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



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

**Short title:** Antiviral therapy for hepatitis B

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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).

Antiviral therapy did not affect mortality in cohort studies, but reduced mortality in case control studies (relative risk 0.71; 95% confidence interval 0.54 to 0.93; test for subgroup differences, P=0.406).

**Conclusions:** The effect of antiviral therapy on clinical outcomes in HBV remains to be established. Although there was a positive effect in the sensitivity analyses, the strength of the evidence does not allow for extrapolation to clinical practice as research design plays an essential role in the overall assessment.

**Trial registration number:** Prospero number CRD42013003881

# 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 hepatitis[1-3]. Globally, HCC is the fifth most common cause of cancer deaths in men, and the sixth in women[4-6]. 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 measures[11-13]. Recommended treatments include interferon and nucleos(t)ide analogues (NA)[14 15]. A viral response may reduce the risk of HCC[12], but the results of clinical studies and meta-analyses on antiviral therapy are not consistent[16-24]. One meta-analysis[25] found that antiviral therapy decreased liver-related mortality whereas a cohort series found decreased overall mortality in patients with a viral response to interferon[26]. 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 Interventions[29] and the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies[30]. 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

mortality. To avoid prevalent cases of HCC the outcomes were assessed after at least 12 months of follow up. The secondary outcome measure was HCC related mortality.

## Data extraction and quality assessment

Two authors extracted data in an independent manner. When data were not available in the published reports, additional information was retrieved through correspondence with the primary investigators.

The Cochrane Collaboration's Tool for Assessing Risk of Bias was used to evaluate bias control in RCTs. The assessment included the randomisation methods (allocation sequence generation and allocation concealment), blinding (of participants, personnel and investigators), the completeness of outcome data, reporting of data and other biases[33]. All observational studies were classed as having a high risk of bias. Based on the MOOSE guidelines, the assessment of potential sources of bias within observational studies included documentation of how data were classified and coded (multiple raters, blinding and interrater reliability), assessment of confounding (comparability of cases and controls in studies where appropriate) and blinding of quality assessors, stratification or regression on possible predictors of study results.

## Data synthesis and analysis

Statistics were performed using Stata Version 12 (Statacorp, College Station, TX, USA) and Trial Sequential Analysis (CTU, Copenhagen, Denmark). Meta-analyses were performed with results expressed as risk ratios, 95% CI and $I^2$ as a marker of heterogeneity. For meta-analyses showing a statistically significant effect the number needed to treat was calculated based on the risk difference. Initial sensitivity analyses

included repeating all meta-analyses using both random and fixed effect models. The results of these analyses were only reported if the conclusions differed. Regression analyses were performed to assess for publication bias and other small study effects (Egger's test). Sequential analyses were performed for meta-analyses showing an intervention effect after adjusting for the risk of bias associated with cumulative testing[34]. The sequential analysis was performed using a random-effects model, alpha (5%), power (80%) and the incidence rates and the intervention effects identified in the meta-analyses. Pre-planned sensitivity analyses were performed with inclusion of observational studies. These analyses were performed stratified by study design (RCT, prospective cohort or case-control study) and with fixed-effect inverse variance models that compared the results of subgroups. The result of the subgroup comparisons was expressed as P values (test for subgroup differences). Additional sensitivity analyses were performed to evaluate the influence of bias control (limiting the analysis to trials with adequate randomisation), the type of antiviral therapy (comparing interferon, NA or both), and the effect HCC screening (comparing the results of trials with or without screening). Finally, subgroup analyses including only patients with cirrhosis were performed.

# 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

HCC (relative risk 1.44; 95% confidence interval 1.06 to 1.95) whereas the case control studies found that antiviral therapy reduced the risk of HCC (relative risk 0.69; 95% confidence interval 0.54 to 0.88). Due to the high heterogeneity, a post-hoc meta-regression analysis was performed. We evaluated study and patient characteristics not accounted for in the sensitivity analyses, which may have influenced the result. No modifiers were found when adjusting for the following variables: proportion of men (coefficient -0.074; P=0.08) mean age of treated patients at inclusion (coefficient 0.020; P=0.94), mean age of untreated patients at inclusion (coefficient 0.121; P=0.65), proportion with cirrhosis at inclusion (coefficient -0.001; P=0.76), and region of trial (coefficient -0.394; P=0.55).

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

## 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

observational studies had little influence on the overall result (41 of 3433 versus 146 of 4625; relative risk 0.83; 95% confidence interval 0.5 to 1.20; $I^2$=0%; Egger's test P=0.248). There was no difference between subgroups of trials stratified by design (test for subgroup differences P=0.481).

## 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 measure[27] 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 measures[68]. Still, our findings are not sufficiently convincing and do not allow for changes in clinical practice.

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

There are several potential explanations for the discrepancies between RCTs and observational studies[69]. The fact that only prospective cohort studies found an increased risk of HCC among patients receiving antiviral therapy opposes speculations that the treatment affected HCC development. The findings are more likely to reflect baseline differences in the viral load, genotype and degree of liver disease. The degree of monitoring in the treatment and control group is also likely to differ and may lead to detection bias. The importance of detection bias is further supported by the subgroup differences observed according to HCC screening. The case control studies are likely to

have an even higher risk of bias, as confounding by indication and ascertainment bias is likely to exist in retrospective studies. Reporting bias should also be considered[33].

The subgroup differences with regards to type of intervention suggests a possible anti-carcinogenic effect of interferon, as seen in HCV[70]. We additionally found a decrease in both HCC incidence and overall mortality in sensitivity analyses of patients with cirrhosis. This could support the case for continued treatment of patients with cirrhosis.

We found a beneficial effect of interferon and/or NA on mortality in HBV when including RCTs and observational studies in chronic HBV patients. The assessment of mortality is robust to bias[71]. Accordingly, our subgroup analysis showed no clear relation between the results and the study design. HCC mortality is more prone to bias. Whether antiviral treatment for HBV decreases mortality except from HCC is unknown.

In conclusion, antiviral treatment for HBV has no proven effect on the clinical outcomes HCC and mortality. Bias has a paramount impact on treatment effect estimates in observational studies and we recommend a critical approach to conclusions drawn in such studies. Future trials on antiviral treatment for HBV should be designed to show an effect on clinical endpoints rather than surrogate markers.

