Antiviral therapy for Prevention of Hepatocellular Carcinoma Chronic Hepatitis C-Related Cirrhosis or Fibrosis: Systematic Review and Meta-Analysis of Randomised Controlled Trials

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Antiviral therapy for Prevention of Hepatocellular Carcinoma Chronic Hepatitis C-Related Cirrhosis or Fibrosis: Systematic Review and Meta-Analysis of Randomised Controlled Trials

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Abstract

Objectives To determine whether antiviral therapy reduces the risk of developing hepatocellular carcinoma in chronic hepatitis C.

Design Systematic review and meta-analyses of randomised controlled trials. Prospective cohort studies were included in sensitivity analyses.

Data Sources Eligible trials were identified through electronic and manual searches.

Review Methods Trials on antiviral therapy versus placebo or no intervention for patients with hepatitis C-related fibrosis or cirrhosis were eligible for inclusion if the number of patients who developed hepatocellular carcinoma was available. Random effects meta-analyses were performed. Subgroup, sensitivity, regression and sequential analyses were performed to evaluate sources of intertrial heterogeneity, the risk of bias and the robustness of the results after adjusting for multiple comparisons.

Results Eight randomised controlled trials comparing antiviral therapy (interferon or pegylated interferon alone or with ribavirin versus placebo or no intervention) were included. Random effects meta-analysis showed that antiviral therapy reduced the risk of hepatocellular carcinoma (81/1156 versus 129/1174; risk ratio 0.53, 95% confidence interval 0.34 to 0.81). In subgroup analyses, antiviral therapy was more beneficial (p=0.03) in virological responders (0.15, 0.05 to 0.45) compared with non-responders (0.57; 0.37-0.85). No evidence of bias was seen in regression analyse. Sequential analysis confirmed the overall result. The result was also confirmed in sensitivity analyses including five cohort studies, but regression analysis showed evidence of bias in the cohort studies (P=0.02).

Interpretation Antiviral therapy may reduce the risk of hepatocellular in hepatitis C-related fibrosis and cirrhosis. Although the effect of interferon on prevention of hepatocellular

carcinoma is greater in virological responders, a beneficial effect may be seen even in patients without a virological response.

What is already known on this topic

Hepatitis C and liver cirrhosis causes Hepatocellular carcinoma

Anti viral therapy eliminates virus RNA, but it remains uncertain whether it decreases the risk of HCC

What this study adds

Significant proof that anti viral therapy reduces the risk of HCC

The most prominent effect is seen in patients with virological response

Introduction

World-wide, hepatocellular carcinoma (HCC) is one of the most common malignant diseases accounting for approximately 90% of primary liver cancers (1;2). Hepatitis C and cirrhosis are two of the most important risk factors for the development of HCC (3). Among patients with hepatitis C and cirrhosis, the incidence of HCC ranges from 1 to 4 % depending on the severity of the underlying liver disease (2).

Hepatitis C is an insidious disease, which often lead to chronic infection. Few patients clear the hepatitis C spontaneously. Antiviral therapy for patients with chronic hepatitis C may lead loss of the virus. A number of patients with an initial response relapse within a few months after treatment. For patients who achieve a six months sustained virological response, the risk of relapse is negligible.

The proportion of patients who achieve a virological response depends on the type of therapy. Interferon was introduced in 1986 and was initially used as monotherapy (4). Subsequent trials showed that the addition of ribavirin and use of a pegylated form of interferon increased the number of sustained virological responders considerably (5-8). Whether a sustained virological response leads to a reduced risk of developing HCC is not known.

Methods

This systematic review was carried and reported out based on a protocol developed using the methods described in the Cochrane Handbook for Systematic Reviews of Interventions and the PRISMA Statement for Reporting Systematic Reviews and Meta-analysis (9;13).

The main objective of the present review was to determine the effect of antiviral therapy versus placebo or no intervention for prevention of HCC in hepatitis C-related cirrhosis or fibrosis.

Our primary analyses included randomised controlled trials. Prospective cohort studies with defined control groups were included in sensitivity analyses. Trials were included irrespective of language or publication status. The dose, type and duration of therapy was not considered in the inclusion criteria. Accordingly, trials on interferon or pegylated interferon alone or with ribavirin were eligible for inclusion. Trials on patients with human immunodeficiency virus and patients with chronic hepatitis B were excluded from the analysis. The primary outcome measure was HCC. Secondary outcomes were overall mortality, HCC-related mortality and liver related mortality (defined as death following variceal bleeding, hepatorenal syndrome, liver failure or spontaneous bacterial peritonitis).

Two authors (NK, AK) participated in the literature searches. Excluded trials were listed with the reason for exclusion. Two authors (NK, ED) extracted data in an independent manner. The extracted data were validated by two authors (AK, LG).

Search strategy identification of eligible trials

Eligible trials were identified through electronic and manual searches. The electronic searches (Appendix 1) were performed in the Cochrane Library (issue 3,2012), Pubmed (1966-March 2012), Embase (1955-March 2012) and Web of Science (1900-March 2012). Additional searches were performed in reference lists from relevant papers (papers on

chronic hepatitis C and HCC), conference proceedings and the World Health Organization Trial Search Portal (www.who.int/trialsearch/).

Assessment of bias control

The quality of bias control was assessed through individual components (9). Based on previous evidence (10), our primary assessment of bias control was based on the randomization methods including the allocation sequence generation (classed as adequate if based on a table of random numbers or similar) or allocation concealment (classed as adequate if based on a central independent unit or similar). Additional components included blinding (performance bias and detection bias), handling of missing outcome data (attrition bias) and selective reporting (reporting bias). We also extracted sample size calculations and whether the sample size was reached or the trial was terminated prematurely. Due to the risk of selection bias associated with the observational design, all cohort studies were classed as having a high risk of bias.

Statistical analysis

The analyses were performed using Revman version 5.1 (Nordic Cochrane Centre, Copenhagen), STATA version 11 (STATA Corp, Texas, USA) and TSA version 9 (Copenhagen Trial Unit, Copenhagen, Denmark). The primary meta-analyses were performed using random effects models due to an expected clinical heterogeneity (differences between patient and intervention characteristics). The results of the analyses were presented as risk ratios with 95% confidence intervals and I^2 as a marker of intertrial heterogeneity. The number needed to treat was calculated as the inverse of the risk difference between the intervention and control group. Fixed effect meta-analyses were

performed to evaluate the robustness of the results. The test of subgroup differences was calculated and the results expressed using the P values (11). The risk of bias and small study effects was assessed through regression analyses (Egger's test). Planned subgroup and sensitivity analyses evaluated the effect of the trial design (randomised trials or observational studies), patient characteristics (virological responders compared with non-responders and patients with cirrhosis compared with fibrosis). A sequential analysis was performed to adjust for the risk of false-positive findings due to repeated tests (12). The sequential analysis was performed for the primary random effects meta-analysis of randomised trials. Based on the results of the primary meta-analysis, the incidence in the control group was set to 12% and the relative risk reduction to 41%. The heterogeneity correction was set to 64% (model-based), power to 80% and alpha to 5%.

Results

Study selection

The electronic searches generated 1711 references (Figure 1). After reading the titles and abstracts, we identified 26 potentially relevant randomised controlled trials and observational studies described in 27 references. Fourteen additional trials and references were identified through the manual searches. Twenty-four references referring to retrospective cohort studies, case control studies or trials that did not assess the risk of HCC. Eight randomised trials (14-21) and five prospective cohort studies (22-26) fulfilled our inclusion criteria.

Characteristics of included trials and patients

All trials were published in English as full paper articles. The trials were conducted in France, Italy, Spain, Japan and USA. All patients underwent ultrasound, serological testing and a liver biopsy. The diagnosis of chronic hepatitis C was verified based on hepatitis C virus RNA for at least six months and active hepatitis on liver histology. Two randomised trials included patients with cirrhosis or fibrosis (table 1). All patients had cirrhosis in the remaining randomised trials and all observational studies. Two randomised trials assessed pegylated interferon (table 1) and one assessed interferon plus ribavirin (14). The remaining randomised trials and all of the observational studies assessed interferon monotherapy. All control groups received no intervention. The duration of therapy varied from 1 to 5 years for the randomised trials and from 0.5 to 1.5 years in the observational studies. The duration of follow up ranged from 2 to 8.7 years for the randomised trials and from 5 to 7 years for the observational studies.

Risk of Bias

Randomisation methods (allocation sequence generation and allocation concealment) were classified as adequate in 6 of the 8 randomised trials (14;16-20). Two trials did not describe how the allocation sequence was generated or the allocation sequence was concealed. None of the trials found discrepancies between baseline patient characteristics in the intervention versus control group. None of the included trials were blinded. No clear evidence of reporting or attrition bias was identified. Five trials reported sample size calculations and that the planned sample size was achieved (16-20). Two trials were registered in clinical trial databases three months after the enrolment of the first patient and before the completion of the trial (16;18).

Intervention effects: HCC

In total, 81 of 1156 patients randomised to antiviral therapy and 129 of 1074 patients in the control group developed HCC. Random effects meta-analysis showed that antiviral therapy reduced the risk HCC (relative risk 0.53, 95% confidence interval 0.34 to 0.81; I^2 50%). The corresponding number needed to treat to prevent one case of HCC was 8 patients. There was evidence of bias or small study effects in regression analysis (Egger's test P=0.931). The sequential analysis revealed that the cumulative Z-curve crossed the monitoring boundary, which confirmed the overall result after adjusting for multiple testing. Excluding trials without adequate randomisation also confirmed the overall result (0.58, 0.37 to 0.95) as did fixed effect model meta-analysis and sensitivity analysis excluding data on patients without cirrhosis (0.51, 0.34 to 0.77). In subgroup analysis (figure 3), the effect of antiviral therapy was more pronounced (P=0.03) among patients with a virological response (0.15, 0.05 to 0.45, Egger's test P=0.543) compared with virological non-responders (0.57; 0.37to 0.85, Egger's test P=0.425).

Sensitivity analyses including the randomised trials and observational studies found that antiviral therapy reduces the risk of HCC compared with no intervention when analysed separately (0.50; 0.36 to 0.71; I^2 56%). However, when observational studies were analysed separately, clear evidence of bias was identified (0.29; 0.12 to 0.69; Egger's test P=0.02).

Intervention effects: mortality and liver-related complications

Four randomised trials reported all-cause mortality (16;18-20). Random effects meta-analysis found no clear difference between the intervention and control group (93/918 versus 90/932; 0.81, 0.33 to 2.03; I^2 84%; Egger's test P=0.348). No beneficial or detrimental effects were identified when analysing liver related mortality (0.82, 0.34 to 1.95; I^2 73%; Egger's test P=0.883, five trials) or liver-related complications (34/400 versus 42/389, 0.73, 0.48 to 1.11, I^2 0%; Egger's test P=0.306).

