BILE ACID AND IMMUNOSUPPRESSIVE THERAPY IN PRIMARY BILIARY CIRRHOSIS

Drukkerij Babeliowsky Amsterdam
drukkers/uitgevers
ISBN 90-9008640-4

De publicatie van dit proefschrift werd financieel mogelijk gemaakt door Zambon Nederland BV.

BILE ACID AND IMMUNOSUPPRESSIVE THERAPY IN PRIMARY BILIARY CIRRHOSIS

DE BEHANDELING VAN PRIMAIRE BILIAIRE CIRROSE MET GALZUREN EN IMMUNOSUPPRESSIE

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof. Dr. P.W.C. Akkermans M.A.
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 20 september 1995 om 15.45 uur

door

Franciscus Hubertus Joseph Wolfhagen

geboren te Spaubeek

Promotiecommissie

Promotores:	Prof. dr. S.W. Schalm
	Prof. dr. G.P. van Berge Henegouwen
Overige Leden:	Prof. dr. J.C. Birkenhäger
	Prof. dr. F.T. Bosman
	Prof. J.H.P. Wilson

συμφερτη δ'αρετη πελει
ανδρων και μαλα λυγρων·

Er ligt kracht in de samenwerking
van zelfs de minder sterken

Homerus, Ilias, XIII, 237

CONTENTS

CHAPTER I

GENERAL INTRODUCTION

1.1 PRIMARY BILIARY CIRRHOSIS

Primary Biliary Cirrhosis (PBC) is a chronic, cholestatic liver disease characterized by non-suppurative destruction of interlobular and septal bile ducts, with subsequent liver damage and eventually development of cirrhosis (1).The disease is relatively rare with an estimated annual incidence and point prevalence in Europe of about 14 and 114 per million population respectively (2).The course of the disease is variable and unpredictable, but potentially fatal.

Clinical features and natural course.

Over 90% percent of the patients are female, predominantly in their middle ages. Pruritus and fatigue are the most common symptoms and are present in about one third of the patients at presentation. Nowadays, jaundice (ca. 4-20%) and other complications of liver diseases e.g ascites and variceal bleeding (ca. 5%) are rather unusual at presentation, but may develop later on (3,4). Other symptoms regularly appearing in the course of the disease are right upper abdominal pain, hepatosplenomegaly, hyperpigmentia and xanthelasmas.

PBC is associated with other disorders, mostly of autoimmune origin, like Raynauds syndrome, sclerodermia, CREST syndrome, hypothyroidism, arthropathy (mainly arthralgias, rarely frank arthritis) and, most frequently, the sicca syndrome. Patients are frequently recognized in the evaluation of these disorders (1,3,4). Furthermore, persistent pruritus after gravidity may indicate PBC (1).

The clinical presentation of PBC has changed during the last decades (5). In 1959, Sherlock reported that 86% of the PBC patients presented with symptoms and a similar percentage was found in a group observed in the sixties (6,7). In contrast, studies in the eighties reported about 30% of the patients to be symptomatic at entry (3,4,8). This increasing recognition of PBC in an earlier, asymptomatic stage is probably due to more frequent routine biochemical screening, the increased availability of assays for antimitochondrial antbodies and greater awareness of the disease (2). Although 5-year survival after

diagnosis remained unchanged (80%), 10-year survival was 20% in the fifties (5,9) compared to over 50% in more recent series (3,10-12). Earlier detection of PBC may have attributed to this change in the long-term prognosis of PBC patients as a group.

Life expectancy is better in patients asymptomatic at presentation than in those who are symptomatic (median 16 years versus 7.5 years (13)), though worse than for a normal age and sex matched population (8,13,14). Once patients develop symptoms the disease runs a course comparable to that of symptomatic patients (8,13). The reported frequencies of asymptomatic patients developing symptoms (i.e. pruritus, fatigue, edema, complications of cirrhosis) are variable (3,8,13,14). The majority of the studies indicate that about 40% of the patients will become symptomatic after a median follow-up of ca. 10 years (3,8,13); this percentage rises to 64% after a median follow-up period of 15 years (13).

Biochemical features.

PBC is characterized by a cholestatic liver test profile, i.e. raised serum alkaline phosphatase and γ-GT with normal or moderately increased transaminases. Bilirubin may be normal or increased. In about 90% of the patients immunoglobulin M is elevated (15). Furthermore cholesterol levels are often high (1). This does not seem to be associated with a higher mortality due to atherosclerosis, probably because of the elevated high density lipoprotein concentrations in PBC (16,17).

The most important diagnostic serological marker is the presence of antimitochondrial antibodies (AMA), found in 95% of the patients. Recent research has disclosed the antigenic site of the PBC specific antibodies (labeled M2) which is the E2 subunit of the 2-oxo-acid dehydrogenase complex located on the inner mitochondrial membrane (18). The relation between disease progression and M2 levels is still controversial (19,20). One study showed the presence of E2 antibodies on biliary epithelial membranes of PBC patients, but not in controls (21). Previously, cross-reactivity of AMA with antigens on the surface of a variety of other cells in PBC had already been found (22). Up till

now, it is still unclear whether the presence of AMA is of pathophysiological significance or an epiphenomenon with only diagnostic importance. Interestingly, based on the presence of other types of antimitochondrial antibodies, the possibility to differentiate early in the disease between patients with a benign (M9 antibodies) or progressive course (M4 and M8 antibodies) has been suggested by one group (23). This finding as well as the identities of M4 and M9 antibodies, however, have not been confirmed by others (24).

Recently some workers (25-28) have compared the features of AMA positive with those of AMA negative patients, for which the term "autoimmune cholangiopathy" has been coined (28). The currently available data do not indicate any major differences between these patient groups with respect to clinical, biochemical and histological features, except for the higher incidence of antinuclear and smooth muscle antibodies and lower IgM levels in the AMA negative group. Whether these groups differ in natural history and response to treatment remains to be established.

Histological features

The characteristic histological lesion of PBC is the destruction of septal and interlobular bile ducts by predominantly mononuclear cell infiltrates (florid duct lesion), often associated with granuloma formation. This leads to ductopenia, progressive cholestasis, liver cell damage, fibrosis and ultimately biliary cirrhosis. However, the florid duct lesion and granulomas are often not identified, partly due to the focal character of the disease. Ductopenia, with or without copper accumulation secondary to cholestasis, may then be highly suggestive, though not specific, for PBC (29).

PBC can histologically be staged according to the method proposed by Scheuer (30), which was slightly modified by Ludwig (31) (table 1).

It should be born in mind that currently only 20-45% of the patients have cirrhosis at the time of diagnosis and the term "primary biliary cirrhosis" is therefore incorrect and misleading (3,8,13)

Table 1. **Histological stages of PBC (31)**

Stage I	Portal hepatitis with little or no periportal inflammation or piece meal necrosis.
Stage II	Periportal hepatitis, usually with piece meal necrosis.
Stage III	Presence of bridging necrosis or septal fibrosis or both.
Stage IV	Cirrhosis with fibrous septa and nodular regeneration.

Pathogenesis

The etiology of the disease is still unclear. PBC is increasingly considered to be an auto-immune disorder as suggested by the presence of autoantibodies, particularly antimitochondrial antibodies, and multiple other humoral and cellular immunological aberrations including increased IgM, complement system activation, circulating immune complex-like material which is ineffectively cleared by Kupffer cells and functional defects of T-lymphocytes and natural killer cells such as reduced lymphokine production (32-36). Histologically, PBC resembles the lesions observed in immunological disorders like graft versus host disease and chronic rejection of a liver transplant (37,38). Increased HLA-I antigens on bile duct and liver cells and the aberrant expression of HLA-II antigens (39-41), as well as the presence of intercellular adhesion molecules (particularly ICAM-1), which are essential in the T lymphocyt-target cell interaction, on biliary epithelium (42) and in serum (43) also suggest an immunological pathogenesis. Furthermore the predominance of the disease in women and the association with other autoimmune diseases strengthen the concept of an autoimmune genesis.

Genetic factors may also play a role in PBC. Recently, genotypic HLA typing methods have confirmed the association with HLA-DR8 as previously noted by phenotypic methods (44-46). Also associations

with several MHC Class-III profiles, encoding complement components, have been reported, although not consistently (47,48). Furthermore familiar PBC has been reported in 2-4% of the patients (2,49), and decreased in vitro suppressor lymphocyte responses have been found in PBC patients as well as in their family members (50).

The pathogenesis of PBC appears to be multifactorial. Supposedly, an at present unknown exogenous factor triggers the immune action directed at the biliary epithelium in predisposed persons, as may be indicated by the above mentioned HLA associations and abnormalities in suppressor cell function and complement action (51). An infectious etiology has been postulated, because in close contacts, relatives and laboratory personel working with PBC blood, as well as during viral infections "naturally occurring antimitochondrial antibodies" (NOMA) have been found. However, these NOMA differ from PBC specific AMA and are rarely present in PBC patients (52). Furthermore cross-reactions between mitochondrial antigens and membrane components of E. Coli Rough mutants have been documented (53,54). In addition, it was shown that 69% of healthy controls with recurrent urinary tract infections show weak mitochondrial antibody reactivity, while Rough bacteria forms were present in equal percentages (40%) of controls and PBC patients with recurrent urinary tract infections (55).

More recently a similar cross-reactivity has been found for Mycobacterium Gordonae, which is particularly interesting in view of the granulomatous character of PBC (56). Although these findings suggest a possible etiological role of these micro-organisms and add to the hope on future unravelling of the PBC enigma, up to now no convincing evidence has been established.

Subsequent to the primary immunological destruction of bile ducts, irreversible bile duct loss leads to cholestasis and accumulation of hepatotoxic primary bile salts such as chenodeoxycholic acid, which is supposed to cause further liver damage (57,58).

Prognosis

Before the advent of the liver transplantation in the beginning of the eighties the ultimate outcome of the disease was fatal in most cases. Liver transplantation, however, has drastically changed this grim prospect. Currently it is the only curative treatment for (end-stage) PBC, with 1- and 5-year survival rates of about 75% and 70% respectively (59). Recurrence of PBC after transplantation has been suggested by several groups (60-62). Difficulties in differentiating PBC-related lesions from features of chronic rejection preclude definite conclusions (63,64). Clinically manifest disease recurrence has not been reported.

The success of liver transplantation is greatly influenced by the disease state at referral, those with least advanced disease faring best (65). Models predicting prognosis may optimalize the decision making on who and when to operate.The (ongoing) development of such models using Cox regression analysis (66-68), recently culminated in sophisticated models which offer the possibility to adjust the estimated prognosis during the course of the disease (69,70). However, the current models may not be applicable in individual patients and in patients under treatment and further validation and refinement is needed (71).

1.2 THE TREATMENT OF PBC - A.D. 1990

For end-stage PBC liver transplantation has become an effective treatment. However, medical treatment, aiming at the prevention of disease progression in patients with non-advanced disease has been disappointing. Cupruretic (72-79), antifibrotic (80-82), anti-inflammatory and immunosuppressive (67,83-87) drugs have been evaluated and were found to be unable to halt disease progression or to be too harmful.These trials have been reviewed in extenso by others (1,51,88) and are summarized in table 2. During the eighties, reports on treatment with ursodeoxycholic acid emerged and gave new hope to workers in the PBC field and their patients.

Table 2. **Controlled drug trials in Primary Biliary Cirrhosis, other than ursodeoxycholic acid: effects on symptoms, liver tests, histology and survival. The degree of intolerance to the drug has been estimated, based on the percentage of withdrawals/drop-outs in the treatment group as compared to the placebo group (++ = >10% and >2x the percentage in the placebo group; + = >10% and >1.5x ; - = ≤10% or ≤1.5 x)**

Drug (ref)	No. of studies	Total no. patients	Improvement in *symptoms*	*liver functions*	*histology*	*survival*	Intolerance
Penicillamine (72-79)	8	740	-	±	-	-	++
Colchicine (80-82)	3	181	-	±	-	±	+
Chlorambucil (84)	1	24	n.e.	+	-	n.e.	++
Cyclosporin (85,86)	2	41	±	+	-	n.e.	+
Prednisolone[1] (87)	1	36	+	+	+	n.e.	- (10mg/d for 1yr)
Azathioprine[1] (83)	2	45	±	-	-	±	+ (2mg/kg/d for 1-6 yrs)
(67)		248	+	-	- (5 yr: 41% ↓†)	+ [2]	- (1mg/kg/d for 6-12 yrs)

n.e. = not evaluable/evaluated + = improvement ± = trend or not consistent - = no improvement ↓† = reduction in death
[1] presented more explicitly in view of the importance for this thesis; [2] after adjustment for unequal bilirubin levels at entry

Ursodeoxycholic acid

In 1981, Leuschner et al. first noted that ursodeoxycholic acid improved transaminases in patients suffering from both chronic active hepatitis and gallstones (89,90). Soon thereafter, Poupon et al. reported the first uncontrolled data in PBC patients showing similar improvements in standard liver tests as well as pruritus (91). Subsequent small controlled trials (92-94) were all encouraging and in agreement with the results of the first large randomized placebo-controlled trial published by Poupon et al. (95) in 1991 (table 3).

Poupon found improvements in fatigue and pruritus which tended to be greater in the treatment group, although they did not reach significance in the intention to treat analysis. UDCA clearly decreased the levels of bilirubin, liver enzymes, IgM and antimitochondrial antibodies. Furthermore, improvement of histological, inflammatory features but no effect on fibrosis was noted in the UDCA group. Although these studies did not show serious side effects, some authors warned that ursodeoxycholic acid may not only be ineffective but could even be toxic in PBC patients with stage IV disease (96-98).

UDCA is the 7β epimer of chenodeoxycholic acid with the chemical structure 3α7β–dihydroxy-5β-cholan-24-oic acid and is naturally occurring in the human bile acid pool in small quantities (<4%) (58). In Asia, for ages already, healing properties in chronic liver disease have been ascribed to the bile of the black bear, which contains large amounts of UDCA (99).

Several mechanisms of action were considered to be of importance in the treatment of PBC with UDCA. Hydrophobic bile acids are more damaging to cells than hydrophilic bile acids. Compared to the more abundant endogenous bile acids, UDCA is less hydrophobic, has a higher critical micellar concentration and lower cell surface activity. These properties result in a lower cell damaging capacity (58). Therefore enrichment of the bile acid pool with UDCA at the expense of hydrophobic bile acids is likely to reduce hepatic damage. During UDCA treatment the proportion of UDCA in bile and serum indeed rises to over 50% (58,100) and it has been shown that UDCA decreases

Table 3. **Controlled trials with ursodeoxycholic acid in Primary Biliary Cirrhosis**

Author year (ref.)	Dose (mg/day)	No. of patients (n=)	Follow up (months)	Symptoms	Liver functions [bilirubin]	Histology *inflammation/fibrosis*	Signs of progression[1]	Survival/ liver Tx
Leuschner 1989 (92)	10/kg	20	9	±	+ [n.e.]	± /-	n.e.	n.e.
Oka 1990 (93)	600	45	6	-	+ [-]	n.e /n.e.	-	n.e.
Hadzyannis 1991 (94)	12-15/kg	50	29	+	+ [±]	- /-	-	-/-
Poupon 1991 (95)	13-15/kg	146	24	±	+ [+]	+ /-	+	-/-

n.e. = not evaluable/evaluated + = improvement ± = trend or not consistent - = no improvement

[1] signs of progression: hepatic decompensation e.g. ascites, variceal bleeding, encephalopathy

the reabsorption of endogenous bile acids from the gut (101-103). However, several workers found the decrease in endogenous bile acids to be mainly at the expense of cholic acid, a relatively hydrophilic bile acid, while chenodeoxycholic acid, the main hydrophobic constituent of the bile acid pool, remained unaltered (101,104). Whether UDCA produced a net shift towards hydrophilicity in the bile acid pool was therefore still unclear.

Another potential mechanism of action is the enhancement of bile flow by UDCA as has been found in rats. This is probably due to the existence of a cholehepatic shunt in which the unconjugated form of UDCA in bile becomes protonated by H_2CO_3 (leading to increased biliary HCO_3- excretion), and thus becomes more lipophilic. This makes UDCA more easily reabsorbed by the biliary epitheliary cells after which it can be resecreted again by the hepatocytes (105,106).

UDCA may have more direct, hepatoprotective effects. It has been shown that UDCA reduces the cytolysis induced by other, hydrophobic bile salts (107-109). Furthermore it was suggested that UDCA might also have immunomodulating properties because UDCA reduced elevated serum IgM levels (95) as well as the aberrant expression of HLA I on hepatocytes (110), although this might be an effect secondary to reduced cholestasis (111).

Considerations on further treatment of PBC

No data concerning the effect of UDCA on disease progression were available in the beginning of the nineties. However, presuming that UDCA mainly interferes with bile acid mediated liver damage secondary to cholestasis, we considered it unlikely that UDCA alone would lead to an inactivation of the disease in a substantial number of patients during short term treatment and to a major slowing of disease progression in the long run. We reasoned that the combination of UDCA with other potentially effective drugs would be more appropiate to reach these aims (112). Therefore studies evaluating additional treatment options in patients who would not achieve a complete disease remission on UDCA alone were considered a logical next step.

Regarding the supposed pathophysiology of PBC the combination of UDCA (mainly protecting the liver from bile acid toxicity) with immunosuppressive drugs directed at the alleged autoimmune attack seems logical (112,113) while another attractive avenue would be the additional use of antifibrotic drugs like colchicine.

A number of drugs have been shown to delay disease progression to some extent or at least to ameliorate liver function abnormalities, which could be candidates to be added to UDCA treatment. The toxicity of the immunosuppressive drugs chlorambucil and cyclosporine, outweighed their beneficial effects and they were therefore considered less suitable options (84,86,87,114).

Limited uncontrolled data indicated a possible beneficial effect (115,116) of low dose methotrexate (15 mg/week) in PBC. However, methotrexate may have potential severe adverse effects such as hepatotoxicity, interstitial pneumonitis and cytopenia and was therefore considered less appropiate (117,118).

Colchicine, a drug with anti-inflammatory and antifibrotic properties, is well tolerated and has been shown to improve biochemistry in PBC. Though some studies suggested a reduced progression (80,82), long term follow up did not show an effect on survival or need of liver transplantation (119). Furthermore a treatment directed against the primary cause of liver damage (autoimmune attack) was considered more attractive than one directed mainly at a mechanism secondary to hepatic damage i.e. fibrogenesis.

Corticosteroids, the mainstay in the treatment of many immune disorders has long been considered contraindicated in PBC, due to fear of its deteriorative effects on the bone status, particularly because osteoporosis is already associated with PBC (29,120). Early uncontrolled experience with corticosteroids in PBC patients with advanced disease and jaundice fed this fear (9,121).

In our centre, uncontrolled studies evaluating prednisone in combination with cyclosporine indicated the potential benefit of corticosteroids in PBC (51) and recently, Mitchison et al. (88) for the first time evaluated the effects of low dose corticosteroids in a placebo-controlled study. They found improvements in biochemical activity

parameters as well as in procollagen-III propeptide, a marker of active fibrogenesis (122) and liver biopsy features. However, the rate of bone loss doubled during the first year of treatment.

It is well known that steroid induced bone loss is highest during the first year of treatment (123,124) and recent studies have indicated that the long term use of low dosages of corticosteroids is not associated with major deterioration of bone status (125,126). Furthermore, the risk of steroid-induced bone loss may be a manageable adverse effect, by using preventive measures such as bisphosphonates (127).

Like prednisone, azathioprine has been used as an immunosuppressive drug on a large scale since several decades. In PBC, two controlled studies with azathioprine have been performed. In the largest and longest trial a small improvement of survival was noted (67), although effects on biochemistry and symptoms were minor (83).

The use of azathioprine in PBC has been limited mainly by the fear for an increased risk of malignancies. This risk seems to be very small and has been mainly documented during the use of high doses of azathioprine in post transplant patients (128). Nowadays, there is vast experience with the combination of prednisone and azathioprine in another autoimmune liver disease i.e. chronic autoimmune hepatitis, in which this combination has been effectively used during more than 20 years (129, 130). Long term follow-up of patients with chronic auto-immune hepatitis, who were treated with prednisone and azathioprine showed only a very slight increase in extrahepatic malignancies as compared to a normal population (131).

The drugs have a mutual dose sparing effect and therefore a decrease in the risk of adverse effects and an increase in immuno-suppressive potential may be achieved by combining low doses of prednisone and azathioprine in PBC.

Clinical investigations in PBC during the last 10-15 years (51,132), have raised several questions. The Dutch Multicenter PBC Project, initiated in 1990, offered the opportunity to study some of these questions. This thesis contains the current results of this ongoing project.

1.3 AIMS OF THIS THESIS

1. To determine how many PBC patients achieve complete disease remission during 1 year of ursodeoxycholic acid (UDCA) therapy.

2. To assess whether patients with compensated stage IV PBC respond less to, or may even deteriorate by UDCA treatment.

3. To compare single dose and multiple dose UDCA treatment with respect to their effects on biliary UDCA enrichment and biochemical response.

4. To study the effects of prednisone/azathioprine or placebo in addition to UDCA with regard to symptoms, serological parameters of cholestasis, liver damage, fibrogenesis and immune activity, and histology.

5. To investigate whether bone loss in corticosteroid treated PBC patients can be reduced by cyclical etidronate.

6. To assess whether the short term benefit-risk ratio of prednisone/azathioprine treatment in addition to UDCA justifies larger, long term studies with this therapeutic regimen.

REFERENCES

1. Kaplan MM. Primary Biliary Cirrhosis. N Engl J Med 1987;316:521-8.
2. James OFW, Myszor M. Epidemiology and genetics of primary biliary cirrhosis. In: Popper H, Schaffner F eds. Progress in liver disease 9, WB Saunders Company, USA, 1990; pp.523-36.
3. Nyberg A, Lööf L. Primary biliary cirrhosis: clinical features and outcome, with special reference to asymptomatic disease. Scand J Gastroenterol 1989;24:75-64.
4. Brenard R, Degos F, Degott C, Lassoued K, Benhamou JP. La cirrhose biliaire primitive: modes actuels de présentation. Gastroenterol Clin Biol 1990;14:307-12.
5. Taal BG, Schalm SW. Primary biliary cirrhosis: a changing clincial presentation. Neth J Med 1981;24:101-8.
6. Sherlock S. Primary Biliary Cirrhosis (chronic intrahepatic obstructive jaundice). Gastroenterology 1959;37:574-86.
7. Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. New Engl J Med 1973;289:674-8.
8. Mitchison HC, Lucey MR, Kelly PJ, Neuberger JM, Williams R, James OFW. Symptom development and prognosis in primary biliary cirrhosis: a study in two centers. Gastroenterology 1990;778-84.
9. Hoffbauer FW. Primary Biliary Cirrhosis: Observations on the natural course of the disease in 25 women. Am J Dig Dis 1960;5:348-83.
10. Rydning A, Schrumpf E, Abdelnoor M, Elgjo K, Jenssen E. Factors of prognostic importance in primary biliary cirrhosis. Scand J Gastroenterol 1990;25:119-26.
11. Goudie BM, Burt AD, MacFarlane GJ, Boyle P, Gillis CR, MacSween RNM et al. Risk factors and prognosis in primary biliary cirrhosis. Am J Gastroenterol 1989;84:713-6.
12. Roll J, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histological features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl J Med 1983;308:1-7.
13. Mahl TC, Shockcor W, Boyer JL. Primary biliary cirrhosis: survival of a large cohort if symptomatic and asymptomatic patients followed for 24 years. J Hepatol 1994;20:707-13.
14. Balasubramiam K, Grambsch PM, Wiesner RH, Lindor KD, Dickson ER. Diminished survival in asymptomatic primary biliary cirrhosis. Gastroenterology 1990;98:1567-71.
15. Taal BG, Schalm SW, De Bruyn AM, De Rooy FWM, Klein F. Clin Chim Acta 1980:108:457-463.
16. Crippin JS, Lindor KD, Jorgensen R, Kottke BA, Harrisson JM, Murtaugh PA, Dickson ER. Hypercholesterolemia and atherosclerosis in primary biliary cirrhosis: what is the risk? Hepatology 1992;15:858-62.
17. Propst A, Propst T, Lechleitner M, Hoppichler F, Kathrein H, Vogel W. Hypercholesterolemie in primary biliary cirrhosis is no risk factor for atherosclerosis. Dig Dis Sci 1993;38:379-80.
18. Yeaman SJ, Danner DJ, Mutimer DJ, Fussey SPM, James OFW, Bassendine MF. Primary biliary cirrhosis: identification of two major M2 antimitochondrial autoantigens. Lancet 1988;1:1067-69.
19. Heseltine L, Turner IB, Fussey SPM, Kelley P, James OFW, Yeaman SJ, Bassendine MF. Primary biliary cirrhosis: quantification of antibodies to purified mitochondrial enzymes and correlation with disease progression. Gastroenterology 1990;99:1786-92.
20. Lindor KD, Therneau TM, Malinchoc M, Dickson ER, Homburger HA. M2 antimitochondrial antibody titers do not correlate with disease progression in patients with primary biliary cirrhosis. Hepatology 1993;18:215A (Abstract)
21. Joplin R, Lindsay JG, Johnson GD, Strain A, Neuberger J. Membrane dihydrolipoamide acetyltransferase (E2) on human biliary epithelial cells in primary biliary cirrhosis. Lancet 1992;339:93-4.
22. Ghadiminejad I, Baum H. Evidence for the cell-surface localization of antigens cross-reacting with the "mitochondrial antibodies" of primary biliary cirrhosis. Hepatology 1987;7:743-9.
23. Klein R, Klöppel G, Garbe W, Fintelmann V, Berg PA. Antimitochondrial antibody profiles

determined at early stages of primary biliasry cirrhosis differentiate between a benign and a progressive course of the disease: a retrospective analysis of 76 patients over 6-18 years. J Hepatol 1991;12:21-7.

24. Palmer JM, Yeaman SJ, Bassendine MF, James OFW. M4 and M9 autoantigens in primary biliary cirrhosis - a negative study. J Hepatol 1993;18:251-4.
25. Michieletti P, Wanless IR, Katz A, Scheuer PJ, Yeaman SJ, Bassendine MF et al. Antimitochondrial antibody negative primary biliary cirrhosis: a distinct syndrome of autoimmune cholangitis. Gut 1994;35:260-5.
26. Lacerda MA, Lindor KD, Jorgensen RA, Ludwig J, Dickson ER. "Autoimmune cholangitis" better defines antimitochondrial antibody negative primary biliary cirrhosis. Gastroenterology 1993;104:A933 (Abstract)
27. Goodman ZD, McNally PR, Davis D, Ishak KG. "Autoimmune cholangitis"-a variant of primary biliary cirrhosis. Hepatology 1993;18:109A(Abstract).
28. Ben-Ari Z, Dhillon AP, Sherlock S. Autoimmune cholangiopathy: part of the spectrum of autoimmune chronic active hepatitis. Hepatology 1993;18:10-5.
29. Sherlock S., Dooley J. Disease of the liver and biliary system. Blackwell Scientific publications 9th ed. 1993 pp.236-48.
30. Scheuer PJ. Primary biliary cirrhosis. Proc R Soc Med 1967;60:1257-60.
31. Ludwig J, Dickson ER, McDonald GSA. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch Path Anat and Histol 1978;379:103-12.
32. Mackay IR, Gershwin M.E. Molecular basis of mitochondrial autoreactivity in primary biliary cirrhosis. Immunol Today 1989;10:315-8.
33. Pape GR, Spengler U, Hoffmann RM, Jung M-C. Pathogenesis of primary ciliary birrhosis. New aspects of the role of T Lymphocytes. In Krawitt EL, Wiesner RH, eds. Autoimmune liver disease. Raven Press, New York, 1991; pp. 43-62.
34. Manns MP, Krüger M. Immunogenetics of chronic liver diseases. Gastroenterology 1994;106:1676-97.
35. Spengler U, Möller A, Jung MC, Messer G, Zachoval R, Hoffmann RM et al. T lymphocytes from patients with primary biliary cirrhosis produce reduced amounts of lymphotoxin, tumor necrosis factor and interferon-gamma upon mitogen stimulation. J Hepatol 1992;15:129-35.
36. Menendez JL, Giron JA, Manzano L, Garrido A, Abreu L, Albillos A et al. Deficient interleukin-2 responsiveness from patients with primary biliary cirrhosis. Hepatology 1992;16:931-6.
37. Bernau D, Feldmann G, Degott MD, Gisselbrecht C. Ultrastructural lesions and bile ducts in primary biliary cirrhosis: a comparison with the lesions observed in graft versus host disease. Hum Pathol 1981;12:782-93.
38. Neuberger J, Portmann B, MacDougall BRD, Calne RY, Williams R. Recurrence of primary biliary cirrhosis after liver transplantation. N Engl J Med 1982;306:1-4.
39. Ballardini G, Bianelli FB, Doniach D, Mirakian R, Pisi E, Bottazo GF et al. Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis; relevance to pathogenesis. Lancet 1984;2:1009-13.
40. Spengler U, Pape GR, Hoffmann RM, Johnson JP, Eisenburg J, Paumgartner G. Differential expression of MHC Class II subregion products on bile duct epithelial cells and hepatocytes in patients with primary biliary cirrhosis. Hepatology 1988;8:459-62.
41. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of Class I and II Major Histocompatibility Complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-15.
42. Adams DH, Hubscher S, Shaw J, Johnson GD, Babbs, Rothlein R, Neuberger JM. Increased expression of ICAM-1 on bile ducts in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 1991;14:426-31.
43. Adams DH, Mainolfi EA, Burra P, Neuberger JM, Ayres R, Elias E, Rothlein R. Detection of

circulating intercellular adhesion molecule-1 in chronic liver diseases. Hepatology 1992;16:810-4.
44. Maeda T, Onishi S, Saibara T, Iwasaki S, Yamamot J. HLA DR8 and primary biliary cirrhosis. Gastroenterology 1992;103:1118-9.
45. Underhill J, Donaldson P, Bray G, Doherty D, Portmann, Wiliams R. Susceptibility to primary biliary cirrhosis is associated with the HLA-DR8-DQB1*0402 haplotype. Hepatology 1993;16:1404-8.
46. Gregory W, Mehal W, Dunn AN, Daly AK, Cavanagh G, Chapman RW. Primary biliary cirrhosis: contribution of HLA Class II allele DR8. Q J Med 1993;86:393-9.
47. Manns MP, Bremm A, Schneider PM, Notghi A, Gerken G, Prager-Eberle M et al. HLA DRw8 and Complement C4 deficiency as risk factors in primary biliary cirrhosis. Gastroenterology 1991;101:1367-73.
48. Briggs DC, Donaldson PT, Hayes P, Welsh KI, Williams R. Neuberger JM. A major histocompatibility complex class III allotype (C4B2) associated with primary biliary cirrhosis. Tissue antigens 1987;29:141-5.
49. Bach N, Schaffner F. Familial primary biliary cirrhosis. J Hepatol 1994;20:698-701.
50. Tsuji H, Murai K, Akagi K, Fujishima M. Familial primary biliary cirrhosis associated with impaired concanavalin A-induced lymphocyte transformation in relatives. Dig Dis Sci 1992;37:353-60
51. Beukers R. Immunosuppressive therapy in primary biliary cirrhosis. Thesis. Rotterdam 1992.
52. Berg PA, Klein R. Antimitochondrial antibodies in primary biliary cirrhosis and other disorders: Definition and clinical relevance Dig Dis 1992;10:85-101.
53. Stemerowicz R, Hopf U, Möller B, Wittenbrink C, Rodloff A, Reinhardt R et al. Are antimitochondrial antibodies in primary biliary cirrhosis induced by R(Rough)-mutants of enterobacteriaceae? Lancet 1988;ii:1166-70.
54. Hopf U, Möller B, Stemerowicz R, Lobeck H, Rodloff A, Freudenberg M et al. Relation between escherichia coli R(Rough)-forms in gut, lipid A in liver, and primary biliary cirrhosis. Lancet 1989; ii:1419-21.
55. Butler P, Valle F, Hamilton-Miller JMT, Brumfitt W, Baum H, Burroughs AK. M2 antimitochondrial antibodies and urinary rough mutant bacteria in patients with primary biliary cirrhosis and in patients with recurrent bacteriuria. J Hepatol 1993;17:408-14.
56. Vilagut L, Vila J, Viñas O, Parés A, Ginés A, Jiménez de Anta MT. Cross-reactivity of anti-Mycobacterium gordonae antibodies with the major mitochondrial autoantigens in primary biliary cirrhosis. J Hepatol 1994;21:673-7.
57. Van Berge Henegouwen GP, Brandt K-H, Eyssen H, Parmentier G. Sulphated and unsulphated bile acids in serum, bile, and urine of patients with cholestasis. Gut 1976;17:861-9.
58. Hofmann AF. Bile acid hepatotoxicity and the rationale of UDCA therapy in chronic cholestatic liver diseases: some hypotheses. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990; pp. 13-33.
59. Markus BH, Dickson ER, Grambsch PM, Fleming TR, Mazzaferro V, Klintmalm GBG et al. Efficacy of liver transplantation in patients with primary biliary cirrhosis. N Engl J Med 1989;320:1709-13.
60. Polson RJ, Portmann B, Neuberger J, Clane RY, Williams R. Evidence for disease recurrence after liver transplantation of primary biliary cirrhosis. Gastroenterology 1989;97:715-25.
61. Balan V, Batts KP, Porayko MK, Krom RAF, Ludwig J, Wisner RH. Histological evidence for recurrence of primary biliary cirrhosis after liver transplantation. Hepatology 1993;18:1392-8.
62. Hubscher SG, Elias E, Buckels JAC, Mayer AD, McMaster P, Neuberger JM. Primary Biliary Cirrhosis. Histological evidence of disease recurrence after liver transplantation. J Hepatol 1993:18;173-84.
63. ASH Gouw, Haagsma EB, Manns M, Klompmaker IJ, Slooff MJH, Gerber MA. Is there recurrence of primary biliary cirrhosis after liver tranplantation? J Hepatol 1994;20:500-7.
64. Anonymous. Is PBC cured by liver tranplantation ? Lancet 1991;337:272-3.