**Contributors:** MT, LLG and AK conceived the idea and design. MT and EKD collected and assembled data. MT, LLG and AK analysed and interpreted the data. MT and LLG drafted the manuscript. LLG and AK revised the manuscript for important intellectual content. All authors discussed and approved of the final version of the manuscript. MT is guarantor.

**Competing interests:** All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

**Ethical approval:** Not required.

**Data sharing:** Dataset available from the corresponding author.

# 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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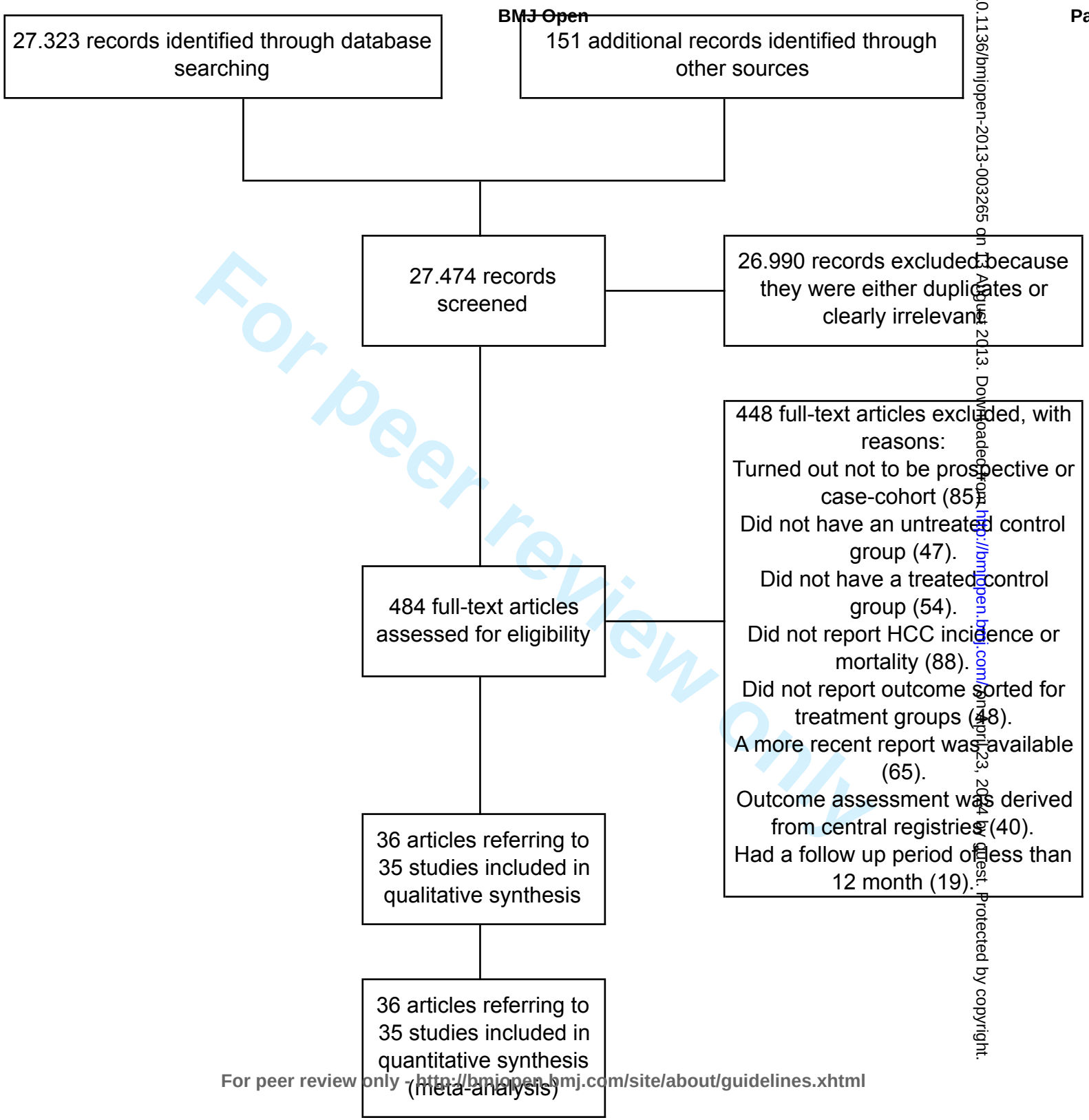