Discussion

The present review suggests that antiviral therapy may prevent HCC in patients with hepatitis C related fibrosis or cirrhosis. The size of the effect was clinically relevant with a number needed to treat of eight patients after a median of five years. Even considering that patients in clinical trials (on average) fare better than patients in clinical practice, the overall estimate still supports the use of antiviral therapy in the patient group assessed. The evidence concerning mortality and complications was less convincing. No beneficial or detrimental effects were identified when assessing all-cause mortality, liver related mortality or clinical complications. Accordingly, additional evidence is needed to assess these outcome measures.

The assessment of intervention effects on clinical outcome measures in randomised controlled trials is difficult in diseases with a protracted course. Complications to hepatitis C including decompensated cirrhosis and HCC take years to develop. We therefore planned to perform sensitivity analyses including observational studies. Our analyses

showed that the duration of follow up was slightly longer in the randomised trials than in the observational studies. Furthermore, clear evidence of bias was identified when analysing the results of the observational studies.

Our subgroup analyses showed that the effect of antiviral therapy was better among sustained virological responders compared with non-responders. Still, there was a clear effect in both patient groups. This finding suggests that the antiviral therapy may have a small beneficial effect, irrespective of the virological response.

As expected, we found clinical heterogeneity between trials. In some of our analyses, clear evidence of statistical intertrial heterogeneity was identified. The differences between trials were related to the type of intervention regimens assessed and patient inclusion criteria. Most of the included trials assessed interferon monotherapy. Since the current practice is to use pegylated interferon and ribavirin, direct extrapolation of the size of the effect is difficult. We cannot exclude that the size of the effect is larger in current antiviral treatment regimens since the proportion of virological responders continues to increase with the ongoing improvements in therapy. On the other hand, several patients are treated early in the course of their disease. Follow up regimens are also improving. Chronic inflammation of the liver is critical to the development of HCC (27). Hepatitis C patients with cirrhosis or fibrosis are likely to have a higher degree of chronic inflammation than patients without these histological changes. It is therefore likely that patients without fibrosis or cirrhosis have a smaller benefit of antiviral therapy than the patient population included in our analyses. The number needed to treat may therefore be higher.

Randomised trials are generally considered as the gold standard for intervention comparisons. In spite of the risk of bias associated with the inclusion of observational studies, we planned to include prospective cohort studies since we expected that we would not be able to identify a sufficient number of trials with adequate statistical power and follow up in randomised controlled trials would be insufficient. However, during recent years, large randomised trials with long term follow up and adequate bias control (based on the assessment used in the present review) have been published. Furthermore, we did find evidence of bias in the analysis of the cohort studies although all used a clearly defined strategy for prospective collection of data. Accordingly, the present review confirms that the results of cohort studies should be adjusted for the risk of bias before the extrapolation to clinical practice. A relatively recent meta-analysis combined the results of three randomised trials and six observational studies with prospective or retrospective collection of data. (28) The overall result of the meta-analysis was that interferon reduces the risk of HCC and that the effect was greater in studies with less than three years of follow up than in studies with longer follow up. Our result add to previous evidence by showing that the effect is stable when assessed in randomised trials with long term follow up. The increased internal validity that is achieved when the results are based on trials with a higher degree of bias control supports the extent to which the overall results may be extrapolated to clinical practice.

The development of HCC involves inflammatory mediators, which promote liver cancer by compensatory proliferation of hepatocytes in response to tissue damage (27). Experimental models show that the cytokine interferon-gamma suppresses chemical carcinogenesis in hepatocytes in spite of concomitant liver injury. Prolonged treatment with

interferon reduces inflammation in liver (17;29). The potential anticarcinogenic effect of interferon could be related to its immunoregulatory and antitumoral effects. The combined evidence suggests that interferon may have other beneficial effects than the direct antiviral activity. Based on the duration of follow up and the lack of clear evidence concerning morbidity or mortality, we cannot exclude that interferon delays rather than prevents carcinogenesis. Additional randomised trials with longer follow up are still warranted.

Contributors

AK conceived the present review and developed the overall design with LG. NK and ED extracted data. AK and LG validated the data extraction. NK and LG performed the statistical analyses and all authors participated in the interpretation of the overall results. NK and LG drafted the protocol and review and all authors participated in the critical revision for important intellectual content. All authors have read and approved of the final version of the protocol and paper. NK had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis and had the final responsibility for the decision to submit for publication.

Funding

No external funding was received for the present study.

Competing interests

All authors have completed the Unified Competing Interest 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 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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Ethical approval

Not required.

Figure Legends

Fig 1 Study selection flow chart

Fig 2 Individual trial and pooled effects of randomised trials and prospective cohort studies on antiviral therapy versus no intervention for development of hepatocellular carcinoma in hepatitis C-related cirrhosis or fibrosis. Random effects model used. RR= relative risks (RR); 95% CI=95% confidence intervals.

Fig 3 Individual and pooled effects of randomised trials for subgroups of patients classed as virological responders or non-responders. The outcome measure is developments of hepatocellular carcinoma. no intervention for development of HCC Random effects model used. RR= relative risks (RR); 95% CI=95% confidence intervals

Table1 **Characteristics of randomised controlled trials and cohort studies**

Trial	Proportion of patients with cirrhosis at baseline	Antiviral therapy administered	Duration of treatment	Maximum duration of follow up	Total number of patients
Randomised controlled trials					
Azzaroli 2004 (14)	100%	Interferon alpha plus ribavirin	1 to 2 years	5 years	101
Bernardinello 1996 (15)	100%	Interferon	1 year	5 years	61
Bruix 2011 (16)	100%	Pegylated interferon	5 years	5 years	626
Fartoux 2007 (17)	100%	Interferon	2 years	2 years	102
Lok 2011 (18)	41%	Pegylated interferon	3.5 years	8.7 years	1048
Nishiguchi 2001 (19)	100%	Interferon	2 years	8.7 years	90
Soga 2005 (21)	0%	Interferon	Unclear	5 years	133
Valla 1999 (20)	100%	Interferon	1 year	4.8 years	99
Cohort studies					

Bruno 1997 (22)	100%	Interferon	0.5 to 1 year	7 years	163
Gramenzi 2001 (23)	100%	Interferon	1 year	5.8 years	144
Mazzella (24)	100%	Interferon	0.5-1 year	6.4 years	193
Serfaty 1998 (25)	100%	Interferon	0.5-1.5 years	6 years	103
Shiratory 2005 (26)	100%	Interferon	39 weeks*	5 years	345

*Mean

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Interferon for Prevention of Hepatocellular Carcinoma in Chronic Hepatitis C; Systematic Review and Meta-Analysis

