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Li WX, Yan X, Shi CR, Zhang AP

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Cochrane Database of Systematic Reviews 2012, Issue 9. Art. No.: CD008714.
DOI: 10.1002/14651858.CD008714.pub2.

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[Intervention Review]

Chlorambucil for patients with primary biliary cirrhosis

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Editorial group: Cochrane Hepato-Biliary Group.
Publication status and date: New, published in Issue 9, 2012.

Citation: Li WX, Yan X, Shi CR, Zhang AP. Chlorambucil for patients with primary biliary cirrhosis. *Cochrane Database of Systematic Reviews* 2012, Issue 9. Art. No.: CD008714. DOI: 10.1002/14651858.CD008714.pub2.

ABSTRACT

Background

Chlorambucil has been used for patients with primary biliary cirrhosis as it possesses immunosuppressive properties. But it is unknown whether it benefits or harms these patients.

Objectives

To evaluate the beneficial and any harmful effects of chlorambucil for primary biliary cirrhosis patients.

Search methods

Eligible trials were identified by searching the Cochrane Hepato-Biliary Group Controlled Trials Register (March 2012), the Cochrane Central Register of Controlled Trials (CENTRAL) in *The Cochrane Library* (2012, Issue 2), MEDLINE (1946 to March 2012), EMBASE (1974 to March 2012), Science Citation Index EXPANDED (1900 to March 2012), The Chinese Biomedical Database (1976 to March 2012), The Chinese Medical Current Contents (1994 to March 2012), The China Hospital Knowledge Database (1994 to March 2012), and a database of ongoing trials (http://www.controlled-trials.com/mrct/) (accessed 6 March 2012). The reference lists of the retrieved publications and review articles were also read through, and pharmaceutical companies known to produce chlorambucil were contacted.

Selection criteria

Randomised clinical trials, irrespective of language, year of publication, and publication status, comparing chlorambucil at any dose versus placebo, no intervention, another active drug, or one dose of chlorambucil with another dose.

Data collection and analysis

We planned to assess continuous data with mean differences (MD), and dichotomous outcomes with relative risk (RR), both with 95% confidence intervals (CI). As we only identified one trial, Fisher's exact tests were employed.

Main results

Only one randomised trial was identified and included in the review. The bias risk in the trial was high. The trial compared chlorambucil versus no intervention in 24 patients with primary biliary cirrhosis. Fisher's exact test did not show a significant reduction of mortality when comparing chlorambucil with no treatment (0/13 (0%) versus (2/11 (18.2%); P = 0.20). There was no significant difference regarding adverse events for chlorambucil compared with no treatment, but all patients receiving chlorambucil experienced adverse events (13/13 (100%) versus (3/11 (27%); P = 0.1). According to the authors of the trial, chlorambucil led to a significant improvement in mean serum levels of bilirubin (P < 0.05), albumin (P < 0.05), immunoglobulin M (P < 0.01), serum aspartate aminotransferase activity (P < 0.01), and hepatic inflammatory infiltrates (P < 0.01).

Authors' conclusions

There is not sufficient evidence to support or reject the use of chlorambucil for patients with primary biliary cirrhosis. Chlorambucil may show benefit in some unvalidated surrogate outcome measures (for example, serum bilirubin and immunoglobulin M levels). Chlorambucil is, however, connected with a number of adverse events. Bone marrow suppression should be noted in particular. Further randomised clinical trials are necessary to assess the benefits and harms of chlorambucil in this indication.

PLAIN LANGUAGE SUMMARY

Chlorambucil for patients with primary biliary cirrhosis

Primary biliary cirrhosis is an autoimmune disease of the liver. Chlorambucil has been used for patients with primary biliary cirrhosis as it possesses immunosuppressive properties. This review aimed to assess the beneficial or harmful effects of chlorambucil for primary biliary cirrhosis. The authors identified only one randomised trial, with 24 participants included. This trial compared chlorambucil with no intervention. The trial is small and at a high risk of bias, which suggests that the results may not be reliable. Meta-analyses were not possible because of the inclusion of one trial only. Fisher's exact test and t-test were used instead. Chlorambucil was not associated with significantly lower mortality when compared with no intervention. All patients on chlorambucil experienced adverse events, especially bone marrow suppression. Chlorambucil led to a significant improvement in mean serum levels of bilirubin, albumin, immunoglobulin M, serum aspartate aminotransferase activity, and hepatic inflammatory infiltrates. However, these outcomes are unvalidated surrogate outcomes for patient-relevant outcomes. This means that improvement of these biochemistry measures cannot be taken as proof of improvement of patient-relevant outcomes. It remains unclear whether chlorambucil can be supported or rejected for use in patients with primary biliary cirrhosis.

BACKGROUND

Description of the condition

Primary biliary cirrhosis (PBC) is a chronic liver disease that is characterised by destruction of the epithelium of the small septal and interlobular bile ducts. It primarily affects middle-aged women (40 to 60 years old). The incidence of primary biliary cirrhosis among adults (more than 20 years old) is about two to three in 100,000 persons per year in both Europe and the United States. The prevalence of the disease varies widely in different geographical regions (Kim 2002; Sood 2004). It is thought that primary biliary cirrhosis is caused by a complex interplay between genetic and environmental risk factors (Gershwin 2008). Some environmental factors (for example, micro-organisms or xenobiotics) may initiate autoimmune mechanisms in combination with the effects of genetic predisposition that leads to immunopathological damage of small bile duct, and eventually to liver fibrosis, cirrhosis, and failure (Kaplan 2005). Antimitochondrial autoantibodies (AMA) are the characteristic serologic hallmark of primary biliary cirrhosis, found in 90% to 95% of patients and less than 1% of normal controls (Gershwin 1987; Invernizzi 1997).

