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[Intervention Protocol]

Granulocyte colony-stimulating factor with or without stem or progenitor cell infusion for people with compensated or decompensated advanced chronic liver disease

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ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To assess the benefits and harms of granulocyte colony-stimulating factor with or without stem or progenitor cell infusion in people with compensated or decompensated advanced chronic liver disease.

BACKGROUND

Description of the condition

Different etiologies of liver disease, such as viral infection, toxin exposure, alcohol abuse, and metabolic, immunological, or genetic diseases, cause continuous and repeated damage to the liver. Persistent injury leads to inflammation, progressive fibrosis, and compensatory hepatocyte hyperplasia, usually culminating in cirrhosis that is characterised by distortion of the hepatic architecture and the formation of regenerative nodules. The histological pattern is generally considered to be irreversible, and the disease is usually asymptomatic until complications develop (Garcia-Tsao 2010; Tsochatzis 2014). The clinical identification of cirrhosis is imperfect and requires a liver biopsy and histology. Liver stiffness measurement (LSM) by transient elastography and use of biomarkers are non-invasive accurate tests for the diagnosis of severe fibrosis and cirrhosis (Foucher 2006; Pavlov 2015). It has been proposed that the term 'advanced chronic liver disease' should be used as an alternative to 'cirrhosis' (de Franchis 2015).

Advanced chronic liver disease is characterised by a long compensated phase, with median survival from diagnosis of around 12 years (d'Amico 2006). This asymptomatic phase, termed 'compensated', is followed by a rapidly progressive phase, termed 'decompensated', which is marked by the development of complications of portal hypertension and/or liver dysfunction. In the compensated phase, portal pressure may be normal. As the disease progresses, portal pressure increases and liver function decreases, resulting in the development of ascites, portal hypertensive gastrointestinal bleeding, encephalopathy, and jaundice. The development of any of these complications marks the transition from a compensated to a decompensated phase. Progression may be accelerated by the development of other complications such as (re)bleeding, renal impairment (refractory ascites, hepatorenal syndrome), hepatopulmonary syndrome, and sepsis (spontaneous bacterial peritonitis). The development of hepatocellular carcinoma (HCC) may accelerate the course of the disease at any stage. When decompensation occurs, expected median survival is around two years (d'Amico 2006; d'Amico 2014).

Globally, advanced chronic liver disease is considered responsible for more than one million deaths annually (Rowe 2017). The geographical distribution of liver disease is non-uniform and reflects the different prevalence of risk factors including alcohol consumption, hepatitis C virus (HCV) infection, hepatitis B virus infection, obesity, and the metabolic syndrome. In 2010, advanced chronic liver disease accounted for approximately 49,500 deaths and was the eighth leading cause of death in the United States (Murray 2013). A recent European Association for the Study of Liver Disease (EASL) report from 35 European countries estimated a median age-adjusted prevalence of chronic liver disease of 833 people per 100,000. Following data from 2017, prevalence ranged from a minimum of 447 people per 100.000 in Iceland to a maximum of 1100 per 100.000 in Romania, with a total of 151,513 deaths from liver disease in European countries (Pimpin 2018). In 2012, in England, people with liver disease admitted to a hospital were more likely to die compared to people classified as all-cause admissions (8.8% versus 1.4%) (NICE 2016).

Description of the intervention

No treatment is available to specifically target fibrosis and cirrhosis, and liver transplantation remains the only curative option (Rossi 2007). Many researchers are investigating strategies to restore liver functionality to avoid or slow progression towards end-stage liver disease, ultimately requiring a rescue liver transplantation.