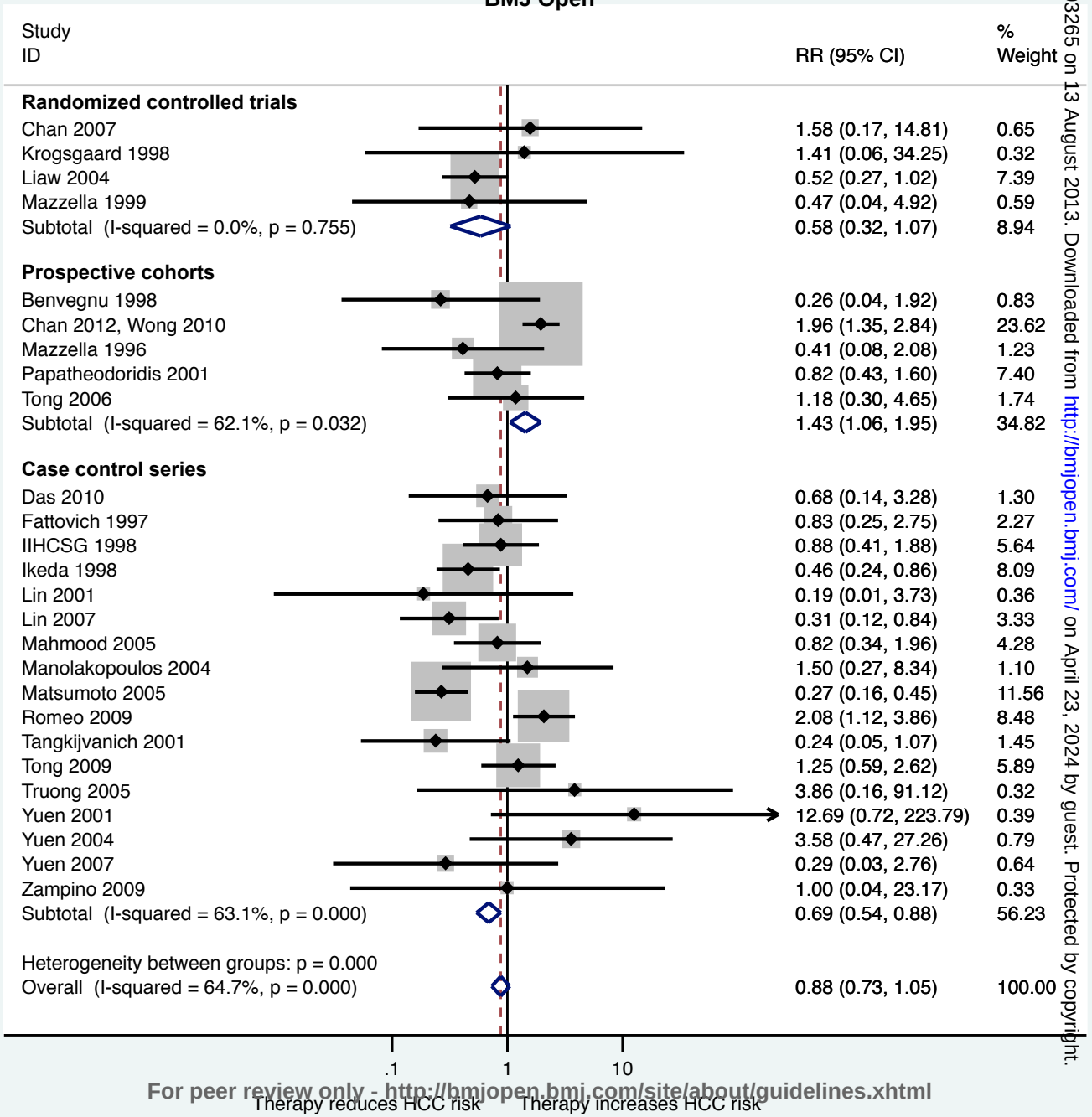

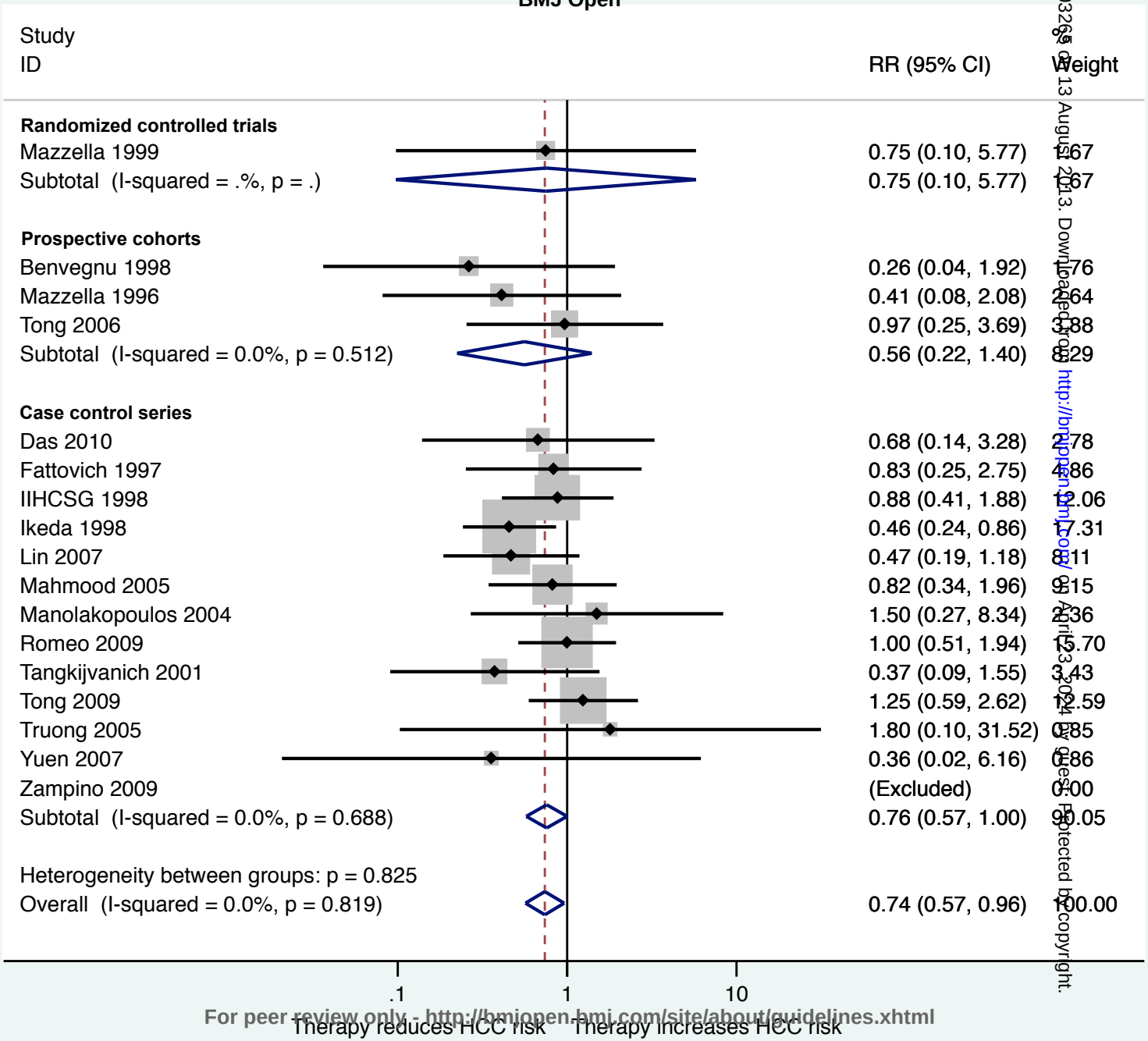

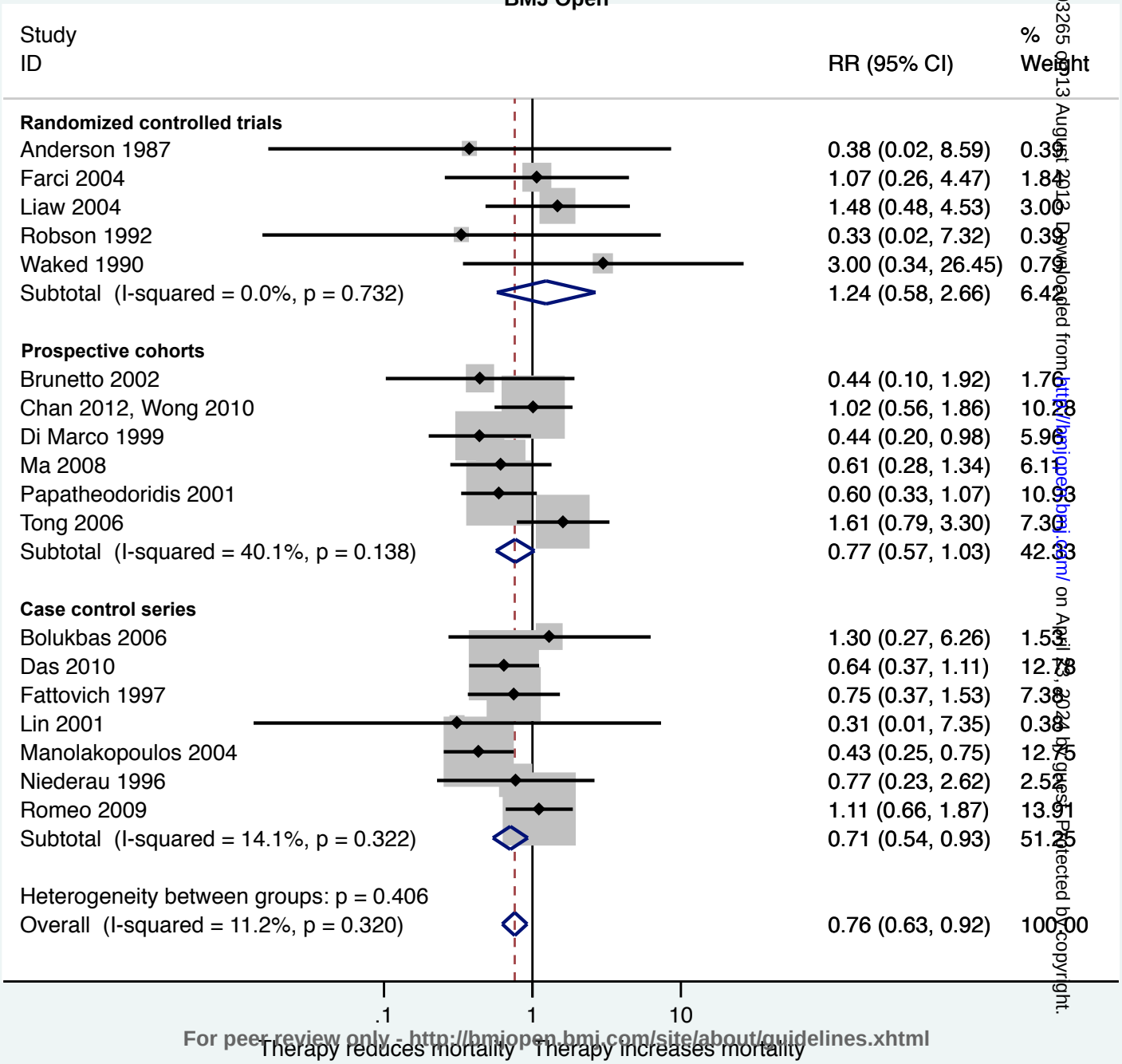

*Table 1.* **Characteristics of Trials**

***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<br>C: 16 | I: 1.0<br>C: 1.0 | No | Overall mortality |
| Chan 2007 (39) | China | Lamivudine (100mg/d) | I: 89<br>C: 47 | I: 2.5<br>C: 2.5 | No | HCC incidence |
| Farci 2004 (43) | Italy | IFN (3-9 MU/x3w) | I: 28<br>C: 10 | I: 10.8<br>C: 10.8 | No | Overall mortality |
| Krogsgaard 1998 (28) | Europe | IFN (1.5-18 MU/x3w) | I: 210<br>C: 98 | I: 1.3<br>C: 1.3 | No | HCC incidence and -mortality |
| Liaw 2004 (27) | Asia | Lamivudine (100mg/d) | I: 436<br>C: 215 | I: 2.7<br>C: 2.7 | Yes | HCC incidence<br>Overall mortality |
| Mazzella 1999 (53) | Italy | IFN (648MU total) | I: 33<br>C: 31 | I: 7.2<br>C: 6.6 | No | HCC incidence and -mortality |
| Robson 1992 (56) | South Africa | IFN (10MU/x3w) | I: 10<br>C: 10 | I: 1.4<br>C: 1.4 | No | Overall and HCC mortality |
| Waked 1990 (62) | Egypt | IFN (5MU/$m^2$/x3w - 5MU/$m^2$/d) | I: 20<br>C: 20 | I: 1.3<br>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 |
|---|---|---|---|---|---|---|
| Benvegnu 1998 (36) | Italy | IFN (5-10 MU/x3w) | I: 13<br>C: 24 | I: 6.0<br>C: 6.0 | Yes | HCC incidence<br>Overall mortality |
| Brunetto 2002 (38) | Italy | IFN (9MU/x3w) | I: 103<br>C: 61 | I: 6.0<br>C: 6.0 | Yes | Overall mortality |
| Chan 2012 (40)<br>Wong 2010 (63) | China | Nucleos(t)ides<br>IFN (NS) | I: 158<br>C: 1271 | I: 10.1<br>C: 10.1 | Yes | HCC incidence, overall and HCC mortality |
| Di Marco 1999 (42) | Italy | IFN (NS) | I: 109<br>C: 193 | I: 7.8<br>C: 7.8 | Yes | Overall mortality |
| Ma 2008 (49) | China | Nucleos(t)ides (NS) | I: 41<br>C: 176 | I: 2.9<br>C: 2.9 | Yes | Overall mortality |
| Mazzella 1996 (52) | Italy | IFN (10MU/x3w) | I: 34<br>C: 28 | I: 4.1<br>C: 4.0 | Yes | HCC incidence |