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Figure 1

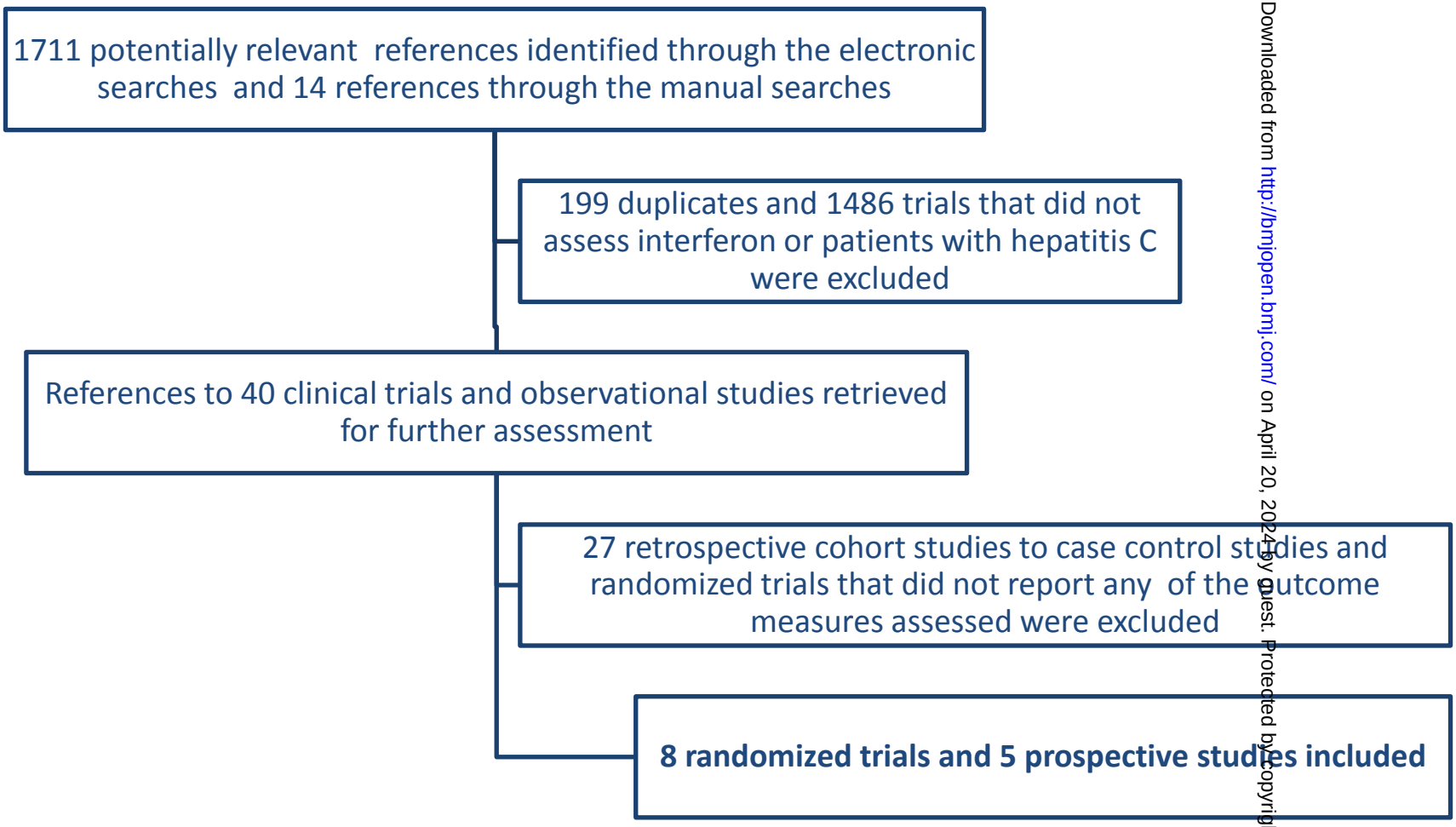


Fig. 2

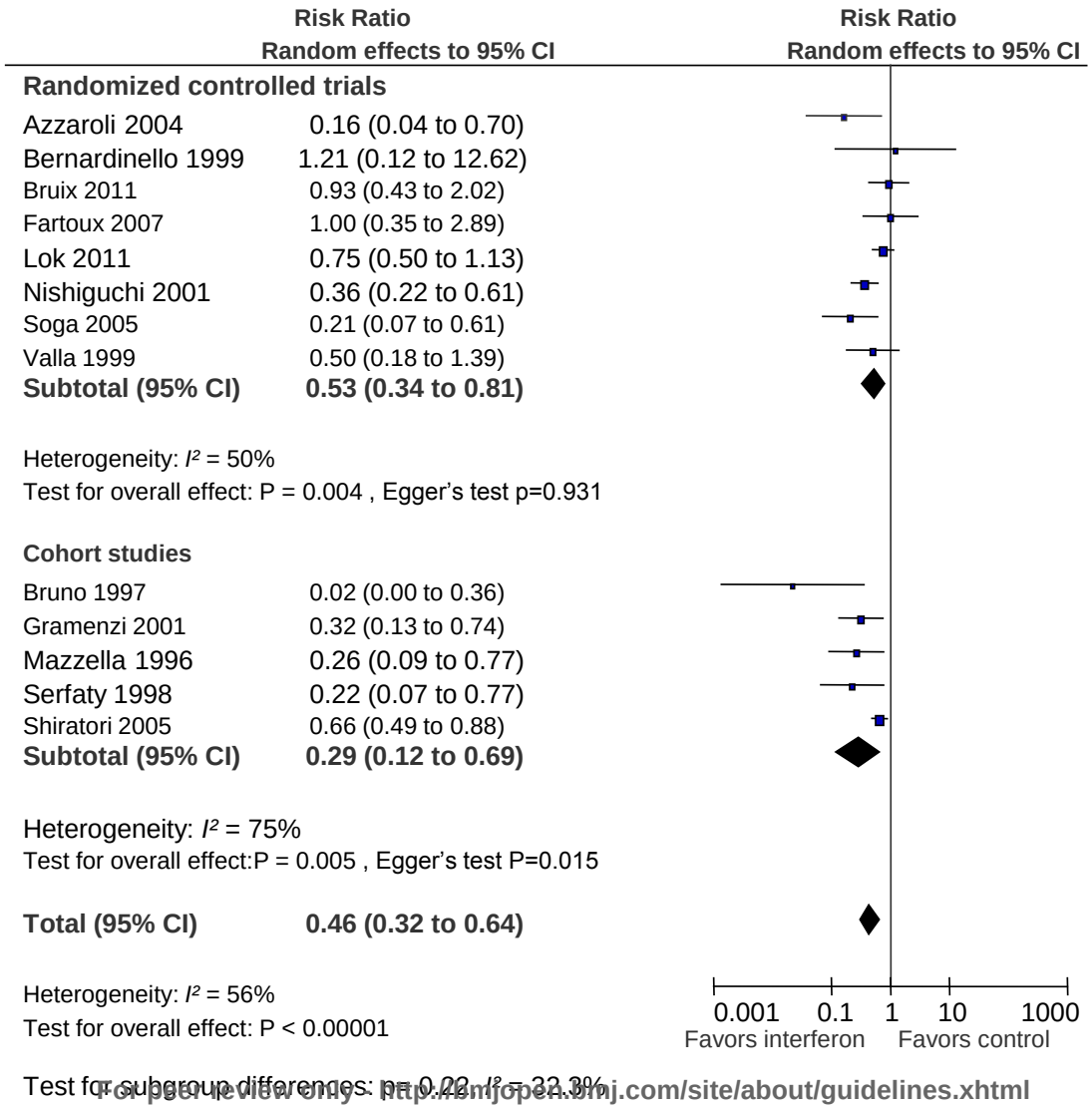
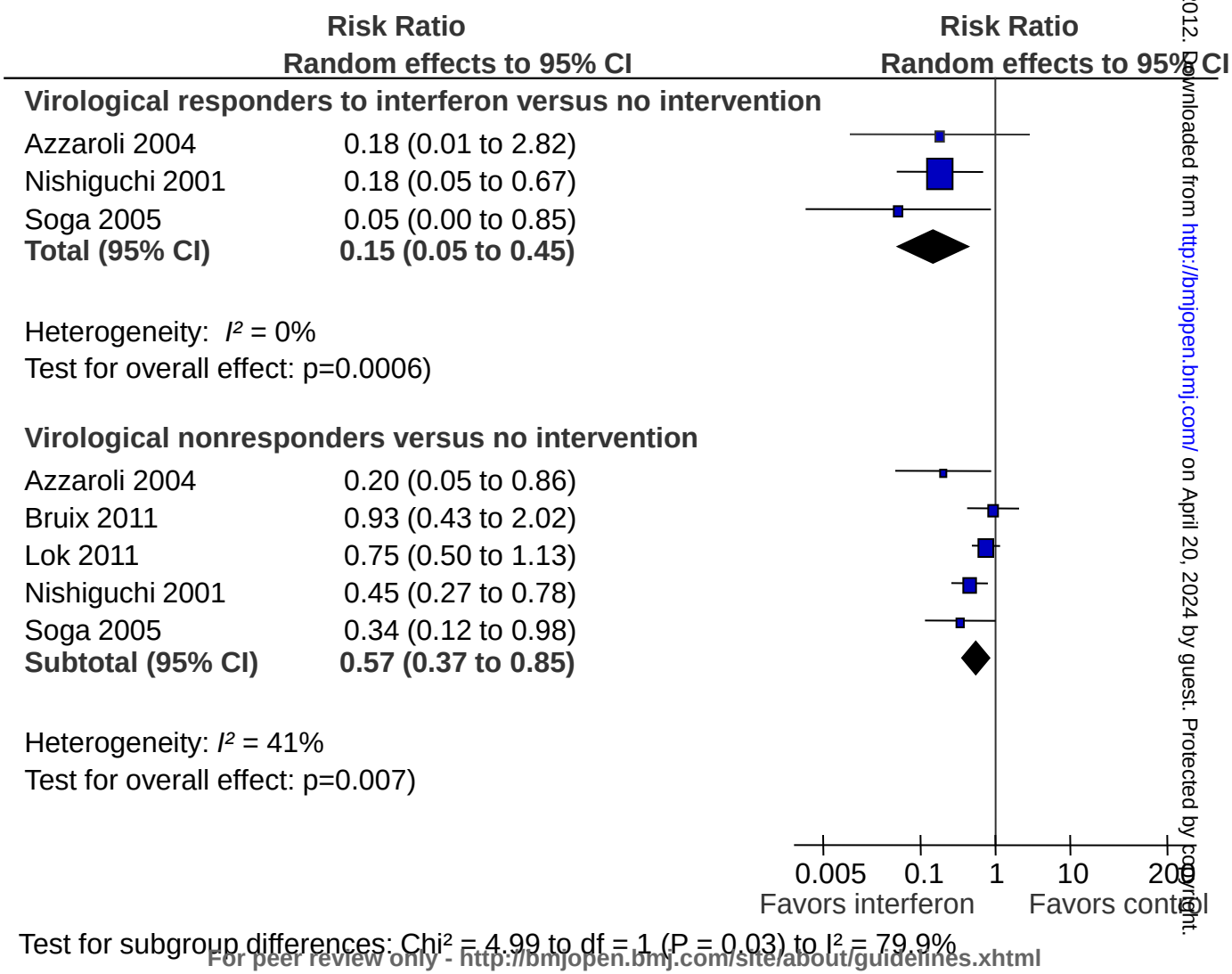


Fig. 3



Interferon for Prevention of Hepatocellular Carcinoma in Patients with Chronic Hepatitis C and Cirrhosis or Fibrosis: Systematic Review and Meta-Analysis

Review information

Review number: NK01

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Background

Chronic infection with hepatitis C virus (HCV) can lead to the development of hepatocellular carcinoma (HCC). The major risk factors is HCV-related HCC is cirrhosis although HCC can also be seen in patients without cirrhosis. Randomized controlled trial of antiviral therapy in patients with hepatitis B show that antiviral therapy prevents disease progression and reduced the incidence of HCC. This effect is also seen in cohort studies. Recent and earlier randomized controlled trials of interferon for HCV have reached inconsistent conclusions although there is a tendency suggesting that patients who are virological responders (patients with a sustained loss of HCV RNA) seem to have a reduced risk of HCC. Since a number of recent trials on interferon have reported long term follow up, we plan to perform a systematic review on this question.

Methods

Objectives

PICOS: The primary objective of the present review will be to assess the effects of interferon versus placebo or no intervention for development of HCC in patients with chronic HCV who have developed cirrhosis or fibrosis of the liver.

Criteria for considering studies for this review

Types of studies

In the primary analyses, randomised trials will be included irrespective of blinding, publication status or language. Since we expect that randomised trials may have insufficient follow up, prospective cohort studies with a clear description of the control group will be included in sensitivity analyses.

Types of participants

Patients with chronic HCV as defined by authors of included trials (normally a positive HCV RNA combined with histological changes suggesting chronic active hepatitis or elevated transaminases including ALT or AST for at least six months). Patients with clinical or histological cirrhosis or histological fibrosis are eligible for inclusion.

Types of interventions

Interferon versus no intervention or placebo. The type and dose of interferon will not be considered in the decision to include trials, nor will the duration of therapy. Accordingly, trials on interferon-alpha 2a or 2b, interferon-beta or pegylated interferon will be eligible for inclusion. Additional treatment with ribavirin will be allowed.

Types of outcome measures

The primary outcome measure will be HCC. Secondary outcome measures will include mortality (all-cause and liver-related) and liver related complications.

Search methods for identification of studies

Electronic searches

The Cochrane Hepato-Biliary Group Controlled Trials Register, The Cochrane Library, MEDLINE, EMBASE, and Science Citation Index Expanded will be searched.

Searching other resources

Additional trials will be identified through scanning of reference lists in relevant papers and conference proceedings. The meta-register http://www.controlled-trials.com/mrct/ will be searched for additional ongoing or unpublished trials.

Data collection and analysis

Selection of studies

All authors will participate in the identification and selection of trials for inclusion. All authors must approve of the trials selected for inclusion. Excluded trials will be listed with the reason for exclusion.

Data extraction and management

Two authors (NK and ED) will extract data in an independent manner. The extracted data will be validated by AK (outcome measures) and LG (bias control). Disagreements will be resolved through discussion before any analyses are made.

Primary authors of the included trials will be contacted for additional information when outcome measures or trial methods are not described in the published trial reports. Additional data will be retrieved through correspondence with experts and other publications such as previous meta-analyses.

Assessment of risk of bias in included studies

Based on previous evidence (Gluud 2006; Wood 2008), the randomisation methods (allocation sequence generation and concealment) will be extracted as the primary measures of bias control.

- The allocation sequence generation will be classified as adequate if based on a computer, random number table, or similar.
- Allocation concealment was classified as adequate if based on a central independent unit, serially numbered opaque sealed envelopes, or on-site locked computer.
- Information regarding comparability between intervention groups at baseline will also be extracted since differences may reflect skewed allocation.

