was no evidence of small
12 study effects (Egger's test $P=0.890$). In trial sequential analysis, the monitoring and alpha-
13 spending boundary did not cross suggesting that the result was not robust to adjustment
14 for multiple testing.
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21 22 **Mortality**

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24 In the RCTs, there was no difference in mortality between the treatment and control group
25 (21 of 840-508 versus 9 of 447-271 patients; relative risk 1.24; 95% confidence interval
26 0.58 to 2.66; $I^2=0\%$). No evidence of small study effects (Egger's test $P=0.783$) and no
27 difference between trials stratified by treatment (test for subgroup differences $P=0.668$) or
28 HCC screening ($P=0.828$). In the observational studies, the number of patients in the
29 treatment and control groups who died was 51 of 689-655 versus 247 of 2283-2231 for
30 prospective cohort studies and 794 of 4904-506 versus 92 of 4895-413 in the cohort
31 studies. When combining RCTs and observational studies, random effects meta-analysis
32 showed that antiviral treatment decreased mortality (relative risk 0.76; 95% confidence
33 interval 0.62 to 0.95; $I^2=14\%$; number needed to treat 77; Egger's test $P=0.487$) (fig 4).
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35 There was no difference between RCTs and observational studies (test for subgroup
36 differences $P=0.406$). In the trial sequential analysis, the monitoring boundary crossed the
37 alpha-spending boundary in 2004 suggesting that the meta-analysis was robust to
38 adjustments for multiple testing.
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8 Only observational studies reported mortality in patients with cirrhosis. The number of
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10 patients who died in the intervention and control groups was 36 of ~~864-298~~ versus 141 of ~~4~~
11 ~~477499~~ (relative risk 0.61; 95% confidence interval 0.44 to 0.86; $I^2=9%$; number needed to
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13 treat 16 patients). There were no small study effects (Egger's test $P=0.533$) and no
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15 difference between prospective cohort and case control studies (test for subgroup
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17 differences $P=0.292$).
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20 **HCC related mortality**

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22 Antiviral therapy had no effect on HCC related mortality (3 of ~~840-282~~ versus 2 of ~~447154~~;
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24 relative risk 0.50; 95% confidence interval 0.10 to 2.44; $I^2=0%$, $n=2$ RCT). Including data
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26 from observational studies had little influence on the overall result (~~41-38~~ of ~~3433-1233~~
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28 versus ~~146-144~~ of ~~46252632~~; relative risk 0.83; 95% confidence interval 0.5 to 1.20;
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30 $I^2=0%$; Egger's test $P=0.248$). There was no difference between subgroups of trials
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32 stratified by design (test for subgroup differences $P=0.481$).
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DISCUSSION

This systematic review found that the evidence of the effect of antiviral therapy on clinical outcomes in HBV is weak. RCTs found no benefit of treatment on HCC, mortality or HCC related mortality in HBV. The total number of patients and duration of follow up may be too small to determine clinical effects. The inclusion of observational studies did not strengthen the overall findings because there was clear evidence of bias suggesting that the study design was closely related to the estimated treatment effects. The prospective cohort studies found that antiviral therapy increased the risk of HCC and had no effect on mortality in HBV. The case control studies found that antiviral therapy reduced both HCC and mortality. These findings suggest that detection and ascertainment bias as well as confounding by indication had a considerable influence on the overall result, which may explain why previous meta-analyses have disagreed in their assessment of the benefit of antiviral therapy^{16-18 20 21 23 24}. The importance of detection bias was underlined in the subgroup analysis of HCC screening. No intervention effect was found in trials that performed systematic HCC screening.

The main limitation of our review is the limited number of RCTs. Only one of the included trials had prevention of HCC as a primary outcome measure²⁷ and none were designed to evaluate the effect on mortality or HCC related mortality. Tests to evaluate the robustness of the results (including Egger's test) were difficult to interpret.

The current recommendation to treat patients with HBV is primarily based on surrogate outcomes. At present the evidence supporting the use of virological markers as surrogate outcomes is weak. The fact that some studies have found a correlation between a

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8 virological response and improved liver histology does not necessarily validate their use as
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10 surrogate outcomes. Previous evidence shows that interventions supported by surrogate
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12 markers may in fact have no benefit or even harmful effects on clinical outcome
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14 measures⁶⁸. Still, our findings are not sufficiently convincing and do not allow for changes
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16 in clinical practice.