Cell therapy is an emerging strategy that aims to restore liver functionality; in particular, bone marrow-derived stem cells (BMSCs) seem to be able to contribute to liver regeneration and to differentiate into hepatocyte-like cells (Forbes 2011; Thomas 2011). These stem cells can be infused, can reach the liver, and can become hepatocytes, improving liver function (Forbes 2016). Furthermore, cytokine mobilisation of BMSCs from the bone marrow to the liver could improve liver function (Alison 2000). Granulocyte colony-stimulating factor (G-CSF) is a 175-amino-acid protein, obtained through recombinant DNA technology and currently available for mobilisation of haematopoietic stem cells (HSCs) from the bone marrow (Moore 2014; Lanthier 2018). The minimum recommended dosage needed to obtain peripheral cell mobilisation is 5 mcg/kg daily, for at least five consecutive days (Alison 2000; Duong 2014).

How the intervention might work

Multiple courses of G-CSF have been shown to modulate inflammation, mobilise HSCs, increase hepatocyte growth factor, and induce hepatic progenitor cells to proliferate within seven days of administration (Spahr 2008; Gilchrist 2010; Gaia 2013). In compensated or decompensated cirrhosis, multiple courses of G-CSF with or without stem or progenitor cell infusion might be associated with accelerated hepatic regeneration and improved liver function and survival (Kedarisetty 2015; Verma 2018a; Verma 2018b). Two randomised clinical trials showed that multiple courses of G-CSF improved survival in people with acute-on-chronic liver failure (Garg 2012; Duan 2013).

Why it is important to do this review

Studies on the effects of multiple courses of G-CSF on hepatic regeneration and function reported conflicting results (Kedarisetty 2015; Lanthier 2018; Newsome 2018; Verma 2018a; Verma 2018b). A 2014 systematic review, including studies up to July 2013, concluded that "further robust clinical trials and collaborative protocols are required" (Moore 2014). We consider it important to summarise the results of studies assessing the benefits and harms of G-CSF with or without stem or progenitor cell infusion in people with chronic advanced liver disease.

OBJECTIVES

To assess the benefits and harms of granulocyte colony-stimulating factor with or without stem or progenitor cell infusion in people with compensated or decompensated advanced chronic liver disease.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised clinical trials, irrespective of publication type, publication status, and language.

Types of participants

Adults (18 years of age and older) with the diagnosis of advanced chronic liver disease (as defined by trialists), either compensated (i.e. without complications such as gastro-oesophageal varices, ascites, jaundice, encephalopathy) or decompensated, with one or more of the above listed complications, or with acute-on-chronic liver failure (ACLF), as defined according to European Association for the Study of the Liver (EASL)-Chronic Liver Failure (CLIF) consortium criteria, that is, "an acute deterioration of pre-existing chronic liver disease, usually related to a precipitating event and associated with increased mortality at three months due to multisystem organ failure" (Arroyo 2017).

Types of interventions

Experimental intervention

G-CSF, independent of the route or schedule of administration, as a single treatment or combined with stem or progenitor cell infusion or with other medical co-interventions.

Control intervention

No intervention or placebo.

We will allow collateral interventions if delivered equally to all participants in the trial groups.

Types of outcome measures

Primary outcomes

- All-cause mortality
- Proportion of participants with one or more serious adverse events. We will consider an event as a serious adverse event if trial authors clearly state that it was due to the experimental or control intervention, and if it fulfils the definition of serious adverse events of the International Conference on Harmonization (ICH) Guidelines (ICH-GCP 1997), that is, any event that leads to death; is life-threatening; requires in-patient hospitalisation or prolongation of existing hospitalisation; or results in persistent or significant disability, congenital birth, or anomaly; and any important medical event that may have jeopardised the patient or required intervention to prevent it. We will consider all other adverse events as non-serious. If an included study reports only a short list of serious adverse events that the trialists deem important, we plan to use the highest reported number. If trialists clearly stated that a death was due to the experimental or control intervention, we plan to consider and use this event as a serious adverse event
- Health-related quality of life (any validated continuous outcome scale used by trialists)

Secondary outcomes

- Proportion of participants with liver disease-related morbidity (i.e. proportion of participants who developed one or more complications such as ascites, variceal bleeding, hepatorenal syndrome, hepatic encephalopathy, jaundice, or portal thrombosis, or who underwent liver transplantation)
- Proportion of participants with adverse events considered to be non-serious
- Proportion of participants without improvement in liver function scores such as Child-Turcotte-Pugh (CTP) or model for end-stage liver disease (MELD) scores as defined by trialists

We plan to assess all outcomes only at 'maximum follow-up'. If the time from randomisation to maximum follow-up differed significantly between the included trials, then we will do a subgroup analysis to assess whether the different follow-up periods affected our results.