| Papatheodoridis 2001 (55) | Greece | IFN (3MU/x3w) | I: 209<br>C: 152 | I: 6.0<br>C: 6.1 | Yes | HCC incidence, overall and HCC mortality |
| Tong 2006 (59) | USA | IFN (NS) | I: 22<br>C: 378 | I: 7.0<br>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<br>C: 15 | I: 1.5<br>C: 2.0 | Yes | Overall and HCC mortality |
| Das 2010 (41) | India | Lamivudine Adefovir (NS) | I: 151<br>C: 102 | I: 4.0<br>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 |
| IIHCSG 1998 (45) | Italy, Argentina | IFN (9MU/w) | I: 49<br>C: 97 | I: 5.8<br>C: 6.9 | Yes | HCC incidence |
| Ikeda 1998 (46) | Japan | IFN (6MU/x2w) | I: 94<br>C: 219 | I: 6.8<br>C: 7.0 | Yes | HCC incidence |
| Lin 2001 (47) | China | IFN (5MU/x3w) | I: 30<br>C: 28 | I: 2.7<br>C: 2.6 | No | HCC incidence, overall and HCC mortality |
| Lin 2007 (48) | China | IFN (6-9 MU/$m^2$/x3w) | I: 233<br>C: 233 | I: 6.8<br>C: 6.1 | Yes | HCC incidence and -mortality |
| Mahmood 2005 (50) | Japan | IFN (6MU/d) | I: 23<br>C:68 | I: 7.0<br>C: 7.0 | Yes | HCC incidence |
| Manolakopoulos 2004 (26) | Greece | Lamivudine (100mg/d) | I: 30<br>C: 30 | I: 1.5<br>C: 1.8 | Yes | HCC incidence, overall and HCC mortality |
| Matsumoto 2005 (51) | Japan | Lamivudine (100mg/d) | I: 508<br>C: 231 | I: 2.7<br>C: 5.3 | No | HCC incidence |
| Niederau 1996 (54) | Germany | IFN (2-5 MU/x3w) | I: 103<br>C: 53 | I: 4.2<br>C: 3.2 | No | Overall mortality |
| Romeo 2009 (57) | Italy | Lamivudine (NS) IFN (6-9 MU) | I: 102<br>C: 135 | I: 22.4<br>C: 16.5 | Yes | HCC incidence, overall and HCC mortality |
| Tangkijvanich 2001 (58) | Thailand | IFN (3-6 MU/x3w) | I: 67<br>C: 72 | I: 4.9<br>C: 4.9 | Yes | HCC incidence |
| Tong 2009 (60) | USA | Lamivudine (NS) | I: 27<br>C: 101 | I: 5.3<br>C: 5.3 | Yes | HCC incidence and -mortality |
