UNIVERSITY OF BELGRADE

MEDICAL FACULTY

Jelena S. Rudić

Interventions for primary biliary cirrhosis and osteoporosis in patients with primary biliary cirrhosis: Cochrane reviews with meta-analyses and trial sequential analyses of randomized clinical trials

doctoral dissertation

Belgrade, 2015

UNIVERZITET U BEOGRADU

MEDICINSKI FAKULTET

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Intervencije za lečenje primarne bilijarne ciroze: Kohranova analiza sistematskih pregleda sa meta-analizama i sekvencijalnim analizama randomizovanih kliničkih studija

doktorska disertacija

Beograd, 2015

Mentor

Prof. Dr. Miodrag Krstić, Faculty of Medicine, University of Belgrade

Co-mentor

Prof. Dr. Christian Gluud, Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Members of the Board

Prof. Dr. Tatjana Pekmezović, Faculty of Medicine, University of Belgrade
Prof. Dr. Đorđe Ćulafić, Faculty of Medicine, University of Belgrade
Prof. Dr. Goran Bjelaković, Faculty of Medicine, University of Niš

Preface

The present doctoral dissertation has been partly conducted during my visit in The Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen University Hospital in Denmark, during the period January – April 2011 and January – February 2012.

I would like to express my special appreciation and thanks to my co-mentor Christian Gluud, you have been a tremendous support for me. Thank you for encouraging my research and for allowing me to grow as a research scientist. I also want to thank you for an enjoyable and unforgettable moments during my stay in The Copenhagen Trial Unit, and for your brilliant comments and suggestions. Without your supervision and constant help this dissertation would not have been possible.

A special thanks to my mentor, professor Dr Miodrag Krstic, who supported me in writing, and incented me to strive towards my goal, your advice on both research as well as on my career have been priceless. Thanks to your great enthusiasm, constant guidance and support throughout all these years.

I am deeply grateful to Dimitrinka Nikolova, The Cochrane Hepato-Biliary Group, for expert assistance during the preparation of the reviews and excellent collaboration. Dima you were more to me than this, thank you for constant support in the moments when there was no one to answer my queries.

Besides, I would like to thank to Sarah Louise Klingenberg, The Cochrane Hepato-Biliary Group, for literature searches, and Mette Hansen for secretary assistance. Also, I would like to thank the staff at the Copenhagen Trial Unit for all their help.

I wish to thank my co-authors Goran Poropat, Vanja Giljača and Goran Bjelaković for their valuable contribution to the publications in this thesis.

Thanks to my family for all of the sacrifices that they've made on my behalf, for their immense love, understanding, support, enthusiasm and faith. They are the best partners on all my adventures.

I would like to express my sincere gratitude for the financial support from Clinic of Gastroenterology, Clinical Centre of Serbia, Belgrade, Ministry of Science (Grant No. 41004) in Belgrade, and The Cochrane Hepato-Biliary Group, Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen University Hospital, Denmark.

Original papers

This doctoral dissertation is based on the following papers:

Paper I. Rudic JS, Poropat G, Krstic MN, Bjelakovic G, Gluud C. Ursodeoxycholic acid for primary biliary cirrhosis. Cochrane Database Syst Rev 2012; 12:CD000551. doi: 10.1002/14651858. CD000551.pub3.

Paper II. Rudic JS, Poropat G, Krstic MN, Bjelakovic G, Gluud C. Bezafibrate for primary biliary cirrhosis. Cochrane Database Syst Rev 2012; 1:CD009145. doi: 10.1002/14651858. CD009145.pub2.

Paper III. Rudic JS, Giljaca V, Krstic MN, Bjelakovic G, Gluud C. Bisphosphonates for osteoporosis in primary biliary cirrhosis. Cochrane Database Syst Rev 2011; 12:CD009144. doi: 10.1002/14651858.CD009144.pub2.

Paper IV. Rudic JS, Poropat G, Krstic MN, Bjelakovic G, Gluud C. Hormone replacement for osteoporosis in women with primary biliary cirrhosis. Cochrane Database Syst Rev 2011; 12:CD009146. doi: 10.1002/14651858.CD009146.pub2.

Abstract

Background

Primary biliary cirrhosis is a chronic autoimmune-mediated liver disease characterised by progressive destruction of intrahepatic bile ducts, resulting in chronic cholestasis, portal inflammation, and fibrosis that can lead to cirrhosis and, ultimately, liver failure and the need for liver transplantation. The disease primarely affects middle-aged women and is associated with osteoporosis - either postmenopausal or secondary to the liver disease. Low bone mass is an important cause of morbidity in patients with primary biliary cirrhosis, leading to an increased risk of fractures, pain, and deformity. Treatment of primary biliary cirrhosis and osteoporosis associated with primary biliary cirrhosis is complicated. A number of drugs have been evaluated for patients with primary biliary cirrhosis (glucocorticosteroids, methotrexat, azathioprine, colchicine, cyclosporin, D-penicillamine, and chlorambucil). Ursodeoxycholic acid is the only drug approved for primary biliary cirrhosis by the U.S. Food and Drug Administration. Bezafibrate may be effective for treatment of primary biliary cirrhosis. Bisphosphonates and hormone replacement may be effective treatment options for osteoporosis in primary biliary cirrhosis, but the effects have only had limited assessment in systematic reviews. Therefore, interventions based on evidence are highly warranted.

Cochrane reviews with meta-analyses and trial sequential analyses of randomised clinical trials generally provide the best available evidence for health care interventions and clinical practice. Such Cochrane reviews are used to assess and summarise benefits and harms of clinical interventions. Furthermore, Cochrane reviews will also reveal lack of evidence, and define the specific need for future randomised clinical trials.

Objectives

To summarize the evidence from Cochrane systematic reviews on treatment

options for patients with primary biliary cirrhosis and osteoporosis associated with primary biliary cirrhosis.

Methods

Four Cochrane systematic reviews of all relevant randomised clinical trials with meta-analyses and trial sequential analyses were conducted using The Cochrane Collaboration methodology, the GRADE, and the PRISMA-guidelines. Three out of four systematic reviews were performed according to published protocols following the recommendations of the Cochrane Handbook for systematic reviews of interventions, and one review was updated according to the same recommendations. Included trials were identified through The Cochrane Library, MEDLINE, EMBASE, Science Citation Index Expanded, LILACS, Clinicaltrials.gov, the WHO International Clinical Trials Registry Platform, manual searches of bibliographies and journals, authors of trials, and pharmaceutical companies. Data extraction and the assessment of risk of bias were conducted by two authors independently of each other.

Results

The four Cochrane systematic reviews included a total of 30 trials with 1,847 participants. Only three trials could be considered low risk of bias regarding all bias types. The reporting of patient-important outcomes was in general sparse.

We included 16 randomised clinical trials with 1447 patients with primary biliary cirrhosis, out of which 14 trials compared ursodeoxycholic acid with placebo and 2 trials compared ursodeoxycholic acid with no intervention. Ursodeoxycholic acid versus placebo or no intervention did not significantly affect all-cause mortality, all-cause mortality or liver transplantation, adverse events, liver transplantation, pruritus, fatigue, or liver-related morbidity in patients with primary biliary cirrhosis. Ursodeoxycholic acid seemed to have a beneficial effect on liver biochemistry measures and on histological progression compared with placebo or no intervention. According to the results of the trial

sequential analyses, there seems to be firm evidence for a beneficial effects of ursodeoxycholic acid on decreasing serum bilirubin concentration and the activity of serum alkaline phosphatases in patients with primary biliary cirrhosis. All the other biochemical markers assessed showed non-significant effect estimates.

We included 6 randomised clinical trials with 151 Japanese patients, out of which 4 trials compared bezafibrate versus no intervention, and 2 trials compared bezafibrate with ursodeoxycholic acid. Bezafibrate did not demonstrate any significant effect on mortality, liver-related morbidity, or adverse events when compared with no intervention, or when compared with ursodeoxycholic acid. Bezafibrate did not demonstrate any significant effect on pruritus compared with no intervention. The results of trial sequential analysis imply that there is firm evidence for a beneficial effect of bezafibrate on decreasing the activity of serum alkaline phosphatases when compared with no intervention, or when compared with ursodeoxycholic acid. The results of trial sequential analysis imply that there is no firm evidence for a beneficial effect of bezafibrate on decreasing plasma immunoglobulin M concentration and serum bilirubin concentration when compared with no intervention. All the other biochemical markers assessed showed non-significant effect estimates.

We included 6 randomised clinical trials with 200 participants, out of which 3 trials with 106 participants compared etidronate or alendronate with placebo or no intervention; 2 trials with 62 participants compared etidronate or alendronate with alendronate or ibandronate; and 1 trial with 32 participants compared etidronate with sodium fluoride. Having conducted statistical analyses, we found no evidence of effect of any of the aforementioned three bisphosphonates on mortality, fractures, adverse events, liver-related mortality, liver transplantation, liver-related morbidity or bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DEXA) in patients with primary biliary cirrhosis. The results of trial sequential analysis imply that there

is firm evidence for a beneficial effect of bisphosphonates on decreasing urinary amino telopeptides of collagen I (NT_x) concentration compared with placebo or no intervention. Etidronate compared with sodium fluoride significantly decreased serum osteocalcin, urinary hydroxyproline, and parathyroid hormone concentration. All the other assessed biochemical markers of bone turnover showed non-significant effect estimates.

We included 2 randomised clinical trials with 49 participants, which compared the effect of hormone replacement in treatment of osteoporosis in women with primary biliary cirrhosis with placebo or no intervention. We found no significant effect of hormone replacement on mortality, fractures, lumbar spine BMD measured by DEXA, liver-related mortality, liver transplantation, or liver-related morbidity in women with primary biliary cirrhosis. Hormone replacement significantly increased adverse events and number of patients having hormone replacement withdrawn due to adverse events. Hormone replacement may decrease BMD at the proximal femur.

Conclusions

We found no reliable evidence of benefit of the assessed treatments used in patients with primary biliary cirrhosis and in osteoporosis associated with primary biliary cirrhosis on patient-important outcomes which were poorly reported in most of the trials. Almost all of the trials had methodological limitations leading to systematic errors, small number of participants increasing the risks of random errors, and short trial duration. None of the treatments can be recommended for general use in clinical practice. Multi-centre randomised clinical trials with larger sample sizes and minimised risk of bias would be appropriate for participant recruitment since primary biliary cirrhosis is a relatively rare disease.

Key words: Cochrane review; primary biliary cirrhosis; osteoporosis

Scientific field: Epidemiology/gastroenterohepatology

Sažetak

Uvod

Primarna bilijarna ciroza je hronična autoimuna bolest jetre koju karakteriše progresivna destrukcija intrahepatičnih žučnih puteva sa posledičnom holestazom, portnom inflamacijom, i fibrozom što dovodi do nastanka ciroze jetre, i hepatičke insuficijencije sa transplantacijom jetre kao jedinom uspešnom terapijskom metodom. Više od 90% bolesnika su žene, prosečne starosti oko 50 godina. Najvažnija komplikacija bolesti vezana za holestazu je osteoporoza gde smanjenje koštane gustine dovodi do velikog rizika za nastanak preloma kostiju, bola i deformiteta. Lečenje primarne bilijarne ciroze, kao i osteoporoze u sklopu primarne bilijarne ciroze je veoma komplikovano. Za sada nema zadovoljavajuće specifične medicinske terapije koja se preporučuje za lečenje ove bolesti. Evaluirani su mnogi lekovi u terapiji ove bolesti (kortikosteroidi, metrotreksat, azatioprin, kolhicin, ciklosporin, D-penicilamin, i hlorambucil), ali do sada prikazani trajali su uglavnom bili kratki, mali i slabo kontrolisani. Ursodeoksiholna kiselina jedini je lek odobren za terapiju primarne bilijarne ciroze. U nekim kontrolisanim studijama konstatovano je da bezafibrat ima višestruka pozitivna dejstva kod bolesnika sa primarnom bilijarnom cirozom. Za bisfosfonate i supstitucionu hormonsku terapiju se očekuje da budu efikasni u terapiji osteoporoze u sklopu primarne bilijarne ciroze, ali ne postoje za sada dokazi efikasnoti u sistematskim pregledima.

Kohranovi sistematski pregledi sa meta-analizama i sekvencijalnim analizama randomizovanih kliničkih studija sintetišu dokaze u cilju dobijanja pouzdanog, validnog i kompletnog pregleda proverenih dokaza o korisnim i štetnim efektima terapijskih procedura koristeći metodologiju u kojoj nema pristrasnosti u tumačenju rezultata i izvođenju zaključaka. Takođe, oni mogu ukazati na nedostatak dokaza i potrebu za budućim dobro dizajniranim randomizovanim kliničkim studijama.

Ciljevi

Identifikovati i objediniti sve postojeće dokaze koji se odnose na procenu povoljnih i štetnih efekata različitih intervencija kod bolesnika sa primarnom bilijarnom cirozom i osteoporozom u sklopu primarne bilijarne ciroze.

Materijal i metode

Četiri Kohranova sistematska pregleda sa meta-analizama i sekvencijalnim analizama randomizovanih kliničkih studija su izrađena koristeći standardizovanu metodologiju Kohranove Kolaboracije, GRADE I PRISMA vodiča. Tri sistematska pregleda su izvedena prema protokolima objavljenim u Kohranovoj bazi sistematskih pregleda, dok je jedan ažuriran. Randomizovane kliničke studije su identifikovane sveobuhvatnom pretragom literature i sledećih baza podataka *The Cochrane Library, Medline, Embase, Science Citation Index Expanded, LILACS, Clinicaltrials.gov, the WHO International Clinical Trials Registry Platform,* ručnim pretraživanjem literature, ličnim kontaktom sa glavnim istraživačima identifikovanih randomizovanih kliničkih studija i farmaceutskim kompanijama koje produkuju ispitivani lek. Ekstrakciju podataka i procenu rizika od pristrasnosti odnosno metodološkog kvaliteta uključenih studija su obavljala dva autora nezavisno jedan od drugog.

Rezultati

U doktorsku tezu su uključena četiri Kohranova sistematska pregleda sa ukupno 30 randomizovanih kliničkih studija i 1.847 ispitanika.

Analiza ursodeoksiholne kiseline je uključila 16 randomizovanih studija sa 1447 pacijenata sa primarnom bilijarnom cirozom, od kojih 14 studija je poredilo ursodeoksiholnu kiselinu sa placebom a 2 studije su poredile ursodeoksiholnu kiselinu sa *'no intervention'*. Primena ursodeoksiholne kiseline nije značajno uticala na ukupnu smrtnost, ukupnu smrtnost ili transplantaciju jetre, neželjena dejstva, transplantaciju jetre, svrab, umor, ili komplikacije bolesti kod pacijenata sa primarnom bilijarnom cirozom. Ursodeoksiholna kiselina može povoljno

uticati na biohemijske parametre jetrine funkcije i histološku progresiju u poređenju sa placebom ili *'no intervention'*.

Analiza bezafibrata je uključila 6 randomizovanih studija sa 151 ispitanika sa primarnom bilijarnom cirozom, od kojih 4 studije je poredilo bezafibrat sa *'no intervention'* a 2 studije su poredile bezafibrat sa ursodeoksiholnom kiselinom. Primena bezafibrata nije pokazala nikakav značajan uticaj na ukupnu smrtnost, komplikacije bolesti, i neželjena dejstva kod pacijenata sa primarnom bilijarnom cirozom u poređenju sa ursodeoksiholnom kiselinom ili *'no intervention'*. Nije pokazano da bezafibrati imaju značajan efekat na svrab u poređenju sa *'no intervention'*. Rezultat sekvencijalne analize studija ukazuje na mogući povoljan efekat bezafibrata na smanjenje aktivnosti serumske alkalne fosfataze u poređenju sa ursodeoksiholnom kiselinom ili *'no intervention'*. Na sve ostale biohemijske markere bezafibrat je bio bez značajnog efekta.

Analiza bisfosfonata je uključila 6 randomizovanih studija sa ukupno 200 ispitanika sa primarnom bilijarnom cirozom i osteoporozom, od kojih 3 studije sa 106 ispitanika su poredile etidronat ili alendronat sa placebom ili *'no intervention'*; 2 studije sa 62 ispitanika su poredile etidronat ili alendronat sa alendronatom ili ibandronatom, i 1 studija sa 32 ispitanika je poredila etidronat sa natrijum fluoridom. Za nijedan od navedena tri bisfosfonata nije dokazano da imaju uticaj na ukupnu smrtnost, nastanak preloma, neželjene efekte, smrtnost vezanu za bolest jetre, transplantaciju jetre, komplikacije bolesti ili koštanu mineralnu gustinu merenu dvostrukom X zračnom apsorpciometrijom kod bolesnika sa primarnom bilijarnom cirozom i osteoporozom. Rezultat sekvencijalne analize studija ukazuje na mogući povoljan efekat bifosfonata na smanjenje urinarnog N-terminalnog telopeptida (NTx) u poređenju sa placebom ili *'no intervention'*. Samo je jedna studija poredila etidronat sa natrijum fluoridom zbog čega meta-analizu nije bilo moguće sprovesti, a opisuje da etidronat značajno smanjuje serumski osteokalcin, urinarni

hidroksiprolin, i koncentraciju paratireoidnog hormona. Na sve druge biohemijske markere koštanog prometa nije bilo značajnih efekata.

Analiza supstitucione hormonske terapije je uključila 2 randomizovane studije sa 49 ispitanica sa primarnom bilijarnom cirozom i osteoporozom, koje su poredile supstitucionu hormonsku terapiju sa placebom ili *'no intervention'*. Dokazano je da supstituciona hormonska terapija ne utiče na smrtnost, nastanak preloma, koštanu mineralnu gustinu lumbalne kičme merenu dvostrukom X zračnom apsorpciometrijom, smrtnost vezanu za bolest jetre, transplantaciju jetre, ili komplikacije bolesti kod bolesnica sa primarnom bilijarnom cirozom i osteoporozom. Pokazano je da supstituciona hormonska terapija može smanjiti koštanu mineralnu gustinu na proksimalnom okrajku butne kosti. Supstituciona hormonska terapija je udružena sa povećanim brojem neželjenih efekata.

Zaključak

Izradom Kohranovih sistematskih pregleda te meta-analizom dostupnih literaturnih dokaza prikazani su podaci efikasnosti i štetnosti primene različitih intervencija kod bolesnika sa primarnom bilijarnom cirozom i osteoporozom u sklopu primarne bilijarne ciroze. Ustanovljeno je da se ne može preporučiti njihova rutinska primena u svakodnevnoj kliničkoj praksi zbog visokog rizika pristranosti i manjkavosti u dizajnu primarnih studija, kao i zbog malog broja randomizovanih ispitanika. Dodatne dobro dizajnirane studije su potrebne s ciljem određivanja njihove stvarne štetnosti, odnosno efikasnosti.

Ključne reči: Kohranov pregled; primarna bilijarna ciroza; osteoporoza

Naučna oblast/uža naučna oblast: Epidemiologija/gastroenterolohepatologija

Contents

INTRODUCTION

Primary biliary cirrhosis is a chronic inflammatory autoimmune liver disease characterised by progressive destruction of intrahepatic bile ducts, resulting in chronic cholestasis, portal inflammation, and fibrosis that can lead to cirrhosis and, ultimately, liver failure. It remains one of the major indications for liver transplantation worldwide.

Epidemiology

The disease was first comprehensively described around 1950 (MacMahon and Thannhauser, 1949; Ahrens et al, 1994). Primary biliary cirrhosis is a rare disease that primarily affects middle-aged women with a sex ratio of 10:1. Data about the incidence and prevalence of primary biliary cirrhosis have generally been obtained passively and might not indicate the true rates of the disease in the general population. Reported annual incidence of primary biliary cirrhosis ranges from 1 to 49 persons per million, and the prevalence has been estimated between 7 to 402 persons per million (Prince and James, 2003; Poupon, 2010). The disease seems to cluster within specific geographical areas, being most prevalent in northern Europe (Prince and James, 2003). Risk factors include history of familial autoimmune disease, history of active or passive smoking and recurrent urinary tract infections. Coexisting autoimmune diseases among patients with primary biliary cirrhosis included Sjogren's syndrome (17.4%), Raynaud's phenomenon (12.5%), and autoimmune thyroid disease (11.5%), with significantly lower frequencies among siblings and healthy persons (Parikh-Patel et al, 2001). Primary biliary cirrhosis is now a frequent cause of liver morbidity, and the patients are significant users of health resources, including liver transplantation (Prince and James, 2003).

Pathogenesis

The etiology of primary biliary cirrhosis is still unclear, but it is thought to involve multiple genetic factors and environmental triggers leading to an

intense autoimmune response against the biliary epithelial cells. Pathogenesis is multi-step that follows from an initial loss of immunologic tolerance to a ubiquitous antigen all the way through to immune mediated inflammation, cholestasis and subsequent fibrosis. Environmental factors such as chemicals likely play a role in causes of the disease. Bacteria have attracted the most attention because of the reported elevated incidence of urinary tract infections in patients with primary biliary cirrhosis. Other potential causes include exposure to environmental chemicals. However, it is unclear whether the chemical immunisation is serendipitous and capable of eliciting antimitochondrial antibodies or whether these antibodies are capable of inducing primary biliary cirrhosis (Leung et al, 2005). Cellular (CD4 and CD8 T cells) and humoral abnormalities have both been noted. The major finding associated with humoral immunity in primary biliary cirrhosis resides with recognition of the antimitochondrial antibody. Formation of this antibody is presented in more than 95% of patients.

Clinical findings and natural history

The clinical features and natural history of primary biliary cirrhosis vary greatly between patients. It may manifest as asymptomatic, slowly progressive, symptomatic, or rapidly evolving. Asymptomatic patients have about equivalent short-term survival compared to an age-matched and sex-matched healthy population (Lee and Kaplan, 2005). Most asymptomatic people with primary biliary cirrhosis will develop symptoms within five years after the diagnosis has been made. The progress to cirrhosis and end stage liver disease may necessitate liver transplantation as the only treatment option (Prince et al, 2004). On the other hand, the overall median survival for symptomatic patients is between 10 and 15 years. Serum bilirubin level is an independent predictor of survival and is used for prognosis in patients with primary biliary cirrhosis (Shapiro et al, 1979). The most common symptoms and findings are fatigue and pruritus, hyperlipidaemia, hypothyroidism, osteoporosis, and coexisting

autoimmune diseases (Kaplan and Gershwin, 2005). Primary biliary cirrhosis is associated with features of autoimmune hepatitis in 10% patients.

Diagnosis

Diagnosis is made upon the following criteria: a) abnormal biochemical tests with preferential elevation of serum alkaline phosphatases and gammaglutamyltranspeptidases activities; b) presence of detectable serum antimitochondrial antibodies with M2 specificity as confirmed by ELISA or immunoblotting; c) evidence of lymphocytic destructive cholangitis (LDC) at histology. Criteria of a and b or c are sufficient for the diagnosis considering the high specificity of anti-M2 antibody and LDC (Heathcote, 2000; EASL, 2009). Characteristic liver histological changes confirm the diagnosis and are used for staging and assessing disease activity before therapeutic intervention, and can identify other co-existent diseases such as steatosis or steatohepatitis (Lindor et al, 2009; Drebber et al, 2009). Histological staging is based on Ludwig's and Scheuer's classifications (Scheuer, 1967), ranging from portal tract inflammation with predominantly lymphoplasmacytoid infiltrates and septal and interlobular bile duct loss (stage I) to frank cirrhosis (stage IV). Focal duct obliteration with granuloma formation has been termed the 'florid duct lesion' and is considered almost pathognomonic for primary biliary cirrhosis when present. Stage II is characterized by portal expansion with periportal inflammation (interface hepatitis) and/or ductular reaction, and stage III is dominated by the existence of bridging fibrosis. Features predictive of a poor outcome include the presence of an established cirrhosis or marked ductopenia. However, according to the latest clinical guidelines (EASL, 2009), a liver biopsy shall not necessarily be used for diagnosis of primary biliary cirrhosis in patients who present with typical biochemical and serological abnormalities. Therefore, liver biopsy is now mainly used as a diagnostic investigation in patients presenting with atypical biochemical or serological findings (e.g. AMA-negative PBC) and those who are suspected to have an 'overlap syndrome'' with autoimmune hepatitis.

Non-invasive markers, including panels of serum markers and transient elastography, have been used to a limited degree in patients with primary biliary cirrhosis to assess disease severity, but further studies are required to determine their diagnostic utility.

Interventions

Treatment for primary biliary cirrhosis remains presently non-specific, having essentially remained unchanged for more than a decade, with standard of care requiring the use of ursodeoxycholic acid. Patients with suboptimal response to ursodeoxycholic acid deserve trials with adjuvant therapies. However there is no consensus how to treat these patients.

Several drugs, glucocorticosteroids, methotrexat, azathioprine, colchicine, cyclosporin, D-penicillamine, and chlorambucil have been evaluated in primary biliary cirrhosis. Cochrane systematic reviews showed that none of them have been effective in patients with primary biliary cirrhosis (Gong and Gluud, 2004a; Gong et al, 2004b; Prince et al, 2005; Gong et al, 2007a; Gong et al, 2007b; Giljaca et al, 2010; Li et al, 2012). Malotilate (1.5 g/day) has been evaluated versus placebo in a doubleblind multicentre randomised clinical trial including 101 patients. After a mean follow-up of 28 months significant beneficial effects were found on liver enzymes, immunoglobulin G and M, liver necrosis and inflammatory cell infiltration, but not on fibrosis, pruritus, disease progression, or survival. The observed benefits appeared too slight to recommend the drug as therapy (A European multicentre study group, 1993). Thalidomide 100 mg/day has been tested against placebo in a small double-blind trial involving 18 patients. Except for a possible effect on pruritus no significant effects of the drug were found, and adverse effects occurred in 40% (McCormick et al, 1994).

Ursodeoxycholic acid

Ursodeoxycholic acid is the only drug approved for primary biliary cirrhosis by the U.S. Food and Drug Administration. Doses of 13 to 15 mg/kg/day seem to

cause significant improvements in liver tests and immunoglobulin levels and reduce titers of antimitochondrial antibodies. The dose of ursodeoxycholic acid appears to be important. A study comparing three different doses showed that a dose of 13 to 15 mg/kg of body weight per day appeared to be optimal, as compared with a dose of either 5 to 7 mg or 23 to 25 mg (Angulo et al, 1999a). Bile duct destruction leads to the retention of hydrophobic bile acids within the liver cell. This most likely contributes to the gradual deterioration of liver function and liver histology observed in patients with primary biliary cirrhosis. Ursodeoxycholic acid increases the transportation of intracellular bile acids across the liver cell and into the canaliculus in patients with primary biliary cirrhosis (Jazrawi et al, 1994). Mechanisms of action of ursodeoxycholic acid in primary biliary cirrhosis remain unclear, yet the hydrophilic nature of this agent could lead to a reduction in amounts of primary bile acids, and the substance might also regulate cellular signalling and protect against apoptosis (Crosignani et al, 1991; Paumgartner and Beuers, 2002). Ursodeoxycholic acid is a secondary bile acid, which is a metabolic byproduct of intestinal bacteria. After oral ingestion and intestinal absorption, the drug enters the portal circulation and is taken up by the hepatocytes where ursodeoxycholic acid is conjugated to glycine or taurine and is subsequently transported into the bile ducts (Kullak-Ublick et al, 2000). Ursodeoxycholic acid undergoes extensive enterohepatic recycling along with the other bile acids (Hofmann, 1994). Because of its high first-pass metabolism (70%), the blood level of ursodeoxycholic acid in the systemic circulation is low (Saksena and Tandon, 1997). In the colon, the unabsorbed ursodeoxycholic acid is transformed to lithocholic acid by colonic microbial flora and is excreted via the faeces (Kullak-Ublick et al, 2000). The half life of ursodeoxycholic acid is about 100 hours (Setchell et al, 1996). The drug acts through several pathways, such as alteration of the bile-acid pool, choleresis (the flow of bile from the liver), immune-modulation effects, and cytoprotective mechanisms. One of the main mechanisms of ursodeoxycholic acid is displacement of endogenous

hepatotoxic bile by expansion of the hydrophilic bile acid pool which may correlate with competitive displacement of endogenous bile acids, either at the level of ileal absorption or at the hepatocyte (Stiehl et al, 1999). Ursodeoxycholic acid treatment in patients with primary biliary cirrhosis might reduce the serum level of IgM class antimitochondrial antibodies and IgG antibodies to pyruvate dehydrogenase. Ursodeoxycholic acid might also reduce the T-cell-mediated hepatocellular damage by decreasing hepatocellular and biliary expression of major histocompatibility complex (MHC) class I and MHC class II molecules (Lazaridis et al, 2001). Ursodeoxycholic acid is theoretically a safe and well tolerated drug but can induce modest weight gain (2 to 3 kg) during the first year of treatment (Siegel et al, 2003). The effect of ursodeoxycholic acid on mortality and histological progression remains still controversial (Goulis et al, 1999; Gluud and Christensen, 2001b; Gong et al, 2008; EASL, 2009; Silveira et al, 2010). Our previously updated Cochrane systematic review did not provide sufficient information on benefits and harms of ursodeoxycholic acid in patients with primary biliary cirrhosis to recommend or reject the drug for this indication (Gong et al, 2008).

Bezafibrate

PPAR alpha agonists (bezafibrate, fenofibrate) are now recognized to have anti-inflammatory and immunomodulatory properties in experimental models of autoimmunity. Bezafibrate was first introduced in 1977 by Boehringer Mannheim Ltd. (Williams et al, 1984). Bezafibrate is a hypolipidaemic agent, which reduces cholesterol and triglyceride synthesis in the liver by inhibiting acetyl coenzyme A carboxylase activity. Fibrates are known to reduce the flow of fatty acids to the liver, decrease very low-density lipoprotein hepatic synthesis, stimulate lipoprotein-lipase activity, and increase the biliary excretion of hepatic cholesterol. Bezafibrate is used in treatment of hypertriglyceridaemia and combined hyperlipidaemia (Vessby et al, 1980). Bezafibrate effectively reduces low-density lipoprotein and triglycerides, and

elevates high-density lipoproteins levels thus improving hyperlipidaemia (The BIP Study Group, 2000). Fibrates are associated with a number of adverse effects, including liver enzyme elevations, gastrointestinal adverse effects, and rhabdomyolysis (Muscari et al, 2002). In patients with metabolic syndrome, bezafibrate decreases the incidence of myocardial infarction and reduces the risk of cardiac mortality (Tenenbaum et al, 2005). Bezafibrate decreases the incidence of type 2 diabetes and may delay the onset of type 2 diabetes in patients with impaired glucose tolerance (Tenenbaum, et al, 2004). Bezafibrate decreases the activity of the cholestatic liver enzymes (alkaline phosphatases and gamma-glutamyl transferase) in asymptomatic patients (Fukuo et al, 1996). In some small studies, biochemical improvement was reported by using bezafibrate alone or in combination with ursodeoxycholic acid (Kurihara et al, 2000; Nakai et al, 2000; Kurihara et al, 2002). There are two possible mechanisms of the bezafibrate effects on primary biliary cirrhosis involving multiple drug-resistant gene (MDR-2) and peroxisome proliferative-activated receptor alpha (PPAR-α) system pathway. Bezafibrate is a ligand of PPAR-α, which is involved in immune function and inflammation control by regulation of leukotriene B4 and through this mechanism it improves lipid serum concentration balance (Devchand et al, 1996; Delerive et al, 2001). Secondly, bezafibrate induces the expression of MDR-2 and thus controls the balance of biliary phospholipids and bile acids which prevents biliary cell damage through activation of the MDR-2 gene of a knockout mice (mimicking the human MDR-3 gene) (Smit et al, 1993; Chianale et al, 1996). In human studies, defects of the MDR-3 gene may produce progressive familial intrahepatic cholestasis, and in advanced primary biliary cirrhosis the expression of MDR-3 messenger RNA and proteins is increased (Jacquemin et al, 2001; Ros et al, 2003). Bezafibrate lowers the proportion of Fas antigen (surface transmembrane protein that mediates apoptosis)-positive T cells in the peripheral blood and suppresses the inflammatory response in patients with primary biliary cirrhosis (Ishimaru and Iino, 2002). Fibrates might inhibit migration of inflammatory cells by RANTES (hepatic regulated upon

activation, normal T-cell expressed and secreted) to the liver in patients with primary biliary cirrhosis (Hirano et al, 2002). The exact mechanisms yielding the therapeutic benefits of bezafibrate in primary biliary cirrhosis are still to be understood.

Disease-related complications

A number of systemic complications associated with primary biliary cirrhosis have been documented that represent disease progression and impair health-related quality of life in some individuals. Disease-specific complications, including fatigue, pruritus, and metabolic bone disease, are important to recognize and treat appropriately.

Metabolic bone disease

Patients with primary biliary cirrhosis are predisposed to develop metabolic bone disease and premature cortical bone thinning. They often suffer from postmenopausal osteoporosis due to their age. Bone disease is a major complication of chronic liver disease with serious clinical consequences, affecting quality of life, morbidity, and mortality (Luxon, 2011). The term 'hepatic osteodystrophy' includes bone disease associated with chronic liver disease (Rouillard and Lane, 2001).

Osteoporosis is a common progressive systemic skeletal disease characterised by low bone strength and increased fracture risk (WHO, 1994; Klibanski et al, 2001). Bone loss among patients with primary biliary cirrhosis is twice that of age and sex-matched controls (Eastell et al, 1991), and the prevalence of osteoporosis among these patients is between 14% and 52% (WHO, 1994). Osteoporotic fractures of the spine and hip contribute importantly to the increased morbidity and mortality (Cooper, 1997; Center et al, 1999). More than 200 million people worldwide have osteoporosis (Cooper et al, 1992). Bone mineral testing by dual-energy X-ray absorptiometry is the current gold standard for measuring bone mineral density in grams per square centimetre

(g/cm^2) in the lumbar spine (L1-L4), proximal femur, the distal one-third of radius, and the total hip. The classification of bone mineral density is determined by the standard deviation difference between the patient's bone mineral density and the mean bone mineral density of a young-adult reference population represented by the T-score (≤ 2.5 'osteoporosis', between 1.0 and 2.5 'low bone mass' or 'osteopenia', and ≥ 1.0 'normal') (Kanis, 1994; WHO, Kanda 1994). Bone mineral density measured by dual-energy X-ray absorptiometry combined with clinical risk factors for fracture (when available, with electronic algorithms such as FRAX ®) are widely used to estimate fracture risk (WHO, 1994). According to the American Gastroenterological Association guidelines bone mineral density should be considered in all patients with primary biliary cirrhosis at diagnosis (AGA, 2003; Leslie et al, 2003).

The pathogenesis of osteoporosis in primary biliary cirrhosis is complex and needs further elucidation, but it is thought to be multifactorial. Bone loss is the result of an imbalance between bone formation and bone resorption (Diamond et al, 1989; Hodgson et al, 1993). The main risk factors for osteoporosis in primary biliary cirrhosis include age and severity of liver disease which is correlated with the severity of bone disease (Menon et al, 2001; Boulton-Jones et al, 2004). Potential factors that may alter bone mass include insulin growth factor-1 deficiency, hyperbilirubinaemia, hypogonadism (oestrogen and testosterone deficiency), alcoholism, excess tissue iron deposition, vitamin D deficiency, vitamin D receptor genotype, osteprotegerin deficiency, and immunosuppressive therapy before and after liver transplantation (McCaughan and Feller, 1994; Sambrook and Cooper, 2006). Furthermore, retained bilirubin and biliary salts, increased production of fibronectin iso-form, increased osteoclast formation, calcium malabsorption, and nutritional status have an influence on the low bone formation (Collier et al, 2002; Smith et al, 2006; Kawelke et al, 2008; Olivier et al, 2008). Osteoporosis is more prevalent in women with primary biliary cirrhosis than in the age and sex-matched general population, and fracture risk in these women is greater than in other patients

with chronic liver disease (Guañabens et al, 2005; Guañabens et al, 2010).

Interventions for osteoporosis

With the increasing prevalence of patients with primary biliary cirrhosis, there will be a large number of people with a potential bone disease. Thus, it is of potential great importance to focus on early recognition of these individuals as well as define the risk of fracture in each patient in order to treat excessive bone loss and prevent osteoporotic fractures. Defining optimal treatment regiments for osteoporosis in primary biliary cirrhosis is a challenge as pathogenesis remains poorly understood. Patients with primary biliary cirrhosis are mainly elderly women who are naturally prone to osteoporosis. In general, the principles of management in postmenopausal osteoporosis also apply in primary biliary cirrhosis.

Agents shown to be useful in preventing or reducing bone loss in postmenopausal women include calcium, cyclical etidronate, alendronate, risedronate, hormone replacement, raloxifene, calcitonin, and combined vitamin D and calcium (Collier et al, 2002; Wells et al, 2008a; Wells et al, 2008b; Wells 2008c; Arteh et al, 2010). Current recommendations are that treatment of osteoporosis should be given for a minimum of five years and bone density repeated after two years and at the end of treatment (Collier et al, 2002). Bisphosphonates should be considered in all patients who have had a fragility fracture or have a T-score below - 2.5 (Collier et al, 2002). Bisphosphonates may be used with hormone replacement or without hormone replacement. Calcitriol and calcitonin should be considered in those patients with osteoporosis who are either intolerant of hormone replacement and bisphosphonates, or whose bone mineral density worsens despite the use of bisphosphonates or treatment of hypogonadism (Collier et al, 2002).

Bisphosphonates

Bisphosphonates are the most often used drugs in the treatment of

postmenopausal osteoporosis. Meta-analyses show that bisphosphonates increase bone mineral density measured by dual-energy X-ray absorptiometry and reduce fracture risk (Wasnich and Miller, 2000). Lumbar spine bone mineral density increased by 8% with bisphosphonate treatment will reduce vertebral fracture risk by 54% (Wasnich and Miller, 2000; Cummings et al, 2002; Lewiecki, 2010). Larger increases in lumbar spine and hip bone mineral density after treatment with bisphosphonates were associated with lower risk of non-vertebral fractures (Hochberg et al, 2002). Cochrane systematic reviews have demonstrated that alendronate and risedronate have statistically significant and clinically important benefit in the secondary prevention of vertebral, non-vertebral, and hip fractures in postmenopausal women (Wells et al, 2008a; Wells et al, 2008c). Reductions in wrist fractures were observed only for alendronate (Wells et al, 2008a). Benefit of etidronate in the secondary prevention of vertebral fractures was demonstrated as well (Wells et al, 2008b). No significant reductions in the primary prevention of vertebral and non-vertebral fractures were observed for alendronate and risedronate with the exception of vertebral fractures for etidronate, for which the reduction was clinically important (Wells et al, 2008a; Wells et al, 2008b; Wells et al, 2008c). Bisphosphonates have proven effective for other forms of osteoporosis (eg, associated with glucocorticoid administration) (Saag et al, 1998; Homik et al, 1999). This evidence is important since corticosteroid use is one of the risk factors associated with osteoporosis among people with primary biliary cirrhosis.

Based on current, limited data, bisphosphonates are the most rational choice for the prevention and treatment of osteoporosis in primary biliary cirrhosis, both spontaneous osteoporosis and glucocorticosteroid induced osteoporosis (Wolfhagen et al, 2000). These drugs have been studied in a small number of patients with primary biliary cirrhosis (Pares et al, 2006). In a head-to-head trial, the alendronate group showed better improvement of bone mineral density compared with the etidronate group (Guanabens et al, 2003). Accordingly, the

harms and benefits of bisphosphonates for osteoporosis are unclear. Patients with primary biliary cirrhosis have an increased risk of fractures compared to the general population (Solaymani-Dodaran et al, 2006). The correlation between vertebral fracture and a T-score below -1.5 suggests that this measurement may be useful to decide when to prescribe agents to prevent bone loss and development of new fractures in patients with primary biliary cirrhosis (Guañabens et al, 2010).

Bisphosphonates (formerly called diphosphonates) are synthetic compounds derived from pyrophosphate characterized by a P–C–P group. Bisphosphonates were synthesised in 1865 in Germany (Menschutkin, 1865). The most important step toward their clinical use is their potential in preventing the dissolution of hydroxylapatite, the principal bone mineral, thus inhibiting bone resorption (Fleisch et al, 1969). Bisphosphonates can be classified into two groups with different molecular modes of action. Non-nitrogen-containing bisphosphonates (eg, etidronate, clodronate) inhibit osteoclasts by producing toxic analogues of adenosine trisphosphate that cause cell death. Nitrogen-containing bisphosphonates (eg, pamidronate, alendronate, risedronate, ibandronate, and zoledronate) inhibit an enzyme called farnesyl pyrophosphate synthase (FPPS), a key branch-point enzyme in the mevalonate pathway. FPPS generates isoprenoid lipids used for the posttranslational modification of small GTP-binding proteins essential for osteoclast function. Inhibition of this enzyme leads to reduced resorptive activity of osteoclasts and accelerated apoptosis (Russell, 2011).

These agents are of value as treatment for various metabolic bone diseases associated with increased bone turnover, such as Paget's disease, osteoporosis, and bone tumours. Bisphosphonates are used for diagnostic purposes as skeletal markers in the form of 99mTc derivatives (Fleisch, 1991; Papapoulos et al, 1992). Bisphosphonates can be administered orally or intravenously with a wide range of doses and dosing intervals, and duration of therapy (Russell,

2006). Less than 1% of an orally administered dose of bisphosphonates is absorbed, 50% of the absorbed dose binds to bone surfaces, and the 50% or so that does not bind to bone is excreted rapidly by the kidneys.

Potential adverse effects of bisphosphonates include upper gastrointestinal disorders (eg, oesophagitis or oesophageal ulcer), influenza-like illness, renal toxicity, and osteonecrosis of the jaw (Bounameaux et al, 1983; Cryer and Bauer, 2002; Chang et al, 2003). Symptoms of influenza-like illness such as fatigue, fever, chills, myalgia, and arthralgia are transitory and mostly observed after the first exposure to nitrogen-containing bisphosphonates (Adami and Zamberlan, 1996; Reid et al, 2002). Osteonecrosis of the jaw can occur with heavy doses of intravenous bisphosphonates in patients with malignancy (Migliorati et al, 2005; Gimsing et al, 2010). Overall, the safety and tolerability of the nitrogen-containing bisphosphonates seem good, and a long-term treatment does not appear to carry a risk of serious adverse events (Strampel et al, 2007).

Hormone replacement

Oestrogen has important effects on bone. Oestrogen deficiency is considered to be a major factor leading to bone loss in postmenopausal women. The mechanism of oestrogen effect on bone is via oestrogen receptors that were identified both on osteoclasts and especially on osteoblasts (Lindsay, 1993). Oestrogen also has an indirect effect by increasing the production of insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), and transforming growth factor-ß (TGF-ß) which also stimulates bone formation (Wren, 1997). Oestrogen replacement reduces bone loss in postmenopausal osteoporosis by inhibiting bone resorption and stimulating new bone formation (Chow et al, 1992; Riggs and Melton, 1993).

Oestrogen, with or without a progesterone, has beneficial effects on surrogate markers of bone turnover and on fracture risk and has been used extensively for the prevention of osteoporosis. There is evidence that hormone replacement

increases bone mineral density in the hip, lumbar spine, and peripheral body sites (Wells et al, 2002). A meta-analysis of randomised clinical trials has shown that hormone replacement reduces the incidence of non-vertebral fractures in women, but the benefit may decrease if it is started after age of 60 years (Torgerson and Bell-Syer, 2001a). Hormone replacement was associated with significant reduction in vertebral fracture as well (Torgerson and Bell-Syer, 2001b).

Hormone replacement generally includes either oestrogen alone or oestrogen combined with progesterone or a chemical analogue, called a progestin. The addition of a progestin reduces the risk of endometrial hyperplasia associated with the use of oestrogen alone in women with a uterus (Lethaby et al, 2004). Progestogens have adverse effects on blood lipids and may cause symptoms such as headache, bloating, and breast tenderness (McKinney and Thompson, 1998). Hormone replacement is used in a variety of formulations which can be taken orally, vaginally, transnasally, as an implant, skin patch, cream, or gel. The transdermal route avoids first-pass metabolism, thus having less metabolic effects on the liver and reducing the cholestatic potential of hormone replacement. Hormone replacement administrated transdermally is potentially safer in patients with chronic liver disease (Ribot et al, 1990; Stevenson et al, 1990). Doses often vary cyclically, with oestrogens taken daily and progesterone or progestins taken for about two weeks every month or two. Clinical effects are different according to the type of hormone replacement and its duration of use.

Hormone replacement has been used worldwide to treat symptoms of menopause and to prevent chronic conditions such as osteoporosis. There is no evidence that hormone replacement could prevent cardiovascular events in postmenopausal women (with or without cardiovascular disease) (Gabriel et al, 2005). On the contrary, a Cochrane review assessing the long-term clinical effects of using hormone replacement for perimenopausal and postmenopausal women reports strong evidence that hormone replacement significantly

increases the risk of venous thromboembolism, fatal or nonfatal heart attacks (after one year's use), stroke (after three years use), breast cancer, gallbladder disease, and in women over 65 years, the risk of dementia (Farquhar et al, 2009). Prolonged use of unopposed oestrogen (that is without progesterone) may carry an increased risk for ovarian and endometrial cancer (Rodriguez et al, 2001; Lacey et al, 2002; Riman et al, 2002; U.S. PSTF 2002).

Beneficial effects of hormone replacement on bone mineral density in primary biliary cirrhosis have been reported (Olsson et al, 1999; Menon et al, 2003). There is a theoretical concern of worsening cholestasis by application of hormone replacement to patients with primary biliary cirrhosis (Schreiber and Simon, 1983). However, in a small retrospective study, hormone replacement resulted in a significant increase in bone mineral density compared to untreated patients, and there was no evidence of worsening cholestasis (Crippin et al, 1994). Furthermore, hormone replacement could also be used to treat postmenopausal symptoms in women with primary biliary cirrhosis, and such trials might have examined the effects of hormone replacement on the bone.

OBJECTIVES

The objective of this PhD thesis was to summarize the evidence from Cochrane systematic reviews on treatment options for patients with primary biliary cirrhosis and osteoporosis associated with primary biliary cirrhosis.

MATERIAL AND METHODS

Cochrane Reviews are systematic reviews of primary research in human health care and health policy, and are internationally recognized as the highest standard in evidence-based health care. They investigate the effects of interventions for prevention and treatment. A Cochrane Review is a scientific investigation in itself, with a pre-planned methods section and an assembly of original studies (predominantly randomised controlled trials and clinical controlled trials) as their 'subjects'. The results of these multiple primary investigations are synthesized by using strategies that limit bias and random error. These strategies include a comprehensive search of all potentially relevant studies and the use of explicit, reproducible criteria in the selection of studies for review. Primary research designs and study characteristics are appraised, data synthesized, and results interpreted.

Criteria for considering reviews for inclusion

Only Cochrane systematic reviews were considered for inclusion in this thesis. We performed four Cochrane systematic reviews of all relevant randomised clinical trials with meta-analyses and trial sequential analyses using The Cochrane Collaboration methodology. Two systematic reviews assessed the effects of ursodeoxycholic acid and bezafibrate in patients with primary biliary cirrhosis, and the other two systematic reviews assessed the effects of bisphosphonates and hormone replacement for osteoporosis in patients with primary biliary cirrhosis. Three out of four systematic reviews were performed according to published protocols following the recommendations of the

Cochrane Handbook for systematic reviews of interventions, and the review assessing the effects of ursodeoxycholic acid in patients with primary biliary cirrhosis was updated according to the same recommendations.

Types of participants

Eligible participants were patients with primary biliary cirrhosis, i.e., patients having at least two of the following: elevated serum activity of alkaline phosphatases, a positive antimitochondrial antibody, and liver biopsy compatible with primary biliary cirrhosis (EASL, 2009; Silveira et al, 2010).
Eligible participants were participants with primary biliary cirrhosis who received bisphosphonates as primary and secondary prevention, and postmenopausal women with primary biliary cirrhosis who received hormone replacement as primary and secondary prevention. A trial was considered as primary prevention if it included patients that had an average T-score of -1.0 or above, or if the prevalence of vertebral fracture at baseline was less than 20%. A trial was considered as secondary prevention if the inclusion criteria were restricted to patients with T-score between -1 and -2.5 or below -2.5, or to patients who had experienced previous fractures. Participants who were liver-transplanted patients were excluded.

Types of interventions

<u>Interventions for primary biliary cirrhosis</u>

Ursodeoxycholic acid administered perorally at any dose versus placebo or no intervention. Bezafibrate administered at any dose or regimen versus placebo or no intervention, or any other drug that is being used for treatment of primary biliary cirrhosis, eg, ursodeoxycholic acid, colchicine, glucocorticoids, azathioprine, d-penicillamine, cyclosporine A, methotrexate, or any other drug that is being compared.

Interventions for osteoporosis in primary biliary cirrhosis

Bisphosphonates administered orally, such as alendronate, etidronate, or any other bisphosphonate that could be identified versus placebo or no intervention, or another bisphosphonate, or any other drug.
Any hormone replacement therapy administered by any route, or regimen, or dose versus placebo or no intervention.

Types of outcomes measures

Ursodeoxycholic acid

Primary outcomes

1. All-cause mortality
2. All-cause mortality or liver transplantation
3. Adverse events: serious adverse events are defined as any untoward medical occurrence that was life threatening, resulted in death, or was persistent or led to significant disability; or any medical event, which had jeopardized the patient or required intervention to prevent it (ICH-GCP, 1997). All other adverse events (that is, any medical occurrence not necessarily having a causal relationship with the treatment) will be considered as non-serious
4. Quality of life

Secondary outcomes

1. Liver transplantation
2. Pruritus: number of patients with pruritus or pruritus score
3. Fatigue: number of patients with fatigue
4. Liver-related morbidity (number of patients who developed jaundice, portal hypertension, oesophageal varices, gastric varices, upper gastrointestinal haemorrhage, ascites, hepatic encephalopathy, hepato-

renal syndrome)

5. Biochemical markers: serum bilirubin, serum alkaline phosphatases, serum gamma-glutamyltransferase, serum aspartate aminotransferase, serum alanine aminotransferase, serum albumin, total cholesterol, plasma immunoglobulins, prothrombin index
6. Liver biopsy findings: worsening of liver histological stage or score
7. Cost-effectiveness: the estimated costs connected with the interventions were weighed against any possible health gains.

Bezafibrate

Primary outcomes

1. All-cause mortality
2. Liver-related morbidity
3. Adverse events
4. Quality of life

Secondary outcomes

1. Pruritus
2. Fatigue
3. Biochemical markers: serum alkaline phosphatases, serum gamma-glutamyltransferase, serum aspartate aminotransferase, serum alanine aminotransferase, plasma immunoglobulin M, total cholesterol, triglyceride, platelet count, and serum bilirubin
4. Liver biopsy findings (histological stage)
5. Number of patients having bezafibrate withdrawn due to adverse events

Bisphosphonates or hormone replacement

Primary outcomes

1. All-cause mortality

2. Fractures (number of participants with new fractures and number of fractures at all sites)
3. Adverse advents
4. Quality of life

Secondary outcomes

1. Bone mineral density measured by dual-energy X-ray absorptiometry (DXA) at the following sites: lumbar spine; proximal femur – hip; radius; and total body
2. Liver-related mortality or liver transplantation
3. Liver-related morbidity
4. Biochemical indices (serum bilirubin, serum alkaline phosphatases, serum alanine aminotransferase, serum aspartate aminotransferase, and albumin) for hormone replacement
5. Biochemical markers of bone turnover (serum osteocalcin and the procollagen type I N-terminal propeptide (PINP) - as indices of bone formation, and urinary hydroxyproline, the amino (NTx), and ß-carboxyterminal (CTx) telopeptides of collagen I - as indices of bone resorption) for bisphosphonates and hormone replacement; and serum alkaline phosphatases; 25-hydroxyvitamin D; and parathyroid hormone (PTH) for bisphosphonates
6. Number of patients having bisphosphonate or hormone replacement withdrawn due to adverse events

Search methods for identification of reviews

Included reviews were published in The Cochrane Library; there was no additional searching.

Data collection and analysis

Selection of reviews

Cochrane systematic reviews addressing treatment of primary biliary cirrhosis and osteoporosis associated with primary biliary cirrhosis were conducted by the same authors and confirmed for inclusion in this analyses. Any disagreement was resolved by discussion with a mentor and co-mentor.

Data extraction and management

One review author (JR) collated results from the four reviews, and another checked them (MK). The following information was extracted from included Cochrane systematic reviews: review objective, search methods for identification of studies, inclusion criteria (study design, participants, intervention, comparator and outcomes), source of funding, and stated conflicts of interest of review authors. From each trial the following information was extracted: first author, country of origin, trial design (parallel or cross-over), inclusion and exclusion criteria, number of patients randomized, characteristics of patients: age range (mean or median) and sex ratio, dose of interventions, duration, frequency and mode of administration, type and dose of additional interventions, and outcomes at the end of treatment. Two review authors (JR and GP) extracted data independently using data extraction forms that were developed for the purpose. If more than one publication of a trial existed, we listed the publications under the publication with the most complete data and marked it as primary. If information was not available in the published trial, in order to obtain missing data and assess the trials correctly, we contacted authors of the trial publications. We added information obtained through correspondence with these authors to the data extraction form. In the 'Notes' section of the respective trial ('Table of included studies'), we provided the date when the information was requested and received. Disagreements were resolved by discussion among the review authors.

Assessment of methodological quality of included reviews

Quality of evidence from primary studies in included reviews

Assessment of risk of bias in primary studies

The confidence that the design and the report of the randomised clinical trial would restrict bias in the comparison of the intervention defines methodological quality, and hence risk of bias, which we assessed using the following domains (Schulz et al, 1995; Moher et al, 1998; Kjaergard et al, 2001; Gluud, 2006; Wood et al, 2008).

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 trials, 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 harms.

Allocation concealment

-Low risk of bias: allocation was controlled by a central and inde-pendent 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 trials will be excluded for the assessment of benefits but not for harms.

Blinding

-Low risk of bias: the trial was described as blinded, the parties that were blinded, and the method of blinding was described, so that knowledge of allocation was adequately prevented during the trial.

-Uncertain risk of bias: the trial was described as blind, but the method of blinding was not described, so that knowledge of allocation was possible during the trial.

-High risk of bias, the trial was not blinded, so that the allocation was known during the trial.

Incomplete outcome data

-Low risk of bias: the numbers and reasons for dropouts and withdrawals in all intervention groups were described or if it was specified that there were no dropouts or withdrawals.

-Uncertain risk of bias: the report gave the impression that there had been no dropouts or withdrawals, but this was not specifically stated.

-High risk of bias: the number or reasons for dropouts and withdrawals were not described.

Selective outcome reporting

- Low risk of bias: pre-defined, or clinically relevant and reasonably expected outcomes are reported on.

-Uncertain risk of bias: not all pre-defined, or clinically relevant and reasonably expected outcomes are reported on or are not re-ported fully, or it is 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 bias

-Low risk of bias: the trial appears to be free of other domains that could put it at risk of bias.

-Uncertain risk of bias: the trial may or may not be free of other domains that could put it at risk of bias.

-High risk of bias: there are other factors in the trial that could put it at risk of bias, eg, for-profit involvement, authors have conducted trials on the same topic etc.

Trials assessed as having 'low risk of bias' in all of the specified individual domains were considered 'trials with low risk of bias'. Trials assessed as having 'uncertain risk of bias' or 'high risk of bias' in one or more of the specified individual domains were considered trials with 'high risk of bias' (Gluud et al, 2011).

We used the GRADE Pro 'Summary of findings' tables from each review to indicate the quality of the evidence for the main comparisons. The following criteria were taken into account: study limitations (that is risk of bias), consistency of effect, imprecision, indirectness and publication bias.

Dealing with missing data and assessment of heterogeneity in included reviews

We performed analyses according to the intention-to-treat method only for dichotomous outcomes. For continuous outcomes we performed available patient analysis and included data only on those whose results were known. Regarding the primary outcome measures, we included patients with incomplete or missing data in sensitivity analyses, by imputing the missing data

following the scenarios below in case of available data (Hollis and Campbell, 1999; Gluud et al, 2011).

-Available patient analysis which simply excludes all patients with the missing outcome from the analysis.

-Extreme-case analysis favoring the experimental intervention ('best-worse' case scenario): none of the dropouts/patients lost from the experimental arm but all of the dropouts/patients lost

We explored the presence of statistical heterogeneity by the chi-squared test with significance less than or equal to P 0.10 and measured the quantity of heterogeneity by I^2 (Higgins et al, 2003). When data were available from one trial only, we used Fisher's exact test (Fisher, 1922) for dichotomous data and Student's t-test (Student, 1908) for continuous data.

Between-trial heterogeneity was explored by meta-regression with STATA 8.2 (STATA Corp, College Station, Tex), depending on the available data. The covariates were: risk of bias of the trials, disease severity of patients at entry, intervention dosage, and trial duration (treatment and follow-up). Univariate and multivariate analyses including all covariates were performed. The results are presented with regression coefficients and 95% CI.

Data synthesis

We combined the reviews in a narrative summary, organised by interventions. There was no pooling of data beyond what was reported in the individual reviews. We performed all included reviews in the thesis according to the recommendations of The Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011) and the Cochrane Hepato-Biliary Group Module (Gluud et al, 2011). For the statistical analyses, we used Review Manager 5.1 (RevMan 2011). We meta-analysed the data with both a random-effects model (DerSimonian and Laird, 1986) and a fixed-effect model (DeMets,

1987) to ensure robustness of the results. In case of significant differences of the results that the two models produced, we presented the result with both methods. We presented the results with the fixed-effect model if the results of the two models did not differ (Higgins and Thompson, 2002).

Data synthesis from primary studies in included reviews

No de novo data analysis of trial level outcomes was conducted for this thesis. For each included review, we extracted all results for the outcomes listed above, and where outcomes were meta-analysed, we have reported pooled effect sizes. Where no quantitative pooling of effect sizes has been reported, or where outcomes are reported descriptively by single studies, we have reported these results by using statistical significance. Dichotomous data were expressed as relative risk (RR) and/or risk difference (RD) with 95% confidence intervals (CI). When continuous scales of measurement were used to assess the treatment effects, we used the mean difference (MD) (Thompson and Higgins, 2002). Mean differences based on changes from baseline can usually be assumed to be addressing exactly the same underlying intervention effects as analyses based on final measurements (Higgins and Green, 2011). Therefore, we combined data reported as change from baseline values with final measurement values in meta-analysis when using the mean difference method in RevMan (RevMan 2011). We did not use standardised mean differences (SMD) when we combined change scores and final measurements. For trials addressing the same outcome but using different scales of measuring, SMD were used.

Trial sequential analysis

In order to control for the risks of random errors due to sparse data and multiplicity, we performed trial sequential analysis (Brok et al, 2008; Wetterslev et al, 2008; Thorlund et al, 2009). We calculated the required information size (ie, the number of participants needed in a meta-analysis to detect or reject a certain intervention effect) (Wetterslev et al, 2008). In our analysis, the required

information size was based on the minimal relevant difference of a half standard deviation of the meta-analysis, the variance of the meta-analysis, a type I error of 5%, and a type II error of 20% (Wetterslev et al, 2008). As default, diversity-adjusted required information size was used unless otherwise stated (Wetterslev et al, 2008; Wetterslev et al, 2009). The underlying assumption of trial sequential analysis is that testing for significance may be performed each time a new trial is added to the meta-analysis. We added the trials according to the year of publication, and if more than one trial was published in a year, trials were added alphabetically according to the last name of the first author (Wetterslev et al, 2008).

On the basis of the required information size, trial sequential monitoring boundaries were constructed (Wetterslev et al, 2008). These boundaries determine the statistical inference one may draw regarding the cumulative meta-analysis that has not reached the required information size; if the trial sequential monitoring boundary is crossed before the required information size is reached, firm evidence may be established and further trials may turn out to be superfluous. On the other hand, if the boundary is not surpassed, it is most probably necessary to continue doing trials in order to detect or reject a certain intervention effect.

Results

Ursodeoxycholic acid (Paper I)

Results of the search

Our search strategy identified 1365 publications, out of which 637 were duplicates. Of the remaining 728 publications, 623 were excluded because they were reviews, because they did not relate to primary biliary cirrhosis, or because they did not describe a randomised clinical trial investigating the effect of ursodeoxycholic acid in patients with primary biliary cirrhosis. The remaining 105 publications referred to 16 randomised clinical trials (Image 1).