65. Neuberger JM, Gunson BK, Buckels JAC, Elias E, McMater P. Referral of patients with primary biliary cirrhosis for liver tranplantation. Gut 1990;31:1069-72.
66. Roll J, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histological features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl J Med 1983;308:1-7.
67. Christensen E, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis: final results of an international trial. Gastroenterology 1985;89:1084-91.
68. Wiesner RH, Porayko MK, Dickson ER, Gores GJ, LaRusso NF, Hay JE et al. Selection and timing of liver transplantation in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 1992; 1290-9.
69. Christensen E, Altman DG, Neuberger J, De Stavola BL, Tygstrup N, Williams R and the PBC1 and PBC2 trial groups. Updating prognosis in primary biliary cirrhosis using a time-dependent Cox Regression Model. Gastroenterology 1993;105:1865-6.
70. Murtaugh PA, Dickson ER, Van Dam GM, Malinchoc M, Grambsch PM, Langworthy AL et al. Primary biliary cirrhosis: prediction of short-term survival based on repeated patient visits. Hepatology 1994;20:126-34).
71. Christensen E. Prognostication in primary biliary cirrhosis: relevance to the individual patient. Hepatology 1989;10:111-3.
72. Triger DR, Manifold IH, Underwood JCE. D-Penicillamine in primary biliary cirrhosis: two year results of a single centre, double blind controlled trial. Gut 1980;21:919-20.
73. Epstein O, Jain S, Lee RG, Cook DG, Boss AM, Scheuer PJ et al. D-penicillamine treatment improves survival in primary biliary cirrhosis. Lancet 1981:i:1275-7.
74. Bassendine MF, Macklon AF, Mulcahy R, James OFW. Controlled trial of high and low dose D-penicillamine in primary biliary cirrhosis: results at 3 years. Gut 1982;23:909 (Abstract)
75. Matloff DS, Alpert E, Resnick RH, Kaplan MM. A prospective trial of D-penicillamine in primary biliary cirrhosis. N Engl J Med 1982;306:319-26.
76. Taal BG, Schalm SW, Ten Kate FWJ, Van Berge Henegouwen GP, Brandt KH. Low therapeutic value of D-penicillamine in a short term prospective trial in primary biliary cirrhosis. Liver 1983;3:345-52.
77. Dickson ER, Fleming TR, Wiesner RH, Baldus WP, Fleming CR, Ludwig J et al. Trial on penicillamine in advanced primary biliary cirrhosis. N Engl J Med 1985;312:1011-5.
78. Neuberger J, Christensen E, Portmann B, Caballeria J, Rodes J, Ranek L et al. Double-blind controlled trial of D-penicillamine in patients with primary biliary cirrhosis. Gut 1985;26:114-9.
79. Bodenheimer HC, Schaffner F, Sternlieb J, Klion FM, Vernace S, Pezzulo J. A prospective clinical trial of D-penicillamine in the treatment of primary biliary cirrhosis. Hepatology 1985;5:1139-42.
80. Kaplan MM, Alling DW, Zimmerman HJ, Wolfe HJ, Speresky RA, Hirsch GS et al. A prospective trial of colchicine for primary biliary cirrhosis. N Engl J Med 1986:315:1448-54.
81. Bodenheimer H, Schaffner F, Pezullo J, Evaluation of colchicine therapy in primary biliary cirrhosis. Gastroenterology 1988;95:124-9.
82. Warnes TW, Smith A. Lee FI, Haboubi NY, Johnson PJ, Hunt L. A controlled trial of colchicine in primary biliary cirrhosis. J Hepatol 1987:5:1-7.
83. Heathcote J, Ross A, Sherlock S. A prospective controlled trial of azathioprine in primary biliary cirrhosis. Gastroenterology 1976;70:656-60.
84. Hoofnagle JH, Davis GL, Schafer DF, Peters M, Avigan MI, Pappas SC et al. Randomized trial of chlorambucil for primary biliary cirrhosis. Gastroenterology 1986;91:1327-34.
85. Minuk GY, Bohme CE, Burgess E, Hershfield NB, Kelly JK, Shaffer EA et al. Pilotstudy of cyclosporine A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988;95:1356-63.
86. Wiesner RH, Ludwig J, Lindor KD, Jorgensen RA, Baldus WP, Homburger HA et al. A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. N Engl J Med 1990;32:119-24.

87. Mitchison HC, Bassendine MF, Malcolm MF, Watson AJ, Record CO, James OFW. A pilot double-blind, controlled trial one year trial of prednisolone treatment in primary biliary cirrhosis: hepatic improvement but greater bone loss. Hepatology 1989;10:420-9.
88. Wiesner RH, Grambsch PM, Lindor KD, Ludwig J, Dickson ER. Clinical and statistical analysis of new and evolving therapies for primary biliary cirrhosis. Hepatology 1988;8:688-76.
89. Leuschner U, Leuschner M, Huebner K. Gallstone dissolution in patients with chronic active hepatitis. Gastroenterology 1981;80:1208(Abstract)
90. Leuschner U, Leuschner M, Sieratzki J, Kurtz W and Huebner K. Gallstone dissolution with ursodeoxycholic acid in patients with chronic active hepatitis and two years follow-up. A pilot-study. Dig Dis Sci, 1985;30:642-9.
91. Poupon R, Chrétien Y, Poupon RE, Ballet F, Calmus Y, Darnis F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis. Lancet 1987;i:834-6.
92. Leuschner U, Fischer H, Kurtz W, Güldütuna S, Hübner k, Hellstern A et al. Ursodeoxycholic acid in primary biliary cirrhosis; results of a controlled double-blind study. Gastroenterology 1989;97:1268-74.
93. Oka H, Toda G, Ikeda Y, Hashimoto N, Hasanuru Y, Kaminura T et al. A multi-center double-blind controlled trial of ursodeoxycholic acid for primary biliary cirrhosis. Gastroenterol Jpn 1990;25:774-80.
94. Hadziyannis SJ, Hadziyannis ES, Lianidou E, Makris A. Long-term treatment of primary biliary cirrhosis with ursodeoxycholic acid: the third year of a controlled trial. In: Bile acids as therapeutic agents. In: Paumgartner G, Stiehl A, Gerok W eds, Bile acids as therapeutic agents, Kluwer, Dordrecht 1991; pp.287-96.
95. Poupon RE, Balkau B, Eschwège E, Poupon R and the UDCA-PBC Study Group. A multicenter controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Engl J Med 1991;324:1548-54.
96. Vogel W, Kathrein J, Judmaier G, Braunsteiner H. Deterioration of primary biliary cirrhosis during treatment with ursodeoxycholic acid. Lancet 1988;i:1163.
97. Kneppelhout JC, Mulder CJJ, Van Berge Henegouwen GP, De Vries RA, Brandt K-H. Ursodeoxycolic acid treatment in primary biliary cirrhosis with the emphasis on late stage disease. Neth J Med 1992;41:11-6.
98. Lotterer E, Stiehl A, Raedsch R, Foelsch UR, Bircher J. Ursodeoxycholic acid in primary biliary cirrhosis: no evidence for toxicity in the stages I-III. J Hepatol 1990;10:284-90.
99. Van Berge Henegouwen GP, Van Erpecum KJ. Galzuurtherapie; nieuwe hoop voor patienten met chronische leverziekten. Nederlands Tijdschrift voor Geneeskunde 1991;135;696-700.
100. Stiehl A, Rudolph G, Raedsch R, Möller B, Hopf U, Lotterer E et al. Ursodeoxycholic acid-induced changes of plasma and urinary bile acids in patients with primary biliary cirrhosis. Hepatology 1990;492-7.
101. Stiehl A, Raedsch R, Rudolph G. Acute effects of ursodeoxycholic acid and chenodeoxycholic acid on the small intestinal absorption of bile acids. Gastroenterology 1990;98:424-8.
102. Marteau P, Chazouillères O, Myara A, Jian R, Rambaud JC, Poupon R. Effect of chronic administration of ursodeoxycholic acid in the ileal absorption of endogenous bile acids in man. Hepatology 1990;12:1206-8.
103. Eusufzai S, Ericsson S, Cederlund T, Einarsson K, Angelin B. Effect of ursodeoxycholic acid treatment on ileal absorption of bile acids in man as determined by the SeHCAT test. Gut 1991;32:1044-48.
104. Crosignani A, Podda M, Battezzati PM et al. Changes in bile acid composition in patients with primary biliary cirrhosis induced by ursodeoxycholic acid administration. Hepatology 1991;14:1000-7
105. Erlinger S, Dumont M. Influence of ursodeoxycholic acid on bile secretion. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic; 1990; pp. 35-42.

106. Yoon YB, Hagey LR, Hoffman AF, Gurantz D, Michelotti EL, Steinbach JH. Effect of side-chain shortening on the physiological properties of bile acids: hepatic transport and effect on bile acid secretion of 23-nor-ursodeoxycholate in rodents. Gasttroenterology 1986;90:837-852.
107. Heuman DM, Mills AS, McCall J, Hylemon PB, Pandak WM, Vlahcevic ZR. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts. Gastroenterology 1991;100:203-11.
108. Galle PR, Theilmann L, Raedsch R, Otto G, Stiehl A. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human cultures. Hepatology 1990;12:486-91.
109. Kitani K. Hepatoprotective effect of ursodeoxycholate in experimental animals. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990; pp.43-60.
110. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-5.
111. Calmus Y, Arvieux C, Gane P, Boucher E, Nordlinger B, Rouger P et al. Cholestasis induces major histocompatibility complex class I expression in hepatocytes. Gastroenterology 1992;102:1371-7.
112. Beukers R, Schalm SW. Immunosuppressive therapy for primary biliary cirrhosis. J Hepatol 1992;14:1-6.
113. De Caestecker JS, Jazrawi RP, Petroni ML, Northfield TC. Ursodeoxycholic acid in chronic liver disease. Gut 1991;32:1061-1065.
114. Beukers R, Schalm SW. Cyclosporin A in primary biliary cirrhosis. effect of cyclosporine and cyclosporine plus prednisone in primary biliary cirrhosis. Transplant Proceed 1988;20(suppl.4): 340-43.
115. Kaplan MM, Knox TA. Treatment for primary biliary cirrhosis with low dose weekly methotrexate. Gastroenterolgoy 1991;101:1332-8.
116. Weber P, Scheurlen M, Wiedmann KH. Methotrexate ameliorates disease in patients with early primary biliary cirrhosis. Gastroenterology 1991;100:A810 (Abstract)
117. Zakim D, Boyer TD, eds. Hepatology. Philadelphia:WB Saunders Company 1990; pp. 773-4.
118. Ridley MG, Wolfe CS, Mathews JA. Life threatening acute pneumonitis during low dose methotrexate treatment for rheumatoid arthritis: a case report and literature review. Ann Rheum Dis 1988;47:784-8.
119. Zifroni A, Schaffner F. Long-term follow-up of patients with primary biliary cirrhosis on colchicine therapy. Hepatology 1991;14:990-3.
120. Long RG, Meinhard E, Skinner RK, Varghese Z, Wills MR, Sherlock S. Clinical, biochemical and histological studies of osteomalacia, osteoporosis, and paratyroid function in chronic liver disease. Gut 1978;19:85-90.
121. Howat HT, Ralston AJ, Varley H, Wilson JAC. The late results of long term treatment of primary biliary cirrhosis by corticosteroids. Rev Int Hepatol 1966;16:227-38.
122. Schuppan D. Connective tissue polypeptides in serum as parameters to monitor antifibrotic treatment in hepatic fibrosis. J Hepatol 1991;13 (suppl.3):S17-26.
123. Locascio V, Bonucci E, Imbimbo B, Ballantani P, Adami S, Milani S et al. Bone loss in response to long term glucocorticoid therapy. Bone Miner 1990;8:39-51.
124. Gennari C, Vitelli R. Glucocorticoid-induced osteoporosis. Clin Rheum Dis 1986;12:637-54.
125. Berkum van FNR, Beukers R, Birkenhäger JC, Kooij PPM, Schalm SW, Pols HAP. Bone mass in women with primary biliary cirrhosis; the relation with histological stage and use of corticosteroids. Gastroenterology 1990;99:1134-9.
126. Sambrook PN, Cohen ML, Eisman JA, Pocock NA, Champion GD, Yeates MG. Effects of low dose corticosteroids on bone mass in rheumatoid arthritis: a longitudinal study. Ann Rheum Dis 1989;48:535-8.
127. Reid IR, Alexander CJ, King AR, Ibbertson HK. Prevention of steroid-induced osteoporosis with

(3-amino-1-hydroxypropylididene)-,1-bisphosphonate (APD). Lancet 1988:i;143-6.
128. Lawson DH, Lovatt GE, Gurton CS, Hennings RC. Adverse effects of azathioprine. Adv Drug React Ac Pois Rev 1984;3:161-71.
129. Soloway RD, Summerskill WHJ, Baggenstoss AH, Geall MG, Gitnick GL, Elveback LR et al. Clinical, biochemical, and histological remission of severe chronic active liver disease : a controlled study of treatments and early prognosis. Gastroenterology 1972;63:820-33.
130. Davis GL, Czaja AJ. Immediate and long term results of corticosteroid therapy for severe idiopathic chronic active hepatitis. In:Chronic Active Hepatitis-The Mayo Clinic Experience. Eds. Czaja AJ, Dickson ER. Marcel Dekker Inc, New York, 1986, pp 269-83.
131. Wang KH, Czaja AJ, Beaver SJ, Go VLW. Extrahepatic malignancies following long term immunosuppressive therapy of severe hepatitis B surface antigen-negative chronic active hepatitis. Hepatology 1989;10:39-43.
132. Taal BG. Studies in primary biliary cirrhosis. Thesis, Rotterdam, 1981.

CHAPTER II

CAN URSODEOXYCHOLIC ACID INDUCE COMPLETE REMISSIONS IN PRIMARY BILIARY CIRRHOSIS ?

Frank H.J. Wolfhagen[1], Henk R. van Buuren[1], Solko W. Schalm[1], Fiebo J.W. ten Kate[2], Jan van Hattum[3], Ferry A.L.M. Eskens[4], Jannie W. den Ouden[5], Richard A. de Vries[6], Jaap Ferwerda[7], Egbert W. van der Hoek[8], Joost Scherpenisse[9], Gerard P. van Berge Henegouwen[3] for the Dutch Multicentre PBC-Study Group.

Affiliations:

1. Dept. of Internal Medicine II, University Hospital Dijkzigt, Rotterdam.
2. Dept. of Pathology, Academic Medical Centre, Amsterdam.
3. Dept. of Gastroenterology, University Hospital Utrecht.
4. Dept. of Internal Medicine, Canisius-Wilhelmina Hospital, Nijmegen.
5. Dept. of Internal Medicine, St. Franciscus Hospital, Rotterdam.
6. Dept. of Internal Medicine, Rijnstate Hospital, Arnhem.
7. Dept. of Internal Medicine, Kennemer Hospital, Haarlem.
8. Dept. of Internal Medicine, Carolus Hospital, Den Bosch.
9. Dept. of Internal Medicine, Reinier de Graaf Hospital, Delft.

Published in adapted form in the Journal of Hepatology 1995;22:381

SUMMARY

To assess the potential of ursodeoxycholic acid (UDCA) to induce complete remissions in Primary Biliary Cirrhosis (PBC) 110 patients with non-advanced PBC entered an open, prospective, multicentre study.

Complete remission was defined as symptomatic remission (no pruritus and fatigue), biochemical remission (normal bilirubin, aspartate aminotransferase and IgM and alkaline phosphatase ≤ 1.5 times the upper limit of normal) and histological remission (no inflammation beyond portal tracts and no active bile duct destruction).

Within 1 year the prevalence of abnormal biochemical parameters as well as the prevalence of pruritus and fatigue decreased significantly. Twelve patients attained a biochemical remission. Seven achieved a biochemical and symptomatic remission. In 6 of these 7 patients a liver biopsy could be performed. The criteria for histological remission were fulfilled in 3 of them.

In conclusion, complete remission is achieved in at most 4% (95% CI: 0-8%) of the patients with non-advanced PBC during UDCA treatment within 1 year. To induce complete inactivation of the disease, combined treatment of UDCA with other drugs appears necessary.

INTRODUCTION

Primary Biliary Cirrhosis (PBC) is a usually slowly progressive disease of unknown cause with many features of an autoimmune disorder. Initially there is mainly involvement of the small intrahepatic bile ducts, in the later stages progressive fibrosis and ultimately cirrhosis with secondary copper overload may develop (1).

Ursodeoxycholic acid (UDCA) is nowadays increasingly considered as the first-line treatment option for PBC. Despite numerous studies the precise mode of action of UDCA remains unclear, and beside replacement of toxic endogenous bile acids by UDCA (2), hepatoprotective (3-5), hypercholeretic (6), and immunological (7,8) mechanisms may be involved.

Several controlled trials have shown clear beneficial effects of UDCA on biochemical and immunological parameters of the disease (9-14). Uncontrolled studies suggested impressive improvement in complaints, but this effect was less convincing in controlled studies (9,14). Some studies have also indicated improvement in liver histology (9,10). Reports of up to 4 years of placebo-controlled follow-up did not show clear improvements in survival (15-17), although in one study significantly less liver transplants were performed in patients receiving UDCA during 4 years compared to patients with 2 years of UDCA (17). Current data suggest that UDCA slows down the progression of the disease but does not seem capable to induce a complete inactivation of the disease in most patients. It seems likely that any drug which potentially could alter the natural course of the disease should exert marked effects on clinical, biochemical and histological parameters. In analogy with other autoimmune diseases, e.g. chronic active autoimmune hepatitis, disease remission has therefore been proposed as the first and main treatment goal in PBC (18,19). Surprisingly, no studies have been published evaluating the potential of UDCA to induce disease remission.This prospective study was initiated to assess how many patients with PBC achieve a complete (symptomatic, biochemical and histological) remission during UDCA treatment.

PATIENTS AND METHODS

In October 1990 a prospective multicentre study was initiated in the Netherlands to evaluate new treatments in PBC. The design of this project aims to reflect the general therapeutic approach to diseases for which no established effective treatment is available: patients are first treated with the current best monotherapy and when this is not sufficiently effective are offered an alternative (or additional) treatment. Patients who do not satisfactorily respond to the standard therapy, *in casu* UDCA, are candidate for subsequent enrollment in controlled evaluations of additional, new treatment options. Up till now 160 patients in 30 centres (4 university and 26 non-university clinics) are participating in this project.

The first 110 patients completing the first phase of the project, i.e. an open 1 year study with UDCA, are subject of this report. Before the start of the study it was determined that the first evaluation would be performed as soon as the complete follow-up data of at least 100 patients would be available.

Patient selection

Newly diagnosed patients as well as patients with a longer-standing diagnosis of PBC were included. Patients who were already being treated with UDCA were withdrawn from this treatment for at least 3 months before entry.

PBC was diagnosed when 2 major and 2 minor diagnostic criteria or 1 major and 4 minor criteria, according to Taal et al., were present (20). Major criteria are: a level of antimitochondrial antibodies 1/20 and a liver biopsy showing duct lesions. Minor criteria are: pruritus, jaundice with normal clotting factors, alkaline phosphatase ≥ 2 x upper limit of normal, serum IgM above normal, a Schirmer test showing < 10 mm tear secretion in 5 minutes. Exclusion criteria were: age >75 years, presence of another life expectancy limiting disease, evidence of extrahepatic bile duct disease on ultrasound, pregnancy or risk of pregnancy due to lack of suitable contraception, absence of manifest disease (= fulfillment of criteria for complete response - see

below) and the use of UDCA within 3 months prior to entry. Furthermore patients with advanced disease (Child-Pugh class B or C) were excluded as these patients are likely candidates for liver transplantation in the near future and in general seem unlikely to benefit from medical treatment (19).

Study design

Patients received UDCA (Ursochol® 300 mg tablets; Zambon B.V., Amersfoort, The Netherlands) in a single late evening gift. The dose was adjusted according to body-weight: 450 mg if < 50 kg, 600 mg if 50-70 kg and 750 mg if >70 kg, thus corresponding to ± 10 mg/kg daily.

At entry and at 3-monthly follow-up visits a clinical examination was performed, including assessment of pruritus and fatigue as either being absent or present, and laboratory investigations (bilirubin, alkaline phosphatase (APh), aspartate aminotransferases (AST), IgM). Furthermore creatinine, albumin, Hb, WBC, platelets, prothrombin time, and antithrombin-III were measured at entry.

For all patients admitted to the study liver biopsies were available for diagnostic review. Considering the non-controlled design of the study it was decided not to insist on performing a new liver biopsy at entry. For this study, evaluation of repeat biopsies was only demanded in patients who had attained both symptomatic and biochemical remission after 1 year of UDCA, as it is unlikely that patients with clinical disease activity will have a histological remission. All biopsies were reviewed by one pathologist (FTJW ten K), who was not aware of the clinical results.

Definitions for remission as proposed by Beukers and Schalm were used (19). These include disappearance of PBC-related complaints, especially pruritus and fatigue, (*symptomatic remission*); normal values for bilirubin, AST and IgM, with APh not exceeding 1.5 x the upper limit of normal (*biochemical remission*), and inflammatory infiltrate absent or restricted to the portal tracts without bile duct destruction and absence of granulomas (*histological remission*). *Complete remission* was defined as symptomatic, biochemical ànd histological remission.

A steering committee was established to address scientific, methodological and ethical problems arising during the study. Informed consent was obtained from each patient and the study was approved by all local ethical committees.

Statistical analysis

Quantitative data are expressed as multiples of the upper limit of normal (ULN) of each centre. Differences between the levels at entry and after 1 year were analysed using the Wilcoxon's signed rank test. Differences in the number of patients with abnormal parameters and complaints between entry and 1 year were compared by the McNemar's-test. A p-value ≤ 0.05 was considered significant.

Table 1. **Patient characteristics at entry.**

	Patients included	Patients evaluated
No. of patients:	110	104
Median [range] age (yrs)	58 [33-75]	58 [33-75]
Median [range] disease duration (yrs):	5 [0-19]	5 [0-17]
No. of males:	14 (13%)	14 (14%)
Histology		
Cirrhosis[1]:	20 (18%)	20 (19%)
No cirrhosis[2]:	38 (35%)	38 (37%)
No recent data:	52 (48%)	46 (44%)

[1] As histologically documented previously.
[2] Liver biopsy performed < 1 year before entry.

RESULTS

At the time of evaluation 104 of the 110 included patients had completed one year of treatment with UDCA . Six patients stopped treatment and were excluded from analysis. Four of them stopped UDCA treatment because of supposed adverse effects: three patients experienced abdominal discomfort (nausea, vomiting) and one had increased itching, fatigue and malaise not responding to dose reduction; two asymptomatic patients were no longer motivated to continue the medication. In 5 of the 6 patients, biochemical abnormalities clearly improved during UDCA and deteriorated after withdrawal. None of them attained a spontaneous clinical remission.The main entry characteristics of the 110 included as well as the 104 evaluated patients are given in table 1.

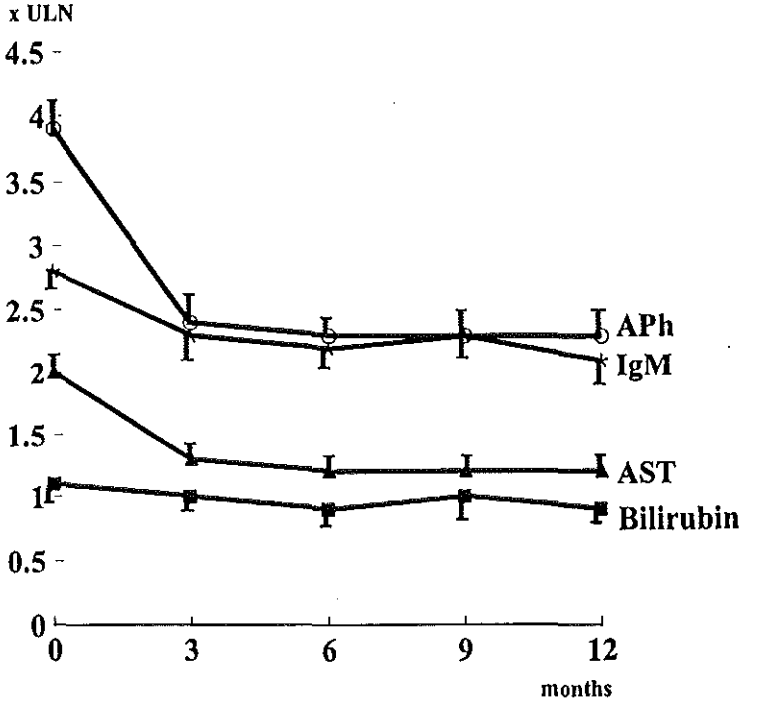


Figure 1.
Mean levels of bilirubin, APh, AST and IgM in 104 patients completing 1 year of UDCA treatment. The bars indicate the standard error of the mean. All parameters improved significantly (bilirubin $p=0.02$, all other parameters $p<0.001$).

Symptomatic remission

The numbers and percentages of patients with pruritus and fatigue at entry and after 1 year are shown in table 2. At entry 63% (65/104) of the patients complained of fatigue and 42% (44/104) of itching. After 1 year this was 49% (51/104) and 29% (30/104) respectively. This decrease was significant for both fatigue ($p<0.03$) and pruritus ($p<0.01$). The percentage of symptomatic patients significantly decreased from 68% (71/104) to 51% (53/104) ($p<0.01$).

Table 2. **Presence of symptoms and abnormal biochemical parameters at entry and 1 year.**

	Entry	1 year	P-value[1]
Symptoms			
Pruritus	42% (44/104)	29% (30/104)	<0.03
Fatigue	63% (65/104)	49% (51/104)	<0.01
Biochemical parameters			
Bilirubin	32% (33/104)	26% (27/104)	>0.1
APh	96% (100/104)	60% (62/104)	<0.001
AST	86% (89/104)	42% (44/104)	<0.001
IgM	93% (97/104)	75% (78/104)	<0.001

[1] P-value with respect to the difference between entry and 1 year of treatment within each group (McNemar's test).

Biochemical remission

After twelve months of treatment the mean levels of bilirubin ($p=0.02$), APh, AST and IgM (all $p<0.001$) were lower than at baseline (figure 1). The number and percentage of patients with abnormal biochemical parameters at entry and after one year is shown in table 2. After 12 months of treatment 29 patients had only 1 abnormal parameter: IgM was still elevated in 19, APh was more than 1.5 times the upper normal value in 8 and AST was still above normal in 2 patients.

Twelve of the 104 (12%) patients achieved a biochemical remission on UDCA. The majority of these patients had mild liver function abnormalities at entry and all had normal bilirubin levels. Characteristics at entry of these patients are given in table 3.

At 12 months 10 of the twelve patients with a biochemical remission were without pruritus and 7 did not have fatigue at 12 months. One patient had developed persistent fatigue after 3 months of treatment, which seemed to be (at least partly) attributable to an intercurrent vital depression. Therefore 7/104 (7%) patients achieved a symptomatic and biochemical remission.