| Truong 2005 (61) | Japan | IFN (174-687 MU total) | I: 27<br>C: 35 | I: 7.0<br>C: 6.2 | Yes | HCC incidence and -mortality |
| Yuen 2001 (64) | China | IFN (2.5-10 MU/ $m^2$/x3w) | I: 208<br>C: 203 | I: 8.9<br>C: 9.0 | Yes | HCC incidence and -mortality |
| Yuen 2004 (65) | China | IFN (NS) | I: 6<br>C: 86 | I: 10.5<br>C: 10.5 | No | HCC incidence |
| Yuen 2007 (66) | China | Lamivudine (100mg/d) | I: 142<br>C: 124 | I: 7.5<br>C: 9.0 | Yes | HCC incidence |
| Zampino 2009 (67) | Italy | IFN (5MU/$m^2$/x3w) | I: 41<br>C: 13 | I: 23<br>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<br>C: 35 | 100 | 20 | 77 | 100 | I: 2; 14%<br>C: 0; 0% |
| Chan 2007 (39) | I: 39<br>C: 39 | 84 | 16 | 77 | 5 | NS |
| Farci 2004 (43) | I: 35<br>C: 38 | 83 | 66 | 100 | 2 | I: NA<br>C: 1; 100% |
| Krogsgaard 1998 (28) | I: 36<br>C: 36 | 81 | 19 | 100 | 100 | NS |
| Liaw 2004 (27) | I: 43<br>C: 44 | 85 | 33 | 78 | 58 | NS |
| Mazzella 1999 (53) | I: 36<br>C: 41 | 78 | 0 | 100 | 100 | I: 30; 91%<br>C: 19; 61% |
| Robson 1992 (56) | I: 33<br>C: 31 | 70 | NS | 100 | 100 | I: 5; 50%<br>C: 1; 10% |
| Waked 1990 (62) | I: 35<br>C: 35 | 78 | 40 | 100 | 100 | I: 13; 81%<br>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<br>C: 60 | 65 | 100 | NS | NS | NS |
| Brunetto 2002 (38) | I: 40<br>C: 40 | 80 | 38 | NS | 0 | NA |
| Chan 2012 (40)<br>Wong 2010 (63) | NS | 67 | 32 | 87 | NS | NS |
| Di Marco 1999 (42) | I: 33<br>C: 35 | 71 | 29 | 100 | 29 | I: 35; 32%<br>C: 29;15% |
| Ma 2008 (49) | I: 54<br>C: 54 | 72 | 100 | NS | 24 | NS |
| Mazzella 1996 (52) | I: 48<br>C: 49 | 73 | 100 | NS | NS | NS |
| Papatheodoridis 2001 (55) | I: 47<br>C: 49 | 83 | 31 | 100 | 0 | NA |
| Tong 2006 (59) | I: 48<br>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<br>C: 46 | 82 | 100 | NS | 0 | NA |
| Das 2010 (41) | I: 42<br>C: 46 | 91 | 100 | NS | 45 | I: 12; 13%<br>C: 3; 10% |