The most common symptoms of primary biliary cirrhosis are fatigue and pruritus (Goldblatt 2002; Bergasa 2005). More than 50% of patients are asymptomatic during the first stage of the disease, but they do not show a better prognosis than symptomatic patients (Prince 2004). The diagnosis is based on abnormal biochemical indicators of cholestasis (mainly raised activity of alkaline phosphatases), AMA-positivity, and exclusion of other causes of chronic cholestatic liver disease. Histologic evidence of impaired bile duct lobular and nonsuppurative destructive cholangitis can help the diagnosis in some patients (Lindor 2009).

Over the past decades, there have been many attempts to improve the management of primary biliary cirrhosis, and it is still one of the major indications for liver transplantation worldwide (Gong 2004a; Gong 2004b; Prince 2005; Gong 2007a; Gong 2007b; Gong 2008; Giljaca 2010).

Description of the intervention

Over the years, many drugs have been used to try to treat primary biliary cirrhosis. These are immunomodulatory drugs as well as other agents such as D-penicillamine (Dickson 1985; Neuberger 1985; Gong 2004a), azathioprine (Christensen 1985; Gong 2007b), colchicine (Poupon 1996; Gong 2004b), cyclosporin A (Wiesner 1990; Gong 2007a), methotrexate (Kaplan 1991; Giljaca 2010), and prednisolone (Mitchison 1992; Prince 2005). However, their usage is limited (Verma 1999). A bile acid ursodeoxycholic acid (UDCA) is the most extensively used drug among them. It is also the only FDA-approved drug in patients with primary biliary cirrhosis. A Cochrane Hepato-Biliary Review did not demonstrate any benefit of UDCA on mortality or liver transplantation (Gong 2008), and UDCA did not improve pruritus, fatigue, autoimmune conditions, liver histology, or portal pressure (Gong 2008). The few observed beneficial effects such as improved biochemical variables, like serum bilirubin, ascites, and jaundice, were uncertain (Gong 2008). The use of UDCA is also associated with weight gain and costs (Gong 2008).

Chlorambucil is an alkylating agent. It is indicated in the treatment of chronic lymphatic (lymphocytic) leukaemia, and malignant lymphomas including lymphosarcoma, giant follicular lymphoma, and Hodgkin's disease but it has also been used for treatment of patients with primary biliary cirrhosis.

How the intervention might work

Autoimmune mechanisms are involved in the pathogenesis of primary biliary cirrhosis. Activated T-lymphocytes attack and destroy epithelial cells in the small intralobular bile ducts of genetically susceptible individuals. Chlorambucil has a high selective inhibition of lymphoid tissue, which can quickly and clearly lead to lymphoid tissue atrophy and to inhibition of antibody synthesis, at a lower dose than than those that cause toxicity. Thus, chlorambucil is a potential agent to modify the immunologic irregularities in primary biliary cirrhosis. It is often combined with glucocorticoids in the therapy of immune-mediated diseases (Miller 1997).

Why it is important to do this review

Chlorambucil may delay the progression of primary biliary cirrhosis and thereby improve serum bilirubin, serum aspartate aminotransferase activity, and albumin levels. Also, it can decrease immunoglobulin M levels and may improve inflammatory cell infiltration in treated patients (Hoofnagle 1986). However, one review article states that chlorambucil is not ideal because of its toxicity (Kaplan 1997). We could find no meta-analyses or systematic reviews studying the beneficial or harmful effects of chlorambucil in patients with primary biliary cirrhosis.

OBJECTIVES

To evaluate the beneficial or harmful effects of chlorambucil for primary biliary cirrhosis patients.

METHODS

Criteria for considering studies for this review

Types of studies

All randomised clinical trials, with no language, year of publication, or publication status restrictions. We planned to also include quasi-randomised clinical and observational studies for the assessment of harm.

Types of participants

Any adult (age > 18 years) with primary biliary cirrhosis regardless of sex or race. Participants with primary biliary cirrhosis were defined by at least two of the following criteria:
- elevated serum activity of alkaline phosphatases (or other markers of intrahepatic cholestasis);
- antimitochondrial autoantibodies (AMA) positive;
- histologic evidence of nonsuppurative destructive cholangitis and destruction of interlobular bile ducts.

Types of interventions

Administration of per oral chlorambucil at any doses compared with placebo, no intervention, another active drug, or one dose of chlorambucil with another dose. Co- interventions were allowed if used equally in both intervention groups.

Types of outcome measures

Primary outcomes

1. All-cause mortality and hepatic-related mortality.
2. Number of patients undergoing liver transplantation.
3. Adverse events: any adverse events correlated to use of chlorambucil or other related treatment, such as bone marrow suppression, anaemia, leukopenia, neutropenia, thrombocytopenia, pancytopenia; nausea, vomiting, or diarrhoea; tremors, muscular twitching, and hallucinations; skin rash, urticaria, angioneurotic edema; interstitial pneumonia, drug fever, etc. The adverse events were subdivided into non-serious adverse events as well as serious adverse events according to the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines (ICH-GCP 1997). Serious adverse events are defined as any event that led to death, was life-threatening, required inpatient hospitalisation or prolongation of existing hospitalisation, resulted in persistent or significant disability or congenital anomaly or birth defect, or any important medical event which might have jeopardized the patient or required intervention to prevent it.
4. Health-related quality of life (using a validated instrument), such as patient-reported health outcomes.