Additional measures of bias control will include

- Blinding (was the trial single or double blind, the method of blinding and testing of blinding. If trials are described as blind, the persons who are blinded e.g., patients or outcome assessors will be registered).
- Handling of missing data (whether all patients randomised were accounted for in the analyses),
- Reporting bias (whether the most clinically relevant outcome measures were reported)
- Sample size calculation
- Whether the preset sample size achieved.
- Whether the trial was registered before start or before termination

All cohort studies will be classed as having a low bias control. For cohort studies, the methods used for the allocation of patients to the intervention and control group will be

extracted. Trials in which patients in the control group had contraindications/comorbidities will be considered as having the highest risk of bias.

Data synthesis

The analyses will be performed in RevMan version 5 (the Nordic Cochrane Centre, Copenhagen Denmark), STATA version 11 (STATA Corp, Texas USA), and TSA, trial sequential analysis (Copenhagen Trial Unit, Copenhagen Denmark). Meta-analyses will be performed using random effects models due to an expected clinical heterogeneity (duration of follow up and intervention regimens). Results will be expressed as relative risks (RR) with 95% confidence intervals (CI) and I-square as a measure of intertrial heterogeneity and test for subgroup differences by P values and I-square. Data on all patients randomised will be sought to perform intention-to-treat analyses. Carry forward of the last observed response will be used for patients with missing data (i.e., patients without HCC at inclusion will be counted as non-events).

Subgroup analysis and investigation of heterogeneity

Subgroup and sensitivity analyses will be performed to evaluate the effect of interferon in

Randomised trials and cohort studies

Patients with cirrhosis and patients without cirrhosis

Patients with a virological response or no virological response.

Trials with adequate randomisation

Trials with a placebo control or blinded outcome assessment

Random-effect metaregression analyses assessing the influence of potential predictors (duration of follow up) will be performed if at least ten randomised trials are identified.

Antiviral therapy for Prevention of Hepatocellular Carcinoma in Chronic Hepatitis C: Systematic Review and Meta-Analysis of Randomised Controlled Trials

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Antiviral therapy for Prevention of Hepatocellular Carcinoma in Chronic Hepatitis C: Systematic Review and Meta-Analysis of Randomised Controlled Trials

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Key words: Hepatocellular carcinoma, Hepatitis C, Interferon, cirrhosis, Meta analysis

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ABSTRACT

Objectives: To determine whether antiviral therapy reduces the risk of developing hepatocellular carcinoma in chronic hepatitis C.

Design: Systematic review and meta-analyses of randomised controlled trials. Prospective cohort studies were included in sensitivity analyses.

Data Sources: Eligible trials were identified through electronic and manual searches.

Study Selection: Eight randomised controlled trials comparing antiviral therapy (interferon or pegylated interferon alone or with ribavirin) versus placebo or no intervention were included.

Data extraction and synthesis: Two independent reviewers assessed the methodological quality of studies and extracted data. Random effects meta-analyses were performed. Subgroup, sensitivity, regression and sequential analyses were performed to evaluate sources of intertrial heterogeneity, the risk of bias and the robustness of the results after adjusting for multiple testing.

Results: Random effects meta-analysis showed that antiviral therapy reduced the risk of hepatocellular carcinoma (81/1156 versus 129/1174; risk ratio 0.53, 95% CI 0.34 to 0.81). In subgroup analyses, antiviral therapy was more beneficial (test for subgroup differences p=0.03) in virological responders (0.15, 0.05 to 0.45) than in non-responders (0.57; 0.37-0.85). No evidence of bias was seen in regression analyses. Sequential analysis confirmed the overall result. The sensitivity analyses showed that the cohort studies found that antiviral therapy reduced the risk of hepatocellular carcinoma. There was clear statistical evidence of bias in the cohort studies (p=0.02).

Conclusion: Antiviral therapy may reduce the risk of hepatocellular carcinoma in hepatitis C-related fibrosis and cirrhosis. The effect may be seen irrespective of the virological response, but is more pronounced among virological responders compared with non-responders.

ARTICLE SUMMARY

Article focus: To determine whether antiviral therapy reduces the risk of developing hepatocellular carcinoma in chronic hepatitis C.

Key Messages:

- Anti viral therapy reduces the risk of HCC
- The preventive effect of antiviral therapy on the development of HCC is seen irrespective of the antiviral response (loss of hepatitis C virus RNA), but is more pronounced among patients who are virological responders.

Strenghts and limitations of this study:

- The review only addresses interferon as monotherapy.
- No points on dose and duration can be made on the available data.
- HCC incidence is diminished in both virological responders and non-responders.
- A thorough systematic review and meta-analysis provide confidence in the findings.

INTRODUCTION

World-wide, hepatocellular carcinoma (HCC) is one of the most common malignant diseases accounting for approximately 90% of primary liver cancers.[1;2] Hepatitis C and cirrhosis are two of the most important risk factors for the development of HCC.[3] Among patients with hepatitis C related cirrhosis the estimated annual incidence of HCC ranges from 1 to 4%[1] depending on the severity of the underlying liver disease and ethnicity of the patient.[1;4]

Hepatitis C is an insidious disease that often leads to chronic infection. Few patients clear the virus spontaneously. Antiviral Therapy for patients with chronic hepatitis C may lead to a sustained loss of the virus.[5;6] A number of patients with an initial response relapse within a few months after treatment. For patients who achieve a 24 weeks sustained virological response, the risk of relapse is negligible.[7] The proportion of patients who achieve a virological response depends on the underlying viral genotype and on the type of therapy. Interferon was introduced in 1986 and initially used as monotherapy.[8] Subsequent trials showed that the addition of ribavirin and the use of a pegylated form of interferon increased the number of sustained virological responders.[5;6;9;10] The effect of antiviral therapy on clinical outcome measures is debated. Some studies have found that interferon increases survival and reduces the incidence of HCC.[11-13] Some data also suggest a reduction in HCC in non-sustained responders[14]. Whether a sustained virological response (SVR) is the key factor leading to a reduced risk of developing HCC is not known. Other studies and randomised trials as well as systematic reviews did not find beneficial effects of antiviral therapy on mortality or morbidity.[15;16]

METHODS

The main objective of the present review was to determine the effect of antiviral therapy versus placebo or no intervention for prevention of HCC in hepatitis C related cirrhosis or fibrosis, and to assess the importance of virological response to treatment in relation to risk of HCC.

The review was carried and reported out based on a protocol developed using the methods described in the Cochrane Handbook for Systematic Reviews of Interventions and the PRISMA Statement for Reporting Systematic Reviews and Meta-analysis.[17;18]

Trials on patients with hepatitis C related cirrhosis or fibrosis treated with antiviral therapy were included if reporting any of the outcome measures assessed. Our primary analyses included randomised controlled trials. Prospective cohort studies with defined control groups were included in sensitivity analyses. Trials were included irrespective of language or publication status. The dose, type and duration of therapy were not considered in the inclusion criteria. Trials on interferon or pegylated interferon alone or with ribavirin were eligible for inclusion. Trials on patients with human immunodeficiency virus and patients with chronic hepatitis B were excluded. The primary outcome measure was HCC. Secondary outcomes were overall mortality, HCC-related mortality, liver related mortality (defined as death following variceal bleeding, hepatorenal syndrome, liver failure or spontaneous bacterial peritonitis) and liver related morbidity (variceal bleeding, hepatorenal syndrome, liver failure or spontaneous bacterial peritonitis).

Two authors (NK, AK) participated in the literature searches. Excluded trials were listed with the reason for exclusion. Two authors (NK, ED) performed independent standardised data extraction. Extracted data were validated by two authors (AK, LG).

Search strategy identification of eligible trials

Eligible trials were identified through electronic searches of the Cochrane Library (issue 3, 2012), Pubmed (1966-August 2012), Embase (1955-August 2012) and Web of Science (1900-August 2012). Additional searches were performed including scanning of reference lists from relevant papers on chronic hepatitis C and HCC, conference proceedings and the World Health Organization Trial Search Portal (www.who.int/trialsearch/). All authors were contacted by email with enquiries of additional data.

Assessment of bias control

The quality of bias control was assessed through individual components.[17] Based on previous evidence,[19] our primary assessment of bias control was based on the

randomization methods including the allocation sequence generation (classed as adequate if based on a table of random numbers or similar) or allocation concealment (classed as adequate if based on a central independent unit or similar). Trials in which randomisation methods were classed as adequate were defined as having a low risk of bias. Additional components included blinding (performance bias and detection bias), handling of missing outcome data (attrition bias) and selective reporting (reporting bias). We also extracted sample size calculations and whether the sample size was reached or the trial was terminated prematurely. Due to the risk of selection bias associated with the observational design, all cohort studies were classed as having a high risk of bias.

Statistical analysis

The analyses were performed using Revman version 5.1 (Nordic Cochrane Centre, Copenhagen), STATA version 11 (STATA Corp, Texas, USA) and TSA version 9 (Copenhagen Trial Unit, Copenhagen, Denmark). The primary meta-analyses were performed using random effects models due to an expected clinical heterogeneity (differences between patient and intervention characteristics). The results of the analyses were presented as risk ratios with 95% confidence intervals and I^2 as a marker of intertrial heterogeneity. We defined I^2 values between 30%-60% as moderate heterogeneity and values >60% as substantial heterogeneity. The number needed to treat was calculated as the inverse of the risk difference. Fixed effect meta-analyses were performed to evaluate the robustness of the results. The results were only reported if the overall conclusion differed from the result of the random effect meta-analysis.To evaluate the risk of bias and the influence of patient characteristics, the results of the analysis was analysed after exclusion of trials without adequate randomisation and trials including patients with fibrosis. The risk of bias and small study effects was assessed through regression analyses (Egger's test). Planned subgroup analyses evaluated the effect of virological response (virological responders compared with non-responders). Differences between subgroups were analysed using the test of subgroup differences and the results expressed using the p values.[20] A sequential analysis was performed to adjust for the risk of false-positive findings due to repeated tests.[21] The sequential analysis was performed for the primary random effects meta-analysis. Based on the results of the primary meta-analysis,

the incidence in the control group was set to 12% and the relative risk reduction to 41%. The heterogeneity correction was set to 64% (model-based), power to 80% and alpha to 5%.

RESULTS

Study selection

The electronic searches generated 1711 references (Figure 1). After reading the titles and abstracts, we identified 26 potentially relevant randomised controlled trials and observational studies described in 27 references. Fourteen additional trials and references were identified through the manual searches. Twenty-four references were retrospective cohort studies, case control studies or trials that did not assess the risk of HCC. Eight randomised trials[15;22-28] and five prospective cohort studies[29-33] were included in our analyses.