| | | | | | | |
|---|---|---|---|---|---|---|
| Fattovich 1997 (44) | I: 47<br>C: 45 | 87 | 100 | 100 | 100 | I: 27; 68%<br>C: 30; 60% |
| IIHCSG 1998 (45) | I: 54<br>C: 54 | 64 | 100 | NS | NS | NS |
| Ikeda 1998 (46) | I: 41<br>C: 44 | 79 | 100 | NS | 52 | NS |
| Lin 2001 (47) | I: 39<br>C: 41 | 95 | 10 | 100 | 0 | NA |
| Lin 2007 (48) | I: 32<br>C: 31 | 94 | 9 | NS | 100 | I: 115; 49%<br>C: 86; 37% |
| Mahmood 2005 (50) | I: 49<br>C: 49 | 69 | 100 | NS | 36 | NS |
| Manolakopoulos 2004 (26) | I: 65<br>C: 63 | 80 | 100 | 100 | 0 | NA |
| Matsumoto 2005 (51) | I: 42<br>C: 41 | 73 | 18 | NS | 55 | NS |
| Niederau 1996 (54) | I: 40<br>C: 41 | 78 | 28 | 100 | 100 | I: 53; 51%<br>C: 7; 13% |
| Romeo 2009 (57) | NS | 77 | 35 | NS | 27 | NS |
| Tangkijvanich 2001 (58) | I: 37<br>C: 40 | 72 | 20 | NS | 100 | I: 24; 36%<br>C: 7; 10% |
| Tong 2009 (60) | I: 46<br>C: 46 | 86 | 100 | 14 | 53 | NS |
| Truong 2005 (61) | I: 33<br>C: 37 | 53 | 2 | 100 | 60 | I: 9; 53%<br>C: 11; 55% |
| Yuen 2001 (64) | I: 27<br>C: 28 | 64 | NS | 32 | 100 | I: 96; 46%<br>C: 93; 46% |
| Yuen 2004 (65) | I: 43<br>C: 43 | 71 | NS | NS | 23 | NS |
| Yuen 2007 (66) | I: 34<br>C: 33 | 74 | 0 | 48 | 100 | NS |
| Zampino 2009 (67) | NS | 67 | 0 | NS | 54 | I: 16; 62%<br>C: NS |

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.

**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.<br>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.<br>Interventions: IFN or NA without restrictions on duration or dose of therapy.<br>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.<br>Keywords for HCC were: HCC, hepatoma, hepatocell*, liver cancer, liver neoplasm*, liver cell carcinoma.<br>Keywords for HBV were: HBV, CHB, hepatitis B, B |

| | | |
|---|---|---|
| | | hepatitis, dane particle, *HBs*, *HBe*, chronic hepatitis B, chronic B hepatitis.<br>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.<br>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.<br>$I^2$ 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


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

**Short title:** Antiviral therapy for hepatitis B

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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).

Antiviral therapy did not affect mortality in cohort studies, but reduced mortality in case control studies (relative risk 0.71; 95% confidence interval 0.54 to 0.93; test for subgroup differences, P=0.406).

**Conclusions:** The effect of antiviral therapy on clinical outcomes in HBV remains to be established. Although there was a positive effect in the sensitivity analyses, the strength of the evidence does not allow for extrapolation to clinical practice as research design plays an essential role in the overall assessment.

**Trial registration number:** Prospero number CRD42013003881

# 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 hepatitis[1-3]. Globally, HCC is the fifth most common cause of cancer deaths in men, and the sixth in women[4-6]. 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 measures[11-13]. Recommended treatments include interferon and nucleos(t)ide analogues (NA)[14 15]. A viral response may reduce the risk of HCC[12], but the results of clinical studies and meta-analyses on antiviral therapy are not consistent[16-24]. One meta-analysis[25] found that antiviral therapy decreased liver-related mortality whereas a cohort series found decreased overall mortality in patients with a viral response to interferon[26]. 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 Interventions[29] and the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies[30]. 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

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

## Data extraction and quality assessment

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

The Cochrane Collaboration's Tool for Assessing Risk of Bias was used to evaluate bias control in RCTs. The assessment included the randomisation methods (allocation sequence generation and allocation concealment), blinding (of participants, personnel and investigators), the completeness of outcome data, reporting of data and other biases[33]. All observational studies were classed as having a high risk of bias. Based on the MOOSE guidelines, the assessment of potential sources of bias within observational studies included documentation of how data were classified and coded (multiple raters, blinding and interrater reliability), assessment of confounding (comparability of cases and controls in studies where appropriate) and blinding of quality assessors, stratification or regression on possible predictors of study results.

## Data synthesis and analysis

Statistics were performed using Stata Version 12 (Statacorp, College Station, TX, USA) and Trial Sequential Analysis (CTU, Copenhagen, Denmark). Meta-analyses were performed with results expressed as risk ratios, 95% CI and $I^2$ as a marker of

heterogeneity. For meta-analyses showing a statistically significant effect the number needed to treat was calculated based on the risk difference. Initial sensitivity analyses included repeating all meta-analyses using both random and fixed effect models. The results of these analyses were only reported if the conclusions differed. Regression analyses were performed to assess for publication bias and other small study effects (Egger's test). Sequential analyses were performed for meta-analyses showing an intervention effect after adjusting for the risk of bias associated with cumulative testing[34]. The sequential analysis was performed using a random-effects model, alpha (5%), power (80%) and the incidence rates and the intervention effects identified in the meta-analyses. Pre-planned sensitivity analyses were performed with inclusion of observational studies. These analyses were performed stratified by study design (RCT, prospective cohort or case-control study) and with fixed-effect inverse variance models that compared the results of subgroups. The result of the subgroup comparisons was expressed as P values (test for subgroup differences). Additional sensitivity analyses were performed to evaluate the influence of bias control (limiting the analysis to trials with adequate randomisation), the type of antiviral therapy (comparing interferon, NA or both), and the effect HCC screening (comparing the results of trials with or without screening). Finally, subgroup analyses including only patients with cirrhosis were performed.