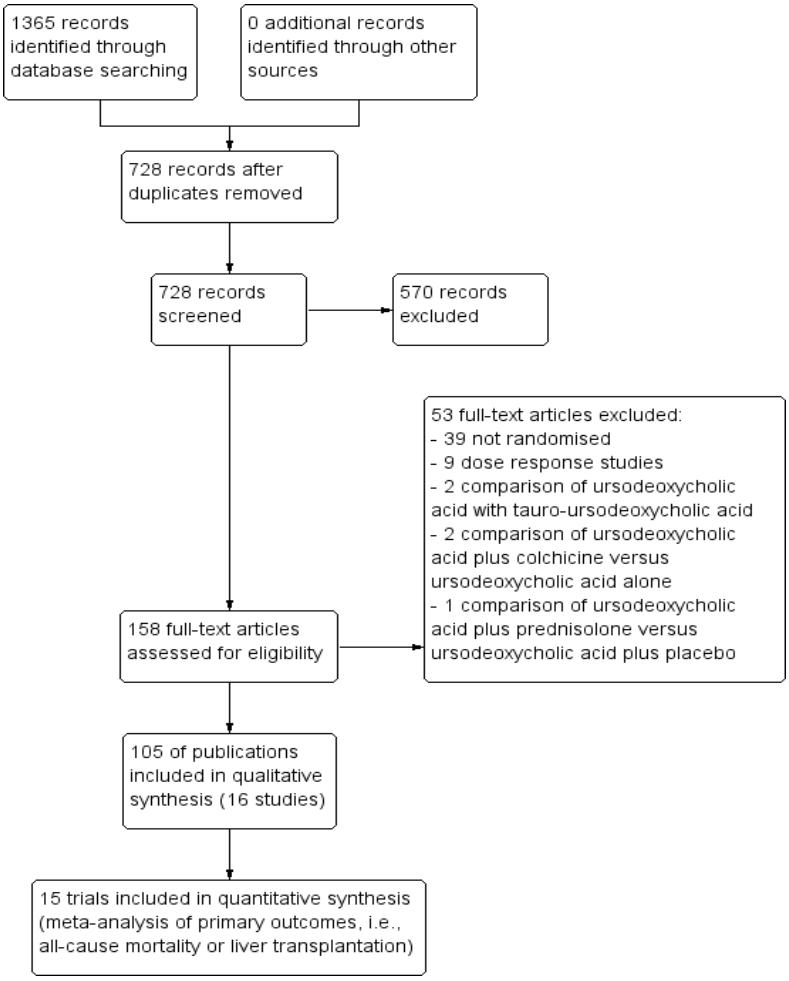

Image 1. Flow chart

Fourteen of the included trials consisted of more than one publication. Two out of the 16 randomised clinical trials were published as abstracts only (De la Mora et al, 1994; Goddard et al, 1994), and the De la Mora 1994 trial provided no extractable data on the trial's characteristics and outcomes. Most of the primary authors and manufacturers of the ursodeoxycholic acid were contacted for further information and data relating to the trials while conducting the previous up-date of this review. Dr. Albert Pares kindly provided data on the method of sequence generation. Through a search for ongoing trials in Clinicaltrials.gov (http://clinicaltrials.gov/) and WHO International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/) we have not identified any registered ongoing or planned trials.

Included studies

A total of 1476 patients with primary biliary cirrhosis were randomised in the 16 randomised clinical trials. Ursodeoxycholic acid dose varied from 7.7 to 15.0 mg/kg/day with a median of 10 mg/kg/day. The duration of the trials varied from 3 to 92 months with a median of 24 months. The percentage of symptomatic patients and patients with advanced primary biliary cirrhosis at baseline varied from 15% to 83% with a median of 51%. The details are displayed in Table 1. From the publications which reported sex of the patients, more than 89.5% were females. Three trials were conducted in United States (Senior and O'Brian, 1991; Lindor et al, 1994; Combes et al, 1995) and two trials were conducted in United Kingdom (Goddard et al, 1994; Turner et al, 1994). Other trials were conducted each in different countries: Italy, Mexico, Sweden, Canada, China, Germany, Japan, Greece, Spain, France, and Finland (Tables of included studies). Fiftheen trials had the parallel group design and one trial had the cross-over group design (Hwang et al, 1993).

Following the stipulated follow-up in the ursodeoxycholic acid-group and the placebo-group, six trials (Poupon et al, 1991; Battezzati et al, 1993; Heathcote et

al, 1994; Lindor et al, 1994; Combes et al, 1995; Eriksson et al, 1997) continued ursodeoxycholic acid treated patients on open label ursodeoxycholic acid (ursodeoxycholic acid→ursodeoxycholic acid) and offered open label ursodeoxycholic acid to the patients originally given placebo (placebo→ursodeoxycholic acid). The Papatheodoridis 2002 trial continued to administer ursodeoxycholic acid to all patients randomised to the ursodeoxycholic acid arm and switched 14/43 'no intervention' patients to ursodeoxycholic acid after they had been followed for a mean duration of 3.5 years. It was not possible to separate the data of the original period (ursodeoxycholic acid versus no intervention) from the total period (ursodeoxycholic acid→ursodeoxycholic acid versus no intervention→ursodeoxycholic acid), as only data from the total period were given.

Table 1 Tables of the included trials

Trial	Risk of bias	Ursodeoxycholic acid dose*	Trial duration (months)	Severity of PBC#¤
Papatheodoridis 2002	High	13.5	92.4	0.6400
Pares 2000	Low	15.0	40.8	0.2708
Combes 1995	High	11.0	24.0	0.6689
Leuschner 1989	High	10.0	9.0	0.1500
Eriksson 1997	High	7.7	24.0	0.3350
Vuoristo 1995	High	13.5	24.0	0.3333
Goddard 1994	High	10.0	15.0	0.3200
Lindor 1994	Low	14.0	48.0	0.6833
Battezzati 1993	Low	8.7	12.0	0.4950

Senior 1991	High	10.0	6.0	0.6666
Turner 1994	Low	10.0	24.0	0.8261
Hwang 1993	High	9.2	3.0	0.5833
Oka 1990	High	9.2	6.0	0.3795
Heathcote 1994	Low	14.0	24.0	0.5270
Poupon 1991	High	14.0	24.0	0.4658

* ursodeoxycholic acid dose in mg/kg/day.

PBC= primary biliary cirrhosis.

¤ proportion of patients with stage III or IV at entry;

or proportion of symptomatic patients at entry.

Excluded studies

The excluded studies are listed under 'Tables of excluded studies' and the reasons for exclusion are given there.

Risk of bias in included studies

Risk of bias was assessed according to six domains: allocation sequence generation; allocation concealment; blinding; handling of incomplete outcome data; selective outcome reporting; and other potential sources of bias. One out of 16 trials was considered as having low risk of bias (Lindor et al, 1994). Our statistical analyses are, therefore, based mainly on trials with high risk of bias. For details of the judgements made for the individual trials, please see Image 2 and Image 3.

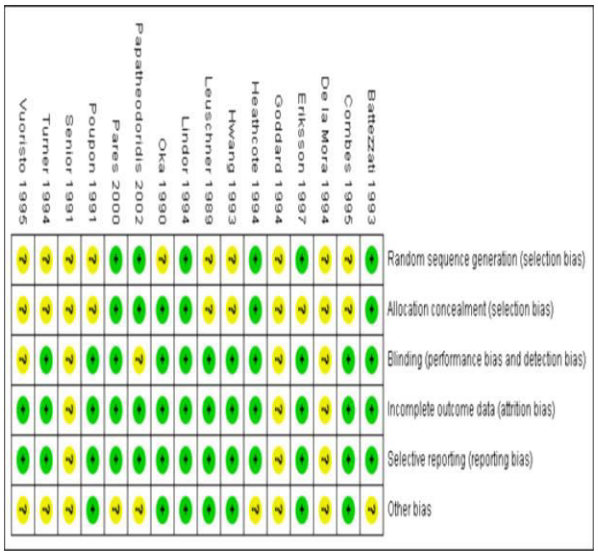

Image 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included trial

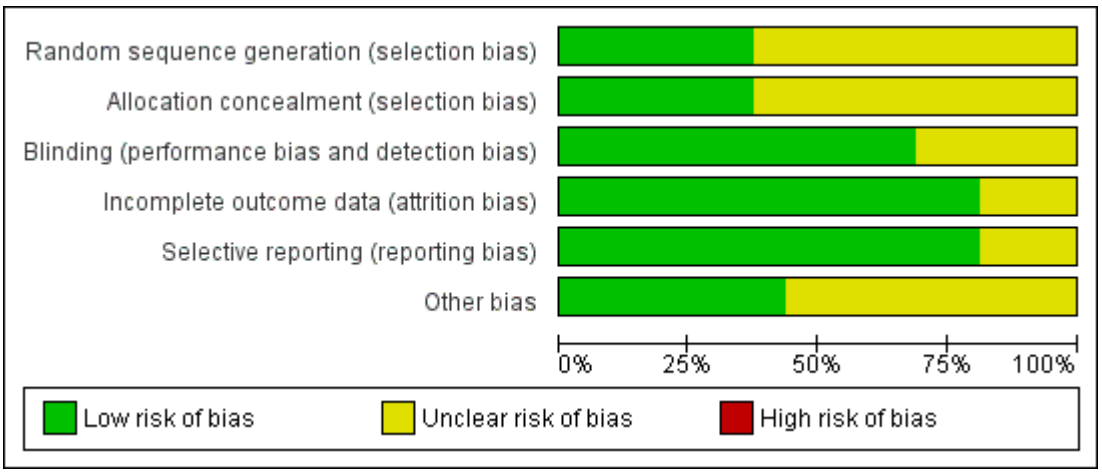

Image 3. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

Allocation

The generation of the allocation sequence was adequately described in six trials (Battezzati et al, 1993; Heathcote et al, 1994; Lindor et al, 1994; Eriksson et al, 1997; Pares et al, 2000; Papatheodoridis et al, 2002). The remaining ten trials

were described as randomised, but the method for sequence generation was not described (Leuschner et al, 1989; Oka et al, 1990; Poupon et al, 1991; Senior and O'Brien, 1991; Hwang et al, 1993; De la Mora et al, 1994; Goddard et al, 1994; Turner et al, 1994; Combes et al, 1995; Vuoristo et al, 1995).

The method used to conceal allocation was adequately described in six trials (Oka et al, 1990; Battezzati et al, 1993; Heathcote et al, 1994; Lindor et al, 1994; Pares et al, 2000; Papatheodoridis et al, 2002). The method for allocation concealment was judged as unclear in 10 trials (Leuschner et al, 1989; Oka et al, 1990; Poupon et al, 1991; Heathcote et al, 1994; Lindor et al, 1994; Turner et al, 1994; Vuoristo et al, 1995; Eriksson et al, 1997; Pares et al, 2000; Papatheodoridis et al, 2002).

Blinding

The method of blinding was adequately described in 11 trials (Leuschner et al, 1989; Oka et al, 1990; Poupon et al, 1991; Battezzati et al, 1993; Hwang et al, 1993; Heathcote et al, 1994; Lindor et al, 1994; Turner et al, 1994; Combes et al, 1995; Eriksson et al, 1997; Pares et al, 2000). The method of blinding was unclear or not used in five trials (Senior and O'Brian, 1991; De la Mora et al, 1994; Goddard et al, 1994; Vuoristo et al, 1995; Papatheodoridis et al, 2002).

Incomplete outcome data

Incomplete data were addressed adequately in the included trials except for three trials (Senior and O'Brian, 1991; De la Mora et al, 1994; Goddard et al, 1994).

Selective reporting

Predefined primary and secondary outcomes were adequately assessed in all included trials except three (Senior and O'Brian, 1991; De la Mora et al, 1994; Goddard et al, 1994). Whenever less than 16 trials reported on an outcome,

there was risk of outcome reporting bias as we had no access to any of the trial protocols.

Other potential sources of bias

Following the information provided in the trial publication, one trial may be free of other causes of bias (Lindor et al, 1994).

Effects of interventions

Primary outcomes

All-cause mortality

Fourteen trials provided information on all-cause mortality and could be included in the analyses. The included trials reported a total of 91 (6.5%) deaths in 1391 patients (Image 4). In the ursodeoxycholic acid group, 45 (6.4%) out of 699 patients died versus 46 (6.6%) out of 692 patients in the control group. Meta-analyses with both the fixed-effect model and random-effects model showed that ursodeoxycholic acid had no effect on all-cause mortality (RR 0.97; 95% CI 0.67 to 1.42, $I^2 = 0\%$) (Image 4).

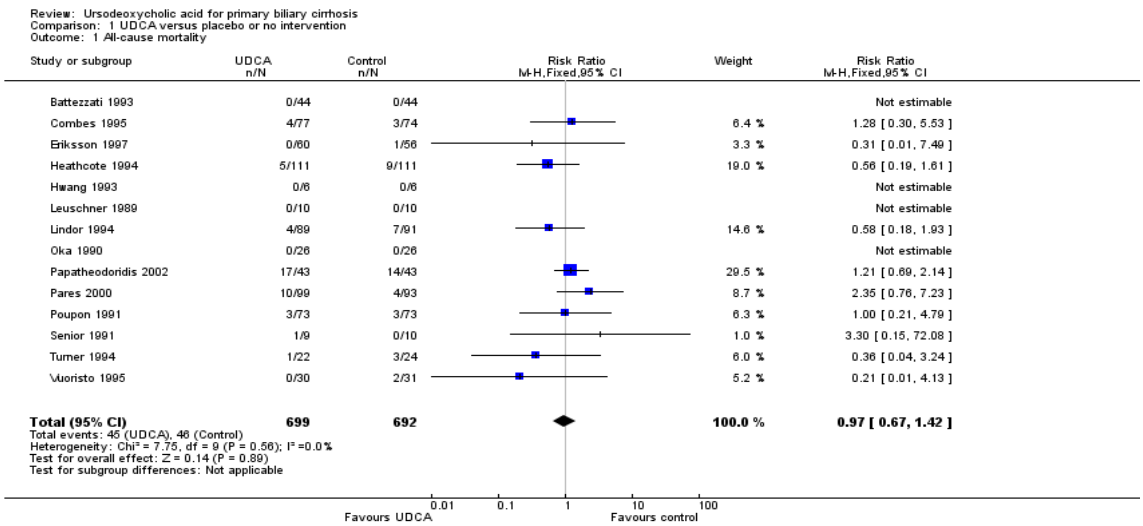

Image 4: UDCA vs placebo/no intervention; outcome: all-cause mortality

Inspection of the funnel plot did not indicate bias (Image 5).

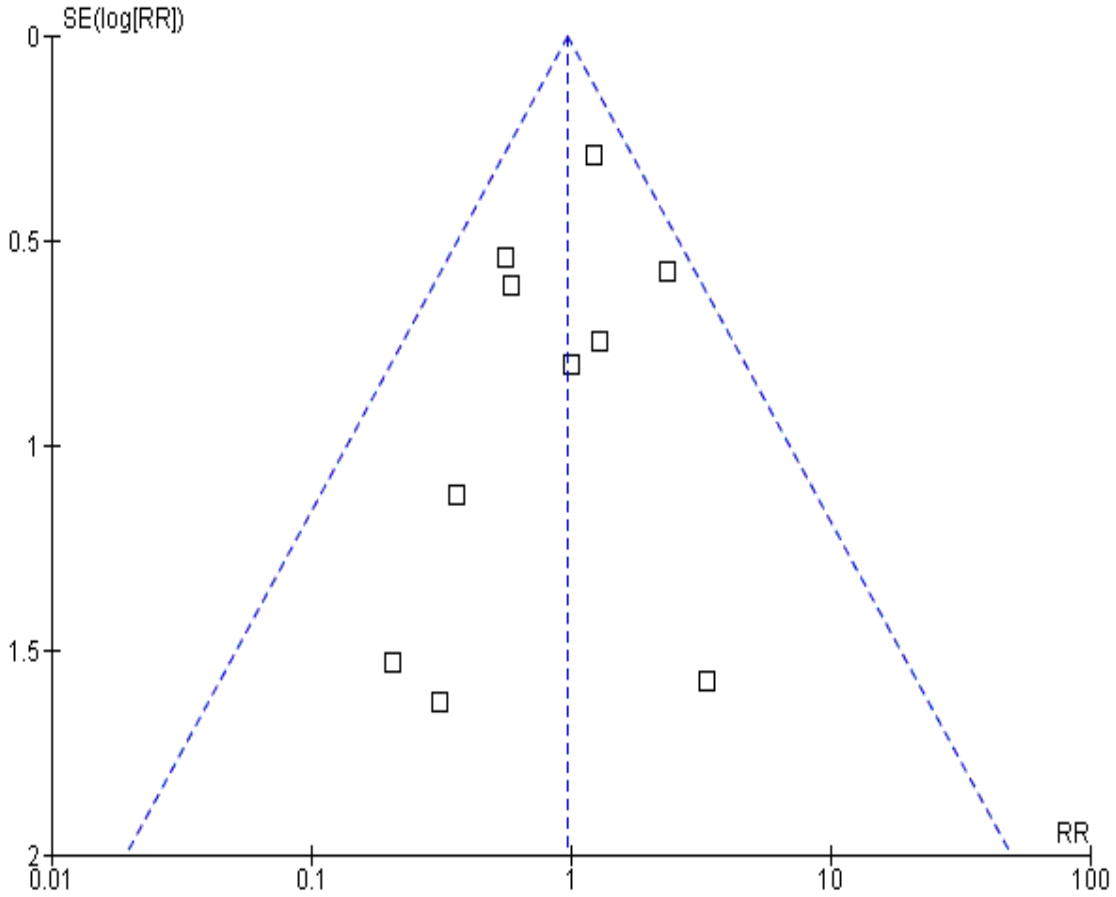

Image 5. Funnel plot of comparison: ursodeoxycholic acid versus placebo or no intervention, outcome: All-cause mortality

The subgroup analyses stratifying the trials according to risk of bias, risk of bias including industry involvement, trial duration, and dose of ursodeoxycholic acid did not reveal any differences in effect on all-cause mortality (Image 6, 7, 8, 9). Heterogeneity was absent ($I^2 = 0\%$, $P = 0.56$).

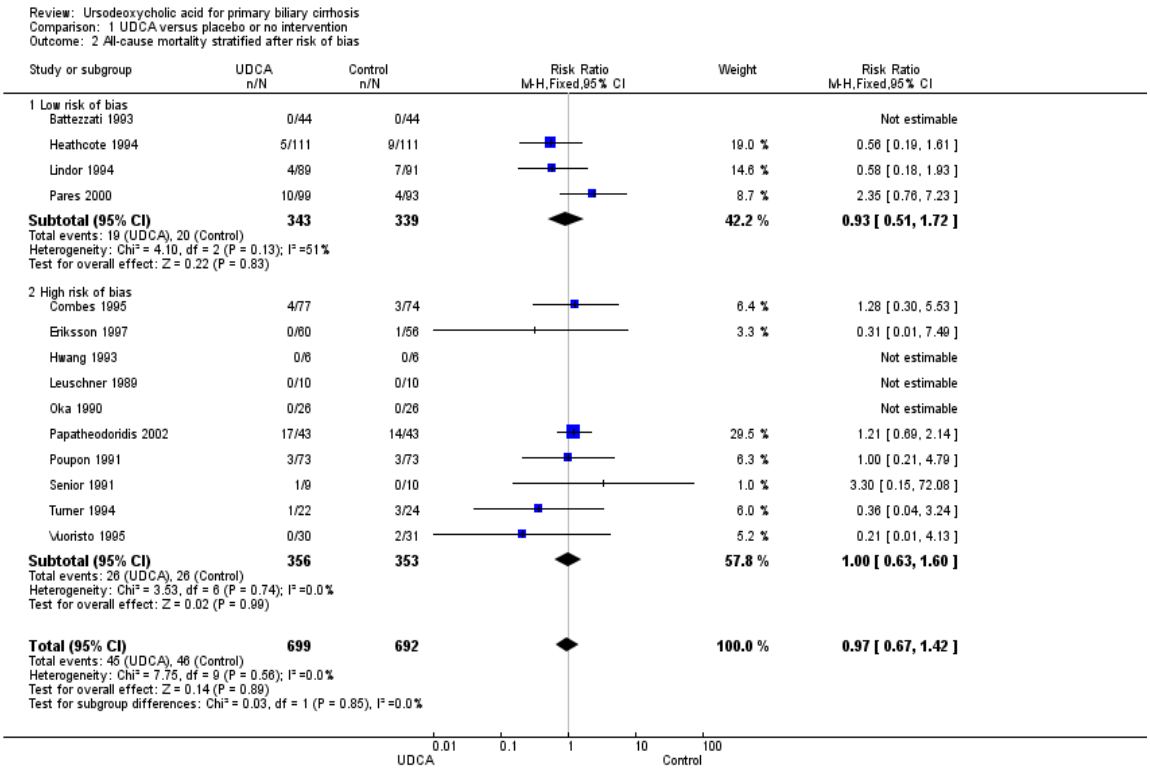

Image 6: UDCA vs placebo/no intervention; outcome: all-cause mortality stratified after risk of bias

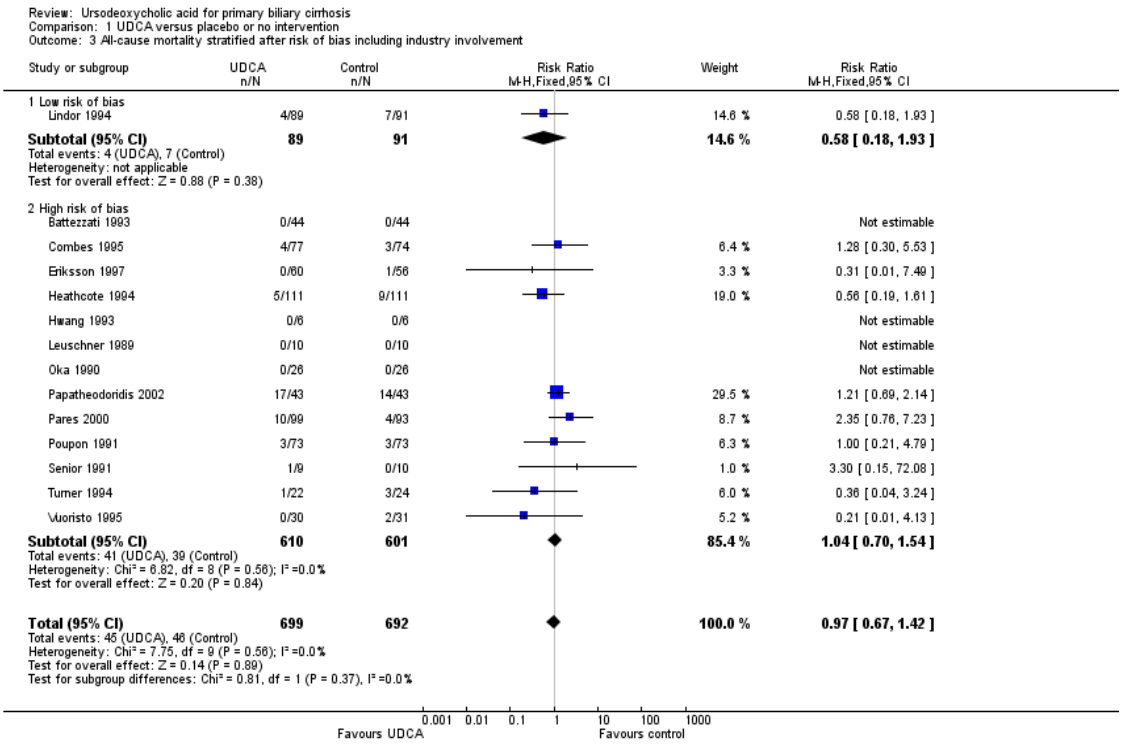

Image 7: UDCA vs placebo/no intervention; outcome: all-cause mortality stratified after risk of bias including industry involvment

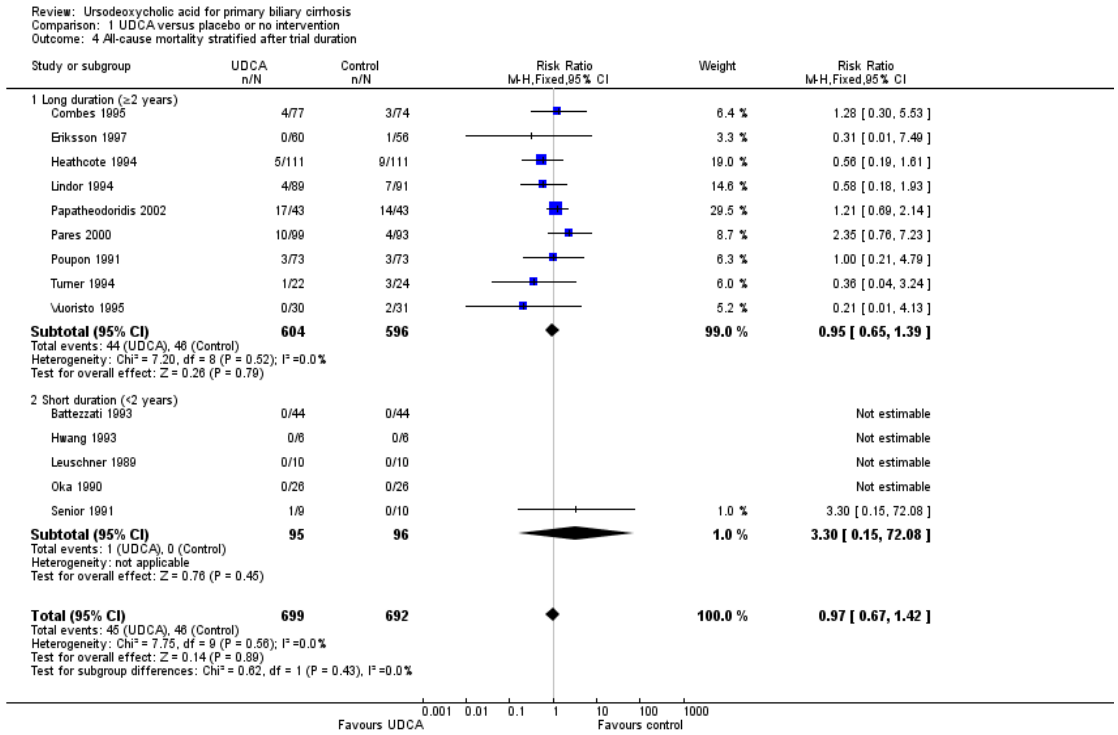

Image 8: UDCA vs placebo/no intervention; outcome: all-cause mortality stratified after trial duration

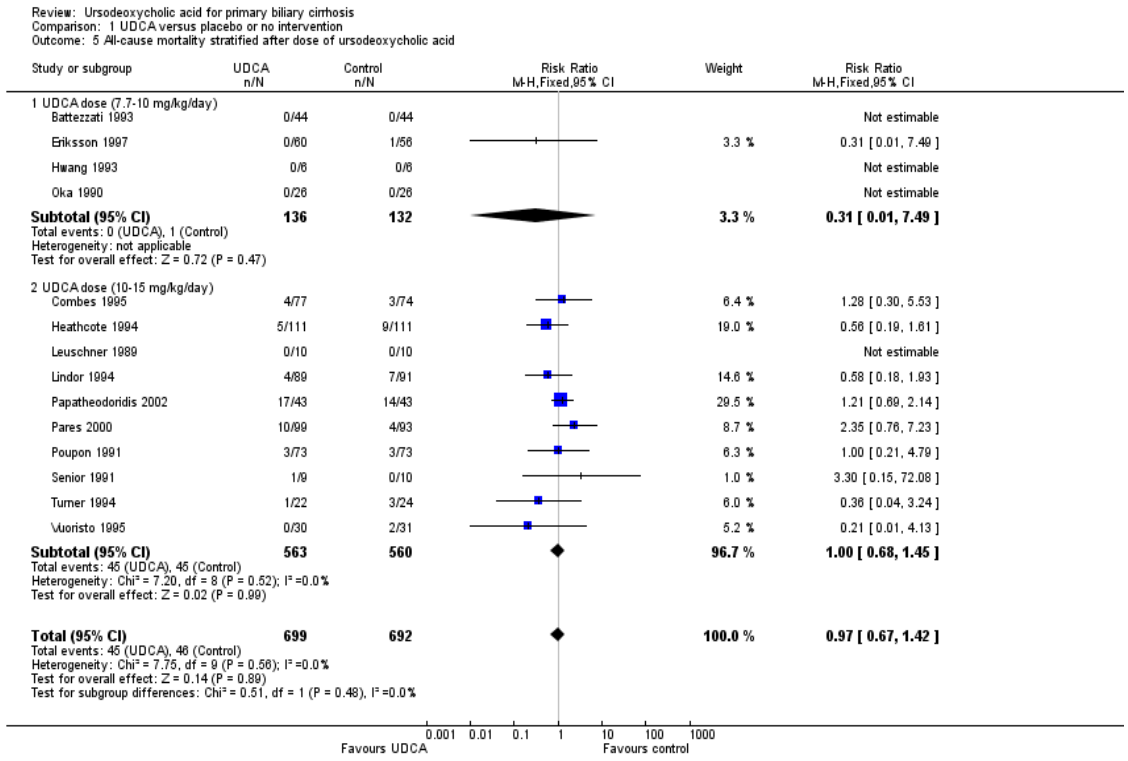

Image 9: UDCA vs placebo/no intervention; outcome: all-cause mortality stratified after dose of ursodeoxycholic acid

Trial sequential analysis with data from all included trials showed that only 1382 patients of the diversity-adjusted required information size of 8539 were accrued (16%) and no firm evidence for benefit or harm was reached (Image 10). The cumulative Z-curve did not cross the red trial sequential alpha-spending monitoring boundaries for benefit or harm. Therefore, there is no evidence to support or reject that ursodeoxycholic acid influences mortality.

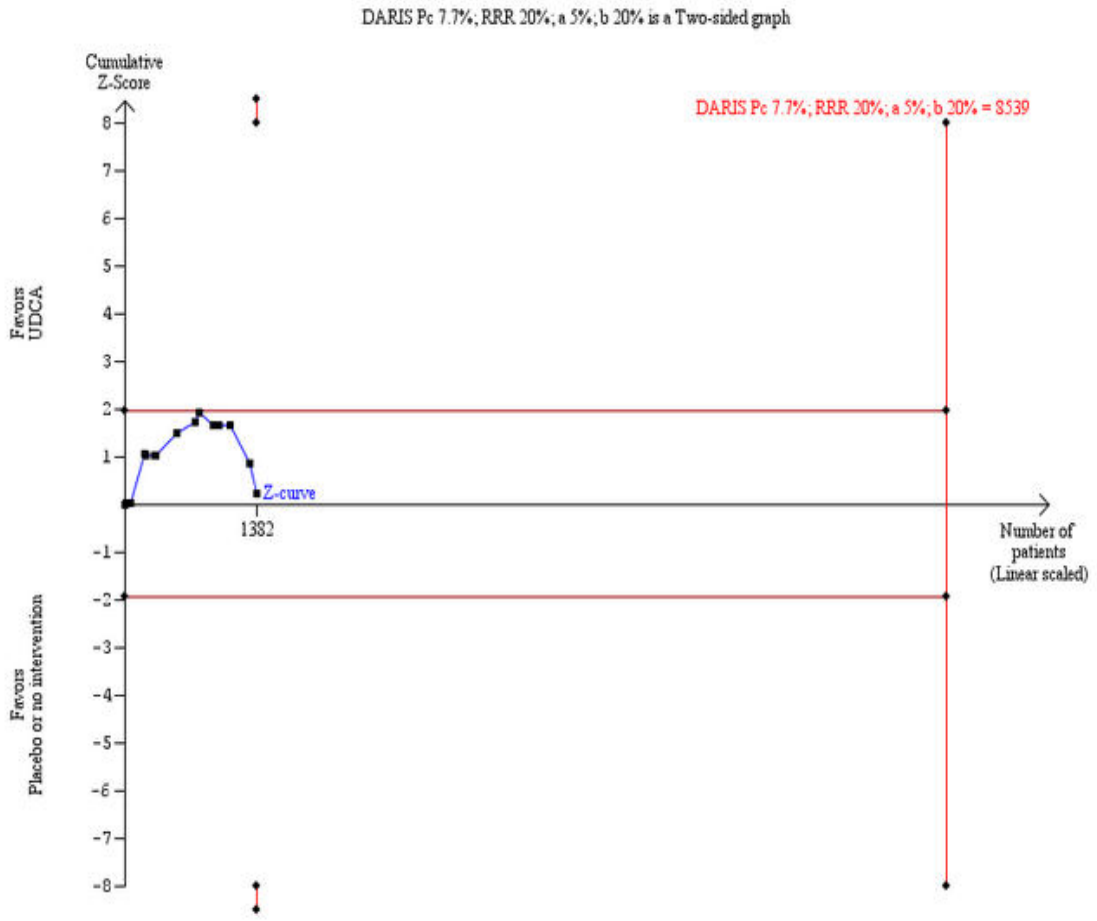

Image 10. Trial sequential analysis of the random-effects meta-analysis of the effect of ursodeoxycholic acid versus placebo or no intervention on all-cause mortality. The trial sequential analysis is performed with an assumed control proportion of death of 7.7%, an anticipated relative risk reduction (RRR) of 20%, a type 1 error risk of 5% (two-sided) (a), and a power of 80% (a type II error risk of 20%) (b). The diversity-adjusted required information size (DARIS) to detect or reject a RRR of 20% with a between trial heterogeneity of 0% is estimated to

8539 patients. The actually accrued number of patients is 1382, which is only 16% of the required information size. The blue cumulative Z-curve does not cross the red trial sequential alpha-spending monitoring boundaries for benefit or harm. Therefore, there is no evidence to support or refute that ursodeoxycholic acid influences mortality with a 20% RRR of mortality. The cumulative Z curve does not reach the futility area delineated by the trial sequential beta-spending monitoring boundaries (which are not even drawn by the program), demonstrating that further randomised trials are needed.

Sensitivity analyses to assess intervention effects of 40% or 30% relative risk reduction of mortality showed that we could exclude a very large intervention effect of 40% relative risk reduction of deaths (Image 11). However, we were unable to prove or disprove a relative risk reduction of 30% (Image 12), and below (data not shown). For such smaller intervention effects, the number of trial patients has to be increased substantially.

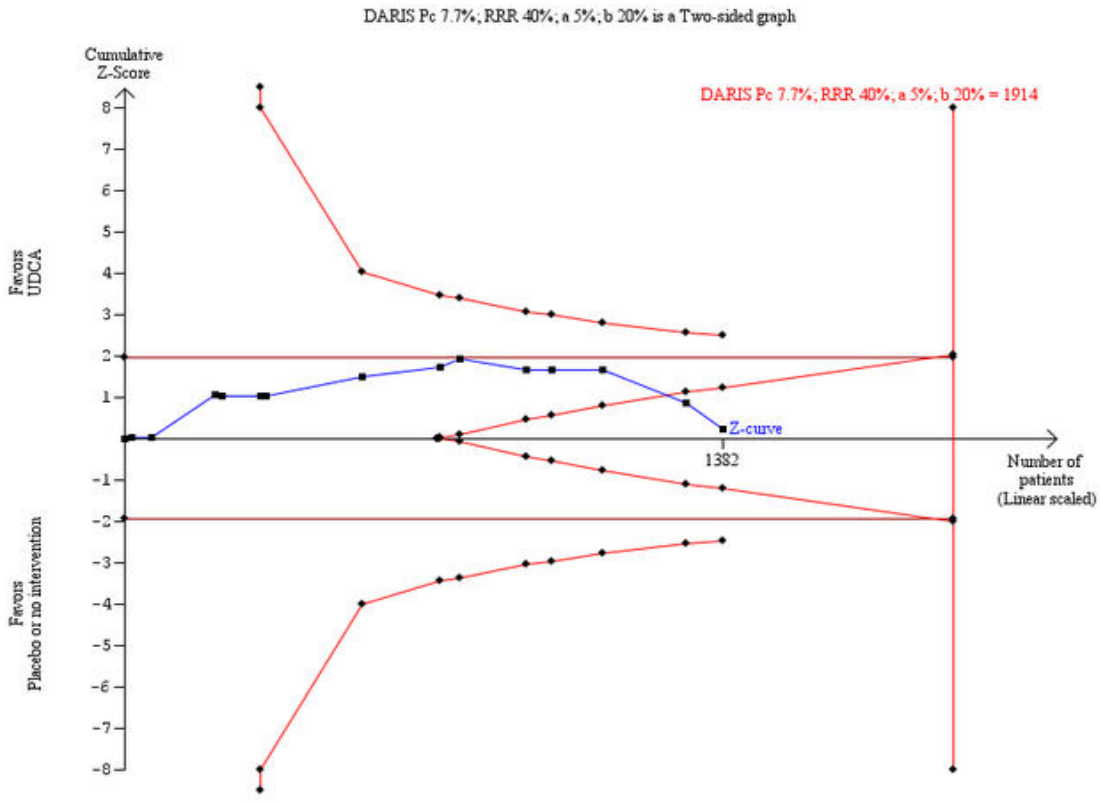

Image 11. Trial sequential analysis of the random-effects meta-analysis of the effect of ursodeoxycholic acid versus placebo or no intervention on all-cause mortality. The trial sequential analysis is performed with an assumed control proportion of death of 7.7%, an anticipated relative risk reduction (RRR) of 40%, a type 1 error risk of 5% (two-sided) (a), and a power of 80% (type 2 error risk of 20%) (b). The diversity-adjusted required information size to detect or reject a RRR of 40% with a between trial heterogeneity of 0% is estimated to 1914 patients. The actually accrued number of patients is 1382, which is 72% of the required information size. The blue cumulative Z-curve does not cross the red trial sequential alpha-spending monitoring boundaries for benefit or harm. However, the boundaries for futility (the red inner wedge boundaries showing the trial sequential beta-spending monitoring boundaries) are crossed. The red conventional boundaries (horizontal line at Z = 1.96 and Z = -1.96) for harm or benefit are not crossed. Therefore, there is no evidence to support ursodeoxycholic acid and we can refute that ursodeoxycholic acid influences mortality by a 40% RRR of mortality with the chosen error risks.

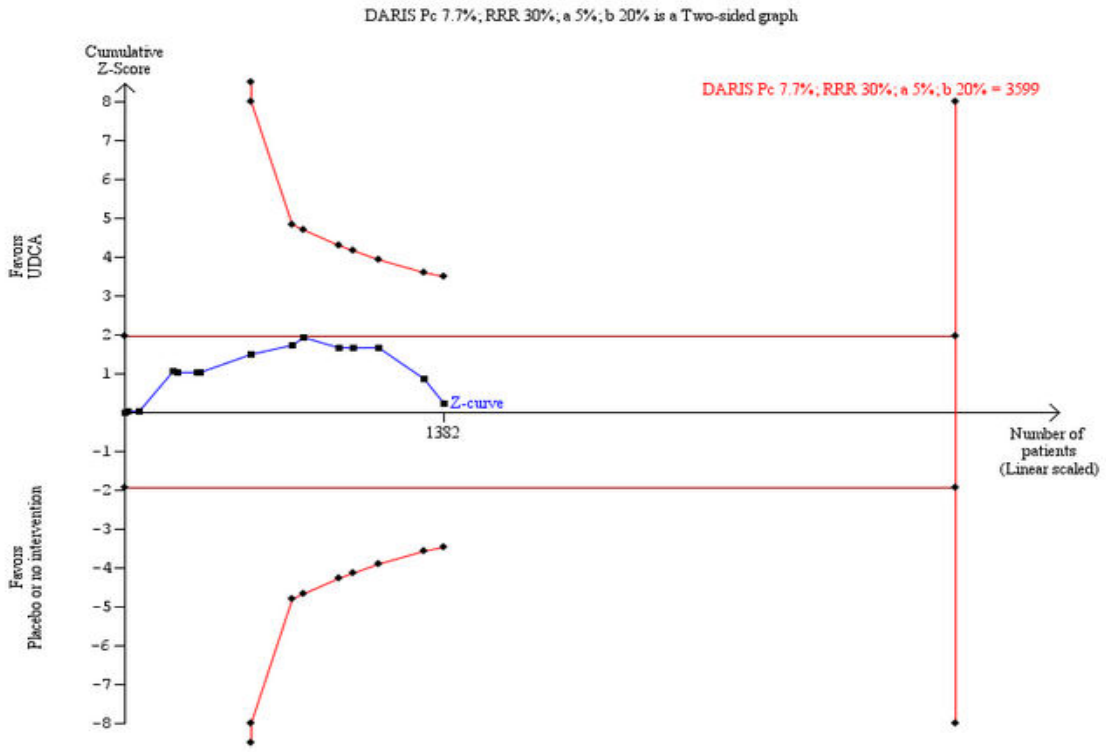

Image 12. Trial sequential analysis of the random-effects meta-analysis of the effect of ursodeoxycholic acid versus placebo or no intervention on all-cause mortality. The trial sequential analysis is performed with an assumed control proportion of death of 7.7%, an anticipated relative risk reduction (RRR) of 30%, a type 1 error risk of 5% (two-sided) (a), and a power of 80% (a type 2 error risk of 20%) (b). The diversity-adjusted required information size (DARIS) to detect or reject a RRR of 30% with a between trial heterogeneity of 0% is estimated to 3599 patients. The actually accrued number of patients is 1382, which is only 38% of the required information size. The blue cumulative Z-curve does not cross the red trial sequential monitoring boundaries for benefit or harm. Therefore, there is no evidence to support that ursodeoxycholic acid influences mortality. The cumulative Z-curve does not reach the futility area delineated by the trial sequential beta-spending monitoring boundaries (which are not even drawn by the program), demonstrating that further randomised trials are needed.

Available patient analysis did not result in any changes of effect estimates (RR 0.98; 95% CI 0.67 to 1.43; I^2 = 0%; 1247 patients, 14 trials (Image 13). Analysing the missing data in the best-case scenario (assuming that patients with unknown vital status receiving ursodeoxycholic acid were alive and that all patients from the control group with unknown vital status were dead) or in the worst-case scenario (assuming that patients with unknown vital status receiving ursodeoxycholic acid were dead and all patients with unknown vital status from the control group were alive) showed statistical significant effects of ursodeoxycholic acid ranging from a beneficial effect (best-case scenario: RR 0.35; 95% CI 0.26 to 0.48; 1 391 patients, 14 trials) to a harmful effect (worst-case scenario: RR 2.16, 95% CI 1.57 to 2.97; 1391 patients, 14 trials) (Image 13).

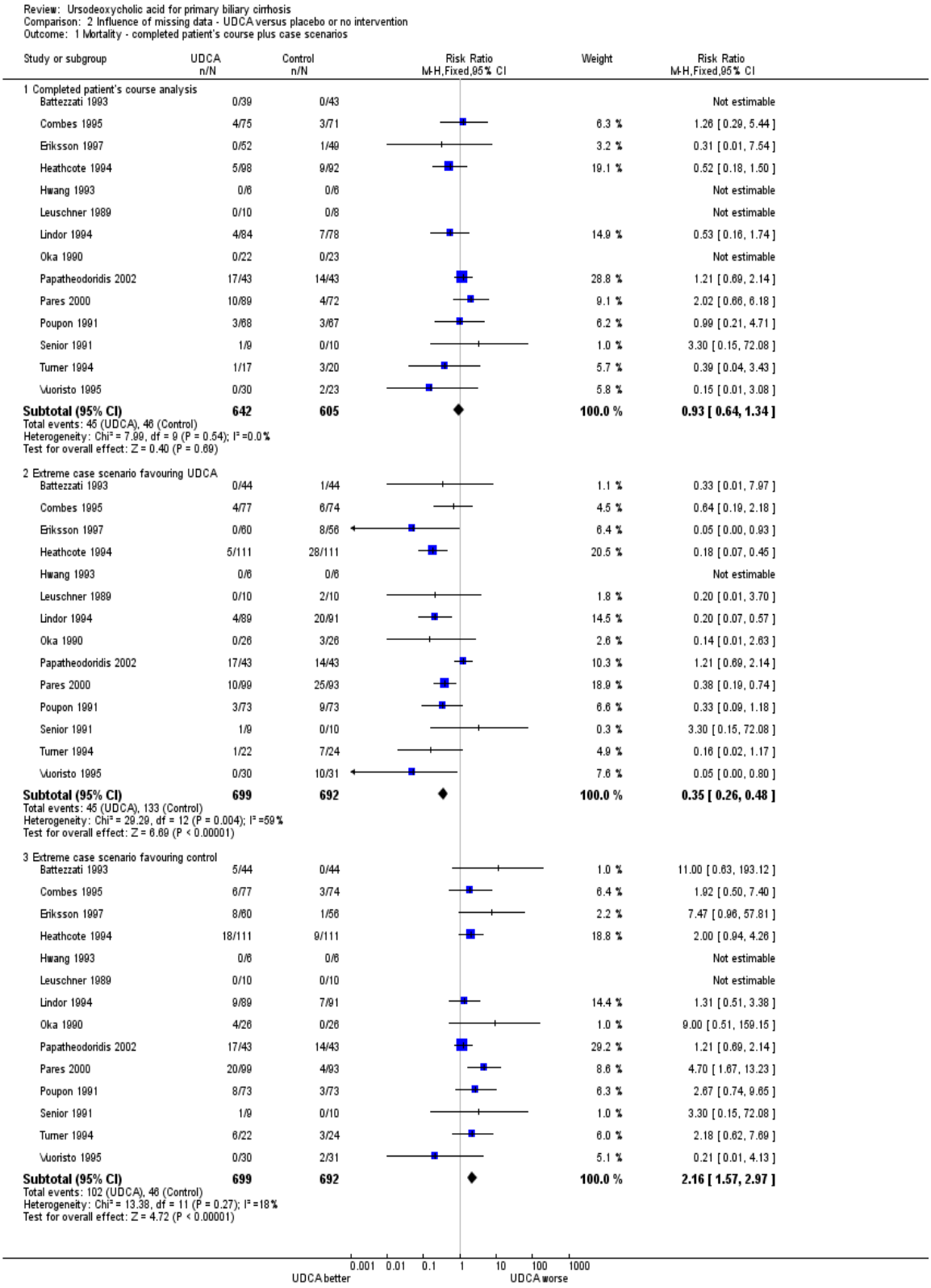

Image 13: Influence of missing data – UDCA vs placebo or no intervention; outcome: mortality – completed patient's course plus case scenarios

Univariate meta-regression analyses revealed that none of examined covariates (risk of bias of the trials, disease severity of patients at entry, ursodeoxycholic acid dosage, and trial duration) were significantly associated with the estimated intervention effect on mortality. In multivariate meta-regression analysis including all covariates, none were significantly associated with the estimated intervention effect on mortality (Table 2).

Table 2 UDCA* effects on mortality adjusted for trial-level covariates

Covariates	Coefficient	95% CI	P-value
Risk of bias (low versus high)	0.225	-1.153 to 1.630	0.749
UDCA* dose (mg/kg/day)	-0.284	-1.004 to 0.437	0.440
Trial duration (year)	0.014	-0.012 to 0.040	0.296
Severity of PBC#	-4.938	-10.459 to 0.582	0.080

* UDCA= ursodeoxycholic acid.

PBC= primary biliary cirrhosis.

Analysis of data from the extended follow-up for ursodeoxycholic acid→ursodeoxycholic acid versus placebo→ursodeoxycholic acid into the analyses demonstrated a RR of 0.97 with 95% CI 0.73 to 1.30 (Image 14). It compared 76 (10.9%) deaths in 699 patients originally randomised to ursodeoxycholic acid with 78 (11.2%) deaths in 692 patients originally randomised to placebo or no intervention.

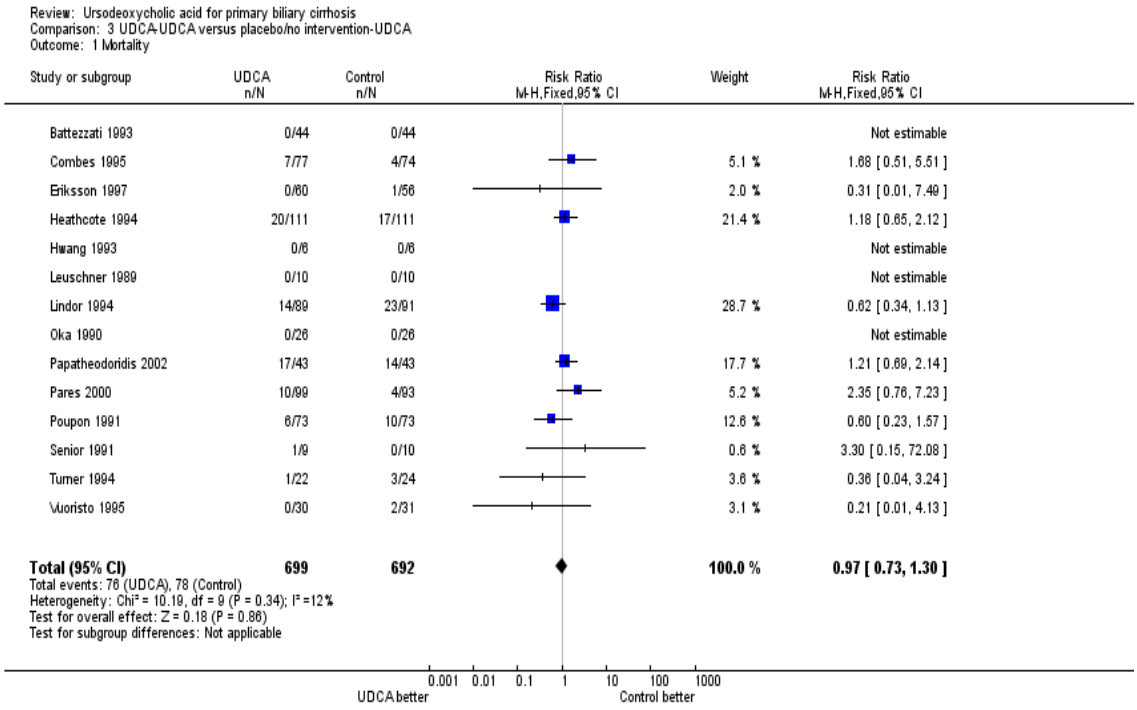

Image 14: extended follow-up for ursodeoxycholic acid→ursodeoxycholic acid versus placebo/no intervention→ursodeoxycholic acid; outcome: mortality

All-cause mortality or liver transplantation

Fifthteen trials provided information on all-cause mortality or liver transplantation and could be included in the analyses. The included trials reported a total of 175 (12.3%) deaths or transplants in 1419 patients (Image 15). In the ursodeoxycholic acid group, 86 (12.0%) out of 713 patients died or were transplanted versus 89 (12.6%) out of 706 patients in the control group. Meta-analyses with both the fixed-effect model and random-effects model showed no significant difference in effect between the compared interventions (RR 0.96; 95% CI 0.74 to 1.25, I^2 = 15%) (Image 15).

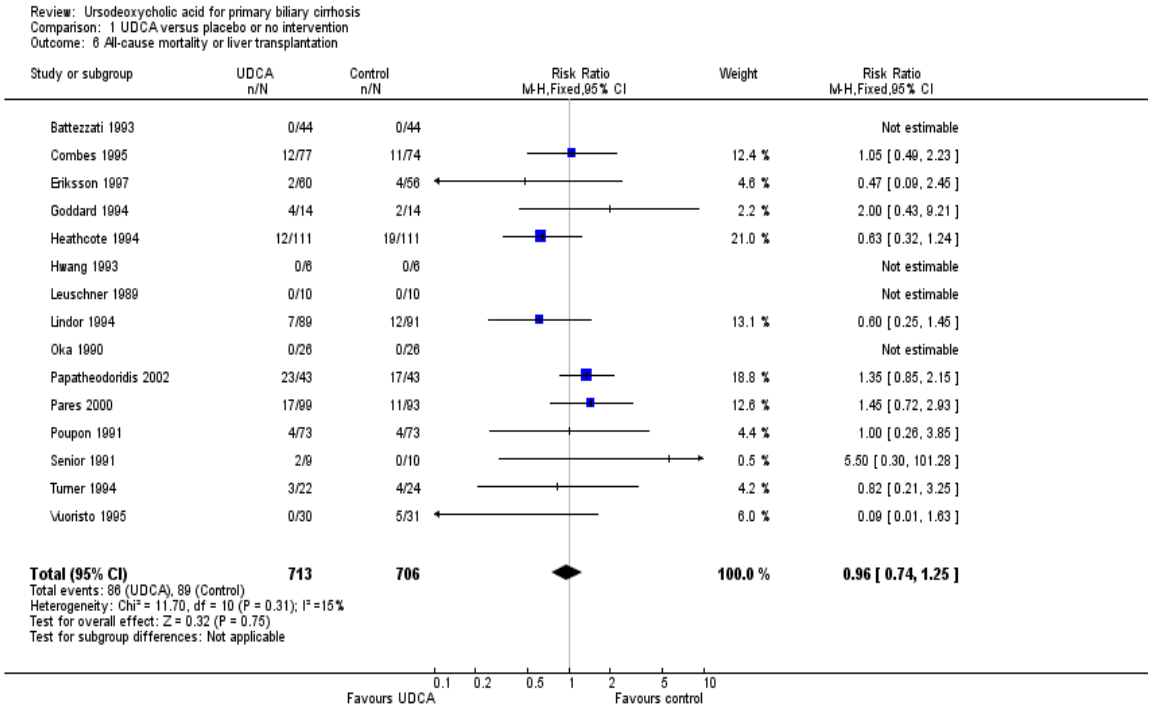

Image 15: UDCA vs placebo or no intervention; outcome: all-cause mortality

Inspection of the funnel plot did not indicate bias (Image 16)

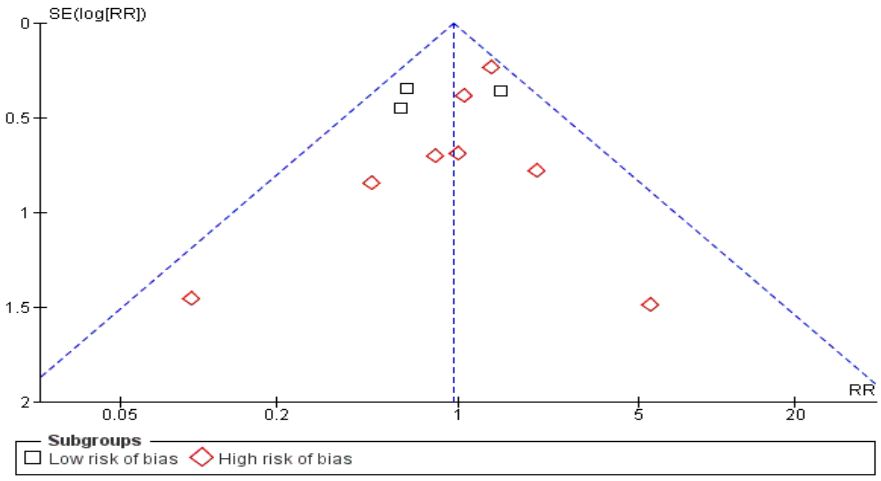

Image 16. Funnel plot of comparison: UDCA versus placebo or no intervention, outcome: All-cause mortality or liver transplantation stratified after risk of bias

The subgroup analyses stratifying the trials according to risk of bias, risk of bias including industry involvement, trial duration, and dose of ursodeoxycholic acid did not reveal any differences in effect estimates in the risk of all-cause mortality or liver transplantation (Image 17, 18, 19, 20). Heterogeneity might not be important (I^2 = 15%, P = 0.31).