The above listed outcomes will nol be used as criteria for including studies.

Search methods for identification of studies

Electronic searches

We plan to search: the Cochrane Hepato-Biliary Group Controlled Trials Register (maintained and searched internally by the CHBG Information Specialist via the Cochrane Register of Studies Web); the Cochrane Central Register of Controlled Trials (CENTRAL; latest issue), in the Cochrane Library; MEDLINE Ovid (1946 to date of search); Embase Ovid (1974 to date of search); Latin American Caribbean Health Sciences Literature (LILACS; Bireme; 1982 to date of search); BIOSIS (Web of Science; 1969 to date of search); Science Citation Index Expanded (Web of Science; 1900 to date of search); and Conference Proceedings Citation Index - Science (Web of Science; 1990 to date of search) (Royle 2003). Appendix 1 presents the preliminary search strategies.

Searching other resources

We also plan to search the bibliographic references of identified randomised clinical trials and review articles to find randomised clinical trials not identified by the electronic searches. We will contact the principal authors of the identified randomised clinical trials to inquire about additional randomised clinical trials that they might know of.

We will search Google Scholar, the Turning Research into Practice (TRIP) database, and on-line trials registries such as ClinicalTrials.gov, European Medicines Agency (EMA) (www.ema.europa.eu/ema/), World Health Organization (WHO) International Clinical Trial Registry Platform (www.who.int/ictrp), and US Food and Drug Administration (FDA) (www.fda.gov). We will endeavour to identify randomised clinical trials referenced in non-English databases, using our personal contacts or local access, or by asking the CHBG Information Specialist to contact Cochrane collaborators from around the world with the same intent.

We will search for grey literature in the System for Information on Grey Literature in Europe - OpenGrey (www.opengrey.eu).

Data collection and analysis

We will prepare the review by following recommendations provided in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2019). We will perform analyses using Review Manager 5 (Review Manager 2014).

If, during the selection of trials, we identify observational studies such as quasi-randomised or controlled clinical studies with the same characteristics of participants and interventions as in our protocol, and reporting adverse events relevant to the outcomes of this review, we will extract the adverse event data for experimental

and control groups separately from data for the randomised clinical trials. We will not specifically search for observational studies for inclusion in this review, which is a known limitation of the study in terms of adverse events. We are aware that the decisions to not search systematically for all observational studies and to extract data on harm only from quasi-randomised and controlled clinical studies might bias our review towards assessment of benefits and might overlook certain harms such as late or rare harms. If we demonstrate benefits from G-CSF in compensated and decompensated chronic advanced liver disease, then a systematic review of the harms of this intervention in observational studies ought to be launched (Storebø 2018).

Review authors working in pairs will independently extract the following information: publication data (i.e. year, country, authors); study characteristics and design; characteristics of trial participants; trial inclusion and exclusion criteria; interventions; outcomes; follow-up; types of data analyses (i.e. intention-to-treat, modified intention-to-treat, per-protocol); and for-profit support. We will contact trial authors for missing information. We will extract data at maximum follow-up.

Review authors will resolve disagreements among themselves.

Selection of studies

Two review authors (AC and DP) will independently select publications on randomised clinical trials relevant to the review. If a trial is identified as relevant by one review author, but not by another, the two review authors will discuss the reasoning behind their decision. If they still disagree, GC will serve as arbitrator.

AC and DP will also scan observational studies retrieved through searches for a report on adverse events due to the experimental intervention in this review. If such data are reported, we will use this information for discussion. None of these studies will be eligible for inclusion in the review.