Histological remission

One patient died of cardiac arrest shortly after her 1-year visit; a liver biopsy was performed in the remaining 6 patients with a symptomatic and biochemical remission. One patient did not display any histological signs of active PBC and presented only aspecific reactive changes (slight portal and periportal fibrosis). In the other 5 patients a predominantly lymphocellular portal infiltrate was found. In 3 of them the infiltrate affected the biliary epithelium, without evidence of actual bile duct destruction; in one of these 3 patients granulomas were found and in another patient granulomas and a periportal infiltrate with piecemeal necrosis. In the remaining 2 patients the biliary epithelium was not affected by the portal infiltrate; in one of them a periportal infiltrate with piecemeal necrosis was found. Thus the criteria for histological remission were fulfilled in 3 of the 6 biopsied patients.

Table 3. **Characteristics at entry of patients attaining biochemical, symptomatic and/or histological remission.**

Nr.	Age (yrs)	Sex (m/f)	Histological stage	Disease duration (yrs)	Bili	APh (x ULN)	AST	IgM	Pruritus (yes/no)	Fatigue (yes/no)
Biochemical remission										
1.	56	f	III	<0.5	0.8	4.1	2.8	1.9	yes	yes
2.	73	f	III	10	0.7	2.0	1.0	0.7	yes	yes
3.	51	f	II	<0.5	0.4	2.0	0.9	4.0	yes	yes
4.	71	f	-[1]	15	0.8	2.2	0.9	1.3	no	no
5.	57	f	II	1	0.3	4.1	0.9	0.6	no	no
Biochemical and symptomatic remission										
6.	56	f	I	5	0.2	1.8	0.6	1.4	no	no
7.	75	f	II	<0.5	0.5	1.7	1.1	1.4	no	yes
8.[2]	61	f	-[1]	9	0.7	2.1	0.9	1.2	no	no
9.	53	f	I	8	0.5	1.8	0.7	1.5	no	yes
Biochemical, symptomatic and histological remission										
10.	66	f	-[1]	10	0.7	1.7	1.3	2.0	no	yes
11.	46	m	IV	<0.5	0.7	1.0	1.4	1.0	no	yes
12.	69	f	I	<0.5	0.5	2.8	1.3	0.6	no	no

[1] No liver biopsy within 1 year before entry available and no cirrhosis established in previous biopsies.
[2] No liver biopsy after UDCA treatment available

Complete (biochemical, symptomatic and histological) remission

Seven of the 110 initial patients (6%; 95% confidence interval: 2-10%) achieved a biochemical and symptomatic remission and 3 patients also attained a histological remission. The patient in whom no biopsy could be performed might also have attained a histological remission; thus, the maximal number of patients attaining a complete remission according to our criteria was 4 (4%; 95% CI: 0-8%) (figure 2).

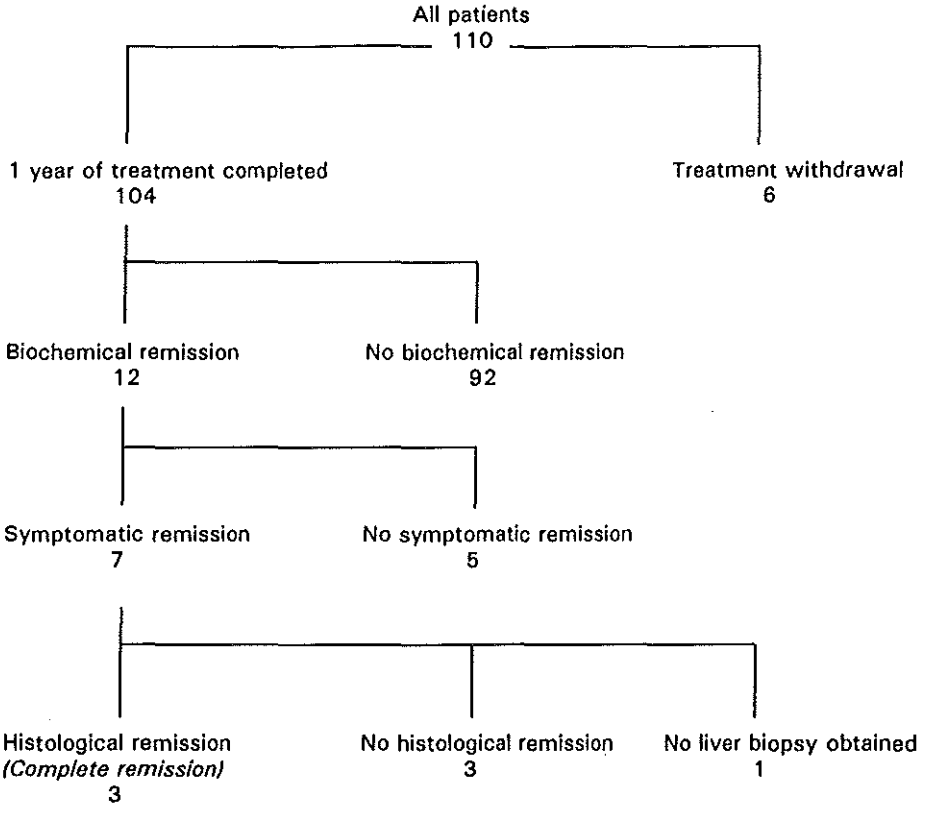


Figure 2.
Flowchart of the 110 patients entering the study, indicating the number of patients stopping treatment and of patients attaining biochemical-, clinical- (biochemical + symptomatic) and complete (biochemical, symptomatic and histological) remissions.

DISCUSSION

The results of this study confirm the already well-established effects of UDCA on biochemical and immunological parameters of the disease in a homogeneous group of patients with non-advanced PBC. However, despite these marked overall improvements in laboratory parameters, an assessment of the effect of UDCA on an individual basis shows that a biochemical, symptomatic and histological inactivation of the disease is rarely achieved. Our results further suggest that a remission may only occur in patients with mild disease activity. It is also clear that, although the number of symptomatic patients decreased, a large number of patients remains symptomatic on UDCA treatment.

To define remission we used recently proprosed, strict criteria (19). These criteria are based on the definitions which have been extensively used in chronic autoimmune hepatitis (21). Since PBC is a cholestatic disorder with primarily involvement of the bile ducts, the criteria were slightly modified, especially with regard to alkaline phosphatase. To the best of our knowledge this is the first study in PBC in which the main outcome variable was remission of disease. This might be due to the absence of generally accepted criteria for remission. Obviously the validity of these criteria and the relation between clinical and histological remission must be further explored in future studies.

With regard to establishing histological remission possible sample errors constitute a problem and may lead to underestimation of disease activity. Therefore, in our study the absence of clear histological activity in 3 of the liver biopsies after UDCA treatment can only be considered as suggestive for complete remission.

We administered a dose of about 10 mg/kg UDCA in this study, in agreement with several other authors (10,14). It could be argued that a higher dose might lead to a higher remission frequency. However, this seems unlikely since the quantitative changes in our study population are comparable with those reported in studies using higher doses (9,15,16). Furthermore, the only dose response study with UDCA performed in chronic liver diseases indicated no extra benefit of a 750 mg dose over a 500 mg dose dose (22).

It may not be surprising that UDCA monotherapy does not appear to be sufficient to induce disease inactivation in PBC. UDCA seems to interfere mainly with the consequences of cholestasis and not, or only to a minor extent, with the underlying immunological disorder. Combined therapy of PBC with bile acids and other treatment modalities therefore seems a logical next step (23-28).

In conclusion, our findings confirm that UDCA improves biochemical parameters and complaints in PBC. However, in spite of overall improvement, complete remission of disease activity in individual patients is rare. Therefore future studies should assess whether the use of additional treatment e.g. immunosuppressive drugs, will increase the number of patients in whom remissions can be achieved.

REFERENCES

1. Kaplan MM. Primary Biliary Cirrhosis. N Engl J Med 1987;316:521-8.
2. Hofmann AF. Bile acid hepatotoxicity and the rationale of UDCA therapy in chronic cholestatic liver diseases: some hypotheses. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990:13-33.
3. Heuman DM, Mills AS, McCall J, Hylemon PB, Pandak WM, Vlahcevic ZR. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts. Gastroenterology 1991;100:203-11.
4. Galle PR, Theilmann L, Raedsch R, Otto G, Stiehl A. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human cultures. Hepatology 1990;12:486-91.
5. Kitani K. Hepatoprotective effect of ursodeoxycholate in experimental animals. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990:43-60.
6. Erlinger S, Dumont M. Influence of ursodeoxycholic acid on bile secretion. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990:35-42.
7. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-5.
8. Yoshikawa M, Tsujii T, Matsumura K, Yamao J, Matsumura Y, Kubo R et al. Immunomodulatory efects of ursodeoxycholic acid on immune responses. Hepatology 1992;16:358-64.
9. Poupon RE, Balkau B, Eschwège E, Poupon R and the UDCA-PBC Study Group. A multicenter controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Engl J Med 1991;324:1548-54.
10. Leuschner U, Fischer H, Kurtz W, Güldütuna S, Hübner K, Helkstern A et al. Ursodeoxycholic acid in primary biliary cirrhosis; results of a controlled double-blind study. Gastroenterology 1989;97:1268-74.
11. Oka H, Toda G, Ikeda Y et al. A multi-center double-blind controlled trial of ursodeoxycholic acid for primary biliary cirrhosis. Gastroenterol Jpn 1990;25:774-80.
12. Combes B, Carithers RL Jr, Maddrey WC, Munoz SJ, McDonald MF, Garcia-Tsao G et al. Ursodeoxycholic acid therapy in patients with primary biliary cirrhosis. Hepatology 1991;14:A174 (Abstract)
13. O'Brien CB, Senior JR, Sternlieb JM, Sample M, Saul SM, Arora R, Batta AK et al. Ursodiol treatment of primary biliary cirrhosis. Gastroenterology 1989;98:A617 (abstract)
14. Battezzati PM, Podda M, Bianchi R and the Italian Multicenter Group for the Study of UDCA in PBC. Ursodeoxycholic acid for symptomatic primary biliary cirrhosis: preliminary analysis of a double-blind controlled multicenter trial. J Hepatol 1993;17:332-8.
15. Lindor KD, Dickson ER, Baldus WB, Jorgensen RA, Ludwig F, Murtaugh PA et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106;1284-90.
16. Heathcote EJL, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN et al. The Canadian multicenter double-blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.
17. Poupon RE, Poupon R, Balkau B and the UDCA-PBC Study Group. Ursodiol for the long term treatment of primary biliary cirrhosis. N Engl J Med 1994;330:1342-7.
18. Wiesner RH. Is continued enthusiasm for ursodeoxycholic acid therapy for the treatment of primary biliary cirrhosis warranted? Hepatology 1992;15:971-3.
19. Beukers R, Schalm SW. Immunosuppressive therapy for primary biliary cirrhosis. J Hepatol 1992;14:1-6.

20. Taal BG, Schalm SW, Kate ten FWJ, Hermans J, Geertzen GM, Feltkamp BEW. Clinical diagnosis of primary biliary cirrhosis: A classification based on major and minor criteria. Hepato-gastroenterol 1983;30:178-82.
21. Davis GL, Czaja AJ. Immediate and long-term results of corticosteroid therapy for severe idiopathic chronic active hepatitis. In: Czaja AJ, Dickson ER eds. Chronic Active Hepatitis - The Mayo Clinic Experience. New York: Marcel Dekker, 1986;269-283.
22. Podda M, Ghezzi C, Battezzati PM, Bertolini E, Crosignani A, Petroni ML, Zuin M. Effect of different doses of ursodeoxycholic acid in chronic liver disease. Dig Dis Sci 1989;34:59S-65S.
23. Caestecker JS de, Jazrawi RP, Petroni ML, Northfield TC. Ursodeoxycholic acid in chronic liver disease. Gut 1991;32:1061-5.
24. Jansen PLM. Antifibrotic therapy of liver cirrhosis, with special reference to primary biliary cirrhosis. Neth J Med 1992;40:209-14.
25. Kaplan MM. New strategies needed for treatment of primary biliary cirrhosis? Gastroenterology 1993;104:651-3.
26. Buscher HP, Zietschmann Y, Gerok W. Positive responses to methotrexate and ursodeoxycholic acid in patients with primary biliary cirrhosis responding insufficiently to ursodeoxycholic acid alone. J Hepatol 1993;18:9-14.
27. Raedsch R, Stiehl A, Walker S, Shermann JM, Kommerel B. Kombinierte Ursodeoxycholsäure plus Colchizinbehandlung bei primär biliärer Zirrhose: Ergebnisse einer Placebo-kontrollierten Doppelblindstudie. Z Gastroenterologie 1992;30 Suppl 1:55-7.
28. Wolfhagen FHJ, Van Buuren HR, Schalm SW. Combined treatment with ursodeoxycholic acid and prednisone in primary biliary cirrhosis. Neth J Med 1994;44:84-90.

CHAPTER III

URSODEOXYCHOLIC ACID IN STAGE IV PRIMARY BILIARY CIRRHOSIS

Frank H.J. Wolfhagen[1], Henk R. van Buuren[1], Gerard P. van Berge Henegouwen[2], Fiebo J.W. ten Kate[3], Janny W. den Ouden[4], Marion J. Kerbert[5], Adelbert M. Smit[6], Richard A. de Vries[7], Ferry A.L.M. Eskens[8], Jan van Hattum[2], Joost Scherpenisse[9], Solko W Schalm[1] and the Dutch Multicenter PBC Study Group.

Affiliations:

1. Dept. of Internal Medicine II, University Hospital Dijkzigt, Rotterdam.
2. Dept. of Gastroenterology, University Hospital Utrecht.
3. Dept. of Pathology, Academic Medical Centre, Amsterdam
4. Dept. of Internal Medicine, St. Franciscus Hospital, Rotterdam
5. Dept. of Internal Medicine, Medical Spectrum, Enschede
6. Dept. of Internal Medicine, Carolus Hospital, Den Bosch
7. Dept. of Internal Medicine, Rijnstate Hospital, Arnhem
8. Dept. of Internal Medicine, Canisius-Wilhelmina Hospital, Nijmegen
9. Dept. of Internal Medicine, Reinier de Graaf Hospital, Delft.

Submitted for publication

SUMMARY

Objective: It has been suggested that patients with primary biliary cirrhosis (PBC) who have developed cirrhosis respond less to treatment with ursodeoxycholic acid (UDCA) than patients with non-cirrhotic disease, and that UDCA may even induce clinical deterioration in these patients. Aim of this study was to compare the response to, and safety of UDCA treatment in PBC-patients with and without cirrhosis.

Design and methods: Twenty cirrhotic patients with compensated (Child-Pugh Class A) PBC and 39 non-cirrhotic patients, were included in a Dutch, prospective, multicenter study. Symptoms (pruritus and fatigue) and biochemical parameters (Bilirubin, Alkaline Phosphatase [APh], AST, IgM and albumin) were assessed 3-monthly during 1 year of UDCA treatment (10 mg/kg/day). Clinical deterioration was defined as de novo appearance of ascites or variceal bleeding, death or transplantation, doubling of bilirubin or a fall in albumin >6 gr/L.

Results: The median percentage decreases from baseline of APh, AST and IgM were significant in both groups. No differences in response were established between cirrhotic and non-cirrhotic patients for bilirubin (-9 vs. -10%), APh (-39 vs. -41%), AST (-38 vs. -45%), IgM (-25 vs. -20%) and albumin (+1 vs. 0%). The decrease from baseline in pruritus and fatigue did not significantly differ between groups. No major adverse effects were noted. Clinical deterioration was observed in one non-cirrhotic patient.

Conclusions: The effects of UDCA on symptoms and biochemical parameters of disease are comparable in PBC patients with non-cirrhotic and compensated cirrhotic disease. Moreover, in patients with compensated cirrhosis UDCA appears to be a safe therapy.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a usually slowly progressive autoimmune disease characterized by non-suppurative destructive cholangitis, cholestatic liver function abnormalities and the presence of antimitochondrial antibodies (1).

Ursodeoxycholic acid (UDCA) is a hydrophilic, non-toxic bile acid which has become the drug of first choice for PBC. The mechanism of action is thought to be based on the displacement of toxic endogenous bile acids from the liver cell, increased choleresis, hepatoprotective- and possibly immunomodulating properties (2-8).

UDCA has documented beneficial effects on biochemical and immunological disease parameters and may ameliorate symptoms (9-16). Also histological improvement has been reported (9,10). Although UDCA seems to slow down the rate of disease progression, a clear effect on histological progression, need of liver transplantation and survival has not been consistently shown (17-19). Furthermore, UDCA rarely induces complete remission of the disease (20).

Despite of its limited effects, the safety and virtual absence of adverse effects of UDCA have contributed to its current widespread use. However, it has been suggested that in patients in the cirrhotic stage of PBC, UDCA is not only ineffective, but may even induce deterioration of the disease (21-25). Therefore a prospective study was initiated to compare the clinical and biochemical response to, and the safety of UDCA in patients with and without cirrhosis.

PATIENTS AND METHODS

Sixty-four patients with PBC, diagnosed according to the criteria of Taal et al. (26) and included in a 1-year, open prospective, multicenter study evaluating UDCA treatment were studied. Patients were excluded in the following cases: age >75 years, presence of another life expectancy limiting disease, evidence of extrahepatic bile duct disease on ultrasound, pregnancy or risk of pregnancy due to lack of suitable contraception and the use of UDCA within 3 months prior

to entry. Patients with advanced disease defined as Child-Pugh class B or C disease (27) were excluded as these patients were considered unlikely to benefit from medical therapy. Thirty-nine patients were classified as "non-cirrhotics" based on a liver biopsy obtained within 1 year before start of the study and 20 as "cirrhotics", as previously established histologically. Liver biopsies were reviewed and classified according to Ludwig et al. (28) by one experienced pathologist (FJW t K.) who was not aware of the clinical state of the patients. The other 5 patients were wrongly included as they already had Child class B cirrhosis. They were followed prospectively in a similar way, but were not included in the comparative study.

Patients received UDCA (Ursochol® 300 mg tablets; Zambon B.V., Amersfoort, The Netherlands) in a single late evening gift. The dose was adjusted according to body-weight: 450 mg if < 50 kg, 600 mg if 50-70 kg and 750 mg if >70 kg (ca. 10 mg/kg/daily).

A clinical examination was performed at entry and at 3-monthly intervals, including assessment of pruritus and fatigue and laboratory investigations (bilirubin, alkaline phosphatase (APh), alanine- and aspartate aminotransferases (AST, ALT), cholesterol, albumin, IgM, IgA and IgG). Furthermore prothrombin time and antithrombin-III activity were measured at entry. Pruritus and fatigue were assessed by semi-quantative scores. Patients were asked to indicate the hours during which they had experienced pruritus and fatigue during the 7 days preceding each visit. The number of hours multiplied by the number of days resulted in a score ranging from 0-168 points.

Hepatic deterioration was defined, using the (slightly modified) criteria of Mitchison et al. (29) as: liver transplantation or hepatic death, *de novo* development of ascites or variceal bleeding, doubling of bilirubin, fall of albumin > 6 g/L (at 2 consecutive measurements). Liver biopsies after 1 year of treatment were not demanded and were not performed in most patients. The criterium of progression to cirrhosis used by Mitchison was therefore not applied.

Laboratory values are expressed as median percentage change from baseline or times the upper limit of normal (ULN). The absolute scores for pruritus and fatigue are given. Differences between values within the groups were analysed using the Wilcoxon's signed

Table 1. **Patient characteristics at entry: cirrhosis vs. non-cirrhosis group (quantative data as medians+range [..]).**

	Cirrhosis	Non-cirrhosis	p =
Number	20	39	
Males	4 (20%)	5 (12%)	0.5
Age (yrs)	60 [37-71]	54 [33-75]	0.07
Disease duration (yrs)	8 [0-17]	1 [0-19]	0.001
Histological stage:			
I	-	13	
II	-	10	
III	-	16	
IV	20	-	
APh	4.0 [1.0-11.0]	3.1 [1.1-12.2]	0.1
AST	2.5 [1.2-7.4]	1.6 [0.5-4.0]	0.09
IgM	2.2 [1.0-16.8]	2.0 [0.6-10.6]	0.6
Bilirubin	1.1 [0.5-4.6]	0.7 [0.1-2.8]	0.009
Bilirubin > ULN (n=..)	11 (55%)	8 (13%)	0.007
Bilirubin if > ULN	1.3 [1.1-4.6]	1.3 [1.1-2.8]	0.9
Albumin in gr/L	41 [29-48]	41 [20-48]	0.3

APh, AST, IgM and Bilirubin given as multiples of the Upper Limit of Normal (ULN).

rank test and differences between groups using the Wilcoxon's ranksum test. Proportions were compared by the X^2-test. A two-sided p-value ≤ 0.05 was considered significant.

RESULTS

Patient characteristics at entry are presented in table 1. In the cirrhotic patients, the duration of disease since diagnosis was longer and bilirubin levels were significantly higher. No other differences were noted. All patients had intact clotting as indicated by a normal prothrombin time.

None of the studied patients withdrew from treatment and no major adverse effects were noted. Two patients reduced the UDCA dosage because of diarrhea: one cirrhotic patient (from 750 to 300 mg) and one non-cirrhotic patient (600 to 300 mg). The diarrhea subsequently subsided and the patients maintained the lowered dose.

The effect of UDCA on pruritus and fatigue did not differ significantly between the cirrhotic and non-cirrhotic patient groups (pruritus p=0.5 and fatigue p=0.3). In view of the substantial number of asymptomatic patients the changes in only those patients who were symptomatic at entry and/or at 1 year are more illustrative (figure 1). In both the cirrhotic and non-cirrhotic patients decreases in pruritus score (median -13 and -15 points, respectively) and in fatigue scores (median -29 vs. -12 points respectively) were observed, which did not differ significantly between both groups (both p=0.6).

In both cirrhotic and non-cirrhotic patients APh (-39% vs. -41% resp.), AST (-38 vs. -45% resp.) and IgM (-25 vs.-20% resp.) significantly decreased (all $p<0.001$); bilirubin (-9 vs. -10%; p=0.7 and p=0.07 resp.) and albumin (+1 vs. 0%; p=1 and 0.7 resp.) remained stable. No significant differences between both groups were found with respect to percentage change from baseline after 1 year for any of the parameters (figure 2).

None of the patients died, required a liver transplantation or developed ascites or variceal bleeding during this study. In the cirrhotic group 2 patients showed an increase in bilirubin. In one of them

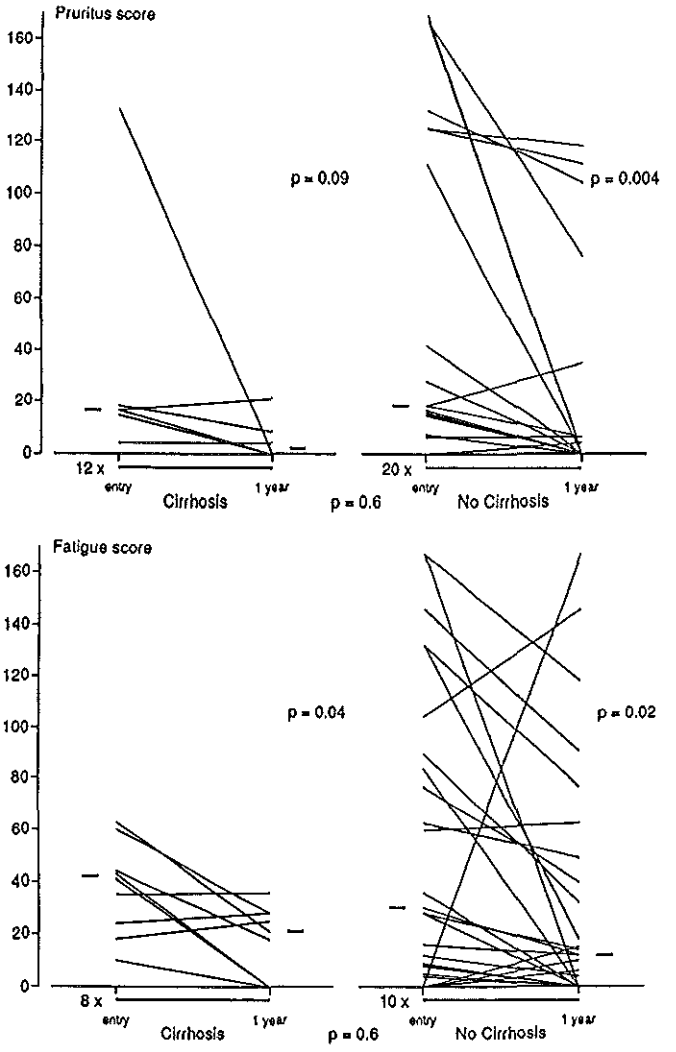


Figure 1.

Scores for pruritus and fatigue at entry and after 1 year of treatment for cirrhotic and non-cirrhotic patients. The horizontal lines represent the median scores of the groups. The p-values in the figure apply to the difference between entry and 1 year within each group; the p-value below the figure applies to the difference in changes between both groups. All p-values and medians indicate the data for patients symptomatic at entry and/or at 1 year. The number of patients who were asymptomatic at entry and at 1 year are indicated below the graph. Two patients in the cirrhotic group (2 symptomatic of which one became asymptomatic) and two in the non-cirrhotic group (1 symptomatic at entry and during the whole follow up period, one becoming symptomatic) were unable to fill in the pruritus score. Three patients in the cirrhotic group (2 symptomatic becoming asymptomatic and one asymptomatic becoming symptomatic) and four in the non-cirrhotic group (all symptomatic at entry and during the whole follow up period) were unable to fill in the fatigue score.

signedbilirubin almost doubled (from 1.2 to 2.2 x ULN). However, this increase only started after 6 months of UDCA. In the non-cirrhotic group 3 patients showed a minor increase of bilirubin (figure 3). One non-cirrhotic patient, in whom serum bilirubin remained stable, showed a decrease in albumin of 10 g/L. A liver biopsy was performed in this patient after 1 year of UDCA which indicated progression from stage III to stage IV disease.

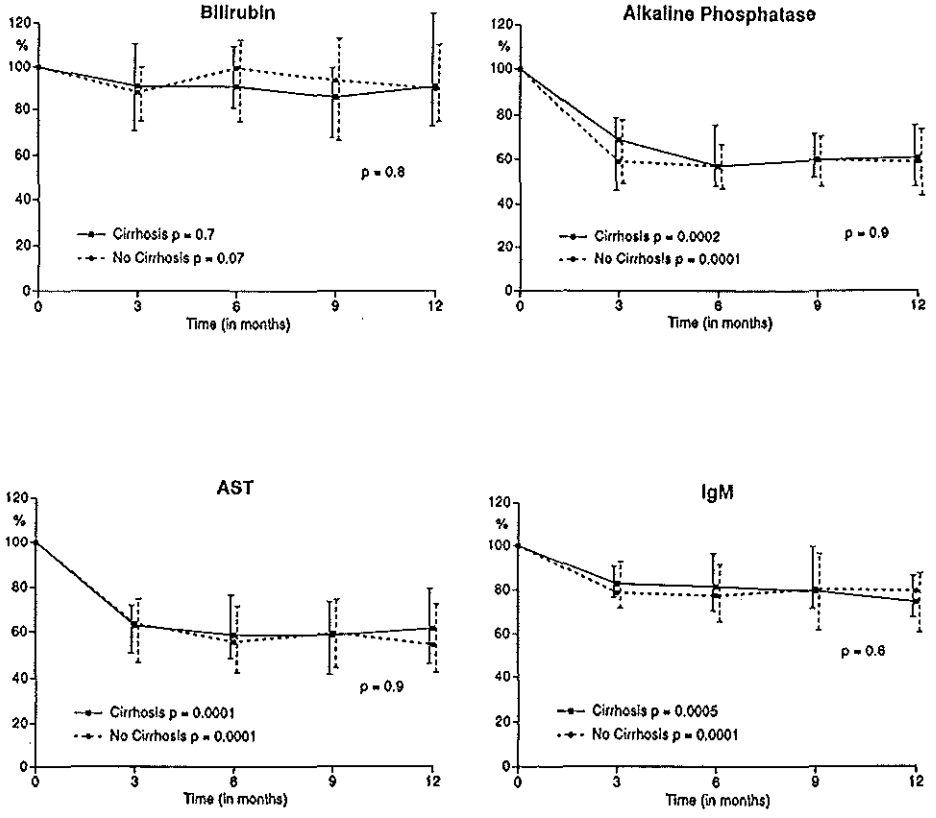


Figure 2.
Percentage of baseline values of bilirubin, alkaline phosphatase, AST and IgM during one year of treatment with UDCA in patients with and without cirrhosis, expressed as medians with interquartiles. Percentage change from baseline after 1 year did not differ between both groups for any of the parameters (all $p \geq 0.6$). P-values for the difference between entry and one year for both groups are given in the legends of the individual figures.

Two of the 5 patients with Child Class B disease died within 1 year from hepatic insufficiency; a third one showed disease progression and was transplanted 1 year after start of treatment. Two patients did not show disease progression and are still alive after respectively 1.5 and 2 years of follow-up (table 2).

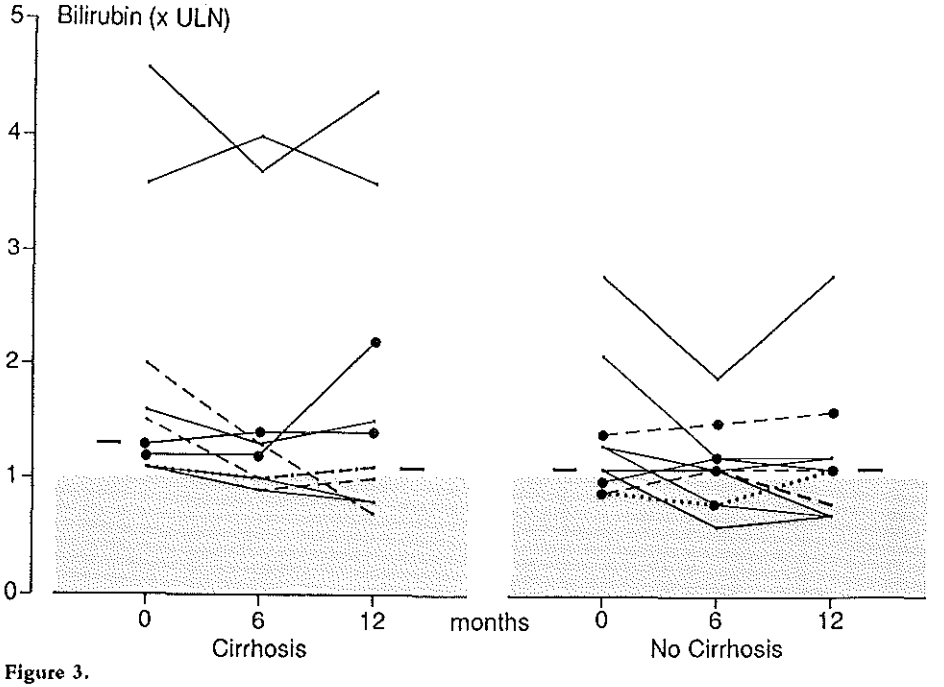


Figure 3.