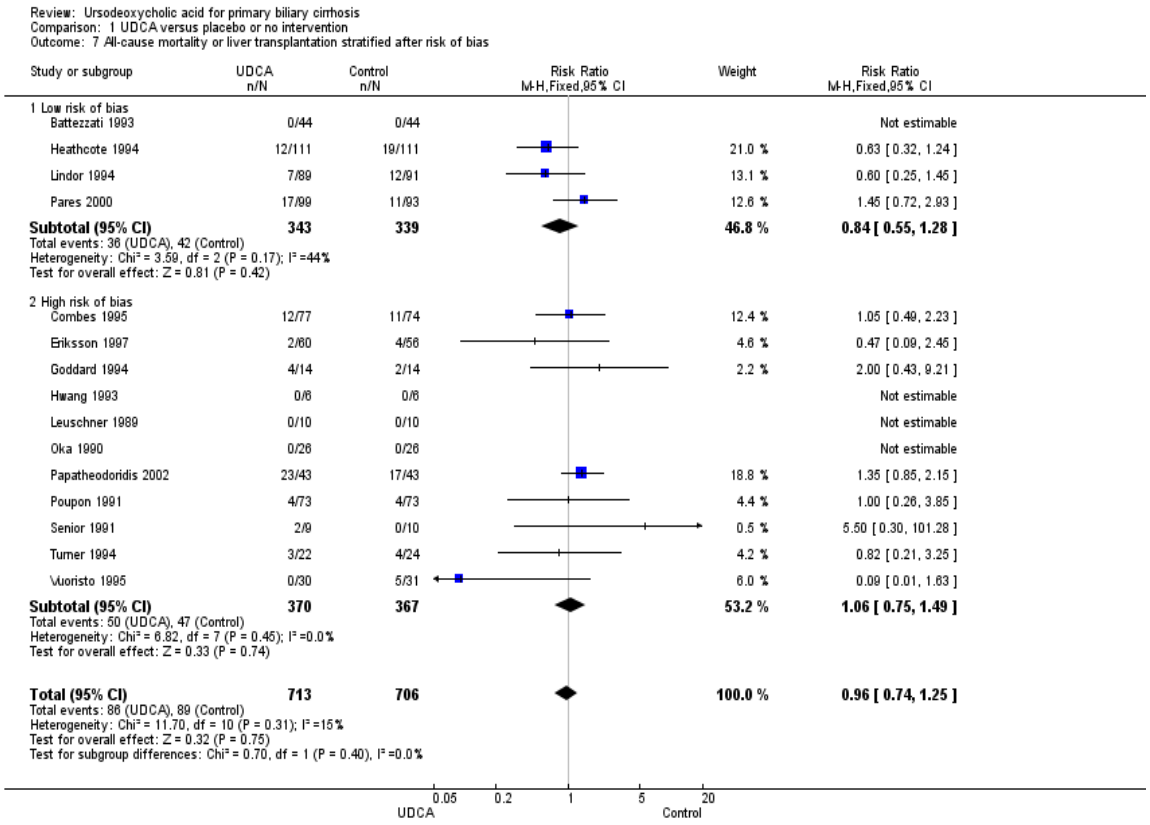

Image 17: UDCA vs placebo/no intervention; outcome: all-cause mortality or liver transplantation stratified after risk of bias

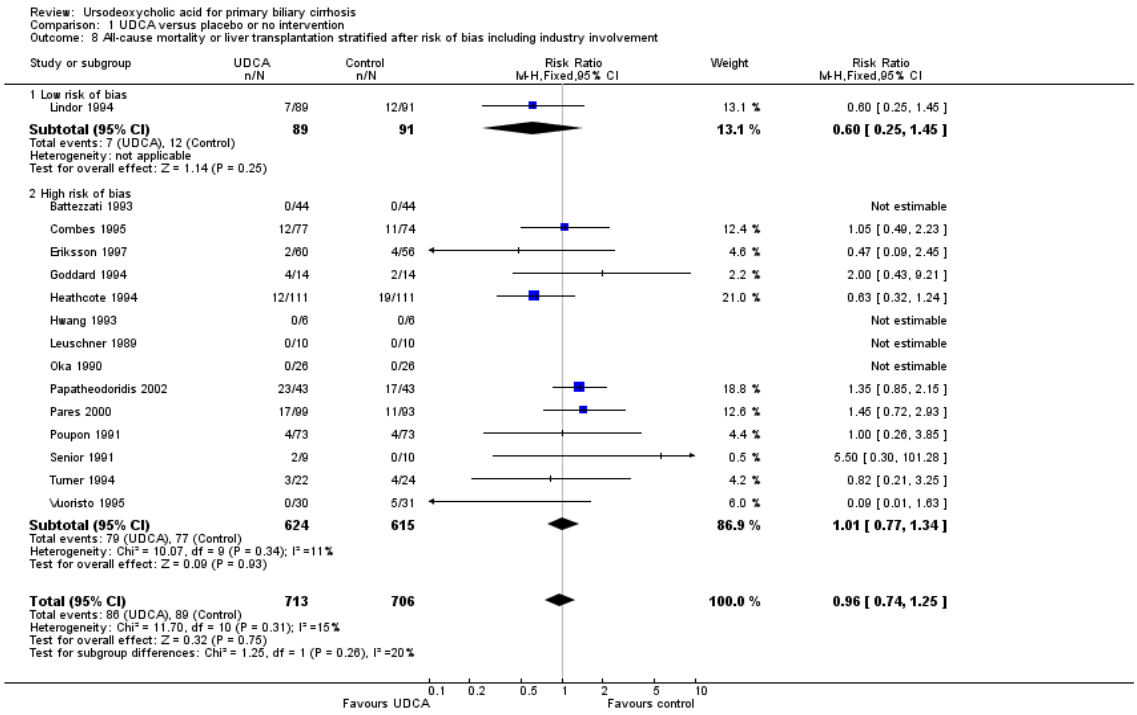

Image 18: UDCA vs placebo/no intervention; outcome: all-cause mortality or liver transplantation stratified after risk of bias including industry involvment

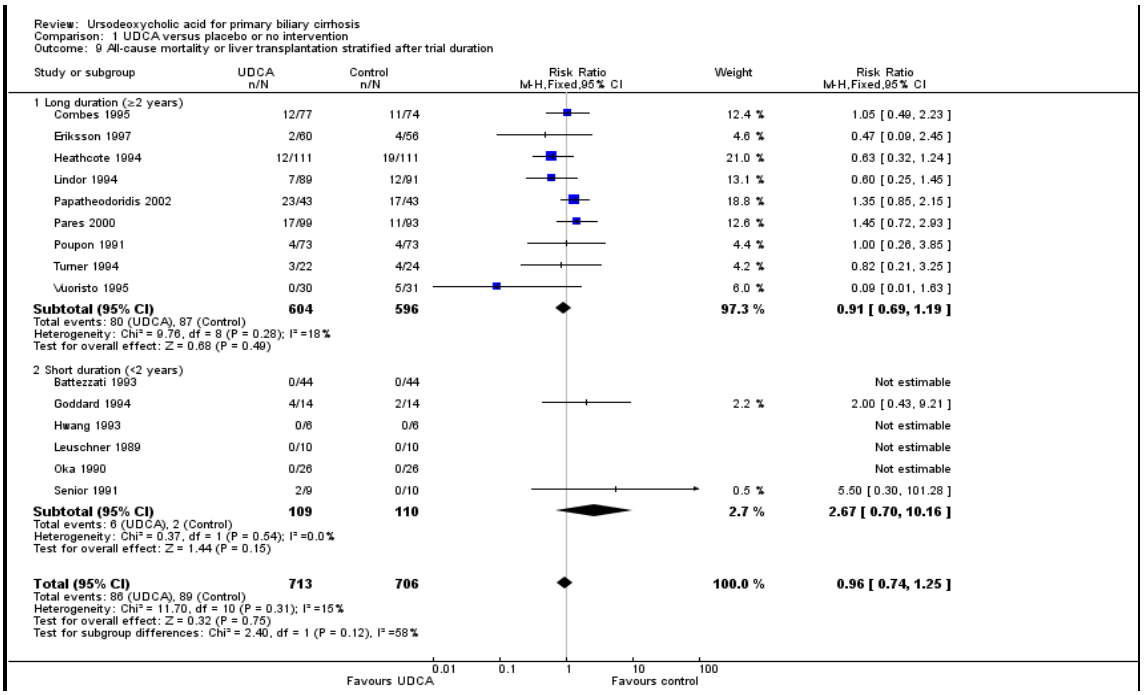

Image 19: UDCA vs placebo/no intervention; outcome: all-cause mortality or liver transplantation stratified after trial duration

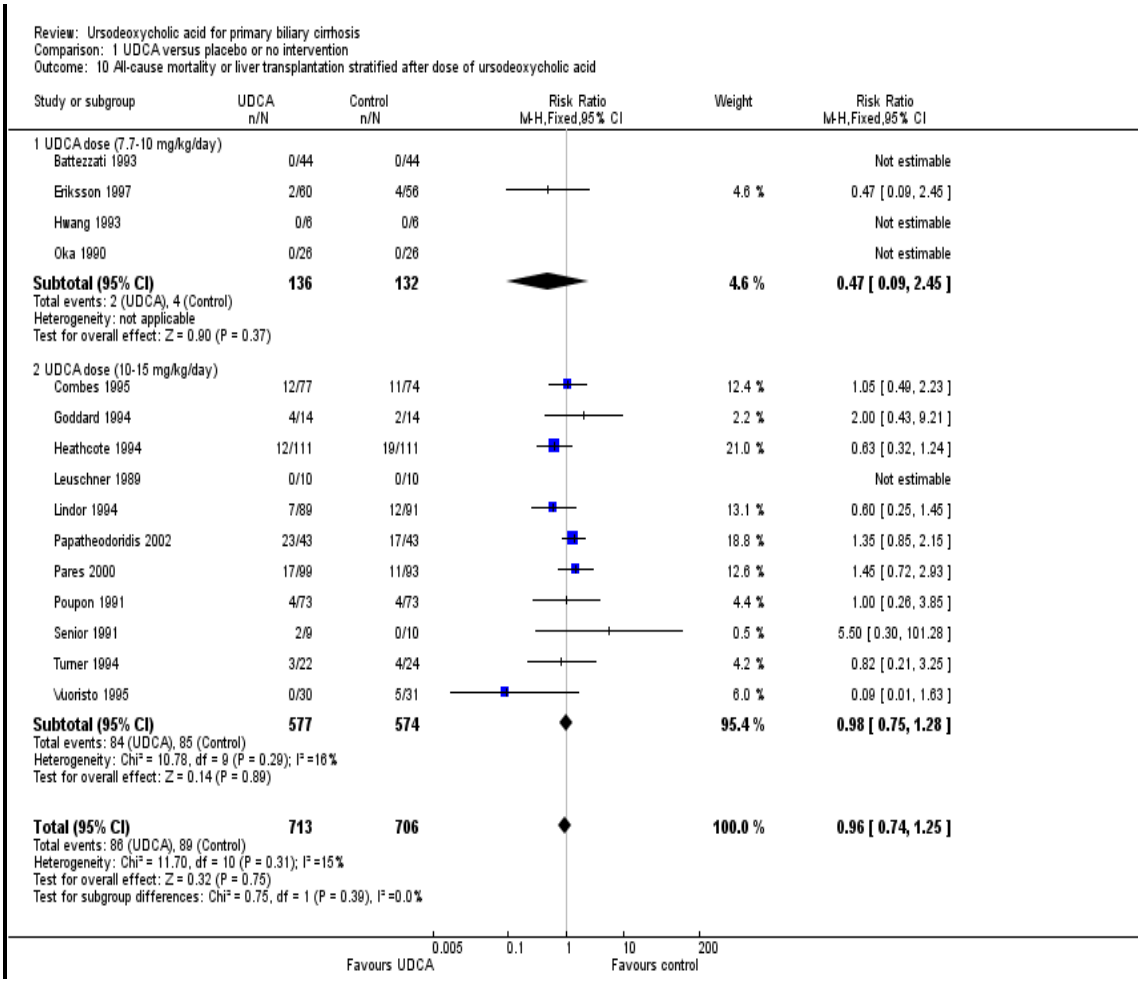

Image 20: UDCA vs placebo/no intervention; outcome: all-cause mortality or liver transplantation stratified after dose of ursodeoxycholic acid

Trial sequential analysis with data from all included trials showed that only 1 410 patients of the required diversity-adjusted information size of 4 043 were accrued (35%) and no firm evidence for benefit or harm was therefore reached (Image 21). The cumulative Z-curve did not cross the red trial sequential alpha-spending monitoring boundaries for benefit or harm. Therefore, there is no evidence to support or refute that ursodeoxycholic acid influences mortality or transplantation. Sensitivity analyses showed that an intervention effect corresponding to a 30% relative risk reduction of all-cause mortality or liver transplantation can be excluded (Image 22).

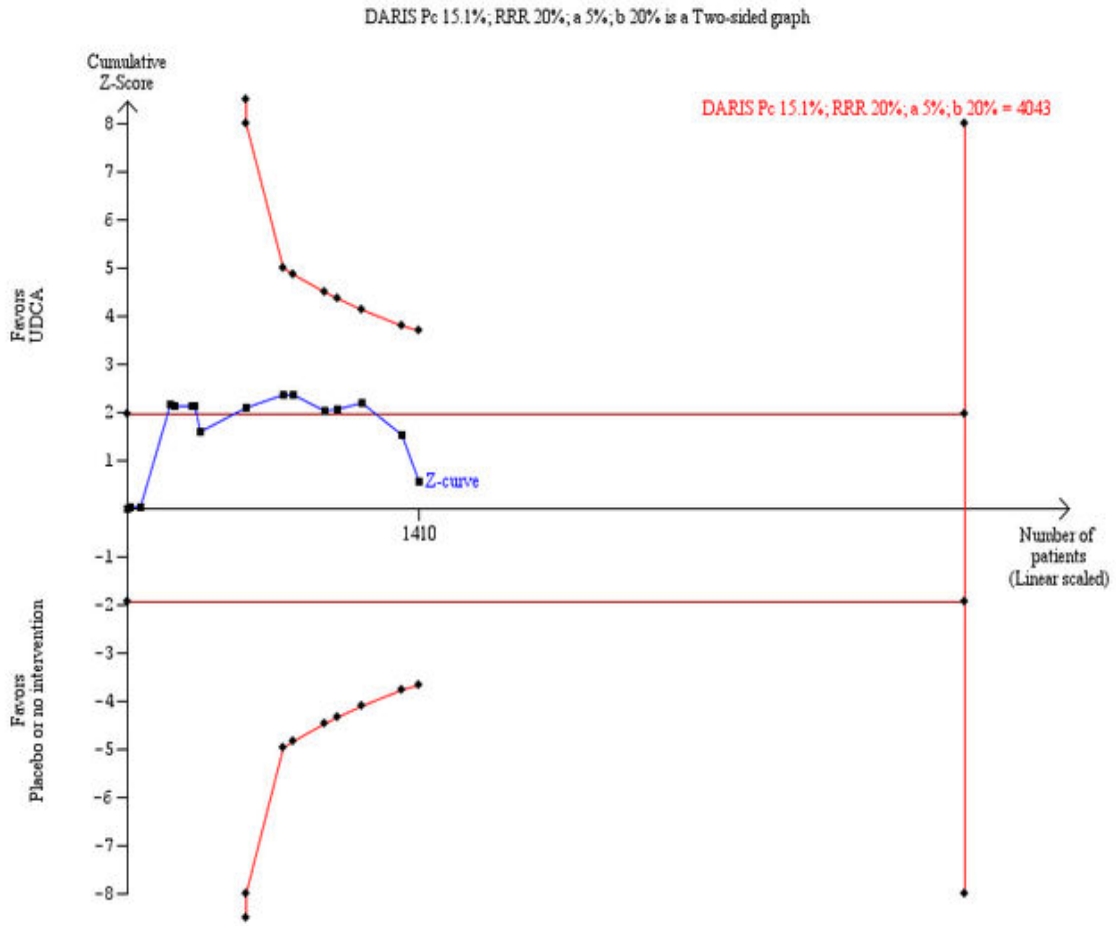

Image 21. Trial sequential analysis of the random-effects meta-analysis of the effect of ursodeoxycholic acid versus placebo or no intervention on all-cause mortality or liver transplantation. The trial sequential analysis is performed with an assumed control proportion of death of 15.1%, an anticipated relative risk reduction (RRR) of 20%, a type 1 error risk of 5% (two-sided), and a power of 80% (a type 2 error risk of 20%) (b). The diversity-adjusted required information size (DARIS) to detect or reject a RRR of 20% with a between trial heterogeneity of 37% is estimated to 4043 patients. The actually accrued number of patients is 1410, which is only 35% of the required information size. The blue cumulative Z-curve does not cross the red trial sequential monitoring boundaries for benefit or harm. Therefore, there is no evidence to support or refute that ursodeoxycholic acid influences mortality or transplantation. The cumulative Z curve does not reach the futility area delineated by the trial

sequential beta-spending monitoring boundaries (which are not even drawn by the program), demonstrating that further randomized trials are needed.

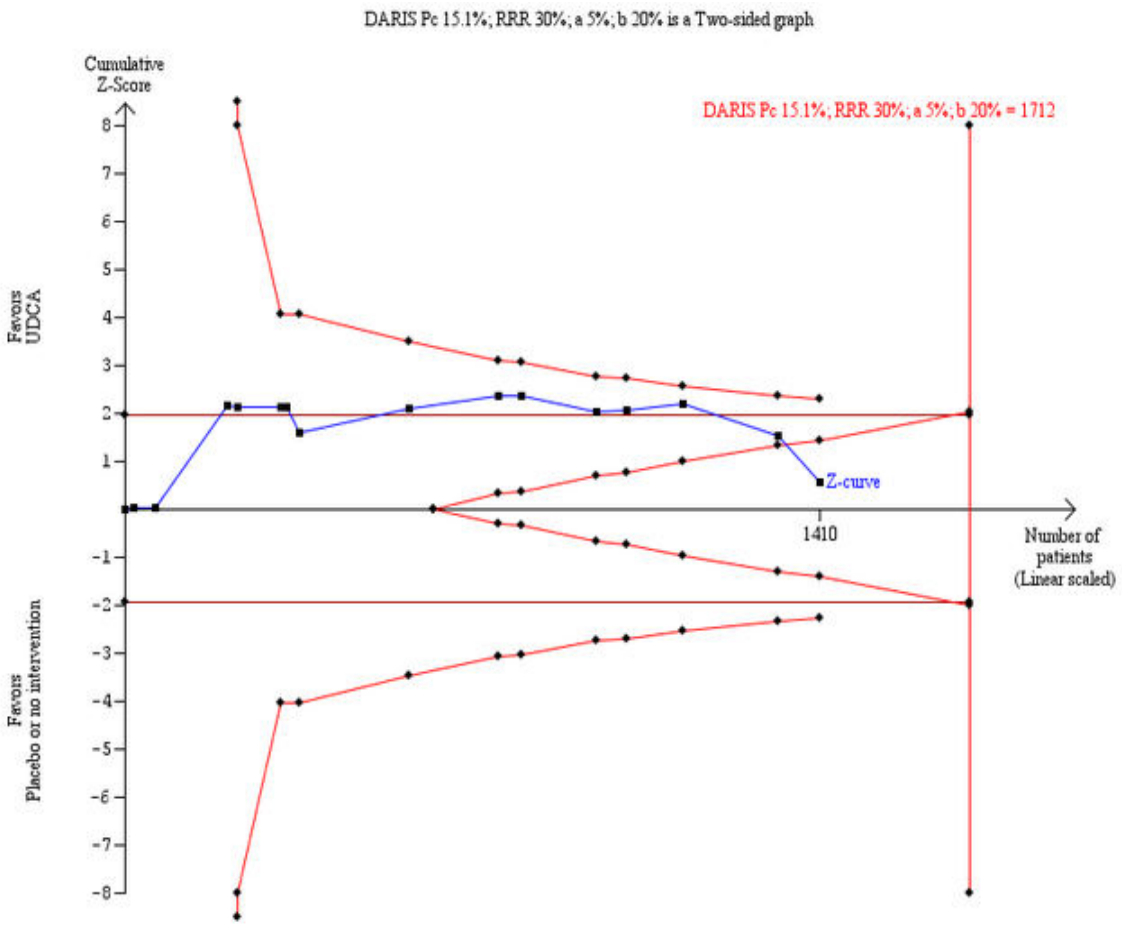

Image 22. Trial sequential analysis of the random-effects meta-analysis of the effect of ursodeoxycholic acid versus placebo or no intervention on all-cause mortality or liver transplantation. The trial sequential analysis is performed with an assumed control proportion of death of 15.1%, an anticipated relative risk reduction (RRR) of 30%, a type 1 error risk of 5% (two-sided), and a power of 80% (a type 2 error risk of 20%) (b). The diversity-adjusted required information size (DARIS) to detect or reject a RRR of 30% with a between trial heterogeneity of 37% is estimated to 1712 patients. The actually accrued number of patients is 1410, which is 82% of the required information size. The blue cumulative Z-curve does not cross the red trial sequential alpha-spending monitoring boundaries for benefit or harm. However, the boundaries for futility

delineated by the trial sequential beta-spending monitoring boundaries (the red inner wedge boundaries) are crossed. Accordingly, the red conventional boundaries (horizontal line at z =1.96 and z =-1.96) for harm or benefit are not crossed. Therefore, there is no evidence to support that ursodeoxycholic acid influences mortality or transplantation. Moreover, a 30% RRR of mortality or transplantation can be rejected with the chosen error risks.

Available patient analysis did not result in any significant changes of effect estimates (RR 0.93; 95% CI 0.64 to 1.34; I^2 = 23%; 1 275 patients, 15 trials) (Image 23). The best-case scenario and worst-case scenario analyses on missing data showed statistical significant effects of ursodeoxycholic acid ranging from a beneficial effects (best-case scenario: RR 0.49; 95% CI 0.30 to 0.80; 1419 patients, 15 trials) to a harmful effects (worst-case scenario: RR 1.60; 95% CI 1.21 to 2.10; 1419 patients, 15 trials) (Image 23). These data show that we have too little knowledge about the true effect of ursodeoxycholic acid on all-cause mortality or liver transplantation, also due to poor outcome reporting of the included trials on mortality and liver transplantation.

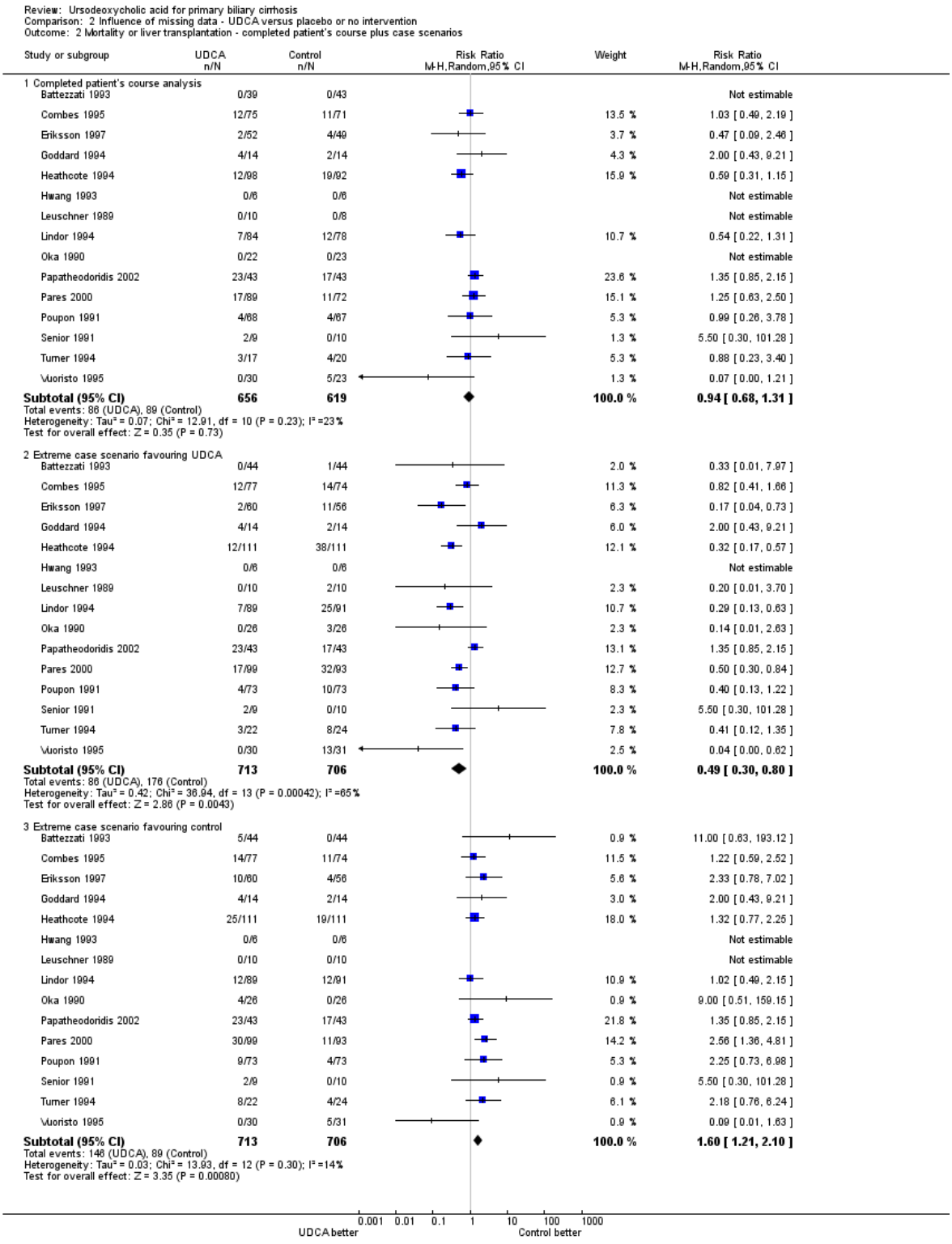

Image 23: Influence of missing data – UDCA vs placebo or no intervention; outcome: mortality or liver transplantation – completed patient's course plus case scenarios

Univariate meta-regression analyses revealed that none of the examined covariates (risk of bias, disease severity of patients at entry; ursodeoxycholic acid dosage, and trial duration) were significantly associated with the estimated intervention effect on mortality or liver transplantation. In multivariate meta-regression analysis including all covariates, none were significantly associated with the estimated intervention effect on mortality or liver transplantation (Table 3).

Table 3 UDCA* effects on mortality or transplantation adjusted for trial-level covariates

Covariate	Coefficient	95% CI	P-value
Risk of bias (low vs. high)	-0.487	-1.484 to 0.510	0.338
UDCA* (mg/kg/day)	0.039	-0.244 to 0.322	0.787
Trial duration (year)	0.008	-0.011 to 0.027	0.408
Severity of PBC#	-1.282	-3.637 to 1.073	0.286

* UDCA= ursodeoxycholic acid.

PBC= primary biliary cirrhosis.

Including data from the extended follow-up for ursodeoxycholic acid→ursodeoxycholic acid versus placebo/no intervention→ursodeoxycholic acid demonstrated a RR of 0.88 with 95% CI from 0.73 to 1.06 (Image 24). The meta-analysis showed 147 (20.6%) deaths or liver transplantations out of 713 patients originally randomised to ursodeoxycholic acid, and 165 (23.3%) deaths or liver transplantations out of 706 patients originally randomised to placebo or 'no intervention'.

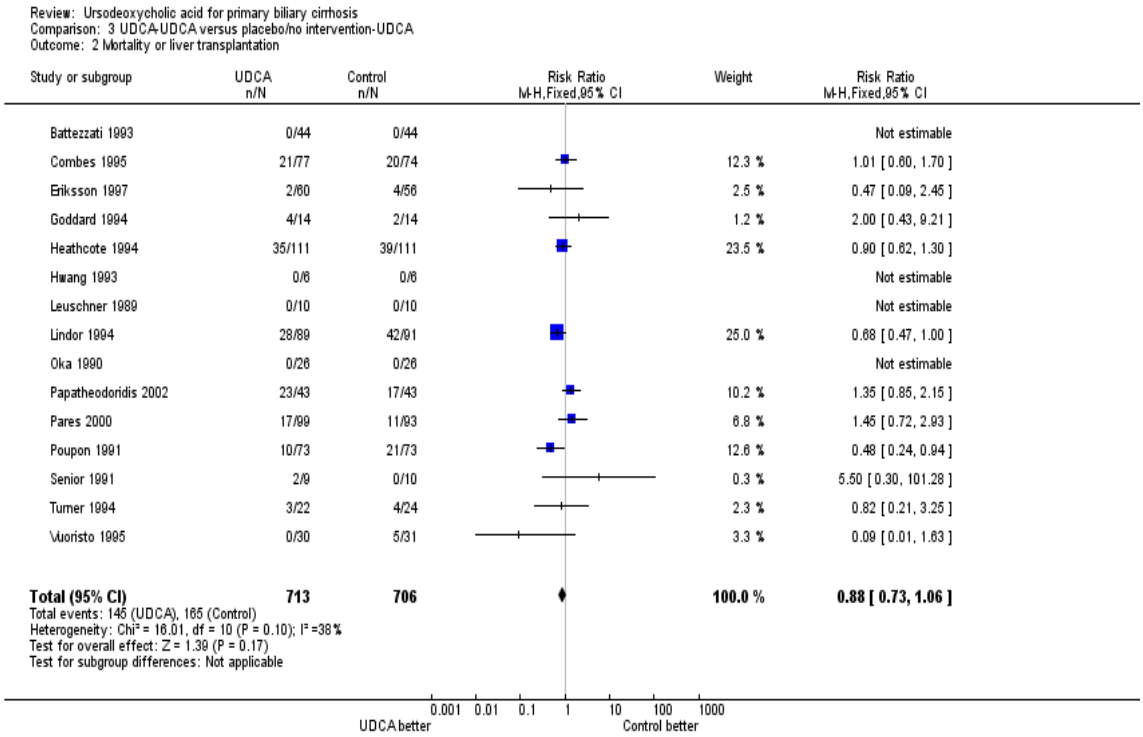

Image 24: extended follow-up for ursodeoxycholic acid→ursodeoxycholic acid versus placebo/no intervention→ursodeoxycholic acid; outcome: mortality or liver transplantation

Adverse events

We divided the reporting of adverse events into the following types: serious adverse events and non-serious adverse events (ICH-GCP 1997).

There was no significant difference in the risk ratio for overall proportion of serious adverse events when comparing ursodeoxycholic acid with placebo or no intervention (RR 0.87; 95% CI 0.68 to 1.12; I^2 = 23%; 1382 patients, 14 trials) (Image 25). In the ursodeoxycholic group 94 serious adverse events were reported versus 107 serious adverse events in the control group of the included trials.

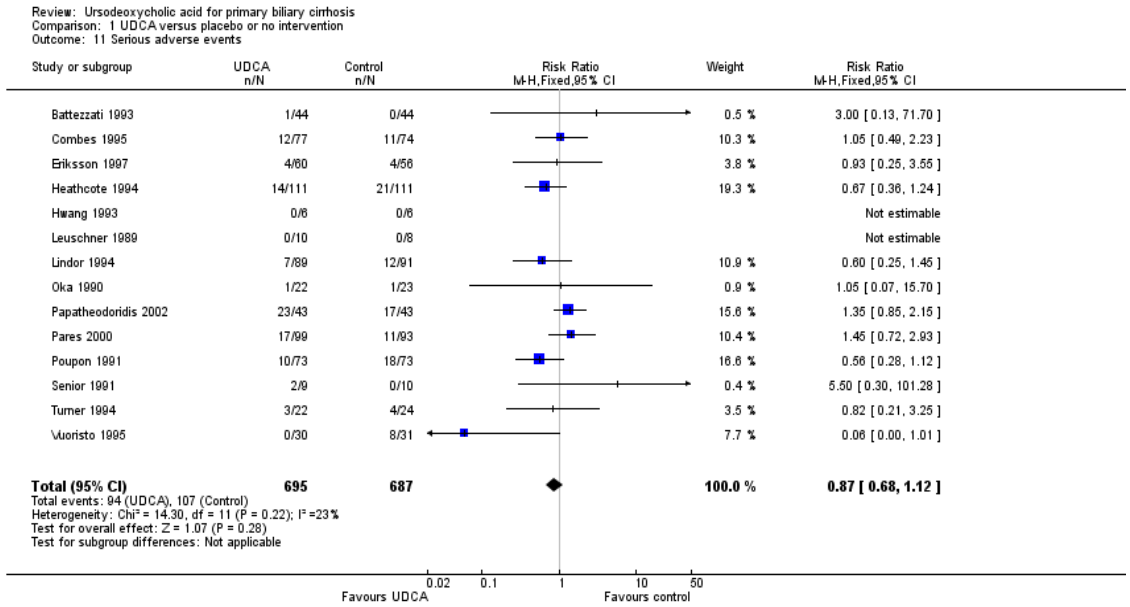

Image 25: UDCA vs placebo or no intervention; outcome: serious adverse advents

There was also no significant difference in the risk ratio for overall incidence of non-serious adverse events when comparing ursodeoxycholic acid with placebo or 'no intervention' (RR 1.46; 95% CI 0.83 to 2.56; I^2 = 0%; 1 277 patients, 12 trials) (Image 26).

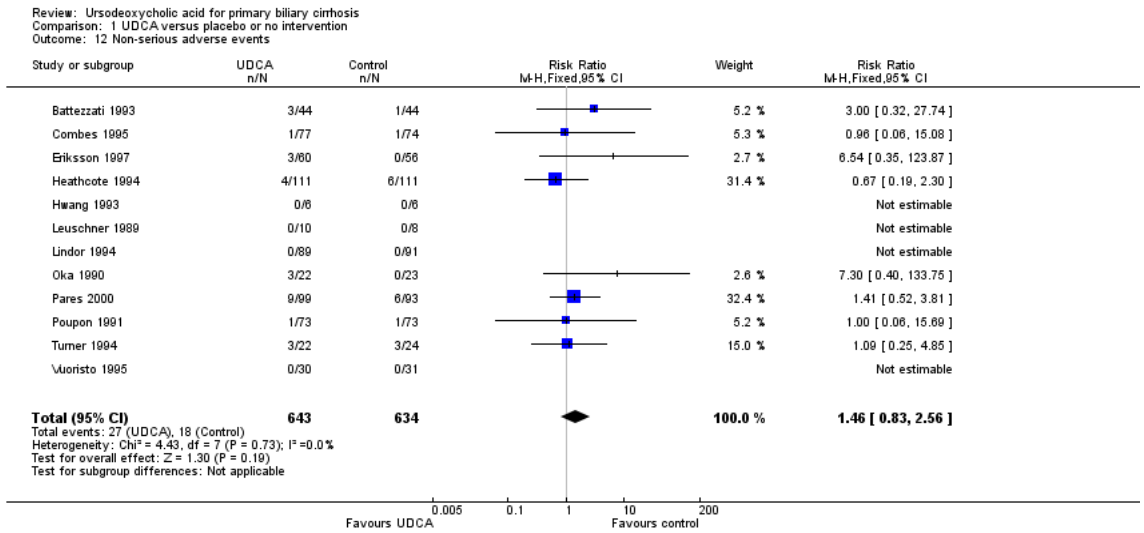

Image 26: UDCA vs placebo or no intervention; outcome: non-serious adverse advents

For assessment of harm, besides the data provided by randomised clinical trials which are included in our analyses (Image 25, 26) we also included data from eleven non-randomised studies which reported on harm (Podda et al, 1989; Lotterer 1990; Kneppelhout 1992; Peridigoto 1992; Shibata 1992; Ikeda 1996; Poupon et al, 1996; Schonfeld 1997; Van Hoogstraten 1998; Angulo et al, 1999a; Verma 1999). For details regarding description of these non-randomised studies see Tables of excluded studies. In Lotterer 1990, there were 7 patients out of 12 who experienced adverse events. One patient died, two patients had acute upper gastrointestinal bleeding, one patient developed ascites, one patient had transient diarrhoea, and one patient had transient exacerbation of pruritus (Table 4).

Table 4 Adverse events (Lotterer 1990)

Adverse event	UDCA*
Death	1/12
Transient exacerbation of pruritus	1/12
Transient diarrhoea	2/12
Ascites	1/12
Acute upper GI bleeding	2/12

* UDCA = ursodeoxycholic acid.

In Ikeda 1996, in the colchicine-ursodeoxycholic acid group, there were 2 patients out of 10 who experienced diarrhoea versus 0 patients out of 12 in the ursodeoxycholic acid group. In Poupon et al, 1996, in the colchicine-ursodeoxycholic acid group, there were 4 patients out of 37 who experienced an

adverse event such as death (2 patients), variceal bleeding (1 patient) and peripheral polyneuropathy (1 patient) versus 2 patients out of 37 in the ursodeoxycholic acid-placebo group (Table 5).

Table 5 Adverse events (Poupon 1996)

Adverse event	Colchicin-UDCA	UDCA-placebo
Variceal bleeding	1/37	2/37
Death	2/37	0/37
Peripheral polyneuropathy	1/37	0/37

The two former studies may say more about adverse events associated with colchicine than with ursodeoxycholic acid. In Angulo et al, 1999a, 155 patients with primary biliary cirrhosis were treated with three different doses of ursodeoxycholic acid, there were 21 patients out of 155 who experienced adverse events such as hypertension (2 patients), creatinine elevation (2 patients), thrombocytopenia (3 patients), leukopenia (1 patient), nausea and vomiting (6 patients), diarrhoea (3 patients), fever (1 patient), and rash (3 patients) (Table 6).

Table 6 Adverse events (Angulo et al, 1999a)

Adverse event	UDCA
Hypertension	2/155
Creatinine elevation	2/155
Thrombocytopenia	3/155

Leukopenia	1/155
Nausea and vomiting	6/155
Diarrhoea	3/155
Fever	1/155
Rash	3/155

In Van Hoogstraten 1998, 61 patients with primary biliary cirrhosis were treated with two different doses of ursodeoxycholic acid, there were 2 patients out of 61 who experienced adverse events such as liver failure (1 patient) and diarrhoea (1 patient) (Table 7).

Table 7 Adverse events (Van Hoogstraten 1998)

Adverse event	UDCA
Liver failure	1/61
Diarrhoea	1/61

In Peridigoto 1992, there were 3 patients who experienced adverse events such as variceal bleeding and ascites and more than one event occurred in some patient (Table 8).

Table 8 Adverse events (Peridigoto 1992)

Adverse event	UDCA
Variceal bleeding	3/3
Ascites	2/3

In Podda 1989, there were 2 patients out of 30 who experienced pruritus. In Kneppelhout 1992, there were 9 patients out of 17 who experienced adverse events such as liver transplantation, ascites, nausea, increased pruritus, increase in pre-existent hyperbilirubinaemia, fever, weakness, and more than one event occurred in some patient (Table 9).

Table 9 Adverse events (Kneppelhout 1992)

Adverse event	UDCA
Nausea	2/17
Increased pruritus	4/17
Increase in pre-existent hyperbilirubinaemia	3/17
Ascites	1/17
Liver transplantation	1/17
Fever	1/17
Weakness	1/17

In Schonfeld 1997, there was one patient out of 15 who experienced severe and progressive fatigue, weight loss, ascites, an increase in serum bilirubin concentration and was liver transplanted. In Shibata 1992, there were 3 patients out of 12 who experienced adverse events such as death, bleeding varices, hepatocellular carcinoma, diarrhoea, gallstones, and more than one event occurred in some patient (Table 10).

Table 10 Adverse events (Shibata 1992)

Adverse event	Colchicin-UDCA
Diarrhoea	1/12
Gallstones	1/12
Bleeding varices	1/12
Death	1/12
Hepatocellular carcinoma	1/12

In Verma 1999, there was one patient out of 24 who experienced severe migraine.

Quality of life

None of the trials used specific quality-of-life scales. Two trials (Turner et al, 1994; Eriksson et al, 1997) evaluated symptoms using visual analogue scales. None of these showed any significant difference between the ursodeoxycholic acid group and placebo group. However, significantly ($P < 0.01$) more patients felt better or much better following ursodeoxycholic acid intervention than after placebo in the Eriksson 1997 trial.

Secondary outcomes

Liver transplantation

Fourteen trials provided information on liver transplantation and could be included in the analyses. The included trials reported 78 (5.6%) transplants in 1391 patients (Image 27). In the ursodeoxycholic acid group, 37 (5.3%) out of 699 patients were transplanted versus 41 (5.9%) out of 692 patients in the control group. Meta-analyses with both the fixed-effect model and random-effects model showed no significant difference in effect of ursodeoxycholic acid on liver transplantation (RR 0.89; 95% CI 0.59 to 1.36, $I^2 = 0\%$) (Image 27).

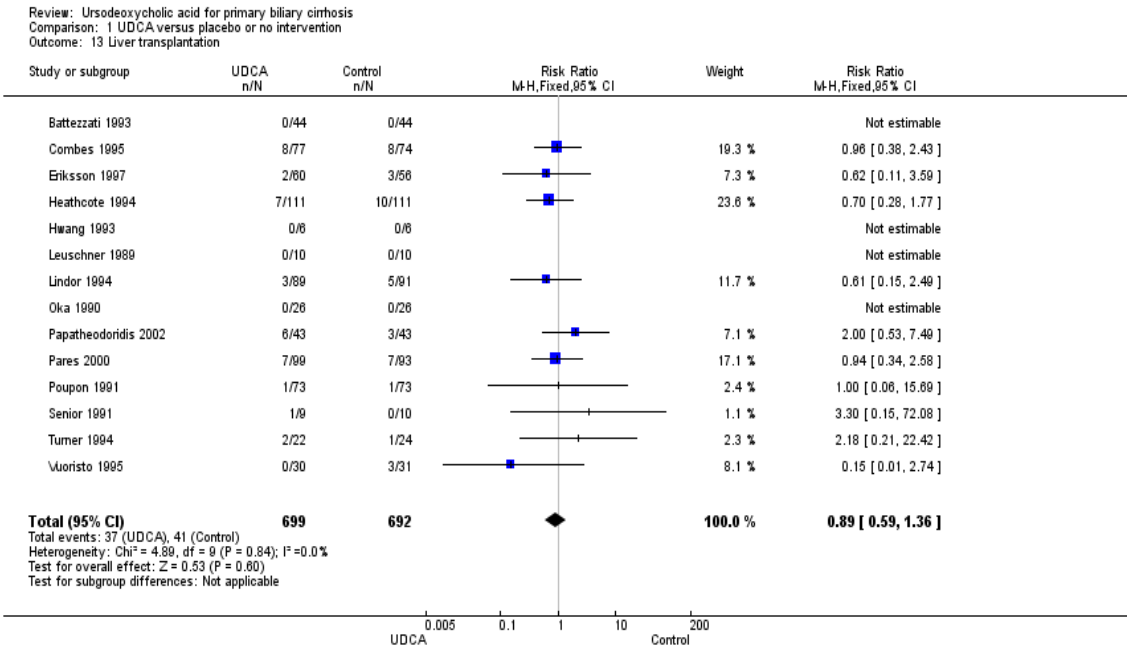

Image 27: UDCA vs placebo or no intervention; outcome: liver transplantation

Including data from the extended follow-up for ursodeoxycholic acid→ursodeoxycholic acid versus placebo/'no intervention'→ursodeoxycholic acid (now comprising 65 (9.3%) liver transplantations in 699 patients originally randomised to ursodeoxycholic acid versus 85 (12.3%) liver transplantations in

692 patients originally randomised to placebo/no intervention) demonstrated an RR of 0.76 with 95% CI from 0.57 to 1.03 (Image 28).

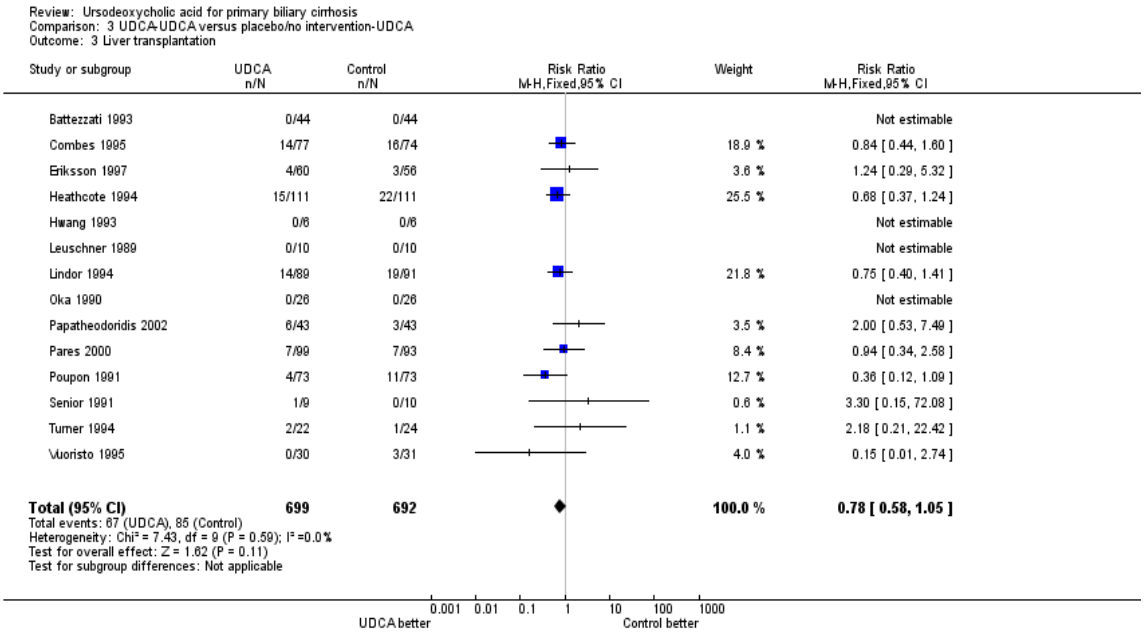

Image 28: extended follow-up for ursodeoxycholic acid→ursodeoxycholic acid versus placebo/'no intervention'→ursodeoxycholic acid; outcome: liver transplantation

Pruritus and fatigue

Ursodeoxycholic acid did not significantly influence neither the number of patients with pruritus (RR 0.96; 95% CI 0.84 to 1.09; I^2 = 0%; 630 patients, 6 trials) (Image 29) nor the pruritus score (SMD -0.10; 95% CI -0.33 to 0.12; I^2 = 0%; 314 patients, 3 trials) (Image 30).

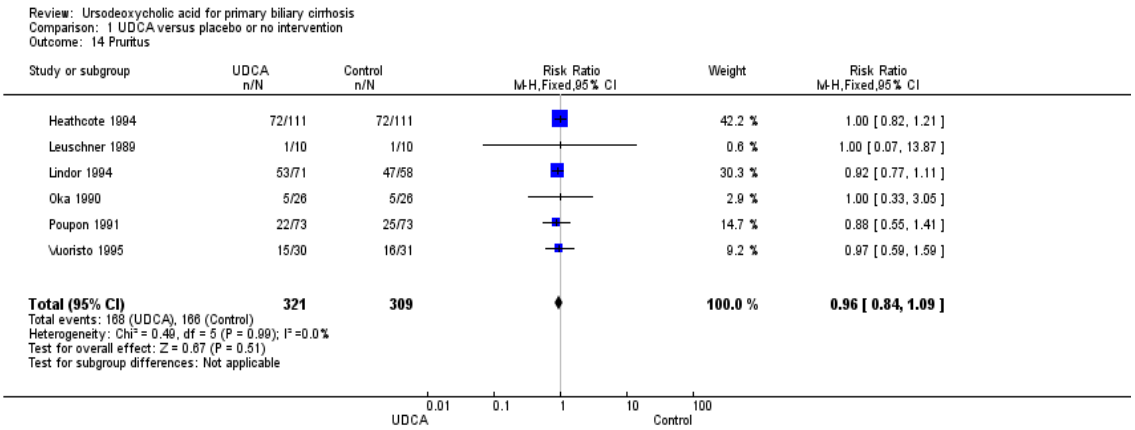

Image 29: UDCA vs placebo or no intervention; outcome: pruritus

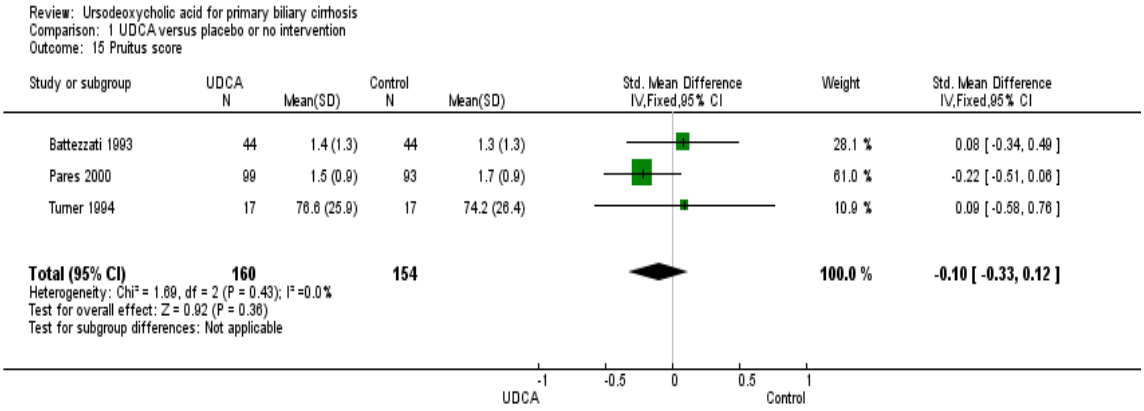

Image 30: UDCA vs placebo or no intervention; outcome: pruritus score

Trial sequential analysis of these data supports the finding in the meta-analysis (Image 31).

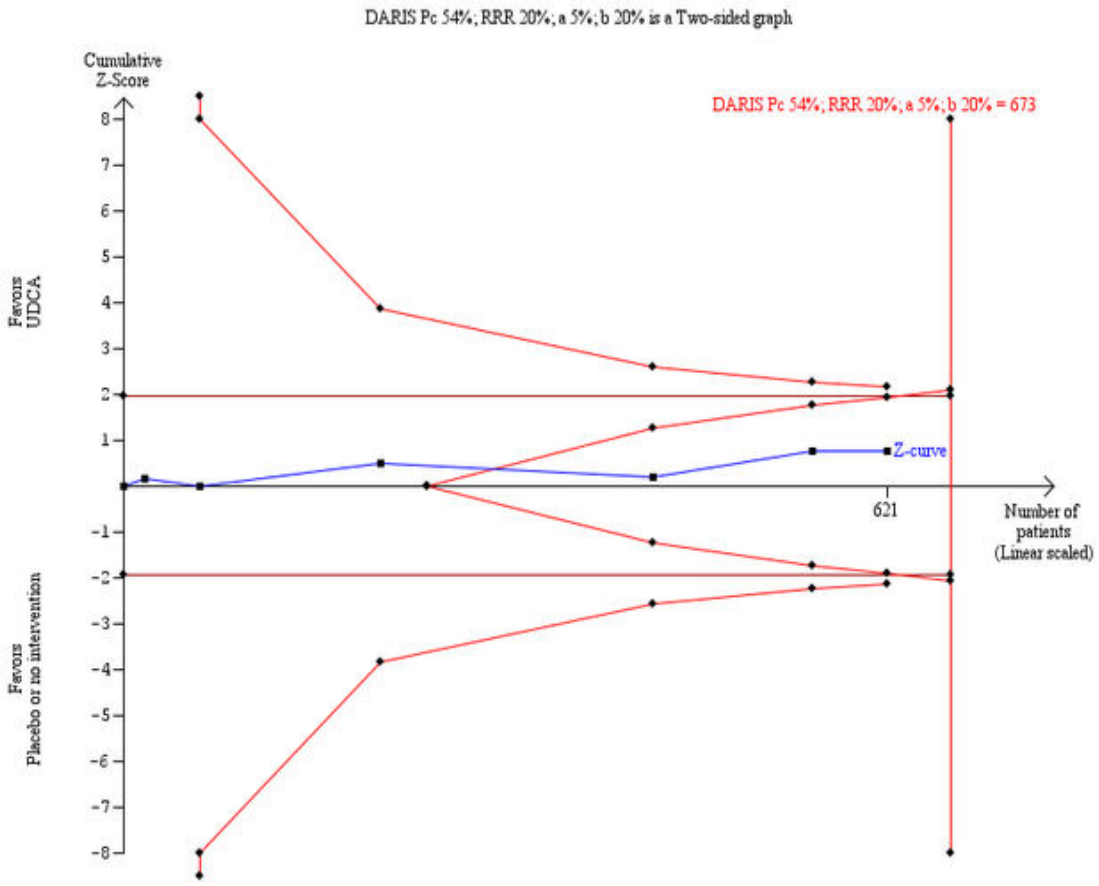

Image 31. Trial sequential analysis of the random-effects meta-analysis of the effect of ursodeoxycholic acid versus placebo or no intervention on pruritus. The trial sequential analysis is performed with an assumed control proportion of pruritus of 54%, an anticipated relative risk reduction (RRR) of 20%, a type 1 error risk of 5% (two-sided), and a power of 80% (a type 2 error risk of 20%) (b). The heterogeneity-adjusted required information size (DARIS) to detect or reject a RRR of 20% with a between trial heterogeneity of 0% is estimated to 673 patients. The actually accrued number of patients is 621, which is 92% of the required information size. The blue cumulative Z-curve does not cross the red trial sequential monitoring boundaries for benefit or harm. However, the boundaries for futility delineated by the trial sequential beta-spending monitoring boundaries (the red inner wedge boundaries) are crossed. Therefore, there is no evidence to support that ursodeoxycholic acid influences pruritus and a 20% RRR of pruritus can be rejected with the chosen error risks.

Fatigue was not significantly improved by ursodeoxycholic acid (RR 0.90; 95% CI 0.81 to 1.00; I^2 = 62%; 506 patients, 4 trials) (Image 32).

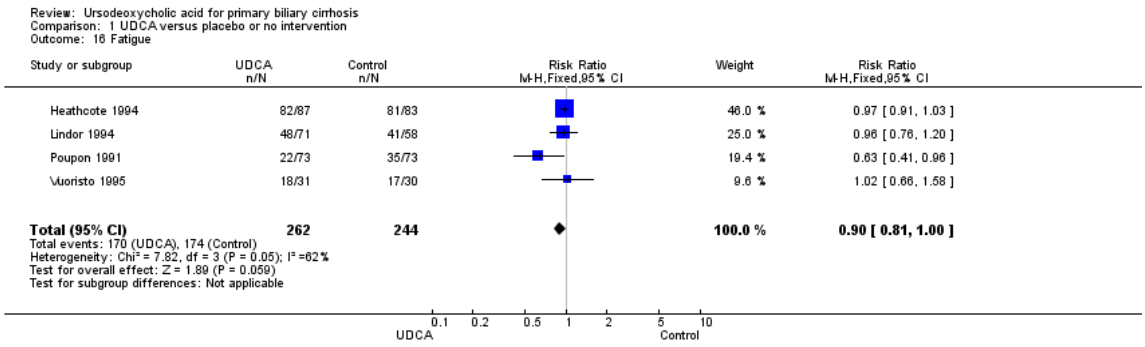

Image 32: UDCA vs placebo or no intervention; outcome: fatigue

Liver-related morbidity

In fixed-effect meta-analysis, two trials in which the number of patients with jaundice was reported led to a significant effect of ursodeoxycholic acid versus placebo or no intervention (RR 0.35; 95% CI 0.14 to 0.90; I^2 = 51%; 198 patients, 2 trials). However, in random-effects meta-analysis, two trials in which the number of patients with jaundice was reported showed no significant effect of ursodeoxycholic acid versus placebo or no intervention (RR 0.56; 95% CI 0.06 to 4.95; I^2 = 51%; 198 patients, 2 trials) (Image 33).

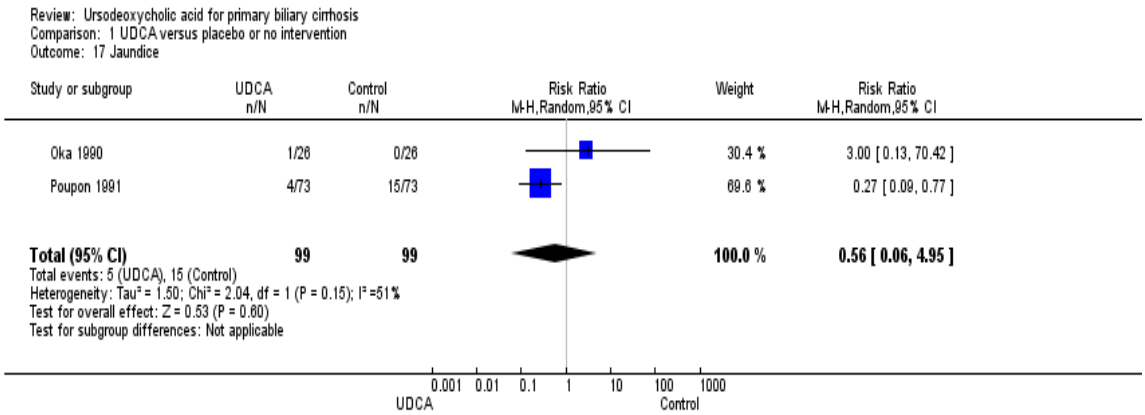

Image 33: UDCA vs placebo or no intervention; outcome: jaundice

Neither portal pressure (MD 0.60 mmHg; 95% CI -2.78 to 3.98; 28 patients, 1 trial) (Image 34), varices (RR 1.16; 95% CI 0.64 to 2.09; I^2 = 0%; 341 patients, 3 trials) (Image 35), bleeding varices (RR 1.05; 95% CI 0.52 to 2.15; I^2 = 0%; 767 patients, 7 trials) (Image 36), ascites (RR 0.55; 95% CI 0.24 to 1.26; I^2 = 0%; 547 patients, 5 trials) (Image 37) nor hepatic encephalopathy (RR 0.47; 95% CI 0.04 to 5.09; 212 patients, 2 trials) (Image 38) were significantly affected by ursodeoxycholic acid treatment.

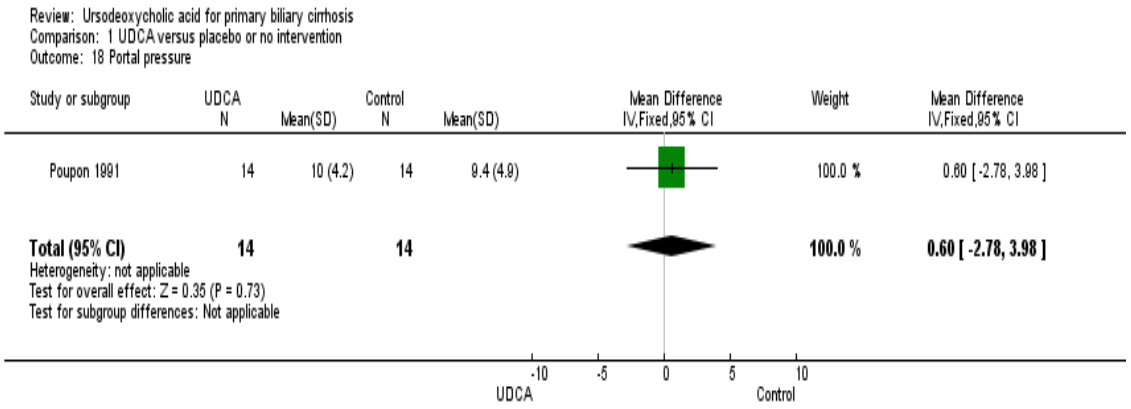

Image 34: UDCA vs placebo or no intervention; outcome: portal pressure

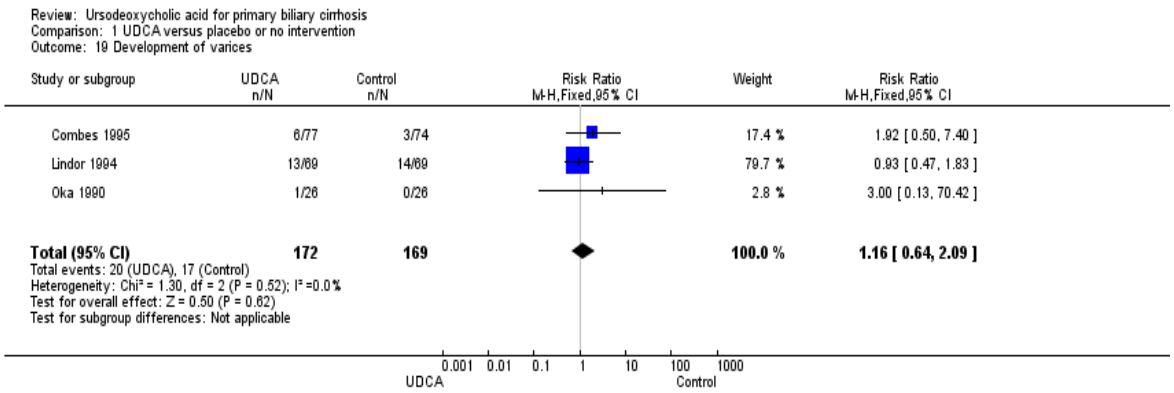

Image 35: UDCA vs placebo or no intervention; outcome: development of varices

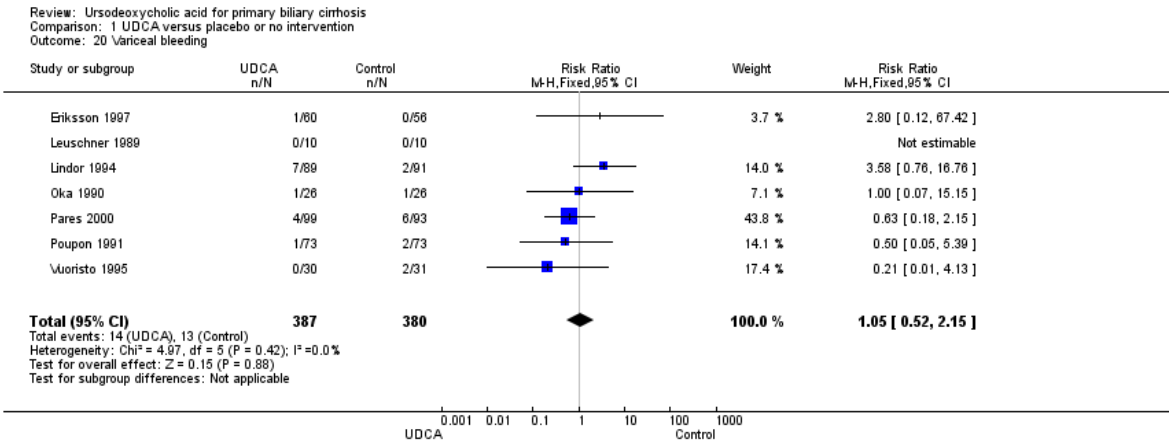

Image 36: UDCA vs placebo or no intervention; outcome: variceal bleeding

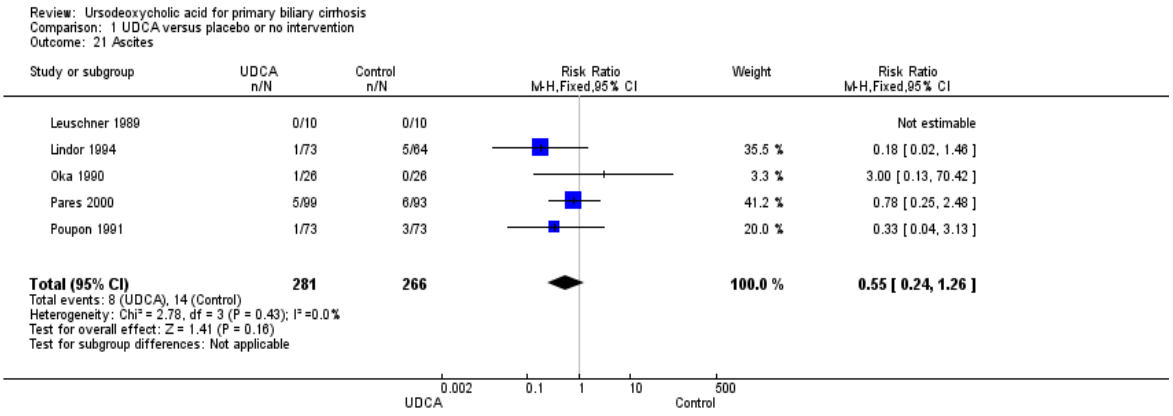

Image 37: UDCA vs placebo or no intervention; outcome: ascites

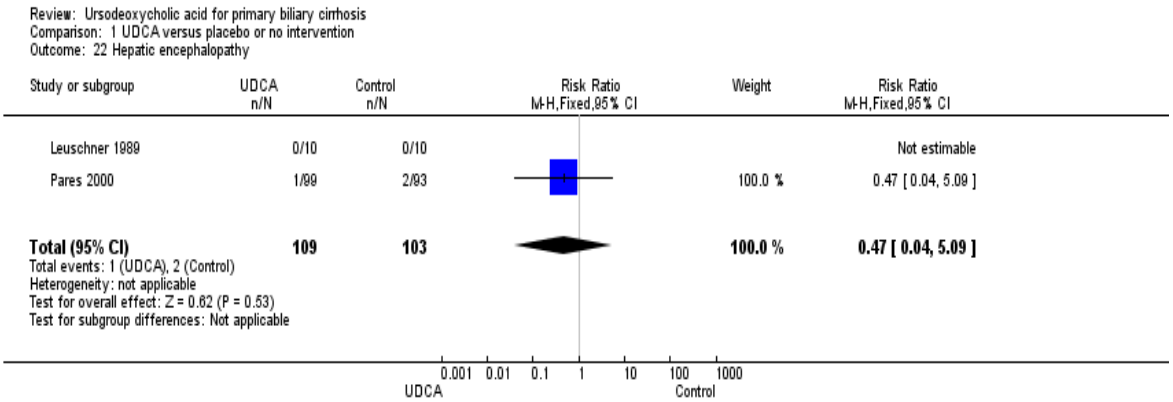

Image 38: UDCA vs placebo or no intervention; outcome: hepatic encephalopathy

Biochemical markers

Ursodeoxycholic acid significantly decreased serum bilirubin concentration (MD -8.69 µmol/l; 95% CI -13.90 to -3.48; I^2 = 0%; 881 patients, 9 trials) (Image 39).