Secondary outcomes

1. Fatigue: number of patients with fatigue.
2. Pruritus: number of patients with pruritus.
3. Other clinical symptoms or signs: number of patients with jaundice or who have developed jaundice, hepatomegaly, xanthoma, ascites, sicca complex, variceal bleeding, hepatic encephalopathy, or hepatorenal syndrome.
4. Liver biochemistry: serum (s)-alkaline phosphatases (ALP); s-bilirubin; s-gamma-glutamyltransferase (ɤ-GT); s-aspartate aminotransferase (AST); s-alanine aminotransferase (ALT); s-albumin; s-cholesterol (total); plasma immunoglobulin M; s-copper, etc.
5. Liver biopsy findings: worsening of liver histological stage or score.
6. Costs.

Search methods for identification of studies

Electronic searches

We searched the Cochrane Hepato-Biliary Group Controlled Trials Register (March 2012), the Cochrane Central Register of Controlled Trials (CENTRAL) in *The Cochrane Library* (2012, Issue 2), MEDLINE (1946 to March 2012), EMBASE (1974 to March 2012), and Science Citation Index EXPANDED (1900 to March 2012) (Royle 2003). We also searched The Chinese Biomedical Database (CBM-CD) (1976 to March 2012), The Chinese Medical Current Contents (CMCC-CD) (1994 to March 2012), The China Hospital Knowledge Database (CHKD) (1994 to March 2012), and the database of ongoing trials (at www.controlled-trials.com/mrct/) (accessed 6 March 2012).

We searched the databases using the search strategies given in Appendix 1.

Searching other resources

Further trials were sought through scanning of reference lists of the included trials and (systematic) reviews and meta-analysis related to the topic. In addition, we contacted the pharmaceutical company (GlaxoSmithKline (GSK)) involved in the production of chlorambucil for information about any published, unpublished, or ongoing trials, at http://www.gsk-clinicalstudyregister.com/) (accessed 6 March 2012).

Data collection and analysis

We followed the instructions given in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011) and the Cochrane Hepato-Biliary Group Module (Gluud 2012) for data collection and analysis.

Selection of studies

Two authors (WX Li and CHR Shi) independently cross-checked the search results, including titles, abstracts, and full texts, according to the inclusion and exclusion criteria. Disagreements were resolved by discussion. In case of disagreements, a third author (X Yan) had to decide whether the trial should be included or not.

Data extraction and management

Two authors (WX Li and AP Zhang) independently extracted the data below from the included trials. Disagreements were resolved in consultation with a third author (CHR Shi).

1. General information: language of publication; authors; article title; journal title, year of publication, volume, issue, and page numbers.
2. Study design: design type, predetermined sample size, generation of randomisation sequence, allocation concealment method, blinding of information, statistical methods, and handling losses to follow-up.
3. Participants: sample size, diagnostic criteria, baseline characteristics (eg, age, sex), inclusion criteria, exclusion criteria, and study settings.
4. Intervention: duration of treatment, dose, co-intervention, control.
5. Outcome measures: observed outcome measures as published at the end of treatment.

Assessment of risk of bias in included studies

The bias risk of the randomised clinical trials was assessed following the guidelines of the Cochrane Hepato-Biliary Group (Schulz 1995; Moher 1998; Kjaergard 2001; Wood 2008; Higgins 2011).

Allocation sequence generation

- Low risk of bias: sequence generation was achieved using computer random number generation or a random number table. Drawing lots, tossing a coin, shuffling cards and throwing dice are adequate if performed by an independent adjudicator.
- Uncertain risk of bias: the trial is described as randomised, but the method of sequence generation was not specified.
- High risk of bias: the sequence generation method is not, or may not be, random. Quasi-randomised studies, those using dates, names, or admittance numbers in order to allocate patients, are inadequate and will be excluded for the assessment of benefits but not for assessment of harms.

Allocation concealment

- Low risk of bias: allocation was controlled by a central and independent randomisation unit, sequentially numbered, opaque and sealed envelopes, or similar, so that intervention allocations could not have been foreseen in advance of, or during, enrolment.
- Uncertain risk of bias: the trial was described as randomised but the method used to conceal the allocation was not described, so that intervention allocations may have been foreseen in advance of, or during, enrolment.
- High risk of bias: if the allocation sequence was known to the investigators who assigned participants or if the study was quasi-randomised. Quasi-randomised studies will be excluded for the assessment of benefits but not for assessment of harms.

Blinding of participants, personnel, and outcome assessors

- Low risk of bias: blinding was performed adequately, or the outcome measurement was not likely to be influenced by lack of blinding.
- Uncertain risk of bias: there was insufficient information to assess whether the type of blinding used was likely to introduce bias on the estimate of effect.
- High risk of bias: no blinding or incomplete blinding, and the outcome or the outcome measurement was likely to be influenced by lack of blinding.

Incomplete outcome data

- Low risk of bias: the underlying reasons for missing data were unlikely to make treatment effects depart from plausible values, or proper methods had been employed to handle missing data.
- Uncertain risk of bias: there was insufficient information to assess whether the missing data mechanism in combination with the method used to handle missing data were likely to induce bias on the estimate of effect.
- High risk of bias: the crude estimate of effects (eg, complete case estimate) was clearly biased due to the underlying reasons for missing data, and the methods used to handle missing data were unsatisfactory.

Selective outcome reporting

- Low risk of bias: predefined or clinically relevant and reasonably expected outcomes (mortality, adverse events, quality of life, and liver transplant) were reported on.
- Uncertain risk of bias: not all predefined or clinically relevant and reasonably expected outcomes were reported on or were not reported fully, or it was unclear whether data on these outcomes were recorded or not.
- High risk of bias: one or more clinically relevant and reasonably expected outcomes were not reported on; data on these outcomes were likely to have been recorded.