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18 Another limitation of the current review is our failure to extract data for analyses of
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20 treatment responders versus non-responders. However, only six cases of HCC were
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22 reportedly diagnosed in patients with biochemical or viral treatment response. This
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24 suggests that treatment response does not lead to elimination of the HCC risk, but
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26 probably decreases HCC incidence compared to non- or partial-responders. This would be
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28 in line with previous findings^{19,25}. The majority of included trials in the current review
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30 assessed first generation NA and interferon, as reflected in low response rates. It was
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32 however not within the scope of the review to investigate modern antiviral treatments, as
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34 we included untreated control groups. Newer treatments will likely result in more patients
35
36 achieving sustained suppression of HBV-DNA. It is therefore possible that the current
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38 review underestimates a potential treatment effect. It would also have been of interest had
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40 we been able to adjust for other common risk factors for HCC such as non-alcoholic
41
42 steatohepatitis, alcoholic liver disease and coinfection with hepatitis C, hepatitis D and
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44 human immunodeficiency virus. Although these data were extracted, there was not
45
46 enough data to allow for analyses.

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48 There are several potential explanations for the discrepancies between RCTs and
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50 observational studies⁶⁹. The fact that only prospective cohort studies found an increased
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52 risk of HCC among patients receiving antiviral therapy opposes speculations that the
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8 treatment affected HCC development. The findings are more likely to reflect baseline
9 differences in the viral load, genotype and degree of liver disease. The degree of
10 monitoring in the treatment and control group is also likely to differ and may lead to
11 detection bias. The importance of detection bias is further supported by the subgroup
12 differences observed according to HCC screening. The case control studies are likely to
13 have an even higher risk of bias, as confounding by indication and ascertainment bias is
14 likely to exist in retrospective studies. Reporting bias should also be considered³³.
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22 The subgroup differences with regards to type of intervention suggests a possible anti-
23 carcinogenic effect of interferon, as seen in HCV⁷⁰. We additionally found a decrease in
24 both HCC incidence and overall mortality in sensitivity analyses of patients with cirrhosis.
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26 This could support the case for continued treatment of patients with cirrhosis.
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30 We found a beneficial effect of interferon and/or NA on mortality in HBV when including
31 RCTs and observational studies in chronic HBV patients. The assessment of mortality is
32 robust to bias⁷¹. Accordingly, our subgroup analysis showed no clear relation between the
33 results and the study design. HCC mortality is more prone to bias. Whether antiviral
34 treatment for HBV decreases mortality except from HCC is unknown.
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40 In conclusion, antiviral treatment for HBV has no proven effect on the clinical outcomes
41 HCC and mortality. Bias has a paramount impact on treatment effect estimates in
42 observational studies and we recommend a critical approach to conclusions drawn in such
43 studies. Future trials on antiviral treatment for HBV should be designed to show an effect
44 on clinical endpoints rather than surrogate markers.
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8 **Contributors:** MT, LLG and AK conceived the idea and design. MT and EKD collected
9 and assembled data. MT, LLG and AK analysed and interpreted the data. MT and LLG
10 drafted the manuscript. LLG, EKD and AK revised the manuscript for important intellectual
11 content. All authors discussed and approved of the final version of the manuscript. MT is
12 guarantor.
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18 **Competing interests:** All authors have completed the ICMJE uniform disclosure form at
19 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author)
20 and declare: no support from any organisation for the submitted work; no financial
21 relationships with any organisations that might have an interest in the submitted work in
22 the previous three years; no other relationships or activities that could appear to have
23 influenced the submitted work.
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30 **Ethical approval:** Not required.
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33 **Data sharing:** Dataset available from the corresponding author.
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36 **Licence statement:** Corresponding Author has the right to grant on behalf of all authors
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FIGURE LEGENDS

Figure 1. Study flow diagram.

Figure 2. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on hepatocellular carcinoma in patients with chronic hepatitis B, subgroups according to trial design.

Figure 3. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on hepatocellular carcinoma in patients with chronic hepatitis B and cirrhosis, subgroups according to trial design.

Figure 4. Random effects inverse variance meta-analysis of antiviral therapy treatment effect on mortality in patients with chronic hepatitis B, subgroups according to trial design.

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