Characteristics of included trials and patients

All trials were published in English as full paper articles. The trials were conducted in France, Italy, Spain, Japan and USA. All patients underwent ultrasound, serological testing and a liver biopsy at baseline. The diagnosis of chronic hepatitis C was based on hepatitis C virus RNA for at least six months and active hepatitis on liver histology. Two randomised trials included patients with cirrhosis or fibrosis (table 1). The remaining trials included patients with cirrhosis. Two randomised trials assessed pegylated interferon[15;25] and one assessed interferon plus ribavirin.[22] The remaining trials assessed interferon monotherapy. All control groups received no intervention. The duration of therapy varied from 1 to 5 years and the duration of follow up ranged from 2 to 8.7 years. The observational studies compared interferon versus no intervention for patients with cirrhosis. The duration of therapy ranged from 0.5 to 1.5 years and the duration of follow up from 5 to 7 years.

Risk of bias

Randomisation methods (allocation sequence generation and allocation concealment) were classified as adequate in 6 trials.[15;22;24-26;28] Two trials did not describe how the allocation sequence was generated or the allocation sequence was concealed. None of

the trials found discrepancies between baseline patient characteristics in the intervention versus control group. None of the included trials were blinded. No clear evidence of reporting or attrition bias was identified. Five trials reported sample size calculations and that the planned sample size was achieved.[15;24-26;28] Two trials were registered in clinical trial databases three months after the enrolment of the first patient and before the completion of the trial.[15;25]

Intervention effects: HCC

In total, 81 of 1156 patients randomised to antiviral therapy and 129 of 1074 patients in the control group developed HCC. Random effects meta-analysis showed that antiviral therapy reduced the risk of HCC (RR 0.53, 95% CI 0.34 to 0.81; I^2 50%) (Figure 2). The corresponding number needed to treat to prevent one case of HCC was 8 patients. There was no evidence of bias or small study effects in regression analysis (Egger's test p=0.931). The sequential analysis revealed that the cumulative Z-curve crossed the monitoring boundary, which confirmed the overall result after adjusting for multiple testing. Similar results were achieved after exclusion of trials without adequate randomisation which confirmed the overall result (RR 0.58, 95% CI 0.37 to 0.95) and trials on patients with fibrosis (RR 0.51, 95% CI 0.34 to 0.77). In subgroup analysis (Figure 3), the effect of antiviral therapy was more pronounced (test for subgroup differences p=0.03) among patients with a virological response (RR 0.15, 95% CI 0.05 to 0.45, Egger's test p=0.543) compared with virological non-responders (RR 0.57; 95% 0.37 to 0.85, Egger's test p=0.425).

Sensitivity analyses were performed to evaluate the results of the observational studies. In agreement with our primary analyses, the observational studies found that antiviral therapy reduces the risk of developing HCC (RR 0.29 95% CI 0.12 to 0.69) (Figure 2). The analysis also found a higher degree of heterogeneity among observational studies (I^2 75%) than among randomised trials (33%). Regression analysis showed clear evidence of bias in the observational studies (Egger's test p=0.02).

Intervention effects: mortality and liver-related complications

Four randomised trials reported all-cause mortality.[15;25;26;28] Random effects meta-analysis found no clear difference between the intervention and control group (93/918 versus 90/932; RR 0.81, 95% CI 0.33 to 2.03; I^2 84%; Egger's test p0.348). No beneficial or detrimental effects were identified when analysing liver related mortality (RR 0.71, 95% CI 0.2 to 2.51; I^2 74%; Egger's test p=0.59, four trials) or liver-related morbidity (34/400 versus 42/389, RR 0.73, 95% CI 0.48 to 1.11, I^2 0%; Egger's test p=0.306).

DISCUSSION

This review found that antiviral therapy may prevent HCC in patients with hepatitis C related fibrosis or cirrhosis. The size of the effect was clinically relevant with a number needed to treat of eight patients after a median of five years. Based on the relatively high event rates, the underlying prognosis of the included patients may differ from the patient population in some clinical settings. However, after considering the risk of detection or ascertainment bias the size of the effect was clinically relevant. The evidence concerning all-cause and liver-related mortality and morbidity was less convincing. Additional evidence is needed to assess these outcome measures.

Our subgroup analyses suggest that the antiviral therapy may have beneficial effects on the risk of developing HCC that are unrelated to the virological response. Although the intervention was more beneficial among sustained virological responders than non-responders, there was a clear effect in both patient groups. A former review [14]reached similar conclusions, but included randomised controlled trials and observational studies in their overall analysis.

The assessment of intervention effects on clinical outcome measures is difficult to assess in trials of a diseases with a protracted course. Complications to hepatitis C including cirrhosis and HCC takes years to develop.[34]

We originally planned to include observational studies in sensitivity analysis because we expected that the randomised controlled trials would be too small or have insufficient follow up. We were surprised to find that the duration of follow up was slightly longer in the randomised trials than in the observational studies. Likewise, the statistical power of the

randomised trials was not weaker than the observational studies. Since we also found a high degree of heterogeneity and evidence of bias in the observational studies, the result of these studies should only be used with caution. Our findings do not support the inclusion of non-randomised studies in systematic reviews on viral hepatitis.

Only two of the included trials evaluated pegylated interferon, which is the current standard treatment for chronic hepatitis C.[7] Two studies have found that prolonged treatment with interferon reduces inflammation in the liver[24;35] and improve the proportion of patients who achieve a sustained virological response.[36] The duration of treatment in some of our included trials was relatively long, which may increase the proportion of responders. Unfortunately, we were unable to perform subgroup analyses on treatment duration or dose due to the variation in these parameters across trials. Our data provide no information on the best standard for duration of treatment or dose.

As expected, we found clinical heterogeneity between trials. The differences between trials were related to the type of intervention regimens and patient inclusion criteria.

Most of the included trials assessed interferon monotherapy. Standard practice is pegylated interferon and ribavirin in combination,[7] and direct extrapolation of the observed effects to clinical practice is difficult. The protection from HCC might be even better among patients in current antiviral therapy since the proportion of virological responders continues to increase with ongoing improvements in therapy[1]. Also, today's patients are diagnosed and treated earlier in the course of their disease.

Chronic inflammation of the liver is critical to the development of HCC.[37] Hepatitis C patients with cirrhosis or fibrosis are likely to have a higher degree of chronic inflammation than patients without these histological changes. It is therefore likely that patients without fibrosis or cirrhosis have a smaller benefit of antiviral therapy than the patient population included in our analyses. The number needed to treat may therefore be higher.

During recent years, large randomised trials with long term follow up and adequate bias control have been published. The overall result of this meta-analysis was that interferon reduces the risk of HCC. Our results add to previous evidence by showing that the reduced risk of HCC is stable when assessed in randomised trials with long term follow up. The increased internal validity that is achieved when the results are based on trials with a

higher degree of bias control supports the extent to which the overall results may be extrapolated to clinical practice.

The development of HCC involves inflammatory mediators, which promote liver cancer by compensatory proliferation of hepatocytes in response to tissue damage.[37] Experimental models show that the cytokine interferon-gamma suppresses chemical carcinogenesis in hepatocytes in spite of concomitant liver injury. Prolonged treatment with interferon reduces inflammation in liver.[24;35] The potential anticarcinogenic effect of interferon could be related to its immunoregulatory and antitumoral effects. The combined evidence suggests that interferon may have other beneficial effects than the direct antiviral activity. Based on the duration of follow up and the lack of clear evidence concerning morbidity or mortality, we cannot exclude that interferon delays rather than prevents carcinogenesis. Additional randomised trials with longer follow up are still warranted to determine whether this is the case.

Contributors

AK conceived the present review and developed the overall design with LG. NK and ED extracted data. AK and LG validated the data extraction. NK and LG performed the statistical analyses and all authors participated in the interpretation of the overall results. NK and LG drafted the protocol and review and all authors participated in the critical revision for important intellectual content. All authors have read and approved of the final version of the protocol and paper. NK had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis and had the final responsibility for the decision to submit for publication.

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Competing interests

All authors have completed the Unified Competing Interest 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 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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Ethical approval

Not required.

Data sharing statement

All original data extraction is available from the corresponding author.

Figure legends

Figure 1 Study selection flow chart

Figure 2 Random effects meta-analysis of randomised trials and cohort studies on antiviral therapy versus no intervention for development of hepatocellular carcinoma in hepatitis C-related cirrhosis or fibrosis.

Figure 3 Random effects meta-analysis of randomised trials on antiviral therapy versus no intervention for prevention of hepatocellular carcinoma among subgroups of sustained virological responders and non-responders.

table 1 Characteristics of randomised controlled trials and cohort studies

Trial	Proportion of patients with cirrhosis at baseline	Antiviral therapy administered	Duration of treatment	Maximum duration of follow up	Total number of patients
Randomised controlled trials					
Azzaroli 2004 [22]	100%	Interferon alpha plus ribavirin	1 to 2 years	5 years	101
Bernardinello 1996 [23]	100%	Interferon	1 year	5 years	61
Bruix 2011 [15]	100%	Pegylated interferon	5 years	5 years	626
Fartoux 2007 [24]	100%	Interferon	2 years	2 years	102
Lok 2011 [25]	41%	Pegylated interferon	3.5 years	8.7 years	1048
Nishiguchi 2001 [26]	100%	Interferon	2 years	8.7 years	90
Soga 2005 [27]	0%	Interferon	Unclear	5 years	133
Valla 1999 [28]	100%	Interferon	1 year	4.8 years	99
Cohort studies					
Bruno 1997 [29]	100%	Interferon	0.5 to 1 year	7 years	163
Gramenzi 2001 [30]	100%	Interferon	1 year	5.8 years	144
Mazzella [31]	100%	Interferon	0.5-1 year	6.4 years	193
Serfaty 1998 [32]	100%	Interferon	0.5-1.5 years	6 years	103
Shiratory 2005 [33]	100%	Interferon	39 weeks*	5 years	345

*Mean

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- Anti viral therapy reduces the risk of HCC
- The preventive effect of antiviral therapy on the development of HCC is seen irrespective of the antiviral response (loss of hepatitis C virus RNA), but is more pronounced among patients who are virological responders.

Strenghts and limitations of this study:

- The review only addresses interferon as monotherapy.
- No points on dose and duration can be made on the available data.
- HCC incidence is diminished in both virological responders and non-responders.
- A thorough systematic review and meta-analysis provide confidence in the findings.