Bilirubin levels (x Upper Limit of Normal) of patients with abnormal bilirubin levels at any time during 1 year of UDCA for those with cirrhosis (n=11) and without cirrhosis (n=11). The horizontal lines represent the median levels for T=0 and T=1 year. The shaded area represents the normal range. No significant differences were found between median levels at entry, 6 months and 1 year. Patients with rising bilirubin levels are marked (●----●).

Table 2. **Data of patients with initial Child B disease at entry (E), and at the end of follow-up (F).**

Patient nr.	Age in yrs	Child-Pugh score at entry	Bilirubin (μmol/L)		Albumin (gram/L)		PT*		Ascites		Encepha-lopathy		Outcome (duration until outcome in yrs)
			E	F	E	F	E	F	E	F	E	F	
1.	54	7	42	180	27	25	0	3	-	+	-	-	LTx (1)
2.	63	7	89	121	30	29	0	3	-	+	-	+	dead (1.5)
3.	74	7	118	82	29	25	81%	3	-	+	-	+	dead (1)
4.	61	7	43	79	26	28	0	1	-(d)	-	-	-	alive (1.5)
5.	62	7	34	32	31	30	45%	50%	-	-	-	-	alive (2)

*: PT = Prothrombin Time as seconds above upper normal limit. When no Prothrombin Time available Antithrombin-III activity is given (normal > 80%)
LTx = Liver transplantation
(d) = on diuretics for oedema

DISCUSSION

This study shows that PBC patients who have developed cirrhosis, but still have compensated disease, respond to UDCA treatment like patients without cirrhosis. Furthermore UDCA is well tolerated and does not induce deterioration of disease in such patients. The near doubling of bilirubin observed in one patient was unlikely to be related to the institution of UDCA treatment as bilirubin started to increase after more than 6 months of treatment.

It should be emphasized that only patients with compensated cirrhosis, defined as Child-Pugh class A disease, were included in the comparative study and that in only three of the twenty cirrhotic patients serum bilirubin was elevated more than twice the upper limit of normal at entry. Our results are in agreement with the findings of Battezzatti et al. (14) who performed a randomized controlled trial in 88 patients with either pruritus (90 %) or a serum bilirubin exceeding 2 mg/dL (33 %). Histological cirrhosis was present in 50 % of the patients. UDCA significantly improved liver biochemistry, including serum bilirubin in patients with elevated levels at entry. These and our results seem to be in conflict with other reports (12,16,25). Floreani et al. (25) recently reported absence of an effect of UDCA on liver biochemical tests in 11 patients with stage IV disease. In 3 patients serum bilirubin was above 2 mg/dL. Hadziyannis reported biochemical improvements in 13 cirrhotic patients, though less pronounced than in non-cirrhotic patients (16). Preliminary data from a controlled study by Combes et al. in 153 patients suggest that the majority of patients with bilirubin $\geq$ 2 mg/dL is unlikely to benefit from UDCA (12). At present there seems to be no obvious explanation for these contrasting results. In these studies individual data on serum bilirubin and Child-Pugh score were not reported and it could be speculated that inclusion of cirrhotic patients with more advanced or decompensated disease accounts for the different results.

Longer follow-up of small numbers of patients with late stage disease has indicated that, after 2-5 years, initial improvements may worsen again and that disease complications occur more often in these

patients than in early stage patients (16,25,30). Our study has a relatively short follow-up but we do not doubt that further follow-up will show the same pattern, considering the minor effect of UDCA on the natural course of the disease. Naturally, signs of progression will first become evident in patients with cirrhosis. Though it could be argued that these signs would have occurred earlier and more frequently without the use of UDCA, this can only be proved by controlled studies in cirrhotic patients.

Is UDCA safe for PBC patients with cirrhosis? Our results indicate that UDCA is well tolerated and safe for patients with compensated disease. Most studies on UDCA treatment included patients with cirrhosis, and in several large series no patients were reported with deterioration due to UDCA (14-20). Nevertheless, occasional patients have been reported with deteriorating disease after introduction of UDCA with amelioration after discontinuation or dose reduction in some cases (21-23). Kneppelhout et al. (22) retrospectively studied 13 patients with stage III and stage IV PBC who were treated with UDCA. The majority (10/13) of these improved both clinically and biochemically. However, in three of the nine patients with stage IV disease bilirubin increased markedly. One patient showed progression despite discontinuation of UDCA and was eventually transplanted, one patient improved after dose reduction and one after interruption of UDCA. In these three patients initial serum bilirubin levels varied from 32-56 μmol/L (normal up to 17 μmol/L) and was highest in the patient requiring liver grafting. Moreover the four patients who deteriorated on UDCA, reported by Hwang et al. and Vogel et al. had initial bilirubin levels varying from 1.5-4.4 times the upper limit and were elevated more than twice in three of them (21,23). Further details regarding the course of the disease and hepatic function before treatment were not provided clearly. Therefore the observed deterioration in these reports may have represented the natural course in patients with already decompensating disease. Our results suggest that in patients with Child-Pugh class B no clinically significant beneficial effect of UDCA can be expected and progression of the disease may even be accelerated. However, differentation between

natural progression of disease and deterioration due to bile acid treatment remains difficult.

We would like to suggest the following policy: patients with Child-Pugh class A, and serum bilirubin levels less than twice the upper limit of normal can be expected to respond to UDCA like non-cirrhotic patients. Special precautions when initiating treatment with UDCA are not necessary.

Patients with compensated Child-Pugh A cirrhosis and bilirubin levels above 2 times the upper limit of normal may be at risk for responding adversely to UDCA. These patients should start UDCA with about 5-6 mg/kg/day, and be reinvestigated with performance of liver biochemical tests after 2, 4 and 8 weeks. In the absence of problems the dose may thereafter be increased to 10-15 mg/kg/day, with further check-ups after 4 weeks and increasing intervals thereafter.

Patients with cirrhosis and evidence of decompensating or severely advanced disease, defined as presence of Child-Pugh class B or C disease, are very unlikely to benefit from any medical treatment, including UDCA. In these patients liver transplantation should be the primary therapeutic consideration.

In conclusion, PBC patients with compensated cirrhosis can be expected to respond clinically and biochemically in the same way as patients without cirrhosis, and UDCA is very well tolerated by such patients. Only subgroup analyses of patients participating in large, long term controlled trials may prove whether UDCA is associated with a beneficial effect on the course of the disease in cirrhotic patients. However, until such information becomes available, we believe that patients with histological cirrhosis should not be routinely denied treatment with UDCA.

REFERENCES

1. Kaplan MM. Primary Biliary Cirrhosis. N Engl J Med 1987;316:521-8.
2. Hofmann AF. Bile acid hepatotoxicity and the rationale of UDCA therapy in chronic cholestatic liver diseases: some hypotheses. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990:pp 13-33.
3. Heuman DM, Mills AS, McCall J, Hylemon PB, Pandak WM, Vlahcevic ZR. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts. Gastroenterology 1991;100:203-11.
4. Galle PR, Theilmann L, Raedsch R, Otto G, Stiehl A. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human cultures. Hepatology 1990;12:486-91.
5. Kitani K. Hepatoprotective effect of ursodeoxycholate in experimental animals. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990:pp 43-60.
6. Erlinger S, Dumont M. Influence of ursodeoxycholic acid on bile secretion. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990:pp 35-42.
7. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-5.
8. Yoshikawa M, Tsujii T, Matsumura, Yamao J, Matsumura Y, Kubo R et al. Immunomodulatory efects of ursodeoxycholic acid on immune responses. Hepatology 1992;16:358-64.
9. Poupon RE, Balkau B, Eschwège E, Poupon R and the UDCA-PBC Study Group. A multicenter controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Engl J Med 1991;324:1548-54.
10. Leuschner U, Fischer H, Kurtz W, Güldütuna S, Hübner K, Hellstern A et al. Ursodeoxycholic acid in primary biliary cirrhosis; results of a controlled double-blind study. Gastroenterology 1989;97:1268-74.
11. Oka H, Toda G, Ikeda Y, Hashimoto N, Hasumura Y, Kamimura T et al. A multi-center double-blind controlled trial of ursodeoxycholic acid for primary biliary cirrhosis. Gastroenterol Jpn 1990;25:774-80.
12. Combes B, Carithers RL, Maddrey WC, Munoz SJ, McDonald MF, Garcia-Tsao G et al. A randomzied double-blind, placebo-controlled trial of ursodeoxycholic acid (UDCA) in primary biliary cirrhosis. Hepatology 1993;18:A175 (Abstract)
13. O'Brien CB, Senior JR, Sternlieb JM, Sample M, Saul SM, Arora R et al. Ursodiol treatment of primary biliary cirrhosis. Gastroenterology 1989;98:A617 (Abstract)
14. Battezzati PM, Podda M, Bianchi FB, Naccarato F, Orlandi F, Surrenti C et al. and the Italian Multicenter Group for the Study of UDCA in PBC. Ursodeoxycholic acid for symptomatic primary biliary cirrhosis: preliminary analysis of a double-blind controlled multicenter trial. J Hepatol 1993;17:332-8.
15. Turner IB, Myszor M, Mitchison HC, Bennett MK, Burt AD, James OFW. A two year controlled trial examining the effectiveness of ursodeoxycholic acid in primary biliary cirrhosis. J Gastroent Hepatol 1994;9:162-8.
16. Hadzyannis SJ, Hadzyannis ES, Lianidou E, Makris A. Long-term treatment of primary biliary cirrhosis with ursodeoxycholic acid: the third year of a controlled trial. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990:pp 287-96.
17. Poupon RE, Poupon R, Balkau B, Niard AM and the UDCA-PBC Study Group. Ursodiol for the long-term treatment of primary biliary cirrhosis. N Engl J Med 1994;330:1342-7.
18. Lindor K, Dickson ER, Baldus WB, Jorgensen RA, Ludwig J, Murtaugh PA et al.

Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-90.
19. Heathcote EJL, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN et al. The Canadian multi-centre double blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.
20. Wolthagen FHJ, Van Buuren HR, Schalm SW, Ten Kate FJW, Van Hattum J, Den Ouden JW et al. and the Dutch Multicenter PBC Study Group. Can ursodeoxycholic acid induce disease remission in primary biliary cirrhosis? J Hepatol 1995;22:381.
21. Vogel W, Kathrein H, Judmaier G, Braunsteiner H. Deterioration of primary biliary cirrhosis during treatment with ursodeoxycholic acid. Lancet 1988;i:1163.
22. Kneppelhout JC, Mulder CJJ, Van Berge Henegouwen GP, De Vries RA, Brandt K-H. Ursodeoxycholic acid treatment in primary biliary cirrhosis with the emphasis on late stage disease. Neth J Med 1992;41:11-6.
23. Hwang S-J, Chan C-Y, Lee S-D, Wu J-C, Tsay S-H, Lo K-J. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis: a short-term, randomized, double-blind controlled, cross-over study with long-term follow-up. J Gastroenterol & Hep 1993;8:217-23.
24. Lotterer E, Stiehl A, Raedsch R, Foelsch UR, Bircher J. Ursodeoxycholic acid in primary biliary cirrhosis: no evidence for toxicity in the stages I to III. J Hepatol 1990;10:284-90.
25. Floreani A, Zappalà F, Mazzetto M, Naccarato R, Plebani M, Chiaramonte M. Different response to ursodeoxycholic acid in primary biliary cirrhosis according to severity of disease. Dig Dis Sci 1994;39:9-14.
26. Taal BG, Schalm SW, Kate ten FWJ, Hermans J, Geertzen GM, Feltkamp TEW. Clinical diagnosis of Primary Biliary Cirrhosis: A classification based on major and minor criteria. Hepato-gastroenterol 1983;30:178-82.
27. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646.
28. Ludwig J, Dickson ER, McDonald GSA. Chronic nonsuppurative Destructive Cholangitis (Syndrome of Primary Biliary Cirrhosis). Virchows Arch (Pathol Anat) 1978;379:103-12.
29. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OWF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. J Hepatol 1992;15:336-44.
30. Leuschner U, Güldütuna S, Imhof M, Hübner K, Benjaminov A, Leuschner M. Effects of ursodeoxycholic acid after 4-12 years of therapy in early and late stages of primary biliary cirrhosis. J Hepatol 1994;21:624-33.

CHAPTER IV

SINGLE OR MULTIPLE DOSE URSODEOXYCHOLIC ACID FOR CHOLESTATIC LIVER DISEASE: RELATION WITH BILIARY ENRICHMENT AND BIOCHEMICAL RESPONSE

Paul C. van de Meeberg[1], Frank H.J. Wolfhagen[2], Gerard P. VanBerge-Henegouwen[1], Jan M.J.I. Salemans[3], Albert Tangerman[3], Henk R. van Buuren[2], Jan van Hattum[1], Karel J. van Erpecum[1]

Affiliations:

1. Dept. of Gateroenterology, University Hospital Utrecht.
2. Dept. of Internal Medicine II,University Hospital Dijkzigt, Rotterdam.
3. Dept. of Gastroenterology. University Hospital Nijmegen.

Submitted for publication

SUMMARY

Background: Ursodeoxycholic acid (UDCA) improves liver biochemistry in primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Since UDCA acts partly by reducing the intestinal absorption of hydrophobic endogenous bile salts (BS) and is poorly absorbed from the intestine, a multiple dose regimen has been advocated. Single dose treatment on the other hand may improve compliance. The effects of a single or multiple dose regimen on liver enzymes and serum and biliary BS composition were evaluated.

Methods: 27 patients (19 PSC, 8 PBC) received during 3 months UDCA (10 $mgkg^{-1}$ day^{-1}) in a single dose at bedtime (n=13) or in 3 divided gifts with meals (n=14).

Results: Liver biochemistry equally improved in both groups. Biliary enrichment (% UDCA of total BS, mean±SEM) was 38.2±2.5 in the single dose group vs 35.5±2.5 in the multiple dose group (p=NS) and positively correlated with biochemical improvement (AP: r=0.47,p=0.02; γ GT: r=0.58,p=0.002; ASAT: r=0.67,p=0.002; ALAT: r=0.52,p=0.01). Biochemical improvement did not correlate with the concentration or % UDCA in serum.

Conclusions: Single and multiple dose UDCA have equal effects on liver biochemistry and biliary enrichment in cholestatic liver disease. Biochemical improvement is related to biliary (but not serum) enrichment with UDCA. Single dose treatment is more convenient, may improve compliance, and is therefore preferable to a multiple dose regimen

INTRODUCTION

The bile salt ursodeoxycholic acid (UDCA) is currently considered the standard therapy for cholestatic liver diseases such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC)(1-6). UDCA is a hydrophilic bile salt and probably protects the hepatocyte directly against the toxicity of accumulated hydrophobic endogenous bile salts such as chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) (7). Other suggested mechanisms of action are an immunomodulatory effect (8,9), the induction of hypercholeresis (10-12) and competitive inhibition of intestinal absorption of toxic endogenous bile salts (13-15). UDCA is a poor micel former and is poorly solubilized in the intestinal lumen (16,17). Consequently, the absorption rate of UDCA is low as compared to other bile salts (18-20). The active transport of conjugated bile salts in the ileum is limited to a maximum. Although higher doses of UDCA lead to higher biliary enrichment, the proportion of UDCA absorbed decreases with higher doses (19,20). Furthermore, since bile is mainly secreted during meals, and UDCA is supposed to inhibit the absorption of endogenous bile salts, it seems more appropriate to administer UDCA with meals. Therefore, a multiple dose rather than a single dose treatment with UDCA, has been advocated for cholestatic liver disease (20). Indeed, preliminary data from the group of Stiehl suggested a higher absorption of UDCA during multiple dose treatment than during single dose treatment in 4 patients with biliary obstruction and external biliary drainage (mean±SEM: 58.7±10.9% vs 44.4±11.2%) (21). On the other hand, a single dose regimen is more comfortable for the patient and will improve treatment compliance. Moreover, a single dose administration at bedtime appeared to be more efficacious than meal time administration for cholesterol gallstone dissolution (22). Whether there is any difference in efficacy between multiple or single dose treatment with UDCA for cholestatic liver diseases has never been studied. We therefore decided to study the improvement of liver enzymes and the bioavailablity of UDCA, as reflected in serum and biliary enrichment, during a single dose and a multiple dose regimen in patients with PSC and PBC.

MATERIALS AND METHODS

Protocol

Nineteen PSC and 8 PBC patients were randomly assigned to a group receiving UDCA 10 $mgkg^{-1}day^{-1}$ (Ursofalk, Falk GMBH, Freiburg Germany) in a single dose, taken with a small snack at bedtime (n=13), or in 3 divided doses with meals (n=14) during 3 months. Both groups (single vs multiple dose) were comparable for age (mean±SD: 40±7 vs 45±15), sex (M/F: 9/4 vs 8/6), disease (PSC/PBC: 9/4 vs 10/4) and disease stage (early stage / late stage disease: 10/3 vs 12/2). Early stage disease was defined as stage I-II, late stage disease as stage III-IV, as described by Ludwig for PBC (23) and PSC (24). Liver biochemistry was comparable between groups (table 1). In each patient at least one of the standard liver tests (bilirubin, Alkaline Phosphatase [AP], γGT, ASAT, ALAT) was elevated more than 1.5 times the upper limit of normal.

Table 1. **Effect of single or multiple dose UDCA (10 $mgkg^{-1}day^{-1}$) on liver enzymes (mean ± SEM).**

	Single dose (n=13)		Multiple dose (n=14)	
	before UDCA	during UDCA	before UDCA	during UDCA
Bilirubin (μmol/L)	16.5 ± 1.8	14.2 ± 1.1	16.4 ± 2.4	12.6 ± 1.9*
AP (U/L)	383.8 ± 63.9	227.3 ± 31.9†	357.4 ± 60.3	203.0 ± 36.9†
γGT (U/L)	448.2 ± 118.6	197.6 ± 56.9†	455.1 ± 78.3	157.5 ± 29.7†
ASAT (U/L)	71.7 ± 17.2	31.3 ± 4.4*	60.7 ± 9.2	32.4 ± 5.4†
ALAT (U/L)	109.1 ± 27.1	42.0 ± 8.2†	88.6 ± 12.6	43.4 ± 6.9†

* $p < 0.05$ versus pretreatment value, no difference between groups.

† $p < 0.01$ versus pretreatment value, no difference between groups.

Normal ranges: Bilirubin: <17 μmol/l; AP: 27-93 U/l; γGT: (male) 8-46 U/l, (female) 7-29 U/l; ASAT: <30 U/l; ALAT: <30 U/l.

All patients were classified as class A according to the Child-Pugh classification. Anti-mitochondrial antibodies were positive in all PBC patients and in none of the PSC patients. In all patients liverbiopsy was compatible with the diagnosis; all PSC patients had an ERCP confirming the diagnosis. Eleven (58%) PSC patients had concomitant inflammatory bowel disease, however, none of them had active disease during the study. Colectomized patients and patients treated with bile salt sequestrants (e.g. cholestyramine) were excluded from the study. All other medication was continued at a fixed dose throughout the study.

Five patients (1 PSC and 4 PBC, 3 early stage and 2 late stage disease) consented to have an additional treatment of 3 months according to a cross-over design, with a 1 month wash-out period in between (initial treatment: single dose in 2, multiple dose in 3). In all patients, laboratory findings returned to pre-treatment values during the wash-out period.

The protocol was approved by the ethical committee of our hospital and all patients gave informed consent.

Bile sampling

Bile samples were collected in fasting patients at 9.00 am. before and after 3 months of UDCA. In the cross-over part bile samples were also taken after 7 months, at the end of the second treatment phase. Patients had their last medication the previous day with dinner (multiple dose) or at 10.00 pm. with a small snack (single dose). A catheter was positioned under fluoroscopic control in the descending part of the duodenum and the gallbladder was stimulated with an intravenous bolus injection of ceruletide (0.3 μg/kg). Aspirated bile was collected in ice-chilled tubes. Three milliliters of the most concentrated bile was directly transferred to the laboratory for further analysis. The remaining bile was returned through the catheter to avoid bile salt depletion.

Bile analysis

Total bile salt concentration was measured in whole bile using 3α-hydroxysteroid dehydrogenase according to Turley (25). Conjugated bile salt species were analyzed in whole bile by isocratic high

performance liquid chromatography on a Waters Bondapak C-18 10 μm column (using methanol/ phosphate buffer as solvent, pH 5.2, flow rate 1 ml/min) and detection at 200 nm (26). Cumulative hydrophobicity indices were calculated for bile salt species according to Heuman (27).

Blood sampling and analysis of serum bile salts

Blood samples were taken for determination of bilirubin, AP, γ GT, ASAT, ALAT and serum bile salts. Serum bile salts were determined before and during UDCA in the first 16 consecutive patients studied (single dose n=7; multiple dose n=9). Of these, 13 had early stage disease and 3 (all single dose) late stage disease. Serum bile salt concentrations were measured by capillary gas-liquid chromatography as described previously (28). 7α,12α-dihydroxy-5β cholanoic acid was added as an internal standard. Bile salts were extracted from serum using C18-bounded silica cartridges (SepPak, Waters Associates, Milford, MA, USA) (29). Separation of conjugated and unconjugated bile salts was carried out by means of column chromatography using the lipophilic anion exchanger diethylaminohydroxypropyl Sephadex LH-20 (Lipidex-DEAP, Packard Instruments, Groningen, The Netherlands) (29). The conjugated fractions were subjected to enzymatic hydrolysis by cholylglycine hydrolase (from Clostridium perfringens). After enzymatic hydrolysis, the deconjugated bile salts were extracted and eluted on Lipidex-1000 columns (Packard Instruments, Groningen, The Netherlands). The bile salts were converted to methyl esters by 2,2-dimethoxypropane. After methylation, trimethylsilyl ether derivates were prepared by addition of a solution of pyridine, hexamethyldisilazane and trimethylchlorosilane (3:2:1 by vol.). Separation and quantification of bile salts was performed on a Packard 430 gas-liquid chromatograph with a flame ionisation detector and equipped with a 25 m x 0.25 mm glass capillary column (CP-Sil-5 CB, Chrompack, Middelburg, The Netherlands).

Data analysis

Results are expressed as means ± SEM. Data were analysed using paired and unpaired *t*-tests for normally distributed data, Wilcoxon rank

sum and signed rank tests for non-parametric data. Correlations were determined with linear regression analysis between biochemical response (% decrease/increase from baseline), serum concentrations of UDCA and % UDCA of total bile salts in bile and serum. Patients with normal pre-treatment liver enzymes were only taken into account for regression analysis when a rise above the upper limit of normal was observed during UDCA treatment. From the 5 patients who participated in the cross-over design only the results of the first treatment phase were used for regression analysis (single dose in 2 and multiple dose in 3). A two-tailed probability of $p \leq 0.05$ was considered significant.

RESULTS

Biochemical response

Treatment with UDCA resulted in a significant decrease of AP, γ GT, ASAT and ALAT both in patients treated with a single dose and multiple dose. Bilirubin also decreased, however significance was reached in the multiple dose group only (table 1). The improvement of liver enzymes, including bilirubin, did not differ significantly between the two groups.

Biliary bile salts

Pre-treatment biliary bile salt composition was comparable in both treatment groups and was modified in a similar way during UDCA (table 2). Cholic acid (CA) was the major biliary bile salt before therapy followed by CDCA. During treatment, in both groups UDCA became the major bile salt with a significant decrease of the proportions of CA and CDCA. Accordingly, the hydrophobicity index decreased from 0.23 ± 0.02 to -0.01 ± 0.02 in the single dose group and from 0.24 ± 0.02 to 0.03 ± 0.02 in the multiple dose group ($p<0.001$ in both groups, difference between groups non significant).

Regression analysis showed a positive correlation between biochemical response and biliary enrichment with UDCA in patients

Table 2. **Effect of single or multiple doses of UDCA (10 mgkg^{-1}day^{-1}) on biliary bile salt species* (% of total ±SEM) in cholestatic liver disease.**

	Single dose (n=13)		Multiple dose (n=14)	
	before UDCA	during UDCA	before UDCA	during UDCA
TCA	20.9 ± 4.1	9.7 ± 1.8†	22.7 ± 4.1	9.4 ± 1.4†
GCA	36.2 ± 3.0	25.2 ± 1.9†	32.4 ± 2.3	24.6 ± 1.8†
CA (T+G)	57.2 ± 3.2	34.9 ± 3.3‡	55.1 ± 4.0	34.0 ± 2.6‡
TCDCA	10.8 ± 1.6	5.3 ± 0.7‡	11.7 ± 1.6	5.4 ± 0.6‡
GCDCA	21.5 ± 2.2	15.5 ± 1.0†	22.6 ± 3.1	17.6 ± 1.1
CDCA (T+G)	32.3 ± 1.1	20.8 ± 1.0‡	34.3 ± 2.7	22.9 ± 0.8‡
TDCA	1.6 ± 0.5	1.0 ± 0.2	1.8 ± 0.6	1.2 ± 0.3
GDCA	6.4 ± 2.0	4.5 ± 1.2	6.9 ± 2.3	6.1 ± 1.8
DCA (T+G)	8.0 ± 2.4	5.6 ± 1.4	8.7 ± 2.7	7.2 ± 2.0
TUDCA	0.6 ± 0.2	4.6 ± 0.5‡	0.4 ± 0.1	4.6 ± 0.7‡
GUDCA	0.9 ± 0.2	33.6 ± 2.4‡	1.1 ± 0.4	30.9 ± 2.3‡
UDCA (T+G)	1.5 ± 0.2	38.2 ± 2.5‡	1.5 ± 0.4	35.5 ± 2.5‡
Hydrophobicity Index ¥	0.23 ± 0.02	- 0.01 ± 0.02‡	0.24 ± 0.02	0.03 ± 0.02‡

* Taurine (T) and glycine (G) conjugated biliary bile salts have been determined with HPLC. LCA accounted for less than 1% of total bile salts both before and during therapy. CA=cholic acid; CDCA=chenodeoxycholic acid; DCA=deoxycholic acid; UDCA=ursodeoxycholic acid.

† $p < 0.01$ versus pre-treatment value, no difference between groups

‡ $p < 0.001$ versus pre-treatment value, no difference between groups

¥ Hydrophobicity Index calculated according to Heuman (27)

with pre-treatment elevated liver enzymes (figure 1). Normal liver enzyme values at entry did not exceed the upper limit of normal during treatment, except in 1 patient participating in the cross-over study, who showed a small rise of AP during multiple dose UDCA.

Biliary enrichment with UDCA (% of total bile salts ± SEM) was not different in patients with early stage disease (37.0±1.9%) or late stage disease (37.9±2.8%) and patients with PSC (37.1±1.9) or PBC (39.2±3.7) .

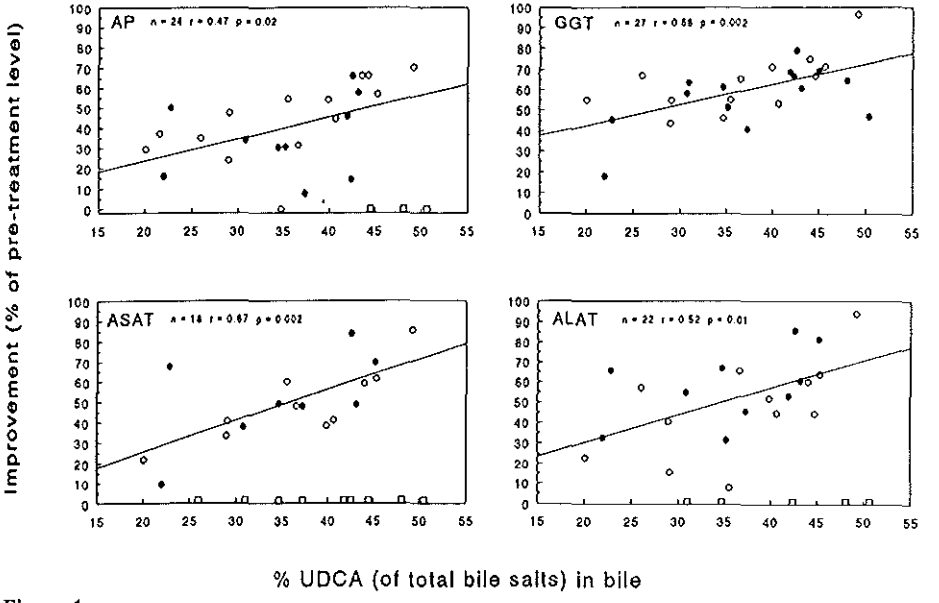


Figure 1.
Improvement of liver enzymes (% of pre-treatment) in relation to biliary enrichment with UDCA (% of total bile salts) as determined with linear regression analysis in patients with pre-treatment elevated liver enzymes, treated with a multiple dose (●) or single dose (○) regimen of UDCA (10 $mgkg^{-1}day^{-1}$). Patients with normal pre-treatment liver biochemistry (□) are shown on the site on the X-axis corresponding to their level of biliary enrichment. In none of these patients, enzymes exceeded the upper limit of normal during treatment.

Serum bile salts

The pre-treatment concentrations of fasting total serum bile salts (sum of conjugated and unconjugated fractions) were much higher than previously described by our group in healthy subjects (n=22, range: 1.4 - 10.7 μmol/l) (28,29). Total serum bile salts increased from 31.9±13.1 to 56.6±19.8 μmol/l during treatment, due to a strong increase of UDCA (0.2±0.1 vs 35.6±13.1 μmol/l, $p<0.05$). UDCA therapy resulted in a significant decrease of % CA and CDCA and a significant increase of % UDCA. These changes were very similar in single and multiple dose groups (table 3). Conjugated UDCA accounted for 92.8% of total UDCA in serum.

A positive correlation was found between % UDCA in serum and bile ($r=0.55$, $p=0.03$). The improvement of liver biochemistry did not correlate with % UDCA ($0.03<r<0.31$ and $0.30<p<0.92$) or concentration UDCA ($-0.02<r<0.29$ and $0.28<p<0.97$) in serum.

Cross-over study

Biochemical response and biliary bile salt composition were comparable during both treatment regimens in 5 patients who participated in the cross-over study (figure 2).