# 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

received antiviral therapy and 4178 patients received no intervention. 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 768 patients in the treatment group versus 19 of 391 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 436 patients in the treatment group and 174 of 1853. In the case control studies the numbers were 99 of 1778 and 201 of 1827 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 HCC (relative risk 1.44; 95% confidence interval 1.06 to 1.95) whereas the case control studies found that antiviral therapy reduced the risk of HCC (relative risk 0.69; 95% confidence interval 0.54 to 0.88). Due to the high heterogeneity, a post-hoc meta-regression analysis was performed. We evaluated study and patient characteristics not accounted for in the sensitivity analyses, which may have influenced the result. No modifiers were found when adjusting for the following variables: proportion of men (coefficient -0.074; P=0.08) mean age of treated patients at inclusion (coefficient 0.020; P=0.94), mean age of untreated patients at inclusion (coefficient 0.121; P=0.65), proportion with cirrhosis at inclusion (coefficient -0.001; P=0.76), and region of trial (coefficient -0.394; P=0.55).

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

Sensitivity analyses were performed to evaluate the risk of HCC among patients with cirrhosis. In the RCTs, one of 20 patients in the treatment group and two of 12 controls developed HCC (relative risk 0.75; 95% confidence interval 0.10 to 5.77). In the prospective cohort studies 32 of 184 versus 142 of 482 patients developed HCC whereas the numbers were 63 of 680 versus 161 of 955, respectively for case control studies.

Overall, antiviral therapy reduced the risk of HCC when including data from RCTs and observational studies (relative risk 0.74; 95% confidence interval 0.57 to 0.96; $I^2$=0%; number needed to treat 28 patients) (fig 3). The results of RCTs and observational studies were similar (test for subgroup differences P=0.159). There was no evidence of small study effects (Egger's test P=0.890). In trial sequential analysis, the monitoring and alpha-spending boundary did not cross suggesting that the result was not robust to adjustment for multiple testing.

## Mortality

In the RCTs, there was no difference in mortality between the treatment and control group (21 of 508 versus 9 of 271 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 655 versus 247 of 2231 for prospective cohort studies and 79 of 506 versus 92 of 413 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 298 versus 141 of 499

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

# 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 measure[27] 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

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 measures[68]. Still, our findings are not sufficiently convincing and do not allow for changes in clinical practice.

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

There are several potential explanations for the discrepancies between RCTs and observational studies[69]. The fact that only prospective cohort studies found an increased risk of HCC among patients receiving antiviral therapy opposes speculations that the

treatment affected HCC development. The findings are more likely to reflect baseline differences in the viral load, genotype and degree of liver disease. The degree of monitoring in the treatment and control group is also likely to differ and may lead to detection bias. The importance of detection bias is further supported by the subgroup differences observed according to HCC screening. The case control studies are likely to have an even higher risk of bias, as confounding by indication and ascertainment bias is likely to exist in retrospective studies. Reporting bias should also be considered[33].

The subgroup differences with regards to type of intervention suggests a possible anti-carcinogenic effect of interferon, as seen in HCV[70]. We additionally found a decrease in both HCC incidence and overall mortality in sensitivity analyses of patients with cirrhosis. This could support the case for continued treatment of patients with cirrhosis.

We found a beneficial effect of interferon and/or NA on mortality in HBV when including RCTs and observational studies in chronic HBV patients. The assessment of mortality is robust to bias[71]. Accordingly, our subgroup analysis showed no clear relation between the results and the study design. HCC mortality is more prone to bias. Whether antiviral treatment for HBV decreases mortality except from HCC is unknown.

In conclusion, antiviral treatment for HBV has no proven effect on the clinical outcomes HCC and mortality. Bias has a paramount impact on treatment effect estimates in observational studies and we recommend a critical approach to conclusions drawn in such studies. Future trials on antiviral treatment for HBV should be designed to show an effect on clinical endpoints rather than surrogate markers.

**Contributors:** MT, LLG and AK conceived the idea and design. MT and EKD collected and assembled data. MT, LLG and AK analysed and interpreted the data. MT and LLG drafted the manuscript. LLG, EKD and AK revised the manuscript for important intellectual content. All authors discussed and approved of the final version of the manuscript. MT is guarantor.

**Competing interests:** All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

**Ethical approval:** Not required.

**Data sharing:** Dataset available from the corresponding author.

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

**Short title:** Antiviral therapy for hepatitis B

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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).

Antiviral therapy did not affect mortality in cohort studies, but reduced mortality in case control studies (relative risk 0.71; 95% confidence interval 0.54 to 0.93; test for subgroup differences, P=0.406).

**Conclusions:** The effect of antiviral therapy on clinical outcomes in HBV remains to be established. Although there was a positive effect in the sensitivity analyses, the strength of the evidence does not allow for extrapolation to clinical practice as research design plays an essential role in the overall assessment.

**Trial registration number:** Prospero number CRD42013003881

# 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 hepatitis[1-3]. Globally, HCC is the fifth most common cause of cancer deaths in men, and the sixth in women[4-6]. 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 measures[11-13]. Recommended treatments include interferon and nucleos(t)ide analogues (NA)[14 15]. A viral response may reduce the risk of HCC[12], but the results of clinical studies and meta-analyses on antiviral therapy are not consistent[16-24]. One meta-analysis[25] found that antiviral therapy decreased liver-related mortality whereas a cohort series found decreased overall mortality in patients with a viral response to interferon[26]. 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 Interventions[29] and the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies[30]. 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

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

## Data extraction and quality assessment

Two authors extracted data ~~in an~~ independentl~~y manner~~. When data were not available in the published reports, additional information was retrieved through correspondence with the primary investigators.

The Cochrane Collaboration's Tool for Assessing Risk of Bias was used to evaluate bias control in RCTs. The assessment included the randomisation methods (allocation sequence generation and allocation concealment), blinding (of participants, personnel and investigators), the completeness of outcome data, reporting of data and other biases[33]. All observational studies were classed as having a high risk of bias. Based on the MOOSE guidelines, the assessment of potential sources of bias within observational studies included documentation of how data were classified and coded (multiple raters, blinding and interrater reliability), assessment of confounding (comparability of cases and controls in studies where appropriate) and blinding of quality assessors, stratification or regression on possible predictors of study results.