Other sources of bias

- Low risk of other bias: the trial appeared to be free of other components that could put it at risk of bias.
- Uncertain risk of other bias: the trial seemed to or seemed not to be free of other components that put it at risk of bias.
- High risk of other bias: there were other factors in the trial that could put it at risk of bias, eg, for-profit involvement, authors had conducted trials on the same topic etc.

Trials with 'low risk of bias' were considered to be those trials that were assessed as trials having 'low risk of bias' in all of the individual domains specified above. Trials with 'high risk of bias' were considered to be those trials that were assessed as trials having 'uncertain risk of bias' or 'high risk of bias' in one or more of the specified individual domains.

Measures of treatment effect

Measurement data were reported as mean difference (MD) and count data were reported as relative risk (RR) for statistical analysis, with 95% confidence intervals (CIs).

Unit of analysis issues

Randomised clinical trials with cross-over or cluster designs may raise statistical problems. If, in the future, we identify randomised trials that are cross-over trials, then data from the first period only will be considered for the review.

Dealing with missing data

We described the numbers and reasons for dropouts and withdrawals in all trial groups. We used the intention-to-treat principle for our analysis.

Assessment of heterogeneity

We planned to explore the presence of statistical heterogeneity by using the Chi^2 test with significance set at $P < 0.10$ and to measure the extent of heterogeneity with the I^2 statistic (Higgins 2003).

Assessment of reporting biases

The presence of publication bias was planned to be assessed by funnel plots (Egger 1997).

Data synthesis

The Review Manager 5 statistical software (RevMan 2011) provided by The Cochrane Collaboration was to be used for statistical analysis. We planned to perform both fixed-effect and random-effects model analyses with the significance level set at $P < 0.05$. We planned to report the fixed-effect model results when there was no difference between the results of the two models.

Subgroup analysis and investigation of heterogeneity

We had planned the following subgroup analyses.

1. Trials with low risk of bias compared to trials with high risk of bias.
2. Different doses of chlorambucil.
3. Difference of clinical stage of primary biliary cirrhosis.
4. Difference of duration of treatment.

Sensitivity analysis

We planned to perform sensitivity analyses for testing the robustness of conclusions.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies.

Results of the search

We identified a total of 146 references through electronic searches of the Cochrane Hepato-Biliary Group Controlled Trials Register (n = 2), the Cochrane Central Register of Controlled Trials (CENTRAL) in *The Cochrane Library* (n = 3), MEDLINE (n = 20), EMBASE (n = 116), Science Citation Index Expanded (n = 5), The Chinese Biomedical CD Database (n = 0), The Chinese Medical Current Contents (CMCC-CD) (n = 0), The China Hospital Knowledge Database (CHKD) (n = 0), and the database of ongoing trials (www.controlled-trials.com/mrct/) (n = 0). No additional studies were retrieved from the references listed in relevant reviews, meta-analysis, included articles, and pharmaceutical companies.

We excluded 143 duplicates, reviews, and clearly irrelevant references. We identified three potentially relevant references. Of the three retrieved publications, two fulfilled the inclusion criteria of the present review (Hoofnagle 1984; Hoofnagle 1986). However, they reported on the same trial. Hoofnagle 1984 was only published as an abstract and it was a preliminary report to Hoofnagle 1986. The third study was an editorial on azathioprine, colchicine, and chlorambucil for primary biliary cirrhosis, so we excluded it. No quasi-randomised clinical or observational studies were found in order to extract data on harm.

Included studies

The identified randomised trial was a prospective clinical trial assessing the efficacy and safety of chlorambucil in patients with primary biliary cirrhosis compared with no intervention.

Patients

The sample size of the trial was 24 patients. The mean age of the patients was 47 years (range 34 to 63 years), and 23 patients were females. All patients had chronic liver disease symptoms (23 pruritus, 23 fatigue, 6 jaundice) and had been symptomatic for a mean of 2.4 years (range 0.4 to 6 years).

Interventions

The patients were randomly assigned to chlorambucil (n = 13) or no therapy (n = 11). The two groups were comparable with regard to clinical, biochemical, and hepatic histologic indices. The initial dose was 10 mg per day for 10 days, then the dose was adjusted to 0.5 to 4 mg (mean 2 mg) per day according to the results of the white blood cell count and the platelet count. In most of the treated patients, the yearly average decrease in lymphocyte count was from 56% to 63%, and the decrease in polymorphonuclear cell counts was from 25% to 46%. The mean lymphocyte count was initially 2.2 $\times 10^{-3}/mm^3$, a year later it was 0.8 $\times 10^{-3}/mm^3$, and two years later it was 0.9 $\times 10^{-3}/mm^3$. The mean polymorphonuclear cell count was initially 4.8 $\times 10^{-3}/mm^3$, a year later it was 3.6 $\times 10^{-3}/mm^3$, and two years later it was 2.9 $\times 10^{-3}/mm^3$. There was no decrease in the white blood cell count in the control group. The platelet count decreased in the treated patients (the initial mean platelet count was $297600/mm^3$ and during therapy it ranged between 159100 to $215900/mm^3$).

Biochemical testing was re-examined every year, and liver biopsies were repeated at years one, two, and four after randomisation.

Both groups of patients continued on their other routine medications.

Follow-up

Only one patient from the no intervention group was lost from follow-up during the third year. The mean duration of follow-up was 52 months. All chlorambucil-treated patients had some degree of bone marrow suppression (for example, decrease in white blood cell count). Severe bone marrow suppression led to permanent discontinuation of chlorambucil in four patients.

Outcome measures

The outcome measures were overall survival; peripheral blood counts; changes in biochemical variables; and changes of hepatic histological indices.