Table 3. Effect of single or multiple doses of UDCA (10 $mgkg^{-1}day^{-1}$) on serum bile salts* (% of total ± SEM) in cholestatic liver disease.

	Single dose (n=7)		Multiple dose (n=9)	
	before UDCA	during UDCA	before UDCA	during UDCA
CA	41.6 ± 2.3	14.7 ± 1.7†	43.0 ± 2.7	19.0 ± 1.9†
CDCA	48.8 ± 3.9	16.8 ± 1.4†	45.8 ± 3.1	19.3 ± 1.7†
DCA	8.4 ± 4.1	4.7 ± 1.7	8.9 ± 3.3	4.1 ± 0.8
UDCA	1.2 ± 0.6	63.8 ± 2.3†	2.3 ± 0.9	57.6 ± 3.1†

* Bile salt concentrations (sum of conjugated and unconjugated) have been determined with gas-liquid chromatography.

† $p < 0.05$ versus pre-treatment value, no difference between groups.

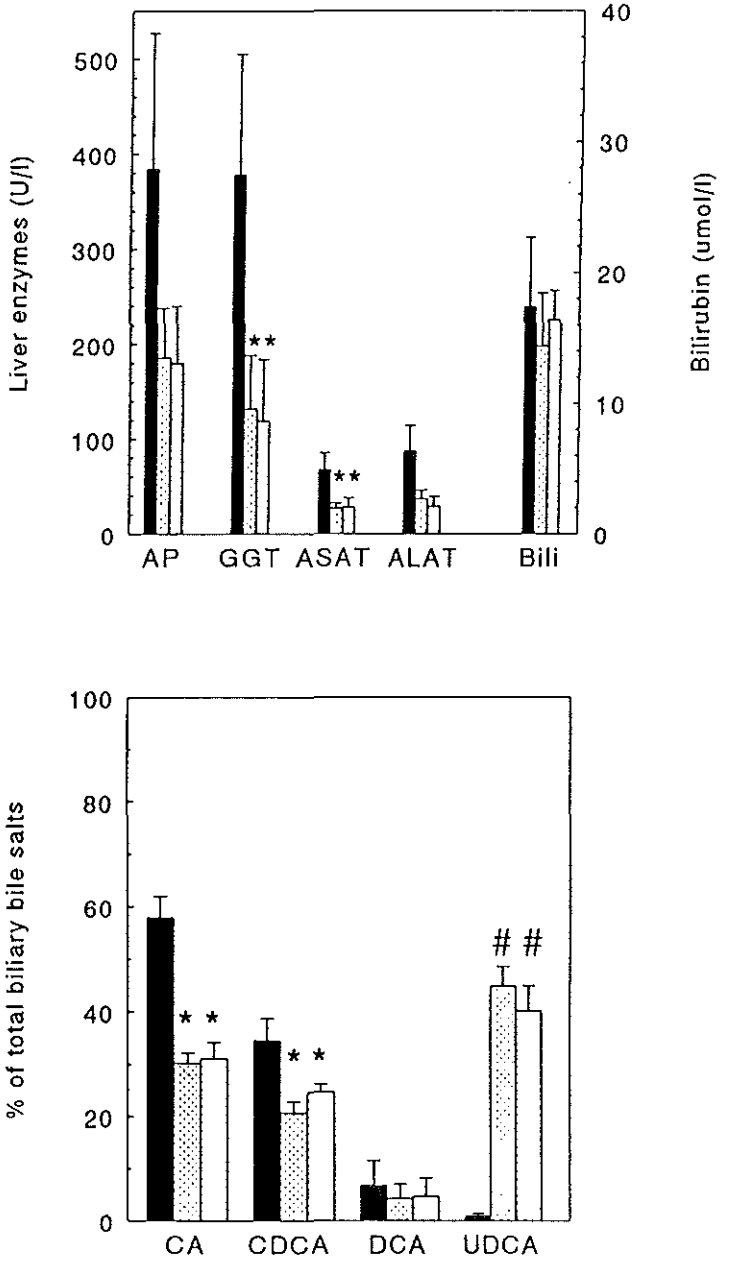


Figure 2.

Changes in liver enzymes (U/L) and bilirubin (μmol/l) (upper panel) and percentages of biliary bile salts (lower panel) in 5 patients treated in cross-over design with UDCA (10 $mgkg^{-1}day^{-1}$) at base-line (left bars, ■), during single dose (middle bars, □) and multiple dose (right bars, □). * $p<0.05$; # $p<0.001$.

DISCUSSION

Our results show that after three months of UDCA at a dose of 10 $mgkg^{-1}day^{-1}$, both biochemical improvement and biliary enrichment are equal during a single and a multiple dose regimen in patients with cholestatic liver disease. Our findings are in contrast with previous studies that have shown a poor intestinal absorption of UDCA particularly when given in a single dose (20,21). However, in these studies the recovery of UDCA in bile was evaluated after a single bolus administration of UDCA. Our results indicate that during chronic treatment with UDCA the mode of administration is neither a determining factor for biliary enrichment, nor for biochemical response. A possible explanation for this finding is the fact that UDCA, once absorbed, will recirculate mainly as conjugated UDCA in the enterohepatic circulation. This is supported by the finding that in our population 92.8% of serum UDCA was conjugated. It can be hypothesized that during chronic treatment most of the UDCA absorbed in the ileum is derived from the circulating pool of conjugated UDCA.

In cholestatic liver disease, not only the intestinal uptake but also the handling of UDCA by the liver is important for biliary enrichment (30). Failure of the liver to excrete the amount of UDCA absorbed from the ileum will be reflected by high concentrations of UDCA in serum (31). The finding of high serum bile salt concentrations in our patients underlines the hypothesis that the excretion of UDCA by the liver is the limiting factor for biliary enrichment in cholestatic liver disease (30,32). Consequently, we believe that the mode of administration of UDCA as a single or a multiple dose regimen is irrelevant.

Biochemical improvement positively correlated with biliary enrichment with UDCA as has been found by others (33-35). On the other hand, no relation was found between biochemical improvement and concentration or % UDCA in serum. These findings suggest that the hepatoprotective properties of UDCA take place at the level of the canalicular membrane rather than the basolateral membrane. This is in line with recent findings of Heuman et al. (36). They showed in vitro

that the membrane stabilizing property of UDCA requires a relatively high (≥0.5) cholesterol / phospholipid (C/P) ratio of the membrane. Since the canalicular membrane has a much higher C/P ratio than the basolateral membrane, they hypothesize that UDCA acts at the canalicular membrane rather than the basolateral membrane. This theory is supported by the fact that UDCA appears to be hepatoprotective in rats with bile salt induced cholestasis (37,38) but not, or to a lesser extent, in rats with completely blocked bile secretion due to bile duct ligation (39,40). These findings are in contrast with recent data from Güldütuna et al. suggesting that UDCA, like cholesterol, stabilises the basolateral rather than the canalicular membrane (41).

The positive correlation between biliary enrichment with UDCA and biochemical response should be regarded with caution. Theoretically, good biochemical response and high biliary enrichment with UDCA may both be secondary to improved liver function. Indirect evidence has been provided that UDCA improves the excretory function of the liver. Jazrawi et al. have shown an improved hepatic excretory rate and transit time of ^{75}SeHCAT during treatment with UDCA in patients with cholestatic liver disease (30). Colombo et al., using hepatobiliary scintigraphy, demonstrated improvement of liver excretion in patients with cystic fibrosis and cholestatic liver disease (42). Further studies are needed to find out whether biliary enrichment, often used as a parameter in dose response studies, is indeed a prerequisite for biochemical improvement.

In conclusion, this study shows that single bedtime administration and multiple (3 times a day) mealtime administration of UDCA have an equal beneficial effect on liver enzymes and lead to similar biliary enrichment with UDCA in patients with cholestatic liver disease. Biochemical improvement is related to the proportion of UDCA in bile but not in serum. In our opinion a single dose treatment is more convenient to the patient, may improve compliance and is therefore to be preferred to a multiple dose regimen.

REFERENCES

1. Lindor KD, Dickson ER, Baldus WP, Jorgensen RA, Ludwig J, Murtaugh PA, Harrison JM, Wiesner RH, Anderson ML, Lange SM, LeSage G, Rossi SS, Hofmann AF: Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-1290.
2. Poupon RE, Poupon R, Balkau B, and the UDCA-PBC Study Group: Ursodiol for the long-term treatment of primary biliary cirrhosis. N Engl J Med 1994;330:1342-1347.
3. Heathcote EJ, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN, Michieletti P, Minuk GY, Pappas SC, Scully LJ, Steinbrecher UP, Sutherland LR, Williams CN, Witt-Sullivan H, Worobetz LJ, Milner RA, Wanless IR: The Canadian multicenter double-blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-1156.
4. O'Brien CB, Senior JR, Arora-Mirchandani R, Batta AK, Salen G: Ursodeoxycholic acid for the treatment of primary sclerosing cholangitis: a 30-month pilot study. Hepatology 1991;14:838-847.
5. Beuers U, Spengler U, Kruis W, Aydemir Ü, Wiebecke B, Heldwein W, Weinzierl M, Pape GR, Sauerbruch T, Paumgartner G: Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo controlled trial. Hepatology 1992;16:707-714.
6. Stiehl A, Walker S, Stiehl L, Rudolph G, Hofmann WJ, Theilmann L: Effect of ursodeoxycholic acid on liver and bile duct disease in primary sclerosing cholangitis. A 3-year pilot study with a placebo-controlled study period. J Hepatol 1994;20:57-64.
7. Queneau PE, Montet JC: Hepatoprotection by hydrophilic bile salts. J Hepatol 1994;21:260-268.
8. Calmus Y, Gane P, Rouger P, Poupon R: Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-15.
9. Kürktschiev D, Subat S, Adler D, Schentke K, U.: Immunomodulating effect of ursodeoxycholic acid therapy in patients with primary biliary cirrhosis. J Hepatol 1993;18:373-377.
10. Dumont M, Erlinger S, Uchman S: Hypercholeresis induced by ursodeoxycholic acid and 7-ketolithocholic acid in the rat: possible role of bicarbonate transport. Gastroenterology 1980;79:82-89.
11. Fracchia M, Setchell KDR, Crosignani A, Podda M, Ferraris R, O'Connel N, Galatola G: Ursodeoxycholic acid induced hypercholeresis in a patient with primary biliary cirrhosis. Hepatology 1993;18:297A
12. Elsing C, Sägesser H, Reichen J: Ursodeoxycholate-induced hypercholeresis in cirrhotic rats: further evidence for cholehepatic shunting. Hepatology 1994;20:1048-1054.
13. Marteau P, Chazouillères O, Myara A, Jian R, Rambaud J, Poupon R: Effect of chronic administration of ursodeoxycholic acid on the ileal absorption of endogenous bile acids in man. Hepatology 1990;12:1206-1208.
14. Eusufzai S, Ericsson S, Cederlund T, Einarsson K, Angelin B: Effect of ursodeoxycholic acid treatment on ileal absorption of bile acids in man as determined by the SeHCAT test. Gut 1991;32:1044-1048.
15. Stiehl A, Raedsch R, Rudolph G: Acute effects of ursodeoxycholic and chenodeoxycholic acid on the small intestinal absorption of bile acids. Gastroenterology 1990;98:424-428.
16. Igimi H, Carey MC: pH-solubility relations of chenodeoxycholic and ursodeoxycholic acids: physical-chemical basis for dissimilar solution and membrane phenomena. J Lipid Res 1980;21:72-90.
17. Parquet M, Metman EH, Raizman A, Ramband JC, Berthaux N, Infante R: Bioavailability, gastrointestinal transit, solubilization and faecal excretion of ursodeoxycholic acid in man. Eur J Clin Invest 1985;15:171-178.
18. Stiehl A, Raedsch R, Rudolph G: Ileal excretion of bile acids: comparison with biliary bile

composition and effect of ursodeoxycholic acid treatment. Gastroenterology 1988;94:1201-1206.
19. Marcus SM, Schteingart CD, Marquez ML, Hofmann AF, Xia Y, Steinbach JH, Ton-Nu H, Lillienau J, Angellotti MA, Schmassmann A: Active absorption of conjugated bile acids in vivo; kinetic parameters and molecular specificity of the ileal transport in the rat. Gastroenterology 1991;100:212-221.
20. Walker S, Rudolph G, Raedsch R, Stiehl A: Intestinal absorption of ursodeoxycholic acid in patients with extrahepatic biliary obstruction and bile drainage. Gastroenterology 1992;102:810-815.
21. Stiehl A, Sauer P, Rudolph G, Klöters-Plachky P: Absorption of ursodeoxycholic acid in man: single dose versus multiple doses versus a microencapsulated, sodium carbonate containing formula. Gastroenterology 1993;104:A999
22. Galatola G, Jazrawi RP, Northfield TC: Low dose ursodeoxycholic acid at bedtime and at mealtimes: effect on mass of lipids within the gallbladder and on gallstone dissolution. Eur J Gastroenterol Hepatol 1992;4:301-306.
23. Ludwig J, Dickson ER, McDonald GS: Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchow's Arch A 1978;379:103-112.
24. Ludwig J, Larusso NF, Wiesner RH: The syndrome of primary sclerosing cholangitis. Prog Liver Dis 1990;9:555-566.
25. Turley SD, Dietschy JM: Reevaluation of the 3-hydroxy-steroid dehydrogenase assay for total bile acids in bile. J Lipid Res 1978;19:924-928.
26. Ruben AT, VanBerge Henegouwen GP: A simple reverse-phase high pressure liquid chromatographic determination of conjugated bile acids in serum and bile using a novel radial compression seperation system. Clin Chim Acta 1982;11:941-950.
27. Heuman DM: Quantitative estimation of the hydrophylic-hydrophobic balance of mixed bile salt solutions. J Lipid Res 1989;30:719-730.
28. Salemans JMJI, Nagengast FM, Tangerman A, Van Schaik A, Hopman WPM, De Haan AFJ, Jansen JBMJ: Effect of ageing on postprandial conjugated and unconjugated serum bile acid levels in healthy subjects. Eur J Clin Invest 1993;23:192-198.
29. Tangerman A, Van Schaik A, Van Der Hoek EW: Analysis of conjugated and unconjugated bile acids in serum and jejunal fluid of normal subjects. Clin Chim Acta 1986;159:123-132.
30. Jazrawi RP, de Caestecker JS, Goggin PM, Britten AJ, Joseph AEA, Maxwell JD, Northfield TC: Kinetics of hepatic bile acid handling in cholestatic liver disease: effect of ursodeoxycholic acid. Gastroenterology 1994;106:134-142.
31. VanBerge Henegouwen GP, Hofmann AF: Clinical aspects of disturbances in the enterohepatic circulation of bile acids in man: the cholanopathies. Neth J Med 1978;21:257-269.
32. VanBerge Henegouwen GP, Brandt KH, Eyssen H, Parmentier G: Sulphated and unsulphated bile acids in serum , bile, and urine of patients with cholestasis. Gut 1976;17:861-869.
33. Lacerda MA, Lindor KD, Jorgensen RA, Rossi S, Hofmann A, Dickson ER: Ursodiol therapy in primary biliary cirrhosis (PBC): enrichment correlates with biochemical improvement. Gastroenterology 1993;104:A933
34. Podda M, Ghezzi C, Battezzati PM, Bertolini E, Crosignani A, Petroni ML, Zuin M: Effect of different doses of ursodeoxycholic acid in chronic liver disease. Dig Dis Sci 1989;34:59S-65S.
35. Colombo C, Crosignani A, Assaisso M, Battezzati PM, Podda M, Giunta A, Zimmer-Nechemias L, Setchell KDR: Ursodeoxycholic acid therapy in cystic fibrosis-associated liver disease: a dose-response study. Hepatology 1992;16:924-930.
36. Heuman DM, Bajaj R: ursodeoxycholate conjugates protect against disruption of cholesterol-rich membranes by bile salts. Gastroenterology 1994;106:1333-1341.
37. Kitani K, Kanai S: Tauroursodeoxycholate prevents taurocholate induced cholestasis. Life Sci 1982;30:515-523.
38. Kitani K, Ohta M, Kanai S: Taroursodeoxycholate prevents biliary protein excretion induced by other bile salts in the rat. Am J Physiol 1985;248:G407-G417.

39. Poo JL, Feldmann G, Erlinger S, Braillon A, Gaudin C, Dumont M, Lebrec D: Ursodeoxycholic acid limits liver histologic alterations and portal hypertension induced by bile duct ligation in the rat. Gastroenterology 1992;102:1752-1759.
40. Zimmerman H, Reichen J: Ursodeoxycholate has no beneficial effects on liver function or histology in biliary cirrhosis in the rat. J Hepatol 1992;16:355-359.
41. Güldütuna S, Zimmer G, Imhof M, Bhatti S, You T, Leuschner U: Molecular aspects of membrane stabilization by ursodeoxycholate. Gastroenterology 1993;104:1736-1744.
42. Colombo C, Castellani MR, Balistreri WF, Seregni E, Assaisso ML, Giunta A: Scintigraphic documentation of an improvement in hepatobiliary excretory function after treatment with ursodeoxycholic acid in patients with cystic fibrosis and associated liver disease. Hepatology 1992;15:677-684.

CHAPTER V

COMBINED TREATMENT WITH URSODEOXYCHOLIC ACID AND PREDNISONE IN PRIMARY BILIARY CIRRHOSIS

Frank H.J. Wolfhagen, Henk R. van Buuren, Solko W. Schalm

Dept. of Internal Medicine II (section Hepatology), University Hospital Dijkzigt, Rotterdam, The Netherlands.

Published in the Netherlands Journal of Medicine 1994:44:84-90.

SUMMARY

Objective: To assess the effect of combined therapy (CT) of ursodeoxycholic acid (UDCA) with prednisone on symptoms and biochemistry in patients with non-advanced Primary Biliary Cirrhosis (PBC), who had responded insufficiently to either drug alone.

Methods: Retrospective evaluation of the effect of 1 year of CT on symptoms (pruritus, fatigue, arthralgia) and biochemical parameters (bilirubin, alkaline phosphatase (APh), Aspartate Aminotransferase (AST) and IgM) in 7 symptomatic patients.

Results: Five of the seven patients became asymptomatic. Pruritus disappeared in 2 of 3 patients, fatigue in 4 of 6 and arthralgia in both symptomatic patients. APh and AST decreased in all patients (median 41% and 59%, respectively). IgM decreased, although to a lesser degree (median 16%), in all but one patient. Normal levels for AST were achieved in 4 patients. In 2 of these APh normalized too. In 2 patients IgM became normal. Bilirubin, only slightly elevated in one patient, remained stable in all. The beneficial effects were maintained during follow-up (median 1.5 years). The treatment was well-tolerated by all patients.

Conclusions: In PBC, combined treatment with UDCA and prednisone appears to improve symptoms and biochemical parameters to a larger extent than either treatment alone; randomized controlled trials should be performed to establish the benefit/risk ratio of this combination therapy.

INTRODUCTION

Primary Biliary Cirrhosis (PBC) is a chronic, usually slowly progressive cholestatic liver disease of unknown cause (1). Efforts to establish medical therapy that prevents patients to progress to liver failure and improve prognosis have been largely unsuccessful (2). Such therapy should ideally be aimed at eliminating the cause of the disease, which, however, is still not elucidated. Therefore no causal, but only symptomatic and supportive therapy is feasible at the moment. In PBC both auto-immune mechanisms and liver damage caused by alterations in bile acid metabolism seem of major pathogenetic importance and consequently most therapeutic studies have focused on modulation of these factors (3).

In general the results obtained with immunosuppressives have been disappointing (4-10). In contrast to chronic auto-immune hepatitis no agent has been shown to be capable to induce complete inactivation of the disease (11). The significant adverse effects of several drugs (e.g. cyclosporine, chlorambucil) further limit the clinical usefulness of immunosuppressive treatment. Recently promising results with methotrexate in PBC have been reported, but data from controlled trials have to be awaited (12).

Prednisolone has been evaluated in one controlled trial. The results were encouraging and especially the absence of a clear adverse effect on bone mineral density was reassuring (13).

Ursodeoxycholic acid (UDCA) has been reported to exert a beneficial effect on symptoms, liver biochemistry and histology, and adverse effects are virtually absent (14,15). Many therefore consider UDCA the first treatment option for PBC. However, progression of the disease has been observed during UDCA treatment and the potential to stop histological progression or improve survival is still unclear (16). Preliminary reports of up to 4 years of follow-up did not show any improvement in survival (17-19), though in one study significantly less liver transplants were performed (19).

The limited benefit of any single drug regimen in PBC, has stimulated investigators to propagate and explore the combination of bile acid treatment with other drugs (20-23). In one trial, adding

colchicine to UDCA did not seem to improve the results obtained with UDCA alone (22). Additive beneficial effects of UDCA and methotrexate, in reducing bilirubin and alkaline phosphatase serum levels were recently described, although methotrexate led to increased ALT levels (23).

We here report our experience with combining bile acid and corticosteroid treatment in patients who had no satisfactory response to either regimen alone.

MATERIALS AND METHODS

In december 1992 we reviewed the records of the 75 PBC patients under surveillance at our clinic. Seven patients with clinically non-advanced disease were found, who were simultaneously treated with UDCA and prednisone. In all the diagnosis of PBC was based on the presence of cholestatic liver function abnormalities (alkaline phosphatase more than 2x the upper limit of normal), an elevated serum IgM, antimitochondrial antibodies (determined by conventional immunoflueorescence techniques using rat kidney tissue) and histologic features of PBC. None of the patients had suffered from complications such as ascites, variceal bleeding or encephalopathy. All were in class A according to the Child-Pugh classification. All patients had a complete follow-up untill this evaluation (median 1.5 years, range 1-4). The main patient characteristics and treatment data are shown in table 1.

Our center is a tertiary referral center for liver diseases. All but one patient were referred to us without prior treatment, after the diagnosis of PBC was made, because of the known interest and expertise in our clinic with respect to this disease. One patient (patient 5) had been tought to suffer from Chronic Autoimmune Hepatitis, and was referred to our center because she was only partially responding to immunosuppressive therapy. In our clinic the diagnosis of PBC was established.

The moment at which combined therapy was instituted, i.e. the moment one drug was added to the other, was considered the inception point for this study. Two patients started UDCA with later

addition of prednisone. The latter was started 6 months after initiating UDCA. This 6-month period may allow the conclusion that clinical changes occurring after initiating combined therapy (CT) are attributable to the prednisone addition because UDCA exerts its maximum effect within the first 3 months (14, own experience). The other five patients had been on long term prednisone treatment before UDCA was started (median 7 years; range 5-14). In each of the 5 patients who were on prednisone previous attempts to withdraw this drug had resulted in clear symptomatic and biochemical deterioration. Three of these patients were also on azathioprine 50 mg daily for at least two years. The main reason to institute additional treatment, UDCA or prednisone, was the persistance of symptoms.

The main complaints in these patients, i.e. pruritus, fatigue and arthralgia were scored as either being present or absent before and after 6 and 12 months of CT. Three monthly laboratory evaluations included

Table 1. **Data on demography, histology and drug administration at the start of combined therapy. Patients 1 and 2 were first treated with UDCA alone. Patients 3-7 were on corticosteroid therapy before UDCA was added.**

Patient nr. (years)	Sex	Age	Histologic stage	Duration imm.sup.	Pred*/Aza dose (mg/day)	UDCA dose (mg/kg/day)	Follow-up on CT (months)
1.	F	41	II	-	15/-	8.6	48
2.	F	68	II	-	10/-	8.6	48
3.	F	49	II	7	10/50	8.8	24
4.	M	70	III	6	10/50	7.5	12
5.	F	67	IV	14	5/50	9.2	18
6.	F	60	II	5	7.5/-	7.5	18
7.	F	67	IV	11	12.5/-	9.2	15
Median:	-	67	-	7	10	8.6	18

* Maintenance doses.

measurement of serum bilirubin, alkaline phosphatase (APh), aspartate aminotransferase (AST) and IgM. The mean of 2 values obtained within 3 months before CT were considered as baseline level.

Considering the number of patients no statistical analyses were performed. This study was performed in adherence with the guidelines of Sackett et al. for studies on clinical course and prognosis (24).

RESULTS

Table 2 provides information on the presence of symptoms in relation to treatment. During the combined prednisone-UDCA therapy five patients became asymptomatic. In two of the three patients with itching and four of the six patients with fatigue these symptoms disappeared. In both patients with complaints of arthralgia the pain disappeared. In two patients symptoms were not relieved.

In table 3 the absolute values as well as the decreases of baseline (in percentages), of APh, AST and IgM on 6 and 12 months of CT are presented. APh and AST decreased in all patients (median after 12 months 41 and 59%, respectively). IgM decreased, although to a minor degree (median after 12 months 16%), in all but one patient. The largest effects on IgM were observed in the two patients in whom prednisone was added to UDCA. The main decreases occurred in the first 3-6 months of treatment.

Bilirubin levels were normal in all but one patient (median 9, range 9-21). Patient 4 had an increased level of 21 μmol/L at entry (normal ≤14 μmol/L). In all patients bilirubin levels remained stable throughout the course of follow-up. Therefore data on bilirubin were not presented.

During follow-up (median 1.5 years, range 1-4 years) the beneficial effects on symptoms and on the laboratory values were maintained. No major adverse effects of the treatment were reported by any of the patients. One patient (patient 3) experienced a short period of increased fatigue and itching after starting UDCA, which subsided within 6 months.

Table 2. **Symptoms before and after institution of combined UDCA- and corticosteroid treatment (+ = present and - = absent).**

Patient nr.	Pruritus			Fatigue			Arthralgia			Total symptoms		
T (mths):	0	6	12	0	6	12	0	6	12	0	6	12
1.	-	-	-	+	-	-	-	-	-	+	-	-
2.	-	-	-	-	-	-	+	-	-	+	-	-
3.	-	-	-	+	-	-	-	-	-	+	-	-
4.	-	-	-	+	+	+	-	-	-	+	+	+
5.[1]	+	+	+	+	+	+	-	-	-	+	+	+
6.	+	-	-	+	-	-	-	-	-	+	-	-
7.	+	-	-	+	-	-	+	+	-	+	+	-
Symptomatic patients (n=):	3	1	1	5	2	2	2	1	0	7	3	2

[1] Interrupted UDCA-therapy after 9 months, situation at 9 months provided at T=12.

Table 3. **Absolute values of APh, AST and IgM and percentual decrease from baseline ( ) after 6 and 12 months of combined therapy.**

Patient nr.	APh (nl ≤75 U/L)			AST (nl ≤30 U/L)			IgM (nl ≤2.8 U/L)		
T (mths):	0	6	12	0	6	12	0	6	12
1.	143	64 (55)	51 (64)	35	14 (60)	12 (66)	7.5	4.2 (44)	3.4 (55)
2.	359	248 (31)	223 (38)	90	41 (54)	37 (59)	3.2	2.4 (25)	1.6 (50)
3.	198	127 (36)	117 (41)	57	31 (46)	19 (67)	4.5	3.8 (16)	3.8 (16)
4.	267	185 (31)	187 (30)	41	27 (34)	31 (24)	10.1	10.2 (+1)	10.7 (+6)
5.[1]	198	64 (68)	71 (64)	39	15 (62)	15 (62)	3.6	3.5 (3)	3.4 (6)
6.	355	194 (45)	188 (47)	61	40 (34)	36 (41)	4.4	3.8 (14)	3.7 (16)
7.	199	159 (20)	127 (36)	39	27 (31)	26 (33)	3.4	-	2.5 (26)
Median:	199	159 (36)	127 (41)	41	31 (46)	26 (59)	4.4	3.8 (15)	3.4 (16)
Normal values (n=):	0	2	2	0	2	4	0	1	2

[1] Interrupted UDCA-therapy after 9 months, values at 9 months provided at T=12.

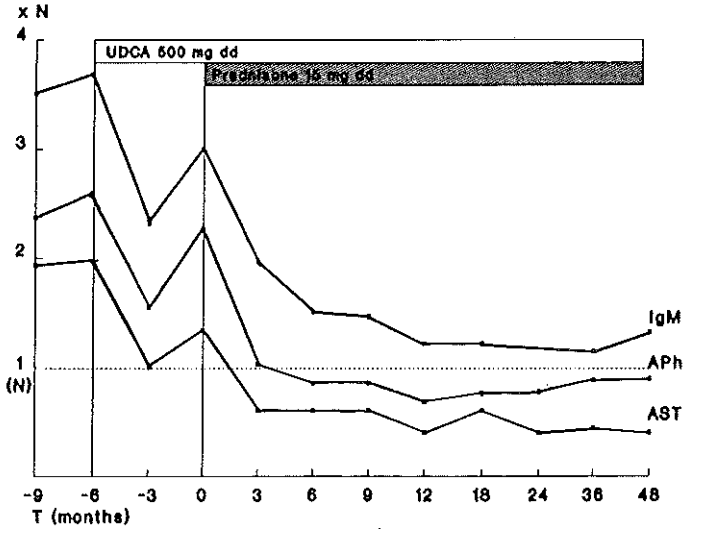


Figure 1.
Patient nr. 1:
APh, AST and IgM, expressed as multiples of the upper limit of normal (N) during 4 years after prednisone treatment was added to UDCA. A normalization of AST and APh was achieved within 3 months.

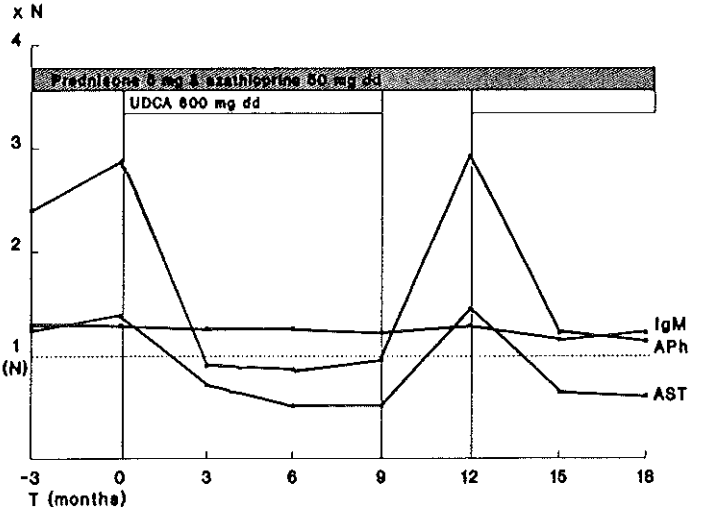


Figure 2.
Patient nr. 5:
APh, AST and IgM, expressed as multiples of the upper limit of normal (N) during 1.5 year after adding UDCA treatment to long-term immunosuppressive therapy. On combined treatment APh and AST normalized within 3 months, immediately rose after stopping UDCA and ameliorated again after recommencing therapy.