INTRODUCTION

World-wide, hepatocellular carcinoma (HCC) is one of the most common malignant diseases accounting for approximately 90% of primary liver cancers.[1;2] Hepatitis C and cirrhosis are two of the most important risk factors for the development of HCC.[3] Among patients with hepatitis C related cirrhosis the estimated annual incidence of HCC ranges from 1 to 4%[1] depending on the severity of the underlying liver disease and ethnicity of the patient.[1;4]

Hepatitis C is an insidious disease that often leads to chronic infection. Few patients clear the virus spontaneously. Antiviral Therapy for patients with chronic hepatitis C may lead to a sustained loss of the virus.[5;6] A number of patients with an initial response relapse within a few months after treatment. For patients who achieve a 24 weeks sustained virological response, the risk of relapse is negligible.[7] The proportion of patients who achieve a virological response depends on the underlying viral genotype and on the type of therapy. Interferon was introduced in 1986 and initially used as monotherapy.[8] Subsequent trials showed that the addition of ribavirin and the use of a pegylated form of interferon increased the number of sustained virological responders.[5;6;9;10] The effect of antiviral therapy on clinical outcome measures is debated. Some studies have found that interferon increases survival and reduces the incidence of HCC.[11-13] Some data also suggest a reduction in HCC in non-sustained responders[14]. Whether a sustained virological response (SVR) is the key factor leading to a reduced risk of developing HCC is not known. Other studies and randomised trials as well as systematic reviews did not find beneficial effects of antiviral therapy on mortality or morbidity.[15;16]

METHODS

The main objective of the present review was to determine the effect of antiviral therapy versus placebo or no intervention for prevention of HCC in hepatitis C related cirrhosis or fibrosis, and to assess the importance of virological response to treatment in relation to risk of HCC.

The review was carried and reported out based on a protocol developed using the methods described in the Cochrane Handbook for Systematic Reviews of Interventions and the PRISMA Statement for Reporting Systematic Reviews and Meta-analysis.[17;18]

Trials on patients with hepatitis C related cirrhosis or fibrosis treated with antiviral therapy were included if reporting any of the outcome measures assessed. Our primary analyses included randomised controlled trials. Prospective cohort studies with defined control groups were included in sensitivity analyses. Trials were included irrespective of language or publication status. The dose, type and duration of therapy were not considered in the inclusion criteria. Trials on interferon or pegylated interferon alone or with ribavirin were eligible for inclusion. Trials on patients with human immunodeficiency virus and patients with chronic hepatitis B were excluded. The primary outcome measure was HCC. Secondary outcomes were overall mortality, HCC-related mortality, liver related mortality (defined as death following variceal bleeding, hepatorenal syndrome, liver failure or spontaneous bacterial peritonitis) and liver related morbidity (variceal bleeding, hepatorenal syndrome, liver failure or spontaneous bacterial peritonitis).

Two authors (NK, AK) participated in the literature searches. Excluded trials were listed with the reason for exclusion. Two authors (NK, ED) performed independent standardised data extraction. Extracted data were validated by two authors (AK, LG).

Search strategy identification of eligible trials

Eligible trials were identified through electronic searches of the Cochrane Library (issue 3, 2012), Pubmed (1966-August 2012), Embase (1955-August 2012) and Web of Science (1900-August 2012). Additional searches were performed including scanning of reference lists from relevant papers on chronic hepatitis C and HCC, conference proceedings and the World Health Organization Trial Search Portal (www.who.int/trialsearch/). All authors were contacted by email with enquiries of additional data.

Assessment of bias control

The quality of bias control was assessed through individual components.[17] Based on previous evidence,[19] our primary assessment of bias control was based on the

randomization methods including the allocation sequence generation (classed as adequate if based on a table of random numbers or similar) or allocation concealment (classed as adequate if based on a central independent unit or similar). Trials in which randomisation methods were classed as adequate were defined as having a low risk of bias. Additional components included blinding (performance bias and detection bias), handling of missing outcome data (attrition bias) and selective reporting (reporting bias). We also extracted sample size calculations and whether the sample size was reached or the trial was terminated prematurely. Due to the risk of selection bias associated with the observational design, all cohort studies were classed as having a high risk of bias.

Statistical analysis

The analyses were performed using Revman version 5.1 (Nordic Cochrane Centre, Copenhagen), STATA version 11 (STATA Corp, Texas, USA) and TSA version 9 (Copenhagen Trial Unit, Copenhagen, Denmark). The primary meta-analyses were performed using random effects models due to an expected clinical heterogeneity (differences between patient and intervention characteristics). The results of the analyses were presented as risk ratios with 95% confidence intervals and I^2 as a marker of intertrial heterogeneity. We defined I^2 values between 30%-60% as moderate heterogeneity and values >60% as substantial heterogeneity. The number needed to treat was calculated as the inverse of the risk difference. Fixed effect meta-analyses were performed to evaluate the robustness of the results. The results were only reported if the overall conclusion differed from the result of the random effect meta-analysis.To evaluate the risk of bias and the influence of patient characteristics, the results of the analysis was analysed after exclusion of trials without adequate randomisation and trials including patients with fibrosis. The risk of bias and small study effects was assessed through regression analyses (Egger's test). Planned subgroup analyses evaluated the effect of virological response (virological responders compared with non-responders). Differences between subgroups were analysed using the test of subgroup differences and the results expressed using the p values.[20] A sequential analysis was performed to adjust for the risk of false-positive findings due to repeated tests.[21] The sequential analysis was performed for the primary random effects meta-analysis. Based on the results of the primary meta-analysis,

the incidence in the control group was set to 12% and the relative risk reduction to 41%. The heterogeneity correction was set to 64% (model-based), power to 80% and alpha to 5%.

RESULTS

Study selection

The electronic searches generated 1711 references (Figure 1). After reading the titles and abstracts, we identified 26 potentially relevant randomised controlled trials and observational studies described in 27 references. Fourteen additional trials and references were identified through the manual searches. Twenty-four references were retrospective cohort studies, case control studies or trials that did not assess the risk of HCC. Eight randomised trials[15;22-28] and five prospective cohort studies[29-33] were included in our analyses.

Characteristics of included trials and patients

All trials were published in English as full paper articles. The trials were conducted in France, Italy, Spain, Japan and USA. All patients underwent ultrasound, serological testing and a liver biopsy at baseline. The diagnosis of chronic hepatitis C was based on hepatitis C virus RNA for at least six months and active hepatitis on liver histology. Two randomised trials included patients with cirrhosis or fibrosis (table 1). The remaining trials included patients with cirrhosis. Two randomised trials assessed pegylated interferon[15;25] and one assessed interferon plus ribavirin.[22] The remaining trials assessed interferon monotherapy. All control groups received no intervention. The duration of therapy varied from 1 to 5 years and the duration of follow up ranged from 2 to 8.7 years. The observational studies compared interferon versus no intervention for patients with cirrhosis. The duration of therapy ranged from 0.5 to 1.5 years and the duration of follow up from 5 to 7 years.

Risk of bias

Randomisation methods (allocation sequence generation and allocation concealment) were classified as adequate in 6 trials.[15;22;24-26;28] Two trials did not describe how the allocation sequence was generated or the allocation sequence was concealed. None of

the trials found discrepancies between baseline patient characteristics in the intervention versus control group. None of the included trials were blinded. No clear evidence of reporting or attrition bias was identified. Five trials reported sample size calculations and that the planned sample size was achieved.[15;24-26;28] Two trials were registered in clinical trial databases three months after the enrolment of the first patient and before the completion of the trial.[15;25]

Intervention effects: HCC

In total, 81 of 1156 patients randomised to antiviral therapy and 129 of 1074 patients in the control group developed HCC. Random effects meta-analysis showed that antiviral therapy reduced the risk of HCC (RR 0.53, 95% CI 0.34 to 0.81; I^2 50%) (Figure 2). The corresponding number needed to treat to prevent one case of HCC was 8 patients. There was no evidence of bias or small study effects in regression analysis (Egger's test p=0.931). The sequential analysis revealed that the cumulative Z-curve crossed the monitoring boundary, which confirmed the overall result after adjusting for multiple testing. Similar results were achieved after exclusion of trials without adequate randomisation which confirmed the overall result (RR 0.58, 95% CI 0.37 to 0.95) and trials on patients with fibrosis (RR 0.51, 95% CI 0.34 to 0.77). In subgroup analysis (Figure 3), the effect of antiviral therapy was more pronounced (test for subgroup differences p=0.03) among patients with a virological response (RR 0.15, 95% CI 0.05 to 0.45, Egger's test p=0.543) compared with virological non-responders (RR 0.57; 95% 0.37 to 0.85, Egger's test p=0.425).

Sensitivity analyses were performed to evaluate the results of the observational studies. In agreement with our primary analyses, the observational studies found that antiviral therapy reduces the risk of developing HCC (RR 0.29 95% CI 0.12 to 0.69) (Figure 2). The analysis also found a higher degree of heterogeneity among observational studies (I^2 75%) than among randomised trials (33%). Regression analysis showed clear evidence of bias in the observational studies (Egger's test p=0.02).

Intervention effects: mortality and liver-related complications

Four randomised trials reported all-cause mortality.[15;25;26;28] Random effects meta-analysis found no clear difference between the intervention and control group (93/918 versus 90/932; RR 0.81, 95% CI 0.33 to 2.03; I^2 84%; Egger's test p0.348). No beneficial or detrimental effects were identified when analysing liver related mortality (RR 0.71, 95% CI 0.2 to 2.51; I^2 74%; Egger's test p=0.59, four trials) or liver-related morbidity (34/400 versus 42/389, RR 0.73, 95% CI 0.48 to 1.11, I^2 0%; Egger's test p=0.306).

DISCUSSION

This review found that antiviral therapy may prevent HCC in patients with hepatitis C related fibrosis or cirrhosis. The size of the effect was clinically relevant with a number needed to treat of eight patients after a median of five years. Based on the relatively high event rates, the underlying prognosis of the included patients may differ from the patient population in some clinical settings. However, after considering the risk of detection or ascertainment bias the size of the effect was clinically relevant. The evidence concerning all-cause and liver-related mortality and morbidity was less convincing. Additional evidence is needed to assess these outcome measures.

Our subgroup analyses suggest that the antiviral therapy may have beneficial effects on the risk of developing HCC that are unrelated to the virological response. Although the intervention was more beneficial among sustained virological responders than non-responders, there was a clear effect in both patient groups. A former review [14]reached similar conclusions, but included randomised controlled trials and observational studies in their overall analysis.

The assessment of intervention effects on clinical outcome measures is difficult to assess in trials of a diseases with a protracted course. Complications to hepatitis C including cirrhosis and HCC takes years to develop.[34]

We originally planned to include observational studies in sensitivity analysis because we expected that the randomised controlled trials would be too small or have insufficient follow up. We were surprised to find that the duration of follow up was slightly longer in the randomised trials than in the observational studies. Likewise, the statistical power of the

randomised trials was not weaker than the observational studies. Since we also found a high degree of heterogeneity and evidence of bias in the observational studies, the result of these studies should only be used with caution. Our findings do not support the inclusion of non-randomised studies in systematic reviews on viral hepatitis.

Only two of the included trials evaluated pegylated interferon, which is the current standard treatment for chronic hepatitis C.[7] Two studies have found that prolonged treatment with interferon reduces inflammation in the liver[24;35] and improve the proportion of patients who achieve a sustained virological response.[36] The duration of treatment in some of our included trials was relatively long, which may increase the proportion of responders. Unfortunately, we were unable to perform subgroup analyses on treatment duration or dose due to the variation in these parameters across trials. Our data provide no information on the best standard for duration of treatment or dose.