Further details are shown in the table 'Characteristics of included studies'.

Excluded studies

The excluded publication was an editorial (Kaplan 1987).

Risk of bias in included studies

See: 'Risk of bias' table in 'Characteristics of included studies'.

Effects of interventions

Mortality

In the chlorambucil group, 0/13 (0%) patients died versus 2/11 (18.2%) patients in the no treatment group. The reasons for the deaths were reported. The two patients from the control group died of hepatic failure. With Fisher's exact test, the two-tailed P = 0.20, which means that the chlorambucil group was not associated with significantly lower mortality as compared with the no intervention group.

Liver transplantation

This outcome was not reported.

Adverse events

One hundred per cent of the participants (13/13) in the chlorambucil group versus 27% (3/11) in the no treatment group experienced adverse events. With Fisher's exact test, the two-tailed P = 0.0957, and hence no significant difference in the adverse events between the chlorambucil and no treatment groups was seen. In addition, four treated patients and three no treatment patients had severe systemic infection, but they all recovered during hospitalisation or by using systemic antibiotic treatment. All chlorambucil-treated patients had decreased white blood cell counts and platelet counts; in four patients the drop in the platelet count was classified as serious. In addition, two patients had menopause, two developed oral herpes simplex, and one patient had localised herpes zoster.

Quality of life

This outcome was not reported.

Clinical findings

The included trial did not report on any clinical findings. However, the authors stated that treatment with chlorambucil was not associated with fatigue, anorexia, nausea, diarrhoea, bitter taste, or hair loss.

Number of patients with fatigue

This outcome was not reported.

Number of patients with pruritus

This outcome was not reported.

Biochemical changes

Following the authors' report, chlorambucil led to a significant improvement in mean serum levels of bilirubin ($P < 0.05$), albumin ($P < 0.05$), immunoglobulin M ($P < 0.01$), and serum aspartate aminotransferase activity ($P < 0.01$) compared with no treatment at the second year or two years later. Serum alkaline phosphatases showed no statistically significant change in either group during the course of the evaluation.

Liver biopsy findings

In the chlorambucil group, the average intensity of the hepatic inflammatory cell infiltrate decreased significantly from year 0 to years 1 and 2 ($P < 0.01$). Six out of 10 (60%) treated patients had a marked decrease in inflammatory infiltrates in comparison with 3/10 (30%) untreated patients. For the average degree of hepatic fibrosis and stage of primary biliary cirrhosis, there was no statistically significant improvement in the chlorambucil group versus the no treatment group.

Costs

This outcome was not reported.

DISCUSSION

Summary of main results

We could only identify one small randomised clinical trial examining the beneficial and harmful effects of chlorambucil for primary biliary cirrhosis patients. The chlorambucil group did not differ significantly regarding mortality compared with the no intervention group. The authors of the trial stated that chlorambucil seemed to improve the mean serum levels of bilirubin, albumin, immunoglobulin M, and serum aspartate aminotransferase activity compared with no treatment. Serum alkaline phosphatases did not change in either group. These biochemical outcomes may be used in the evaluation of primary biliary cirrhosis evolution or prognosis (Dickson 1989; Pasha 1997; Kaplan 2002; Alempijevic 2009) but it is questionable whether the biomarkers can be used as proxies for a clinical benefit of the intervention (Baker 2003; Gluud 2005).

The included trial observed significant effects on the degree of hepatic histological inflammation, but the average degree of hepatic fibrosis and stage of primary biliary cirrhosis had no statistically significant improvement in the chlorambucil group versus the no intervention group. Primary biliary cirrhosis is a pathological process starting with portal inflammation which progresses towards liver fibrosis and cirrhosis, and eventually liver failure. Chlorambucil in this small trial might not have slowed down the progression towards the cirrhotic stage or other more advanced stages. As the included trial had so few patients included and a short follow-up, it is impossible to determine if chlorambucil could delay the natural progression of primary biliary cirrhosis.

The included trial did not report on any clinical features, such as fatigue and pruritus. The significance of the biochemical and histological changes identified are uncertain, probably due to the small sample size of this trial. Thus, the chance of demonstrating or excluding any benefit of the intervention is almost nil.

Meta-analyses were not possible as there is only one trial that fulfilled the inclusion criteria of this review. The result on adverse events obtained through the Fisher's exact test shows that the use of chlorambucil is not significantly different from the no treatment group. It must be noted, however, that bone marrow suppression is of concern as it leads to an increased risk of infection from the drop of white blood cells.

The one included trial with 24 patients did not report any results on the number of patients undergoing liver transplantation, health-related quality of life, and costs. In addition, the methodological quality of the trial is low, which makes it impossible to make any firm conclusion on the benefits or harms of chlorambucil.

Overall completeness and applicability of evidence

We have been unable to draw any conclusion about the benefits or harms of chlorambucil in patients with primary biliary cirrhosis.

Quality of the evidence

The identified randomised trial had a very small sample size and a high risk of bias (Figure 1; Figure 2). This opens the possibility for both random errors and systematic errors (Keus 2009).