The figures 1 and 2 illustrate the course of APh, AST and IgM before and during combined therapy. In patient 1 (figure 1) all laboratory parameters, except IgM, normalized after addition of prednisone to UDCA, and this was maintained up till the writing of this report, 4 years later. In patient 5 (figure 2) a complete biochemical normalization was achieved after addition of UDCA to prednisone. The patient interrupted UDCA intake because of lower abdominal pain, which she attributed to the UDCA treatment. A clear relapse occurred. Reinstitution of UDCA again resulted in biochemical improvement.

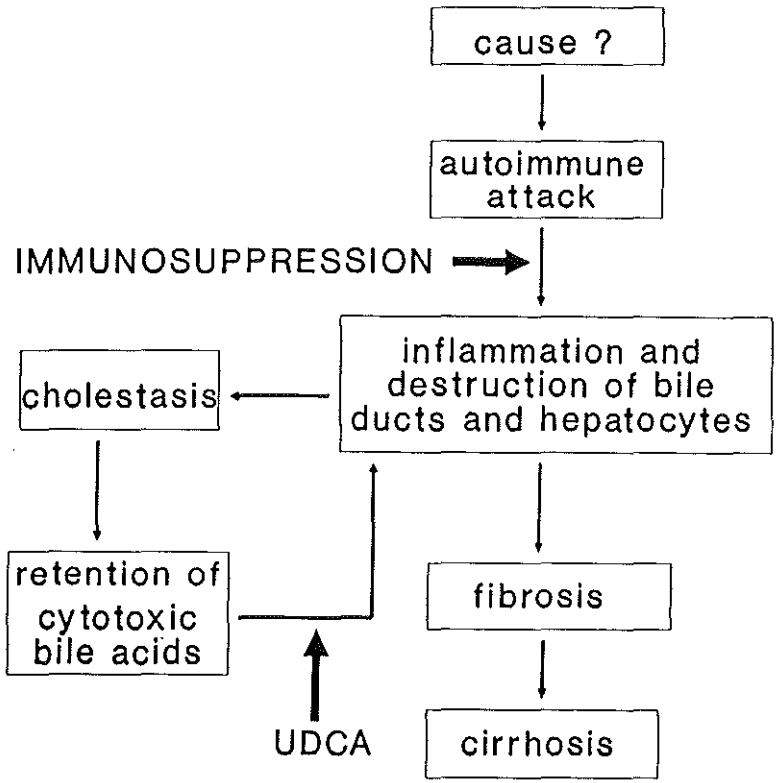


Figure 3.
Hypothesis on the pathophysiology of Primary Biliary Cirrhosis and sites of action of UDCA and immunosuppressive therapy. From this model the synergistic effect of combined immunosuppressive- and bile acid treatment is apparent.

DISCUSSION

The results of this study suggest that the efficacy of either treatment with UDCA or prednisone in PBC can be enhanced by combining these two agents. All patients showed marked amelioration of biochemical abnormalities after initiation of combined therapy. The effect on bilirubin, a major prognostic variable, could not be evaluated because all patients had (near-)normal bilirubin levels at baseline which remained stable during follow-up. Furthermore, in this non-controlled study a remarkable effect on symptoms was suggested.

As far as we know, this is the first report describing the results of combined bile acid- and corticosteroid treatment. Both UDCA and prednisone have been evaluated in controlled trials and have documented beneficial effects in PBC (13-15). From these studies, and our own experience, we can conclude that a complete remission of the disease activity can rarely be achieved using single treatment with UDCA or prednisone (25).

Our observations support the hypothesis that in PBC two distinct pathogenetic mechanisms are involved (figure 3). A hitherto unknown factor triggers a chronic auto-immune attack mainly directed against small bile ducts. Bile duct damage and eventually ductopenia result in cholestasis and, due to the toxic effect of retained bile acids, to secondary liver damage.

Prednisone may modulate the primary auto-immune mediated bile duct inflammation while UDCA probably mainly interferes at the level of the secondary bile-acid mediated liver damage by displacing endogenous toxic bile acids (26) as well as by protecting hepatocytes from bile acid toxicity (27,28). UDCA may also exert immunomodulating effects (29,30).

Immunosuppression alone might diminish or prevent further damage to bile ducts. However, due to existing irreversible structural liver damage, the process of secondary bile acid mediated liver damage could continue. When, on the other hand, administration of UDCA reduces mainly the damage caused by endogenous bile acids, the primary process leading to bile duct inflammation may remain operative. In general, patients in the pre-cirrhotic stages of PBC are likely to

benefit most by immunosuppressive therapy. However, in patients with compensated cirrhosis and normal bilirubin levels, suggesting the existence of residual bile ducts, immunosuppressive therapy may still be useful. This model might provide an explanation for the relatively low response to immunosuppressive therapy in PBC, for the partial effectivity of UDCA and for the synergistic effects of UDCA and prednisone.

Our observations have a preliminary character. Further long term controlled studies in larger groups of patients are indicated to confirm these results. It is also obvious that in further studies the effects on liver histology should be documented as well as the long term benefit-risk ratio of prednisone treatment. Although recent reports indicate that the danger of prednisone induced osteoporosis may have been overestimated in the past (13,31), the influence of long term prednisone treatment on bone mineral density and the prevention of osteoporosis should remain subjects for further studies.

REFERENCES

1. Beukers R. Immunosuppressive therapy for primary biliary cirrhosis. Thesis 1992, Rotterdam:8-15.
2. Neuberger J, Lombard M, Galbraith R. Primary biliary cirrhosis. Gut 1991;32 Suppl:S73-8.
3. De Caestecker JS, Jazrawi RP, Petronei ML, Northfield TC. Ursodeoxycholic acid in chronic liver disease. Gut 1991;32:1061-5.
4. Beukers R, Schalm SW. Effect of cyclosporine and cyclosporine plus prednisolone in primary biliary cirrhosis. Transplant Proc 1988;20 Suppl 4:340-3.
5. Beukers R, Schalm SW. Immunosuppressive therapy for primary biliary cirrhosis. J Hepatol 1992;14:1-6.
6. Minuk GY, Bohme CE, Burgess E et al. Pilotstudy of cyclosporine A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988;95:1356-63.
7. Wiesner RH, Ludwig J, Lindor KD et al. A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. N Engl J Med 1990;32:119-24.
8. Lombard M, Portmann B, Neuberger J et al. Cyclosporin A treatment in primary biliary cirrhosis: results of a long-term placebo controlled trial. Gastroenterology 1993;104:519-26.
9. Hoofnagle JH, Davis GL, Schafer DF et al. Randomized trial of chlorambucil for primary biliary cirrhosis. Gastroenterology 1986;91:137-34.
10. Christensen E, Neuberger J, Crowe J et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Gastroenterology 1985;89:1084-91.
11. Mackay IR. Treatment of chronic active hepatitis and other liver diseases with corticosteroid agents. Med J Aust 1987;146:370-4.
12. Kaplan MM, Knox TA. Treatment of primary biliary cirrhosis with low-dose weekly methotrexate. Gastroenterology 1991;101:1332-8.
13. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OFW. A controlled trial of prednisolone treatment in primary biliary cirrhosis: three-year results. J Hepatol 1992;15:336-44.
14. Poupon RE, Balkau B, Eschwège E, Poupon R and the UDCA-PBC Study group. A multicenter, controlled trial of ursodiol for the treatment of Primary Biliary Cirrhosis. N Engl J Med 1991;324:1548-54.
15. Leuschner U, Fischer H, Kurtz W et al. Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. Gastroenterology 1989;97:1268-74.
16. Perdigoto R, Wiesner RH. Progression of primary biliary cirrhosis with ursodeoxycholic acid therapy. Gastroenterology 1992;102:1389-91.
17. Lindor K, Baldus WB, Jorgensen RA, Ludwig F, Murtaugh PA. Ursodeoxycholic acid (UDCA) is beneficial therapy for patients with primary biliary cirrhosis [abstract]. Hepatology 1992;16:91A.
18. Heathcote EJL, Cauch K, Walker V et al. The Canadian multi-centre double blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis [abstract]. Hepatology 1992;16:91A.
19. Poupon RE, Chrétien Y, Balkau B, Niard AM and the UDCA-PBC Study Group. Ursodeoxycholic acid therapy for primary biliary cirrhosis: a four year controlled study [abstract]. Hepatology 1992;16;91A.
20. Jansen PLM. Antifibrotic therapy of liver cirrhosis, with special reference to primary biliary cirrhosis: promises and limitations. Neth J Med 1992;40:209-214.
21. Kaplan MM. New strategies needed for treatment of primary biliary cirrhosis? Gastroenterology 1993;104:651-3.
22. Raedsch R, Stiehl A, Walker S, Scherrmann JM, Kommerell B. Kombinierte Ursodeoxycholsäure plus Colchizinbehandlung bei primär biliärer Zirrhose: Ergebnisse einer Placebo-kontrollierten Doppelblindstudie. Z Gastroenterol 1992;30 Suppl 1:55-57.

23. Kaplan MM.The therapeutic effects of ursodiol and methotrexate are additive and well tolerated in primary biliary cirrhosis [abstract]. Hepatology 1992;16:92A.
24. Sackett DL, Haynes RB, Guyatt GH, Tugwell P, editors. Clinical epidemiology. Boston: Little, Brown and Company, 1991.
25. Wolfhagen FHJ,Van Buuren HR,Van Berge Henegouwen GP and the Dutch Multicenter PBC Study Group. Can ursodeoxycholic acid induce complete remissions in primary biliary cirrhosis? [abstract]. J Hepatol 1993;18:S43.
26. Hofmann AF. Bile acid hepatotoxicity and the rationale of UDCA therapy in chronic cholestatic liver disease: some hypotheses. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer, 1990:13-33.
27. Heumann DM, Mills AS. McCall J, Hylemon PB, Pandak WM,Vlahcevic ZR. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts. Gastroenterology 1991;100:203-11.
28. Galle PR,Theilmann L, Raedsch R, Otto G, Stiehl A. Ursodeoxycholate reduces hepatoxicity of bile salts in primary human hepatocytes. Hepatology 1990;12:486-91.
29. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-5.
30. Yoshikawa M,Tsuji T, Matsumura K et al. Immunomodulatory effects of ursodeoxycholic acid on immune respons. Hepatology 1992;16:358-64.
31. Van Berkum FNR, Beukers R, Birkenhäger JC, Kooij PPM, Schalm SW, Pols HAP. Bone mass in women with primary biliary cirrhosis: the relation with histological stage and use of glucocorticoids. Gastroenterology 1990;99:1134-9.

CHAPTER VI

TRIPLE THERAPY WITH URSODEOXYCHOLIC ACID, PREDNISONE AND AZATHIOPRINE IN PRIMARY BILIARY CIRRHOSIS: BENEFITS AND RISKS
-an interim analysis-

Frank H.J. Wolfhagen[1], Henk R. van Buuren[1], Gerard P. van Berge-Henegouwen[2], Fiebo J.W. ten Kate[3], Wim C.J. Hop[4], Marion J. Kerbert[5], Janny W. den Ouden[6], Adelbert M. Smit[7], Paul H.G.M. Stadhouders[8], Jan C. Thijs[9], Richard A. de Vries[10], Solko W. Schalm[1] for the Dutch Multicenter PBC Study Group.

Affiliations:

1. Dept. of Internal Medicine II, University Hospital Dijkzigt, Rotterdam
2. Dept. of Gastroenterology, University Hospital Utrecht
3. Dept. of Pathology, Academic Medical Center, Amsterdam
4. Dept. of Biostatistics, Erasmus University Rotterdam
5. Dept. of Internal Medicine, Medical Center Twente, Enschede
6. Dept. of Internal Medicine, St. Franciscus Hospital, Rotterdam
7. Dept. of Internal Medicine, Carolus Hospital, Den Bosch
8. Dept. of Internal Medicine, Canisius Wilhelmina Hospital, Nijmegen
9. Dept. of Internal Medicine, Bethesda Hospital, Hoogeveen
10. Dept. of Internal Medicine, Rijnstate Hospital, Arnhem

SUMMARY

Patients with compensated PBC (Child class B and C excluded), who had not achieved a disease remission after one year of 10 mg/kg ursodeoxycholic acid (UDCA) daily, were randomized to additional treatment with prednisone 10 mg plus azathioprine 50 mg daily (n=18) or placebo (n=18) for one year. The aim of this double-blind trial was to assess the short term benefits and risks of this combination therapy. Pruritus ($p=0.01$), alkaline phosphatase, AST, IgM, procollagen-III-propeptide improved significantly (all $p<0.001$) in the combination treatment group (UDCA/Prednisone/Azathioprine) as compared to the placebo group (UDCA/placebo/placebo). A cumulative histological score was used which decreased significantly within the combination treatment group ($p=0.05$). Lumbar Bone Mineral Content did not change in prednisone treated patients that were using cyclical etidronate. Three patients in the combination treatment group and two in the placebo group were withdrawn from treatment. Thus, in PBC, combination treatment of prednisone with azathioprine, improved pruritus, biochemical, fibrogenetic and histological parameters, on top of improvements already achieved with UDCA. Cyclical etidronate seems to prevent steroid induced bone loss. These results strongly encourage the evaluation of this triple treatment regimen in larger, long-term controlled trials.

INTRODUCTION

Ursodeoxycholic acid (UDCA) is currently considered the treatment of choice for Primary Biliary Cirrhosis (PBC) since it is safe, improves liver tests and slows disease progression (1-3). However, the potential of UDCA to alter the natural course of the disease seems modest and complete remissions are achieved in less than 5% of patients (4). Therefore the combination of UDCA with other drugs has been suggested as a logical next step (5,6). Theoretically, the addition of immunosuppressive drugs to UDCA is attractive because UDCA, interfering mainly with endogenous bile acid mediated liver damage, is unlikely to stop the primary immune damage (5,6).

Both prednisone (7,8) and azathioprine (9,10) have been shown to be of some benefit in PBC. Prednisone has been deemed contraindicated due to its negative effect on bone status, which has been mainly documented in patients with advanced PBC and jaundice (11). Long term experience in our center, however, has documented that the negative effect of low dose prednisone on bone density is limited in patients with compensated PBC (12). Moreover, drugs like bisphosphonates may prevent steroid associated bone loss (13-15).

Azathioprine has not been accepted as therapy for PBC since the observed small benefit in life expectancy did not neutralize the fear for an increased risk of neoplasms (10). Long term follow up of patients with autoimmune hepatitis treated with low dose prednisone/azathioprine indicates that this fear is unwarranted (16).

Combining low doses of prednisone and azathioprine may lead to an increased immunosuppressive potential without enhancing the risk of side effects (6). To investigate the efficacy of combined UDCA, prednisone and azathioprine treatment, a 1-year, multicenter, double-blind, placebo-controlled trial was initiated in PBC patients who had not completely responded to UDCA alone (6). This analysis of the first 36 patients was performed to assess the potential benefits and risks of the triple treatment regimen as compared to monotherapy with UDCA.

PATIENTS AND METHODS

The main endpoint in this study is the achievement of a complete disease remission. To assess a difference in success rate of 25% between both groups, with α=0.05 and a power of 80%, 60 patients are needed. However, in view of the prevailing reluctance regarding the use of corticosteroids in PBC, a preliminary report was planned, to document the potential benefits and risks of this treatment.

As 32 evaluable patients are needed to detect a difference between means of one standard deviation and assuming a drop-out rate of 10%, it was determined that the first 36 patients entering the study would be subject of this interim analysis.

All patients had an established diagnosis of PBC (17). Exclusion criteria were age >75 years, extrahepatic bile duct disease, (risk of) pregnancy, the use of other potential disease modifying drugs within 6 months prior to entry, known intolerance for prednisone or azathioprine, osteoporotic spinal fractures, systemic infections, a psychiatric history or cytopenia (defined as WBC<2.5 x 10.9/L, platelets < 70 x 10.9/L or Hb < 6 mmol/L). Patients with Child-Pugh classification B or C (18) were excluded since they were considered unlikely to benefit from medical treatment.

All patients had been treated with UDCA (ca. 10 mg/kg/day) and after 1 year none of them had achieved a remission of the disease, according to the criteria of Beukers and Schalm (6). After stratification (presence versus absence of cirrhosis and center), patients were allocated at random to additional prednisone/azathioprine or placebo. Both clinicians and patients were unaware of the treatment allocation.

Prednisone and identical looking placebo tablets were taken in a dose of 30 mg daily during the first month, 20 mg in the second month and 10 mg for the remaining period. One tablet containing 50 mg azathioprine or placebo (Wellcome Pharmaceuticals, Utrecht, The Netherlands) was taken daily.

All patients received calciumcarbonate 500 mg daily. Furthermore all, but 12 randomly assigned patients, were treated with 3 monthly cycles of etidronate 400 mg daily for 2 weeks (Procter & Gamble Pharmaceuticals, Rotterdam, The Netherlands). Patients with subnor-

mal 25-OH Vitamin D levels received 600.000 IU colecalciferol intramuscularly once.

Patients visited the outpatient clinics monthly during the first 3 months and at 3-monthly intervals thereafter. A physical examination including weight and blood pressure measurements was performed at each visit and patients were asked to grade itching and fatigue on a scale from 0-4 for the morning, afternoon, evening and night during the 5 subsequent days.

Hemoglobin, white blood count, platelets, serum bilirubin, alkaline phosphatase (APh), aspartate aminotransferase (AST), albumin and immunoglobulin M (IgM) were assessed at each visit. In 24 patients fasting serum aliquots were taken before start of the immunosuppressive treatment and at 3, 6 and 12 months for radioimmunoassay of procollagen-III aminoterminal propeptide concentrations (P-III-P) using a commercially available kit (Orion Diagnostica, Espoo, Finland). All measurements were performed in the local laboratory except P-III-P (central laboratory University Hospital Rotterdam).

Prior to the study and after 1 year Dual Energy X-ray Absorptiometry (DXA) measurements of Bone Mineral Content (BMC) of the lumbar spine (L2-L4) and femur neck as well as liver biopsies were performed. The liver biopsies were reviewed and staged according to Ludwig (19) by one pathologist (FJWtK), who was unaware of the treatment allocation and clinical state of the patients. Furthermore an overall histological score according to the method of Poupon et al. was used (20).

Data are presented as means with standard errors of the mean, unless indicated otherwise. Laboratory data are expressed as multiples of the upper limit of normal (ULN) or percentage change from baseline. Changes in symptoms and laboratory parameters were analysed using Repeated Measurements ANOVA (RmAnova) (21), after logarithmical transformation for bilirubin. BMC and histological scores were compared by non-parametric tests. X^2-tests were used for qualitative data. A two-sided p value ≤ 0.05 was considered significant. All analyses were performed on an intention-to-treat basis. The study was approved by all local Medical Ethical Committees. All patients gave written informed consent.

RESULTS

Entry characteristics in the UDCA/prednisone/azathioprine (U-PA group, n=18) and the UDCA/placebo group (U-PL, n=18) were comparable (table 1).

Table 1. **Characteristics at entry. Data are given as numbers of patients or means ± Standard Deviations.**

		UDCA + Prednisone/azathioprine (n=18)	UDCA + Placebo (n=18)
Female		15	17
Age	(yrs)	53 ± 9	52 ± 11
Bilirubin	(x ULN)	1.1 ± 0.6	1.3 ± 0.7
Albumin	(x ULN)	1.2 ± 0.2	1.1 ± 0.1
APh	(x ULN)	3.0 ± 1.8	3.2 ± 1.6
AST	(x ULN)	1.3 ± 0.7	1.3 ± 0.7
IgM	(x ULN)	1.8 ± 1.0	2.0 ± 2.1
P-III-P (N<4.2)*	µgr/L	6.1 ± 3.5	4.9 ± 3.0
Fatigue		12	16
Pruritus		7	10
Histological stage	I	2	1
	II	5	5
	III	6	7
	IV	5	5
Bone mineral density			
- L2-L4	gr/cm²	1.017 ± 0.213	1.038 ± 0.177
- femur neck	gr/cm²	0.863 ± 0.105	0.813 ± 0.156
On etidronate		12	12

*n = 12 in each group.

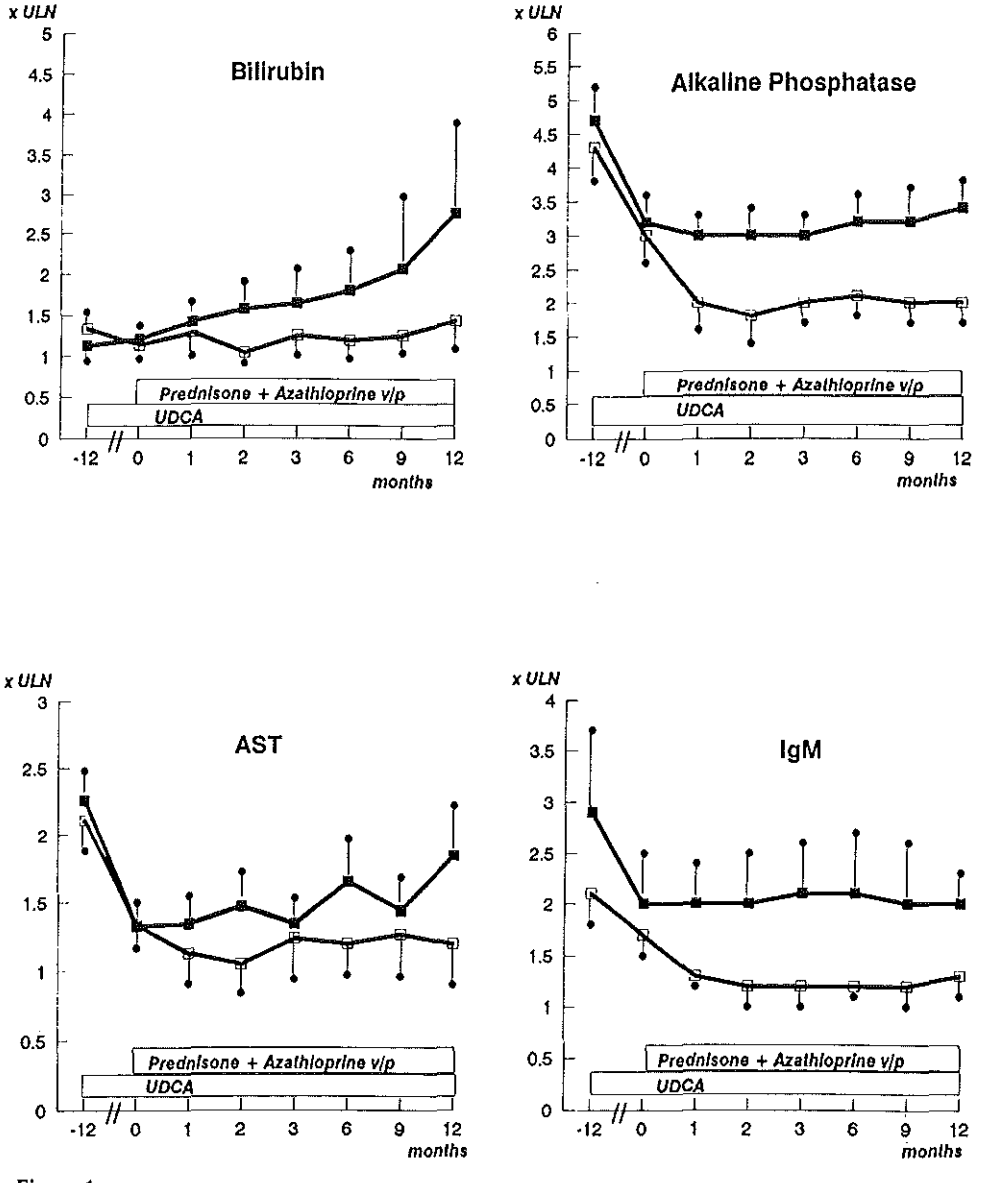


Figure 1.
Mean serum bilirubin, APh, AST and IgM during 1 year of UDCA+prednisone/azathioprine (open squares) versus UDCA+placebo treatment (filled squares). Data are expressed as multiples of the upper limit of normal (ULN). Error bars indicate standard error of the mean. Changes from levels at randomization were significantly different ($p<0.001$) throughout the study for all parameters but bilirubin ($p=0.3$). Changes during the previous treatment year with UDCA alone are also shown. V/p in the medication bar indicates verum or placebo.

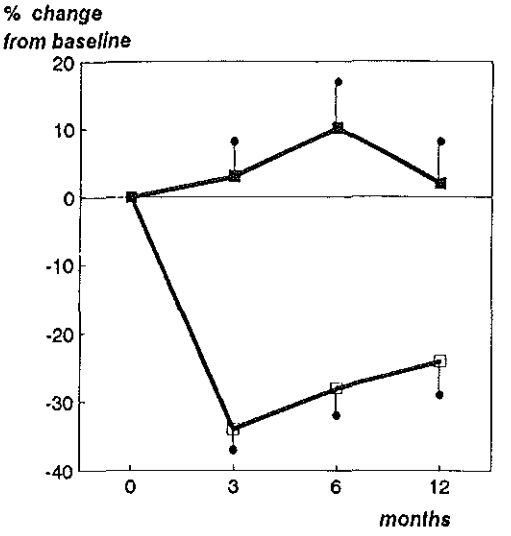


Figure 2.
Mean percentage change from baseline of serum procollagen-III aminoterminal propeptide concentrations during UDCA+prednisone/azathioprine (open squares) or UDCA+placebo treatment (filled squares). Changes are significantly different throughout the study period ($p<0.001$). Error bars indicate standard error of the mean.

Benefits

A small though significant amelioration of itching in the U-PA group (-2.2 ± 3.1 points) was observed in comparison with the U-PL group (+6.8 ± 2.4; $p=0.01$). All patients with arthralgias in the U-PA group (n=4) noted amelioration during treatment and/or deterioration after stopping treatment while no change was reported in 6 patients with arthralgias receiving U-PL.

Biochemical markers in the U-PA group improved significantly as compared to U-PL: APh -32 ± 5% vs.+4 ± 6%, AST -20 ± 7% vs. +24 ± 12% and IgM -21 ± 5% vs. -1 ± 3% (all $p<0.001$). The differences between changes were constant during the whole study period (figure 1). Bilirubin remained stable in the U-PA group while it appeared to rise in the U-PL group, though the difference did not reach significance ($p=0.3$). The degree of the improvements did not differ between early (I-II) and late stage (III-IV) disease. These effects were achieved on top of decreases in APh, AST and IgM induced by the preceding UDCA treatment as compared to values at the start of UDCA (35, 42 and 25% respectively; all $p<0.001$).

Biochemical remission, defined as normal AST, IgM and bilirubin, and APh 1.5x ULN) was achieved in 3 U-PA patients and 1 U-PL patient. In the U-PA group the incidence of normalizations of APh (6 vs. 3), AST (4 vs. 1) and IgM (4 vs. 1) tended to be higher but only reached significance for APh ($p=0.04$). In none of the patients (7 in each group) elevated bilirubin levels normalized.

P-III-P levels improved in the U-PA (-24 ± 6%) but not in the U-PL group (+2 ± 6%). The difference in change between both groups was significant ($p<0.001$) (figure 2). Abnormal P-III-P levels normalized in 4 of 8 U-PA patients but in none of 5 U-PL patients. Changes in P-III-P did not correlate with changes in bilirubin, AST or APh. Paired biopsies were available in 27 of the 36 patients (14 U-PA, 13 U-PL). Six patients refused a follow up biopsy and 3 were referred for transplantation. Histological stage did not change significantly in either group. The cumulative histological score decreased significantly in the U-PA group by 1.8 ± 0.7 points ($p=0.05$), and remained stable in the U-PL group (decrease 0.3 ± 1.1 point, $p=0.8$) (figure 3). These chan-

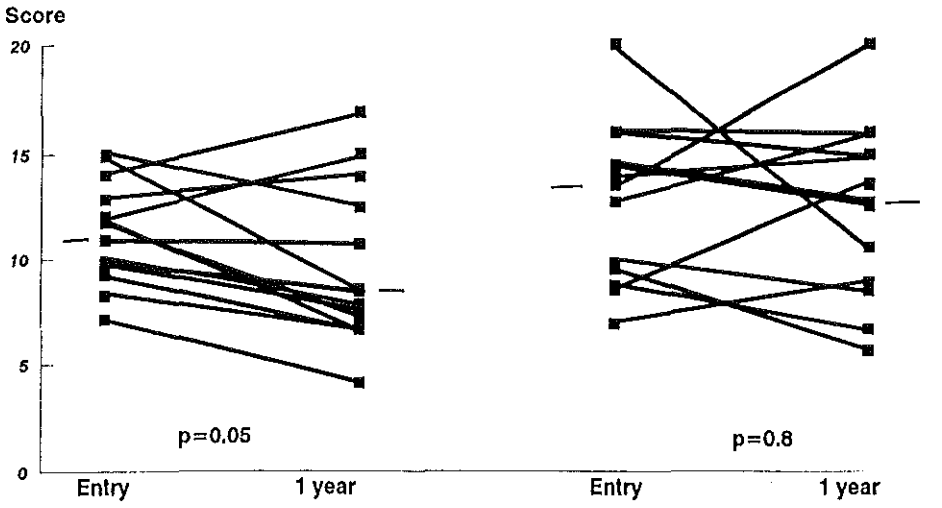


Figure 3.
Individual histological scores before and after 1 year of UDCA+prednisone/azathioprine (left) or UDCA+placebo treatment (right). Bars indicate medians. The sum of the following items constitutes the histological score: fibrosis (graded 0-5), (peri)portal inflammation (0-3), piece meal necrosis (0-3), bile duct proliferation (0-3), lobular inflammation (0-3), focal necrosis (0-2), cholestasis (0-3) and the ratio of the number of portal tracts without pre-existent bile ducts and the total number of portal tracts.

ges, however, did not significantly differ between both study groups. Of the individual constituents of the score the degree of lobular (p=0.03) and portal infiltrates (p=0.01) improved significantly in the U-PA group.

There was no clear difference between the groups in the number of patients showing hepatic deterioration using the criteria of Mitchison (7) (table 2).