Data extraction and management

We plan that two review authors (AC and DP) will independently extract and validate data. We will use data extraction forms that we designed and pre-piloted for the purpose. The two review authors will discuss any disagreement concerning extracted data. If the review authors still disagree, GC will serve as arbitrator. In cases where relevant data are not available, we plan to contact the trial authors.

Assessment of risk of bias in included studies

We plan to assess risk of bias for each primary and secondary outcome of the included trials (Higgins 2011a; Higgins 2011b). Two review authors (AC and MF) will independently assess the risk of bias of each included trial in keeping with recommendations provided in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011b), and according to methodological studies (Schultz 1995; Moher 1998; Kjaergard 2001; Rücker 2008; Wood 2008; Higgins 2019; Savovic 2012a; Savovic 2012b; Savović 2018). We will use the following definitions in our assessment of risk of bias.

Allocation sequence generation

- Low risk of bias: study authors performed sequence generation using computer random number generation or a random numbers table. Drawing lots, tossing a coin, shuffling cards, and throwing dice are adequate if performed by an independent person not otherwise involved in the study. In general, we will classify risk of bias as low if the method used for allocation concealment suggested that it was extremely likely that the sequence was generated randomly (e.g. use of interactive voice response system)
- Unclear risk of bias: study authors did not specify the method of sequence generation
- High risk of bias: the sequence generation method was not random

Allocation concealment

- Low risk of bias: participant allocations could not have been foreseen in advance of, or during, enrolment. A central and independent randomisation unit controlled allocation. Investigators are unaware of the allocation sequence (e.g. if the allocation sequence was hidden in sequentially numbered, opaque, and sealed envelopes)
- Unclear risk of bias: study authors did not describe the method used to conceal the allocation, so intervention allocations may have been foreseen before, or during, enrolment
- High risk of bias: it is likely that investigators who assigned participants knew the allocation sequence. We will exclude such quasi-randomised studies

Blinding of participants and personnel

- Low risk of bias: blinding of participants and key study personnel was ensured, and it was unlikely that the blinding could have been broken; or rarely, no blinding or incomplete blinding, but review authors judged that the outcome was not likely to be influenced by lack of blinding
- Unclear risk of bias: either of the following: insufficient information to permit judgement of 'low risk' or 'high risk'; or the trial did not address this outcome
- High risk of bias: either of the following: no blinding or incomplete blinding, and the outcome was likely to be influenced by lack of blinding; or blinding of key study participants and personnel attempted, but likely that the blinding could have been broken, and the outcome was likely to be influenced by lack of blinding

Blinded outcome assessment

- Low risk of bias: blinding of outcome assessment ensured, and unlikely that the blinding could have been broken; or rarely, no blinding of outcome assessment, but review authors judged that the outcome measurement was not likely to be influenced by lack of blinding
- Unclear risk of bias: either of the following: insufficient information to permit judgement of 'low risk' or 'high risk'; or the trial did not address this outcome
- High risk of bias: either of the following: no blinding of outcome assessment, and the outcome measurement was likely to be influenced by lack of blinding; or blinding of outcome assessment, but likely that the blinding could have been broken, and the outcome measurement was likely to be influenced by lack of blinding

Incomplete outcome data

- Low risk of bias: missing data were unlikely to make treatment effects depart from plausible values. The study used sufficient methods, such as multiple imputation, to handle missing data
- Unclear risk of bias: information was insufficient to assess whether missing data in combination with the method used to handle missing data were likely to induce bias on the results
- High risk of bias: results were likely to be biased due to missing data

Selective outcome reporting

- Low risk: the trial reported as a primary outcome all-cause mortality, which is the main reason for treatment with G-CSF for people with chronic advanced liver disease. If the original trial protocol was available, the outcomes should have been those called for in that protocol. If the trial protocol was obtained from a trial registry (e.g. ClinicalTrials.gov), the outcomes sought should have been those enumerated in the original protocol if the trial protocol was registered before or at the time the trial was begun. If the trial protocol was registered after the trial was begun, those outcomes will not be considered to be reliable
- Unclear risk of bias: not all pre-defined or clinically relevant and reasonably expected outcomes were reported fully, or it was unclear whether or note data on these outcomes were recorded
- High risk of bias: all-cause mortality or one or more pre-defined outcomes were not reported, despite the fact that data on these outcomes should have been available and even recorded

Bias at outcome level

We will classify an outcome in a trial to be at low risk of bias if allocation sequence generation; allocation concealment; blinding of participants, healthcare professionals, and outcome assessors; incomplete outcome data; and selective outcome reporting (at the outcome level) are at low risk of bias for objective and subjective outcomes (Savović 2018).