Figure 1. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

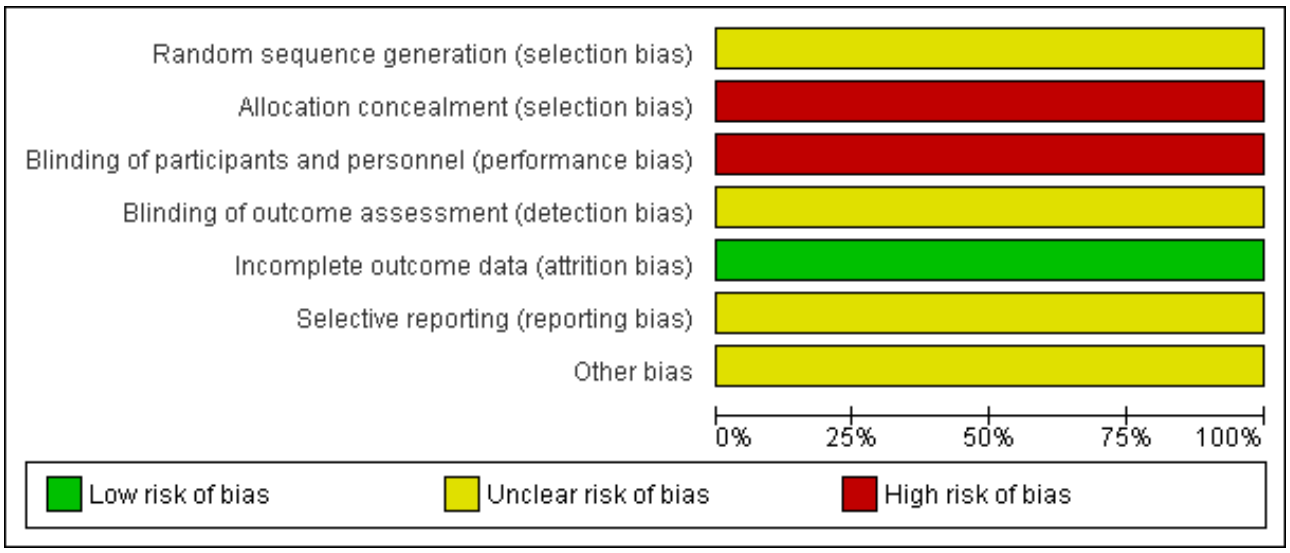
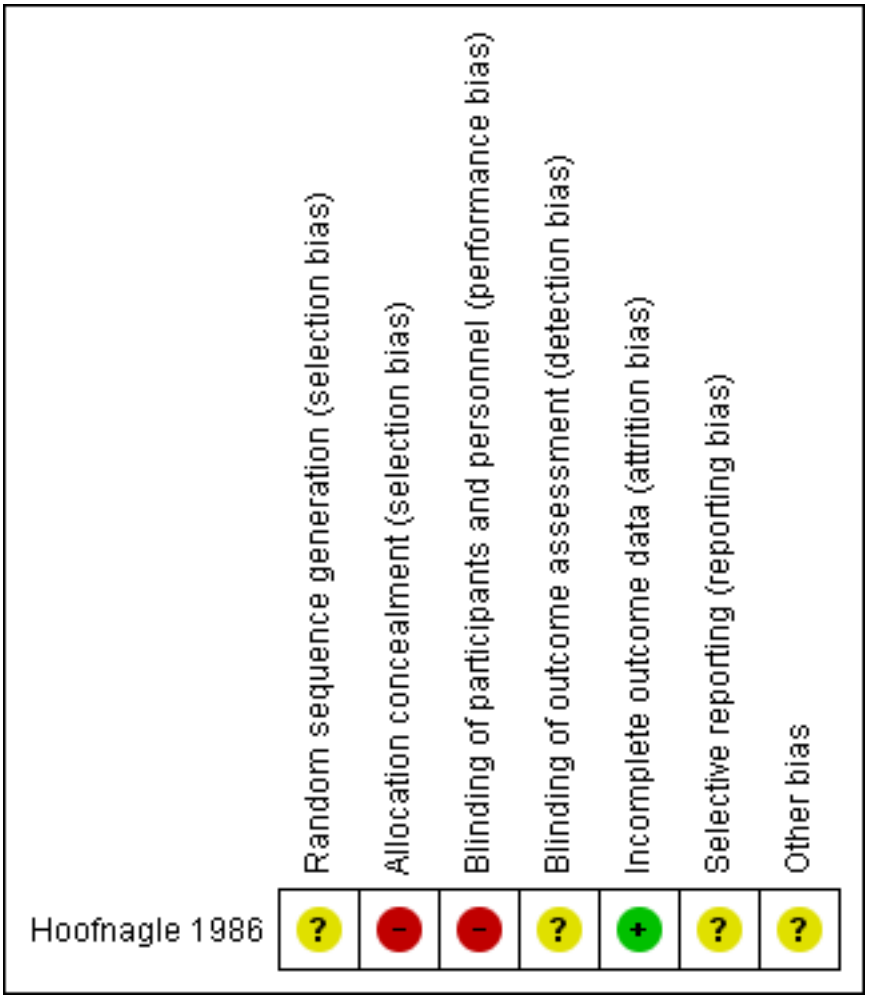


Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Potential biases in the review process

The existence of only one randomised trial, the lack of grey literature (for example, presentations, unpublished data, government reports, and other traditional or non-traditional sources of evidence), the incomplete number of outcomes and the lack of standard deviations in the included trial lead to the limitations of this review. We cannot exclude publication bias. We do not think we have been biased during the review process, and all decisions were reached through consensus between at least two authors.

Agreements and disagreements with other studies or reviews

Chlorambucil has been used to treat many diseases, such as chronic lymphocytic leukaemia (Robak 2000; Knauf 2009), low grade non-Hodgkin's lymphoma (Chisesi 1991; Taylor 2006), Waldenstrom's macroglobulinaemia (Kyle 2000; Johnson 2005), breast cancer (Senn 1997), idiopathic glomerulonephritis (Nand 1997), membranous nephropathy (Ponticelli 1995), systemic sclerosis (Clements 1993), ovarian cancer (Tattersall 1992), children's idiopathic nephrotic syndrome (Niaudet 1992), and rheumatoid arthritis (Cannon 1985). In several studies, chlorambucil achieved beneficial effects to a certain extent and did not clearly show more adverse events (for example, infection) compared with other drugs (Niaudet 1992; Robak 2000; Knauf 2009).