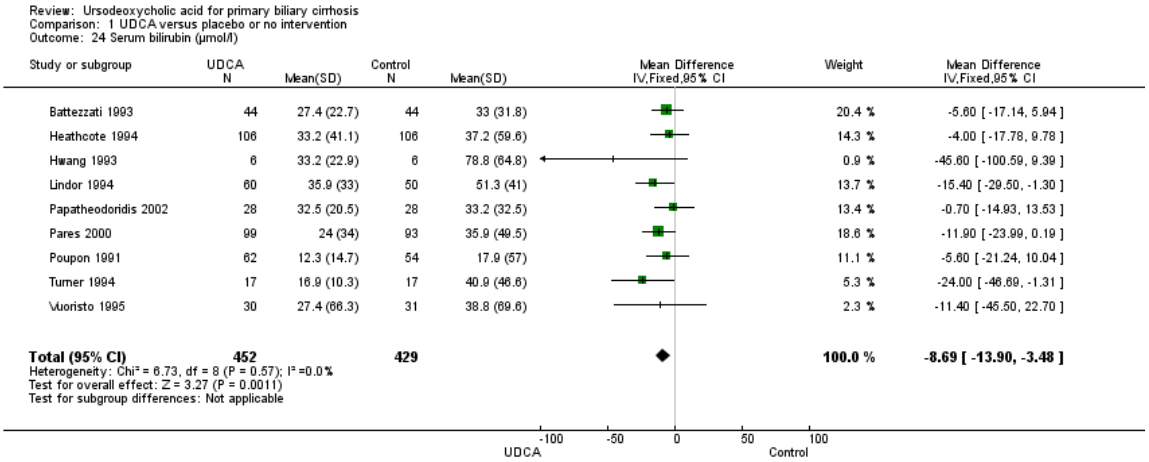

Image 39: UDCA vs placebo or no intervention; outcome: serum bilirubin

Trial sequential analysis of these data supports the finding in the meta-analysis (Image 40).

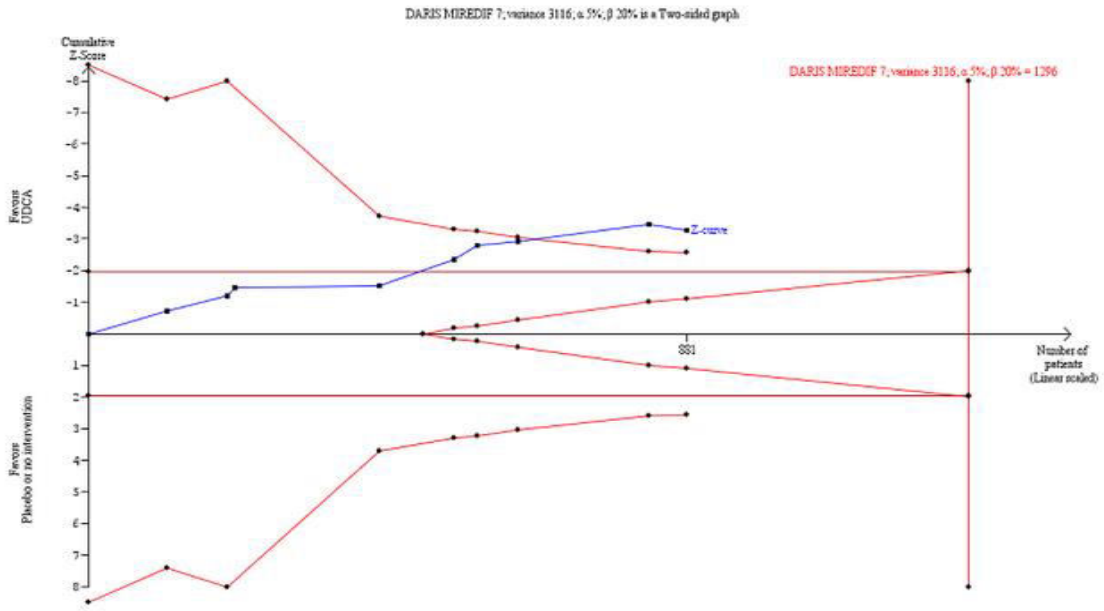

Image 40. Trial sequential analysis of the cumulative meta-analysis of the effect of ursodeoxycholic acid versus placebo or no intervention on serum bilirubin concentration in patients with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 1296 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 7 µmol/l, a standard deviation of 56 µmol/l (variance 3116), a risk of type I error of 5%, a power of 80% (a type 2 error risk of 20%) (b), and a diversity of 0%. The cumulated Z-curve (blue curve) crosses the trial sequential monitoring boundary (red curve) implying that there is evidence for a beneficial effect of 7 µmol/l decrease in the serum bilirubin concentration when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

Ursodeoxycholic acid significantly decreased the activity of serum alkaline phosphatases (MD -257.09 U/l; 95% CI -306.25 to -207.92; I^2 = 0%; 754 patients, 9 trials) (Image 41).

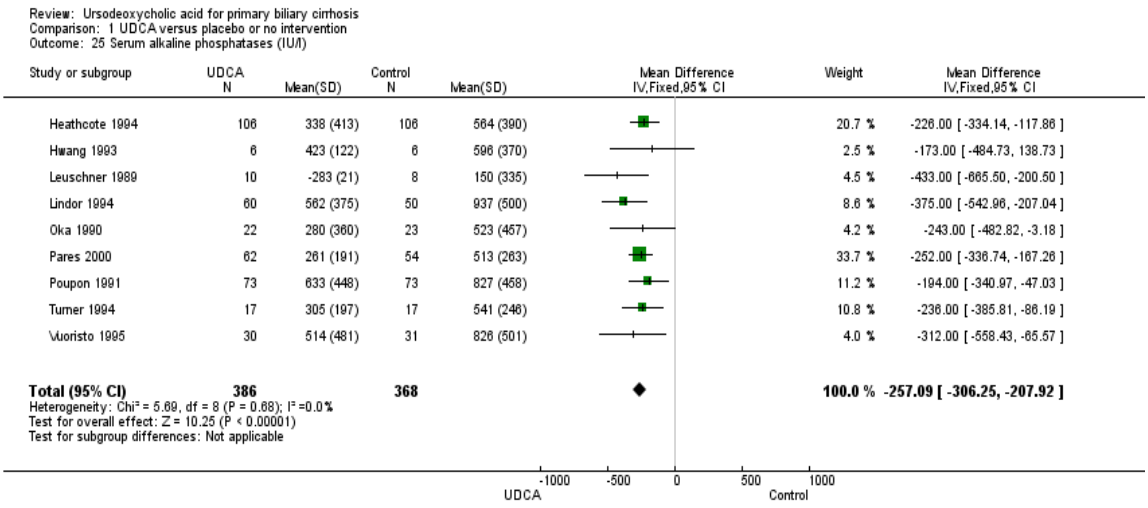

Image 41: UDCA vs placebo or no intervention; outcome: serum alkaline phosphatases

Trial sequential analysis of these data supports the finding in the meta-analysis (Image 42).

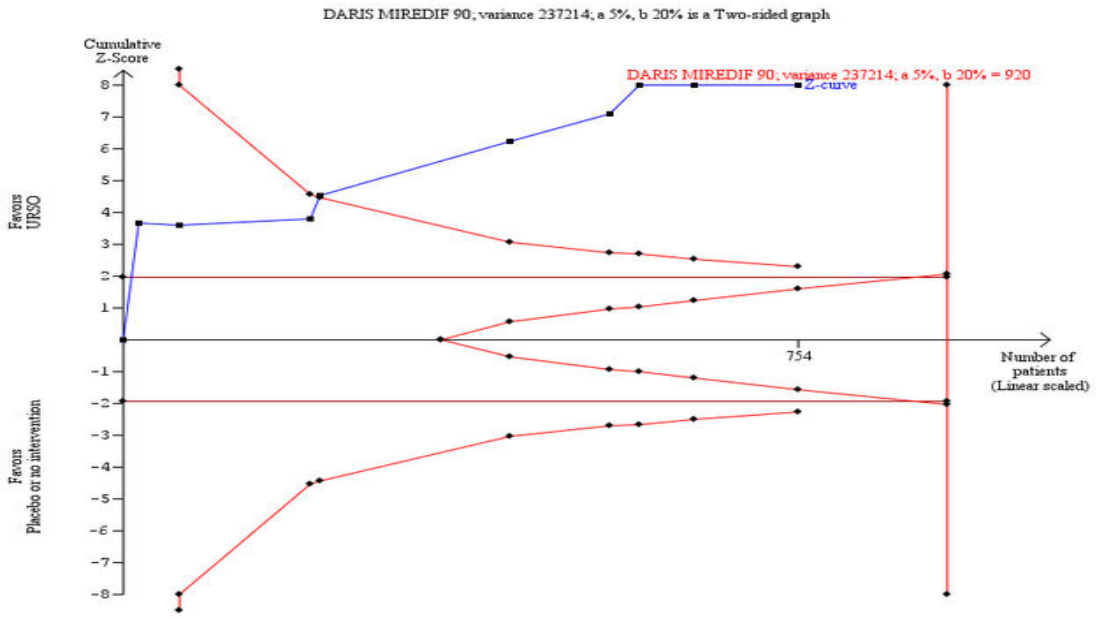

Image 42. Trial sequential analysis of the cumulative meta-analysis of the effect of ursodeoxycholic acid versus placebo or no intervention on the activity of serum alkaline phosphatases in patients with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 920 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 90 IU/L, a standard deviation of 487 IU/L (variance 237214), a risk of type I error of 5%, a power of 80% (a type 2 error risk of 20%) (b), and a diversity of 0%. The cumulated Z-curve (blue curve) crosses the trial sequential monitoring boundary (red curve) implying that there is evidence for a beneficial effect of 90 IU/L decrease in the activity of serum alkaline phosphatases when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

Ursodeoxycholic acid significantly decreased the activity of serum gamma-glutamyltransferase (MD -277.57 U/l; 95% CI -337.84 to -217.30; I^2 = 52%; 426 patients, 5 trials) (Image 43), serum aspartate aminotransferase (MD -35.59 U/l; 95% CI -42.88 to -28.30; I^2 = 0%; 782 patients, 8 trials) (Image 44), serum alanine aminotransferase (MD -34.68 U/l; 95% CI -43.04 to -26.33; I^2 = 32%; 712 patients, 8 trials) (Image 45), total cholesterol (MD -0.78 mmol/l; 95% CI -1.04 to -0.52; I^2

= 19%; 712 patients, 9 trials) (Image 46), and plasma immunoglobulin M concentration (MD -1.33 g/l; 95% CI -1.81 to -0.86; I^2 = 0%; 704 patients, 7 trials) (Image 47).

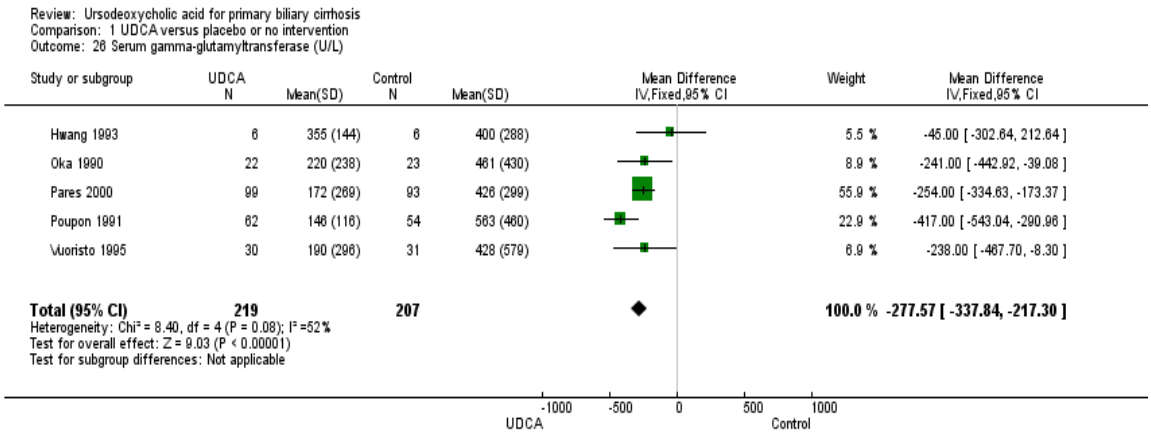

Image 43: UDCA vs placebo or no intervention; outcome: serum gamma-glutamyltransferase

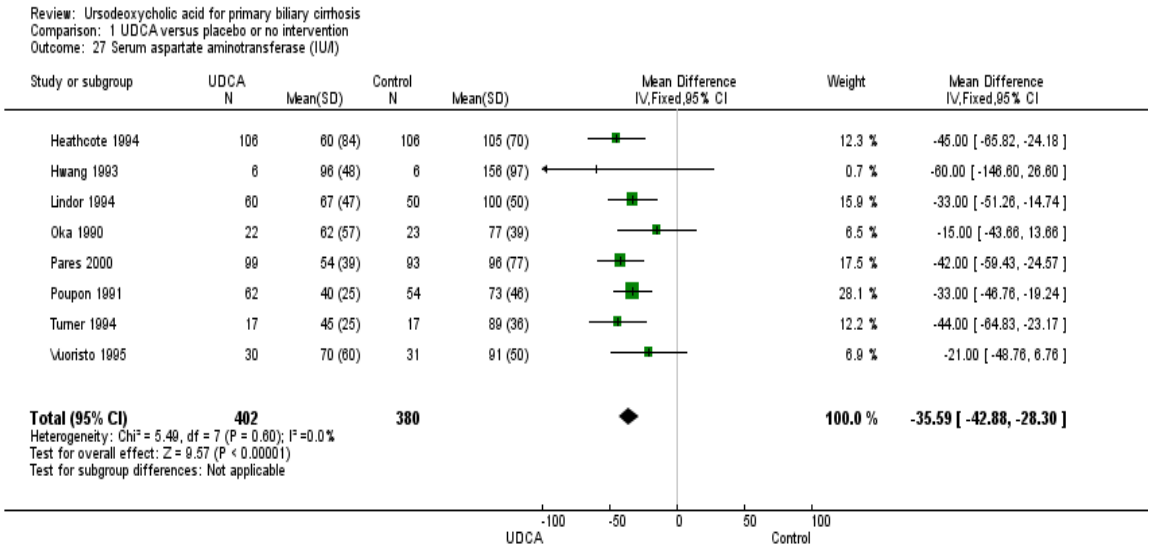

Image 44: UDCA vs placebo or no intervention; outcome: serum aspartate aminotransferase

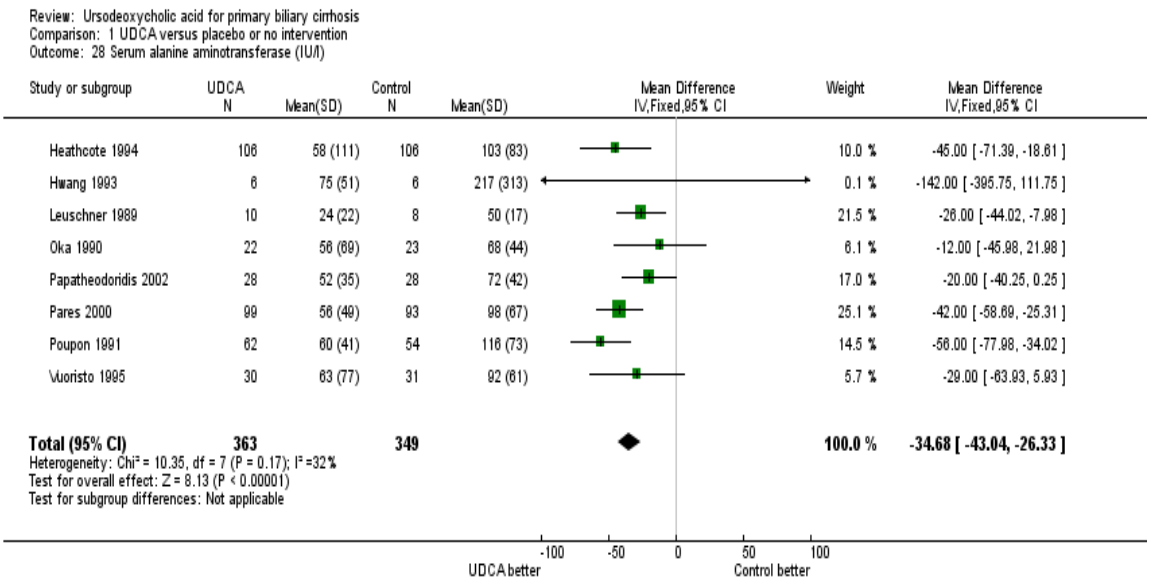

Image 45: UDCA vs placebo or no intervention; outcome: serum alanin aminotransferase

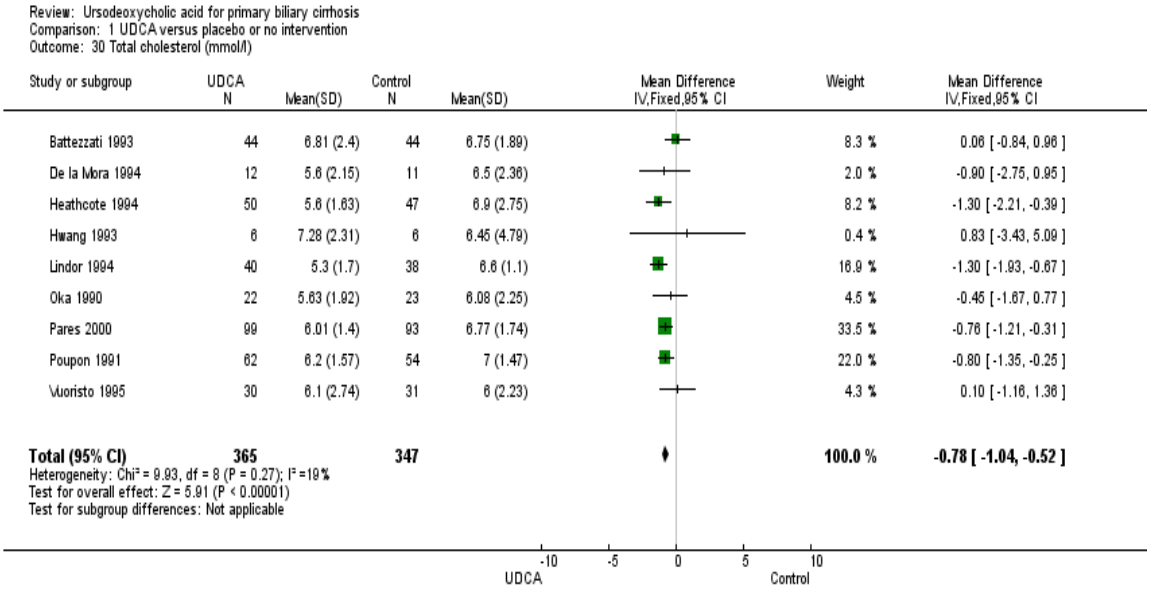

Image 46: UDCA vs placebo or no intervention; outcome: total choletserol

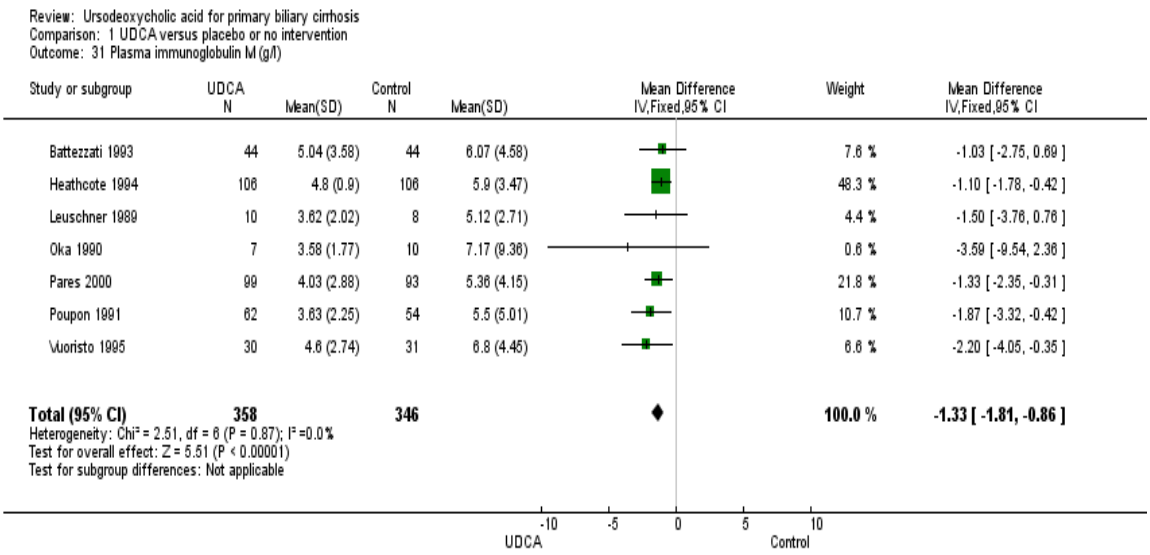

Image 47: UDCA vs placebo or no intervention; outcome: plasma immunoglobulin M

Ursodeoxycholic acid had no significant effect on serum albumin concentration (MD 0.34 mmol/l; 95% CI -0.45 to 1.13; I^2 = 0%; 457 patients, 4 trials) (Image 48) and on prothrombin index (MD 2.05 %; 95% CI -0.62 to 4.71; I^2 = 0%; 308 patients, 2 trials) (Image 49).

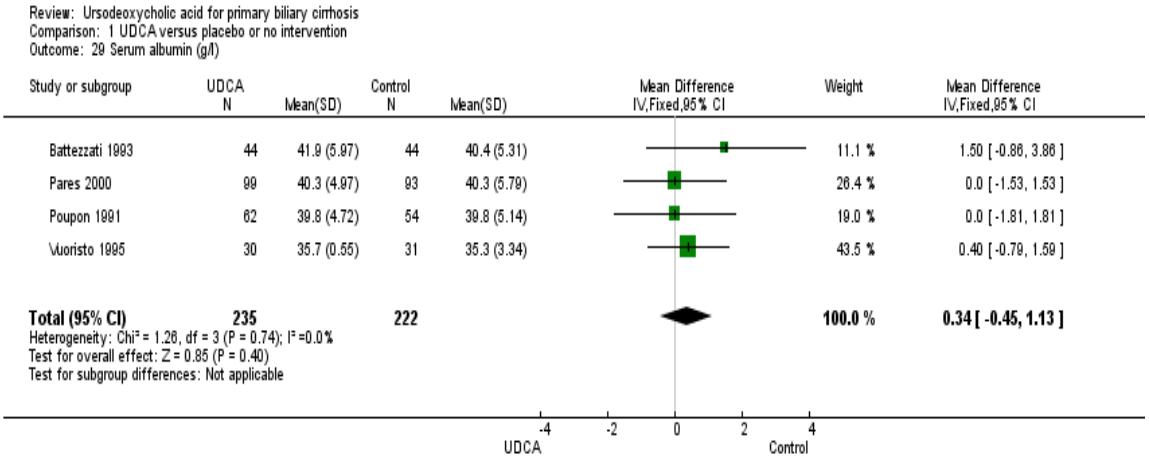

Image 48: UDCA vs placebo or no intervention; outcome: serum albumin

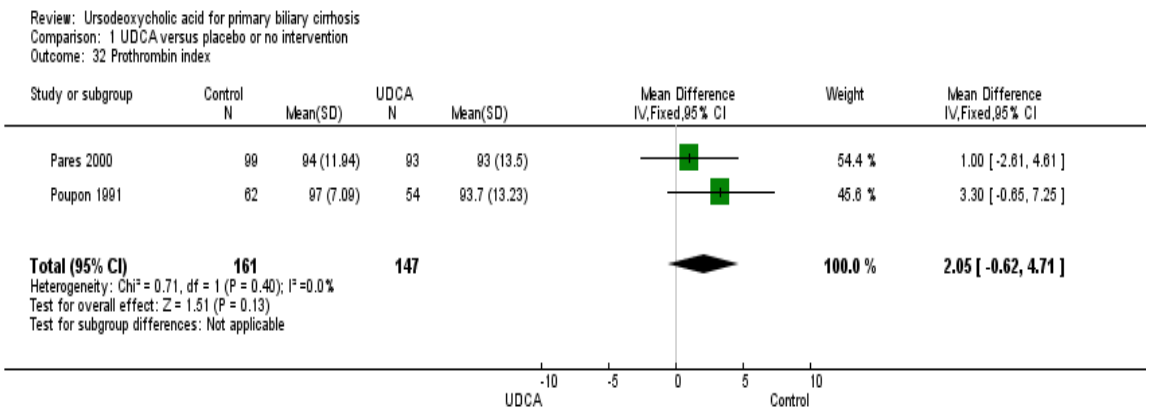

Image 44: UDCA vs placebo or no intervention; outcome: prothrombin time

Liver histology

Liver biopsies at the end of treatment were performed and reported in seven (Leuschner et al, 1989; Poupon et al, 1991; Lindor et al, 1994; Turner et al, 1994; Combes et al, 1995; Pares et al, 2000; Papatheodoridis et al, 2002) out of 16 trials. Ursodeoxycholic acid had statistically significant effect on histological stage (random, RR 0.62; 95% CI 0.44 to 0.88; I^2 = 35%; 551 patients, 7 trials) (Image 50). There was no effect of ursodeoxycholic acid on fibrosis (RR 0.88, 95% CI 0.57 to 1.38; 139 patients, 1 trial) or on florid duct lesions (RR 0.84, 95% CI 0.40 to 1.76; 115 patients, 1 trial). About half of the patients in the Pares et al, 2000 trial observed statistically significant improvements in histological stage, portal inflammation, and piecemeal necrosis in the ursodeoxycholic acid group, but not regarding ductular proliferation or cholestasis. The placebo group had significantly fewer bile ducts per portal tract. Our analyses were based on presented available patient data at the end of treatment.

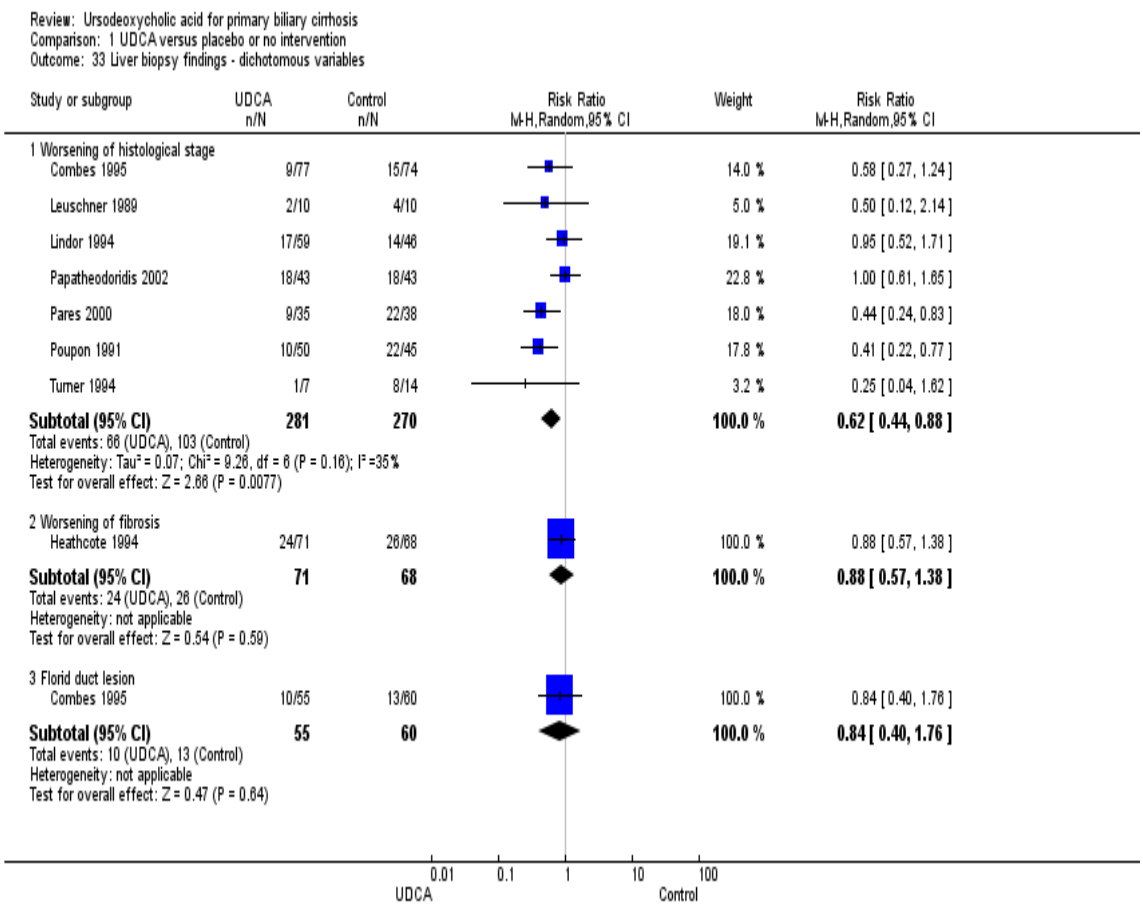

Image 50: UDCA vs placebo or no intervention; outcome: liver biopsy findings

Publication bias and other biases

Neither the Egger's nor the Begg's graphs and their corresponding tests on mortality provided evidence for asymmetry (Egger's test, P = 0.47; Begg's test, P = 0.83).

Description of studies: tables of included studies (Table 11) and tables of excluded studies (Table 12).

Table 11. Tables of included studies

Battezzati 1993

Methods	Multicenter double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration 1 year (six months treatment and six months follow-up). Follow-up: 5 patients receiving ursodeoxycholic acid and 1 placebo dropped out.
Participants	Country: Italy. Number of patients randomised: 88, mean age 54.5 years (88.5% females), histological stage IV 49%. Inclusion criteria: Primary biliary cirrhosis (PBC) defined as: - positive AMA ≥ 1:40 and liver biopsy compatible with PBC. If one of these were missing, patients could enter provided they had three of the following: - serum alkaline phosphatase > 2.0 times upper normal limit; - immunoglobulin M ≥ 280 mg/l; - pruritus; - serum bilirubin > 2 mg/l; - a positive Schyrimer's test plus absence of extrahepatic obstruction. Exclusion criteria: - serum bilirubin levels > 10 mg/dl; - ascites; - previous episodes of variceal bleeding or encephalopathy;

	- evidence of malignant conditions; - alcohol abuse.	
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 500 mg daily in two dived doses at mealtime (˜8.7 mg/kg/day; range 5.4-11.6 mg/kg/day), n = 44; Intervention group 2: placebo, n = 44. No patient was taking any medication known to be hepatotoxic nor had been treated with corticosteroids, immunosuppressant agents, colchicine, penicillamine or ursodeoxycholic acid in the previous six months.	
Outcomes	Symptoms. Liver biochemistry. Serum bile acids. Serum cholesterol.	
Notes	Patients switched onto ursodeoxycholic acid at the end of the trial.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Sequence generation was achieved using computer random number generation.
Allocation concealment	Low risk	Allocation was controlled by a central pharmacy.
Blinding	Low risk	It was reported that the trial was double-

All outcomes		blinded, that placebo was 'identical in appearance', and outcome assessment was performed centrally.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Unclear risk	It was reported that ursodeoxycholic acid and placebo were obtained through the courtesy of ABC Farmaceutici, Torino, Italy.

Combes 1995

Methods	Multicenter double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration 2 years. Follow-up: 2 patients from the ursodeoxycholic acid and 3 patients from the placebo groups withdrew from the trial during the placebo controlled period (0 to 2 year).
Participants	Country: USA Number of patients randomised: 151, from six centres, mean age 49.2 years (89% females), histological stage I-

	II 32.5%, III-IV 67.5%. Inclusion criteria: - cholestatic liver disease for at least six months; - serum alkaline phosphatase > 1.5 times upper normal limit; - positive AMA; - no biliary obstruction; - liver biopsy compatible with PBC. Exclusion criteria: - PBC treatment during the last three months; - recurrent bleeds from varices; - spontaneous encephalopathy; - diuretic-resistant ascites; - serum bilirubin ≥ 20 mg/dl; - pregnancy; - age < 19 years; - other cause of liver disease.
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 10 to 12 mg/kg/day once at bedtime (Ciba-Geigy Corporation), n = 77; Intervention group 2: placebo (2 years) and open-label ursodeoxycholic acid (4 years), n = 74.
Outcomes	Mortality free of liver transplantation. Liver transplantation. Symptoms. Liver biochemistry. Liver histology.

	ursodeoxycholic acid enrichment in bile.	
Notes	Three patients randomised to receive placebo had high bile-ursodeoxycholic acid concentrations, suggesting ursodeoxycholic acid intake. All patients were offered open label ursodeoxycholic acid following completion of the first 2-year of the trial.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Low risk	Described as double-blind, placebo described as 'comparable-appearing' and it was reported that 'coded medications were provided'. All investigators remained blinded throughout the trial to the treatment allocation for each patient.
Incomplete outcome data	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention

All outcomes		groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	The trial appears to be free of information that could put it at risk of bias.

De la Mora 1994

Methods	Randomised trial. Follow-up: information not provided.	
Participants	Patients with PBC (n = 28) from one centre in Mexico.	
Interventions	Experimental: ursodeoxycholic acid (details were not given). Control: placebo.	
Outcomes	Serum cholesterol.	
Notes		
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Unclear risk	'Placebo' employed, but it is not known if it was indeed double blind.
Incomplete outcome data All outcomes	Unclear risk	The report gave the impression that there had been no dropouts or withdrawals, but this was not specifically stated.
Selective reporting	Unclear risk	Not all pre-defined, or clinically relevant and reasonably expected outcomes are reported on or are not reported fully, or it is unclear whether data on these outcomes were recorded or not.
Other bias	Unclear risk	The trial may or may not be free of information that could put it at risk of bias.

Eriksson 1997

Methods	Multicenter double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration 2 years. Follow-up: 8 patients from the ursodeoxycholic acid and 7 patients from the placebo withdrew. Patients were stratified into symptomatic and asymptomatic.

Participants	Country: Sweden. Number of patients randomised: 116, from six centres in Sweeden, mean age 57 years (85.5% females). Inclusion criteria: PBC defined as chronic cholestatic liver disease of more than six months duration with histology typical of or compatible with PBC plus at least two of the following: - positive anti-mitochondrial antibodies; - alkaline phosphatases > 1.5 times the upper reference value; - IgM > 1.5 times the upper reference value during the year preceding the entry into the trial. Exclusion criteria: - patients with severe end-stage liver disease; - diuretic-resistant ascites; - repeated variceal bleeding in spite of sclerosing treatment; - patients waiting for liver transplantation; - pregnancy; - alcohol or drug abuse.
Interventions	Patients were randomly assigned to receive: Intervention group 1: 500 mg ursodeoxycholic acid (˜7.7 mg/kg/day) as two capsules in the evening, n = 60; Intervention group 2: placebo, n = 56.
Outcomes	Mortality. Liver transplantation.

	Symptoms - pruritus, fatigue, ascites, jaundice. Liver biochemistry and bile acids. Histology - portal inflammation, spill-over, interface hepatitis, bile duct proliferation, portal fibrosis. Quality of life.	
Notes	At 24 months, 32 of 49 patients allocated to placebo and still remaining in the trial were switched to ursodeoxycholic acid and 42 of 52 patients allocated to ursodeoxycholic acid and still remaining in the trial continued with ursodeoxycholic acid. Anti-hepatitis C virus tests not performed.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Sequence generation was achieved using a randomisation list which was produced for every clinic.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Low risk	Described as 'double-blind', and placebo looked identical to ursodeoxycholic acid, but details on taste and smell not given. However outcome assessment was blinded

		and the possible non-blinding of others unlikely to introduce bias.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	Trial appears to be free of information that could put it at risk of bias.

Goddard 1994

Methods	Double-blind, placebo controlled randomised clinical trial with parallel group design (three interventions groups and one control group). Mean follow-up: 15 months (range: 0 to 30 months).
Participants	Country: UK. Number of patients randomised: 57, mean age and sex ratio not provided. Inclusion criteria:patients with PBC. Exclusion criteria: none listed. Diagnostic criteria (data being sought).
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 10mg/kg/day. Intervention group 2: colchicine 1 mg/day. Intervention group 3: ursodeoxycholic acid plus colchicine.

	Control: placebo.	
Outcomes	Mortality (being sought). Liver transplantation (being sought). Liver biochemistry.	
Notes	No exact data on number of patients randomised to each arm. Data on mortality and liver transplantation are not given separately.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Unclear risk	'Placebo' employed, but it is not known if it was indeed double blind.
Incomplete outcome data All outcomes	Unclear risk	Treatment failures were reported but the exact numbers and reasons for dropouts and withdrawals were not described in all intervention groups.
Selective reporting	Unclear risk	One or more clinically relevant and reasonably expected outcomes were not reported fully, or

		it is unclear whether data on these outcomes were recorded or not.
Other bias	Unclear risk	The trial may or may not be free of information that could put it at risk of bias.

Heathcote 1994

Methods	Multicenter double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration 2 years. Follow-up: 13 patients receiving ursodeoxycholic acid and 19 placebo withdrew.
Participants	Country: Canada. Number of patients randomised: of 408 patients assessed, 222 patients were randomised (1:1) during a 26 months period, mean age 56.3 years (93% females), histological stage I 18.5%, II 27%, III 29%, IV 25.5%. Inclusion criteria: - positive AMA; - serum alkaline phosphatase > 1.0 times upper normal limit; - liver biopsy compatible with PBC; - age > 18 years. Exclusion criteria: - patients on liver transplant list; - patients needed to take enzyme-inducing drugs; - pregnancy; - severe coexisting condition that was likely to affect survival within five years of trial entry.

Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 14mg/kg/day swallowed with the evening meal, n = 111; Intervention group 2: placebo, n = 111.	
Outcomes	Mortality. Liver transplantation. Symptoms - pruritus, fatigue. Liver biochemistry and bile acids. Histology.	
Notes	Patients offered ursodeoxycholic acid at the end of the trial for 6 to 24 months. Data for serum cholesterol were extracted from Heathcote 1993 (Heathcote 1994).	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	The method of sequence generation was generated using consecutive identification numbers.
Allocation concealment	Low risk	Allocation was controlled separately at each centre by the trial pharmacist stratified for symptomatic/asymptomatic.
Blinding All outcomes	Low risk	Described as double-blind, and the placebo tablets were identical and 'equally bitter tasting', this was confirmed by the research coordinator. Also, outcome assessment was blinded.

Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Unclear risk	It was reported that trial medications were kindly provided by Interfalk and Jouveinal Inc., Canada.

Hwang 1993

Methods	Double-blind, placebo controlled randomised clinical trial with cross-over group design (two interventions groups). Trial duration: 3 months. Follow-up: no patients withdrew.
Participants	Country: China. Number of patients randomised: 12, mean age 58 years (100% females). Inclusion criteria: - elevated serum alkaline phosphatase and gamma-glutamyl transferase with lack of large bile duct abnormalities; - positive AMA with elevated immunoglobulin M, G or A; - liver biopsy compatible with PBC. Exclusion criteria: - previous PBC treatment.
Interventions	Patients were randomly assigned to receive:

	Intervention group 1: ursodeoxycholic acid 600 mg/day. Intervention group 2: placebo.	
Outcomes	Mortality. Symptoms. Liver biochemistry.	
Notes	All patients switched to ursodeoxycholic acid on completion of the six months cross-over trial.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Low risk	It was reported that placebo was 'identical tablet form containing starch'.
Incomplete outcome data All outcomes	Low risk	It was specified that there were no dropouts or withdrawals, and that all 12 patients completed a six month course of treatment.
Selective reporting	Low risk	All expected outcomes are reported.

Other bias	Low risk	The trial appears to be free of information that could put it at risk of bias.

Leuschner 1989

Methods	Double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration: 9 months. Follow-up: 2 patients from placebo arm left the trial.
Participants	Country: Germany. Number of patients randomised: 20, mean age not provided (90% females). Inclusion criteria: PBC defined as at least three of the following: - alkaline phosphatase > 1.7 times upper normal limit; - gamma-glutamyl transferase > 5.0 times upper normal limit; - immunoglobulin M > 2.0 times upper normal limit; - positive AMA plus no obstruction of the extrahepatic biliary tract. Exclusion criteria: - oesophageal varices; - ascites; - pancreatitis; - cardiac failure or renal failure; - pregnancy; - age < 30 years; - any previous PBC treatment within the four weeks;

	- alcohol or drug abuse.	
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 10 mg/kg/ day, divided into two doses, n = 10. Intervention group 2: placebo, n = 10.	
Outcomes	Outcome measure(s): - mortality; - symptoms; - liver biochemistry; - liver histology.	
Notes	Two patients from the placebo arm left the trial for reasons unrelated to the trial and are not considered in the analysis of the results.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Low risk	It was reported that placebo was 'identical tablet'.

Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	All expected outcomes are reported.
Other bias	Low risk	The trial appears to be free of information that could put it at risk of bias.

Lindor 1994

Methods	Multicenter double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration: 4 years. Follow-up: five voluntary withdrawals in ursodeoxycholic acid arm and 13 voluntary withdrawals in the placebo arm.
Participants	Country: USA. Number of patients randomised: 180, enrolled from four USA centres, mean age 53 years (89% females). However, 162 patients (90%) came from one centre. Inclusion criteria: PBC defined as: - chronic cholestatic liver disease for at least six months; - serum alkaline phosphatase level > 1.5 times upper normal limit; - antimitochondrial antibody positivity; - absence of biliary obstruction;

	- liver biopsy compatible with PBC. Exclusion criteria: - previous PBC treatment in preceding 3 months; - anticipated need for liver transplantation within one year; - recurrent variceal haemorrhage; - spontaneous encephalopathy, or diuretic resistant ascites; - pregnancy; - age less than 18 or more than 70 years; - other co-existent liver disease.
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid in the form of 250 mg tablets at a dose of 13 to 15mg/kg/day in four divided doses, n = 89; Intervention group 2: placebo, n = 91.
Outcomes	Outcome measure(s): - mortality; - liver transplantation; - symptoms; - autoimmune conditions; - liver biochemistry; - liver histology; - adverse events.
Notes	Patients originally receiving placebo switched to ursodeoxycholic acid after four years and were followed for an additional eight years.

	Data for the following outcomes were extracted from (Lindor 1994): - development of varices (Angulo et al, 1999); - bleeding varices (Lindor et al, 1997); - ascites (Lindor et al, 1997); - cholesterol (Balan et al, 1994).	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Randomisation was performed separately for each strata using 'a blocked, randomised assignment schedule'.
Allocation concealment	Low risk	Allocation was controlled so that intervention allocations could not have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Low risk	The trial was described as blinded, the parties that were blinded, and the method of blinding was described, so that knowledge of allocation was adequately prevented during the trial.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	The trial appears to be free of other

	information that could put it at risk of bias.

Oka 1990

Methods	Multicenter double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration: 24 weeks. Follow-up: 4 patients receiving ursodeoxycholic acid and 3 placebo dropped out.
Participants	Country: Japan. Number of patients randomised: 52, from 13 departments in Japan, mean age 59 years (91% females). Inclusion criteria: - PBC was diagnosed clinically and histologically. Exclusion criteria: - patients with severe symptoms or having received other medications for their PBC within the last three months.
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 600 mg/day in three divided doses, n = 26; Intervention group 2: placebo, n = 26.
Outcomes	Symptoms (itching). Complications (oesophageal varices). Liver biochemistry. Serum cholesterol. Serum bile acids.

Notes		
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Low risk	Allocation was controlled by a single monitor according to a randomisation scheme (1:1), so that intervention allocations could not have been foreseen in advance of, or during, enrolment.
Blinding All outcomes	Low risk	"Placebo tablets could not be distinguished from ursodeoxycholic acid tablets".
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	The trial appears to be free of information that could put it at risk of bias.

Papatheodoridis 2002

Methods	Randomised clinical trial with parallel group design (two

	interventions groups). Trial duration: 92 months. Follow-up: no patients lost to follow-up.
Participants	Country: Greece. Number of patients randomised: 86, mean age 54 years (89% females). Inclusion criteria: - liver histology compatible with PBC; - positive antimitochondrial antibodies; - alkaline phosphatase levels more than twice the upper limit of normal. Exclusion criteria: - extrahepatic biliary obstruction or other cause of liver disease; - patients aged > 70 years; - patients treated with any immunosuppressive agent within the 12 months before entry; - patients with decompensated cirrhosis (Child class B or C); - baseline bilirubin levels ≥ 3 mg/dl.
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 12 to 15 mg/kg/day, n = 43; Intervention group 2: no intervention, n = 43.
Outcomes	Liver decompensation. Mortality or liver transplantation. Symptoms.

	Liver biochemistry. Liver histology.	
Notes	14/43 control patients were crossed-over to ursodeoxycholic acid at their own request at a median of 3.5 years (range 2 to 8 years) after entry in the trial. Mean follow-up was 7.3 ± 3.0 years in the ursodeoxycholic acid group and 8.1 ± 3.1 years in the control group. The authors did both intention-to-treat analysis and treatment-as-received analysis. Data for the following outcomes were extracted from graphs from Hadziyannis 1990 (Papatheodoridis et al, 2002): - serum bilirubin; - serum alanine aminotransferase.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Sequence generation was achieved using random number table.
Allocation concealment	Low risk	Allocation was controlled by serially numbered sealed envelopes.
Blinding All outcomes	Unclear risk	The trial did not address this component and it was likely unblinded.
Incomplete outcome data All outcomes	Low risk	It was specified that there were no dropouts or withdrawals.
Selective reporting	Low risk	Pre-defined, or clinically relevant and

		reasonably expected outcomes are reported on.
Other bias	Unclear risk	The trial reported a grant from the pharmaceutical company Galenica Hellas.

Pares 2000

Methods	Double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration: at least 2 years (median follow-up was 3.4 years). Follow-up: 10 ursodeoxycholic acid treated patients and 21 placebo treated patients discontinued.
Participants	Country: Spain. Number of patients randomised: 192, from 16 hospitals in Spain, mean age 54 years (93% females). Inclusion criteria: - compatible liver biopsy; - alkaline phosphatase > 2 upper normal limit; - positive antimitochondrial antibodies; - patients with negative antimitochondrial antibodies were accepted if there was no evidence of extrahepatic biliary obstruction. Exclusion criteria: - age > 72 years; - previous PBC treatment in the 6 months before entry; - life expectancy less than 6 months; - drug addiction; - pregnancy;

	- other cause of liver disease.	
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 14 to 16 mg/kg/day in three divided doses, n = 99; Intervention group 2: no intervention, n = 93.	
Outcomes	Mortality. Liver transplantation. Symptoms. Complications. Liver biochemistry. Liver histology. Adverse events.	
Notes	Data for liver biopsy findings - dichotomous variables outcome were extracted from Pares 2001 (Pares et al, 2000). Additional information requested on 26th January 2012 and reply received on 31st January 2012 through personal communication with the principal author Dr. Albert Pares who provided data on the method of sequence generation.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Patients were randomised to take ursodeoxycholic acid or placebo (ratio 1: 1), using a randomisation code generated by computer.
Allocation	Low risk	Allocation was controlled by serially

concealment		numbered sealed and opaque envelopes.
Blinding All outcomes	Low risk	The trial was described as blinded, the parties that were blinded, and the method of blinding was described ('placebo was identical in appearance, smell, and taste'), so that knowledge of allocation was adequately prevented during the trial.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Unclear risk	It was reported that trial medications were provided by Zambon S. A., Laboratorio Farmaceutico.

Poupon 1991

Methods	Multicenter double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups). Trial duration: 2 years. Follow-up: 5 patients receiving ursodeoxycholic acid and 6 placebo withdrew.
Participants	Country: France and Canada. Number of patients randomised: 146, from 22 centres in France and Canada, mean age 56 years (92% females).

	Inclusion criteria: - liver biopsy compatible with PBC; - serum alkaline phosphatase > 2.0 upper normal limit; - positive AMA. Exclusion criteria: - PBC treatment within last six months; - serum bilirubin > 150 µmol/l; - serum albumin < 25 g/l; - past or active bleeding oesophageal varices; - presence of extrahepatic obstruction; - excessive alcohol consumption; - positive hepatitis B surface antigen.
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 13 to 15 mg/kg/day, n = 73; Intervention group 2: placebo, n = 73.
Outcomes	Mortality. Liver transplantation. Symptoms. Liver biochemistry. Liver histology.
Notes	All patients treated for two years with placebo were offered ursodeoxycholic acid and further followed-up for another two years together with patients continuing on ursodeoxycholic acid. One patient, included in the publications of the study up to 1993, was excluded from the 1994 publication due to a raised

	serum bilirubin at entry, which violated the entry criteria. Data were extracted at the maximum follow-up where applicable, if not the end of treatment was used for data extraction.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Low risk	The trial was described as blinded, the parties that were blinded, and the method of blinding was described - placebo was 'identical capsule', so that knowledge of allocation was adequately prevented during the trial.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	The trial appears to be free of information that could put it at risk of bias.

Senior 1991

Methods	Double-blind randomised clinical trial with parallel group design (two interventions groups). Trial duration: six months. Follow-up: no patients withdrew.
Participants	Country: USA. Number of patients randomised: 19, mean age 53 years (75% females). Inclusion criteria: - PBC confirmed by liver biopsy and supporting clinical ests within six months of entry into the trial. Exclusion criteria - none listed.
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 10 mg/kg/ day, n = 9; Intervention group 2: placebo, n = 10.
Outcomes	Mortality. Symptoms. Liver biochemistry.
Notes	Data for the following outcomes were extracted from O'Brian 1990 (Senior and O'Brian, 1991): - mortality; - liver transplantation.
Risk of bias	

Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Unclear risk	The trial was described as double-blind, but the method of blinding was not described, so that knowledge of allocation was possible during the trial.
Incomplete outcome data All outcomes	Unclear risk	The report gave the impression that there had been no dropouts or withdrawals, but this was not specifically stated.
Selective reporting	Unclear risk	Not all pre-defined, or clinically relevant and reasonably expected outcomes are not reported fully and properly.
Other bias	Unclear risk	The trial reported partial support for ursodiol supplies by Ciba-Geigy Corporation.

Turner 1994

Methods	Double-blind, placebo controlled randomised clinical

	trial with parallel group design (two interventions groups). Trial duration: 2 years. Follow-up: 5 patients receiving ursodeoxycholic acid and 4 placebo withdrew.
Participants	Country: UK. Number of patients randomised: 46, mean age 57 years (96% females). Inclusion criteria: - liver biopsy compatible with PBC; - positive AMA; - abnormal liver function tests; - no medication within six months of trial entry. Exclusion criteria: - none listed.
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 10mg/kg/day (mean actual dose (+/-SD): 11.4+/-0.9 mg/kg/day), n = 22; Intervention group 2: placebo, n = 24.
Outcomes	Mortality. Liver transplantation. Symptoms. Liver biochemistry. Liver histology. Quality of life.
Notes	Data for the following outcomes were extracted from the

	preliminary report of the included trial (Myszor 1990): - pruritus score; - serum bilirubin; - serum alkaline phosphatases; - serum aspartate aminotransferase. Number of patients randomised 34, follow-up 1 year.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Low risk	The trial was described as blinded, the parties that were blinded, and the method of blinding was described, so that knowledge of allocation was adequately prevented during the trial.
Incomplete outcome data	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.

Other bias	Unclear risk	It was reported that trial medications were generously donated by Thames Laboratories, Wrex-ham, Wales.

Vuoristo 1995

Methods	Double-blind, placebo controlled randomised clinical trial with parallel group design (two interventions groups and one control group). Trial duration: 2 years. Follow-up: 0 patients receiving ursodeoxycholic acid and 8 placebo withdrew.
Participants	Country: Finland. Number of patients randomised: 90, from four centres in Finland, mean age 55 years (82% females). Inclusion criteria: - elevated serum alkaline phosphatases activity; - liver biopsy compatible with PBC; - positive AMA. Exclusion criteria: - other cause of liver disease; - positive hepatitis B surface antigen and hepatitis C antibodies; - end-stage PBC; - patients treated with drugs that might affect prognosis; - serum bilirubin level > 150 μmol/L; - serum albumin level < 25 g/L; - drug-resistant ascites; - patients in whom liver transplantation was indicated;

	- previous PBC treatment for 6 months before the trial.	
Interventions	Patients were randomly assigned to receive: Intervention group 1: ursodeoxycholic acid 12 to 15 mg/kg/day in two doses, n = 30; Intervention group 2: colchicine 1 mg/day, n = 29; Control: placebo, n = 31.	
Outcomes	Mortality. Liver transplantation. Symptoms. Liver biochemistry. Liver histology. Adverse events.	
Notes		
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	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.
Blinding All outcomes	Unclear risk	The trial was described as blind, but the method of blinding was not described fully

		(it was only reported that placebo was used, but no mention on appearance), so knowledge of allocation was possible during the trial. The outcome assessment was blinded.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Unclear risk	It was reported that ursodeoxycholic acid tablets were donated by Leiras Oy, Helsinki, Finland.

Table 12. Tables of excluded studies

Study	Reason for exclusion
Angulo 1999	This is not a randomised trial, but a comparison of liver histology of 16 ursodeoxycholic acid treated patients from one randomised trial to the liver histology of 51 patients from another randomised trial.
Angulo 1999a	There is no placebo or no intervention group in this randomised trial, which compares low (5 to 7 mg/kg/day), standard (13 to 15 mg/kg/day), and high (23 to 25 mg/kg/day) doses of ursodeoxycholic acid in 155 patients with PBC. The improvements in alkaline

	phosphatase, aspartate aminotransferase, Mayo risk score, and biliary ursodeoxycholic acid enrichment were significantly greater in the standard- and high-dose groups compared to the low-dose group, but not between the standard- and high-dose group. No significant effects were noted on symptoms with any dose.
Bateson 1998	This is a case series of 40 PBC patients with symptomatic disease treated with ursodeoxycholic acid. The results were compared to 12 historic ursodeoxycholic acid-untreated PBC patients.
Brodanova1997	This is a case series of 13 PBC patients treated with ursodeoxycholic acid.
Cauch-Dudek 1998	This is a case series of 88 patients with PBC evaluating fatigue. A self-rated fatigue. Severity score did not correlate with ursodeoxycholic acid use.
Crippa 1995	The trial is not randomised, but compares 18 ursodeoxycholic acid treated PBC patients to eight untreated PBC patients.
Crosignani 1996	This is a dose-response study examining the effects of three doses of tauro-ursodeoxycholic acid in 24 patients with PBC.
Eisenburg 1988	This is a case series of 21 PBC patients during ursodeoxycholic acid administration.
Ferri 1993	This is a controlled comparison of ursodeoxycholic acid with tauro-ursodeoxycholic acid for PBC.
Grippa 1995	This is a non-randomised study comparing 18

	ursodeoxycholic acid treated PBC patients to eight ursodeoxycholic acid-untreated PBC patients.
Ideo 1990	Out of three PBC patients treated with ursodeoxycholic acid (600 mg/day), ursodeoxycholic acid was stopped in one of these patiens 'randomly selected'.
Ikeda 1996	This is a randomised trial comparing ursodeoxycholic acid plus colchicine versus ursodeoxycholic acid alone in 22 patients with PBC.
Kehagioglou1991	The study is not described as randomised, but compares 16 PBC patients treated with ursodeoxycholic acid (14 mg/kg/day for a mean period of 22 months (range 3 months to 35 months) to a control group consisting of 10 PBC patients treated with placebo.
Kim 1997	This is a case series of eight ursodeoxycholic acid-treated PBC patiens who lacked antimitochondrial antibodies.
Kneppelhout1992	This is a case series of 19 patients with PBC during ursodeoxycholic acid administration.
Krzeski 1999	This is a case series of 60 PBC patients treated with ursodeoxycholic acid.
Larghi 1997	This is a randomised trial with crossover design comparing ursodeoxycholic acid versus tauro-ursodeoxycholic acid.
Leuschner 1996	This randomised trial compared ursodeoxycholic acid plus prednisolone versus ursodeoxycholic acid plus placebo for PBC.

LONDON 1998	This trial compared placebo to different doses of URSO (300 mg/day, 600 mg/day, 900 mg/day and 1200 mg/day) in 23 biopsy proven early stage PBC patients. There is no mention of randomisation. Patients were followed for eight weeks with a four week washout period between doses. A significant trend toward normalising of abnormal liver function tests was observed together with a significant increase in lethargy, irrespective of ursodeoxycholic acid dose, compared to placebo.
Lotterer 1990	This is a case series of twelve PBC patients during ursodeoxycholic acid administration.
Matsuzaka 1994	This is a case series of three PBC patients during ursodeoxycholic acid administration.
Matsuzaki 1990	This is a case series of ten PBC patients during ursodeoxycholic acid administration.
MAYO-II 1997	This trial randomised 150 PBC patients to three doses of ursodeoxycholic acid (5 to 7 mg/kg/day; 13 to 15 mg/kg/day; 22 to 25 mg/kg/day) and followed the patients for one year. No differences were observed between the medium and the high dose with respect to liver biochemistry changes, but both these dose groups had significantly greater improvement of liver biochemistry compared to the low dose group. Clinical events such as death, transplantation, or complications of liver disease were rare and were not different between the three dose groups.

NEWARK-I	The study is not randomised. The study included only four patients with PBC and apparently these were treated first with placebo for three months and then with ursodeoxycholic acid (10-15 mg/kg/day) for three-six months. No major outcome variables are reported.
NEWARK-III	This study investigated biochemical features, including biliary bile acids, in 14 patients with PBC using a paired design. First, all patients received placebo for three months. Then, the patients were treated with 900 mg ursodeoxycholic acid (10-12 mg/kg/day) for six months (n = 11) to 12 months (n = 8). The latter patients were then treated with placebo for three months and restarted on ursodeoxycholic acid for another 12 months. Due to the paired design, the observed improvements may be due to the fluctuating course of PBC.
Ogino 1993	This is a case series of 28 PBC patients treated with ursodeoxycholic acid and compared to seven PBC patiens not treated with ursodeoxycholic acid.
Okuyama 1988	This is a study of a single PBC patient during ursodeoxycholic acid administration.
Osuga 1989	This is a case series of eight PBC patients during ursodeoxycholic acid administration.
Peridigoto 1992	This is a study of three PBC patiens during ursodeoxycholic acid administration.
Podda 1989	This is a randomised trial examining three doses of ursodeoxycholic acid in PBC patients and patients with

	primary sclerosing cholangitis and chronic hepatitis.
Poupon 1987	This is a case series of 15 PBC patients during ursodeoxycholic acid administration.
Poupon 1989	This study is not randomised.
Poupon 1996	This is a randomised trial comparing ursodeoxycholic acid plus colchicine versus ursodeoxycholic acid in 74 patients with PBC.
Schonfeld 1997	This is a case series of 15 PBC patients during ursodeoxycholic acid administration.
Shibata 1992	This is a case series of 12 PBC patients during ursodeoxycholic acid administration.
Stiehl 1990	This is a case series of 29 patients with PBC during ursodeoxycholic acid administration.
Taha 1994	This is a case series of patients with PBC during different drug administrations (cholestyramine, wash out, ursodeoxycholic acid, and ursodeoxycholic acid plus cholestyramine).
Takezaki 1991	This is a study of a single PBC patient during ursodeoxycholic acid administration.
Toda 1998	No placebo or no intervention group are included. The trial compares the efficacy of three doses of ursodeoxycholic acid (150 mg/day; 600 mg/day; 900 mg/day) in 82 PBC patients for 24 months.
Unoura 1990	Not a randomised trial, but compares 16 ursodeoxycholic acid treated PBC-patients to eight patients without ursodeoxycholic acid treatment.

Van de Meeberg 1996	No placebo or no intervention group. Five patients treated 'in random order' with 10 mg ursodeoxycholic acid/kg/day in either a single or in three divided doses - no difference in liver biochemistry improvement.
Van Hoogstraten 1998	This RCT compares 10 versus 20 mg ursodeoxycholic acid/kg/day during six months in 61 PBC patients. Liver biochemistry improved in PBC patients receiving 20 mg/kg/day compared to a dose of 10 mg/kg/day.
Verma 1999	This cross-over RCT compares different doses of ursodeoxycholic acid in twenty-four biopsy-proven early-stage PBC patients (one male, 23 female) who received five doses of ursodeoxycholic acid (0, 300, 600, 900, 1200 mg/day) each for eight weeks with four-week washout periods between doses. Symptoms (pruritus, fatigue, diarrhoea) were assessed on a four-point scale (none, mild, moderate, severe). Liver function tests were performed using conventional methods, and serum bile acids were measured using gas liquid chromatography. There was a trend towards normalization of the abnormal LFTs in a dose-dependent manner (for Y-glutamyl transferase (yGT), alkaline phosphatase (ALP), alanine transaminase (ALT) and IgM). Multi-factorial analysis showed that ursodeoxycholic acid treatment, irrespective of dose, was significantly better than placebo for all the variables. The 900 mg and 1200 mg doses were better than both 300 mg and 600 mg using gamma-glutamyl transpeptidase and total bilirubin as variables, better than 300 mg using alkaline phosphatase

	and IgM as variables, and better than 600 mg using albumin as a variable. No variables showed a significant difference between 900 and 1200 mg. The study concluded that the optimum dose of ursodeoxycholic acid is 900 mg/day (equivalent to 13.5 mg/kg/day). This trial is excluded due to the cross-over design and due to the fact that it did not provide any data on the primary outcome variables.
Wirth 1994	This is a case series of 14 patients with PBC examined before and during ursodeoxycholic acid administration.
Wirth 1995	This is a case series of 22 patients with PBC, who have their subtypes of antimitochondrial antibodies examined and related to response to ursodeoxycholic acid administration.
Wolfhagen 1994	No randomisation, combination therapy with ursodeoxycholic acid and prednisone in seven patients.
Yamazaki 1992	This is a study of a single PBC patient with eosinophilic infiltration.
Yamazaki 1996	This is a case series of 38 PBC patients, of which 55 per cent exhibited eosinophilia. The eosinophilia was reduced during ursodeoxycholic acid treatment.