Overall bias assessment

- Low risk of bias: all domains in a trial are classified at low risk of bias according to the definitions described above
- High risk of bias: one or more of the bias domains in a trial are classified at unclear or high risk of bias

Measures of treatment effect

We plan to present risk ratios (RRs) with 95% confidence intervals (CIs) for dichotomous outcomes. For continuous outcomes, we will use mean differences (MDs) between experimental and control groups with their 95% CIs. If studies report quality of life measurements using different tools, we will calculate the standardised mean difference (SMD) with 95% CI, rather than the MD. For interpreting SMDs, we plan to use the Cohen's effect sizes rule, which considers 0.2 as a small effect, 0.5 as a moderate effect, and 0.8 as a large effect (Cohen1988).

Unit of analysis issues

We do not expect to find trials of parallel-group design with more than two intervention groups. However, if we find trials with more than two intervention groups, we will combine groups, as sensible, to create a single pair-wise comparison (Higgins 2019). We do not expect to find cluster-randomised or cross-over trials. However, if we find cluster-randomised trials, we will analyse and assess the risk of bias of cluster-randomised trials separate from that of the randomised parallel-group clinical trials included in our review (Higgins 2019). If we find cross-over trials, we will use for analysis only the first trial period to avoid the cross-over effect of the intervention (Higgins 2019).

Dealing with missing data

We plan to contact investigators of the trials to request missing data. We will perform our analyses according to the intention-to-treat analysis method, that is, by analysing participants in the trials in the groups to which they were randomised, regardless of whether they had received or adhered to the allocated intervention. If data are not available and we receive no reply from study authors, we will use the data as reported.

For our dichotomous outcome 'all-cause mortality', we plan to conduct the sensitivity analyses described below.

- 'Extreme-case' analysis favouring the experimental intervention ('best-worse' case scenario): none of the participants who dropped out from the experimental group experienced the outcome, but all participants who dropped out from the control group experienced the outcome; including all randomised participants in the denominator.
- 'Extreme-case' analysis favouring the control ('worst-best' case scenario): all participants who dropped out from the experimental group, but none from the control group, experienced the outcome; including all randomised participants in the denominator.

For the continuous outcome 'health-related quality of life', if the trial does not report standard deviations, we plan to impute standard deviations according to Higgins 2019.

Assessment of heterogeneity

We plan to explore the presence of statistical heterogeneity by using the Chi^2 test, with significance set at P value less than 0.10. In addition, we will use the I^2 statistic to quantify heterogeneity according to the following classification: from 0% to 40%, heterogeneity may not be important; from 30% to 60%, heterogeneity may be moderate; from 50% to 90%, heterogeneity may be substantial; and from 75% to 100%, heterogeneity may be considerable (Deeks 2019).

Assessment of reporting biases

For any considered outcome when at least 10 trials are included in the meta-analysis, we will test for funnel plot asymmetry (Higgins 2019).

Data synthesis

Meta-analysis

We plan to perform meta-analyses in keeping with recommendations provided in the *Cochrane Handbook for Systematic Reviews of Intervention* (Deeks 2019). We will use the statistical software Review Manager 5 provided by Cochrane to analyse data (Review Manager 2014). We will apply both fixed-effect (DeMets 1987) and random-effects models (DerSimonian 1986) meta-analyses. We will consider the fixed-effect model only as a sensitivity analysis.

Subgroup analysis and investigation of heterogeneity

We plan to conduct the following subgroup analyses.