The exact cause of primary biliary cirrhosis is unknown, but the dynamics of the disease resemble the group of 'autoimmune diseases'. The natural history and progression of the disease varies greatly among individual patients (Ishibashi 2011). Some patients live long without any symptoms while other patients present with jaundice and develop hepatic failure in the early phases of the disease. The bile acid ursodeoxycholic acid (UDCA) is the only FDA-approved drug in patients with primary biliary cirrhosis, but it is uncertain if UDCA has beneficial effects on mortality or liver transplantation (Gong 2008). Some patients do not respond to UDCA, and have advanced histologic disease at presentation. These patients require different or adjuvant treatment. However, cyclosporin A (Gong 2007a), methotrexate (Giljaca 2010), D-penicillamine (Gong 2004a), and azathioprine (Gong 2007b) have no significant effects on survival or progression of the disease (cirrhosis development), and cause more adverse events than placebo. No convincing evidence exists to support or refute the use of glucocorticosteroids (Prince 2005) and colchicine (Gong 2004b) in patients with primary biliary cirrhosis.

A case report (Hughes 1983) suggested that four months of chlorambucil treatment improved the clinical symptom (for example, pruritus and jaundice), laboratory parameters, and hepatic granulomas of patients with sarcoidosis presenting as biliary cirrhosis. In the Israel 1991 trial, chlorambucil treatment was a useful alternative in patients with severe sarcoidosis that was unresponsive, or had developed tolerance, to corticosteroids. Severe hepatic sarcoidosis and primary biliary cirrhosis may have similar clinical and pathologic findings. This is why researchers have hypothesised that chlorambucil may also be an useful adjunct in the treatment of primary biliary cirrhosis when the disease is unresponsive to UDCA or to glucocorticosteroids or other interventions.

We cannot rule out a possible role of chlorambucil in a subset of primary biliary cirrhosis patients with a very aggressive disease and in whom adverse effects would not be a major problem. But based on the available evidence, we cannot identify any evidence to support or reject the use of chlorambucil for patients with primary biliary cirrhosis.

Further research should focus on understanding the pathogenic process of the disease and finding an effective medical treatment for patients with primary biliary cirrhosis.

AUTHORS' CONCLUSIONS

Implications for practice

Based on the evidence from one trial with 24 participants only, we cannot come to any firm conclusions on the clinical importance of chlorambucil for primary biliary cirrhosis. Adverse events may be frequent, and hence we cannot advocate the use of chlorambucil for patients with primary biliary cirrhosis outside randomised clinical trials.

Implications for research

Only 24 patients have been investigated in this review. There is insufficient evidence to support chlorambucil for primary biliary cirrhosis. Therefore, we recommend that placebo-controlled randomised trials should be considered. Such trials ought to be conducted and repeated accordingly to the CONSORT guidelines.

ACKNOWLEDGEMENTS

We primarily extend our acknowledgements to the patients who took part in the trial and the investigators who designed and conducted the reviewed trial. We thank all the people who were involved in the preparation of this review, especially to Sarah Louise Klingenberg and Dimitrinka Nikolova for the expert assistance that they have provided.

Review
Peer Reviewers: Pietro Invernizzi, Italy; Luit Penninga, Denmark.
Contact Editor: Ronald L Koretz, USA; Christian Gluud, Denmark.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Hoofnagle 1986

Methods	Prospective, randomised, clinical trial.
Participants	Inclusion criteria: Patients were between 30 and 65 years old and had symptomatic primary biliary cirrhosis. The diagnosis of primary biliary cirrhosis was based on: the presence of marked elevations in serum alkaline phosphatase and compatible liver biopsy histopathology. Exclusion criteria: Patients with a history of ascites, oesophageal variceal haemorrhage, or hepatic decompensation (bilirubin more than 12 mg/dl, prothrombin time more than 3 s prolonged). Characteristics of the included patients: N = 24 (intervention:13; control: 11). Mean age: 47 years. Proportion of females: 95.8%. Race: 21 white, 1 black, 2 Asian. All patients had symptoms attributable to their chronic liver disease: 23 pruritus, 23 fatigue, 6 jaundice. Twenty-two patients had positive serum mitochondrial antibody. The serum alkaline phosphatase levels in all patients were elevated more than three-fold.
Interventions	Experimental group: chlorambucil. Initial dose of chlorambucil: 10 mg/day for 10 days followed by mean 2 mg/day. The dose was further adjusted to keep the lymphocyte count at half of pretreatment levels. The drug dose was halved if the white blood count was less than 3000/mm^3 or the platelet count was less than 100000/mm^3. Chlorambucil was stopped if leucopenia or thrombocytopenia persisted at a dose of only 0.5 mg/day. Control group: no treatment. Co-intervention: the two groups of patients were continued on their other routine medications.
Outcomes	Mortality. Adverse events. Changes in biochemical indices (eg, bilirubin, alkaline phosphatase, aspartate aminotransferase). Changes in immunoglobulins and mitochondrial antibody levels. Changes in the intensity of hepatic inflammation, the degree of hepatic fibrosis, and the hepatic histologic stage of primary biliary cirrhosis.
Notes	Language: English. Study settings: United Kingdom.