Bezafibrate (Paper II)

Results of the search

Our search strategy identified 95 publications, out of which 26 were duplicates. Of the remaining 69 publications, 57 were excluded, either because they were reviews or because they did not relate to primary biliary cirrhosis or because they did not describe a randomised clinical trial investigating the effect of bezafibrate in patients with primary biliary cirrhosis. Twelve full text articles were assessed for eligibility, out of which five were excluded with listed reasons (Image 51).

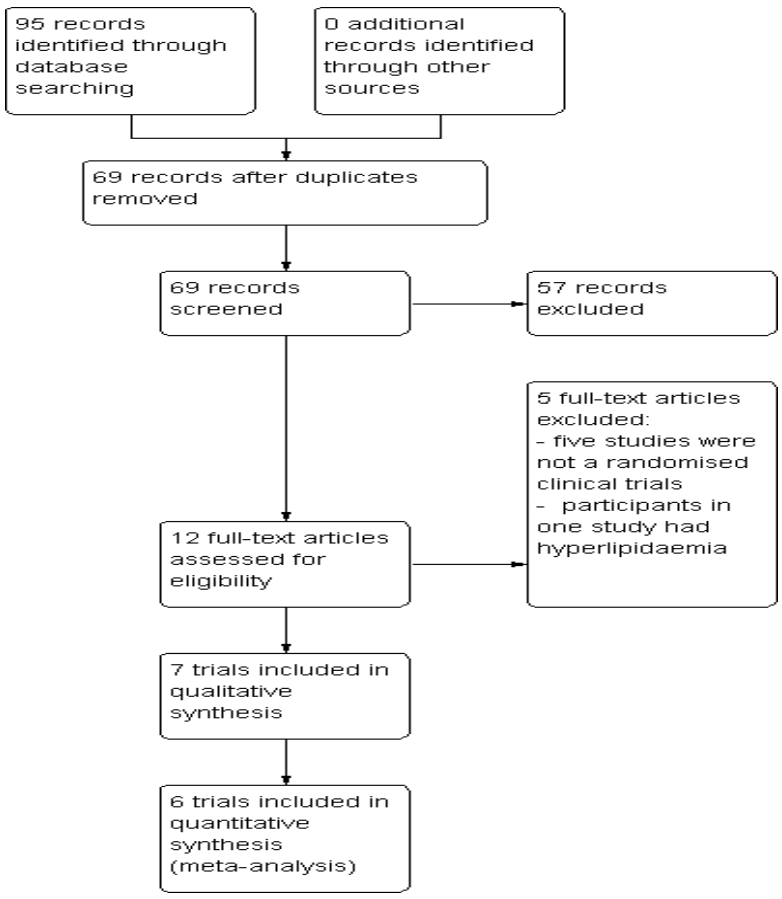

Image 51. Flow chart

We identified a total of seven publications referring to six randomised clinical trials (Table 13). Four trials were published as full text articles (Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008a; Iwasaki et al, 2008b). One trial was published as an abstract and as a letter to the editor (Nakai et al, 1999). Another trial was published only as a letter to the editor (Kurihara et al, 2000). The primary authors were contacted for further information and data relating to the trials. Dr. Shinji Iwasaki, kindly provided data on the method of sequence generation, the number of patients in each intervention group at the end of treatment, adverse events, and outcome measures (Iwasaki et al, 2008a; Iwasaki et al, 2008b). No other responses have so far been received. We contacted manufacturers of bezafibrate and asked for any information about unpublished or on-going trials using bezafibrate involving patients with primary biliary cirrhosis. No responses have so far been received. Through a search for ongoing trials in Clinicaltrials.gov (http://clinicaltrials.gov/) we have not identified any registered ongoing or planned trials. However, through a search for ongoing trials in the WHO International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/), we identified one ongoing trial. This trial has been classified as an ongoing trial (Table 15).

Included studies

A total of 151 patients with primary biliary cirrhosis were randomised in the six randomised clinical trials. All trials were conducted in Japan. From the publications which reported sex of the patients, more than 86% were females. In four trials, all patients had non-advanced primary biliary cirrhosis according to inclusion and exclusion criteria (Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008a; Iwasaki et al, 2008b). In two trials, no data about severity of primary biliary cirrhosis among the patients and the exclusion criteria were provided (Nakai et al, 1999; Kurihara et al, 2000). Five trials had the parallel group design (Nakai et al, 1999; Kurihara et al, 2000; Kanda et al, 2003; Iwasaki et al, 2008a; Iwasaki et al, 2008b), and one trial had the cross-over group design (Itakura et

al, 2004). Four trials assessed bezafibrate plus ursodeoxycholic acid versus no intervention plus ursodeoxycholic acid (referenced as bezafibrate versus no intervention in the following) (Nakai et al, 1999; Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008b), and two trials assessed bezafibrate versus ursodeoxycholic acid (Kurihara et al, 2000; Iwasaki et al, 2008a). Bezafibrate was given in a dose of 400 mg daily and ursodeoxycholic acid in a dose of 600 mg daily in all trials. In two trials duration of administration of bezafibrate was six months (Kanda et al, 2003; Itakura et al, 2004), and in four trials duration of administration of bezafibrate was 12 to 13 months (Nakai et al, 1999; Kurihara et al, 2000; Iwasaki et al, 2008a; Iwasaki et al, 2008b). All the trials reported similar outcome measures: clinical events, changes in biochemical and immunological variables, and adverse events. None of the trials reported on quality of life or fatigue.

Excluded studies

Five studies were excluded; four studies were not randomised clinical trials (Iwasaki et al, 1999; Miyaguchi et al, 2000; Ohmoto et al, 2001; Hazzan and Tur-Kaspa, 2010), and in one study patients had hyperlipidaemia, not primary biliary cirrhosis (Fukuo et al, 1996) (Table 14).

Risk of bias in included studies

Risk of bias was assessed according to six components: allocation sequence generation; allocation concealment; blinding; handling of incomplete outcome data; selective outcome reporting; and other potential sources of bias. Of the six included trials, all trials were assessed as having high risk of bias (Nakai et al, 1999; Kurihara et al, 2000; Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008a; Iwasaki et al, 2008b) (Image 52). Our statistical analyses are, therefore, based only on trials with high risk of bias (Image 53).

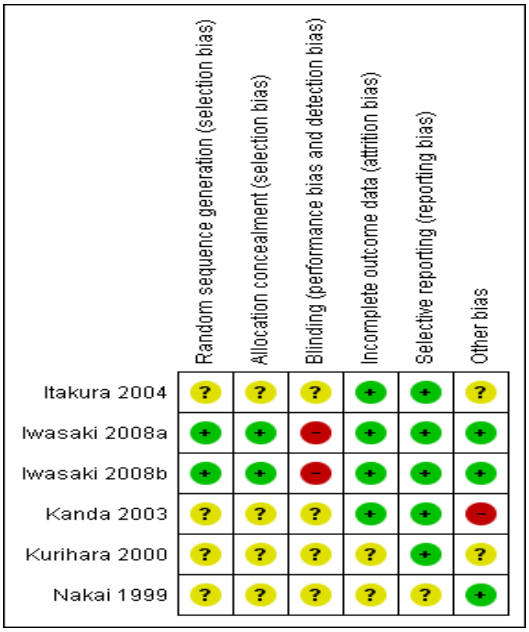

Image 52. Risk of bias summary: review authors' judgements about each risk of bias item for each included study

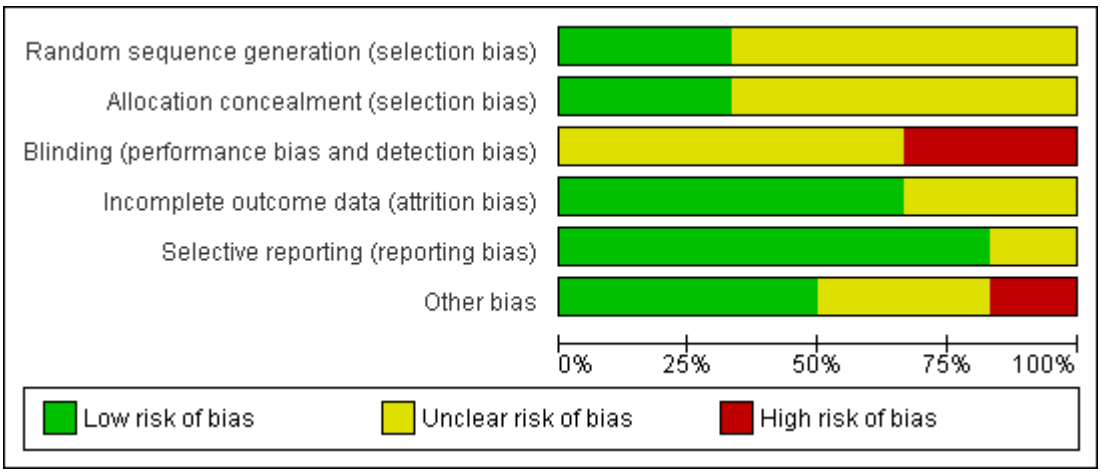

Image 53. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

Allocation

Two trials described a "computer-generated random digits" block method for the generation of the randomisation allocation sequence (Iwasaki et al, 2008a; Iwasaki et al, 2008b). We judged the risk of bias due to the generation of the

randomisation sequence as unclear in the remaining four trials (Nakai et al, 1999; Kurihara et al, 2000; Kanda et al, 2003; Itakura et al, 2004). In two trials allocation concealment was controlled by a central and independent randomisation unit (Iwasaki et al, 2008a; Iwasaki et al, 2008b). Concealment of allocation and hence risk of bias was unclear in the other four trials (Nakai et al, 1999; Kurihara et al, 2000; Kanda et al, 2003; Itakura et al, 2004).

Blinding

Four trials did not address this component and likely have not been blinded (Nakai et al, 1999; Kurihara et al, 2000; Kanda et al, 2003; Itakura et al, 2004). Two trials reported that there was no suitable placebo for bezafibrate available, so the allocation was known during the trial (Iwasaki et al, 2008a; Iwasaki et al, 2008b). Accordingly, all six trials were considered of high risk of bias regarding this domain.

Incomplete outcome data

Four trials described withdrawals or dropouts from treatment (Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008a; Iwasaki et al, 2008b). In two trials it was not specifically stated if there had been no dropouts or withdrawals (Nakai et al, 1999; Kurihara et al, 2000).

Selective reporting

The trial protocols were not available for any of the trials. However, five trials included expected outcomes (Kurihara et al, 2000; Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008a; Iwasaki et al, 2008b). In one trial we considered positively their reporting equalizing the term ''no adverse reaction'' with ''no adverse event'' (Kurihara et al, 2000). Also, in three trials (Kurihara et al, 2000; Kanda et al, 2003; Itakura et al, 2004), in their reporting about adverse events, we considered positively that no one died or developed liver-related

complications when they reported "no other adverse event was noted". Only in one trial, it was reported that no side effects of bezafibrate had been noted, so we could not consider positively their reporting equalizing the term ''side effects'' with ''adverse events'' (Nakai et al, 1999).

Other potential sources of bias

Three trials reported the following support: Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (Nakai et al, 1999), The Ministry of Health, Labour and Welfare of Japan with a Health Science Research Grant on a Specific Disease (Study of Intractable Liver Diseases) to chief scientist Gotaro Toda (Iwasaki et al, 2008a; Iwasaki et al, 2008b). In one trial it was reported that Kissei Pharmaceutical, Matsumoto, Japan provided bezafibrate, and Mitsubishi-Tokyo Pharmaceuticals, Tokyo, Japan supplied with ursodeoxycholic acid (Kanda et al, 2003). Industrial sponsorship was not addressed in two trials (Kurihara et al, 2000; Itakura et al, 2004).

Bezafibrate versus no intervention (Table 16)

Three trials provided data on all-cause mortality, liver morbidity, adverse events, and number of patients having bezafibrate withdrawn due to adverse events (Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008b). Two trials provided data on the number of patients with pruritus (Kanda et al, 2003; Itakura et al, 2004). Four trials reported on the activity of serum alkaline phosphatases and serum gamma-glutamyltransferase (Nakai et al, 1999; Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008b). Three trials reported on plasma immunoglobulin M concentration (Nakai et al, 1999; Itakura et al, 2004; Iwasaki et al, 2008b). Two trials provided data on the activity of serum alanine aminotransferase, total cholesterol, triglycerides, and serum bilirubin concentration (Itakura et al, 2004; Iwasaki et al, 2008b).

Primary outcomes

All-cause mortality

Bezafibrate did not demonstrate any significant effect on all-cause mortality (RD 0.00, 95% CI -0.11 to 0.11, I^2 = 0%) (Image 54). No deaths were reported in any of the two groups (0/32 versus 0/28 patients).

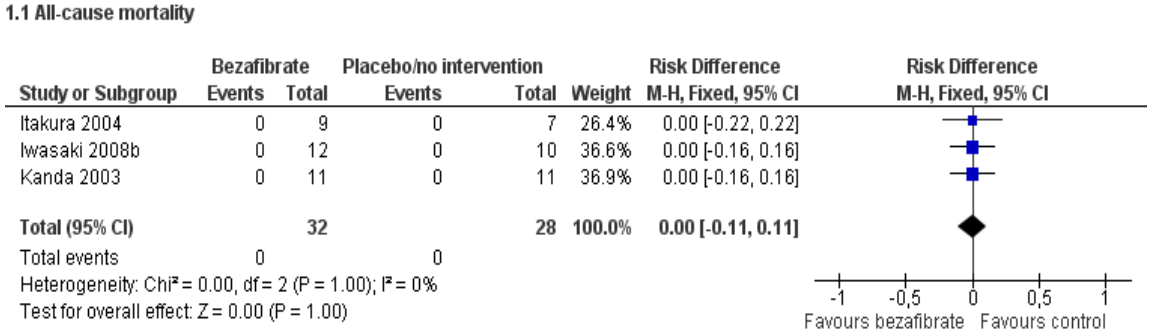

Image 54: bezafibrate vs placebo or no intervention; outcome: all-cause mortality

Liver-related morbidity

Bezafibrate had no significant effect on liver-related morbidity (RD 0.00, 95% CI -0.11 to 0.11, I^2 = 0%) (Image 55). Jaundice, upper gastrointestinal haemorrhage, ascites, hepatic encephalopathy, or hepato-renal syndrome occurred in 0/32 versus 0/28 patients in the bezafibrate and control groups.

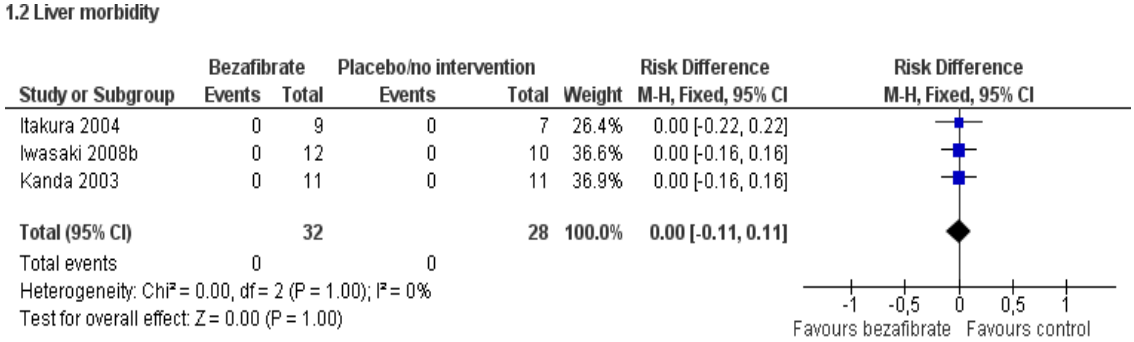

Image 55: bezafibrate vs placebo or no intervention; outcome: liver morbidity

Adverse events

Several adverse events were reported in the bezafibrate group of the included trials (polydipsia (Kanda et al, 2003), serum creatine phosphokinase elevation, and myalgia (Iwasaki et al, 2008b). However, there was no statistically significant difference in the occurrence of adverse events in patients in the bezafibrate group versus the control group (5/32 versus 0/28 patients) (RR 5.40, 95% CI 0.69 to 42.32, I^2 = 0%) (Image 56).

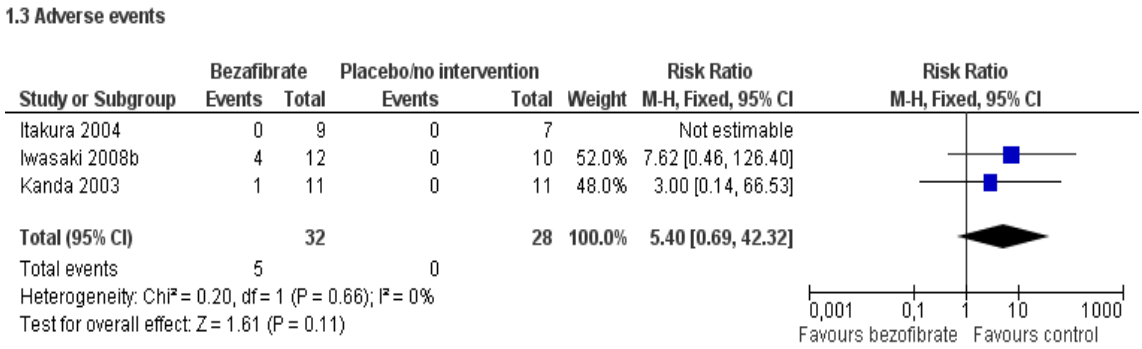

Image 56: bezafibrate vs placebo or no intervention; outcome: adverse events

For assessment of harm, besides the data provided by the three randomised trials (Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008b), we also considered the data from four non-randomised studies which reported on harm (Iwasaki et al, 1999; Miyaguchi et al, 2000; Ohmoto et al, 2001; Hazzan and Tur-Kaspa, 2010). In each of four studies it was reported that there were no adverse effects or side effects attributable to treatment.

Quality of life

No quality of life measurements were reported.

Secondary outcomes

Pruritus

Bezafibrate did not significantly influence the number of patients with pruritus (RR 1.12, 95% CI 0.50 to 2.53, I^2 = 0%) (Image 57).

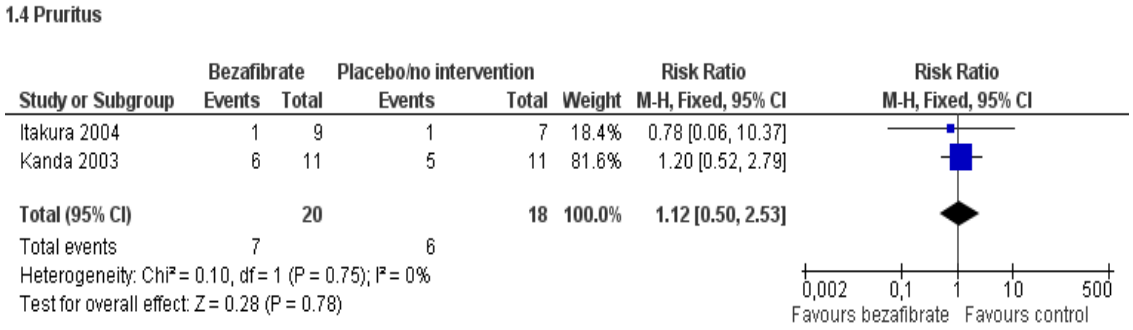

Image 57: bezafibrate vs placebo or no intervention; outcome: pruritus

Fatigue

None of the trials reported data regarding fatigue.

Biochemical indices

These data were reported either as change from baseline (Itakura et al, 2004) or final values (Nakai et al, 1999; Kanda et al, 2003; Iwasaki et al, 2008b). The data were reported either as means with standard deviations (Kanda et al, 2003; Iwasaki et al, 2008b) or as standard error of the mean; therefore, we converted them to standard deviation (Itakura et al, 2004). In one trial we have judged whether standard error of the mean or standard deviation is reported in a data table in the trial report, based on the standard deviations for laboratory values at randomisation given in a data table from the other trial reports we included (Nakai et al, 1999). The results reported in one trial were depicted graphically, and we extracted data from the graphs (Kanda et al, 2003).

In fixed-effect meta-analysis, bezafibrate significantly decreased the activity of serum alkaline phosphatases (MD -186.04 U/L, 95% CI -249.03 to -123.04, I^2 = 34%) (Image 58).

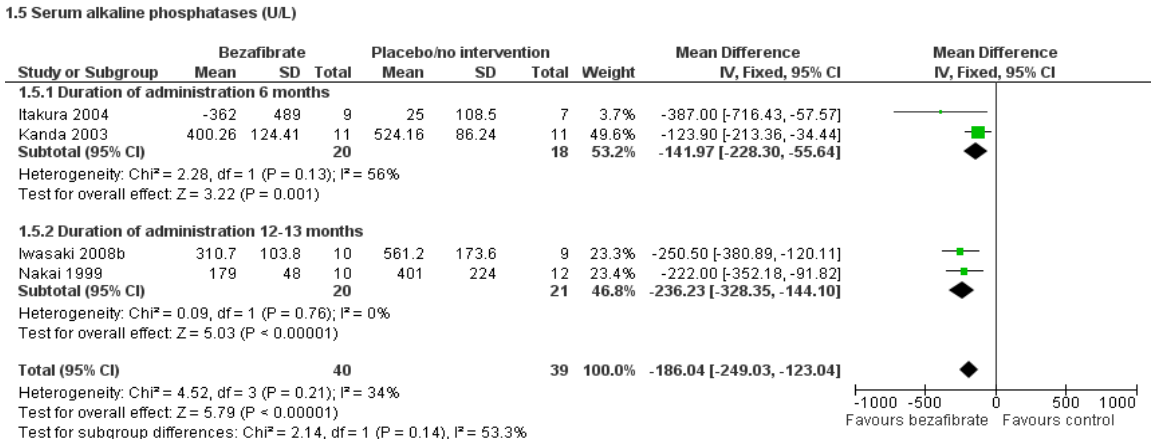

Image 58: bezafibrate vs placebo or no intervention; outcome: serum alkaline phosphatases

Trial sequential analysis of these data supports the finding in the meta-analysis (Image 59). The result of the trial sequential analysis is shown by the cumulated Z-curve (blue curve) which crosses the trial sequential monitoring boundary (red curve) implying that there is firm evidence for a beneficial effect of 100 U/L decrease in the activity of serum alkaline phosphatases in the bezafibrate group (Image 59).

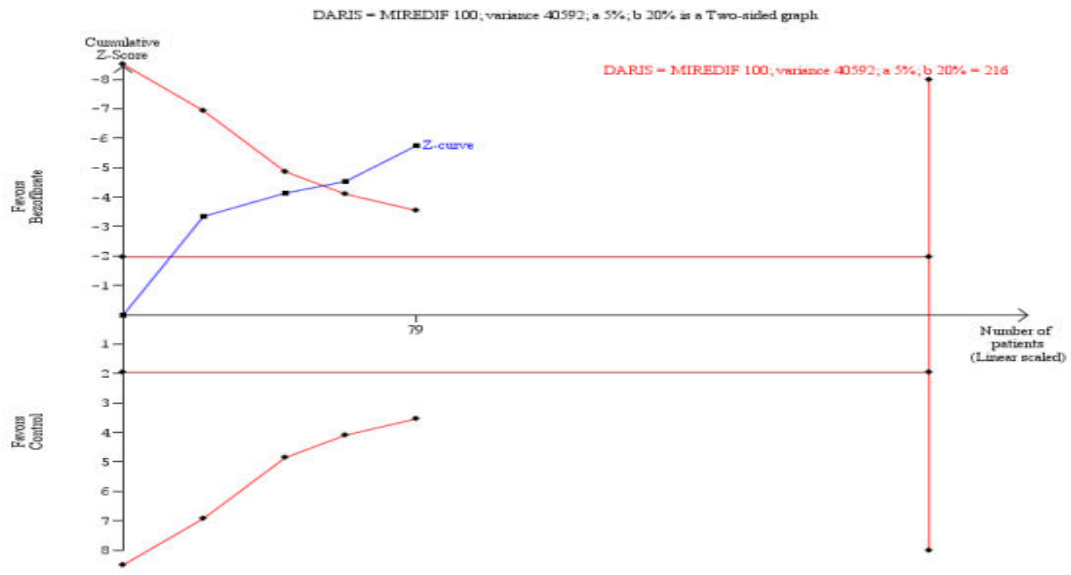

Image 59 . Trial sequential analysis of the cumulative meta-analysis of the effect of bezafibrate versus no intervention on the activity of serum alkaline phosphatases in patients with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 216 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 100 U/L, a standard deviation of 200 U/L, a risk of type I error of 5%, a power of 80%, and a diversity of 41%. The cumulated Z-curve (blue curve) crosses the trial sequential monitoring boundary (red curve) implying that there is firm evidence for a beneficial effect of 100 U/L decrease in the activity of serum alkaline phosphatases when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

In fixed-effect meta-analyses, bezafibrate significantly decreased plasma immunoglobulin M (MD -164.00 mg/dl, 95% CI -259.47 to -68.53, I^2 = 46%) (Image 60) and serum bilirubin concentration (MD -0.19 mg/dl, 95% CI -0.38 to -0.00, I^2 = 0%) (Image 61).

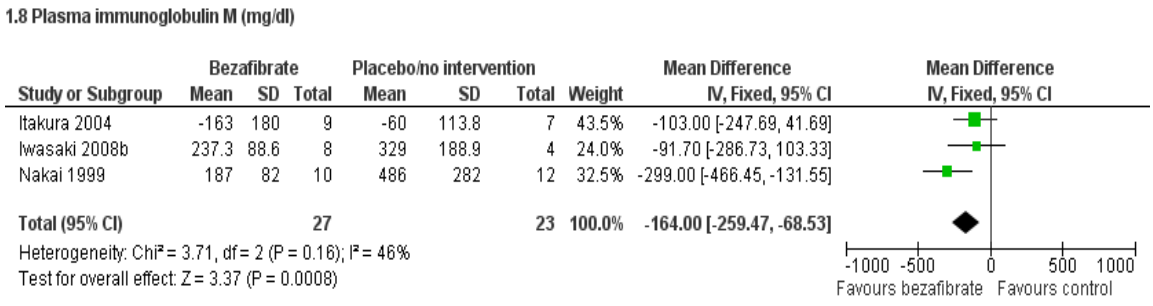

Image 60: bezafibrate vs placebo/no intervention; outcome: immunoglobulin M

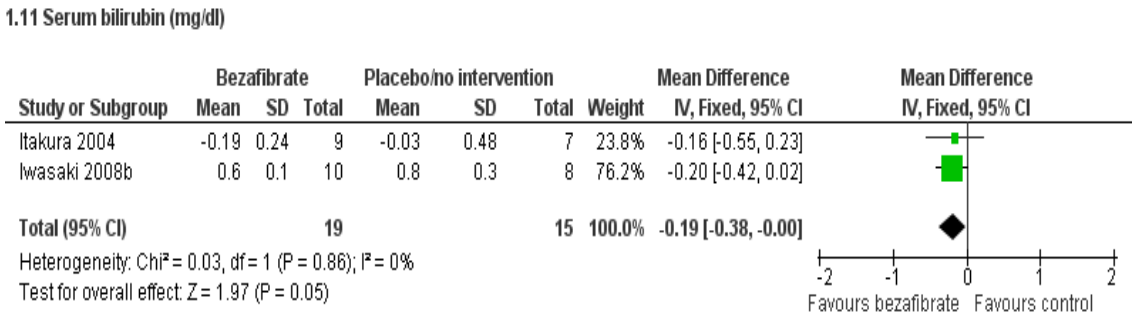

Image 61: bezafibrate vs placebo or no intervention; outcome: serum bilirubin

Trial sequential analyses on these data do not support the findings in Analysis 1.8 and Analysis 1.11. Even though the Z-curve (blue curve) lies in the direction of a decrease in plasma immunoglobulin M and serum bilirubin concentration in the bezafibrate group, it does not cross the trial sequential monitoring boundary, implying that there is no firm evidence for a beneficial effect of 121.5 mg/dl decrease in plasma immunoglobulin M concentration (Image 62) and of 0.20 mg/dl decrease in serum bilirubin concentration (Image 63).

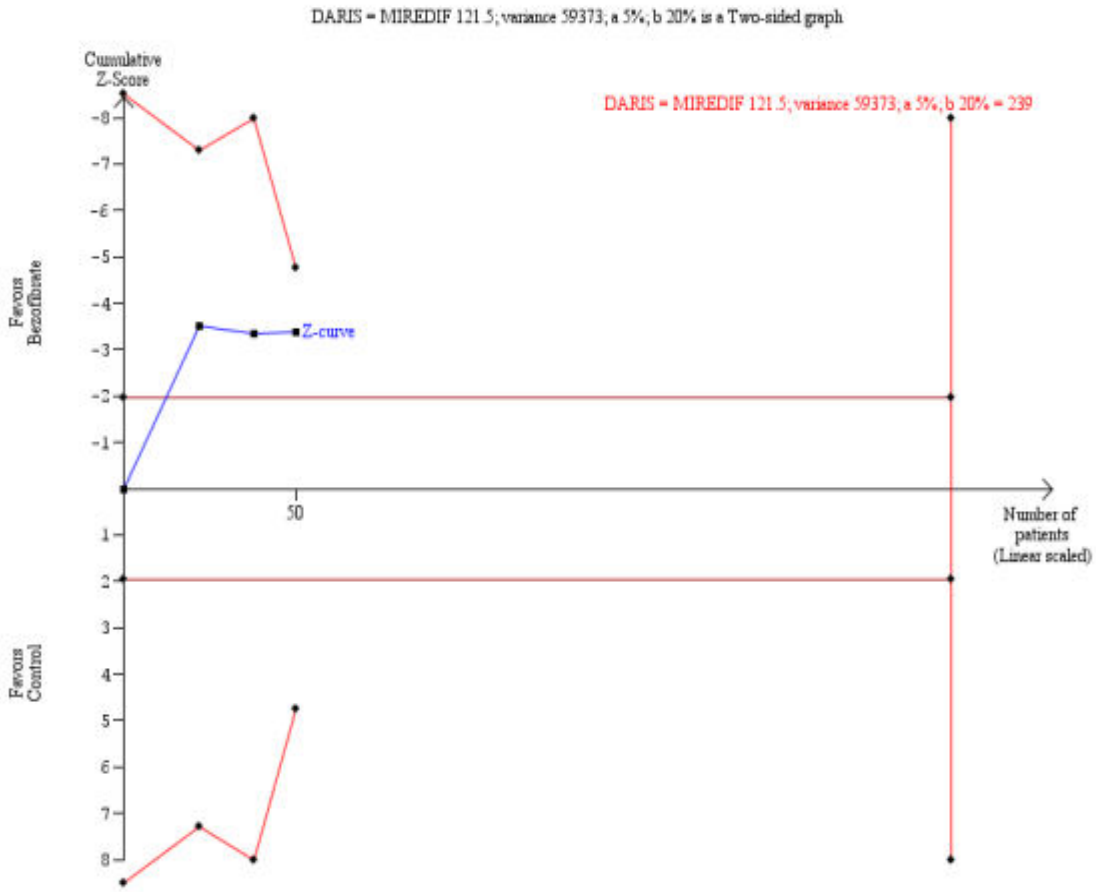

Image 62. Trial sequential analysis of the cumulative meta-analysis of the effect of bezafibrate versus no intervention on concentration of plasma immunoglobulin M in patients with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 239 patients is calculated based

on a minimal relevant intervention effect (MIREDIF) of 121.5 mg/dl, a standard deviation of 243 mg/dl, a risk of type I error of 5%, a power of 80%, and a diversity of 47%. The cumulated Z-curve (blue curve) does not cross the trial sequential monitoring boundary implying that there is no firm evidence for a beneficial effect of 121.5 mg/dl decrease in plasma immunoglobulin M concentration when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

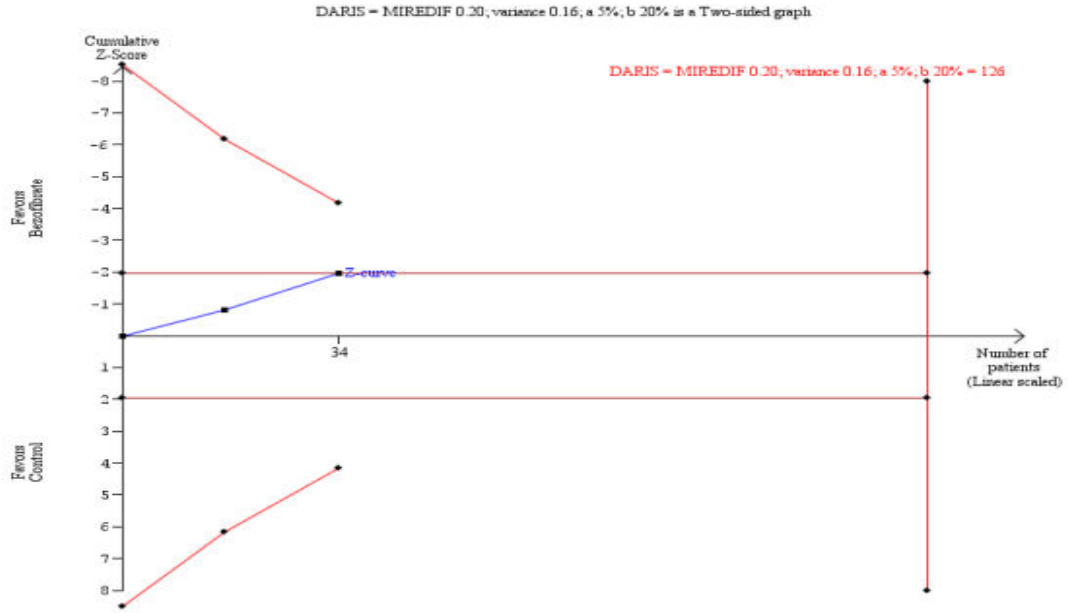

Image 63. Trial sequential analysis of the cumulative meta-analysis of the effect of bezafibrate versus no intervention on concentration of serum bilirubin concentration in patients with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 126 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 0.20 mg/dl, a standard deviation of 0.40 mg/dl, a risk of type I error of 5%, a power of 80%, and a diversity of 0%. The cumulated Z-curve (blue curve) does not cross the trial sequential monitoring boundary implying that there is no firm evidence for a potentially beneficial effect of 0.20 mg/dl decrease in serum bilirubin concentration when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

In fixed-effect meta-analyses, bezafibrate had no significant effect on the activity of serum gamma-glutamyltransferase (MD -1.22 U/L, 95% CI -11.97 to 9.52, I^2 = 42%) (Image 64), serum alanine aminotransferase (MD -5.61 U/L, 95% CI -24.50 to 13.27, I^2 = 34%) (Image 65), total cholesterol (MD -12.51 mg/dl, 95% CI -32.65 to 7.64, I^2 = 82%) (Image 66), and triglyceride concentration (MD -20.12 mg/dl, 95% CI -47.73 to 7.49, I^2 = 1%) (Image 67).

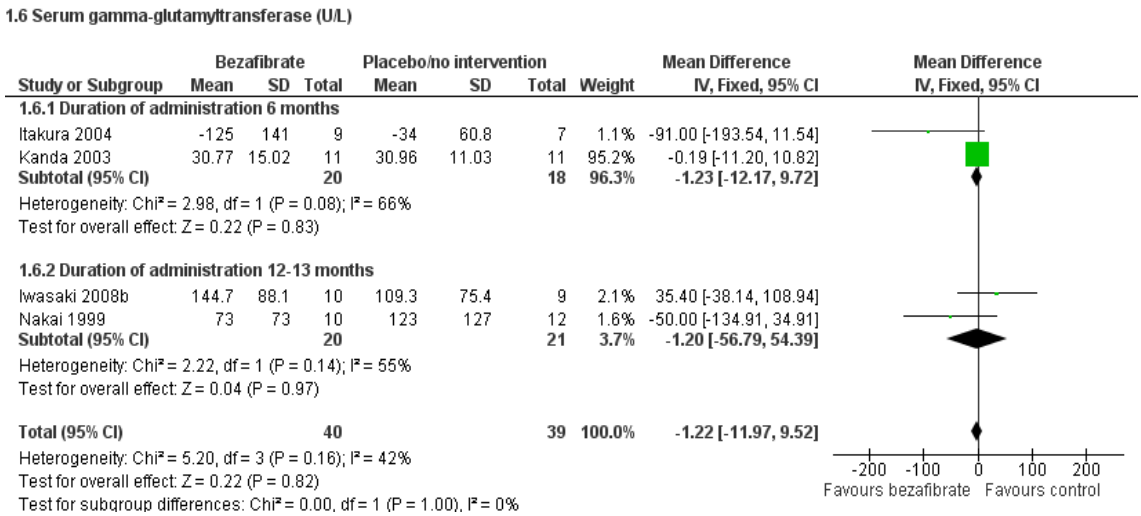

Image 64: bezafibrate vs placebo or no intervention; outcome: serum gamma-glutamyltransferase

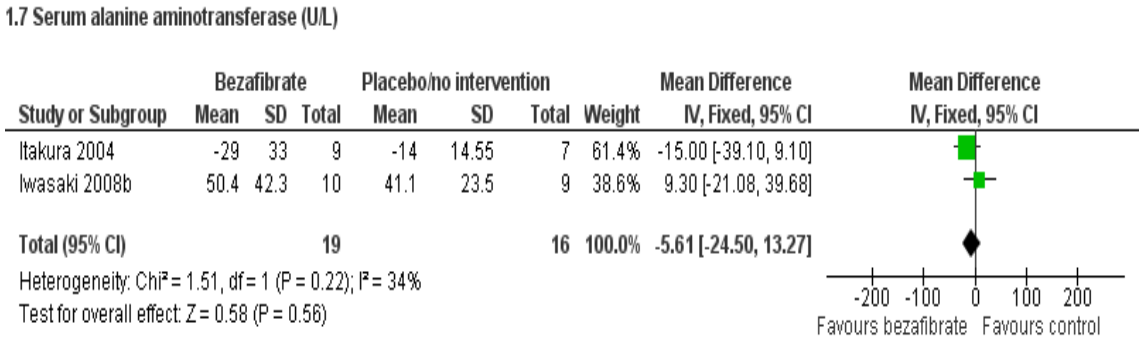

Image 65: bezafibrate vs placebo or no intervention; outcome: serum alanin aminotransferase

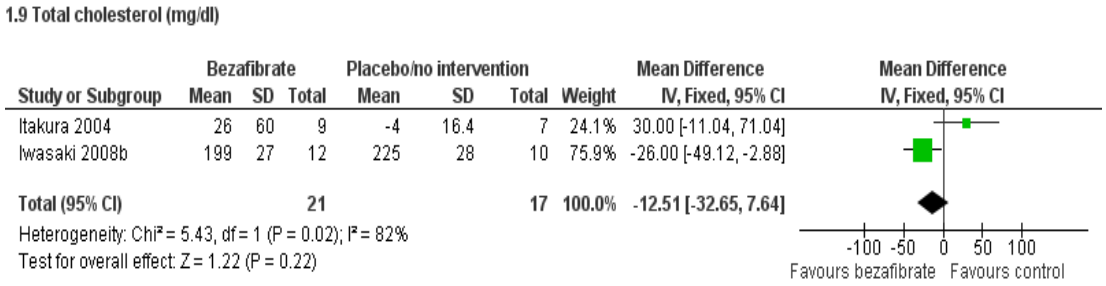

Image 66: bezafibrate vs placebo or no intervention; outcome: total cholesterol

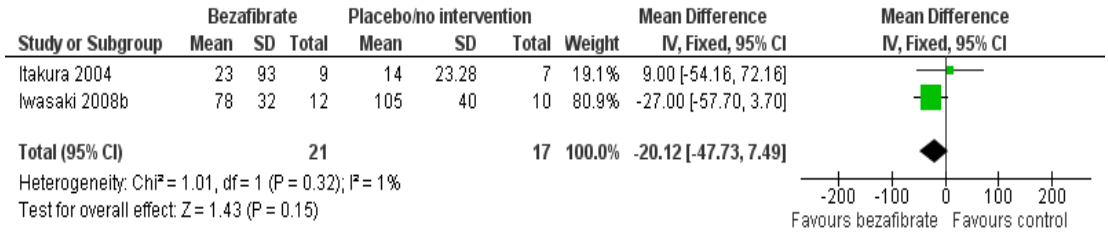

Image 67: bezafibrate vs placebo or no intervention; outcome: triglycerides

Liver biopsy findings (histological stage of primary biliary cirrhosis)

No data about liver biopsy findings after bezafibrate administration were reported.

Number of patients having bezafibrate withdrawn due to adverse events

One patient had bezafibrate withdrawn due to an adverse event (RD 0.03, 95% CI -0.09 to 0.16, I^2 = 0%) (Image 68).

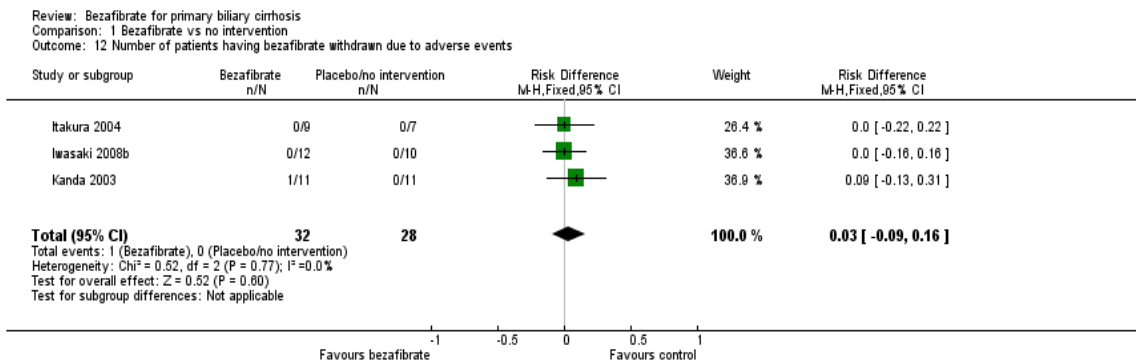

Image 68: bezafibrate vs placebo or no intervention; outcome: number of patients having bezafibrate withdrawn due to an adverse events

Bezafibrate versus ursodeoxycholic acid (Table 17)

Two trials provided data on all-cause mortality, liver-related morbidity, adverse events, number of patients having bezafibrate withdrawn due to adverse events, the activity of serum alkaline phosphatases, serum gamma-glutamyltransferase, serum alanine aminotransferase, and plasma immunoglobulin M concentration (Kurihara et al, 2000; Iwasaki et al, 2008a).

Primary outcomes

All-cause mortality

Bezafibrate did not demonstrate any significant effect on all-cause mortality (RD 0.00, 95% CI -0.08 to 0.08, I^2 = 0%) (Image 69). No deaths were reported in the bezafibrate or ursodeoxycholic acid groups (0/32 versus 0/37 patients).

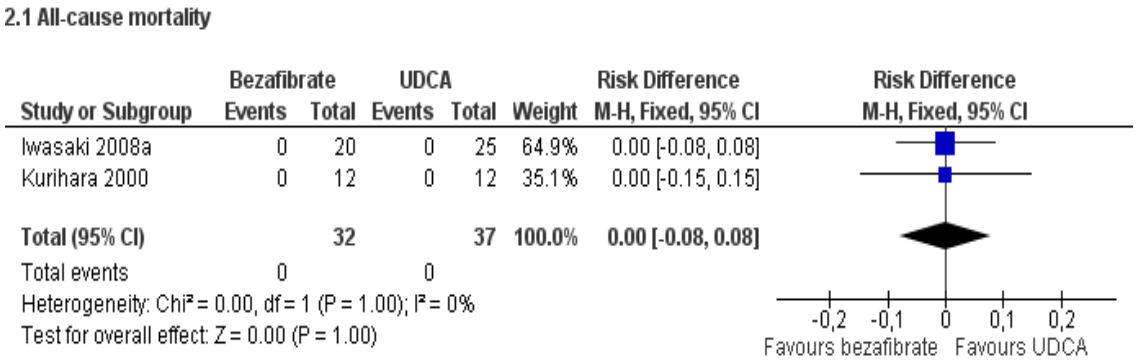

Image 69: bezafibrate vs UDCA; outcome: all-cause mortality

Liver-related morbidity

Bezafibrate had no significant effect on liver morbidity (RD 0.00, 95% CI -0.08 to 0.08, I^2 = 0%) (Image 70). Jaundice, upper gastrointestinal haemorrhage, ascites,

hepatic encephalopathy, or hepato-renal syndrome occurred in 0/32 (0%) versus 0/37 (0%) patients in the bezafibrate and ursodeoxycholic acid groups.

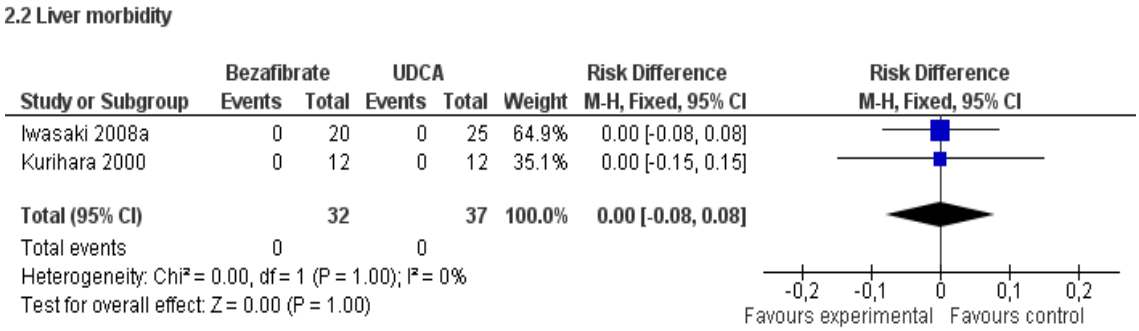

Image 70: bezafibrate vs UDCA; outcome: liver morbidity

Adverse events

A mild upper gastrointestinal pain was reported in the bezafibrate group (Iwasaki et al, 2008a), but no discontinuation of bezafibrate administration occurred. However, there was no statistically significant difference in the occurrence of adverse events in patients in the bezafibrate group versus the ursodeoxycholic acid group (2/32 versus 0/37 patients) (RR 6.19, 95% CI 0.31 to 122.05) (Image 71).

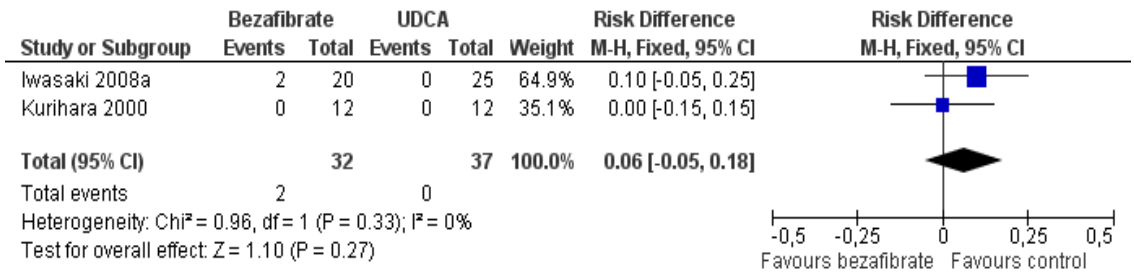

Image 71: bezafibrate vs UDCA; outcome: adverse events

Quality of life

No quality of life measurements were reported.

Secondary outcomes

Pruritus and fatigue

None of the trials reported data regarding pruritus and fatigue.

Biochemical indices

These data were reported either as change from baseline (Kurihara et al, 2000) or final values (Iwasaki et al, 2008a). The data were reported as means with standard deviations (Iwasaki et al, 2008a) or as standard error of the mean; therefore, we converted them to standard deviation (Kurihara et al, 2000). The results reported in one trial were depicted graphically, and we extracted data from the graphs (Kurihara et al, 2000). The data were reported as the degree of change from baseline (%) (Kurihara et al, 2000), and we extracted data as final values from the graphs. In fixed-effect meta-analyses, bezafibrate significantly decreased the activity of serum alkaline phosphatases (MD -162.90 U/L, 95% CI -199.68 to -126.12, I^2 = 0%) (Image 72), serum gamma-glutamyltransferase (MD -58.18 U/L, 95% CI -76.49 to -39.88, I^2 = 89%) (Image 73), serum alanine aminotransferase (MD -58.18 U/L, 95% CI -76.49 to -39.88, I^2 = 95%) (Image 74), and plasma immunoglobulin M concentration (MD -99.90 mg/dl, 95% CI -130.72 to -69.07, I^2 = 90%) (Image 75).

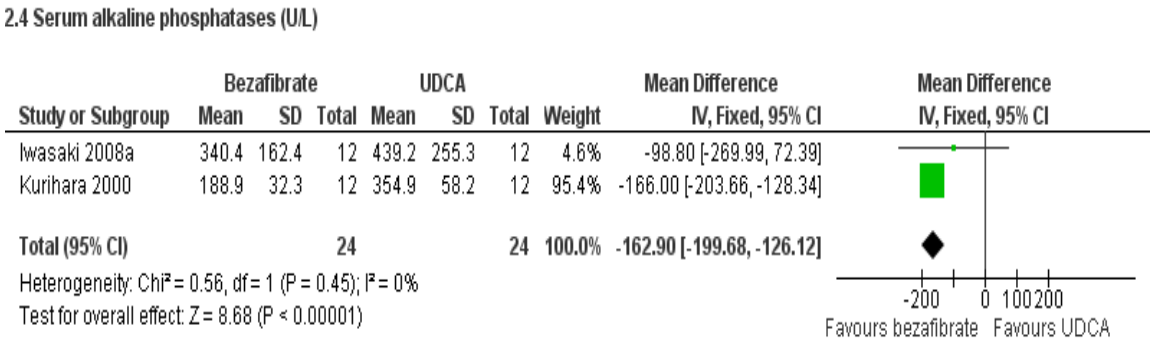

Image 72: bezafibrate vs UDCA; outcome: serum alkaline phosphatases

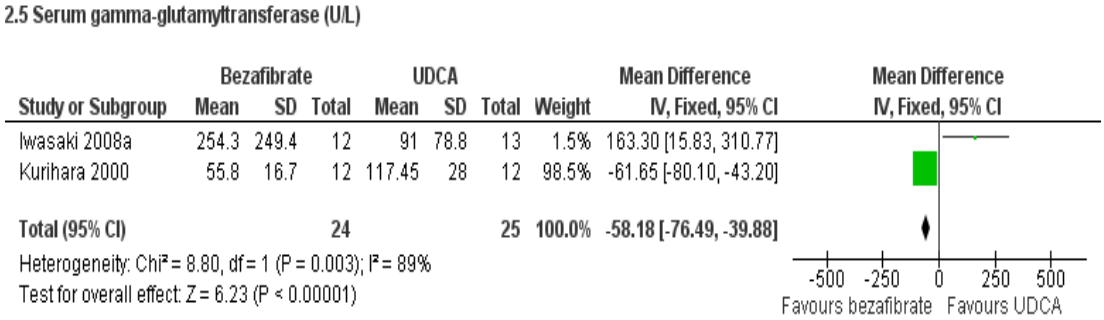

Image 73: bezafibrate vs UDCA; outcome: serum gamma-glutamyltransferase

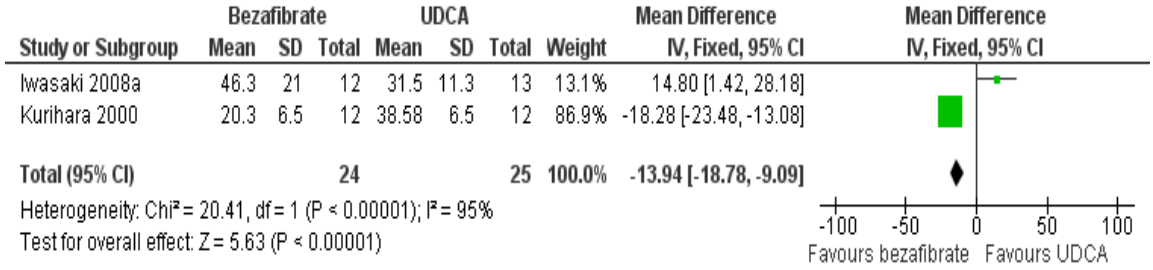

Image 74: bezafibrate vs UDCA; outcome: serum alanin aminotransferase

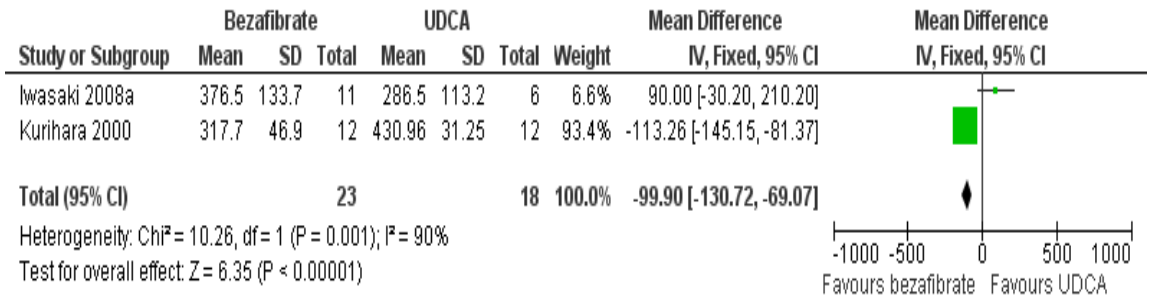

Image 75: bezafibrate vs UDCA; outcome: plasma immunoglobulin M

Trial sequential analysis of these data supports the finding in the meta-analysis of activity of serum alkaline phosphatases (Image 72). The result of the trial sequential analysis is shown by the cumulated Z-curve (blue curve) which crosses the trial sequential monitoring boundary (red curve) implying that there

is firm evidence for a beneficial effect of 45.5 U/L decrease in the activity of serum alkaline phosphatases in the bezafibrate group (Image 76).

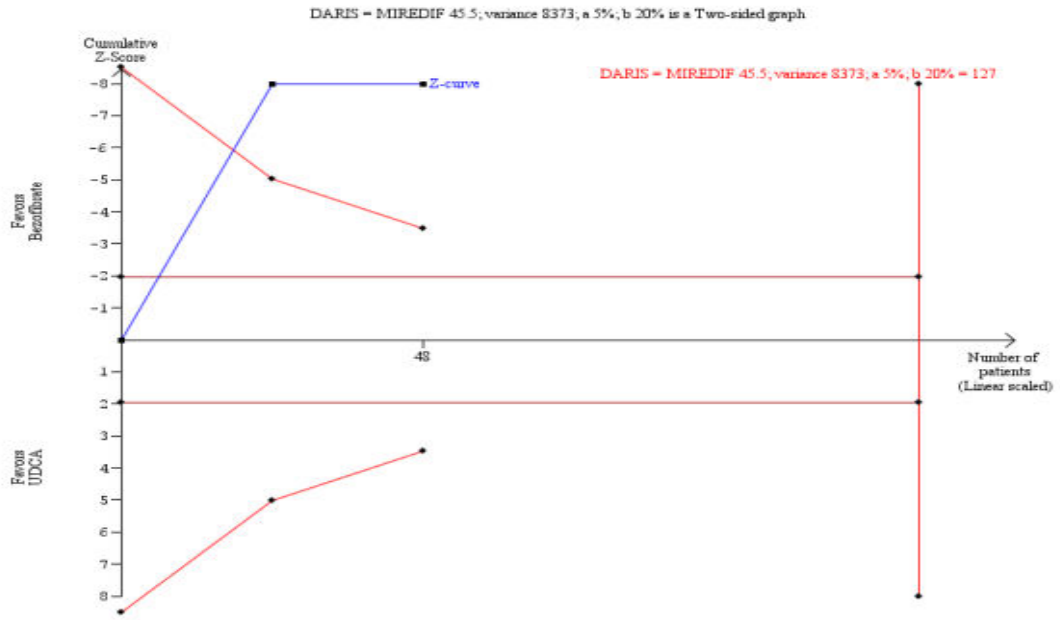

Image 76. Trial sequential analysis of the cumulative meta-analysis of the effect of bezafibrate versus ursodeoxycholic acid on the activity of serum alkaline phosphatases in patients with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 127 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 45.5 U/L, a standard deviation of 91 U/L, a risk of type I error of 5%, a power of 80%, and a diversity of 0%. The cumulated Z-curve (blue curve) crosses the trial sequential monitoring boundary (red curve) implying that there is firm evidence for a beneficial effect of 45.5 U/L decrease in the activity of serum alkaline phosphatases when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

In random-effect meta-analyses, bezafibrate had no significant effect on the activity of serum gamma-glutamyltransferase (MD 38.44 U/L, 95% CI -180.67 to

257.55, I^2 = 89%), serum alanine aminotransferase (MD -2.34 U/L, 95% CI -34.73 to 30.06, I^2 = 95%), and plasma immunoglobulin M concentration (MD -20.23 mg/dl, 95% CI -218.71 to 178.25, I^2 = 90%).

Liver biopsy findings (histological stage of primary biliary cirrhosis)

No data about liver biopsy findings after bezafibrate administration were reported.

Number of patients having bezafibrate withdrawn due to adverse effects

No patient had bezafibrate withdrawn due to adverse effects (RD 0.00, 95% CI -0.08 to 0.08, I^2 = 0%) (Image 77).

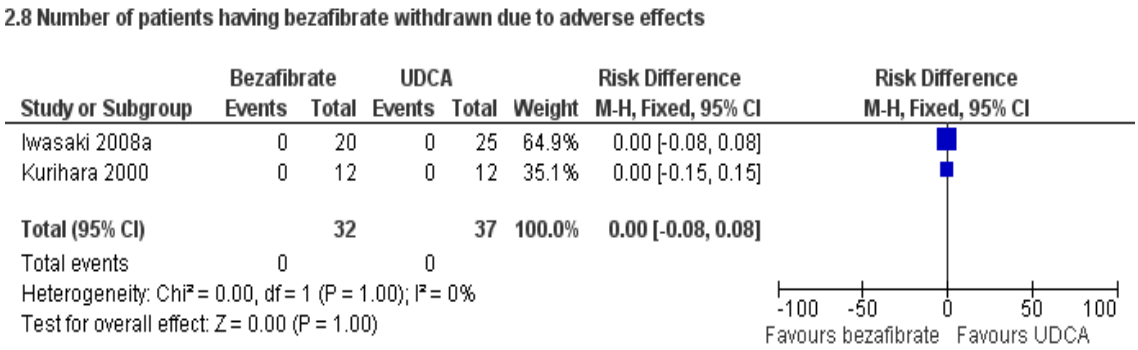

Image 77: bezafibrate vs UDCA; outcome: number of patients having bezafibrate withdrawn due to adverse effects

Subgroup analyses

Only a subgroup analysis on different durations of administration of bezafibrate was performed. Due to the paucity of trials none of the other planned analyses could be conducted.

Subgroup analysis on trials with low risk of bias compared to trials with high risk of bias

All included trials were judged to be at high risk of bias (Image 53). As such, a subgroup analysis comparing trials with low risk of bias to trials with high risk of bias was not possible.

Subgroup analysis on different doses of bezafibrate

Bezafibrate was given as one single dose of 400 mg in four trials; three trials assessing bezafibrate versus no intervention (Nakai et al, 1999; Itakura et al, 2004; Iwasaki et al, 2008b) and in one trial assessing bezafibrate with ursodeoxycholic acid (Iwasaki et al, 2008a). Bezafibrate was divided into two orally administered doses, a post-breakfast and a post-dinner dose of 200 mg, in one trial assessing bezafibrate versus no intervention (Kanda et al, 2003) and in another trial assessing bezafibrate with ursodeoxycholic acid (Kurihara et al, 2000). As such, a subgroup analysis comparing different doses of bezafibrate was not possible.

Subgroup analysis on duration of administration of bezafibrate

Subgroup analysis was performed in order to compare the duration of bezafibrate administration. Bezafibrate was administered for six months in two trials (Kanda et al, 2003; Itakura et al, 2004) and for 12 to 13 months in another two trials (Nakai et al, 1999; Iwasaki et al, 2008b).

According to our subgroup analyses, the duration of bezafibrate administration did not influence the serum alkaline phosphatases activity (MD -141.97 U/L, 95% CI -228.30 to -55.64, I^2 = 56% compared to MD -236.23 U/L, 95% CI -328.35 to -144.10, I^2 = 0%; test of interaction Chi^2 = 2.14; P = 0.14) (Image 78), nor did it influence the serum gamma-glutamyltransferase activity (MD -1.23 U/L, 95% CI -12.17 to 9.72, I^2 = 66% compared to MD -1.20 U/L, 95% CI -56.79 to 54.39, I^2 = 55%; test of interaction Chi^2 = 0.00; P = 1.00) (Image 79).