Table 2. **Incidence of hepatic deterioration, using the Mitchison criteria (7).**

	UDCA + Prednisone/azathioprine (n=18)	UDCA + Placebo (n=18)
Hepatic death	0	0
Accepted for liver transplantation	1	2
Doubling of bilirubin	1	3
Albumin decrease of >6 g/L	0	0
De novo signs of portal hypertension (in casu ascites)	2	1
Development of cirrhosis	2	3
No. of patients fulfilling one or more of these criteria:	4	6

Risks

Thirty-one patients were included in the evaluation of bone mineral content because in 5 patients no paired DXA measurements were available. In the U-PA group mean spinal BMC decreased significantly in the patients without cyclical etidronate (-3.5 ± 0.6%, p=0.03, n=6), while no significant change was seen in the patients receiving this profylaxis (-1.1 ± 0.9, n=11) and in U-PL treated patients with (+0.3 ± 1.5, n=8) or without etidronate (-1.2 ± 2.4, n=6) (figure 4).
No significant changes in femoral BMC were noted. Data on other adverse effects are summarized in table 3. In the U-PA group more patients showed weight gain. Evident cosmetic changes were encoun-

tered in 3 patients (marked weight gain, buffalo hump, ecchymoses, hair loss) and was coupled with increases in blood pressures in 2 of them. This led to lowering of the prednisone dose in 2 patients. Other intercurrent medical problems were not clearly predominant in any group.

Within one month after entry 2 patients in the U-PA group were withdrawn from treatment because of general malaise and 1 after an episode of spontaneous bacterial peritonitis; the latter had progressed from Child-Pugh class A cirrhosis to class B in the month between establishing eligibility and the actual start of medication. Her disease remained progressive after treatment withdrawal and she was eventually referred for liver transplantation. In the U-PL group 2 patients were withdrawn because they showed disease progression and became eligible for liver transplantation (6 and 9 months).

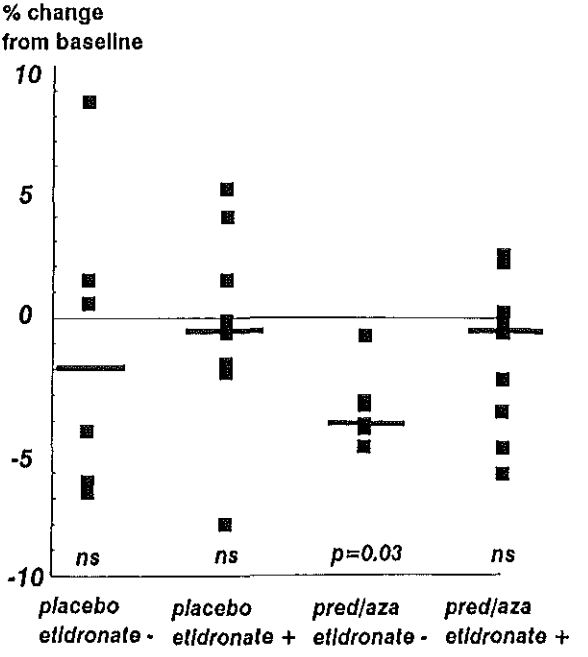


Figure 4.
Individual data on percentage change in bone mineral content of the lumbar vertebrae L2-L4 after 1 year of treatment for patients treated with UDCA+prednisone/azathioprine or UDCA+placebo, treated with or without etidronate. Bars indicate medians.

Table 3. **Intercurrent and/or adverse events**

	UDCA + Prednisone/azathioprine (n=18)	UDCA + Placebo (n=18)
Weight gain ≥2.5 kg	11	3
≥5 kg	8	1
Hypertension*	3	0
Ecchymoses	2	1
Hirsutism	2	0
Hairloss	2	0
Non-insulin dependent Diabetes Mellitus	0	1
Cytopenia**	2	1
Infections***	2	4
- Pulmonary	0	1
- Urinary tract	1	1
- Parotis	0	1
- Spontaneous bacterial peritonitis	1	0
Increased or *de novo* gastrointestinal complaints	7	5
Peritoneal guarding e.c.i.(>laparotomy)	0	1
Partial portomesenterial thrombosis	1	0
Transient ischemic attack	0	1
Instable angina pectoris	0	1
Traumatic vertebral fracture	0	1
General malaise	4	4
Events leading to dose reduction of		
prednisone	2	0
azathioprine	1	1
Events leading to treatment withdrawal	3	2

* Diastolic pressure increased to ≥95 mm Hg or by ≥20 mm Hg on 2 consecutive visits.

** WBC <2.5x10.9/L and/or Platelets <70x10.9/L.

*** Antibiotic treatment instituted.

DISCUSSION

This study shows an additional effect of combination treatment of low dose prednisone and azathioprine on itching, serum markers of liver cell damage, cholestasis, immune activity, fibrogenesis and histology in PBC patients who had been receiving UDCA during at least one year. Our data indicate that side effects of this triple therapy are acceptable and steroid induced bone loss can be prevented by cyclical etidronate.

The effects of the combined immunosuppressive treatment were more pronounced than those reported for azathioprine alone (9,22), and were comparable to those found by Mitchison et al. in a controlled trial with prednisolone (23). The observed effects were achieved in addition to marked improvements already obtained with UDCA, confirming our earlier uncontrolled data (8).

Bilirubin, a major prognostic parameter in PBC, remained stable during triple treatment. P-III-P, a serum marker of fibrogenesis (24-26) which has also been reported by several groups to be of prognostic significance in PBC (27-31) normalized in 50% of patients receiving triple therapy. In patients receiving UDCA, P-III-P may be a more appropiate prognostic marker than bilirubin since UDCA may directly increase bilirubin excretion (29), but does not seem to affect P-III-P levels (32).

The observed histological improvement of liver inflammation is in agreement with previous findings with prednisolone alone (23). Obviously larger trials of longer duration are required to establish whether the triple regimen can prevent histological progression.

The adverse effect of corticosteroids on bone mass has limited their use in PBC, although low dose prednisone has been shown to be relatively safe in patients with non-advanced disease (7,12). The maximal bone loss during corticosteroid treatment occurs during the first year of therapy (23,33,34). Our data indicate that etidronate may prevent this initial bone loss which is in agreement with studies in other diseases (13-15). Whether this approach will prevent bone loss during long term corticosteroid treatment remains to be established.

In the triple treatment group weight gain occurred frequently, which may have been related to the relatively high initial prednisone dose. A minority of these patients experienced significant cosmetic changes and increased blood pressures. The immunosuppressive treatment was not clearly associated with infections or other intercurrent medical problems. As all adverse effects leading to treatment withdrawal occurred during the first weeks, diminishing the high induction doses of prednisone in future studies should be considered. Other measures such as appropiate dietary advice and timely dose adjustments could further decrease the incidence of adverse effects.

Previous studies, combining UDCA with colchicine, have failed to show additional benefit of colchicine (35,36). Additive beneficial effects of methotrexate to UDCA have been reported but the serious side effects of methotrexate, especially interstitial pneumonitis, limit further exploration of this combination (37-39).

We conclude that there is a synergistic beneficial effect of low dose prednisone-azathioprine treatment with UDCA in PBC. The short term benefit/risk ratio appears positive and justifies continued studies to establish the efficacy of the triple regimen on the long term course of the disease.

REFERENCES

1. Poupon RE, Poupon R, Balkau B, Niard AM and the UDCA-PBC Study Group. Ursodiol for the long term treatment of primary biliary cirrhosis. N Engl J Med 1994;330:1342-7.
2. Lindor KD, Dickson ER, Baldus WB, Jorgensen RA, Ludwig J, Murtaugh PA et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-90.
3. Heathcote EJL, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN et al. The Canadian multicenter double-blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.
4. Wolfhagen FHJ, Van Buuren HR, Schalm SW, Ten Kate FJW, Van Hattum J, Eskens FALM et al. and the Dutch Multicenter PBC Study Group. Can ursodeoxycholic acid induce disease remissions in primary biliary cirrhosis? 1995;22:381.
5. Kaplan MM. Primary biliary cirrhosis - a first step in prolonging survival. N Engl J Med 1994;330:1386-7.
6. Beukers R, Schalm SW. Immunosuppressive therapy for primary biliary cirrhosis. J Hepatol 1992;14:1-6.
7. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OWF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. J Hepatol 1992;15:336-44.
8. Wolfhagen FHJ, Van Buuren HR, Schalm SW. Combined treatment with ursodeoxycholic acid and prednisone in primary biliary cirrhosis. Neth J Med 1994;44:84-90.
9. Heathcote J, Ross A, Sherlock S. A prospective controlled trial of azathioprine in primary biliary cirrhosis. Gastroenterology 1976;70:656-60.
10. Christensen E, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Gastroenterology 1985;89:1084-91.
11. Long RG, Meinhard E, Skinner RK, Varghese Z, Wills MR, Sherlock S. Clinical, biological, and histological studies of osteomalacia, osteoporosis and parathyroid function in chronic liver disease. Gut 1978;19:85-90.
12. Berkum van FNR, Beukers R, Birkenhäger JC, Kooij PPM, Schalm SW, Pols HAP. Bone mass in women with primary biliary cirrhosis: the relation with histological stage and use of glucocorticoids. Gastroenterology 1990;99:1134-9.
13. Reid IR, Alexander CJ, King AR, Ibbertson HK. Prevention of steroid-induced osteoporosis with (3-amino-1-hydroxypropylidene)-, 1-bisphosphonate (APD). Lancet 1988;i:143-6.
14. Mulder H, Struys A. Intermittent cyclical etidronate in the prevention of corticosteroid-induced bone loss. Br J Rheumatol 1994;33:348-50.
15. Gallagher SJ, Fenner JA, Anderson K, Bryden FM, Banham SW, Logue FC et al. Intravenous pamidronate in the treatment of osteoporosis associated with corticosteroid dependent lung disease: an open pilot study. Thorax 1992;47:932-6.
16. Wang KH, Czaja AJ, Beaver SJ, Go VLW. Extrahepatic malignancies following long term immunosuppressive therapy of severe hepatitis B surface antigen-negative chronic active hepatitis. Hepatology 1989;10:39-43.
17. Taal BG, Schalm SW, Kate ten FWJ, Hermans J, Geertzen GM, Feltkamp BEW. Clinical diagnosis of primary biliary cirrhosis: A classification based on major and minor criteria. Hepato-gastroenterol 1983;30:178-82.
18. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646.
19. Ludwig J, Dickson ER, McDonald GSA. Chronic nonsuppurative destructive cholangitis (Syndrome of Primary Biliary Cirrhosis). Virchows Arch (Pathol Anat) 1978;379:103-12.

20. Poupon RE, Balkau B, Eschwège E, Poupon R and the UDCA-PBC Study Group. A multicenter controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Engl J Med 1991;324:1548-54.
21. Schluchter MD. Unbalanced Repeated Measures. In: BMDP Statistics Software Manual Volume 2. Eds. WJ Dixon, University of California Press, Berkely, Los Angeles, 1990:1207-44.
22. Crowe J, Christensen E, Smith M, Cochrane M, Ranek L, Watkinson G et al. Azathioprine in primary biliary cirrhosis. A preliminary report of an international trial. Gastroenterology 1980;78:1005-10.
23. Mitchison HC, Bassendine MF, Malcolm AJ, Watson AJ, Record CO, James OFW. A pilot double-blind, controlled 1-year trial of prednisolone treatment in primary biliary cirrhosis: hepatic improvement but greater bone loss. Hepatology 1989;10:420-9.
24. Schuppan D. Connective tissue polypeptides in serum as parameters to monitor antifibrotic treatment in hepatic fibrogenesis. J Hepatol 1991;13 (suppl.3):S17-25.
25. Plebani M, Burlina A. Biochemical markers of hepatic fibrosis. Clin Biochem 1991;24:219-39.
26. Frei A, Zimmermann A, Weigand K. The N-terminal propeptide of collagen type III in serum reflects activity and degree of fibrosis in patients with chronic liver disease. Hepatology 1984;4:830-4.
27. Eriksson S, Zetterval O. The N-terminal propeptide of collagen type III in serum as prognostic indicator in primary biliary cirrhosis. J Hepatol 1986;2:370-78.
28. Babbs C, Hunt LP, Haroubi NY, Smith A, Rowas BP, Warnes TW. Type III procollagen peptide: a marker of disease activity and prognosis in primary biliary cirrhosis. Lancet 1988;i:1021-4.
29. Poupon RE, Balkau B, Guéchot J, Heintzmann F. Predictive factors in ursodeoxycholic acid-treated patients with primary biliary cirrhosis: role of serum markers of connective tissue. Hepatology 1994;19:635-40.
30. Mutimer DJ, Bassendine M, P Kelly F, James OFW. Is measurement of type III procollagen aminopeptide useful in primary biliary cirrhosis? J Hepatol 1989;9:184-9.
31. Teare JP, Sherman D, Greenfield SM, Simpson J, Bray G, Catterall AP et al. Comparison of serum procollagen III peptide concentrations and PGA index for assessment of hepatic fibrosis. Lancet 1993;342:895-98.
32. Floreani A, Zappalà F, Mazzetto M, Naccarato R, Plebani M, Chiaramonte M. Different response to ursodeoxycholic acid in primary biliary cirrhosis according to severity of disease. Dig Dis Sci 1994;39:9-14.
33. Locascio V, Bonucci E, Imbimbo B, Ballantani P, Adami S, Milani S et al. Bone loss in response to long term glucococorticoid therapy. Bone Miner 1990;8:39-51.
34. Gennari C, Vitelli R. Glucocorticoid-induced osteoporosis. Clin Rheum Dis 1986;12:637-54.
35. Raedsch R, Stiehl A, Rudi J, Schlenker T, Gerteis C, Kommerell B et al. Combined urso plus colchicine in primary biliary cirrhosis: efficacy, pharmacology and urso-colchicine drug interactions. Proceedings of the XIIth International Falk bile acid meeting. Basel, 1992:48-9 (abstract).
36. Poupon RE, Niard AM, Huet PM, Miguet JP, Mathieu-Chandelier C, Doffoël M et al. A randomized trial comparing the combination ursodeoxycholic acid (UDCA) and colchicine to UDCA alone in primary biliary cirrhosis. Hepatology 1994;20:151 (Abstract).
37. Buscher H-P, Zietschmann Y, Gerok W. Positive responses to methotrexate and ursodeoxycholic acid in patients with primary biliary cirrhosis responding insufficiently to ursodeoxycholic acid alone. J Hepatol 1993;18:9-14.
38. Kaplan MM. The therapeutic effects of ursodiol and methotrexate are additive and well tolerated in primary biliary cirrhosis. Hepatology 1992;16:92 (Abstract).
39. Sharma A, Provenzale D, McKusick A, Kaplan MM. Interstitial pneumonitis after low-dose methotrexate therapy in primary biliary cirrhosis. Gastroenterology 1994;107:266-70.

CHAPTER VII

SOLUBLE INTERCELLULAR ADHESION MOLECULE 1 IN PRIMARY BILIARY CIRRHOSIS: EFFECT OF URSODEOXYCHOLIC ACID AND IMMUNOSUPPRESSIVE THERAPY

Frank H.J. Wolfhagen[1], A.Guan Lim[2], Ashwin Verma[2], Henk R. van Buuren[1], Riadh P. Jazrawi[2], Johanna H. Levy[2], Tim C. Northfield[2], Solko W. Schalm[1]

Affiliations:

1. Dept. of Internal Medicine II, University Hospital Dijkzigt, Rotterdam, The Netherlands
2. Dept. of Medicine, St. George's Hospital Medical School, London, United Kingdom

Submitted for publication in adapted form, with the same authors.

SUMMARY

It has been suggested that soluble intracellular adhesion molecule-1 (sICAM-1) may be a useful marker of disease activity in inflammatory and immune diseases. Here we determined sICAM-1 levels in 24 patients with Primary Biliary Cirrhosis (PBC) during 12 months of ursodeoxycholic acid (UDCA; 10 mg/kg/day) monotherapy followed by a 6-month period of, randomly assigned, additional treatment with a combination of azathioprine (50 mg/day) and prednisone (30 mg/day first month, 20 mg/day second month, 10 mg/day maintenance) or matching placebos. sICAM-1 levels were assessed by ELISA, at baseline, 12, 15 and 18 months. Baseline levels of sICAM-1 correlated with histological stage, bilirubin and ASAT. sICAM-1 concentrations fell by a median of 20% during UDCA monotherapy ($p<0.0004$). The addition of azathioprine and prednisone during 6 months resulted in further reduction of sICAM-1 levels by a median of 25%, while they remained stable in the placebo group ($p<0.01$). Reductions in sICAM-1 were accompanied by improvements in biochemical liver tests but not by changes in the lymphocyte activation marker, soluble interleukin-2 receptor. We conclude that sICAM-1 levels reflect hepatic inflammatory activity and fall during UDCA monotherapy. Additional treatment with a combination of low dose prednisone and azathioprine leads to further decreases in sICAM-1. Larger, long term studies should further define the usefulness of monitoring sICAM-1 levels in the treatment of PBC, particularly with regard to disease progression.

INTRODUCTION

Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family of adhesion molecules (1). It is expressed on the surface membranes of a variety of cells in many different inflammatory and immunological disorders (2). Structurally, it is a sialylated glycoprotein with a molecular weight of about 95 kDa, consisting of five extracellular immunoglobulin like domains, a single transmembrane region and a short cytoplasmic tail (3).

Functionally, ICAM-1 binds to its corresponding ligands, LFA-1 (CD11a/CD18) and MAC-1(CD11b/CD18) (4,5). In doing so, ICAM-1 acts as a co-stimulatory factor for T-cell activation (6) and also mediates the migration of leucocytes from the circulation, through the endothelium and basement membrane, to home in, and to adhere to target structures or other immune cells (7).

In health, there is minimal membrane surface expression of ICAM-1 in the liver (8,9). In PBC, this expression is increased on interlobular bile ducts, proliferating bile ductules, hepatocytes and infiltrating leucocytes (8,10).

A soluble form of ICAM-1 (sICAM-1), identical in extracellular domains but lacking the transmembrane components of membrane bound ICAM-1, has been identified (11). Significant elevations of sICAM-1 levels have been described in a variety of inflammatory and autoimmune diseases including PBC (12-15). In PBC, correlations of sICAM-1 levels with biochemical liver tests, histological stage and disease severity, as assessed using the Child-Pugh classification have been found (13,14). It has been proposed that measurement of circulating sICAM-1 levels may be useful in the investigation and monitoring of immune and inflammatory disease such as PBC (13,16). Despite uncertainty about its long term efficacy, the current medical treatment of choice for PBC is ursodeoxycholic acid (UDCA)(17-19). The exact mechanisms of action of UDCA remain unclear. It has been suggested that UDCA acts by reducing the damage caused by toxic endogenous bile salts (20,21). There is also some evidence that UDCA may have immunomodulating properties (22-25). As both immune

Table 1. **Patient characteristics at entry for the two groups to be randomized to treatment with prednisone/azathioprine or placebos at T=12. Laboratory parameters given as mean ± SEM.**

To be randomized to:	UDCA/PRED/AZA n=12	UDCA/placebos n=12
Age in years, mean (range)	54 (41-66)	55 (31-66)
Male / female	3 / 9	1 / 11
Stage I	0	1
II	2	4
III	5	3
IV	5	4
Bilirubin (μmol/L)	18 ± 3	15 ± 2
Normal range 4-14		
Alkaline Phosphatase (U/L)	306 ± 35	335 ± 49
Normal range 25-75		
Alanine aminotransferase (U/L)	86 ± 8	95 ± 14
Normal range 5-30		
Aspartate aminotransferase (U/L)	60 ± 5	68 ± 8
Normal range 5-30		
IgM (g/L)	4.8± 0.5	6.1 ± 1.7
Normal range 0.6-2.8		
Albumin (g/L)	43 ± 1	42 ± 1
Normal range 36-48		

mechanisms and bile salt mediated damage are likely to play a central role in the pathogenesis of PBC (26), it is not unreasonable to suggest that a combination of UDCA with immunosuppressive agents may be more efficacious (27). Corticosteroids and azathioprine have both been shown to be of some benefit in PBC (28,29). Combining low doses of prednisone and azathioprine is thought to lead to an enhanced efficacy and a lower risk of side effects (30).

The aims of this study were first to determine the effects of UDCA and immunosuppressive treatment (prednisone and azathioprine) on sICAM-1 levels in PBC. Secondly, to compare the effects on sICAM-1 with those on other parameters of disease activity (biochemical liver tests, IgM) and soluble interleukin-2 receptor (sIL-2R), a marker of lymphocyte activation.

SUBJECTS AND METHODS

Twenty-four patients, with an established diagnosis of PBC according to Taal et al.(31) were recruited at the University Hospital Rotterdam, in the context of a multicentric project aiming at the systematic, prospective evaluation of new treatment options in PBC. All patients had positive antimitochondrial antibodies, liver biopsies consistent with PBC and absence of biliary obstruction on ultrasonography. Patients with Child-Pugh class B and C disease were excluded from the study. The patients received UDCA (10 mg/kg) for the 18 month duration of the study. After 12 months of UDCA, none of the patients had achieved inactivation of the disease, i.e. absence of symptoms and biochemical abnormalities (30), and were subsequently randomized to receive additional treatment with a combination of azathioprine (50 mg/day) and prednisone (30 mg/day for the first month, 20 mg/day for the second, followed by 10 mg/day maintenance), or matching placebos. None of the patients were treated with any other drugs known to affect sICAM-1 at the time of the study.

The study protocol was approved by the local hospital ethical committee and informed written consent was obtained from all subjects.

Table 2. **Biochemical parameters during 1 year of UDCA and during 6 months of additional treatment with prednisone/azathioprine or placebos. Values given as mean±SEM.**

	1 year UDCA alone (n=24)		UDCA/PRED/AZA (n=12)			UDCA/placebos (n=12)		
	Baseline	T=12	T=12	T=15	T=18	T=12	T=15	T=18
Bilirubin	16±2	15±1.3	16±2	15±2	16±2	16±2	16±3	17±3
APh	319±30	234±27[1]	239±46	154±34[2]	152±32[2]	241±32	212±26	235±34
ALAT	90±8	46±6[1]	46±3	41±6	40±4	49±12	46±9	52±12
ASAT	63±5	35±3[1]	36±3	29±3[3]	27±3[2]	36±6	36±6	40±8
IgM	5.5±0.9	3.9±0.7[1]	4.0±0.6	3.1±0.4[4]	2.9±0.3[3]	4.4±1.3	4.5±1.3	4.6±1.4

[1] $p<0.001$ for difference from baseline;
[2] $p<0.005$ for difference from baseline and $p<0.001$ for difference in percentage change between groups;
[3] $p<0.01$ for difference from baseline and $p<0.001$ for difference in percentage change between groups;
[4] $p<0.03$ for difference in percentage change between both groups.

Serum bilirubin, alkaline phosphatase (APh), aspartate and alanine aminotransferases (ASAT and ALAT), Immunoglobulin M (IgM) and albumin were determined at baseline, and at 12, 15 and 18 months. Serum aliquots were stored at -20° C untill the assessment of sICAM-1 and sIL-2R levels. In 5 patients, baseline serum aliquots were not available for analysis. sICAM-1 and sIL-2R levels were also measured in 17 healthy controls.

Measurement of sICAM-1 and sIL-2R

Follow up sera that belonged to the same patient were tested together. The investigators performing the measurements were unaware of the treatment allocation. Levels of sICAM-1 were measured in duplicate using an enzyme linked immunosorbent assay (ELISA) kit, commercially available from British Bio-technology Products (Oxford,UK). In brief, the method involved the binding of sICAM-1 present in serum or standard to antibodies absorbed on to microwells. Unreacted sample components were removed by washing. A horseradish peroxidase-conjugated monoclonal antibody with neutralizing properties against ICAM-1 was used to bind to ICAM-1 captured by the first antibody. After washing, and addition of tetra-methylbenzidine, the reaction was stopped and the absorbance of samples and ICAM-1 standards were read using an Anthos 2001 reader (Denley Instruments, Billinghurst, UK) set at 450 nm with a correction wave length of 620 nm. Inter and intra assay variation was less than 10%.

Levels of sIL-2R in sera were measured in duplicate by ELISA (Genzyme Diagnostics, Cambridge, MA, USA). The method involved the binding of sIL-2R present in serum or standard to antibodies absorbed on to microwells. Unreacted sample components were removed by washing. A peroxidase-labelled streptavidin reagent was added which attaches to biotin in the immune complex on the plate. After incubation, further washing and addition of tetra-methylbenzidine, the reaction was stopped and the absorbance of samples and IL-2R standards were read using an Anthos 2001 reader (Denley Instruments, Billinghurst UK) set at 450 nm. Inter and intra assay variation was less than 10%.

Statistical methods

Differences within and between groups were tested by Wilcoxon's signed rank and rank-sum tests. Correlations were examined by Spearman's rank correlation. A $p \leq 0.05$ was considered significant. The analysis was performed according to the intention to treat principle.

RESULTS

Patient characteristics at entry of the patients to be randomized to prednisone/azathioprine and placebo were comparable (table 1). One patient in the prednisone/azathioprine group stopped this treatment after 1 month because of general malaise.

When dividing patients in early (stage I/II), stage III and stage IV disease (32), baseline sICAM-1 levels were higher in all stages (I/II: median 484 ng/ml, range 298 to 2039, $p<0.001$; III: 878, range 266 to 1348, $p<0.001$; IV: 1663, range 746 to 2344, $p<0.0001$) as compared to healthy controls (median 259 ng/ml, range 156 to 499). sICAM-1 levels correlated with the stage of the disease (R_s 0.54, $p<0.01$) (figure 1), bilirubin (R_s 0.53, $p<0.02$), ASAT (R_s 0.60, $P<0.005$) and ALAT ($R_s=0.44$, $p<0.05$).

sICAM-1 levels fell during the 12 months of UDCA therapy by a median of 20% (range -55 to +6%) from a median of 1137 (range 266 to 2344) to 895 (range 214-2389) ng/ml ($p<0.0004$) as shown in figure 2. APh, ASAT, ALAT and IgM also improved during this period (table 2). The observed changes in sICAM-1, liver tests and IgM during UDCA monotherapy were comparable for the patients subsequently randomized to either prednisone/azathioprine or placebos.

Further decreases in sICAM-1 levels were found in patients receiving the additional immunosuppressive treatment. In this group, levels at both 15 (median 588 ng/ml, range 228 to 2579, $p<0.05$) and 18 months (554, range 238 to 2080, $p<0.01$) were significantly lower than at 12 months (828, range 214 to 3190). In the group on continued UDCA monotherapy, levels were not significantly different at 12 (median 939 ng/ml, range 239 to 2526), 15 (1080, range 225 to 3385) and 18 (966, range 224 to 3174) months. These results are shown in

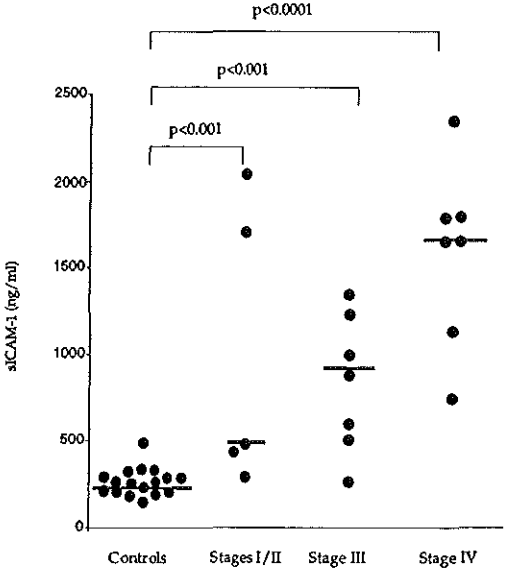


Figure 1.
Serum sICAM-1 levels in healthy controls, patients with early stage (stages I/II), stage III and stage IV PBC. The bars indicate the medians. sICAM-1 levels correlated with histological stage of disease (R_s=0.54, $p<0.01$).

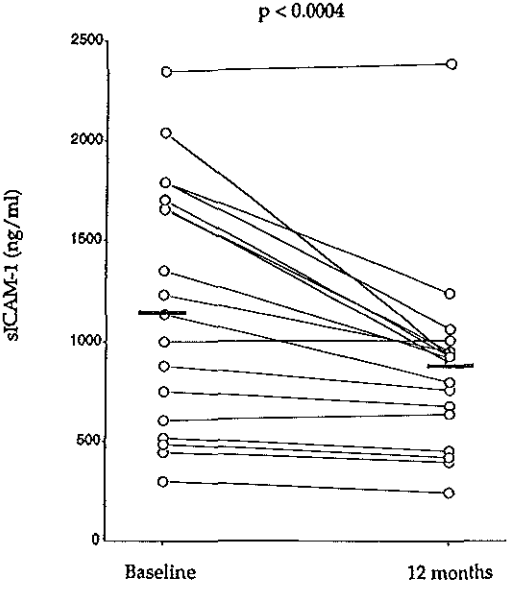


Figure 2.
Effect of 12 months UDCA therapy on sICAM-1 levels in PBC. The bars indicate the medians.

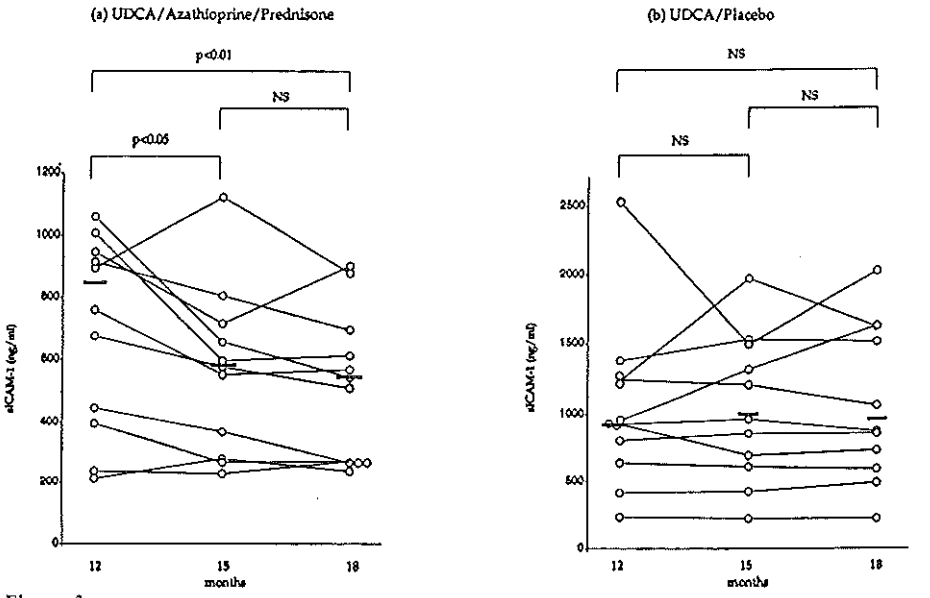


Figure 3.