## Data synthesis and analysis

Statistics were performed using Stata Version 12 (Statacorp, College Station, TX, USA) and Trial Sequential Analysis (CTU, Copenhagen, Denmark). Meta-analyses were performed with results expressed as risk ratios, 95% CI and $I^2$ as a marker of

heterogeneity. For meta-analyses showing a statistically significant effect the number needed to treat was calculated based on the risk difference. Initial sensitivity analyses included repeating all meta-analyses using both random and fixed effect models. The results of these analyses were only reported if the conclusions differed. Regression analyses were performed to assess for publication bias and other small study effects (Egger's test). Sequential analyses were performed for meta-analyses showing an intervention effect after adjusting for the risk of bias associated with cumulative testing[34]. The sequential analysis was performed using a random-effects model, alpha (5%), power (80%) and the incidence rates and the intervention effects identified in the meta-analyses. Pre-planned sensitivity analyses were performed with inclusion of observational studies. These analyses were performed stratified by study design (RCT, prospective cohort or case-control study) and with fixed-effect inverse variance models that compared the results of subgroups. The result of the subgroup comparisons was expressed as P values (test for subgroup differences). Additional sensitivity analyses were performed to evaluate the influence of bias control (limiting the analysis to trials with adequate randomisation), the type of antiviral therapy (comparing interferon, NA or both), and the effect HCC screening (comparing the results of trials with or without screening). Finally, subgroup analyses including only patients with cirrhosis were performed.

# 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

received antiviral therapy and 4178 patients received no intervention. 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~~ 768 patients in the treatment group versus 19 of ~~447~~ 391 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~~ 436 patients in the treatment group and 174 of ~~2283~~1853. In the case control studies the numbers were 99 of ~~1904~~ 1778 and 201 of ~~1895~~ 1827 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 HCC (relative risk 1.44; 95% confidence interval 1.06 to 1.95) whereas the case control studies found that antiviral therapy reduced the risk of HCC (relative risk 0.69; 95% confidence interval 0.54 to 0.88). Due to the high heterogeneity, a post-hoc meta-regression analysis was performed. We evaluated study and patient characteristics not accounted for in the sensitivity analyses, which may have influenced the result. No modifiers were found when adjusting for the following variables: proportion of men (coefficient -0.074; P=0.08) mean age of treated patients at inclusion (coefficient 0.020; P=0.94), mean age of untreated patients at inclusion (coefficient 0.121; P=0.65), proportion with cirrhosis at inclusion (coefficient -0.001; P=0.76), and region of trial (coefficient -0.394; P=0.55).

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

Sensitivity analyses were performed to evaluate the risk of HCC among patients with cirrhosis. In the RCTs, one of 20 patients in the treatment group and two of 12 controls developed HCC (relative risk 0.75; 95% confidence interval 0.10 to 5.77). In the prospective cohort studies 32 of 184 versus 142 of 482 patients developed HCC whereas the numbers were 63 of 680 versus 161 of 955, respectively for case control studies.

Overall, antiviral therapy reduced the risk of HCC when including data from RCTs and observational studies (relative risk 0.74; 95% confidence interval 0.57 to 0.96; $I^2$=0%; number needed to treat 28 patients) (fig 3). The results of RCTs and observational studies were similar (test for subgroup differences P=0.159). There was no evidence of small study effects (Egger's test P=0.890). In trial sequential analysis, the monitoring and alpha-spending boundary did not cross suggesting that the result was not robust to adjustment for multiple testing.

## Mortality

In the RCTs, there was no difference in mortality between the treatment and control group (21 of ~~840~~ 508 versus 9 of ~~447~~ 271 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~~ 655 versus 247 of ~~2283~~ 2231 for prospective cohort studies and 791 of ~~1904~~ 506 versus 92 of ~~1895~~ 413 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~~ 298 versus 141 of ~~1~~ ~~477~~499 (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~~ 282 versus 2 of ~~447~~154; relative risk 0.50; 95% confidence interval 0.10 to 2.44; $I^2$=0%, n=2 RCT). Including data from observational studies had little influence on the overall result (~~41~~ 38 of ~~3433~~ 1233 versus ~~146~~ 144 of ~~46252~~2632; relative risk 0.83; 95% confidence interval 0.5 to 1.20; $I^2$=0%; Egger's test P=0.248). There was no difference between subgroups of trials stratified by design (test for subgroup differences P=0.481).

## 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 measure[27] 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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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 measures[68]. Still, our findings are not sufficiently convincing and do not allow for changes in clinical practice.

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

There are several potential explanations for the discrepancies between RCTs and observational studies[69]. The fact that only prospective cohort studies found an increased risk of HCC among patients receiving antiviral therapy opposes speculations that the

treatment affected HCC development. The findings are more likely to reflect baseline differences in the viral load, genotype and degree of liver disease. The degree of monitoring in the treatment and control group is also likely to differ and may lead to detection bias. The importance of detection bias is further supported by the subgroup differences observed according to HCC screening. The case control studies are likely to have an even higher risk of bias, as confounding by indication and ascertainment bias is likely to exist in retrospective studies. Reporting bias should also be considered[33].

The subgroup differences with regards to type of intervention suggests a possible anti-carcinogenic effect of interferon, as seen in HCV[70]. We additionally found a decrease in both HCC incidence and overall mortality in sensitivity analyses of patients with cirrhosis. This could support the case for continued treatment of patients with cirrhosis.

We found a beneficial effect of interferon and/or NA on mortality in HBV when including RCTs and observational studies in chronic HBV patients. The assessment of mortality is robust to bias[71]. Accordingly, our subgroup analysis showed no clear relation between the results and the study design. HCC mortality is more prone to bias. Whether antiviral treatment for HBV decreases mortality except from HCC is unknown.

In conclusion, antiviral treatment for HBV has no proven effect on the clinical outcomes HCC and mortality. Bias has a paramount impact on treatment effect estimates in observational studies and we recommend a critical approach to conclusions drawn in such studies. Future trials on antiviral treatment for HBV should be designed to show an effect on clinical endpoints rather than surrogate markers.

**Contributors:** MT, LLG and AK conceived the idea and design. MT and EKD collected and assembled data. MT, LLG and AK analysed and interpreted the data. MT and LLG drafted the manuscript. LLG, EKD and AK revised the manuscript for important intellectual content. All authors discussed and approved of the final version of the manuscript. MT is guarantor.

**Competing interests:** All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

**Ethical approval:** Not required.

**Data sharing:** Dataset available from the corresponding author.

## 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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