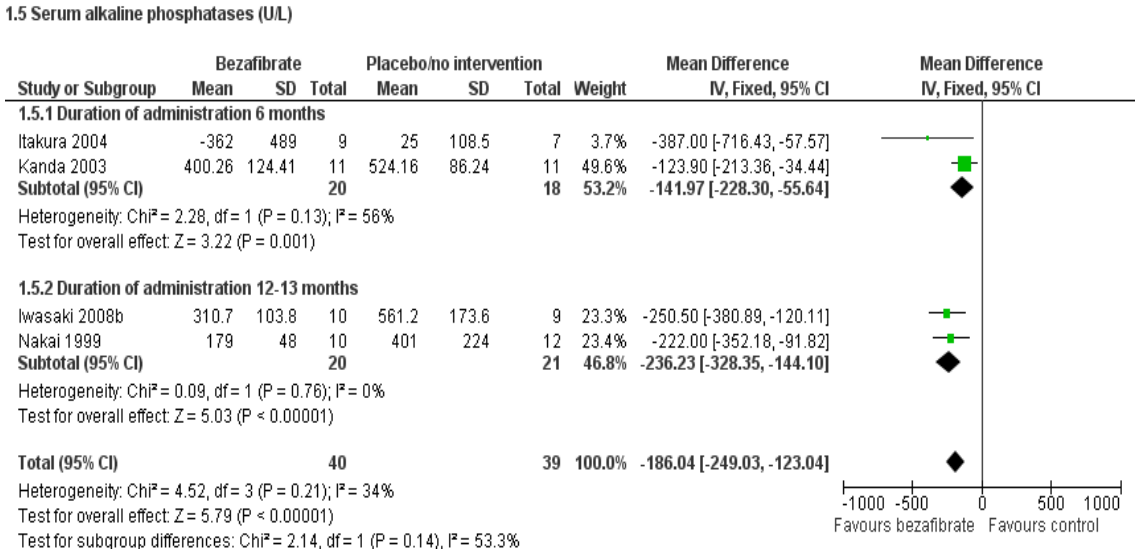

Image 78: subgroup analysis: bezafibrate vs placebo or no intervention; outcome: serum alkaline phosphatases

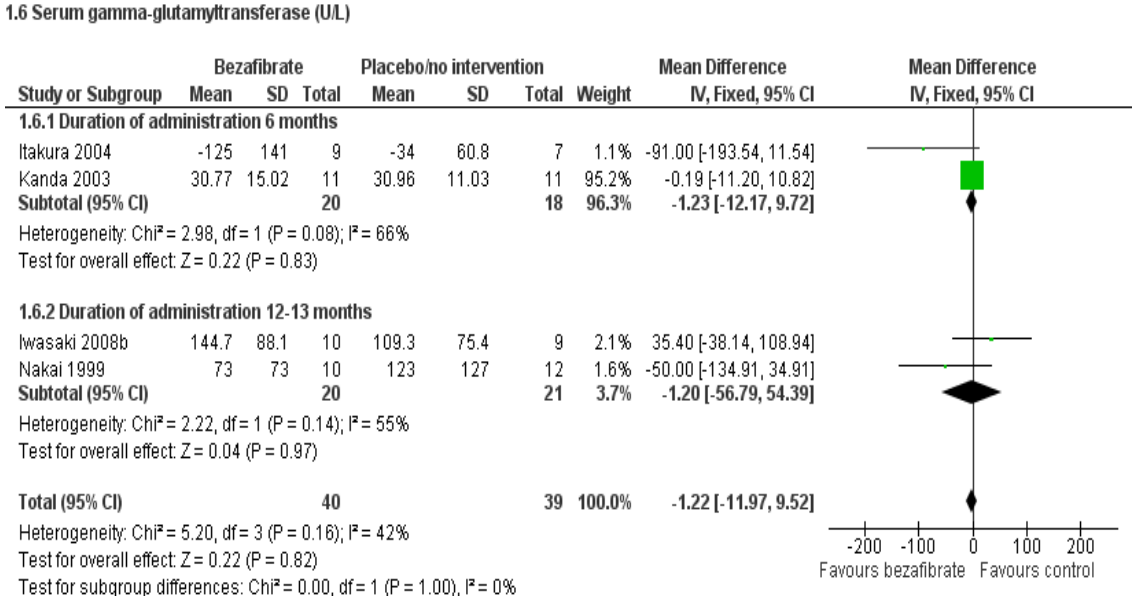

Image 79: subgroup analysis: bezafibrate vs placebo or no intervention; outcome: serum gamma-glutamyltransferases

Subgroup analysis on patients treated for primary biliary cirrhosis with a different drug before bezafibrate administration compared to patients with no pretreatment

In five trials patients were treated with ursodeoxycholic acid before bezafibrate was administrated (Nakai et al, 1999; Kanda et al, 2003; Itakura et al, 2004; Iwasaki et al, 2008a; Iwasaki et al, 2008b). In one trial there are no data about pretreatment of patients (Kurihara et al, 2000). As such, a subgroup analysis on patients treated for primary biliary cirrhosis with a drug different than bezafibrate before bezafibrate administration compared to patients with no pretreatment was not possible. Duration of ursodeoxycholic acid administration was different in each trial: one year or more (Nakai et al, 1999); at least six months (Kanda et al, 2003); and more than 26 weeks (Iwasaki et al, 2008b). In one trial three patients received treatment with ursodeoxycholic acid for 2 to 11 years, but before entry into this trial, patients discontinued the use of ursodeoxycholic acid for at least three months (Itakura et al, 2004). In one trial it was only reported that not all patients had been treated with ursodeoxycholic acid or bezafibrate within the previous four weeks (Iwasaki et al, 2008a).

Subgroup analysis on patients with advanced compared to patients with non-advanced primary biliary cirrhosis

A subgroup analysis on patients with advanced primary biliary cirrhosis compared to patients with non-advanced primary biliary cirrhosis was not possible.

Description of studies: tables of included studies (Table 13); tables of excluded studies (Table 14); tables of ongoing studies (Table 15).

Table 13. Tables of included studies

Itakura 2004

Methods	Randomised clinical trial with cross-over group design (two interventions groups).

	Trial duration: six months.
Participants	Country: Japan. Number of patients randomised: 16, median age 54/61 years (89%/57% females). Inclusion criteria: - at least a 1.3-fold elevated alkaline phosphatase level; - at least a 40-fold positive excess of anti-mitochondrial antibodies; - liver-biopsy proven primary biliary cirrhosis. Exclusion criteria: - histological overlapping with autoimmune hepatitis; - positive serum antigen or antibody associated with the hepatitis B virus; - positive serum antibody of hepatitis C virus; - positive serum antibody of human immunodeficiency virus; - history of drinking excessive amounts of alcohol or drug use; - ascites or oesophageal varices; - renal insufficiency; - cardiac failure; - hepatocellular carcinoma.
Interventions	Patients were randomly assigned to receive: Intervention group 1: bezafibrate (400 mg per day) and ursodeoxycholic acid (600 mg per day), n = 9; Intervention group 2: ursodeoxycholic acid alone (600 mg per day), n = 7. Three patients received treatment with ursodeoxycholic

	acid for 2 to 11 years, but before entry into the trial, they had discontinued the use of ursodeoxycholic acid for at least three months.	
Outcomes	Outcome measure(s): - clinical events; - laboratory data (serum alkaline phosphatases, serum gamma-glutamyltransferase, serum alanine aminotransferase, IgM, total serum bilirubin, and total cholesterol and triglyceride levels); - adverse events.	
Notes	Additional information requested on 17th February 2011, but no response has been received so far. We have used the data from the first period of the cross-over trial.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	The method used to conceal the allocation was not described, so that intervention allocations may have been foreseen in advance of or during enrolment.
Blinding All outcomes	Unclear risk	The trial did not provide information for assessment of this domain, but it is not likely to have been blinded.
Incomplete	Low risk	The numbers and reasons for dropouts and

outcome data All outcomes		withdrawals in all intervention groups were described.
Selective reporting	Low risk	All expected outcomes are reported.
Other bias	Unclear risk	Industrial sponsorship was not addressed.

Iwasaki 2008a

Methods	Multicenter randomised clinical trial with parallel group design (two interventions groups). Trial duration: 52 weeks.
Participants	Country: Japan. Number of patients randomised: 45, mean age 55 years (82% females). Inclusion criteria: - a medical history and laboratory tests consistent with chronic cholestatic liver disease; - positive antimitochondrial antibody or antipyruvate dehydrogenase complex (PDC); - serum alkaline phosphatases elevation of at least 1.5 times the upper limit of normal; - the absence of biliary tract obstruction on imaging results; - hyperlipoproteinaemia. Exclusion criteria: - treatment with D-penicillamine, corticosteroids, colchicine or immunosuppressive agents within 4 weeks; - diagnosis of cirrhosis; - diuretic-resistant ascites, hepatic encephalopathy, haemorrhage from oesophageal or gastric varices;

	- hyperbilirubinaemia (greater than 5.0 mg/dL); - serum albumin level less than 3.0 g/dL; - renal insufficiency; - malignancy; - pregnancy; - below 19 years of age.
Interventions	Patients were randomly assigned to receive: Intervention group 1: bezafibrate (400 mg daily orally), n = 20; Intervention group 2: ursodeoxycholic acid (orally at a dose of 600 mg daily), n = 25. All patients had not been treated with ursodeoxycholic acid or bezafibrate within the previous four weeks.
Outcomes	Outcome measure(s): - clinical events; - laboratory data (serum alkaline phosphatases, serum gamma-glutamyltransferase, serum alanine aminotransferase, IgM, total serum bilirubin, and total cholesterol and triglyceride levels); - adverse events.
Notes	Additional information requested on 14th February 2011 and reply received on 16th February 2011 through personal communication with the principal author Dr. Shinji Iwasaki. Dr. Shinji Iwasaki, provided data on the following: - the method of sequence generation; - the number of patients in each intervention group at the end of treatment; - tables with numeric values for biochemical indices; - adverse events; - all-cause mortality and liver-related morbidity.

Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	It was generated by block method using computer-generated random digits.
Allocation concealment	Low risk	Allocation was controlled by a central and independent randomisation unit, so that intervention allocations could not have been foreseen in advance of, or during enrolment.
Blinding All outcomes	High risk	The trial was not blinded, so that the allocation was known during the trial.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	All expected outcomes are reported.
Other bias	Low risk	The trial appears to be free of other components that could put it at risk of bias.

Iwasaki 2008b

Methods	Multicenter randomised clinical trial with parallel group design (two interventions groups). Trial duration: 52 weeks.
Participants	Country: Japan. Number of patients randomised: 22, mean age 54 years

	(86.4% females). Inclusion criteria: - a medical history and laboratory tests consistent with chronic cholestatic liver disease; - positive antimitochondrial antibody or antipyruvate dehydrogenase complex (PDC); - serum alkaline phosphatases elevation of at least 1.5 times the upper limit of normal after treatment with ursodeoxycholic acid for more than 26 weeks before the study started; - the absence of biliary tract obstruction on imaging results; - hyperlipoproteinaemia. Exclusion criteria: - treatment with D-penicillamine, corticosteroids, colchicine or immunosuppressive agents within 4 weeks; - diagnosis of cirrhosis; - diuretic-resistant ascites, hepatic encephalopathy, haemorrhage from oesophageal or gastric varices; - hyperbilirubinaemia (greater than 5.0 mg/dL); - serum albumin level less than 3.0 g/dL; - renal insufficiency; - malignancy; - pregnancy; - below 19 years of age.
Interventions	Patients were randomly assigned to receive: Intervention group 1: bezafibrate plus ursodeoxycholic acid, n = 12; Intervention group 2: ursodeoxycholic acid, n = 10.

	Ursodeoxycholic acid was given orally at a dose of 600 mg daily, and bezafibrate was given at a dose of 400 mg daily for 52 weeks. All patients were treated with ursodeoxycholic acid for more than 26 weeks before the trial start.	
Outcomes	Outcome measure(s): - clinical events; - laboratory data (serum alkaline phosphatases, serum gamma-glutamyltransferase, serum alanine aminotransferase, IgM, total serum bilirubin, and total cholesterol and triglyceride levels); - adverse events.	
Notes	Additional information requested on 14th February 2011 and reply received on 16th February 2011 through personal communication with the principal author Dr. Shinji Iwasaki. Dr. Shinji Iwasaki, provided data on the following: - the method of sequence generation; - the number of patients in each intervention group at the end of treatment; - tables with numeric values for biochemical indices; - adverse events; - all-cause mortality and liver-related morbidity.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random	Low risk	It was generated by block method using

sequence generation		computer-generated random digits.
Allocation concealment	Low risk	Allocation was controlled by a central and independent randomisation unit, so that intervention allocations could not have been foreseen in advance of, or during enrolment.
Blinding All outcomes	High risk	The trial was not blinded, so that the allocation was known during the trial.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	All clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	The trial appears to be free of other components that could put it at risk of bias.

Kanda 2003

Methods	Randomised clinical trial with parallel group design (two interventions groups). Trial duration: six months.
Participants	Country: Japan. Number of patients randomised: 22, mean age 56 years (86% females). Inclusion criteria: elevated serum alkaline phosphatases level despite receiving 600 mg/day of ursodeoxycholic

	acid, liver-biopsy proven primary biliary cirrhosis, no positive serum antigen or antibody associated with the hepatitis B virus, no positive serum antibody of hepatitis C virus, human immunodeficiency virus negativity, no other cause of liver disease (such as excessive amount of alcohol use, metabolic disorders or drug-induced liver injury), no ascites, hepatic encephalopathy, oesophageal varices, or hyperbilirubinaemia (total bilirubin ≥ 2.0 mg/dl), no previous treatment with colchicine, corticosteroids, or immunosuppressive drugs, no thyroid dysfunction or renal insufficiency (serum creatine level ≥ 2.0 mg/dl), and prior compliance with ursodeoxycholic acid therapy. Exclusion criteria: none listed.
Interventions	Patients were randomly assigned to receive: Intervention group 1: bezafibrate (400 mg per day of bezafibrate divided into two orally administered doses, post-breakfast and post-dinner), plus 600 mg per day of ursodeoxycholic acid divided into three orally administered post-meal doses), n = 11. Bezafibrate was administrated for a period of six months. Intervention group 2: 600 mg per day of ursodeoxycholic acid divided into three orally administered post-meal doses, n = 11. All patients had been treated with 600 mg per day of ursodeoxycholic acid for at least six months. All patients were given 600 mg per day of ursodeoxycholic acid in the same manner before, during, and after the 6-month period of administration of bezafibrate.

Outcomes	Outcome measure(s): - clinical variables (pruritus, ascites, upper gastrointestinal bleeding, and hepatic encephalopathy); - biochemical variables (serum alkaline phosphatases and serum gamma-glutamyltransferase levels); - adverse events.	
Notes	Additional information requested on 16th February 2011, but no response has been received so far.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	The method used to conceal the allocation was not described, even though the trial was described as randomised and intervention allocations may have been foreseen in advance of, or during, enrolment.
Blinding All outcomes	Unclear risk	The trial did not provide information for assessment of this domain, but it is not likely to have been blinded.
Incomplete outcome data All outcomes	Low risk	It was specified that all patients participated until the end of the trial.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported

		on.
Other bias	High risk	It was reported that Kissei Pharmaceutical, Matsumoto, Japan provided bezafibrate, and Mitsubishi-Tokyo Pharmaceuticals, Tokyo, Japan supplied with ursodeoxycholic acid.

Kurihara 2000

Methods	Randomised clinical trial with parallel group design (two interventions groups). Trial duration: 12 months.
Participants	Country: Japan. Number of patients randomised: 24, mean age 60 years (95.8% females). Inclusion criteria: patients with liver biopsy proven primary biliary cirrhosis. Exclusion criteria: none listed.
Interventions	Patients were randomly assigned to receive: Intervention group 1: bezafibrate (400 mg per day of bezafibrate divided into two orally administered doses, 200 mg was taken in the morning and 200 mg in the evening), n = 12; Intervention group 2: 600 mg per day of ursodeoxycholic acid divided into three orally administered doses (200 mg was taken in the morning, afternoon, and evening), n = 12. Both drugs were taken for 12 months.

Outcomes	Outcome measure(s): - biochemical variables (serum alkaline phosphatases, serum gamma-glutamyltransferase levels, serum alanine aminotransferase, and IgM levels); - adverse events.	
Notes	Additional information requested on 18th February 2011, and no response has been received so far.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	The method used to conceal the allocation was not described, so that intervention allocations may have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Unclear risk	The trial did not provide information for assessment of this domain, but it is not likely to have been blinded.
Incomplete outcome data All outcomes	Unclear risk	It was not specifically stated if there had been no dropouts or withdrawals.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on. We considered positively their reporting equalising the term "no adverse

		reaction'' with ''no adverse event''.
Other bias	Unclear risk	Industrial sponsorship was not addressed.

Nakai 1999

Methods	Randomised clinical trial with parallel group design (two interventions groups). Trial duration: 12 months.
Participants	Country: Japan. Number of patients randomised: 22, mean age 58 years (90.9% females). Inclusion criteria: patients with primary biliary cirrhosis who had positive mitochondrial antibody test and liver biopsy-proven diagnosis. Exclusion criteria: none listed.
Interventions	Patients were randomly assigned to receive: Intervention group 1: 400 mg per day of bezafibrate and 600 mg per day of ursodeoxycholic acid, n = 10; Intervention group 2: 600 mg per day of ursodeoxycholic acid, n = 12. All patients had been treated with ursodeoxycholic acid for one year or more.
Outcomes	Outcome measure(s): changes in biochemical and immunological variables (serum alkaline phosphatases, serum gamma-glutamyltransferase levels, and IgM levels after 3, 6, 9, and 12 months of treatment).
Notes	Additional information requested on 18th February 2011, but no response has been received so far.

Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	The method used to conceal the allocation was not described, so that intervention allocations may have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Unclear risk	The trial did not provide information for assessment of this domain, but it is not likely to have been blinded.
Incomplete outcome data All outcomes	Unclear risk	It was not specifically stated if there had been dropouts or withdrawals.
Selective reporting	Unclear risk	Not all pre-defined expected outcomes are reported fully, or it is unclear whether data on these outcomes were recorded or not.
Other bias	Low risk	The trial appears to be free of other components that could put it at risk of bias.

Table 14. Tables of excluded studies

Study	Reason for exclusion

Fukuo 1996	Patients had hyperlipidaemia, not primary biliary cirrhosis.
Hazzan 2010	Not a randomised clinical trial. The study group included 8 patients with primary biliary cirrhosis, 52 to 76 years old, who had been treated with ursodeoxycholic acid (900 to 1500 mg per day) for 2 to 11 years with only a partial response (19% to 56% reduction in alkaline phosphatase level). Bezafibrate (400 mg per day) was added to ursodeoxycholic acid, and the patients were followed for 4 to 12 months. There were no adverse effects attributable to the treatment.
Iwasaki 1999	Not a randomised clinical trial. The aim of this study was to evaluate the efficacy of bezafibrate in primary biliary cirrhosis (11 pre-cirrhotic patients with primary biliary cirrhosis were treated with 400 mg per day of bezafibrate for 12 to 21 months). Bezafibrate was co-administered in seven patients who had been treated with ursodeoxycholic acid but shown incomplete responses. There were no side effects attributable to the treatment.
Miyaguchi 2000	Not a randomised clinical trial. Bezafibrate was administered additionally to 13 out of 21 patients with primary biliary cirrhosis who were treated by monotherapy of ursodeoxycholic acid for 18 months and whose liver enzymes did not remain within normal range. There were no adverse effects attributable to the treatment.
Ohmoto 2001	Not a randomised clinical trial. The aim of this study was to evaluate the efficacy of bezafibrate in ten patients with primary biliary cirrhosis (two men and

	eight women aged 43 to 66 years at the start of treatment: five in stage I of Scheuer's classification, two in stage II, two in stage III, and one in stage IV), who had shown an inadequate response to ursodeoxycholic acid monotherapy. There were no adverse effects attributable to the treatment.

Table 15. Tables of ongoing studies

JPRN-C000000225

Trial name or title	Randomised clinical trial of ursodeoxycholic acid with or without bezafibrate in primary biliary cirrhosis.
Methods	Randomised trial with parallel design.
Participants	Primary biliary cirrhosis.
Interventions	Intervention: ursodeoxycholic acid plus bezafibrate. Control: ursodeoxycholic acid only.
Outcomes	Primary outcome(s): serum alkaline phosphatases and serum gamma-glutamyltransferases. Secondary outcome(s): cytokines.
Starting date	December 2003.
Contact information	http://apps.who.int/trialsearch/Trial.aspx?TrialID=JPRN-C000000225.
Notes	Sponsor is Gunma Liver Study Group. Open public recruiting.

Table 16. Summary of findings table**: b**ezafibrate compared with no intervention for primary biliary cirrhosis

Bezafibrate compared to no intervention for primary biliary cirrhosis

Patient or population: patients with primary biliary cirrhosis
Settings: All trials from Japan
Intervention: Bezafibrate
Comparison: no intervention

Outcomes	Illustrative comparative risks* (95% CI) Assumed risk / Corresponding risk		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	No intervention	Bezafibrate				
All-cause mortality	**Study population**		See comment	60 (3 studies)	See comment	Risks were calculated from pooled risk differences
	See comment	See comment				
	Medium risk population					
	0 per 1000	**0 per 1000** (0 to 0)[1]				
Liver morbidity	**Study population**		See comment	60 (3 studies)	See comment	Risks were calculated from pooled risk differences
	See comment	See comment				
	Medium risk population					
	0 per 1000	**0 per 1000** (0 to 0)[1]				
Adverse advents	**Study population**		**RR 5.4** (0.69 to 42.32)	60 (3 studies)	⊕⊕⊖⊖ **low**[2,3]	
	0 per 1000	**0 per 1000** (0 to 0)				
	Medium risk population					
	0 per 1000	**0 per 1000** (0 to 0)				
Serum alkaline phosphatases (U/L)		The mean Serum alkaline phosphatases (U/L) in the intervention groups was **186.04 lower** (249.03 to 123.04 lower)		79 (4 studies)	⊕⊕⊖⊖ **low**[4,5,6]	
Serum alkaline phosphatases (U/L) - Duration of administration 6 months		The mean Serum alkaline phosphatases (U/L) - Duration of administration 6 months in the intervention groups was **141.97 lower** (228.3 to 55.64 lower)		38 (2 studies)	⊕⊖⊖⊖ **very low**[4,6,7]	
Serum alkaline phosphatases (U/L) - Duration of administration 12-13 months		The mean Serum alkaline phosphatases (U/L) - Duration of administration 12-13 months in the intervention groups was **236.23 lower** (328.35 to 144.1 lower)		41 (2 studies)	⊕⊕⊖⊖ **low**[6,8]	
Serum bilirubin (mg/dl)		The mean Serum bilirubin (mg/dl) in the intervention groups was **0.19 lower** (0.38 lower to 0 higher)		34 (2 studies)	⊕⊕⊖⊖ **low**[3,8]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] This dichotomous outcome was expressed as risk difference (RD) with 95% confidence intervals (CI).

[2] The main limitations in design was the lack of clarity of reporting on adverse events, the lack of clarity of the generation of allocation sequence, the concealment of allocation, blinding, and the length of follow up

[3] Included trials in our meta-analysis include few participants and few events indicating that we have little knowledge about the intervention effect, and that further information is needed.

[4] The main limitations in design was the lack of clarity of the generation of allocation sequence, the concealment of allocation, blinding, and the length of follow up

[5] Heterogeneity is 34%

[6] According to the results of trial sequential analysis there is firm evidence for a beneficial effect of bezafibrate versus no intervention on the activity of serum alkaline phosphatases when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data. Therefore there is no risk for random error.

[7] Heterogeneity is 56%

[8] The main limitations in design was the lack of clarity of the generation of allocation sequence and the concealment of allocation in one trial, one trial was unblinded and another was likely unblinded

Table 17. Summary of findings table: Bezafibrate compared with ursodeoxycholic acid for primary biliary cirrhosis

Bezafibrate compared to ursodeoxycholic acid for primary biliary cirrhosis

Patient or population: patients with primary biliary cirrhosis
Settings: All trials from Japan
Intervention: Bezafibrate
Comparison: ursodeoxycholic acid

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk Ursodeoxycholic acid	Corresponding risk Bezafibrate				
All-cause mortality	**Study population**		See comment	69 (2 studies)	See comment	Risks were calculated from pooled risk differences
	See comment	See comment				
	Medium risk population					
	0 per 1000	0 per 1000 (0 to 0)[1]				
Liver morbidity	**Study population**		See comment	69 (2 studies)	See comment	Risks were calculated from pooled risk differences
	See comment	See comment				
	Medium risk population					
	0 per 1000	0 per 1000 (0 to 0)[1]				
Adverse advents	**Study population**		RD 0.06 (-0.05 to 0.18)	69 (2 studies)	⊕⊕⊖⊖ low[2,3]	
	0 per 1000	0 per 1000 (0 to 0)				
	Medium risk population					
	0 per 1000	0 per 1000 (0 to 0)[1]				
Serum alkaline phosphatases (U/L)		The mean Serum alkaline phosphatases (U/L) in the intervention groups was **162.9 lower** (199.68 to 126.12 lower)		48 (2 studies)	⊕⊕⊕⊖ moderate[2,4]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] This dichotomous outcome was expressed as risk difference (RD) with 95% confidence intervals (CI).

[2] The main limitations in design was the lack of clarity of the generation of allocation sequence and concealment of allocation in one trial. One trial was not blinded, and another one was likely unblinded

[3] Included trials in our meta-analysis include few participants and few events indicating that we have little knowledge about the intervention effect, and that further information is needed

[4] According to the results of trial sequential analysis there is no risk for random error for a beneficial effect of bezafibrate versus UDCA on the activity of serum alkaline phosphatases when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

Bisphosphonates (Paper III)

Results of the search

Our search strategy identified 77 publications, out of which 28 were duplicates. Of the remaining 49 publications, 35 were excluded, either because they were reviews or because they did not relate to primary biliary cirrhosis or because they did not describe a randomised clinical trial investigating the effect of bisphosphonates in participants with primary biliary cirrhosis. Fourteen full-text articles were assessed for eligibility, out of which four were excluded with listed reasons (Image 80).

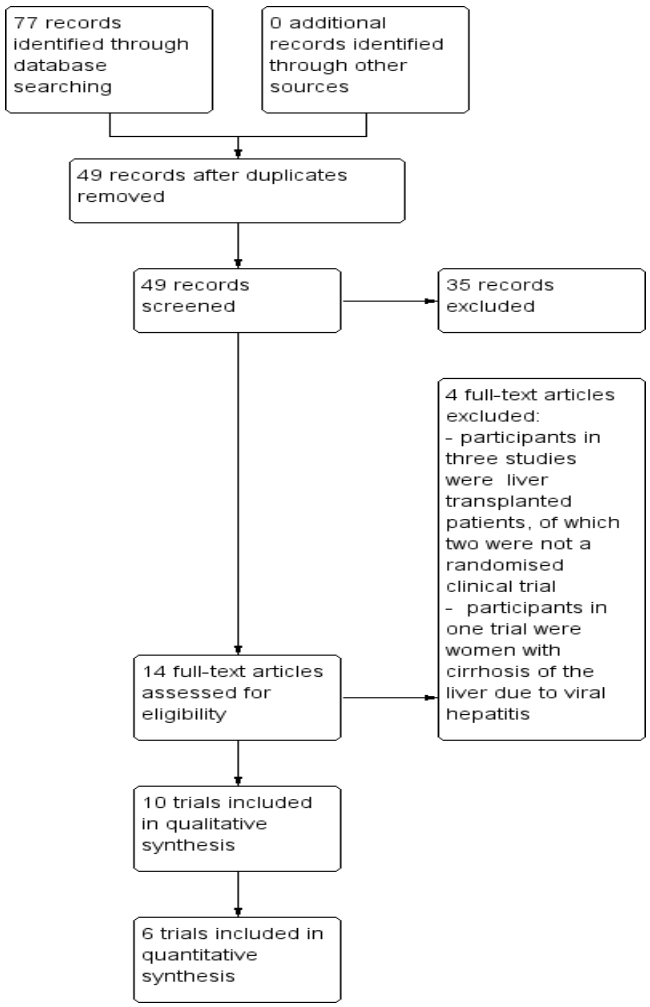

Image 80. Flow chart

We identified a total of 10 publications referring to six randomised clinical trials (Tables of included studies). Four trials were all published as abstracts and as full text articles (Guañabens et al, 1997; Wolfhagen et al, 1997; Guañabens et al, 2003; Zein et al, 2005). One trial was published only as a full text article (Lindor et al, 2000), and another one was published only as an abstract (Pares et al, 2010). The primary authors were contacted for further information and for more data relating to the trials. Dr. Albert Pares kindly provided data on the method of sequence generation, blinding, mortality, fractures, and provided table with numeric values of bone mineral density and markers of bone turnover in both groups of treated participants (Pares et al, 2010). Dr. Frank Wolfhagen kindly provided data on the method of sequence generation, allocation concealment, blinding, and fractures (Wolfhagen et al, 1997). No other responses have been received during the conductance of this review.

We contacted manufacturers of bisphosphonates and asked for any information about unpublished or on-going trials on bisphosphonates in participants with primary biliary cirrhosis. Louise M. Hageman from Warner Chilcott Nederland B.V. replied on knowledge of trials.

A search for ongoing or planned trials in Clinicaltrials.gov (http://clinicaltrials.gov/) and the WHO International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/) did not retrieve any trials.

Included studies

We identified and included six randomised clinical trials which assessed the effect of alendronate, etidronate, and ibandronate (all of them bisphosphonates), in a total of 207 participants with primary biliary cirrhosis. The trials were conducted in Spain, the USA, and the Netherlands. From the publications which reported sex of the participants, more than 92% were females. Two trials were classified as primary prevention trials (Guañabens et

al, 1997; Wolfhagen et al, 1997). Four trials were classified as secondary prevention trials (Lindor et al, 2000; Guañabens et al, 2003; Zein et al, 2005; Pares et al, 2010). In five trials, all patients had non-advanced primary biliary cirrhosis according to inclusion and exclusion criteria (Wolfhagen et al, 1997; Lindor et al, 2000; Guañabens et al, 2003; Zein et al, 2005; Pares et al, 2010). Data about severity of primary biliary cirrhosis among patients and the exclusion criteria were not reported in one trial (Guañabens et al, 1997).

All the six trials used parallel group designs. Three trials assessed a bisphosphonate (etidronate or alendronate) versus placebo or no intervention in 106 participants (Wolfhagen et al, 1997; Lindor et al, 2000; Zein et al, 2005). Two trials assessed a bisphosphonate (etidronate or alendronate) versus another bisphosphonate (alendronate or ibandronate) in 62 participants (Guañabens et al, 2003; Pares et al, 2010). One trial assessed a bisphosphonate (etidronate) versus sodium fluoride in 32 participants (Guañabens et al, 1997). Alendronate was given in a dose of 10 mg/day in one trial (Guañabens et al, 2003) and in a dose of 70 mg weekly in two trials (Zein et al, 2005; Pares et al, 2010). Etidronate was given in a dose of 400 mg/day (Guañabens et al, 1997; Wolfhagen et al, 1997; Lindor et al, 2000; Guañabens et al, 2003). Ibandronate was given monthly in a dose of 150 mg (Pares et al, 2010). In four trials, the duration of administration of bisphosphonates was 12 months (Wolfhagen et al, 1997; Lindor et al, 2000; Zein et al, 2005; Pares et al, 2010), and in the remaining two trials the duration of administration of bisphosphonates was two years (Guañabens et al, 1997; Guañabens et al, 2003). In one trial, patients were previously given immunosuppressive treatment consisting of 30 mg prednisone during the first 4 weeks, 20 mg during the following 4 weeks, and 10 mg daily thereafter for 40 weeks, combined with 50 mg azathioprine daily (Wolfhagen et al, 1997). In five trials, patients were not previously treated with glucocorticosteroids (Guañabens et al, 1997; Lindor et al, 2000; Guañabens et al, 2003; Zein et al, 2005; Pares et al, 2010). Also, in all included trials patients were

not previously treated with sodium fluoride, bisphosphonates, or oestrogens. In one trial most of the patients were treated previously with bisphosphonates, but there was a washout period of at least one year before entering into the trial (Pares et al, 2010).

All the trials reported similar outcome measures such as mortality, fractures, bone mineral density, measurements of biochemical markers of bone turnover, and adverse events. In one trial it was not reported in which participant group a death occurred (Lindor et al, 2000). Fractures were not reported in one trial (Wolfhagen et al, 1997). All trials reported on bone mineral density at lumbar spine and proximal femur, and different markers of bone turnover.

Excluded studies

Four trials were excluded (Table 27). In three trials participants were patients having liver transplantation for chronic liver disease (Valero et al, 1995; Millonig et al, 2005; Crawford et al, 2006), and two out of the three trials were not a randomised clinical trial (Valero et al, 1995; Millonig et al, 2005). One trial was a randomised trial but evaluated the effects of cyclical etidronate on osteopenia in 50 women with cirrhosis of the liver who had underlying hepatitis viral infection (Shiomi et al, 2002).

Risk of bias in included studies

Risk of bias was assessed according to six bias risk domains: sequence generation; allocation concealment; blinding; handling of incomplete outcome data; selective outcome reporting; and other potential sources of bias. Of the six included trials, five were assessed as having high risk of bias, and one as having a low risk of bias (Zein et al, 2005) (Image 81).

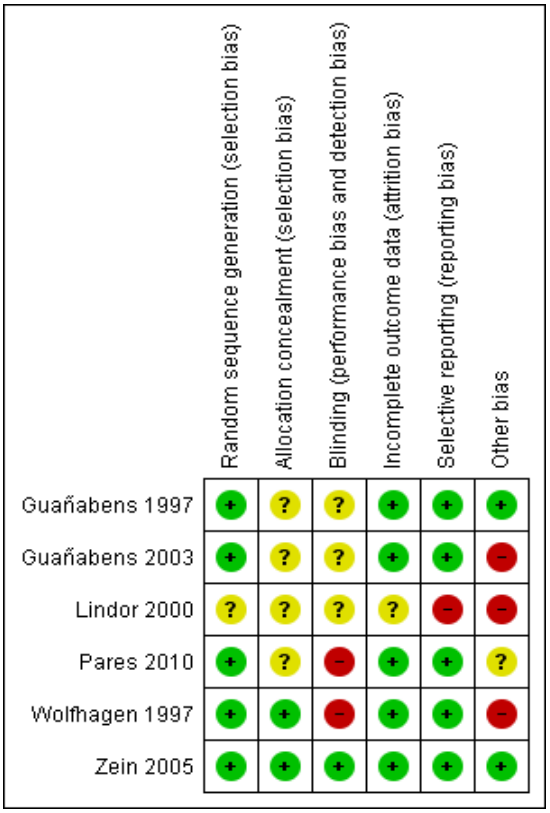

Image 81. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Therefore, the statistical analyses are based mostly on trials with high risk of bias (Image 82).

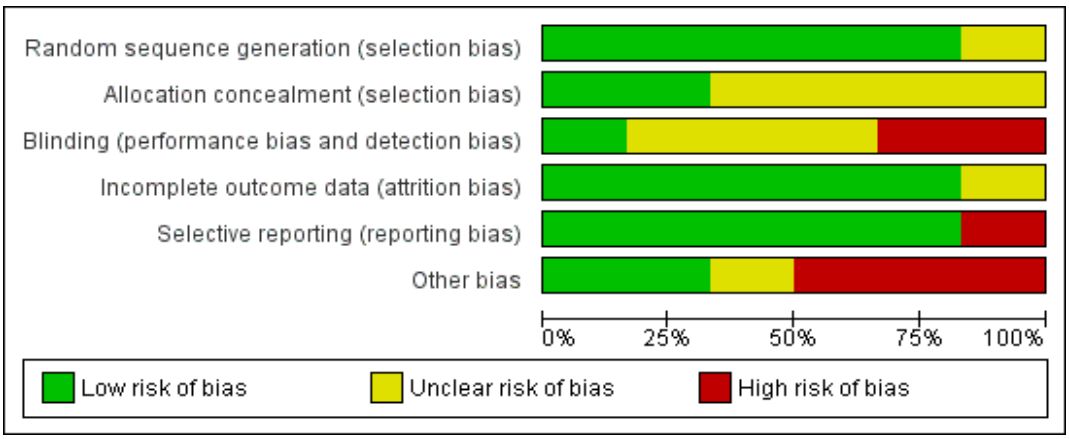

Image 82. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Allocation

In three trials assessing a bisphosphonate versus placebo or no intervention, sequence generation was achieved using a computer random number table in two trials (Wolfhagen et al, 1997; Zein et al, 2005), and in one trial the method of sequence generation was not specified (Lindor et al, 2000). Allocation concealment was controlled by a central and independent randomisation unit (Zein et al, 2005), opaque and sealed envelopes (Wolfhagen et al, 1997), and the method used to conceal the allocation was not described in one trial (Lindor et al, 2000).

In two trials assessing a bisphosphonate versus another bisphosphonate, sequence generation was achieved using computer random number generation (Guañabens et al, 2003; Pares et al, 2010). The method used to conceal the allocation was not described.

In a trial assessing etidronate versus sodium fluoride, sequence generation was achieved using computer random number generation, and the method used to conceal the allocation was not described (Guañabens et al, 1997).

Blinding

From the three trials assessing a bisphosphonate versus placebo or no intervention, only one trial was blinded (Zein et al, 2005). One trial was not blinded (Wolfhagen et al, 1997), and in another one blinding was not reported but it was unlikely to be blinded (Lindor et al, 2000).

From the two trials assessing two different bisphosphonates versus another bisphosphonate, one trial was not blinded (Pares et al, 2010), and another one did not report on blinding and was likely unblinded (Guañabens et al, 2003).

In the trial assessing etidronate versus sodium fluoride, blinding was not reported, so it was likely unblinded (Guañabens et al, 1997).

Incomplete outcome data

Two trials assessing a bisphosphonate versus placebo or no intervention described withdrawals or dropouts from treatment (Wolfhagen et al, 1997; Zein et al, 2005). The number of patients randomised in each group in the beginning of the trial was not reported in one trial; only the number of patients randomised in each group that completed one year therapy was reported, and it was not stated in which group of patients withdrawals or dropouts from treatment or adverse events occurred (Lindor et al, 2000).

Two trials assessing a bisphosphonate versus another bisphosphonate, described withdrawals or dropouts from treatment (Guañabens et al, 2003; Pares et al, 2010).

The trial assessing etidronate versus sodium fluoride described withdrawals or dropouts from treatment (Guañabens et al, 1997).

Selective reporting

The protocols were not available for any of the trials.

From the three trials assessing a bisphosphonate versus placebo or no intervention, two trials reported on expected outcomes (Wolfhagen et al, 1997; Zein et al, 2005), and in one trial, one or more clinically relevant and reasonably expected outcomes were not reported on (Lindor et al, 2000).

The reports included expected outcomes for two trials assessing a bisphosphonate versus another bisphosphonate (Guañabens et al, 2003; Pares et al, 2010).

The trial assessing etidronate versus sodium fluoride reported on expected outcomes (Guañabens et al, 1997).

Other potential sources of bias

The three trials assessing a bisphosphonate versus placebo or no intervention reported the following support: Procter & Gamble Pharmaceuticals BV, The Netherlands (Wolfhagen et al, 1997), Proctor and Gamble (Cincinnati, OH, USA) (Lindor et al, 2000), and Merck Medical School grant (C.O.Z., K.D.L) (Zein et al, 2005).

From the two trials assessing a bisphosphonate versus another bisphosphonate, one trial reported that Merck Sharp & Dohme, Madrid, Spain supplied the alendronate for the trial (Guañabens et al, 2003), and industrial sponsorship was not addressed in another trial (Pares et al, 2010).

In the trial assessing etidronate versus sodium fluoride, it was reported that the work was partly supported by The Field-Initiated Studies Program (FIS) grant (Guañabens et al, 1997).

Risk of bias in assessed comparisons

Out of the three trials assessing a bisphosphonate versus placebo or no intervention, only one trial was with low risk of bias with adequate allocation sequence generation, allocation concealment, blinding, handling of incomplete outcome data, and reporting (Zein et al, 2005). The other two trials were with high risk of bias (Wolfhagen et al, 1997; Lindor et al, 2000) as well as the trials assessing a bisphosphonate versus another bisphosphonate (Guañabens et al, 2003; Pares et al, 2010) and the trial assessing etidronate versus sodium fluoride (Guañabens et al, 1997).

For an overview of the risk of bias of the included trials see image 82.

Effects of interventions (Table 18, 19)

Bisphosphonates versus placebo or no intervention

Two trials assessed etidronate or alendronate versus placebo (Lindor et al, 2000; Zein et al, 2005). One trial assessed etidronate versus no intervention (Wolfhagen et al, 1997) (Table 18)

Primary outcomes

All-cause mortality

We could combine data from two trials (Wolfhagen et al, 1997; Zein et al, 2005). However, there were no deaths reported for either group (0/23 versus 0/23 participants) (RD 0.00; 95% CI -0.12 to 0.12, I^2 = 0%) (Image 83).

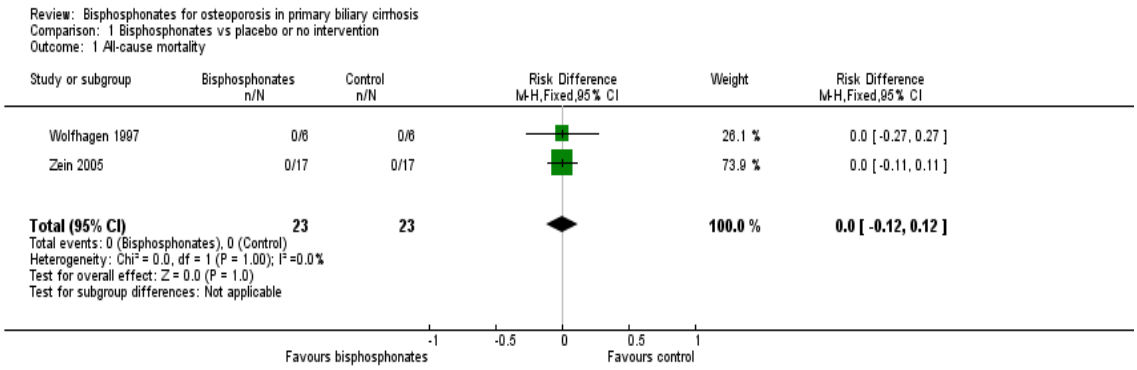

Image 83: bisphosphonates vs placebo or no intervention; outcome: all-cause mortality

New fractures

Three trials reported on fractures (Wolfhagen et al, 1997; Lindor et al, 2000; Zein et al, 2005). There was no statistically significant difference in the number of participants with new fractures in the treatment group compared with the participants in the control group (5/52 versus 6/54 participants) (RR 0.87; 95% CI 0.29 to 2.66, I^2 = 0%) (Image 84).

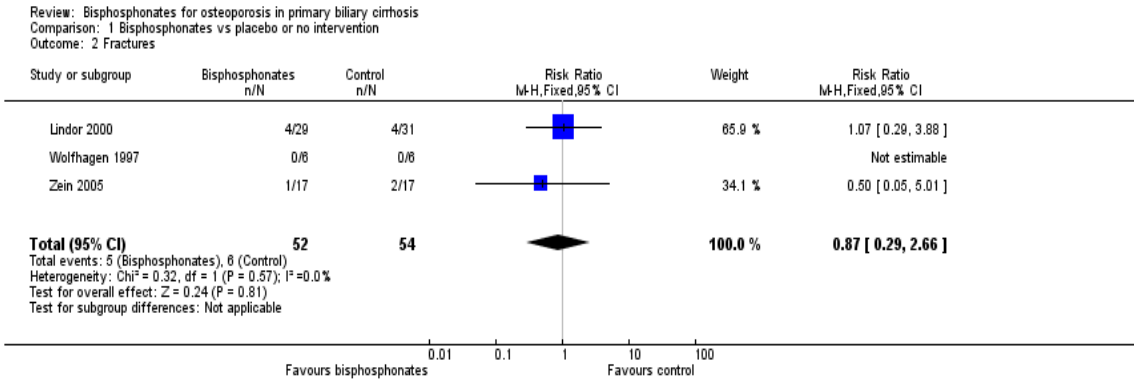

Image 83: bisphosphonates vs placebo or no intervention; outcome: fractures

Adverse events

Two trials reported on adverse events (Wolfhagen et al, 1997; Zein et al, 2005). There was no statistically significant difference in the occurrence of adverse events in participants in the bisphosphonates group (8/23) versus the control group (8/23) (RR 1.00; 95% CI 0.49 to 2.04) (Image 84).

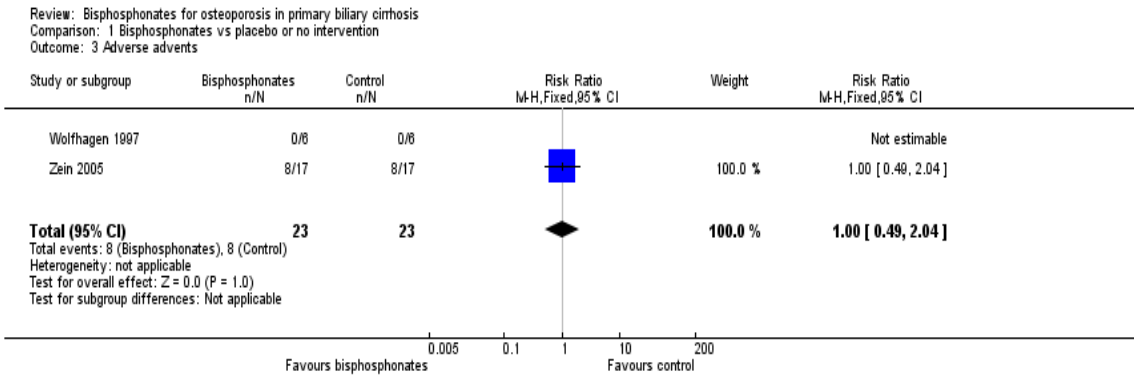

Image 84: bisphosphonates vs placebo or no intervention; outcome: adverse advent

In the alendronate group 7 out of 17 participants compared with 8 out of 17 participants in the placebo group reported gastrointestinal manifestations (eg, abdominal pain, nausea, abdominal distention, heartburn, antral erosions and anaemia, flatulence, or any other gastrointestinal adverse event), and only one

patient in the alendronate group reported concurrent musculoskeletal pain (Zein et al, 2005). One patient in the alendronate group and two patients in the placebo group discontinued therapy as a result of adverse events (Zein et al, 2005). Data from the Wolfhagen trial did not show any adverse events in either treatment or control group (Wolfhagen et al, 1997).

Quality of life

No quality of life measurements were reported.

Secondary outcomes

Bone mineral density (g/cm²)

Three trials reported on the bone mineral density measured at lumbar spine and proximal femur by dual-energy X-ray absorptiometry (Wolfhagen et al, 1997; Lindor et al, 2000; Zein et al, 2005). Bisphosphonates had no significant effect on the bone mineral density measured at the lumbar spine (MD 0.01 g/cm², 95% CI -0.00 to 0.03, I^2 = 8%) (Image 85) and proximal femur (MD 0.00 g/cm², 95% CI -0.01 to 0.02, I^2 = 0%) (Image 86) compared with placebo or no intervention.

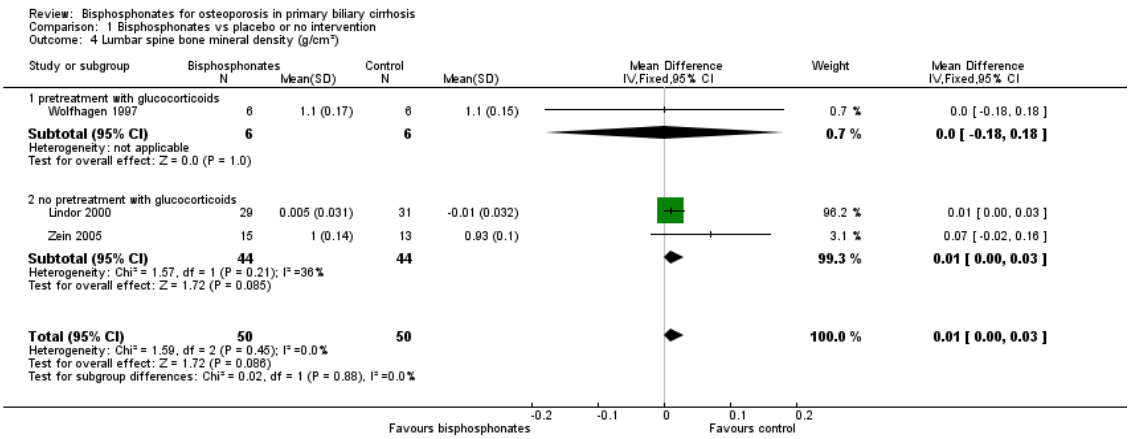

Image 85: bisphosphonates vs placebo or no intervention; outcome: lumbar spine bone mineral density

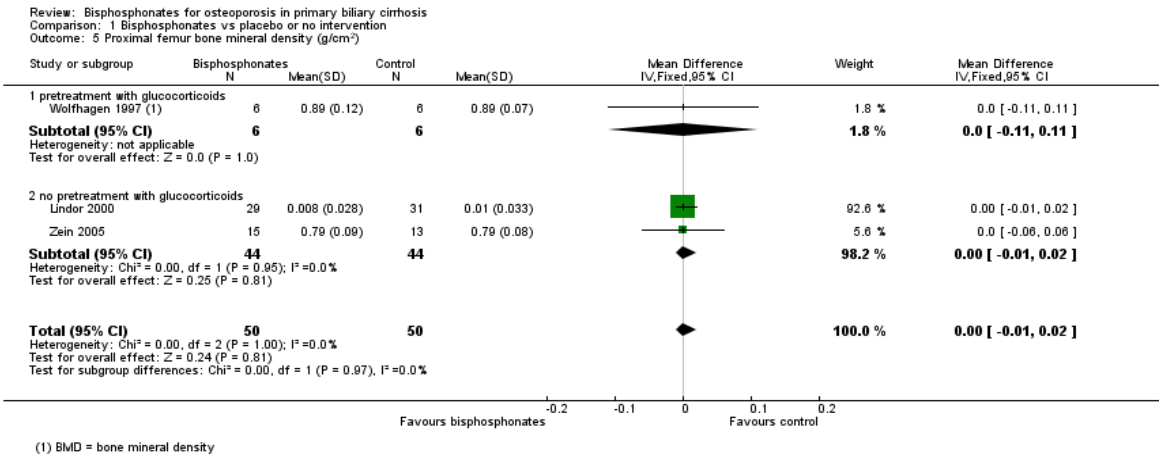

Image 86: bisphosphonates vs placebo or no intervention; outcome: proximal femur bone mineral density

Liver-related mortality or liver transplantation

There were no liver-related deaths reported for any of the two groups (0/23 versus 0/23 participants), and none of the patients underwent liver transplantation (RD 0.00; 95% CI -0.12 to 0.12, $I^2 = 0\%$) (Image 87).

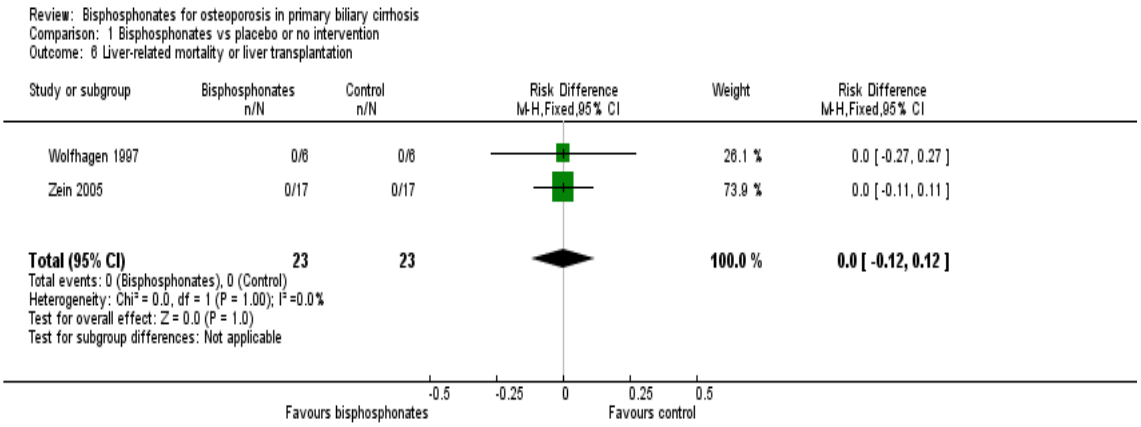

Image 87: bisphosphonates vs placebo or no intervention; outcome: liver mortality or liver transplantation

Liver-related morbidity

Bisphosphonates had no significant effect on liver morbidity (RD 0.00; 95% CI -0.12 to 0.12, I^2 = 0%) (Image 88). Jaundice, upper gastrointestinal haemorrhage, ascites, hepatic encephalopathy, or hepato-renal syndrome occurred in 0/23 (0%) versus 0/23 (0%) participants in the bisphosphonate and control groups.

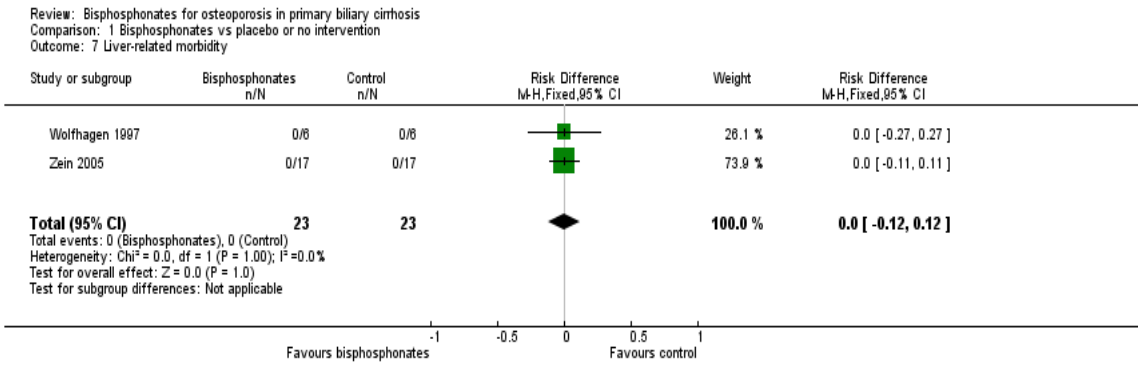

Image 88: bisphosphonates vs placebo or no intervention; outcome: liver-related morbidity

Biochemical markers of bone turnover

Three trials reported on serum osteocalcin (Wolfhagen et al, 1997; Lindor et al, 2000; Zein et al, 2005), and two trials reported on NTx (Lindor et al, 2000; Zein et al, 2005).

These data were reported either as change from baseline (Lindor et al, 2000) or final values (Wolfhagen et al, 1997; Zein et al, 2005). In two trials the data were reported as means with standard deviations (Lindor et al, 2000; Zein et al, 2005). In one trial only standard error of the mean was reported; therefore, we converted it to standard deviation (Wolfhagen et al, 1997). To assess the effect of bisphosphonates on serum osteocalcin concentration, we used the standardised mean difference (SMD) because one trial (Wolfhagen et al, 1997) reported different measure unit for serum osteocalcin compared to the other two trials (Lindor et al, 2000; Zein et al, 2005).

In fixed-effect meta-analyses, bisphosphonates significantly decreased serum osteocalcin (SMD -0.81; 95% CI -1.22 to -0.39, I^2 = 34 %) (Image 89) and NTx concentration (MD -16.93 nmol bone collagen equivalents (BCE)/mmol creatinine (Cr), 95% CI -23.77 to -10.10, I^2 = 0%) (Image 90) compared with placebo or no intervention.

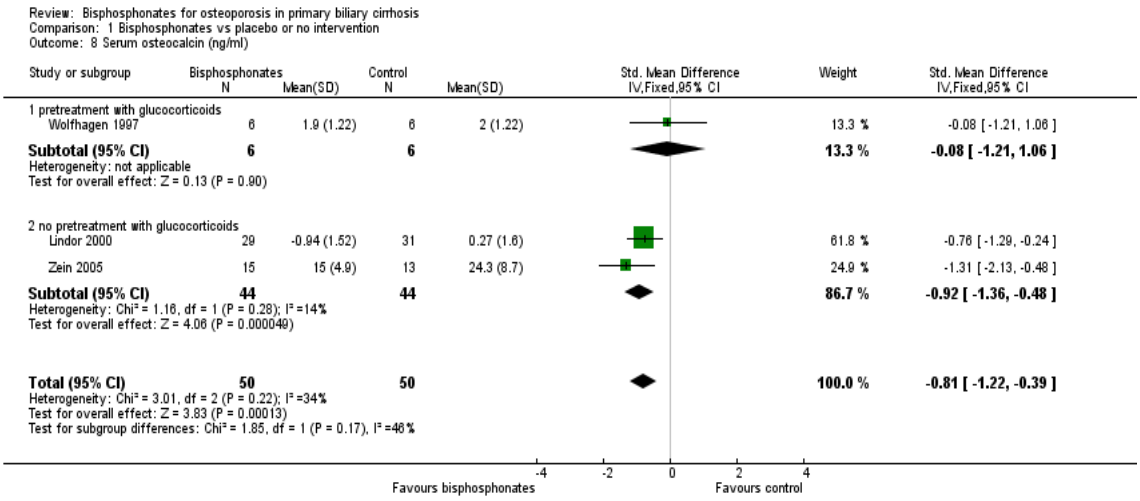

Image 89: bisphosphonates vs placebo or no intervention; outcome: serum osteocalcin

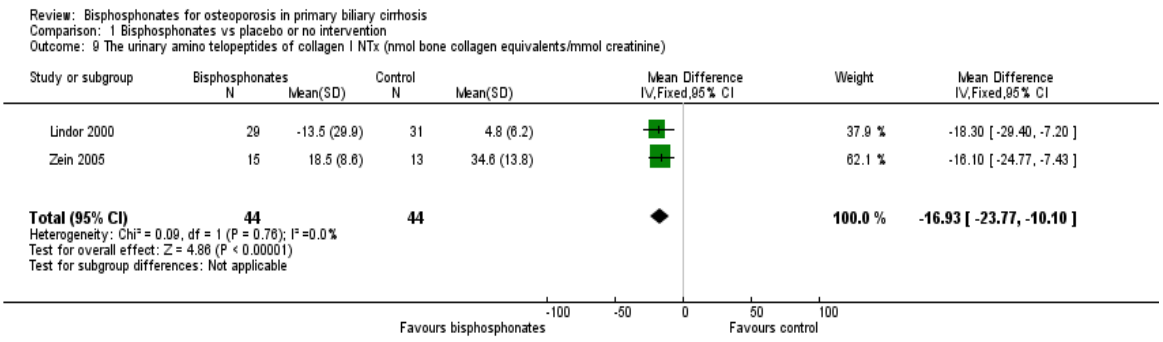

Image 90: bisphosphonates vs placebo or no intervention; outcome: NTx concentration

Trial sequential analysis supports the finding in Analysis 1.9 (Image 91). The result of the trial sequential analysis is shown by the cumulated Z-curve (blue

curve) which crosses the trial sequential monitoring boundary (red curve) implying that there is firm evidence for a beneficial effect of 11.5 nmol BCE/mmol Cr decrease in NTx concentration in the bisphosphonates group (Image 91).

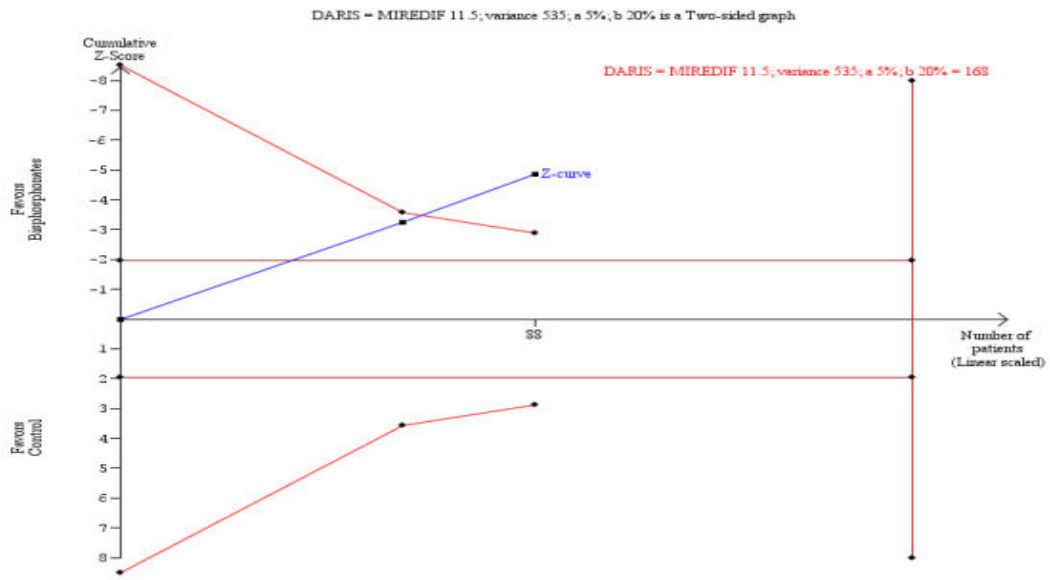

Image 91. Trial sequential analysis of the cumulative meta-analysis of the effect of bisphosphonates versus placebo or no intervention on the urinary amino telopeptides of collagen I (NTx) concentration in participants with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 168 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 11.5 nmol bone collagen equivalents (BCE)/mmol creatinine (Cr), a standard deviation of 23 nmol bone collagen equivalents/mmol creatinine, a risk of type 1 error of 5%, a power of 80%, and a diversity of 0%. The cumulated Z-curve (blue curve) crosses the trial sequential monitoring boundary (red curve) implying that there is firm evidence for a beneficial effect of 11.5 nmol bone collagen equivalents/mmol creatinine decrease in NTx concentration when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

Number of patients having bisphosphonates withdrawn due to adverse events

Discontinuation of bisphosphonate administration occurred in 1/23 patients in bisphosphonates group versus 2/23 patients in the control group due to adverse events (RD -0.04; 95% CI -0.21 to 0.12, I^2 = 0%) (Image 92).