- Trials at low risk of bias compared to trials at high risk of bias (because trials at high risk of bias may overestimate or underestimate intervention effects).
- Trials at risk of for-profit support compared to trials without for-profit support (because trials with for-profit support may overestimate or underestimate intervention effects).
- Trials including decompensated cirrhosis compared to trials including only compensated chronic liver disease (because effects of treatment might vary according to the severity of liver dysfunction).
- Trials including only or mainly participants with alcoholic liver disease compared to trials including only or mainly participants with other liver disease of other origins (because effects of treatment might vary according to the cause).
- Trials using dosage below the recommended dosage of G-CSF for peripheral cell mobilisation (5 mcg/kg daily for at least five consecutive days) compared to trials using equivalent or higher dosage (lower dosages are expected to be ineffective).
- Trials using daily dosage of G-CSF > 10 mcg/kg daily compared to trials using lower dosage (because below this dose, effects of treatment might be impaired).
- Trials using short-term treatment schedule shorter than seven days compared to trials using longer treatment schedules (longer than seven days) (because shorter treatment might reduce effects of treatment).
- Trials combining G-CSF with other medical co-intervention compared to trials using only G-CSF (because any co-intervention might influence effects of treatment).
- Trials combining G-CSF with stem or progenitor cell infusion compared to trials using only G-CSF (because infusion of stem or progenitor cells might modify effects of treatment).

Additional subgroup analyses may be considered at the review stage. Due to the large number of subgroup analyses, we will interpret them conservatively.

Sensitivity analysis

In addition to the two sensitivity analyses specified in Dealing with missing data, we plan to perform the following sensitivity analyses.

- Assessing the robustness of our results by including only trials reporting intention-to-treat analyses.
- Assessing effects of risk of bias of included trials by performing a sensitivity analysis from which we will exclude trials classified at high risk of bias.
- Comparing fixed-effect and random-effects estimates of the intervention effect to assess the influence of small-study effects on the results of our meta-analysis.
- Comparing our assessment of imprecision with GRADE to that performed with the Trial Sequential Analysis (see below) (Castellini 2018; Gartlehner 2018).

Trial Sequential Analysis

We plan to perform Trial Sequential Analysis on the primary outcomes to calculate the cumulative sample size of the meta-analysis (information size) and to reduce the risk of random errors due to sparse data and repetitive testing of accumulating data (Wetterslev 2008; Thorlund 2011). We will calculate the information size adjusted for heterogeneity (diversity, D^2) between trials using the following parameters (Wetterslev 2009): proportion of events in the control group estimated from the included trials (overall mean value); anticipated intervention effect (relative risk reduction, RRR) of 15%; alpha of 2.5%, as we use three primary outcomes; and beta of 10% (Jakobsen 2014; Wetterslev 2017). We will add trials to the analysis according to the year of publication. If more than one trial was published in a year, we will add the trials in alphabetical order, according to the name of the first author. On the basis of the required information size, we will construct the trial sequential monitoring boundaries for benefits and futility using the O'Brien-Fleming alpha spending (for benefit) and beta-spending (for futility) functions. The boundaries for benefit are used for meta-analyses that have not reached the required information size to conclude when statistical significance is reached. If the trial sequential monitoring boundary is crossed before the required information size is reached, a sufficient level of evidence is reached, results of the meta-analysis can be considered conclusive if bias can be excluded, and no additional trials may be needed. Conversely, if the boundary is not crossed, the meta-analysis is inconclusive, and more trials may be needed to detect or reject a certain intervention effect. When the cumulative Z-curve crosses the futility boundaries, a sufficient level of evidence is reached that the two treatments do not differ by more than 15% (anticipated intervention effect used in information size estimation), and no additional trials may be needed. In all situations where no trial sequential monitoring boundaries are reached, further studies may be needed until the information size is reached, or until monitoring boundaries are crossed.

We will perform Trial Sequential Analysis with Trial Sequential Analysis software, version 0.9.5.10 beta (TSA 2011).