As expected, we found clinical heterogeneity between trials. The differences between trials were related to the type of intervention regimens and patient inclusion criteria.

Most of the included trials assessed interferon monotherapy. Standard practice is pegylated interferon and ribavirin in combination,[7] and direct extrapolation of the observed effects to clinical practice is difficult. The protection from HCC might be even better among patients in current antiviral therapy since the proportion of virological responders continues to increase with ongoing improvements in therapy[1]. Also, today's patients are diagnosed and treated earlier in the course of their disease.

Chronic inflammation of the liver is critical to the development of HCC.[37] Hepatitis C patients with cirrhosis or fibrosis are likely to have a higher degree of chronic inflammation than patients without these histological changes. It is therefore likely that patients without fibrosis or cirrhosis have a smaller benefit of antiviral therapy than the patient population included in our analyses. The number needed to treat may therefore be higher.

During recent years, large randomised trials with long term follow up and adequate bias control have been published. The overall result of this meta-analysis was that interferon reduces the risk of HCC. Our results add to previous evidence by showing that the reduced risk of HCC is stable when assessed in randomised trials with long term follow up. The increased internal validity that is achieved when the results are based on trials with a

higher degree of bias control supports the extent to which the overall results may be extrapolated to clinical practice.

The development of HCC involves inflammatory mediators, which promote liver cancer by compensatory proliferation of hepatocytes in response to tissue damage.[37] Experimental models show that the cytokine interferon-gamma suppresses chemical carcinogenesis in hepatocytes in spite of concomitant liver injury. Prolonged treatment with interferon reduces inflammation in liver.[24;35] The potential anticarcinogenic effect of interferon could be related to its immunoregulatory and antitumoral effects. The combined evidence suggests that interferon may have other beneficial effects than the direct antiviral activity. Based on the duration of follow up and the lack of clear evidence concerning morbidity or mortality, we cannot exclude that interferon delays rather than prevents carcinogenesis. Additional randomised trials with longer follow up are still warranted to determine whether this is the case.

Contributors

AK conceived the present review and developed the overall design with LG. NK and ED extracted data. AK and LG validated the data extraction. NK and LG performed the statistical analyses and all authors participated in the interpretation of the overall results. NK and LG drafted the protocol and review and all authors participated in the critical revision for important intellectual content. All authors have read and approved of the final version of the protocol and paper. NK had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis and had the final responsibility for the decision to submit for publication.

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Competing interests

All authors have completed the Unified Competing Interest 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 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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Ethical approval

Not required.

Data sharing statement

All original data extraction is available from the corresponding author.

Figure legends

Figure 1 Study selection flow chart

Figure 2 Random effects meta-analysis of randomised trials and cohort studies on antiviral therapy versus no intervention for development of hepatocellular carcinoma in hepatitis C-related cirrhosis or fibrosis.

Figure 3 Random effects meta-analysis of randomised trials on antiviral therapy versus no intervention for prevention of hepatocellular carcinoma among subgroups of sustained virological responders and non-responders.

table 1 Characteristics of randomised controlled trials and cohort studies

Trial	Proportion of patients with cirrhosis at baseline	Antiviral therapy administered	Duration of treatment	Maximum duration of follow up	Total number of patients
Randomised controlled trials					
Azzaroli 2004 [22]	100%	Interferon alpha plus ribavirin	1 to 2 years	5 years	101
Bernardinello 1996 [23]	100%	Interferon	1 year	5 years	61
Bruix 2011 [15]	100%	Pegylated interferon	5 years	5 years	626
Fartoux 2007 [24]	100%	Interferon	2 years	2 years	102
Lok 2011 [25]	41%	Pegylated interferon	3.5 years	8.7 years	1048
Nishiguchi 2001 [26]	100%	Interferon	2 years	8.7 years	90
Soga 2005 [27]	0%	Interferon	Unclear	5 years	133
Valla 1999 [28]	100%	Interferon	1 year	4.8 years	99
Cohort studies					
Bruno 1997 [29]	100%	Interferon	0.5 to 1 year	7 years	163
Gramenzi 2001 [30]	100%	Interferon	1 year	5.8 years	144
Mazzella [31]	100%	Interferon	0.5-1 year	6.4 years	193
Serfaty 1998 [32]	100%	Interferon	0.5-1.5 years	6 years	103
Shiratory 2005 [33]	100%	Interferon	39 weeks*	5 years	345

*Mean

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Interferon for Prevention of Hepatocellular Carcinoma in Chronic Hepatitis C; Systematic Review and Meta-Analysis