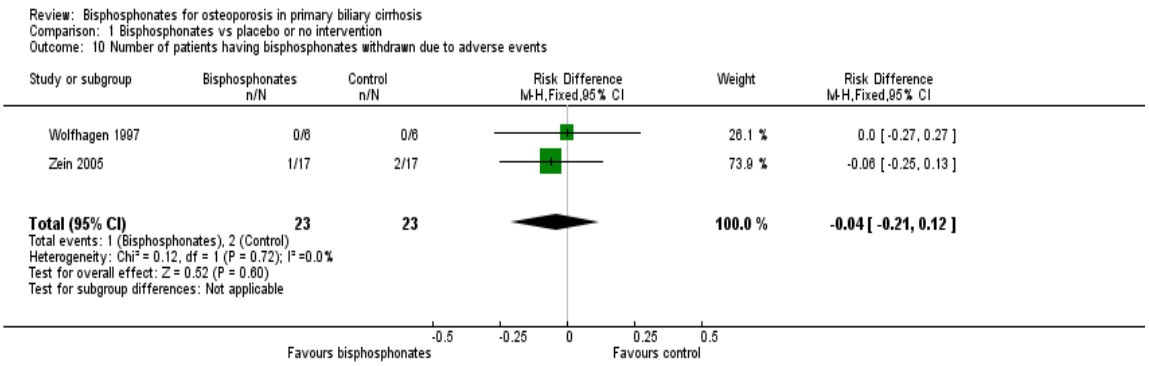

Image 92: bisphosphonates vs placebo or no intervention; outcome: number of patients having bisphosphonates withdrawn due to adverse events

Bisphosphonates versus another bisphosphonate

One trial assessed alendronate versus etidronate (Guañabens et al, 2003), and another trial assessed alendronate versus ibandronate (Pares et al, 2010) (Table 19).

Primary outcomes

All-cause mortality

Two trials reported on mortality (Guañabens et al, 2003; Pares et al, 2010); 0 out of 32 patients died in the bisphosphonates group versus 1 out of 30 patients in the control group (RD -0.03; 95% CI -0.14 to 0.07, I^2 = 0%) (Image 93).

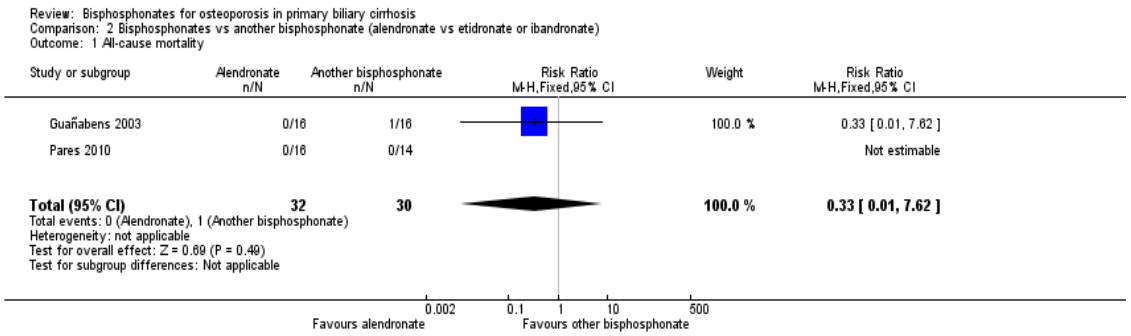

Image 93: bisphosphonates versus another bisphosphonate; outcome: all-cause mortality

One patient who died as a consequence of liver failure was in the etidronate group in the trial assessing alendronate versus etidronate (Guañabens et al, 2003).

New fractures

Two trials reported on fractures (Guañabens et al, 2003; Pares et al, 2010). There was no statistically significant difference in the number of participants with new fractures in the alendronate group compared with the participants in the control group (2/32 versus 2/30 participants) (RR 0.95; 95% CI 0.18 to 5.06, I^2 = 0%) (Image 94).

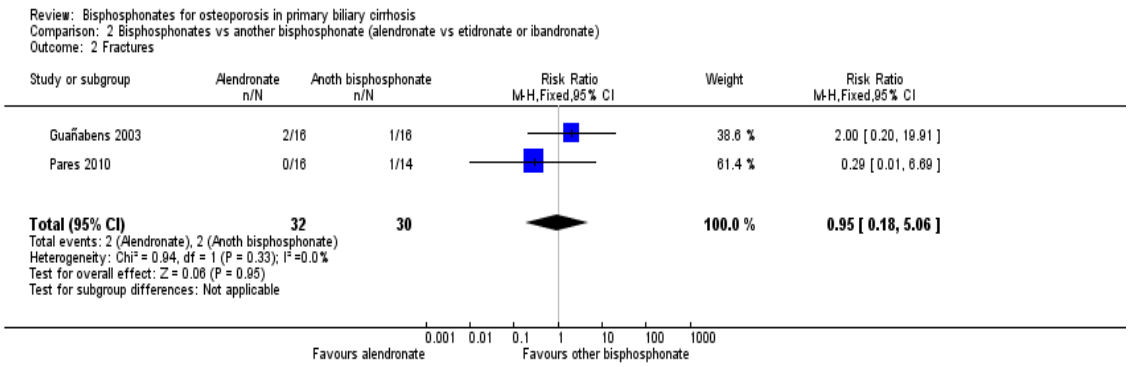

Image 94: bisphosphonates versus another bisphosphonate; outcome: fractures

Adverse events

Two trials reported on adverse events (Guañabens et al, 2003; Pares et al, 2010). There was no statistically significant difference in the occurrence of adverse events among the participants in the bisphosphonates group (5/32) versus the participants in the control group (5/30) (RR 0.95; 95% CI 0.31 to 2.94, I^2 = 0%) (Image 95).

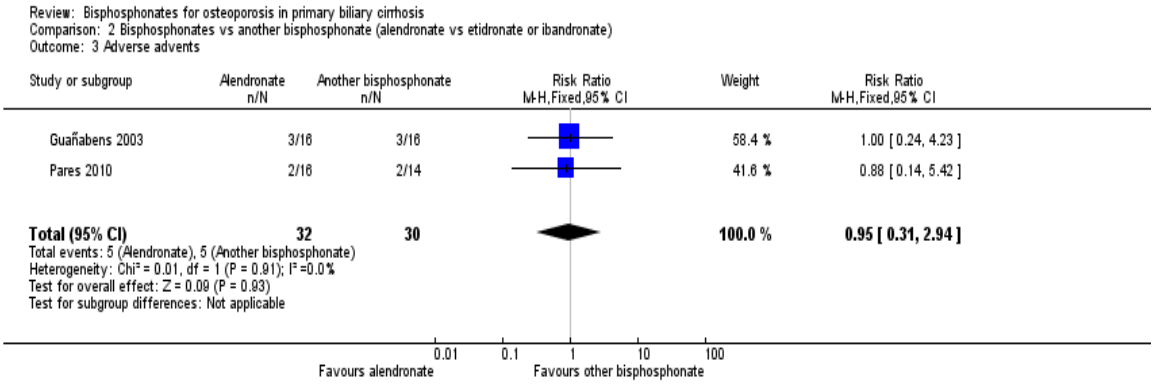

Image 95: bisphosphonates versus another bisphosphonate; outcome: adverse advents

One patient in the etidronate group died during the first year of treatment as a consequence of liver failure; one patient in the alendronate and two patients in the etidronate group left the trial because of gastrointestinal symptoms; and two patients in the alendronate group left the trial within the first six months because they wanted to withdraw (Guañabens et al, 2003).

Two patients in the alendronate group discontinued treatment because of minor gastrointestinal events; two patients in the ibandronate group discontinued because of osteoarticular pain and minor gastrointestinal symptoms; and other two patients discontinued treatment because of violation of the protocol and a coincident disorder (Pares et al, 2010).

Quality of life

No quality of life measurements were reported.

Secondary outcomes

Bone mineral density (g/cm²)

Two trials reported on bone mineral density measured at the lumbar spine and proximal femur by dual-energy X-ray absorptiometry (Guañabens et al, 2003; Pares et al, 2010). Alendronate had no significant effect on the bone mineral density measured at the lumbar spine (MD 0.02 g/cm², 95% CI -0.05 to 0.10, I^2=0%) (Image 96) and proximal femur (MD 0.01 g/cm², 95% CI -0.03 to 0.05, I^2=40%) (Image 97) compared with another bisphosphonate.

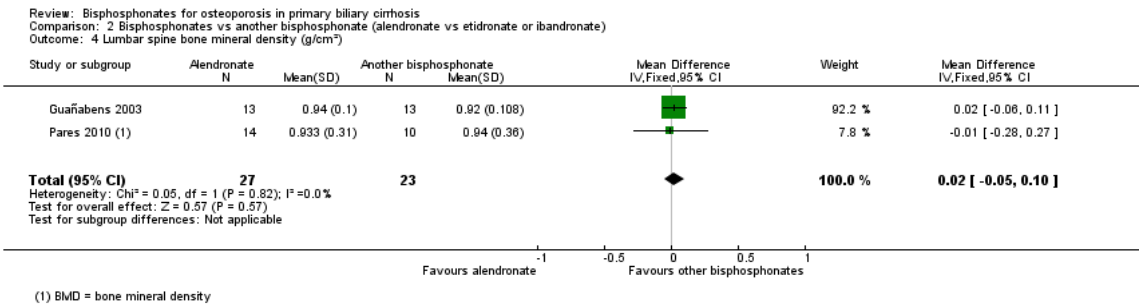

Image 96: bisphosphonates versus another bisphosphonate; outcome: lumbar spine bone mineral density

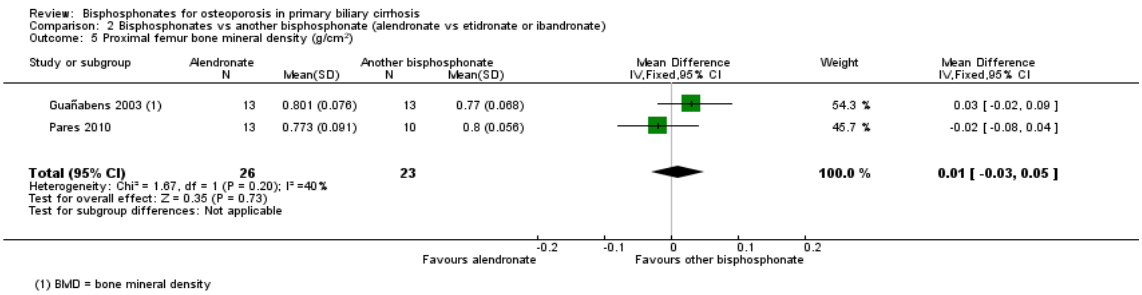

Image 97: bisphosphonates versus another bisphosphonate; outcome: proximal femur bone mineral density

Liver-related mortality or liver transplantation

Alendronate had no significant effect on liver-related mortality or liver transplantation compared with another bisphosphonate. One patient died due to liver failure in the etidronate group versus 0/32 in the alendronate group (RD -0.03; 95% CI -0.14 to 0.07, I^2 = 0%) (Image 98).

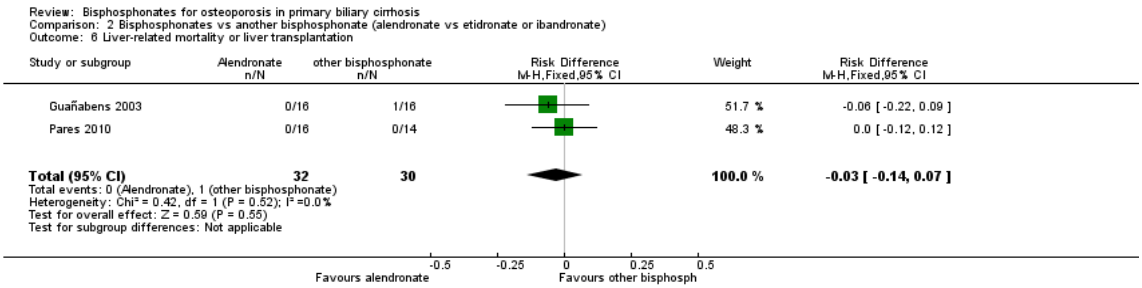

Image 98: bisphosphonates versus another bisphosphonate; outcome: liver-related mortality or liver transplantation

Liver-related morbidity

Bisphosphonates had no significant effect on liver morbidity (RD 0.00; 95% CI -0.09 to 0.09, I^2 = 0%) (Image 99). Jaundice, upper gastrointestinal haemorrhage, ascites, hepatic encephalopathy, or hepato-renal syndrome occurred in 0/32 (0%) versus 0/30 (0%) participants in the alendronate and control groups.

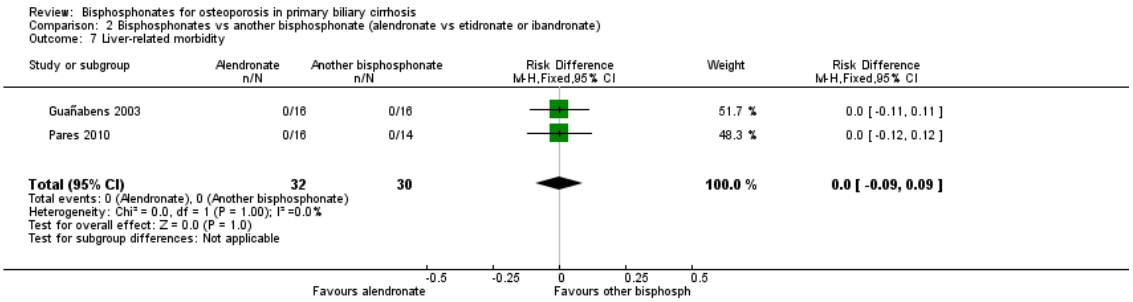

Image 99: bisphosphonates versus another bisphosphonate; outcome: liver-related mortality

Biochemical markers of bone turnover

Two trials reported data on serum osteocalcin, PINP, and NTx (Guañabens et al, 2003; Pares et al, 2010).

These data were reported as final values. In one trial the data were reported as means with standard deviations (Pares et al, 2010). The results reported in another trial regarding markers of bone turnover were depicted graphically, and we extracted data from the graphs (Guañabens et al, 2003). Data were reported as standard error of the mean; therefore, we converted these data to standard deviation (Guañabens et al, 2003).

In fixed-effect meta-analyses, alendronate significantly decreased serum osteocalcin (MD -4.40 ng/ml, 95% CI -6.75 to -2.05, I^2 = 82%) (Image 100), PINP (MD -8.79 ng/ml, 95% CI -15.96 to -1.63, I^2 = 38%) (Image 101), and NTx concentration (MD -14.07 nmol BCE/mmol Cr, 95% CI -24.23 to -3.90, I^2 = 0%) (Image 102) when compared with another bisphosphonate.

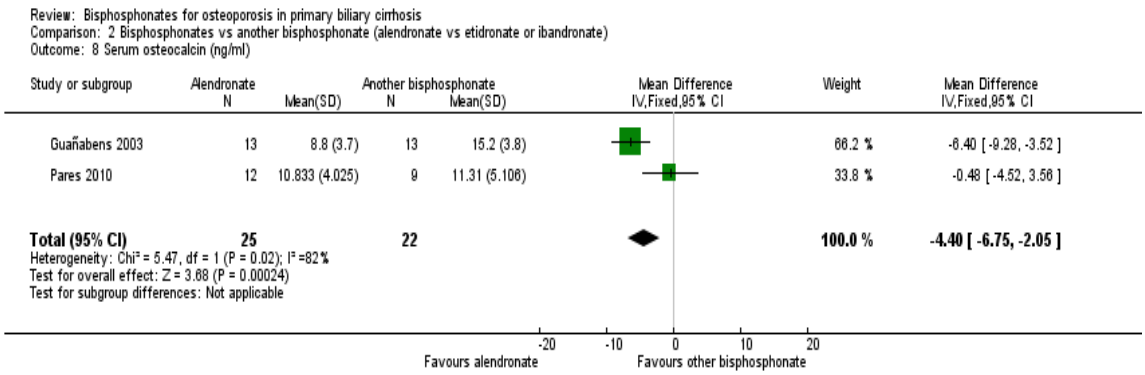

Image 100: bisphosphonates versus another bisphosphonate; outcome: serum osteocalcin

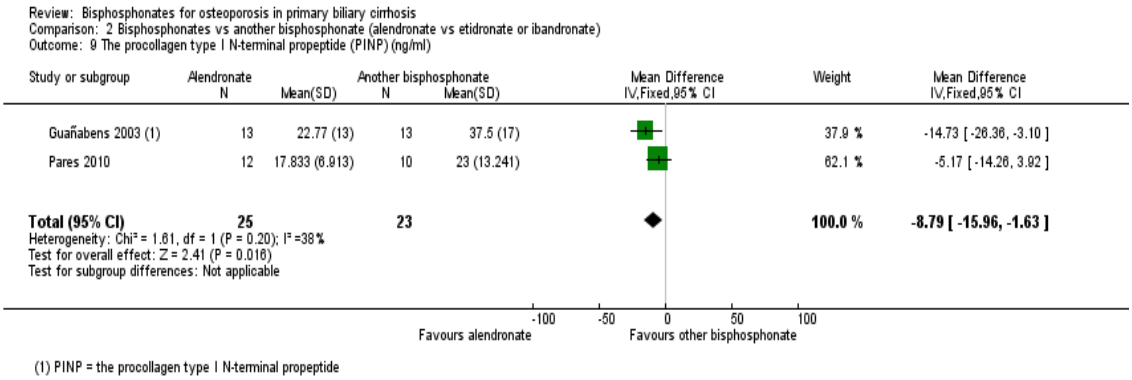

Image 101: bisphosphonates versus another bisphosphonate; outcome: PINP concentration

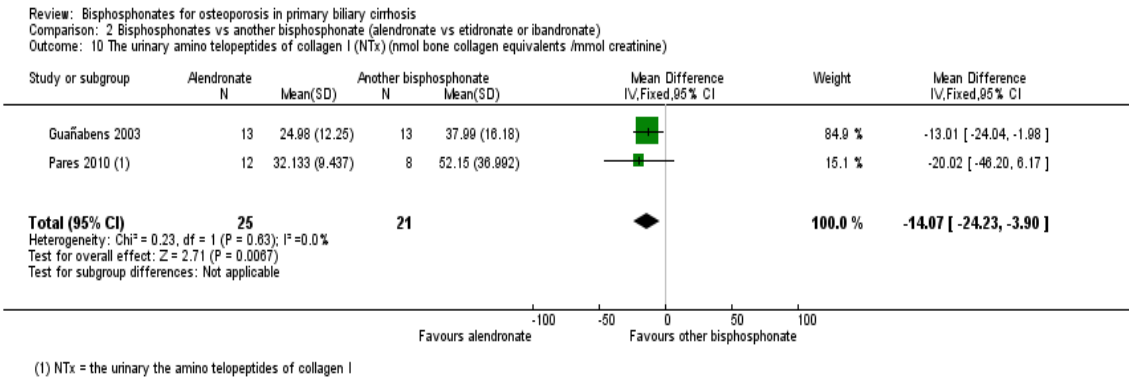

Image 102: bisphosphonates versus another bisphosphonate; outcome: NTx concentration

In random-effect meta-analyses, alendronate had no significant effect on serum osteocalcin concentration (MD -3.61 ng/ml, 95% CI -9.41 to 2.18, I^2 = 82%) when compared with another bisphosphonate.

Trial sequential analyses on these data do not support the finding (image 101, 102). Eventhough the Z-curves (blue curves) lie in the direction of a decrease in PINP and NTx concentrations in the alendronate group, they do not cross the trial sequential monitoring boundaries, implying that there is no firm evidence

for a beneficial effect of 9 ng/ml decrease in PINP concentration (Image 103) and of 12.5 nmol BCE/mmol Cr decrease in NTx concentration (Image 104).

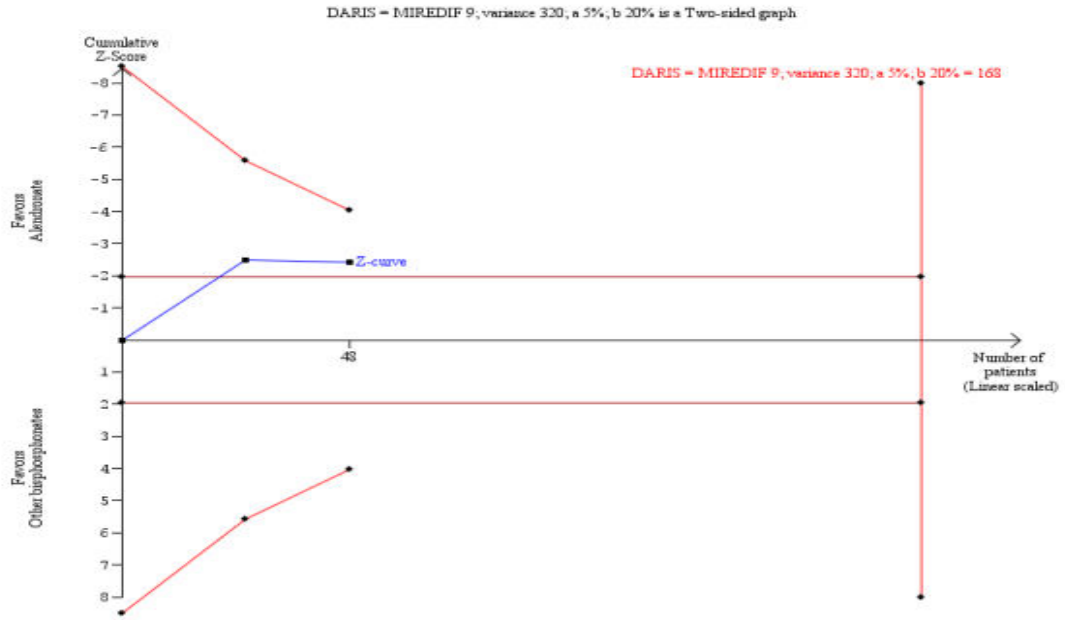

Image 103. Trial sequential analysis of the cumulative meta-analysis of the effect of alendronate versus another bisphosphonate on concentration of the procollagen type I N-terminal propeptide (PINP) in participants with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 168 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 9 ng/ml, a standard deviation of 18 ng/ml, a risk of type 1 error of 5%, a power of 80%, and a diversity of 38%. The cumulated Z-curve (blue curve) does not cross the trial sequential monitoring boundary implying that there is no firm evidence for a beneficial effect of 9 ng/ml decrease in PINP concentration when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

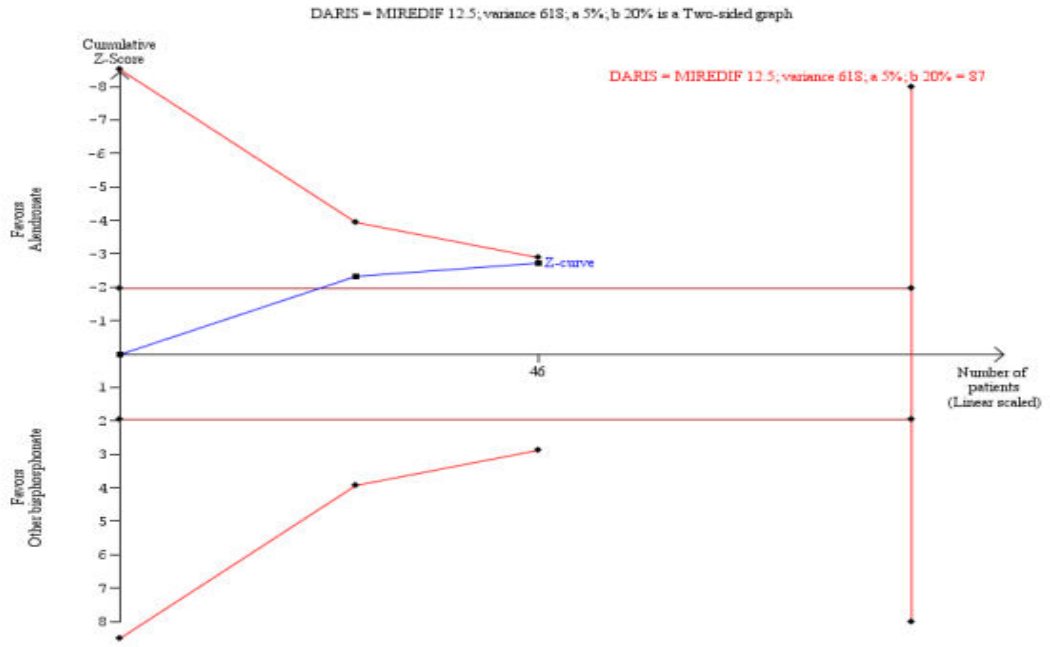

Image 104. Trial sequential analysis of the cumulative meta-analysis of the effect of alendronate versus another bisphosphonate on concentration of the urinary amino telopeptides of collagen I (NTx) in participants with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 87 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 12.5 nmol bone collagen equivalents/mmol creatinine, a standard deviation of 25 nmol bone collagen equivalents/mmol creatinine, a risk of type 1 error of 5%, a power of 80%, and a diversity of 0%. The cumulated Z-curve (blue curve) does not cross the trial sequential monitoring boundary implying that there is no firm evidence for a beneficial effect of 12.5 nmol bone collagen equivalents/mmol creatinine decrease in NTx concentration when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

Number of patients having alendronate withdrawn due to adverse events

Discontinuation of alendronate administration occurred in 3/32 patients in alendronate group versus 5/30 patients in the control group due to adverse events (RR 0.56; 95% CI 0.14 to 2.17, $I^2 = 0\%$) (Image 105).

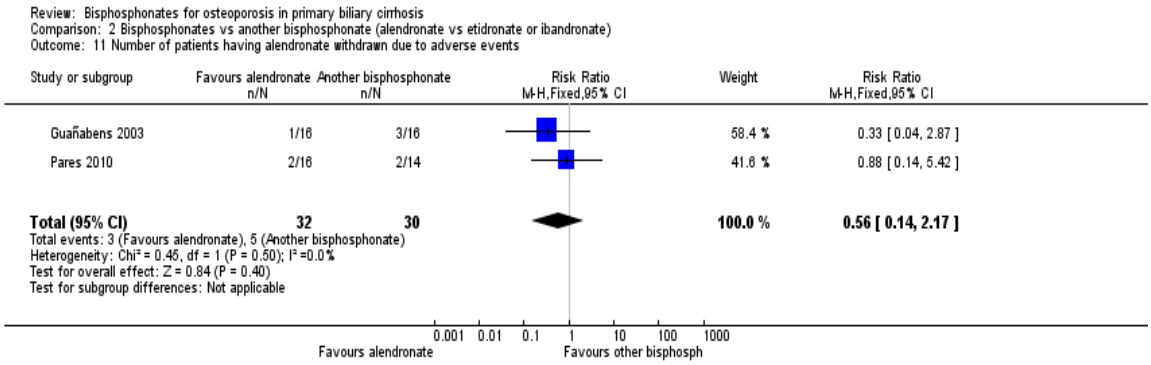

Image 105: bisphosphonates versus another bisphosphonate; outcome: number of patients having alendronate withdrawn due to adverse events

Bisphosphonates versus any other drug

One trial assessed etidronate versus sodium fluoride in 32 patients (Guañabens et al, 1997).

Primary outcomes

All-cause mortality

Death occurred in 1/16 (6.25%) and 0/16 (0%) participants in the etidronate and sodium fluoride groups. There was no significant difference using Fisher's exact test (P = 0.50) (Table 20).

Table 20 Etidronate versus sodium fluoride.

Outcome measures	Type of data	Etidronate group	Sodium fluoride group	Statistical test	P value
All-cause mortality	Dichotomous	1/16 (6.25%)	0/16 (0%)	Fisher's exact test	0.50
Fractures	Dichotomous	3/16 (18.75%)	4/16	Fisher's	0.30

			(25%)	exact test	
Adverse events	Dichotomous	0/16 (0%)	3/16 (18.75%)	Fisher's exact test	0.11
Liver-related mortality or liver transplantation	Dichotomous	1/16 (6.25%)	0/16 (0%)	Fisher's exact test	0.50

New fractures

New fractures occurred in 3/16 (18.75%) and 4/16 (25%) participants in the etidronate and sodium fluoride groups. There was no significant difference using Fisher's exact test (P = 0.30) (Table 21).

Table 21 Etidronate versus sodium fluoride.

Outcome measures	Type of data	Etidronate group	Sodium fluoride group	Statistical test	P value
All-cause mortality	Dichotomous	1/16 (6.25%)	0/16 (0%)	Fisher's exact test	0.50
Fractures	Dichotomous	3/16 (18.75%)	4/16 (25%)	Fisher's exact test	0.30
Adverse events	Dichotomous	0/16 (0%)	3/16 (18.75%)	Fisher's exact test	0.11
Liver-related	Dichotomous	1/16	0/16 (0%)	Fisher's	0.50

mortality or liver transplantation	(6.25%)	exact test

Adverse events

Adverse events occurred in 0/16 (0%) and 3/16 (18.75%) participants in the etidronate and sodium fluoride groups. There was no significant difference using Fisher's exact test (P = 0.11) (Table 22).

Table 22 Etidronate versus sodium fluoride.

Outcome measures	Type of data	Etidronate group	Sodium fluoride group	Statistical test	P value
All-cause mortality	Dichotomous	1/16 (6.25%)	0/16 (0%)	Fisher's exact test	0.50
Fractures	Dichotomous	3/16 (18.75%)	4/16 (25%)	Fisher's exact test	0.30
Adverse events	Dichotomous	0/16 (0%)	3/16 (18.75%)	Fisher's exact test	0.11
Liver-related mortality or liver transplantation	Dichotomous	1/16 (6.25%)	0/16 (0%)	Fisher's exact test	0.50

Quality of life

No quality of life measurements were reported.

Secondary outcomes

Bone mineral density (g/cm²)

Etidronate compared with sodium fluoride had no significant effect on the bone mineral density measured at the lumbar spine, proximal femur, Ward's triangle (area having the lowest bone mineral density in the femoral head), or trochanter. There was no significant difference using the independent groups T-test (Table 23).

Table 23 Etidronate versus sodium fluoride.

Outcome measure Bone mineral density (g/cm²)	Type of data	Etidronate group (mean ± SD)	Sodium fluoride group (mean ± SD)	Statistical test	Degrees of freedom	T value	P value
Lumbar spine	Continuous	0.904 ± 0.14	0.869 ± 0.08	T test	21	0.7047	0.49
Proximal femur	Continuous	0.712 ± 0.11	0.765 ± 0.07	T test	21	1.3271	0.20
Ward's triangle	Continuous	0.585 ± 0.15	0.616 ± 0.07	T test	21	0.6026	0.55
Trochanter	Continuous	0.607 ± 0.10	0.655 ± 0.09	T test	21	1.1907	0.25

Liver-related mortality or liver transplantation

Liver-related death occurred in 1/16 (6.25%) and 0/16 (0%) participants in the etidronate and sodium fluoride groups. There was no significant difference using Fisher's exact test (P = 0.50) (Table 24).

Table 24 Etidronate versus sodium fluoride.

Outcome measures	Type of data	Etidronate group	Sodium fluoride group	Statistical test	Pvalue
All-cause mortality	Dichotomous	1/16 (6.25%)	0/16 (0%)	Fisher's exact test	0.50
Fractures	Dichotomous	3/16 (18.75%)	4/16 (25%)	Fisher's exact test	0.30
Adverse events	Dichotomous	0/16 (0%)	3/16 (18.75%)	Fisher's exact test	0.11
Liver-related mortality or liver transplantation	Dichotomous	1/16 (6.25%)	0/16 (0%)	Fisher's exact test	0.50

Liver-related morbidity

Data on liver-related morbidity were not provided.

Biochemical markers of bone turnover

The trial reported data on serum osteocalcin, urinary hydroxyproline, and parathyroid hormone. Data were reported as standard error of the mean; therefore, we converted them to standard deviation (Higgins and Green, 2011). The results for serum osteocalcin and urinary hydroxyproline are depicted graphically, and we extracted data from the graphs.

Etidronate compared with sodium fluoride significantly decreased serum osteocalcin, urinary hydroxyproline, and parathyroid hormone concentration (Table 25).

Table 25 Etidronate versus sodium fluoride.

Outcome measure Markers of bone turnover	Type of data	Etidronate group (mean ± SD)	Sodium fluoride group (mean ± SD)	Statistical test	Degrees of freedom	T value	P value
Serum osteocalcin (ng/ml)	Continuous	13.81 ± 6.56	24.66 ± 16.06	T test	21	2.219	0.04
Urinary hydroxyproline (nmol/mmol creatinine)	Continuous	59.5 ± 23.05	103.89 ± 49.37	T test	21	2.8742	0.009
Parathyroid hormone (pg/ml)	Continuous	27.4 ± 13.34	40.7 ± 14.55	T test	21	2.2795	0.03

Number of patients having etidronate withdrawn due to adverse events

It was not possible to evaluate this outcome as it was only reported in the etidronate group; one patient died because of liver failure, and two patients were withdrawn with no reasons listed. For the sodium fluoride group it was reported that 6 out of 16 patients were withdrawn (three had gastrointestinal symptoms, one withdrew voluntarily, and for two patients, there were no reasons listed).

Subgroup analyses

Subgroup analysis on trials with low risk of bias compared to trials with high risk of bias

We had insufficient data to perform a subgroup analysis comparing trials with low risk of bias with trials with high risk of bias per each comparison (Image 82).

Subgroup analysis on different doses of a bisphosphonate

Alendronate was given in a dose of 10 mg/day only in one trial (Guañabens et al, 2003) and in a dose of 70 mg weekly in two trials (Zein et al, 2005; Pares et al, 2010). In four trials, etidronate was given in the same dose of 400 mg/day (Guañabens et al, 1997; Wolfhagen et al, 1997; Lindor et al, 2000; Guañabens et al, 2003). Ibandronate was given in one trial monthly in a dose of 150 mg (Pares et al, 2010). Sodium fluoride was given in a dose of 50 mg/day (as 25 mg enteric-coated tablets twice a day) in another trial (Guañabens et al, 1997). A subgroup analysis comparing the different doses of bisphosphonates was not possible.

Subgroup analysis on different duration of administration of a bisphosphonate

Duration of all trials assessing a bisphosphonate versus placebo or no intervention was 12 months (Wolfhagen et al, 1997; Lindor et al, 2000; Zein et al, 2005). We only included two trials assessing a bisphosphonate versus another bisphosphonate, and the duration of administration of alendronate was 2 years and 12 months, respectively (Guañabens et al, 2003; Pares et al, 2010). A subgroup analysis comparing different durations of administration of a bisphosphonate was not possible.

Subgroup analysis on patients treated for primary biliary cirrhosis with glucocorticoids before administration of a bisphosphonate compared to patients with no pretreatment with glucocorticoids

A subgroup analysis was performed to compare patients treated for primary biliary cirrhosis with glucocorticoids before administration of a bisphosphonate to patients with no pretreatment with glucocorticoids. From three trials assessing a bisphosphonate versus placebo or no intervention, only in one trial patients were previously treated with glucocorticoids (Wolfhagen et al, 1997), and in the other two trials, patients were not (Lindor et al, 2000; Zein et al, 2005).

According to our subgroup analyses, pretreatment with glucocorticoids did not influence the bone mineral density measured at lumbar spine (MD 0.00; 95% CI -0.18 to 0.18 compared to MD 0.01; 95% CI -0.00 to 0.03, I^2 = 36%; test of interaction Chi^2 = 0.02; P = 0.88) (Image 85) and proximal femur (MD 0.00; 95% CI -0.11 to 0.11 compared to MD 0.00; 95% CI -0.01 to 0.02, I^2 = 0%; test of interaction Chi^2 = 0.00; P = 0.97) (Image 86). Furthermore, according to our subgroup analysis, pretreatment with glucocorticoids did not influence serum osteocalcin (SMD -0.08; 95% CI -1.21 to 1.06 compared to SMD -0.92; 95% CI -1.36 to -0.48, I^2 = 14%; test of interaction Chi^2 = 1.85; P = 0.17) (Image 89).

Description of studies: tables of included studies (Table 26) and tables of excluded studies (Table 27)

Table 26 tables of included studies

Guañabens 1997

Methods	Randomised clinical trial with parallel group design (two interventions groups). Trial duration: two years.
Participants	Country: Spain. Number of participants randomised: 32, mean age 57 years (100% females). Inclusion criteria: women with primary biliary cirrhosis. Exclusion criteria: none listed. There were no significant differences between the two groups in age, severity of cholestasis, postmenopausal status, and bone mineral density at baseline.
Interventions	Participants were randomly assigned to receive: Intervention group 1: etidronate (400 mg/day orally, taken on an empty stomach followed by a 13-week period without etidronate), n = 16; Intervention group 2: sodium fluoride (given as 25 mg enteric-coated tablets twice a day), n = 16. All patients received calcium supplements (1000 to 1500 mg/day) and low doses of vitamin D orally (266 µg of 25-hydroxyvitamin D every 2 week), except for the patients in the etidronate group on the days they took this treatment. None of the patients had previously received sodium

	fluoride, bisphosphonates, oestrogens, or glucocorticosteroids. Fourteen patients received 15 mg/kg/day of ursodiol during the trial. Patients did not receive any other treatment that could influence calcium metabolism.	
Outcomes	Outcome measures: - mortality; - fractures; - bone mineral density at the lumbar spine and femur; - measurements of biochemical markers of bone turnover; - adverse events.	
Notes	Additional information was requested on 22nd February 2011, but no response was received.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Sequence generation was achieved using computer random number generation.
Allocation concealment	Unclear risk	The method used to conceal the allocation was not described, so that intervention allocation may have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Unclear risk	The trial did not provide information on this domain, but it is not likely to have been blinded.
Incomplete	Low risk	The numbers and reasons for dropouts

outcome data All outcomes		and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	The trial appears to be free of other components that could put it at risk of bias.

Guañabens 2003

Methods	Randomised clinical trial with parallel group design (two interventions groups). Trial duration: two years.
Participants	Country: Spain. Number of participants randomised: 32, mean age 59 years (100% females). Inclusion criteria: - women with primary biliary cirrhosis and osteopenia. Osteopenia was defined as a bone mineral density value ≥ 1 SD below the young normal mean. Exclusion criteria: - previous gastrointestinal bleeding; - known peptic ulcer; - hiatal hernia; - renal failure (serum creatinine > 1.5 mg/dl); - bilirubin concentration > 10 mg/dl.
Interventions	Participants were randomly assigned to receive:

	Intervention group 1: etidronate (400 mg/day orally, taken on an empty stomach (at the midpoint of a 4-h fast) for 2 weeks, followed by a 13-week period without etidronate), n = 16; Intervention group 2: alendronate (10 mg/day orally, taken on rising in the morning with a glass of water, before the first food or beverage of the day), n = 16. All patients received calcium supplements (1000 to 1500 mg/day) and low doses of vitamin D orally (266 µg of 25-hydroxyvitamin D every 2 week), except for patients in the etidronate group on the days they took this treatment. None of the patients had previously received sodium fluoride, bisphosphonates, estrogens, or glucocorticosteroids. All patients received 14 to 16 mg/ kg/day of ursodeoxycholic acid during the study and did not receive any other treatment that could influence calcium metabolism.
Outcomes	Outcome measures: - mortality; - liver transplantations; - fractures; - bone mineral density at the lumbar spine and femur; - measurements of biochemical markers of bone turnover; - adverse events.
Notes	Additional information requested on 22nd February 2011, but no response was received.

Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Sequence generation was achieved using computer random number generation.
Allocation concealment	Unclear risk	The method used to conceal the allocation was not described, so that intervention allocations may have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Unclear risk	The trial did not provide information on this domain, but the trial is not likely to have been blinded.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	High risk	Alendronate was supplied by Merck Sharp & Dohme, Madrid, Spain.

Lindor 2000

Methods	Randomised, placebo-controlled trial with parallel group design (two interventions groups). Trial duration: one year.

Participants	Country: USA. Number of participants randomised: 67, mean age 61 years (85% females). Inclusion criteria: - well-established diagnosis of primary biliary cirrhosis (positive antimitochondrial antibodies and histologic confirmation of primary biliary cirrhosis); - bone mineral density of the lumbar spine (L2-L4) less than a T-score of -2.0; - an estimated survival based on a Mayo risk score of more than 80% at two years; - age between 18 and 70 years; - a negative pregnancy test prior to entry or needed to use adequate contraceptive measures for women of childbearing age. Exclusion criteria: - a history of peptic ulcer disease; - renal insufficiency (creatinine concentration of more than 2.0 mg/dL); - thyroid disease; - treatment with drugs that are known to affect bone metabolism (including calcitonin, sodium fluoride, bisphosphonates, glucocorticosteroids, testosterone, vitamin D in excess of 1000 units per day, chronic heparin, diphenyl hydantoin, carbamazepine, or phenobarbital therapy) within six months of entry into the trial; - oestrogen use within one year or stopping estrogens within the previous six months.
Interventions	Participants were randomly assigned to receive:

	Intervention group 1: etidronate (oral dose of 400 mg per day for 14 days followed by 76 days of 500 mg of calcium carbonate: the 90-day cycle was repeated 4 times each year), n = 29; Intervention group 2: placebo (placebo regimen was identical and a placebo was substituted for the etidronate), n = 31. Supplemental calcium (500 mg elemental calcium)) was administered on the days patients did not receive etidronate. All patients were treated with ursodeoxycholic acid (13 to 15 mg/kg/day) for their underlying liver disease.	
Outcomes	Outcome measures: - fractures; - bone mineral density of the spine and femur; - measurements of biochemical markers of bone turnover; - adverse events.	
Notes	Of the 67 patients entered, 60 completed at least one year of therapy. The number of patients that completed one year of therapy were randomised as follows: etidronate group n = 29; and placebo group n = 31. The trial did not report on number of patients randomised in each group at the beginning of the trial. Additional information requested on 21st February 2011, but no response was received.	
Risk of bias		
Bias	Authors' judgement Support for judgement	

Random sequence generation	Unclear risk	The trial is described as randomised, but the method of sequence generation was not specified.
Allocation concealment	Unclear risk	The trial was described as randomised, but the method used to conceal the allocation was not described, so that intervention allocation may have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Unclear risk	The trial did not provide information on this domain, but it is not likely to have been blinded.
Incomplete outcome data All outcomes	Unclear risk	The report showed that there had been dropouts, but the number of patients who dropped-out was not specifically stated for each of the two groups.
Selective reporting	High risk	One or more clinically relevant and reasonably expected outcomes were not reported on.
Other bias	High risk	There are other factors in the trial that could put it at risk of bias (baseline imbalance in bone mineral density in the proximal femur), and the drugs and placebo were supplied by Proctor and Gamble (Cincinnati, OH, USA).

Pares 2010

Methods	Randomised clinical trial with parallel group design (two interventions groups). Trial duration: 12 months.
Participants	Country: Spain. Number of participants randomised: 30, mean age 63 years (100% females). Inclusion criteria: postmenopausal women with primary biliary cirrhosis if they had a bone mineral density of osteoporosis or osteopenia and fragility fractures. Exclusion criteria: none listed.
Interventions	Participants were randomly assigned to receive: Intervention group 1: weekly alendronate (70 mg), n = 16; Intervention group 2: monthly ibandronate (150 mg), n = 14.
Outcomes	- bone mineral density of the lumbar spine and proximal femur; - liver function tests, 25-hydroxyvitamin D, and parathyroid hormone; - markers of bone turnover; - adherence assessed by the Morisky-Green score.
Notes	Additional information requested on 23rd February 2011 and reply was received on 1st March 2011 through personal communication with the principal author Dr. Albert Pares. Dr. Albert Pares provided data on the following: - the method of sequence generation (sequence generation was achieved using computer random number

	generation); - blinding (the trial was not blinded); - mortality (no one died); - fractures (only one patient in ibandronate group developed fractures); - bone mineral density and markers of bone turnover in both groups of treated participants (the tables with numeric values were provided). Regarding the severity of primary biliary cirrhosis and patients pre-treatment, Dr. Albert Pares provided the following data: - all patients received ursodeoxycholic acid (14 to 16 mg/kg/day) and there was no other specific treatment for primary biliary cirrhosis nor for the bone disease; - most of the patients were treated previously with bisphosphonates, but there was a washing period of at least one year before entering into the trial; - no patients received hormone replacement or calcitonin, nor glucocorticoids; - no patient had cirrhosis, and most of them were in stages I-II, as this was in agreement with the liver elasticity assessment performed within six months to enrolment.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence	Low risk	Sequence generation was achieved using

generation		computer random number generation.
Allocation concealment	Unclear risk	The method used to conceal the allocation was not described, so that intervention allocations may have been foreseen in advance of, or during enrolment.
Blinding All outcomes	High risk	The trial was not blinded.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for drop-outs and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Unclear risk	Industrial sponsorship was not addressed.

Wolfhagen 1997

Methods	Randomised clinical trial with parallel group design (two interventions groups). Trial duration: one year.
Participants	Country: Netherlands. Number of participants randomised: 12 (6/6), mean age 57/49 years (83%/66% females). Inclusion criteria: - patients with an established diagnosis of primary biliary cirrhosis, participating in a double-blind, placebo controlled trial with prednisone/azathioprine.

	Exclusion criteria: - patients with Child-Pugh Class B or C disease; - previous treatment with oestrogen replacement, bisphosphonates, sodium fluoride or calcitonin; - renal impairment; - other gastrointestinal diseases; - insulin-dependent diabetes mellitus; - pituitary dysfunction; - hyperparathyroidism; - alcoholism; - immobility; - age over 70 years; - presence of osteoporotic vertebral fractures (ie, > 20% reduction in vertebral height).
Interventions	Participants were randomly assigned to receive: Intervention group 1: etidronate (3-monthly cycles of etidronate 400 mg daily during 2 weeks, taken with water with two hours intervals between meals, alternated with 11 weeks of 1250 mg calcium carbonate (500 mg elementary calcium), n = 6; Intervention group 2: calcium alone 500 mg, n = 6. Both regimens were started one month before entry in the trial with immunosuppressives and maintained during the whole study period. The immunosuppressive treatment consisted of 30 mg prednisone during the first four weeks, 20 mg during the following four weeks, and 10 mg daily thereafter for 40 weeks, combined with 50 mg azathioprine daily. All patients had been receiving ursodeoxycholic acid

	during at least one year, and this treatment was continued. One patient stopped the prednisone/azathioprine medication one month after the start of the immunosuppressives because of general malaise.	
Outcomes	Outcome measures: - bone mineral density of the spine and femur; - measurements of biochemical markers of bone turnover.	
Notes	Additional information requested on 21st February 2011 and reply was received on 12th March 2011 through personal communication with the principal author Dr. Frank Wolfhagen. Dr. Frank Wolfhagen provided data on: - the method of sequence generation (sequence generation was achieved using a random number table); - allocation concealment (allocation was controlled by opaque and sealed envelopes); - blinding (the trial was not blinded); - fractures (no fractures were found in either group of treated patients).	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Sequence generation was achieved using a random number table.

Allocation concealment	Low risk	Allocation was controlled by opaque and sealed envelopes so intervention allocations could not had been foreseen in advance of, or during enrolment.
Blinding All outcomes	High risk	The trial was not blinded, so that the allocation was known during the trial.
Incomplete outcome data All outcomes	Low risk	It was specified that there were no dropouts or withdrawals (''all patients completed the study and no adverse effects of etidronate were noted'').
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	High risk	It was stated that grant support was received from Procter & Gamble Pharmaceuticals BV, The Netherlands.

Zein 2005

Methods	Randomised, double-blind, placebo-controlled trial with parallel group design (two intervention groups). Trial duration: one year.
Participants	Country: USA. Number of participants randomised: 34, mean age 61 years (94% females). Inclusion criteria: - well-established diagnosis of primary biliary cirrhosis (positive antimitochondrial antibodies (≥ 1: 40) and liver

	biopsy proven primary biliary cirrhosis); - bone loss evidenced by a lumbar spine (L2-L4) bone mineral density T-score below -1.5; - an estimated survival based on a Mayo risk score of more than 80% at two years; - age between 18 and 70 years; - written informed consent. Exclusion criteria: - a history of peptic ulcer disease; - oesophageal varices; - creatinine concentration of more than 1.8 mg/dL; - thyroid disease; - treatment with drugs that are known to affect bone metabolism (including calcitonin, sodium fluoride, glucocorticosteroids, testosterone, vitamin D in excess of 1,000 IU/d, chronic heparin, diphenyl hydantoin, carbamazepine, or phenobarbital) within six months of entry into the trial; - oestrogen use within one year or stopping estrogens within the previous six months; - patients in whom the decreased bone density could be due to osteomalacia; - patients with low serum 25-OH vitamin D or elevated parathyroid hormone; - decompensated liver disease (ascites, hepatic encephalopathy, or significant coagulopathy indicated by INR > 1.8).
Interventions	Participants were randomly assigned to receive: Intervention group 1: alendronate (oral dose of 70 mg per

	week), n = 17; Intervention group 2: placebo, n = 17. Both formulations were white, oblong pills with no markings, no discernible odour, and no difference to taste. All patients received calcium (1,000 mg/day orally) and vitamin D (5,000 U/wk orally).	
Outcomes	Outcome measures: - efficacy of alendronate in comparison with placebo in patients with primary biliary cirrhosis-associated bone loss; - vertebral fractures; - measurements of biochemical markers of bone turnover; - adverse events.	
Notes		
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Sequence generation was achieved using a random number table.
Allocation concealment	Low risk	Allocation was controlled by a central and independent randomisation unit so that intervention allocations could not have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Low risk	The trial was described as blinded, the parties that were blinded, and the method of blinding was described, so that knowledge of allocation was adequately prevented during

		the trial.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	The trial appears to be free of other components that could put it at risk of bias.

Table 27 tables of excluded studies

Study	Reason for exclusion
Crawford 2006	It is a randomised, double-blind, placebo-controlled trial, but it assesses zoledronic acid in 62 participants having liver transplantation for chronic liver disease.
Millonig 2005	It is not a randomised trial, and participants were patients waiting for liver transplantation; 10 out of 136 with primary biliary cirrhosis and primary sclerosing cholangitis. A total of 98 patients (72%) received alendronate after liver transplantation.
Shiomi 2002	It is a randomised trial that evaluated the effects of cyclical etidronate on osteopenia in 50 women with cirrhosis of the liver who had underlying hepatitis viral infection.
Valero 1995	It is not a randomised trial, and participants were liver-transplanted patients, 12 out of 120 with primary biliary cirrhosis.

Table 18. Summary of findings table: Bisphosphonates compared to placebo or no intervention for osteoporosis in primary biliary cirrhosis

Bisphosphonates compared to placebo or no intervention for osteoporosis in primary biliary cirrhosis

Patient or population: patients with osteoporosis in primary biliary cirrhosis
Settings:
Intervention: Bisphosphonates
Comparison: placebo or no intervention

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo or no intervention	Bisphosphonates				
All-cause mortality	**Study population**		See comment	46 (2 studies)	See comment	Risks were calculated from pooled risk differences
	See comment	See comment				
	Medium risk population					
	0 per 1000	**0 per 1000** (0 to 0)[1]				
Fractures	**Study population**		See comment	106 (3 studies)	⊕⊕⊖⊖ **low**[2,3]	Risks were calculated from pooled risk differences
	111 per 1000	**98 per 1000** (-19 to 221)				
	Medium risk population					
	118 per 1000	**104 per 1000** (-20 to 235)				
Adverse advents	**Study population**		**RR 1** (0.49 to 2.04)	46 (2 studies)	⊕⊕⊖⊖ **low**[3,4]	
	348 per 1000	**348 per 1000** (171 to 710)				
	Medium risk population					
	235 per 1000	**235 per 1000** (115 to 479)				
Lumbar spine bone mineral density (g/cm²)		The mean Lumbar spine bone mineral density (g/cm²) in the intervention groups was **0.01 higher** (0 to 0.03 higher)		100 (3 studies)	⊕⊕⊖⊖ **low**[2,3]	
Proximal femur bone mineral density (g/cm2)		The mean Proximal femur bone mineral density (g/cm2) in the intervention groups was **0 higher** (0.01 lower to 0.02 higher)		100 (3 studies)	⊕⊕⊖⊖ **low**[2,3]	
Serum osteocalcin (ng/ml)		The mean Serum osteocalcin (ng/ml) in the intervention groups was **0.81 standard deviations lower** (1.22 to 0.39 lower)		100 (3 studies)	⊕⊕⊖⊖ **very low**[2,3,5]	SMD -0.81 (-1.22 to -0.39)
The urinary the amino telopeptides of collagen I NTx (nmol bone collagen equivalents/mmol creatinine)		The mean The urinary the amino telopeptides of collagen I NTx (nmol bone collagen equivalents/mmol creatinine) in the intervention groups was **16.93 lower** (23.77 to 10.1 lower)		88 (2 studies)	⊕⊕⊕⊖ **moderate**[4,6]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Dichotomous outcome was expressed as risk difference (RD) with 95% confidence intervals (CI).

[2] The main limitations in design was the lack of clarity of the generation of allocation sequence and concealment of allocation in one trial,, blinding in two trials, and selective reporting in one trial.

[3] Included trials in our meta-analysis include few participants and few events indicating that we have little knowledge about the intervention effect, and that further information is needed.

[4] The main limitations in design was the lack of blinding in one trial. Generation of allocation sequence and concealment of allocation was adequate for both trials.

[5] Statistical heterogeneity I2 = 34%.

[6] According to the results of trial sequential analysis there is firm evidence for a beneficial effect of bisphosphonates versus no placebo or intervention on the urinary amino telopeptides of collagen I (NTx) when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data. Therefore there is no risk for random error.

Table 19. Summary of findings table: Bisphosphonates compared to another bisphosphonates (Alendronate vs etidronate or ibandronate) for osteoporosis in primary biliary cirrhosis

Bisphosphonates compared to another bisphosphonates (Alendronate vs etidronate or ibandronate) for osteoporosis in primary biliary cirrhosis

Patient or population: patients with osteoporosis in primary biliary cirrhosis
Settings:
Intervention: Bisphosphonates
Comparison: another bisphosphonates (Alendronate vs etidronate or ibandronate)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk **Another bisphosphonates (Alendronate vs etidronate or ibandronate)**	Corresponding risk **Bisphosphonates**				
All-cause mortality	**Study population**		See comment	62 (2 studies)	⊕⊕⊖⊖ **low**[1,2]	Risks were calculated from pooled risk differences
	33 per 1000	**1 per 1000** (-106 to 102)				
	Medium risk population					
	31 per 1000	**1 per 1000** (-99 to 96)				
Fractures	**Study population**		**RR 0.95** (0.18 to 5.06)	62 (2 studies)	⊕⊕⊖⊖ **low**[1,2]	
	67 per 1000	**64 per 1000** (12 to 339)				
	Medium risk population					
	67 per 1000	**64 per 1000** (12 to 339)				
Adverse advents	**Study population**		**RR 0.95** (0.31 to 2.94)	62 (2 studies)	⊕⊕⊖⊖ **low**[1,2]	
	167 per 1000	**159 per 1000** (52 to 491)				
	Medium risk population					
	165 per 1000	**157 per 1000** (51 to 485)				
Lumbar spine bone mineral density (g/cm²)		The mean Lumbar spine bone mineral density (g/cm²) in the intervention groups was **0.02 higher** (0.05 lower to 0.1 higher)		50 (2 studies)	⊕⊕⊖⊖ **low**[1,2]	
Proximal femur bone mineral density (g/cm2)		The mean Proximal femur bone mineral density (g/cm2) in the intervention groups was **0.01 higher** (0.03 lower to 0.05 higher)		49 (2 studies)	⊕⊖⊖⊖ **very low**[1,2,3]	
The procollagen type I N-terminal propeptide (PINP) (ng/ml)		The mean The procollagen type I N-terminal propeptide (PINP) (ng/ml) in the intervention groups was **8.79 lower** (15.96 to 1.63 lower)		48 (2 studies)	⊕⊖⊖⊖ **very low**[1,2,4]	
The urinary the amino telopeptides of collagen I (NTx) (nmol bone collagen equivalents /mmol creatinine)		The mean The urinary the amino telopeptides of collagen I (NTx) (nmol bone collagen equivalents /mmol creatinine) in the intervention groups was **14.07 lower** (24.23 to 3.9 lower)		46 (2 studies)	⊕⊕⊖⊖ **low**[1,2]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] The main limitations in design was the lack of clarity of concealment of allocation. One trial was not blinded, and another one was likely unblinded.

[2] Included trials in our meta-analysis include few participants and few events indicating that we have little knowledge about the intervention effect, and that further information is needed.

[3] Statistical heterogeneity I2 = 40%

[4] Statistical heterogeneity I2 = 38%

Hormone replacement (Paper IV)

Results of the search

Our search strategy identified 42 publications, out of which 16 were duplicates. Of the remaining 26 publications, 22 were excluded, either because they were reviews, or because they did not relate to primary biliary cirrhosis, or because they did not describe a randomised clinical trial investigating the effect of hormone replacement in women with primary biliary cirrhosis (Image 106).

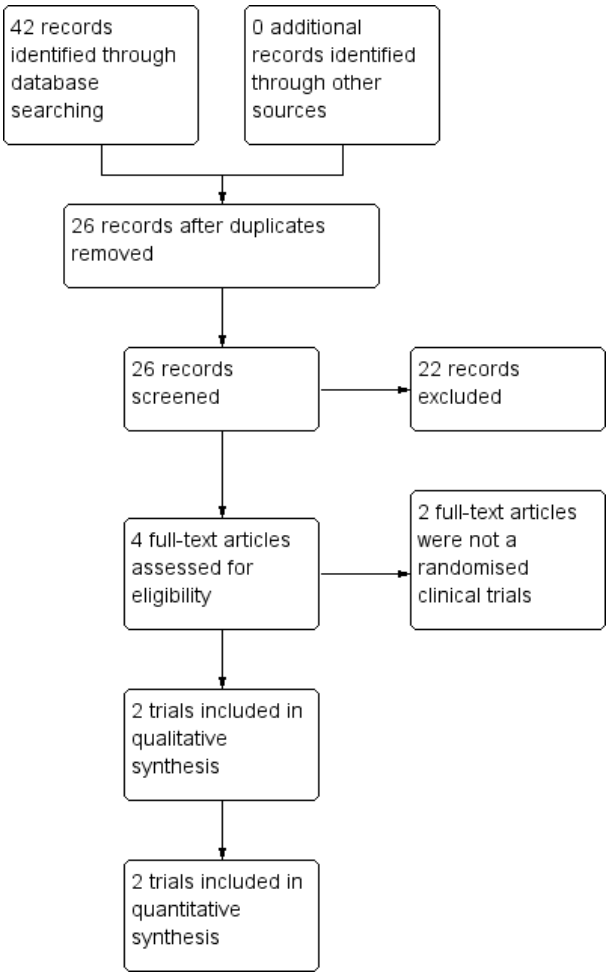

Image 106. Study flow diagram

We identified a total of two publications referring to two randomised clinical trials (Table 35). The two trials were published as full text articles (Ormarsdottir et al, 2004; Boone et al, 2006). The primary authors were contacted for data and other information on the trials. Dr. Jenny Heathcote kindly responded to our inquiry, but she could not provide data on the trial that had been initiated almost 20 years ago (Boone et al, 2006). No other responses were received.

We contacted manufacturers of oestrogens and progestins and asked for any information about unpublished or on-going trials using oestrogens and progestins involving participants with primary biliary cirrhosis. Novartis, Novo Nordisk, and Noven Pharmaceuticals kindly replied that they knew only of two trials we had already included.

We have not identified any registered ongoing or planned trials through Searching Clinicaltrials.gov (http://clinicaltrials.gov/) and the WHO International Clinical Trials Registry Platform (http://www.who.int/ictrpen/).