Summary of findings

We will create a 'Summary of findings' table including the following outcomes: all-cause mortality; proportion of participants with one or more serious adverse events; health-related quality of life; and proportion of participants with liver disease-related morbidity. We will report the longest follow-up with a range of follow-up for each outcome.

We will use the GRADE approach and software to assess the quality of a body of evidence (GRADEpro GDT). GRADE considers the following criteria: study risk of bias (methodological quality); inconsistency of results (unexplained heterogeneity); indirectness of evidence (population, intervention, comparator, or outcome); imprecision of results (wide CIs); and publication bias. We will define levels of certainty as 'high', 'moderate', 'low', or 'very low' as follows.

- High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
- Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

- Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

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APPENDICES

Appendix 1. Search strategies

Database	Time span	Search strategy
The Cochrane Hepato-Biliary Group Controlled Trials Register	Date will be given at review stage	(colony-stimulating factor or G-CSF or GCSF or CSF3 OR CSF 3 or filgrastim or lenograstim) and (advanc* and chronic and (liver* or hepat*))

(Continued)

Cochrane Central Register of Controlled Trials (CENTRAL), in the Cochrane Library	Latest issue	#1 MeSH descriptor: [Granulocyte Colony-Stimulating Factor] explode all trees #2 colony-stimulating factor or G-CSF or GCSF or CSF3 OR CSF 3 or filgrastim or lenograstim #3 #1 or #2 #4 (advanc* and chronic and (liver* or hepat*)) #5 #3 and #4
MEDLINE Ovid	1946 to date of search	1. exp Granulocyte Colony-Stimulating Factor/ 2. (colony-stimulating factor or G-CSF or GCSF or CSF3 or CSF 3 or filgrastim or lenograstim).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] 3. 1 or 2 4. (advanc* and chronic and (liver* or hepat*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] 5. 3 and 4
Embase Ovid	1974 to date of search	1. exp granulocyte colony stimulating factor/ 2. (colony-stimulating factor or G-CSF or GCSF or CSF3 or CSF 3 or filgrastim or lenograstim).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] 3. 1 or 2 4. (advanc* and chronic and (liver* or hepat*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] 5. 3 and 4
LILACS (Bireme)	1982 to date of search	colony-stimulating factor or G-CSF or GCSF or CSF3 OR CSF 3 or filgrastim or lenograstim) [Words] and (advanc$ and chronic and (liver$ or hepat$)) [Words]
BIOSIS (Web of Science)	1969 to date of search	#3 #2 AND #1 #2 TS=(advanc* and chronic and (liver* or hepat*)) #1 TS=(colony-stimulating factor or G-CSF or GCSF or CSF3 OR CSF 3 or filgrastim or lenograstim)
Science Citation Index Expanded (Web of Science)	1900 to date of search	#3 #2 AND #1 #2 TS=(advanc* and chronic and (liver* or hepat*)) #1 TS=(colony-stimulating factor or G-CSF or GCSF or CSF3 OR CSF 3 or filgrastim or lenograstim)
Conference Proceedings Citation Index – Science (Web of Science)	1990 to date of search	#3 #2 AND #1 #2 TS=(advanc* and chronic and (liver* or hepat*))

(Continued)

	#1 TS=(colony-stimulating factor or G-CSF or GCSF or CSF3 OR CSF 3 or filgrastim or lenograstim)

CONTRIBUTIONS OF AUTHORS

AC wrote the protocol, will perform searches for references, will evaluate references to obtain full reports, will evaluate studies for inclusion, will extract data from studies, will assess risk of bias, and will write the final review.
DP co-ordinated protocol design, will evaluate references to obtain full-text reports, and will write the final review.
MF commented on the protocol, will evaluate studies for inclusion, will extract data from studies, will assess risk of bias, and will design the final review.
GC wrote the protocol and will critically comment on the final review, will conduct statistical analyses, will act as arbiter if review authors cannot reach a consensus, and will critically comment on the final review.

All authors approved the protocol for publication.

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AC: nothing to declare.
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