Hoofnagle 1986 *(Continued)*

Sample size calculations: not reported.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described.
Allocation concealment (selection bias)	High risk	Patients were randomised to the chlorambucil therapy or the untreated control group using a set of coded envelopes.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Neither the participants nor the personnel were blinded.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	The trial only indicated that the liver biopsy histology outcome assessors were blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	The number of participants and reasons for dropouts in the trial were described.
Selective reporting (reporting bias)	Unclear risk	We did not find the trial protocol.
Other bias	Unclear risk	Not reported, we could not find whether the trial funded by the industry.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Kaplan 1987	Editorial, not a clinical trial.

APPENDICES

Appendix 1. Search strategies

Database	Time span	Search strategy
The Cochrane Hepato-Biliary Group Controlled Trials Register	March 2012.	(chlorambucil* OR chloroambucil* OR Chloraminophen* OR chlorbutin* OR chlorobutin* OR ambochlorin OR amboclorin OR elcloril OR ecloril OR leukeran OR leukersan OR linfolizin OR linfolysin OR pepstatin) AND ('primary biliary cirrhosis' OR PBC)
The Cochrane Central Register of Controlled	Issue 2, 2012.	#1 MeSH descriptor Chlorambucil explode all trees

(Continued)

Trials (CENTRAL) in *The Cochrane Library*		#2 chlorambucil* OR chloroambucil* OR Chloraminophen* OR chlorbutin* OR chlorobutin* OR ambochlorin OR amboclorin OR elcloril OR ecloril OR leukeran OR leukersan OR linfolizin OR linfolysin OR pepstatin #3 (#1 OR #2) #4 MeSH descriptor Liver Cirrhosis, Biliary explode all trees #5 primary biliary cirrhosis OR PBC 666 #6 (#4 OR #5) #7 (#3 AND #6)
MEDLINE (Ovid SP)	1946 to March 2012.	1. exp Chlorambucil/ 2. (chlorambucil* or chloroambucil* or Chloraminophen* or chlorbutin* or chlorobutin* or ambochlorin or amboclorin or elcloril or ecloril or leukeran or leukersan or linfolizin or linfolysin or pepstatin).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 3. 1 or 2 4. exp Liver Cirrhosis, Biliary/ 5. (primary biliary cirrhosis or PBC).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier] 6. 4 or 5 7. 3 and 6
EMBASE(Ovid SP)	1974 to March 2012.	1. exp chlorambucil/ 2. (chlorambucil* or chloroambucil* or Chloraminophen* or chlorbutin* or chlorobutin* or ambochlorin or amboclorin or elcloril or ecloril or leukeran or leukersan or linfolizin or linfolysin or pepstatin).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] 3. 1 or 2 4. exp primary biliary cirrhosis/ 5. (primary biliary cirrhosis or PBC).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] 6. 4 or 5 7. 3 and 6
SCI-EXPANDED (http://apps.isiknowledge.com)	1900 to March 2012.	#1 TS=(chlorambucil* or chloroambucil* or Chloraminophen* or chlorbutin* or chlorobutin* or ambochlorin or amboclorin or elcloril or ecloril or leukeran or leukersan or linfolizin or linfolysin or pepstatin) #2 TS=(primary biliary cirrhosis or PBC) # 3 #2 AND #1

(Continued)

Chinese Biochemical CD Database(CBM-CD)	1976 to March 2012.	cirrhosis AND (chlorambucil* or chloroambucil* or Chloraminophen* or chlorbutin* or chlorobutin* or ambochlorin or amboclorin or elcloril or ecloril or leukeran or leukersan or linfolizin or linfolysin or pepstatin) /
Chinese Medical Current Contents (CMCC-CD)	1994 to March 2012.	Same as CBM-CD
China Hospital Knowledge Database (CHKD)	1994 to March 2012.	Same as CBM-CD
the database of ongoing trials (at http://www.controlled-trials.com/mrct/)	March 6, 2012.	'Chlorambucil' AND 'primary biliary cirrhosis' or ('leukeran' AND 'primary biliary cirrhosis') or ('Amboclorin' AND 'primary biliary cirrhosis') or ('elcloril' AND 'primary biliary cirrhosis') or ('linfolizin' AND 'primary biliary cirrhosis') or ('pepstatin' AND 'primary biliary cirrhosis')

CONTRIBUTIONS OF AUTHORS

Obtain copies of studies: WX Li.
Select which studies to include: WX Li, CHR Shi, and X Yan.
Extract data from studies: WX Li, AP Zhang, and CHR Shi.
Enter data into RevMan: WX Li and AP Zhang.
Carry out the analysis: WX Li and X Yan.
Interpret the analysis: WX Li and X Yan.
Draft the final review: WX Li and X Yan.
Updates of the review: WX Li and X Yan.

DECLARATIONS OF INTEREST

None known

SOURCES OF SUPPORT

Internal sources

- First Hospital of Lanzhou University, Not specified.

  Lanzhou University, China

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Two additional Chinese databases (Chinese Medical Current Contents (CMCC-CD), China Hospital Knowledge Database (CHKD), and a database of ongoing trials (at http://www.controlled-trials.com/mrct/) were also searched in this review.

Two bias risk domains were removed as the domains 'baseline imbalance' and 'early stopping of trials' are not routinely judged when assessing the risk of bias in an included trial of a systematic review.

We had planned to use RevMan 5 for statistical analysis. However, as only one trial fulfilled the inclusion criteria of our review, and as the trial was small, we could not use RevMan. Instead, we used Fischer's exact test to calculate and present our data.

INDEX TERMS

Medical Subject Headings (MeSH)

Chlorambucil [*therapeutic use]; Immunosuppressive Agents [*therapeutic use]; Liver Cirrhosis, Biliary [*drug therapy]; Randomized Controlled Trials as Topic

MeSH check words

Humans