Included studies

We identified and included two randomised clinical trials which assessed the effect of hormone replacement in a total of 49 participants with primary biliary cirrhosis. The trials were conducted in Canada and Sweeden. Both trials were multicenter trials with parallel group design (Ormarsdottir et al, 2004; Boone et al, 2006). Hormone replacement versus placebo was assessed in 31 participants in one trial (Boone et al, 2006), and hormone replacement versus no intervention was assessed in 18 participants in another trial (Ormarsdottir et al, 2004). Participants in both trials were postmenopausal women with primary biliary cirrhosis. Those women had previously not been treated with drugs known to affect the bone metabolism. In both trials, hormone replacement was given transdermally. In one trial hormone replacement was given as oestradiol patch in combination with medroxyprogesterone (Ormarsdottir et al, 2004).

Oestradiol patch was given in a dose of 50 μg per day twice weekly, and medroxyprogesterone in a dose of 2.5 mg daily continuously (if more then 2 years from menopause), or in a dose of 10 mg daily for 12 days per month (if less then 2 years from menopause) (Ormarsdottir et al, 2004). In the other trial, hormone replacement was given as 7β-estradiol for two weeks followed by two weeks of combined transdermal norethisterone acetate and 17β-estradiol (Boone et al, 2006). 7β-estradiol was given in a dose of 0.05 mg daily and norethisterone acetate in a dose of 0.25 mg daily. The duration of administration of hormone replacement was two years in both trials. All patients received vitamin D and calcium. In one trial, vitamin D was given in a dose of 0.25 μg daily, and calcium in a dose of 1 g daily (Ormarsdottir et al, 2004). In the other trial, vitamin D was given in a dose of 1000 IU daily, and calcium in a dose of 1500 mg daily (Boone et al, 2006). Both trials reported similar outcome measures: bone mineral density measured at the lumbar spine and proximal femur, clinical events, fractures, changes in biochemical variables, and adverse events.

Excluded studies

We excluded two studies because they were not randomised clinical trials (Menon et al, 2003; Pereira et al, 2004) (Table 36).

Risk of bias in included studies

Risk of bias was assessed according to six domains: sequence generation; allocation concealment; blinding; handling of incomplete outcome data; selective outcome reporting; and other potential sources of bias. One was assessed as having a low risk of bias (Boone et al, 2006), and the other as having a high risk of bias (Ormarsdottir et al, 2004) (Image 107).

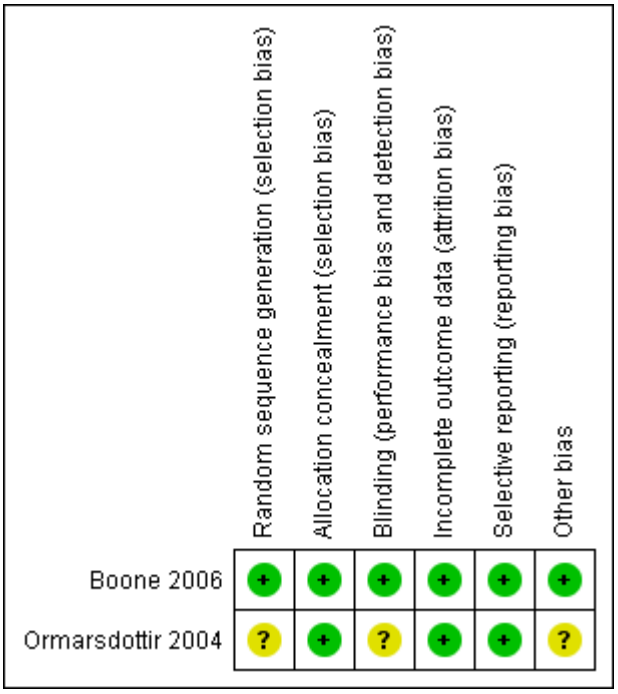

Image 107. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Statistical analyses, which include both trials, are, therefore, based on trials with high risk of bias (Image 108; Table 37)

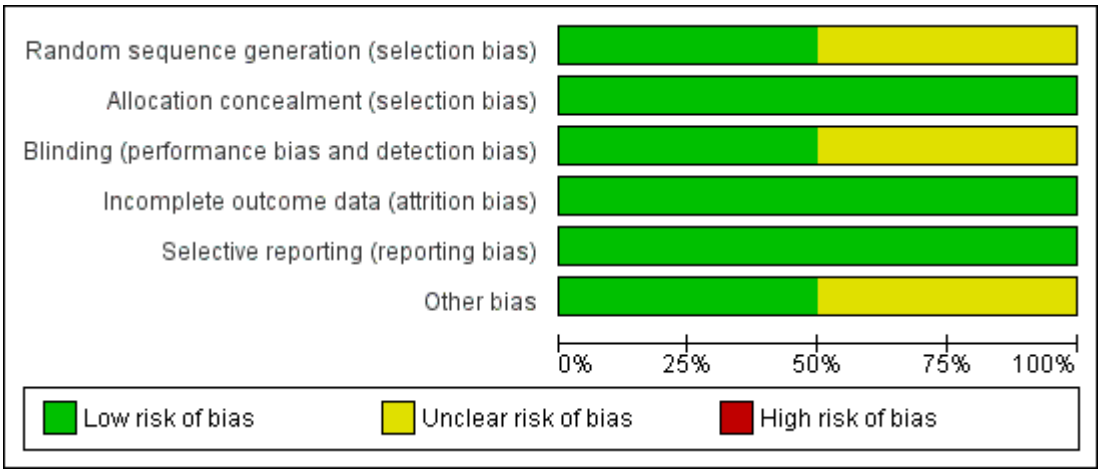

Image 108. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Allocation

In the trial assessing hormone replacement versus placebo, sequence generation was achieved using a randomisation table (Boone et al, 2006). The method of sequence generation was not specified In the trial assessing hormone replacement versus no intervention (Ormarsdottir et al, 2004). Allocation concealment was performed by independent pharmacist who had no role in patient contact or follow-up, nor did he/she participate in data analysis (Boone et al, 2006) and control by sealed envelopes (Ormarsdottir et al, 2004).

Blinding

One trial was blinded (Boone et al, 2006). The other trial did not report on blinding and was likely unblinded (Ormarsdottir et al, 2004).

Incomplete outcome data

The numbers and reasons for dropouts and withdrawals in all intervention groups were described in both included trials.

Selective reporting

The protocols were not available for any of the trials, but pre-defined, or clinically relevant and reasonably expected outcomes were reported.

Other potential sources of bias

The trial assessing hormone replacement versus placebo seems to be free from other potential sources of bias, apart from the fact that it reported that transdermal oestrogen/progestin and placebo were supplied by Novartis (Boone et al, 2006). Novartis was not involved in the collection, analysis, or presentation of the data (Boone et al, 2006). The trial assessing hormone replacement versus no intervention reported sponsorship from Novartis, but it

did not report if Novartis was involved in the collection and data analysis in presentation of the results (Ormarsdottir et al, 2004).

Effects of interventions (Table 37)

Primary outcomes

All-cause mortality

No deaths were reported for any of the two groups (0/24 versus 0/25 participants) (RD 0.00; 95% CI -0.11 to 0.11; I^2 = 0%) (Image 109).

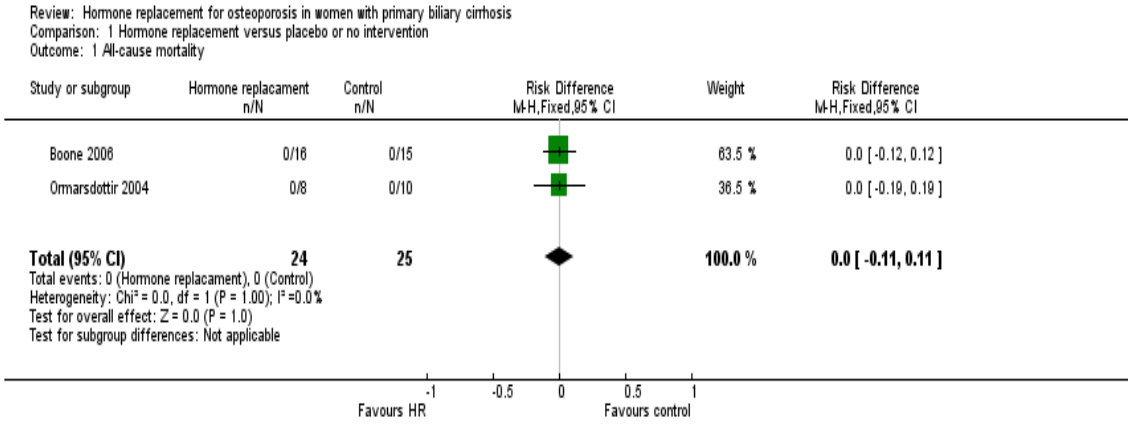

Image 109: hormone replacement versus placebo or no intervention; outcome: all-cause mortality

New fractures

In the trial assessing hormone replacement versus no intervention, no fractures were found in either groups (Ormarsdottir et al, 2004). In the trial assessing hormone replacement versus placebo, 2/15 participants in the placebo group reported fractures compared with 0/16 participants in the treatment group (Boone et al, 2006). There was no statistically significant difference in the number of participants with new fractures in the treatment group compared with controls (RD -0.08; 95% CI -0.24 to 0.07; I^2 = 0%) (Image 110).

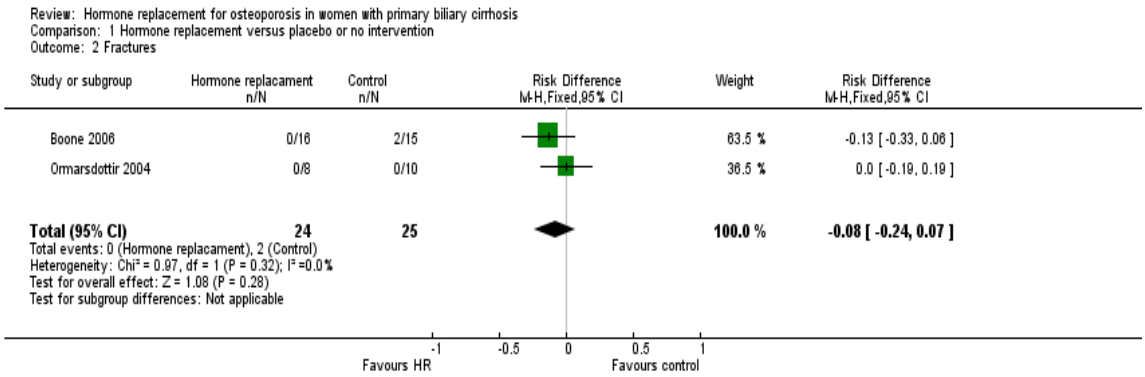

Image 110: hormone replacement versus placebo or no intervention; outcome: fractures

Adverse events

There was a statistically significant increase in the occurrence of adverse events in the hormone replacement group (10/24) versus the control group (2/25) (RR 5.26; 95% CI 1.26 to 22.04; $I^2 = 0\%$) (Image 111).

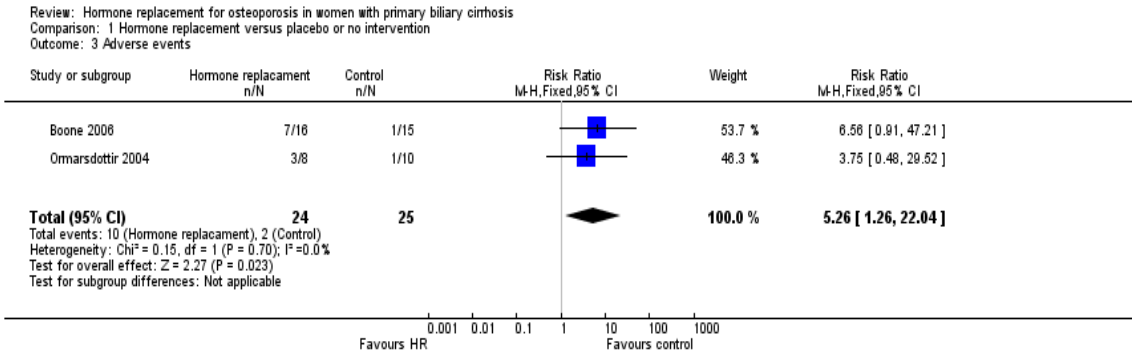

Image 110: hormone replacement versus placebo or no intervention; outcome: adverse advents

Reasons for withdrawal of participants due to the occurrence of adverse events are provided in Table 28 and Table 29.

Table 28 Reasons for withdrawals from treatment due to adverse events (Ormarsdottir 2004)

Adverse events	Hormone replacement	Placebo
Temporary spotty vaginal bleeding	1/8	0/10
Slight increase in systolic blood pressure	1/8	0/10
Increase in liver enzymes	1/8	0/10
Increase in bilirubin concentration	0/8	1/10

Table 29 Reasons for withdrawals from treatment due to adverse events (Boone 2006)

Adverse event	Hormone replacement	Placebo
Generalised pruritus	1/16	0/15
Pneumonia, pulmonary embolism	1/16	0/15
Abdominal pain, headache	1/16	0/15
Local pruritus at patch site	1/16	0/15
Heavy vaginal bleeding	1/16	0/15
Breast pain, chest pain, generalised pruritus, dysuria	1/16	0/15
Local pruritus at patch site	1/16	0/15
Diffuse painful rash of lower back	0/16	1/15

For assessment of harm, besides the data provided by the two randomised trials (Ormarsdottir et al, 2004; Boone et al, 2006) (Table 28, 29) we also considered the data from two non-randomised studies which reported on harm (Menon et al, 2003; Pereira et al, 2004). In Menon 2003, in the hormone replacement group, there were 6 patients out of 46 who experienced adverse events versus 0 patients out of 46 in the control group (Table 30).

Table 30 Adverse events (Menon 2003)

Adverse event	Hormone replacement	No intervention
Breast tenderness	1/46	0/46
Vaginal spotting	1/46	0/46
Increase in bilirubin concentration	4*/46	0/46

*In three of the four patients with increase in bilirubin concentration, this was because of worsening liver function, as manifest by worsening ascites and development of oesophageal varices. The remaining patient developed elevations in her serum bilirubin and alkaline phosphatase after stopping ursodeoxycholic acid therapy.

In Pereira 2004, in the hormone replacement group, there were 2 patients out of 21 who experienced an adverse event versus 0 patients out of 21 in the control group (Table 31).

Table 31 Adverse events (Pereira 2004)

Adverse events	Hormone replacement patches	No intervention
Monthly bleeding	2/21	0/21

Quality of life

No quality of life measurements were reported.

Secondary outcomes

Change in per cent in bone mineral density per year (g/cm^2 year^{-1})

Hormone replacement had no significant effect on bone mineral density measured at the lumbar spine compared with placebo or no intervention (MD 1.25 g/cm^2 year^{-1}; 95% CI -0.91 to 3.42; I^2 = 0%) (Image 111).

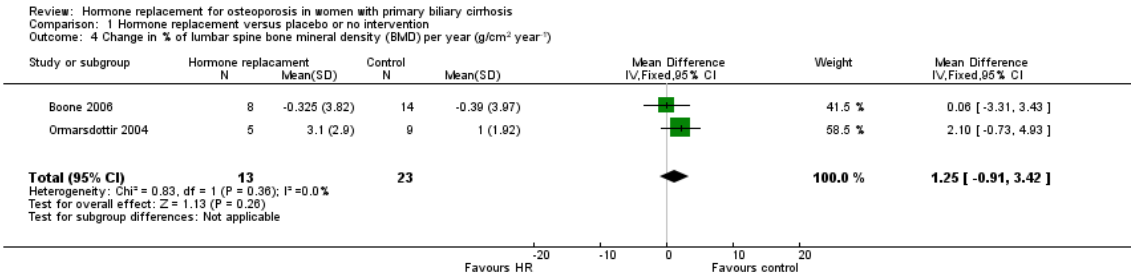

Image 111: hormone replacement versus placebo or no intervention; outcome: change in per cent of lumbar spine bone mineral density per year

Hormone replacement seemed to significantly decrease bone mineral density at the proximal femur (MD 2.24 g/cm^2 year^{-1}; 95% CI 0.74 to 3.74; I^2 = 0%) (Image 112).

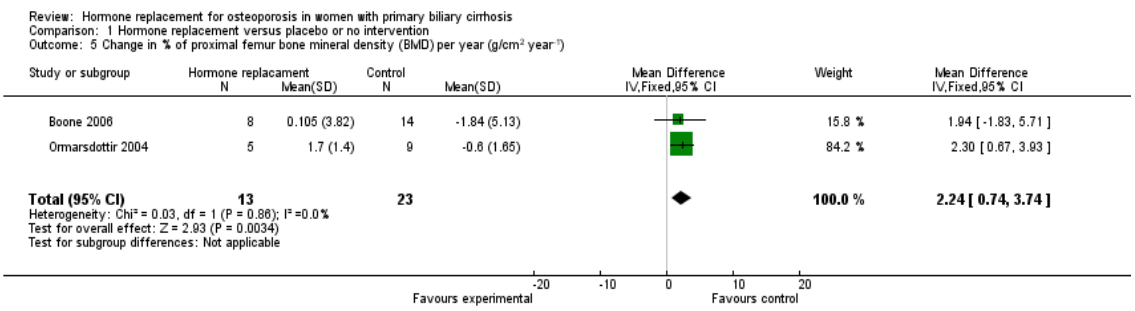

Image 112: hormone replacement versus placebo or no intervention; outcome: change in per cent of proximal femur bone mineral density per year

Trial sequential analysis on data for bone mineral density at the proximal femur does not support the findings in Analysis 1.5. The cumulated Z-curve (blue curve) did not cross the trial sequential monitoring boundary (red curve) implying that there is no firm evidence that hormone replacement decreases bone mineral density measured at proximal femur (Image 113).

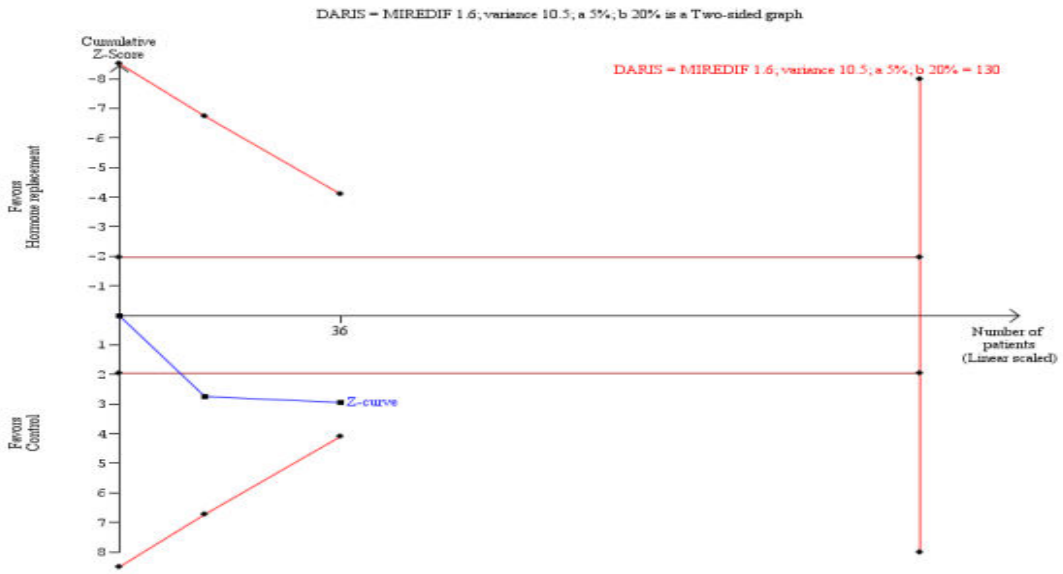

Image 113. Trial sequential analysis of the cumulative meta-analysis of the effect of hormone replacement versus control on bone mineral density measured at proximal femur in women with primary biliary cirrhosis. The diversity-adjusted required information size (DARIS) of 130 patients is calculated based on a minimal relevant intervention effect (MIREDIF) of 1.6 g/cm² yearˉ¹, a standard deviation of 3.2 g/cm² yearˉ¹, a risk of type 1 error of 5%, a power of 80%, and a diversity of 0%. The cumulated Z-curve (blue curve) did not cross the trial sequential monitoring boundary (red curve) implying that there is no firm evidence for an effect of 1.6 g/cm² yearˉ¹ decrease in bone mineral density measured at proximal femur when the cumulative meta-analysis is adjusted for sparse data and multiple testing on accumulating data.

Liver-related mortality or liver transplantation

Hormone replacement had no significant effect on liver-related mortality or liver transplantation. There were no liver-related deaths reported for any of the two groups (0/24 versus 0/25 participants) (RD 0.00; 95% CI -0.11 to 0.11; I^2 = 0%) (Image 114).

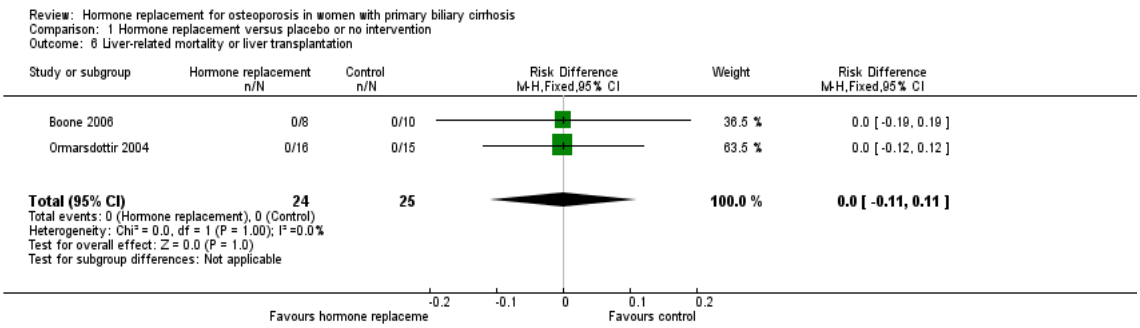

Image 114: hormone replacement versus placebo or no intervention; outcome: liver-related mortality or liver transplantation

Liver-related morbidity

Hormone replacement did not seem to have significant effect on liver-related morbidity. Liver-related complications occurred in 1/24 participants in the hormone replacement group versus 1/25 participants in the control group (RR 1.07; 95% CI 0.15 to 7.63; I^2 = 0%) (Image 115).

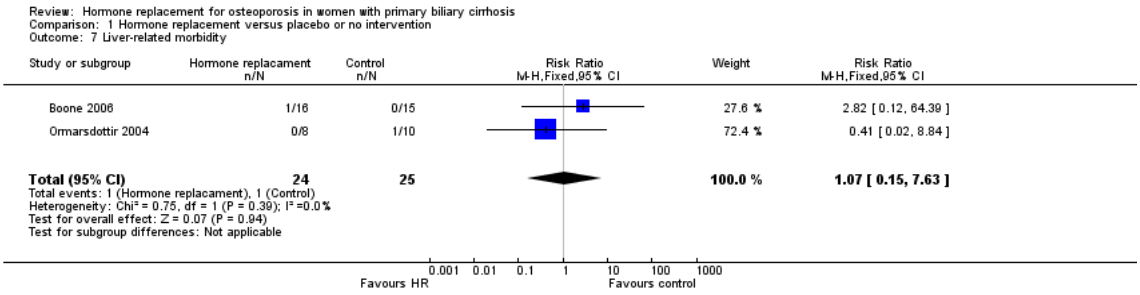

Image 115: hormone replacement versus placebo or no intervention; outcome: liver-related morbidity

One woman in the control group had an increase in bilirubin after twelve months (> 100% increase from baseline) and developed ascites afterwards in the following six months (Ormarsdottir et al, 2004). One women in the treatment group experienced two episodes of variceal haemorrhage (at months 4 and 17 of the trial period) requiring hospital admission, blood transfusion, and band ligation.

Biochemical indices

Two trials reported on serum bilirubin concentration. In one trial the data were reported as percentage change from baseline presented as median with ranges, and in addition they provided the table with final values presented as median with ranges (Ormarsdottir et al, 2004). We used only data presented as final values. In another trial, the data were reported as final values presented as means with ranges (Boone et al, 2006). In order to perform our meta-analysis, we estimated standard deviation to be approximately one quarter of the typical range of data values (Higgins and Green, 2011). In fixed-effect meta-analysis, hormone replacement versus placebo or no intervention had no significant effect on serum bilirubin concentration (MD 4.60 µmol/L; 95% CI -3.42 to 12.62; $I^2 = 0\%$) (Image 116).

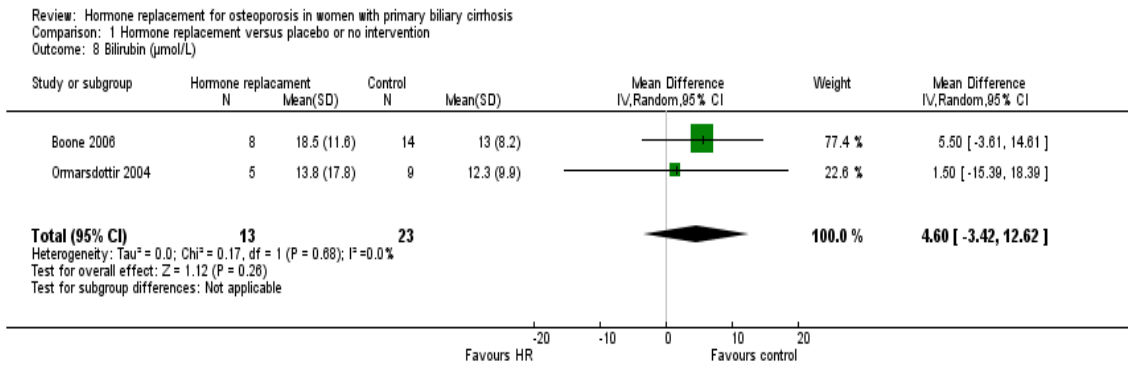

Image 116: hormone replacement versus placebo or no intervention; outcome: bilirubin

One trial reported that the relative change of serum alkaline phosphatases, serum alanine aminotransferase, and albumin concentration over baseline values did not differ when the two treatment groups were compared (Ormarsdottir et al, 2004). The data were reported as percentage change from baseline presented as median with ranges (Table 32).

Table 32 Biochemical indices (Ormarsdottir 2004)

Outcome measure (maximum change % from baseline value)	Type of data	Oestrogen + vitD + Ca (median(range)) n = 7	vitD + Ca (median(range)) n = 10	P
Serum alkaline phosphatases (µkat/L)	Continuous	-4 (-34 to 29)	-2 (-10 to 35)	NS
Serum alanine aminotransferase (µkat/L)	Continuous	-5 (-24 to 483)	8 (-7 to 140)	NS
Albumin (g/L)	Continuous	-5 (-12 to 0)	-5 (-14 to 5)	NS

µkat/L = 60 U/L

No trial reported on serum aspartate aminotransferase activity and biochemical markers of bone turnover.

Number of patients having hormone replacement withdrawn due to adverse events

There was a statistically significant increase in the number of patients having hormone replacement withdrawn due to adverse events in the hormone replacement group (10/24) versus the control group (2/25) (RR 5.26; 95% CI 1.26 to 22.04, $I^2 = 0\%$) (Image 117).

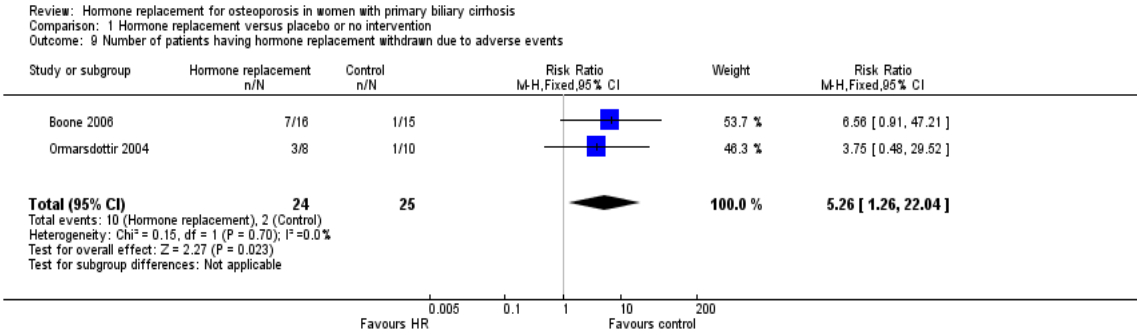

Image 117: hormone replacement versus placebo or no intervention; outcome: number of patients having hormone replacement withdrawn due to adverse events

Reasons for withdrawal of participants due to the occurrence of adverse events are provided in Table 33, 34.

Table 33 Reasons for withdrawals from treatment due to adverse events (Ormarsdottir 2004)

Adverse events	Hormone replacement	Placebo
Temporary spotty vaginal bleeding	1/8	0/10
Slight increase in systolic blood pressure	1/8	0/10
Increase in liver enzymes	1/8	0/10
Increase in bilirubin concentration	0/8	1/10

Table 34 Reasons for withdrawals from treatment due to adverse events (Boone 2006)

Adverse event	Hormone replacement	Placebo
Generalised pruritus	1/16	0/15
Pneumonia, pulmonary embolism	1/16	0/15
Abdominal pain, headache	1/16	0/15
Local pruritus at patch site	1/16	0/15
Heavy vaginal bleeding	1/16	0/15
Breast pain, chest pain, generalised pruritus, dysuria	1/16	0/15
Local pruritus at patch site	1/16	0/15
Diffuse painful rash of lower back	0/16	1/15

Subgroup analyses

It was not possible to perform the planned subgroup analyses due to the paucity of trials.

Description of studies: tables of included studies (Table 35) and tables of excluded studies (Table 36).

Table 35. tables of included studies

Ormarsdottir 2004

Methods	Multicentre randomised clinical trial with parallel group design (two interventions groups). Trial duration: two years.
Participants	Country: Sweden. Number of participants randomised: 18, median age 57 years. Inclusion criteria: - postmenopausal women between the age of 40 and 70 years with the diagnosis of primary biliary cirrhosis (presence of anti-mitochondrial antibodies and liver histopathology compatible with primary biliary cirrhosis), and Child-Pugh score A. * postmenopausal status was defined as loss of menstruations for at least one year and elevated follicle-stimulating hormone compatible with a postmenopausal status. Exclusion criteria: - other bone disorders than osteoporosis related to liver disease or postmenopausal status; - history of cancer; - unexplained vaginal bleeding; - unexplained uterus enlargement or lump in the breasts; - history of thromboembolic disorder; - hyperthyroidism; - impairment of the renal function; - severe heart disease; - uncontrolled hypertension (diastolic blood pressure > 100 mmHg); - history of drug or alcohol abuse;

	- treatment with calcitonin, high-dose vitamin D (more than 50,000 IU weekly), systemic corticosteroids, high dose heparin, oestrogen (except for local preparations not containing oestradiol), progestagens, fluorides, or bisphosphonates.	
Interventions	Participants were randomly assigned to receive: Intervention group 1: transdermal hormone replacement (oestradiol patch, 50 µg per day twice weekly in combination with medroxyprogesterone), n = 8. Duration of administration of hormone replacement was two years. Intervention group 2: no hormone replacement, n = 10. A dose for medroxyprogesterone was 2.5 mg daily continuously if more than two years from menopause, and 10 mg daily for 12 days per month if less than two years from menopause. All patients received vitamin D (alfacalcidol) 0.25 µg daily and calcium 1 g daily.	
Outcomes	Outcome measure(s): - bone mineral density of the lumbar spine and proximal femur; - fractures; - biochemical variables (serum bilirubin, liver enzymes, albumin); - adverse events.	
Notes	Additional information requested on 18th March 2011, but no response was received.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random	Unclear risk	The trial is described as randomised, but the

sequence generation		method of sequence generation was not specified.
Allocation concealment	Low risk	Allocation was controlled by sealed envelopes so that intervention allocation could not have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Unclear risk	The trial did not discuss this domain and was likely unblinded.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Unclear risk	The trial reported sponsorship from Novartis, but it did not report if Novartis was involved in the collection and analysis of the data.

Boone 2006

Methods	Multicentre randomised clinical trial with parallel group design (two interventions groups). Trial duration: two years.
Participants	Country: Canada. Number of participants randomised: 31, mean age 55 years. Inclusion criteria: - postmenopausal women ≤ 65 years with primary biliary cirrhosis (alkaline phosphatases > 110 U/L, positive anti-mitochondrial antibody, and/or compatible liver biopsy).

	* postmenopausal status was defined as no menstrual periods for at least six consecutive months, or a hysterectomy with conservation of at least one ovary and the typical symptoms of oestrogen deficiency, and an elevated follicle-stimulating hormone in the postmenopausal range (> 34.4 IU/L); - a normal pelvic examination, normal Papanicolaou test, and breast examination; - haemoglobin > 80 mg/L; - voluntary informed consent. Exclusion criteria: - patients who did not meet the inclusion criteria; - a liver transplanted patients; - serum bilirubin > 120 μmol/L; - current treatment with oestrogen or progestin (or patients that had received treatment for more then six months since the onset of menopause); - vitamin D deficiency; - contraindications to oestrogen use; - treatment with drugs known to affect bone metabolism; - other chronic disease affecting bone metabolism; - severe spinal deformities that would preclude accurate BMD measurement; - patients that had been immobile for more then three months in the preceding year; - allergy to components of the patch or bandages.
Interventions	Participants were randomly assigned to receive: Intervention group 1: 17β-estradiol (0.05 mg daily) for 14 days followed by 14 days of combined transdermal norethisterone acetate (0.25 mg daily) and 17β-estradiol (0.05 mg daily) transdermally, n =

	16. Duration of administration of hormone replacement was two years. Intervention group 2: identical placebo patches applied in the same manner, dose, and frequency, n = 15. All patients received vitamin D 1000 IU daily and elemental calcium 1500 mg daily.	
Outcomes	Outcome measure(s): - clinical variables; - fractures; - bone mineral density of the lumbar spine and proximal femur; - measurements of biochemical markers of bone turnover (bone alkaline phosphatases and the amino telopeptides of collagen I); - biochemical variables (serum bilirubin, liver enzymes, lipid profile, prothrombin time, etc); - adverse events.	
Notes	Additional information requested on 21st March 2011. Dr. Jenny Heathcote kindly responded on 24th March but she could not provide data on the trial that had been initiated almost 20 years ago.	
Risk of bias		
Bias	Authors' judgement	Support for judgement
Random sequence generation	Low risk	Sequence generation was achieved using randomisation table.
Allocation concealment	Low risk	Allocation was performed by independent pharmacist who had no role in patient contact or follow-up, nor did he/she participate in data analysis, so the intervention

		allocation could not have been foreseen in advance of, or during enrolment.
Blinding All outcomes	Low risk	The trial was described as blinded, the parties that were blinded, and the method of blinding was described, so that knowledge of allocation was adequately prevented during the trial.
Incomplete outcome data All outcomes	Low risk	The numbers and reasons for dropouts and withdrawals in all intervention groups were described.
Selective reporting	Low risk	Pre-defined, or clinically relevant and reasonably expected outcomes are reported on.
Other bias	Low risk	The trial seems to be free from other potential sources of bias. The trial reported that transdermal oestrogen/progestin and placebo were supplied by Novartis, and that Novartis was not involved in the collection, analysis, or presentation of these data.

Table 36. tables of excluded studies

Study	Reason for exclusion
Menon 2003	Not a randomised clinical trial. The aim of this study was to determine the safety and the efficacy of oestrogen replacement therapy in postmenopausal women with primary biliary cirrhosis. Forty-six unselected postmenopausal women with primary biliary cirrhosis receiving oestrogens for at least six months before being included in this study were randomly matched for age, gender, and ethnic group with another patient with primary biliary cirrhosis but not receiving oestrogen therapy. All patients were taking ursodeoxycholic acid (13 to 15 mg/kg/day) during the study. Thirty-five women were taking estrogens alone, and 11 women were taking a combined oestrogen/progesterone regimen. Twenty-one women were receiving oral replacement therapy, 23 topical replacement therapy, and two women long-acting parenteral therapy.

Pereira 2004	Not a randomised clinical trial. Forty-two post-menopausal women with primary biliary cirrhosis were treated with calcium and vitamin D. They could choose to receive it either alone (n ¼ 21) or together with transdermal hormone replacement therapy (n ¼ 21). The two groups were well matched for age, duration of menopause (mean, 10.7 years; range, 1 to 26 years), body mass index (mean, 24.2 kg/m2; range, 17.3 to 31.8 kg/m2), histological stage, serum bilirubin level (mean, 16.9 lm; range, 4 to 65 lm) and Mayo Clinic R score (mean, 3.3; range, 1.0 to 4.6). There were no adverse events attributable to treatment, apart from two patients who stopped HRT because of monthly bleeding and declined continuous combination therapy

Table 37. Summary of findings table: Hormone replacement vs placebo or no intervention for osteoporosis in primary biliary cirrhosis

Hormone replacement versus placebo or no intervention for osteoporosis in women with primary biliary cirrhosis

Patient or population: patients with osteoporosis in women with primary biliary cirrhosis
Settings:
Intervention: Hormone replacement versus placebo or no intervention

Outcomes	Illustrative comparative risks* (95% CI) Assumed risk Control	Corresponding risk Hormone replacement versus placebo or no intervention	Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
All-cause mortality	**Study population**		See comment	49 (2 studies)	See comment	Risks were calculated from pooled risk differences
	See comment	See comment				
	Medium risk population					
	0 per 1000	**0 per 1000** (0 to 0)[1]				
Fractures	**Study population**		See comment	49 (2 studies)	⊕⊕⊖⊖ **low**[2,3]	Risks were calculated from pooled risk differences
	80 per 1000	**-5 per 1000** (-160 to 150)				
	Medium risk population					
	67 per 1000	**-4 per 1000** (-134 to 126)				
Adverse events	**Study population**		**RR 5.26** (1.26 to 22.04)	49 (2 studies)	⊕⊕⊖⊖ **low**[2,3]	
	80 per 1000	**421 per 1000** (101 to 1000)				
	Medium risk population					
	83 per 1000	**437 per 1000** (105 to 1000)				
% change of lumbar spine bone mineral density (BMD) per year (g/cm2 year-1)		The mean % change of lumbar spine bone mineral density (BMD) per year (g/cm2 year-1) in the intervention groups was **1.25 higher** (0.91 lower to 3.42 higher)		36 (2 studies)	⊕⊕⊖⊖ **low**[2,3]	
% change of proximal femur bone mineral density (BMD) per year (g/cm2 year-1)		The mean % change of proximal femur bone mineral density (BMD) per year (g/cm2 year-1) in the intervention groups was **2.24 higher** (0.74 to 3.74 higher)		36 (2 studies)	⊕⊕⊖⊖ **low**[2,3]	
Liver-related morbidity	**Study population**		**RR 1.07** (0.15 to 7.63)	49 (2 studies)	⊕⊕⊖⊖ **low**[2,3]	
	40 per 1000	**43 per 1000** (6 to 305)				
	Medium risk population					
	50 per 1000	**54 per 1000** (8 to 382)				
Bilirubin (µmol/L)		The mean Bilirubin (µmol/L) in the intervention groups was **4.6 higher** (3.42 lower to 12.62 higher)		36 (2 studies)	⊕⊕⊖⊖ **low**[2,3]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Dichotomous outcome was expressed as risk difference (RD) with 95% confidence intervals (CI).

[2] The main limitations in design was the lack of clarity of the generation of allocation sequence and blinding in one trial.

[3] Included trials in our meta-analysis include few participants and few events indicating that we have little knowledge about the intervention effect, and that further information is needed.

Discussion

Summary of main results

Cochrane systematic reviews included in this doctoral thesis investigated the benefits and harms of interventions in patients with primary biliary cirrhosis and osteoporosis in primary biliary cirrhosis. Data from 30 randomised clinical trials with a total of 1847 participants were included. Twenty seven trials were with high risk of bias. Our key findings, in each of the systematic reviews, are that there is lack of statistical significant difference between the interventions we investigated versus control interventions regarding all-cause mortality or liver-related morbidity. However, the trials and meta-analyses of the investigated interventions are under-powered to draw firm conclusions on patient-important outcomes.

Ursodeoxycholic acid is the only drug approved by the U.S. Food and Drug Administration for primary biliary cirrhosis, but the effects of ursodeoxycholic acid remain controversial. Sixteen randomised clinical trials, with 1447 patients included, provided an updated evidence for the systematic review which assessed effects of ursodeoxycholic acid on patients with primary biliary cirrhosis. All but one of the included trials had high risk of bias. With the inclusion of updated data from 2007 to January 2012, this systematic review did not demonstrate any significant benefits of ursodeoxycholic acid on all-cause mortality, all-cause mortality or liver transplantation, or symptoms (pruritus and fatigue). Portal pressure, varices, bleeding varices, ascites, and hepatic encephalopathy were not significantly affected by ursodeoxycholic acid. Ursodeoxycholic acid seemed to have a beneficial effect on liver biochemistry measures and on histological progression compared with placebo or no intervention. According to the results of the trial sequential analyses, there seems to be firm evidence for a beneficial effects of ursodeoxycholic acid on decreasing serum bilirubin concentration and the activity of serum alkaline

phosphatases in patients with primary biliary cirrhosis compared with placebo or 'no intervention'. However, these beneficial effects may still be due to systematic errors (bias), as estimated intervention effects were calculated using data from trials assessed as having ’high risk of bias’ except one. The relationship between ursodeoxycholic acid effect and the severity of primary biliary cirrhosis was indicated in the classical meta-regression (Sharp, 1998), suggesting that ursodeoxycholic acid effect on mortality (if any) is more likely to be observed in patients with more severe primary biliary cirrhosis. However, this relationship was not supported by our univariate and multivariate meta-regression analyses, which included 'severity' as a co-variate. Therefore, whether the intervention effect of ursodeoxycholic acid (if any) is related to the severity of primary biliary cirrhosis should be investigated further.

Six randomised clinical trials, with 151 Japanese patients included, all with high risk of bias, provided information for the systematic review which looked at the effect of bezafibrate in patients with primary biliary cirrhosis. Four trials compared bezafibrate with no intervention, and two trials compared bezafibrate with ursodeoxycholic acid. Bezafibrate did not demonstrate any significant effect on mortality, liver-related morbidity, or adverse events when compared with no intervention, or when compared with ursodeoxycholic acid. Bezafibrate did not demonstrate any significant effect on pruritus compared with no intervention. It was not possible to evaluate changes in quality of life and fatigue since none of the trials reported these outcome measures. A possible positive intervention effect of bezafibrate versus no intervention on liver biochemistry measures can be real but could also be due to systematic errors or random errors. The results of trial sequential analysis imply that there is firm evidence for a beneficial effect of bezafibrate on decreasing the activity of serum alkaline phosphatases when compared with no intervention, or when compared with ursodeoxycholic acid. The results of trial sequential analysis imply that there is no firm evidence for a beneficial effect of bezafibrate on

decreasing plasma immunoglobulin M concentration and serum bilirubin concentration when compared with no intervention.

Six randomised clinical trials, with 200 participants included, provided information for the review which looked at the effect of bisphosphonates for osteoporosis in patients with primary biliary cirrhosis. Three trials with 106 participants, of which two trials with high risk of bias, compared etidronate or alendronate with placebo or no intervention; two trials with 62 participants with high risk of bias compared etidronate or alendronate with alendronate or ibandronate; and one trial with 32 participants and with high risk of bias compared etidronate with sodium fluoride. Having conducted statistical analyses, we found no evidence of effect of any of the aforementioned three bisphosphonates on mortality, fractures, adverse events, liver-related mortality, liver transplantation, liver-related morbidity or bone mineral density measured by dual-energy X-ray absorptiometry in patients with primary biliary cirrhosis. The data seem to indicate a possible positive intervention effect of bisphosphonates on decreasing urinary amino telopeptides of collagen I (NT_x) concentration compared with placebo or no intervention with no risk of random error. The results of trial sequential analysis imply that there is no firm evidence for a beneficial effect of alendronate on decrease in the procollagen type I N-terminal propeptide (PINP) and NTx concentration compared with another bisphosphonate. Serum osteocalcin concentration was measured in a different units, so the standardised mean differences was used in meta-analysis of the data from these trials. Therefore we could not apply trial sequentially analysis to confirm or reject a beneficial effect of bisphosphonates on decrease in serum osteocalcin concentration, and exclude the risk of random error, as trial sequential analysis has not been developed for standardised mean difference. Etidronate compared with sodium fluoride significantly decreased serum osteocalcin, urinary hydroxyproline, and parathyroid hormone concentration.

Two randomized clinical trials, with 49 participants included, of which one trial

with low risk of bias, assessed the effect of hormone replacement on treatment of osteoporosis in women with primary biliary cirrhosis. Hormone replacement had no significant effect on mortality, fractures, liver-related mortality, liver transplantation, or liver-related morbidity compared with placebo or no intervention in women with primary biliary cirrhosis. It seems that hormone replacement given to women with primary biliary cirrhosis is connected with a significant increase in the occurrence of adverse events compared with placebo or no intervention. Hormone replacement had no significant effect on lumbar spine bone mineral density measured by dual-energy X-ray absorptiometry compared with placebo or no intervention. On the other hand, hormone replacement seemed to significantly decrease bone mineral density measured at the proximal femur compared with the control group, and this result was not supported by trial sequential analysis. It seems that hormone replacement had no significant effect on serum bilirubin concentration compared with placebo or no intervention. However, the data are scarce, and we cannot exclude substantial risks of type II errors.

Overall completeness and applicability of evidence

To identify all available evidence from randomised clinical trials, we conducted an extensive search for trials, included publications in all languages, and had no restriction on the outcomes reported in the trials. We could not obtain all relevant data regarding all reasonably expected outcomes, as the trials identified insufficiently addressed all of the objectives of our Cochrane reviews.

The lack of significant differences in mortality, mortality or liver transplantation, liver morbidity, and adverse events may be related to the small number of patients involved and the short duration of the trials. Most of the included trials in our Cochrane reviews reported on biochemical and immunological indices. These data were reported either as change from baseline or final values, so we combined them in our meta-analysis using mean

difference method in RevMan. Mean differences based on changes from baseline can usually be assumed to be addressing exactly the same underlying intervention effects as analyses based on final measurements (Higgins and Green, 2011). Ursodeoxycholic acid and bezafibrate seemed to improve biochemical outcomes, but there is no evidence favouring the ursodeoxycholic acid and bezafibrate interventions for the disease because it is not based on results from randomised trials using clinically and patient relevant outcomes (Gluud et al, 2007).

There is a theoretical possibility that ursodeoxycholic acid may still delay progression from early stage disease to late stage disease and then ultimately prolong survival. However, the effects of ursodeoxycholic acid should primarily be assessed via patient relevant outcomes.

The Mayo Risk Score Model has identified several prognostic biomarkers for primary biliary cirrhosis, e.g., serum bilirubin. These biomarkers may respond to ursodeoxycholic acid and may be predictive of survival (Dickson et al, 1989). But they do not necessarily predict clinical benefit of the intervention in question because 'a perfect correlation does not a surrogate make' (Baker and Kramer, 2003). In the absence of validated surrogate outcomes in ursodeoxycholic acid for primary biliary cirrhosis, confirmatory trials assessing the ursodeoxycholic acid effect should only be based on clinical outcomes, e.g., mortality. We believe that evaluation based on such clinical outcomes-based evaluation will benefit patients in the long run (Gluud et al, 2007).

Other two systematic reviews examined the evidence for bisphosphonates or hormone replacement treatment of osteoporosis in patients with primary biliary cirrhosis. We could not obtain all relevant data regarding all reasonably expected outcomes, as the trials identified were insufficient to address all of the objectives of these reviews.

Unfortunately, not all trials per each comparison reported on mortality and fractures, and the results were inconclusive. The lack of significant differences in mortality or fractures may be related to the small numbers of participants involved and the short duration of the trials. It is important to evaluate the effects of bisphosphonates on fracture prevention in patients with primary biliary cirrhosis. Cochrane systematic reviews have demonstrated that bisphosphonates have statistically significant and clinically important benefit in the secondary prevention of fractures in postmenopausal women (Wells et al, 2008a; Wells et al, 2008c). Since fractures occur at a variable length of time after the onset of osteoporosis, it is not surprising that clinical trials of one year duration are unable to show significant differences between treatment groups. Longer follow-up of much larger patient groups is required to ascertain the efficacy of bisphosphonates in fracture prevention.

From a bisphosphonate safety perspective, we could not find any statistically significant difference in the occurrence of adverse events between the bisphosphonates and control groups. Regarding safety of hormone replacement in women with primary biliary cirrhosis, we found statistically significant difference in the occurrence of adverse events between the treatment and control groups. It seems that hormone replacement given to women with primary biliary cirrhosis is connected with a significant increase in the occurrence of adverse events compared with placebo or no intervention. On the other hand, when participants are aware of the treatment they are receiving, they may be more or less likely to report adverse events. The judgment of individuals who collect and interpret patient data may be affected when the assessor is aware of the treatment a participant is receiving. Lack of blinding in half of the trials included in both reviews that reported on adverse events as well as short follow-up and small numbers of participants may result in biased results, so no conclusions can be drawn regarding adverse events of

bisphosphonates or hormone replacement for osteoporosis in patients with primary biliary cirrhosis (Ioannidis, 2009).

In the absence of fracture outcome data in most clinical trials of osteoporosis, the intermediate outcome of bone mineral density may give fair information regarding fracture risk. It appears that bisphosphonates have no significant effect on the lumbar and proximal femur bone mineral density compared with placebo or no intervention, or another bisphosphonate in patients with primary biliary cirrhosis. It should be noted that the correlation between bone mineral density and fracture risk has been established in post-menopausal osteoporosis and not osteoporosis in primary biliary cirrhosis. Therefore, we do not yet know if bone mineral density is a valid surrogate outcome measure in patients with primary biliary cirrhosis (Gluud et al, 2007).

Most of the included trials reported on serum or urine markers of bone turnover, or both. The clinical significance and utilisation of these biochemical markers of bone turnover are not universally utilised; however, the assumption is that they act as a surrogate outcome measure for efficacy of therapy. This assumption, however, needs to be confirmed (Gluud et al, 2007).

There is a theoretical concern of worsening cholestasis by application of hormone replacement to patients with primary biliary cirrhosis (Schreiber and Simon, 1983). Both included trials reported on serum bilirubin concentration to reflect their concern of possible worsening of cholestasis by application of hormone replacement to women with primary biliary cirrhosis. These data were reported using ranges rather than standard deviations, and we considered this as an indicator that the outcome distribution in trials is possibly skewed. Even though ranges should not be used to estimate the standard deviations, we used an approach which estimates the standard deviation to be approximately one quarter of the typical range of data values. Accordingly, the result of our meta-analysis for this outcome is not a robust result, and we cannot conclude that

hormone replacement influences serum bilirubin concentration in women with primary biliary cirrhosis.

Quality of the evidence and potential biases in the review process

All Cochrane systematic reviews included in this doctoral thesis were conducted according to The Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011) and the Cochrane Hepato-Biliary Group Module (Gluud et al, 2011). The results of our meta-analyses, however, are only as strong as the primary trials included. For the different comparisons in our Cochrane systematic reviews, a large proportion of the trials had methodological limitations, small number of participants, small number of events, and short trial duration. The different comparisons did not have sufficient power to draw firm conclusions.

Risk of bias is known to impact on the estimated intervention effect, with trials with high risk of bias tending to overestimate beneficial intervention effects and underestimate harmful intervention effects. The risk of bias was high in twenty seven trials in our Cochrane systematic reviews. Among the 30 trials included in our reviews, three trials were classified as having low risk of bias according to all bias domains (generation of the randomisation sequence, concealment of the randomisation sequence, blinding of patients and personnel, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, for profit bias). The main limitations in the design and implementation was the lack of clarity of the generation of allocation sequence, concealment of allocation, blinding, and the small number of patients enrolled in the trials and this might have influenced the outcomes of the trials. Therefore, the estimated intervention effect may possibly be due to systematic errors, and our evidence base is therefore severely limited even when trial sequential analyses did not show risk of random errors.

We explored the presence of statistical heterogeneity by the chi-squared test and measured the quantity of heterogeneity by I^2 (Higgins et al, 2003). The chi-squared test has low power in the situation of a meta-analysis when trials have small sample size or are few in number as in our included trials. This means that while a statistically significant result may indicate a problem with heterogeneity, a non-significant result must not be taken as evidence of no heterogeneity. This is also why we used a P value of 0.10 to determine statistical significance regarding heterogeneity. To reflect our concern with heterogeneity, we looked at both fixed-effect and random-effects models in order to provide more conservative estimates of effect. Indeed, our reviews showed some significant results when the fixed-effect model was applied, which were not statistically significant when the random-effects model was applied. This makes our findings less robust. Available case analysis was performed for all continuous outcomes including data only on those patients whose results were known. Variation in the degree of missing data may also be considered as a potential source of bias and heterogeneity in our analyses. Regarding precision of our results, included trials in our meta-analysis include few patients and few events and thus have wide confidence intervals around the estimate of effect which might both hide beneficial and harmful effects.

Random errors are unpredictable variations in outcome measures, i.e., the play of chance. The risk of random error is higher when data come from small information sizes (or 'sample sizes' for individual trials), so information sizes need to be sufficiently large for the risk of random error to be reduced and the chance of observing a true intervention effect to be increased. To reduce the risk of random errors we applied trial sequential analysis on the different outcomes for the different comparisons, and found that we lack firm evidence to draw firm conclusions both regarding benefits and harms of aforementioned interventions in patients with primary biliary cirrhosis and osteoporosis in primary biliary cirrhosis. Therefore, we conclude that there is a need for well-

designed, randomised clinical trials with larger sample sizes and minimised risk of bias. Multi-centre trials would be appropriate for patient recruitment as primary biliary cirrhosis is a relatively rare disease. Such trials ought to be reported according to the CONSORT guidelines (http://www.consort-statement.org/). We also realise that the challenge of performing a new trial on intervention for primary biliary cirrhosis is high. The estimated median survival of primary biliary cirrhosis is 10 to 15 years. To spend 15 years planning and carrying out a trial for each new potential treatment of primary biliary cirrhosis would consume many patients' lifetimes, not to mention the expense and difficulty of retaining patients in such a long trial (Mayo, 2005). Nevertheless, there are at least an estimated one million patients with primary biliary cirrhosis world-wide. Therefore, it is possible to conduct large trials with appropriate statistical power if international groups of primary biliary cirrhosis investigators collaborate. Such large trials do not need to be conducted for more than two to four years.

Agreements and disagreements with other studies or reviews

In consistency with previous meta-analyses and reviews (Goulis et al, 1999; Gluud and Christensen, 2001b; Gong et al, 2008), an updated systematic review assessing the effects of ursodeoxycholic acid in patients with primary biliary cirrhosis did not demonstrate any benefit of ursodeoxycholic acid on all-cause mortality, and all-cause mortality or liver transplantation in these patients. This observation is in contrast to some previous attempts to aggregate data from studies assessing ursodeoxycholic acid interventions for primary biliary cirrhosis (Simko et al, 1994; Poupon et al, 1997; Poupon, 2000). However, Simko et al included non-randomised studies in their meta-analysis that are more liable to bias, that is systematic overestimation of benefit (Simko et al, 1994). Poupon only included three and five out of the 16 randomised clinical trials in their meta-analyses, respectively (Poupon et al, 1997; Poupon, 2000). Such meta-analyses largely run the risk of trial selection bias (Gluud and Christensen,

2001a). Furthermore, updated evidence from randomised clinical trials and analyses on longer follow-up data from our previous review (Gong et al, 2008) did not seem to support long-term ursodeoxycholic acid treatment for primary biliary cirrhosis. The main finding in our present updated review does not seem to support long-term ursodeoxycholic acid intervention, which was suggested in observational studies (Rust and Beuers, 2005; Pares et al, 2006). Thus, the results suggest no benefit of ursodeoxycholic acid on mortality.

On the other hand, ursodeoxycholic acid seemed to improve biochemical outcomes. This seems to place clinicians and researchers in a dilemma: if therapeutic decisions are based on clinical outcomes (e.g., mortality), there is insufficient evidence to support the use of ursodeoxycholic acid in primary biliary cirrhosis, but if based on non-validated 'surrogate' outcomes (e.g., serum bilirubin level or serum alkaline phosphatases), there is evidence favouring the ursodeoxycholic acid interventions for the disease (Gluud et al, 2007). We believe that clinical practice should be based on results from randomised trials using clinically and patient relevant outcomes.

We could not compare our results with the results from other systematic reviews or meta-analysis, as we could not identify any meta-analyses or systematic reviews assessing bezafibrate in primary biliary cirrhosis, nor bisphosphonates or hormone replacement for osteoporosis in people with primary biliary cirrhosis that have summarised the evidence in a systematic way. Cochrane systematic reviews have demonstrated that bisphosphonates have statistically significant and clinically important benefit in the secondary prevention of vertebral, non-vertebral, and hip fractures in postmenopausal women (Wells et al, 2008a; Wells et al, 2008c). In the review assessing effects of bisphosphonates for osteoporosis in primary biliary cirrhosis, two trials were classified as primary prevention trials, and the remaining four trials as secondary prevention trials. More randomised clinical trials on participants receiving bisphosphonates as secondary prevention are needed in order to

conclude whether there is an effect of bisphosphonates for secondary prevention of osteoporosis in patients with primary biliary cirrhosis. If an effect exists, then primary prevention trials could be conducted. There is evidence that hormone replacement increases bone mineral density (Wells et al, 2002) and reduces the incidence of vertebral and non-vertebral fractures (Torgerson and Bell-Syer, 2001a; Torgerson and Bell-Syer, 2001b) in postmenopausal women. On the other hand, there is an increasing concern about the adverse events of hormone replacement among women. Apart from the fact that oestrogen deficiency is considered to be a major factor leading to bone loss in postmenopausal women, there is strong evidence that hormone replacement significantly increases the risk of venous thromboembolism, heart attack, stroke, breast cancer, gallbladder disease, and in women over 65 years, the risk of dementia (Farquhar et al, 2009).

One could argue that patients with primary biliary cirrhosis plus osteoporosis should be treated as women without primary biliary cirrhosis having osteoporosis. This may turn out to be correct. However, we do not know if this is so. First, the pathogenesis of osteoporosis in patients with primary biliary cirrhosis may be different from osteoporosis in patients without cirrhosis. Second, the metabolism and effects of antiosteoporotic drugs may change in patients with primary biliary cirrhosis. Accordingly, without proper trials we cannot assure ourselves that data from osteoporotic patients can be transferred to osteoporotic patients with primary biliary cirrhosis. Without solid evidence patients may not get the appropriate treatment they need.

Recommendations for future research

Randomised clinical trials which assess ursodeoxycholic acid or bezafibrate versus placebo in primary biliary cirrhosis with larger sample sizes, long-term follow-up and minimised risk of bias are needed. Trials should mainly be based on clinical outcomes, e.g., mortality. Outcome measures should include quality

of life.

In order to have evidence on whether bisphosphonates or hormone replacement should be used for treating osteoporosis in primary biliary cirrhosis or not, randomised clinical trials which assess bisphosphonates as secondary prophylaxis in primary biliary cirrhosis, or hormone replacement in primary biliary cirrhosis with larger sample sizes and varying degrees of osteoporosis, and minimised risk of bias are needed. Multi-centre trials would be appropriate for participant recruitment as primary biliary cirrhosis is a relatively rare disease, and such trials ought to be reported according to the CONSORT Statement (www.consort-statement.org/).

CONCLUSIONS

Updated Cochrane review confirms and extends previous observations showing no benefit of ursodeoxycholic acid on all-cause mortality and on all-cause mortality or liver transplantation. Although based on a small number of trials with risk of bias, ursodeoxycholic acid seems to improve liver biochemical variables, including serum bilirubin concentration, and liver histology. This review does not support or refute short-term or long-term use of ursodeoxycholic acid.

Bezafibrate has no statistically significant effects on mortality, liver-related morbidity, adverse events, and quality of life of patients with primary biliary cirrhosis. A possible positive intervention effect of bezafibrate on liver biochemistry measures can be real but could also be due to systematic errors or random errors.

We found no evidence of effect of bisphosphonates on mortality, fractures, adverse events, quality of life, and bone mineral density in patients with primary biliary cirrhosis. Bisphosphonates seem to decrease NTx concentration in patients with primary biliary cirrhosis with no risk of random error, but we lack data from low risk of bias trials, so we do not have enough evidence in order to draw practical conclusions from the data.

Hormone replacement has no statistically significant effects on mortality, fractures, and on the lumbar bone mineral density in women with primary biliary cirrhosis. It seems that hormone replacement given to women with primary biliary cirrhosis is connected with a significant increase in the occurrence of adverse events. On the other hand, hormone replacement may decrease bone mineral density measured at the proximal femur.

Accordingly, treatment of primary biliary cirrhosis with ursodeoxycholic acid, bezafibrate, bisphosphonates, and hormone replacement can neither be supported nor refuted based on the best current evidence available.

The benefits and harms of interventions for patients with primary biliary cirrhosis and osteoporosis in primary biliary cirrhosis need further assessment in randomised clinical trials. Such trials ought to be conducted with impeccable methodology to reduce the risks of random errors and sufficiently large patient groups to reduce the risks of random errors.

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