Nina Kimer to Lise Lotte Gluud to Emilie Dahl to Aleksander Krag

Figure 1

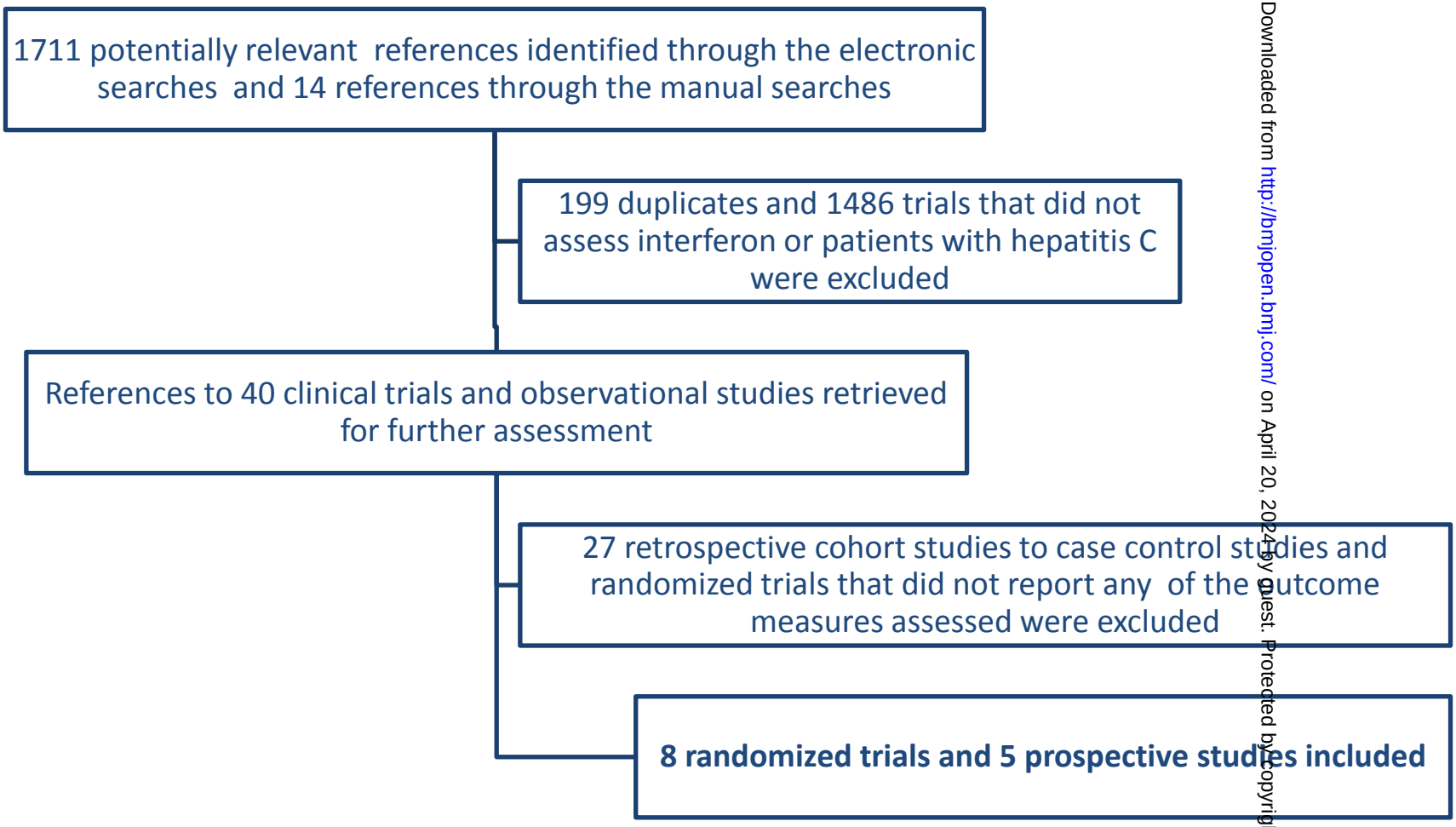


Fig. 2

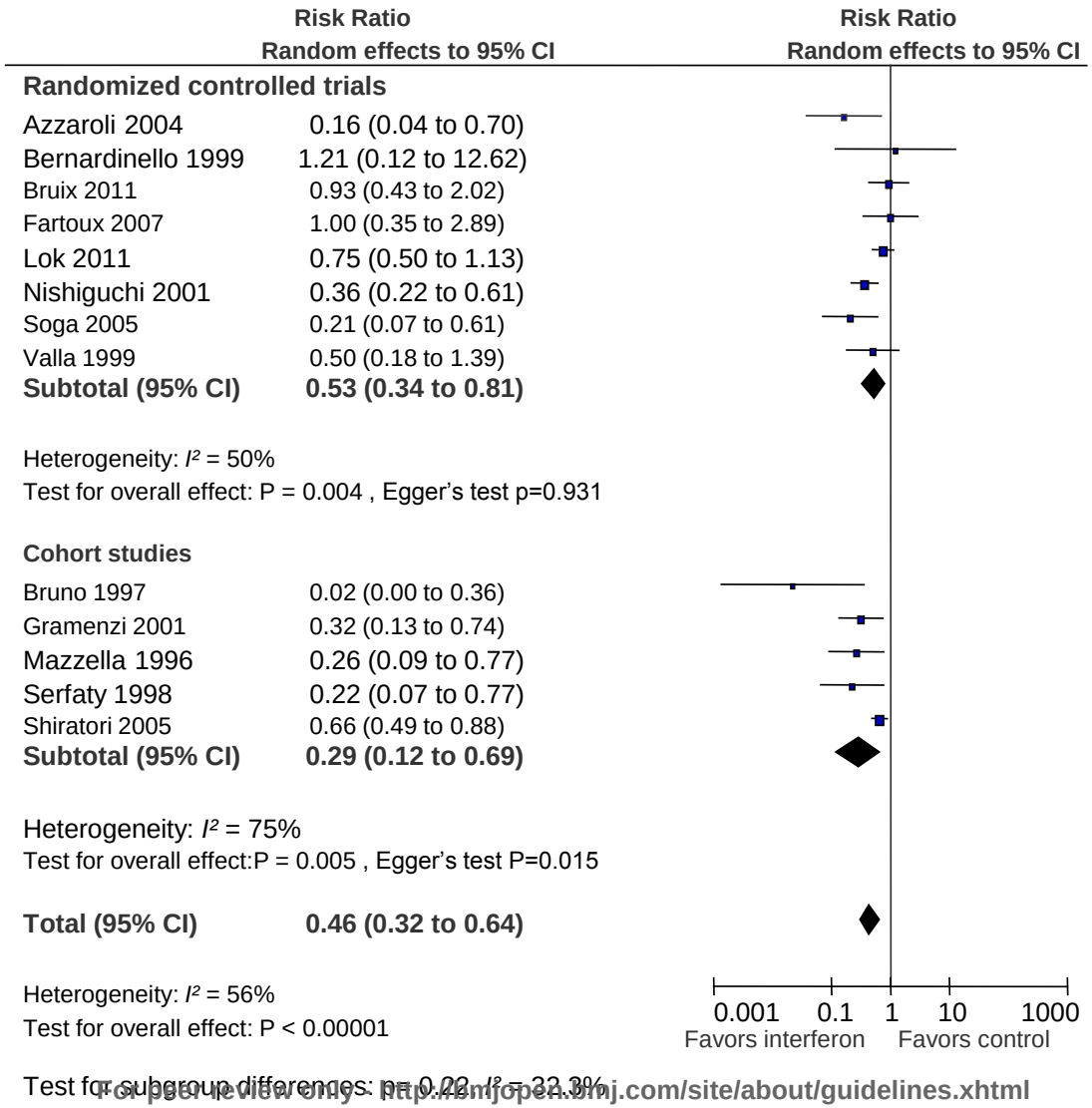
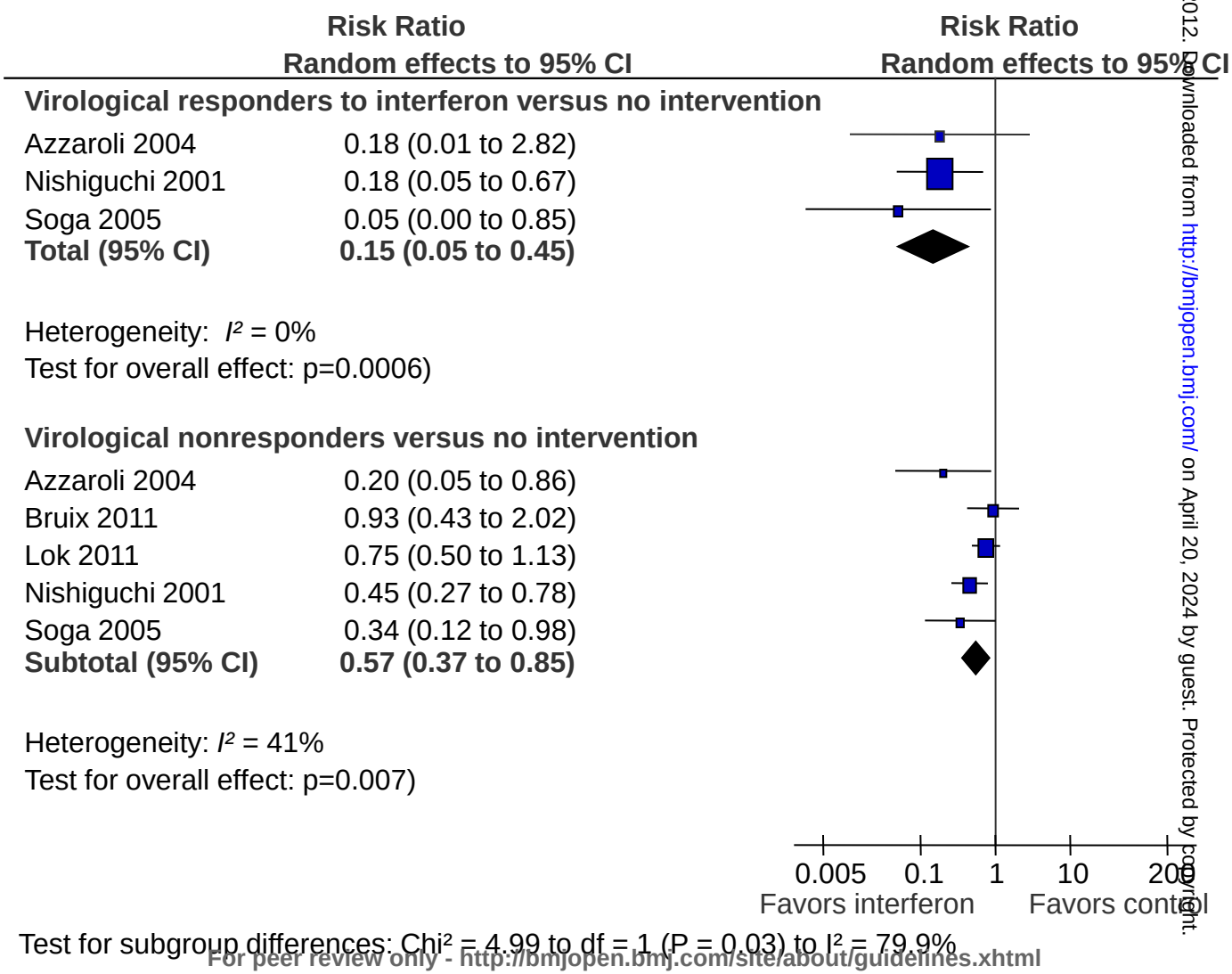


Fig. 3



Interferon for Prevention of Hepatocellular Carcinoma in Patients with Chronic Hepatitis C and Cirrhosis or Fibrosis: Systematic Review and Meta-Analysis

Review information

Review number: NK01

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Background

Chronic infection with hepatitis C virus (HCV) can lead to the development of hepatocellular carcinoma (HCC). The major risk factors is HCV-related HCC is cirrhosis although HCC can also be seen in patients without cirrhosis. Randomized controlled trial of antiviral therapy in patients with hepatitis B show that antiviral therapy prevents disease progression and reduced the incidence of HCC. This effect is also seen in cohort studies. Recent and earlier randomized controlled trials of interferon for HCV have reached inconsistent conclusions although there is a tendency suggesting that patients who are virological responders (patients with a sustained loss of HCV RNA) seem to have a reduced risk of HCC. Since a number of recent trials on interferon have reported long term follow up, we plan to perform a systematic review on this question.

Methods

Objectives

PICOS: The primary objective of the present review will be to assess the effects of interferon versus placebo or no intervention for development of HCC in patients with chronic HCV who have developed cirrhosis or fibrosis of the liver.

Criteria for considering studies for this review

Types of studies

In the primary analyses, randomised trials will be included irrespective of blinding, publication status or language. Since we expect that randomised trials may have insufficient follow up, prospective cohort studies with a clear description of the control group will be included in sensitivity analyses.

Types of participants

Patients with chronic HCV as defined by authors of included trials (normally a positive HCV RNA combined with histological changes suggesting chronic active hepatitis or elevated transaminases including ALT or AST for at least six months). Patients with clinical or histological cirrhosis or histological fibrosis are eligible for inclusion.

Types of interventions

Interferon versus no intervention or placebo. The type and dose of interferon will not be considered in the decision to include trials, nor will the duration of therapy. Accordingly, trials on interferon-alpha 2a or 2b, interferon-beta or pegylated interferon will be eligible for inclusion. Additional treatment with ribavirin will be allowed.

Types of outcome measures

The primary outcome measure will be HCC. Secondary outcome measures will include mortality (all-cause and liver-related) and liver related complications.

Search methods for identification of studies

Electronic searches

The Cochrane Hepato-Biliary Group Controlled Trials Register, The Cochrane Library, MEDLINE, EMBASE, and Science Citation Index Expanded will be searched.

Searching other resources

Additional trials will be identified through scanning of reference lists in relevant papers and conference proceedings. The meta-register http://www.controlled-trials.com/mrct/ will be searched for additional ongoing or unpublished trials.

Data collection and analysis

Selection of studies

All authors will participate in the identification and selection of trials for inclusion. All authors must approve of the trials selected for inclusion. Excluded trials will be listed with the reason for exclusion.

Data extraction and management

Two authors (NK and ED) will extract data in an independent manner. The extracted data will be validated by AK (outcome measures) and LG (bias control). Disagreements will be resolved through discussion before any analyses are made.

Primary authors of the included trials will be contacted for additional information when outcome measures or trial methods are not described in the published trial reports. Additional data will be retrieved through correspondence with experts and other publications such as previous meta-analyses.

Assessment of risk of bias in included studies

Based on previous evidence (Gluud 2006; Wood 2008), the randomisation methods (allocation sequence generation and concealment) will be extracted as the primary measures of bias control.

- The allocation sequence generation will be classified as adequate if based on a computer, random number table, or similar.
- Allocation concealment was classified as adequate if based on a central independent unit, serially numbered opaque sealed envelopes, or on-site locked computer.
- Information regarding comparability between intervention groups at baseline will also be extracted since differences may reflect skewed allocation.

Additional measures of bias control will include

- Blinding (was the trial single or double blind, the method of blinding and testing of blinding. If trials are described as blind, the persons who are blinded e.g., patients or outcome assessors will be registered).
- Handling of missing data (whether all patients randomised were accounted for in the analyses),
- Reporting bias (whether the most clinically relevant outcome measures were reported)
- Sample size calculation
- Whether the preset sample size achieved.
- Whether the trial was registered before start or before termination

All cohort studies will be classed as having a low bias control. For cohort studies, the methods used for the allocation of patients to the intervention and control group will be

extracted. Trials in which patients in the control group had contraindications/comorbidities will be considered as having the highest risk of bias.

Data synthesis

The analyses will be performed in RevMan version 5 (the Nordic Cochrane Centre, Copenhagen Denmark), STATA version 11 (STATA Corp, Texas USA), and TSA, trial sequential analysis (Copenhagen Trial Unit, Copenhagen Denmark). Meta-analyses will be performed using random effects models due to an expected clinical heterogeneity (duration of follow up and intervention regimens). Results will be expressed as relative risks (RR) with 95% confidence intervals (CI) and I-square as a measure of intertrial heterogeneity and test for subgroup differences by P values and I-square. Data on all patients randomised will be sought to perform intention-to-treat analyses. Carry forward of the last observed response will be used for patients with missing data (i.e., patients without HCC at inclusion will be counted as non-events).

Subgroup analysis and investigation of heterogeneity

Subgroup and sensitivity analyses will be performed to evaluate the effect of interferon in

Randomised trials and cohort studies

Patients with cirrhosis and patients without cirrhosis

Patients with a virological response or no virological response.

Trials with adequate randomisation

Trials with a placebo control or blinded outcome assessment

Random-effect metaregression analyses assessing the influence of potential predictors (duration of follow up) will be performed if at least ten randomised trials are identified.