Serum sICAM-1 following the addition of prednisone/azathioprine (a) or placebos (b) to UDCA at 12 months. The very high values of one patient in each group have not been shown for reasons of scale; the values for the patient in the treatment group at 12, 15 and 18 months were 3190, 2579 and 2080 ng/ml respectively. The values for the patient in the placebo group were 2389, 3385 and 3174 ng/ml respectively. The bottom curve in figure a represents two patients; one of them withdrew from treatment after 1 month. Please note the unequal scales in figure a and b. The bars indicate the medians.

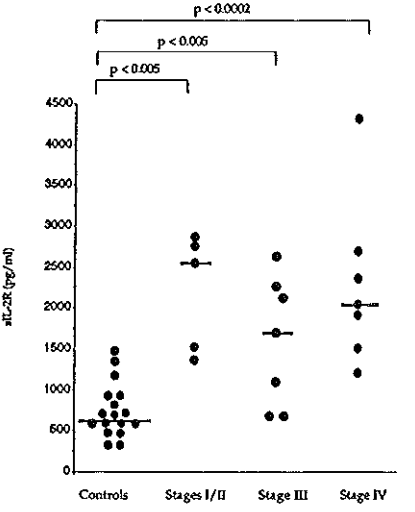


Figure 4.

Serum sIL-2R levels in healthy controls, patients with early stage (stages I/II), stage III and stage IV PBC. The bars indicate the medians. There was no correlation between sIL-2R levels and histological stage.

figure 3. The percentage change between the treatment and placebo groups differed significantly at T=15 (-19%, range -41 to +30% vs. +3, range -41 to +60%, $p<0.03$) and T=18 (-25%, range -49 to +13% vs. 0%, range -21 to +71%, $p<0.01$). In the treatment group ASAT, APh and IgM also improved significantly (table 2).

sIL-2R levels were significantly higher in early (median 2562 pg/ml, range 1378-2882, $p<0.005$), stage III (1709, range 669-2639, $p<0.005$) and stage IV (2046, range 1289 to 4326, $p<0.0002$) PBC compared to healthy controls (692, range 250 to 1500) (figure 4). sIL-2R levels did not correlate with sICAM-1 levels in either PBC patients or healthy controls. Also no correlations with stage or biochemical disease markers were detected. Treatment with UDCA did not significantly affect sIL-2R levels (median 2046 pg/ml, range 669 to 4326 at baseline vs. 1845, range 587 to 10.123 at 12 months). Addition of prednisone/azathioprine led to a reduced sIL-2R level at 15 months (1249 pg/ml, range 683 to 2253, $p<0.05$) but not at 18 months (1429, range 851 to 2628, p=NS) as compared to 12 months (1523, range 587 to 2842). Levels in the continued UDCA monotherapy remained stable (T=12: median 2050 pg/ml, range 888 to 10.123; T=15: 1924, range 786 to 6564; T=18: 1979, range 624 to 6042). Differences in percentage change between both groups were not significant at any moment.

No correlations were found between the percentage change in the standard disease parameters, sICAM-1 and sIL-2R during UDCA or prednisone/azathioprine treatment. Alterations in sICAM-1 and sIL-2R were independent of histological stage or initial liver test results.

DISCUSSION

We found that treatment of PBC patients with UDCA during 12 months resulted in a significant lowering of circulating sICAM-1 levels. Further reduction in sICAM-1 was achieved by the addition of immunosuppressive therapy in the form of prednisone and azathioprine. Decreases in sICAM-1 were accompanied by improvements in serum liver tests and IgM but not in the T-lymphocyte activation marker, sIL-2R.

The observed correlation of sICAM-1 with histological stage and transaminases in untreated PBC patients were consistent with our previous observations (13). However, in the present study, APh did not correlate with sICAM-1, while bilirubin did. These differences may be accounted for by the number and the disease stage of the patients studied; a higher proportion of patients with late stage disease was included in this study.

Uncontrolled data had already indicated a reduction of sICAM-1 levels by immunosuppressive treatment - mostly consisting of corticosteroids with or without another immunosuppressant - in a variety of other non-hepatic (33,34) as well as hepatic immune disorders, such as hepatic allograft rejection and autoimmune hepatitis (35,36). In PBC, Thomson et al reported a fall in sICAM-1 in 2 patients during immunosuppression with FK506 (14). In contrast, no effect of methotrexate on sICAM-1 was found in 8 patients (37). In all of these conditions falls in sICAM-1 indicated a favourable treatment response. As for UDCA, we know of only one abstract reporting a fall in sICAM-1 levels during therapy in 8 of 14 patients with various cholestatic disorders, including PBC (38).

The serum level of sICAM-1 represents an equilibrium between its formation and its excretion. The production of sICAM-1 is stimulated by a variety of cytokines and mediators including lipopolysaccharide, IL-1α, TNF-β and Interferon-γ (39-43). The observed fall in sICAM-1 during the treatment with immunosuppressive agents probably represents a decrease in inflammatory activity. This may also be true in the case of UDCA, as there is some evidence

that UDCA may have immunomodulatory properties (22-25). As for excretion, it has been demonstrated that sICAM-1 is present in bile (28) and that UDCA can improve hepatobiliary excretion in PBC (44). It is conceivable that UDCA may also decrease sICAM-1 levels by reducing cholestasis and increasing hepatic excretion of this molecule. However, the lack of a correlation between changes in bilirubin and APh and sICAM-1 during UDCA therapy suggests that this is not the sole explanation.

In contrast to membrane bound ICAM-1, the source, mechanism of formation and pathophysiological role of sICAM-1 are less clear. With regard to its pathophysiological role, it has been proposed that sICAM-1 may regulate leucocyte-endothelial interaction by competing for binding ligands with membrane bound ICAM-1 (11). It is also possible that sICAM-1 may be acting like a cytokine, providing a signalling effect similar to the co-stimulatory effect of membrane bound ICAM-1 (34). This being the case, the fall of sICAM-1 during treatment may be of direct pathogenetic relevance. It has previously been demonstrated that sICAM-1 can be biologically active. *In vitro*, recombinant forms of sICAM-1 are capable of interfering with antigen specific T cell proliferation in a dose dependent fashion (45). Moreover, sICAM-1 has been found to interfere with the recognition of tumour cells by IL-2 activated lymphocytes (39) and to inhibit the infection of cells by rhinovirus (46).

With regard to its formation, a couple of mechanisms have been proposed. sICAM-1 may be a product of proteolytic cleavage of membrane bound ICAM-1 close to the cell membrane, as has previously been shown for other cell surface molecules (48). This may be supported by the finding that ICAM-1 expression on Interferon-γ stimulated tumour cells declined after an initial increase and the decline was accompanied by an increase in the soluble ICAM-1 form (49). An alternative hypothesis is that sICAM-1 is formed from an alternatively spliced messenger RNA (11). However, there is as yet no experimental evidence to support this hypothesis.

There are several possible sources of sICAM-1 in PBC. While release of sICAM-1 by cultured biliary epithelial cells could not be

substantiated (15), human hepatocytes have recently been shown to first express ICAM-1 and subsequently sICAM-1, during cytokine stimulation (14). Furthermore, activated endothelial cells near the inflammatory sites may also shed sICAM-1 (42).

Activated lymphocytes, which can also be induced to produce sICAM-1 *in vitro*, may be yet another source (15). However, the lack of correlation between the levels of sICAM-1 with markers of lymphocyte activation such as lymphocyte IL-2R expression (13), sIL-2R and β2-microglobulin (15), as documented by us as well as by others, argue against this hypothesis. Moreover, in the present study, falls in sICAM-1 were not parallelled by reductions in sIL-2R levels during either UDCA or immunosuppressive therapy.

The absence of an effect of immunosuppressive therapy on sIL-2R was unexpected. However, sIL-2R, although a marker of systemic lymphocyte activation, may not accurately reflect the degree of local (hepatic) activity (50). Furthermore, in PBC there appears to be an aberrant IL-2R regulation as indicated by the finding of an increased IL-2R expression on unactivated T lymphocytes (51).

In PBC, circulating sICAM-1 is a marker of disease severity and appears to reflect the degree of hepatic inflammatory activity. sICAM-1 levels decreased during UDCA therapy, suggesting an immunomodulatory effect. Further reduction was achieved by the addition of immunosuppressive therapy with combined prednisone and azathioprine. Further clarification of the significance of sICAM-1 in PBC is needed, particularly as an indicator of prognosis and treatment efficacy in the long run.

REFERENCES

1. Marlin SD, Springer TA. Intercellular adhesion molecule 1 (ICAM-1) is a ligand for lymphocyte function associated antigen-1 (LFA-1). Cell 1987;51:831-41.
2. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA. Induction by IL-1 and interferon-γ: tissue distribution, biochemistry, and function of a natural adherence molecule. J Immunol 1986;137:245-54.
3. Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA. Primary structure of intercellular adhesion molecule 1 (ICAM-1) demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 1988;52:925-33.
4. Makgoba MW, Sanders ME, Ginther LGE, Dustin ML, Springer TA, Clark EA et al. ICAM-1, a ligand for LFA-1 dependent adhesion of B,T and myeloid cells. Nature 1988;331:86-8.
5. Diamond MS, Staunton DE, De Fourgerolles AR, Stacker SA, Garcia-Aguilar J, Hibbs ML et al. ICAM-1 (CD54) a counter receptor for MAC-1 (CD11a/CD18). Cell Biol 1990;3:3129-41.
6. Van Seventer GA, Shimizu Y, Horgan KJ, Shaw S. Rhe LFA-1 ligand ICAM-1 provides an important costimulatory signal for T-cell receptor mediated activation of resting T-cells. J Immunol 1990;144:4579-89.
7. Wawryck SO, Novotny JR, Wicks IP, Wilkinson WD, Maher D, Salvaris E et al. The role of the LFA-1/ICAM-1 interaction in human leucocyte homing and adhesion. Immunol Rev 1989;108:135-61.
8. Adams DH, Hubscher SG, Shaw J, Johnson GD, Babbs C, Rothlein R et al. Increased expression of intercellular adhesion molecule 1 on bile ducts in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 1991;14:426-31.
9. Steinhoff G, Behrend M, Schrader B, Pichlmayr R. Intercellular immune adhesion molecules in human liver transplants: overview on expression patterns of leucocyte receptor and ligand molecules. Hepatology 1993;18:440-53.
10. Volpes R, Van den Oord JJ, Desmet VJ. Immunohistochemical study of adhesion molecules in liver inflammation. Hepatology 1990;12:59-65.
11. Rothlein R, Mainolfi EA, Czaikowski M, Marlin SD. A form of circulating ICAM-1 in human serum. J Immunol 1991;47:3788-93.
12. Gearing AJH, Newman W. Circulating adhesion molecules in disease. Immunology Today 1993;14:506-12.
13. Lim AG, Jazrawi RP, Ahmed HA, Levy JH, Zuin M, Douds AC et al. Soluble intercellular adhesion molecule-1 in primary biliary cirrhosis; relationship with disease stage, immune activity and cholestasis. Hepatology 1994;20:882-8.
14. Thomson AW, Satoh S, Nussler AK, Tamura K, Woo W, Gavaller J et al. Circulating intercellular adhesion molecule-1 (ICAM-1) in autoimmune liver disease and evidence for the production of ICAM-1 by cytokine stimulated human hepatocytes. Clin Exp Immunol 1994;95:83-90.
15. Adams DH, Mainolfi E, Burra P, Neuberger JM, Ayres R, Elias E et al. Detection of circulating intercellular adhesion molecule-1 in chronic liver diseases. Hepatology 1992;16:810-4.
16. Seth R, Raymond FD, Makgoba MW. Circulating ICAM-1 isoforms: diagnostic prospects for inflammatory and immune disorders. Lancet 1001;338:83-4.
17. Poupon RE, Poupon R, Balkau B, Niard AM and the UDCA-PBC Study Group. Ursodiol for the long-term treatment of primary biliary cirrhosis. N Engl J med 1994;106:1284-90.
18. Lindor KD, Dickson ER, Baldus WP, Jorgensen RA, Ludwig J, Murtaugh PA et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;108:1284-94.
19. Heathcote EJ, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN et al. The Canadian multicenter double blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.

20. Lim AG, Northfield TC. Ursodeoxycholic acid and primary biliary cirrhosis. Br Med J 1994;309:491-2.
21. Poupon R, Poupon RE. Mechanisms of action of ursodeoxycholic acid in cholestasis. In: Cholestatic liver diseases - new strategies for prevention and treatment of hepatobiliary and cholestatic liver diseases. Eds. Van Berge Henegouwen GP, Van Hoek B, Matern S, Stockbrügger RW. Kluwer Academic Publishers, Dordrecht, The Netherlands. 1994;211-7.
22. Calmus Y, Gane P, Roger P, Poupon R. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effects of ursodeoxycholic acid. Hepatology 1990;11:12-5.
23. Yoshikawa M, Tsuji T, Matsuumara K, Yamao J, Matsumura Y, Kubo R et al. Immunomodulatory effects of ursodeoxycholic acid on immune responses. Hepatology 1992;16:358-64.
24. Kurktschiev D, Subat S, Adler D, Schentke K-U. Immunomodulating effects of ursodeoxycholic acid therapy in patients with primary biliary cirrhosis. J Hepatol 1993;18:373-7.
25. Lacaille F, Paradis K. The immunomodulatory effect of ursodeoxycholic acid: a comparative in vitro study on human peripheral blood mononuclear cells. hepatology 1993;18:165-72.
26. De Caestecker JS, Jazrawi RP, Petroni ML, Northfield TC. Ursodeoxycholic acid in chronic liver disease. Gut 1991;32:1061-5.
27. Wolfhagen FHJ, Van Buuren HR, Schalm SW. Combined treatment with ursodeoxycholic acid and prednisone in primary biliary cirrhosis. Neth J Med 1994;44: 84-90.
28. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OFW. A controlled trial of prednisolone treatment in primary biliary cirrhosis. J Hepatol 1992;15:336-44.
29. Christensen E, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Gastroenterology 1985;89:1084-91.
30. Beukers R, Schalm SW. Immunosuppressive therapy for primary biliary cirrhosis. J Hepatol 1992;14:1-6.
31. Taal BG, Schalm SW, Ten Kate FWJ, Hermans J, Geertzen GM, Feltkamp BEW. Clinical diagnosis of primary biliary cirrhosis: a classification based on major and minor criteria. Hepato-gastroenterol 1983;30:178-82.
32. Ludwig J, Dickson ER, McDonald GSA. Staging of non-suppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchow's Arch Path Anat and Histol 1978;379:103-12.
33. Kling E, Bieg S, Boehme M, Scherbaum WA. Circulating intercellular adhesion molecule 1 as a new activity marker in patients with systemic lupus erythematosus. Clin Investig 1993;71:299-304.
34. Heufelder AE, Bahn RS. Soluble intercellular adhesion molecule-1 in sera of patients with Graves ophtalmopathy. Clin Exp Immunol 1993;92:296-302.
35. Adams DH, Mainolfi E, Elias E, Neuberger JM, Rothlein R. Detection of circulating intercellular adhesion molecule-1 after liver transplantation- evidence of local release within the liver during graft rejection. Transplantation 1993;55:83-7.
36. Zöhrens G, Armbrust T, Pirzer U, Meyer zum Buschenfelde K-H, Ramadori G. Intercellular adhesion molecule-1 concentration in sera of patients with acute and chronic liver disease: relationship to disease activity and cirrhosis. Hepatology 1993;18:798-802.
37. Bergasa NV, Newman W, Rothlein R, Jones EA, Adams DH. Serum levels of soluble adhesion molecules (I-CAM-1, V-CAM-1, and E-selectin) are markedly elevated in primary biliary cirrhosis and unaffected by low dose oral methotrexate treatment (Abstract). Hepatology 1993;18:A877.
38. Zöhrens G, Armbrust T, Polzien F, Ramadori G. ICAM-1 serum concentration in cholestasis. Indications for a biliary elimination [abstract]. J Hepatol 1993;18:S43.
39. Becker JC, Dummer R, Hartmann AA, Burg G, Schmidt R. Shedding of ICAM-1 from human melanoma cell lines induced by IFN γ and tumor necrosis factor α J Immunol 1991;147:4398-401.

40. Giavazzi R, Chirivi RGS, Garofalo A, Rambaldi A, Hemingway IK, Pigott R, Gearing AJH. Soluble intercellular adhesion molecule 1 is released by human melanoma cells and is associated with tumour growth in nude mice. Cancer Res 1992;52:2628-30.
41. Pigott R, Dillon LP, Hemingway IH, Gearing AJH. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Biochem Biophys res Commun 1992;187:584-9.
42. Leeuwenberg JFM, Smeets EFM, Neefjes JJ, Shaffner MA, Cinek T, Jeunhomme TMAA et al. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. Immunology 1992;77:543-9.
43. Harning R, Mainolfi E, Bystryn JC, Henn M, Meluzzi VJ, Rothlein R. Serum levels of circulating intercellular adhesion molecule-1 in human malignant melanoma. Cancer Res 1991;51:5003-5.
44. Jazrawi RP, De Caestecker JS, Goggin PM, Britten AJ, Joseph AEA, Maxwell JD et al. Kinetics of hepatic bile acid handling in cholestatic liver disease: effect of ursodeoxycholic acid. Gastroenterology 1994;106:134-42.
45. Roep BO, Heidenthal E, De Vries RRP, Kolb H, Martin S. Soluble forms of intercellular adhesion molecule-1 in insulin dependent diabetes mellitus. Lancet 1994;343:1590-3.
46. Marlin SD, Staunton DE, Springer TA, Stratowa C, Sommergruber W, Merluzzi VJ. A soluble form of circulating adhesion molecule-1 inhibits rhinovirus infection. Nature 1990;344:70-2.
47. Adams DH, Burra P, Hubscher SG, Elias E, Newman W. Endothelial activation and circulating vascular adhesion molecules in alcoholic liver disease. Hepatology 1994;19:588-594.
48. Kishimoto TK, Jutila MA, Berg EL, Butcher EC. Neutrophil Mac-1 and mel-14 adhesion proteins inversely regulated by chemotactic factors. Science 1989;245:1238-41.
49. Jackson AM, Alexandrov AB, Gribben SC, Esuvarnathan K, James K. Expression and shedding of ICAM-1 in bladder cancer and its immunotherapy. Int J Cancer 1993;55:921-25.
50. Symons JA, Wood NC, Di Giovine FS, Duff GW. Soluble IL-2 receptor in rheumatoid arthritis: correlation with disease activity, IL-1 and IL-2 inhibition. J Immunol 1988;141:2612-8.
51. Menendez JL, Girón JA, Manzano L, Garrido A, Abreu L, Albillos A et al. Deficient Interleukin-2 responsiveness of T lymphocytes from patients with primary biliary cirrhosis. Hepatology 1992;16:931-6.

CHAPTER VIII

CYCLICAL ETIDRONATE IN THE PREVENTION OF BONE LOSS IN CORTICOSTEROID TREATED PRIMARY BILIARY CIRRHOSIS.

Frank H.J. Wolfhagen[1], Henk R. van Buuren[1], Janny W. den Ouden[2], Wim C.J. Hop[3], Johannes P.T.M. van Leeuwen[4], Solko W. Schalm[1], Huib A.P. Pols[4].

Affiliations:

1. Dept. of Internal Medicine II (section Hepatology), University Hospital Dijkzigt, Rotterdam
2. Dept. of Internal Medicine, St.Franciscus Hospital, Rotterdam
3. Dept. of Epidemiology and Biostatistics, Erasmus University, Rotterdam
4. Dept. of Internal Medicine III (section Clinical Endocrinology), University Hospital Dijkzigt, Rotterdam.

Submitted for publication

SUMMARY

Recently promising disease modifying effects of low dose corticosteroid treatment in Primary Biliary Cirrhosis (PBC) have been reported. However, steroid-induced bone loss constitutes a potential drawback of this treatment option.

To assess whether etidronate can reduce bone loss during corticosteroid treatment, 12 PBC patients (all Child-Pugh Class A), treated with prednisone in the context of a 1-year placebo-controlled trial with prednisone (maintenance dose 10 mg daily) and azathioprine (50 mg daily), were randomized to receive either cyclical etidronate (400 mg daily, during 2 weeks) alternated with calcium 500 mg daily during 11 weeks, or calcium alone. Bone Mineral Content (BMC) was measured in the lumbar spine and the femoral neck by Dual Energy X-ray Absorptiometry before and after 3 and 12 months of treatment. Markers of bone formation (serum osteocalcin, procollagen-I-propeptide) and bone resorption (urinary deoxypyridinoline and calcium) were also monitored.

The mean lumbar BMC did not decrease significantly in the patients taking etidronate+calcium, in contrast to patients treated with calcium alone (-0.8 vs. -3.5%; p=0.03). Changes in femoral BMC and markers of bone turnover did not significantly differ between both groups. No adverse effects of etidronate were noted.

In conclusion, cyclical etidronate appears to prevent bone loss associated with prednisone treatment in patients with PBC. These results may encourage further evaluation of long-term prednisone treatment in PBC.

INTRODUCTION

Primary Biliary Cirrhosis (PBC) is a chronic, potentially fatal cholestatic liver disease characterized by non-suppurative destruction of small and intermediate bile ducts, predominantly affecting middle-aged women (1).The disease is associated with osteoporosis and patients seem to lose bone at an annual rate twice that of healthy controls (2 vs. 1%) (2). Impaired osteoblast function as well as increased bone resorption have been proposed as potential underlying mechanisms (3).

Based on findings in patients with advanced disease, corticosteroid treatment is considered contraindicated for PBC, due to fear of further acceleration of bone loss (4). However, the interest in corticosteroids as a treatment option in PBC has been revived. In accordance with our experience (5), Mitchison et al., who performed a placebo-controlled study with corticosteroids in PBC, recently reported beneficial effects regarding disease activity, although at the cost of a doubling of bone loss during the first year of treatment (6,7).

To enable the safe use of corticosteroids in PBC, measures to prevent bone loss acceleration are needed. Bisphosphonates, e.g etidronate, are inhibitors of bone resorption (8) and have been shown to prevent bone loss in postmenopausal women (9,10) as well as in patients using corticosteroids for other non-hepatic diseases (11-13).

This controlled pilot study was initiated to assess whether cyclical etidronate can reduce bone loss in corticosteroid treated PBC patients.

PATIENTS AND METHODS

Twenty-four patients with an established diagnosis of PBC (14), participating in a double-blind, placebo-controlled trial with prednisone/azathioprine were eligible for this study. Exclusion criteria were: Child-Pugh Class B or C; previous treatment with estrogen replacement, bisphosphonates, 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 (i.e. >20%

reduction in vertebral height).

The study was approved by the local medical ethical committees of both participating hospitals and patients gave written informed consent.

As we did not know which patients received prednisone/azathioprine or placebo, all patients were randomly allocated, after stratification for sex and pre/postmenopausal state, to receive either 3-monthly cycles of etidronate 400 mg daily during 2 weeks (taken with water with 2 hour intervals from meals) alternated with 11 weeks of 1250 mg calciumcarbonate (=500 mg elementary calcium) or calciumcarbonate alone (Procter & Gamble Pharmaceuticals, Rotterdam, The Netherlands). Both regimens were started 1 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 4 weeks, 20 mg during the following 4 weeks and 10 mg daily thereafter for 40 weeks, combined with 50 mg azathioprine daily (Wellcome Pharmaceuticals, Utrecht, The Netherlands). All patients had been receiving 10 mg/kg/day ursodeoxycholic acid (UDCA) for at least 1 year and this treatment was continued.

An overview of the study is presented in figure 1. At entry and 12 and 52 weeks thereafter bone mass measurements were performed by Dual Energy X-ray Absorptiometry (DXA) of the 2nd, 3th and 4th lumbar vertebrae and the left femoral neck using a Lunar DPX-L scanner (Lunar Radiation, Madison, WIS, USA). In our laboratory the coefficients of variation in healthy females for the lumbar and femoral measurement are 1.1 and 1.4% respectively. All measurements were performed by one technician. Before and after the study radiological examinations of the thoracic and lumbar spine were performed confirming the absence of fractures in the regions of measurement.

Fasting blood and 2-hour urine samples were collected according to the study overview (figure 1). Apart from standard liver tests, serum albumin and calcium, two serum markers of bone formation, i.e. procollagen I carboxyterminal propeptide (PICP) and osteocalcin, were radioimmunologically assayed by means of commercially available kits

(Orion Diagnostica, Espoo, Finland; Incstar Corporation, Stillwater, MN, USA respectively). As markers of bone resorption the urinary concentrations of deoxypyridinoline cross-links (d-pyr) (competitive enzyme immunoassay kits, Metra Biosystems, Palo Alto, CA, USA) and calcium were determined and corrected for urinary creatinine concentrations (15). Serum 25-hydroxyvitamin D3 [25-(OH)D], 1,25-dihydroxyvitamin D3 [1,25$(OH)_2$D] and parathyroid hormone (PTH) were determined with commercially available kits (Incstar Corporation, Stillwater, MN, USA) at entry and at 1 year. Vitamin D supplementation was to be administered in case of subnormal 25-(OH)D at entry, but levels were normal in all patients.

Bone mass data are given as Bone Mineral Content (BMC) in gram Hydroxyapatite per centimeter (gHA/cm), Bone Mineral Density (BMD) in gHA/cm^2 and Z-scores, i.e. the number of standard

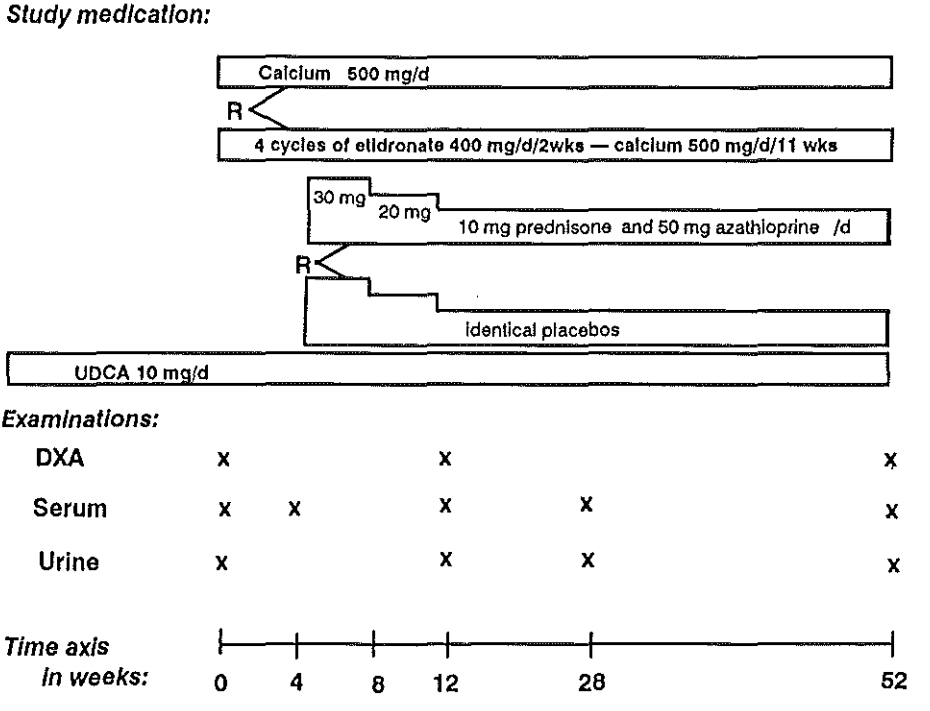


Figure 1.
Study overview. "R" indicates randomization.

Table 1. **Characteristics at entry of prednisone/azathioprine treated patients (means ± Standard Deviation or number of patients).**

		etidronate (n=6)	no etidronate (n=6)
Female/Male		5/1	4/2
Pre/Postmenopausal		1/4	2/2
Histological Stage	I/II	-	1
	III/IV	6	5
Age (yrs)		57 ± 11	49 ± 6
Bilirubin	(N 2-14 μmol/L)	17 ± 8	15 ± 5
APh	(N 25-75 U/L)	147 ± 44	338 ± 131 [1]
Albumin	(N 36-48 g/L)	42 ± 3	44 ± 2
BMD L2-L4	(gr HA/cm^2)	1.096 ± 0.20	1.132 ± 0.17
Z-score		0.0 ± 0.9	-0.5 ± 1.2
BMD femoral neck	(gr HA/cm^2)	0.885 ± 0.10	0.884 ± 0.07
Z-score		0.0 ± 0.5	-0.5 ± 0.8

[1] $p<0.01$ N = normal range

deviations that the individual BMD differs from the mean BMD of age and sex matched healthy controls.

Follow up data are presented as the means with standard errors of the mean of the actual values and/or percentual changes from baseline, unless indicated otherwise. Paired and unpaired t-tests were used to compare differences. A two-sided p-value ≤ 0.05 was considered significant. The data were analysed according to the intention-to-treat principle.

RESULTS

Characteristics at entry of the prednisone/azathioprine treated patients are presented in table 1. There were 6 patients in each group. Bone status was relatively normal, as apparent from the Z-scores. The

Table 2. **Bone Mineral Content (grams hydroxyapatite/cm) and percentage change from baseline (in italics) in the lumbar spine and femoral neck during 1 year of prednisone treatment.**

T in weeks	etidronate (n=6)	no etidronate (n=6)
BMC L2-L4		
Baseline	47.6 ± 6.2	50.9 ± 4.4
12	47.6 ± 5.8	50.0 ± 3.8
% change	*+ 1.0 ± 1.0*	*- 1.3 ± 1.3*
52	47.2 ± 6.0	49.0 ± 4.0‡
% change	*- 0.8 ± 0.9**	*- 3.5 ± 0.6**
BMC femoral neck		
Baseline	4.5 ± 0.4	4.7 ± 0.1
12	4.6 ± 0.5	4.6 ± 0.2
% change	*+ 0.1 ± 3.2*	*- 2.4 ± 2.5*
52	4.4 ± 0.4	4.6 ± 0.1
% change	*- 2.3 ± 3.3*	*- 2.6 ± 2.5*

Data given as means ± SEM
‡ Significantly different from baseline (p=0.006)
* Significantly different between groups (p=0.03)

patients were mostly stage III/IV, but had rather mild disease as indicated by the biochemical disease markers. The mean alkaline phosphatase (APh) was lower in the group receiving etidronate. One patient in the etidronate group stopped the prednisone/azathioprine medication 1 month after the start of the immunosuppressives, because of general malaise. All patients completed the study and no adverse effects of etidronate were noted.

In table 2 changes in BMC during prednisone treatment are reported. Vertebral bone loss was less in patients taking etidronate compared to those without etidronate (-0.8% ± 0.9 vs. -3.5% ± 0.6; p=0.03) (Figure 2). The mean difference in change between both groups was 2.7% (95%-confidence interval: 0.4 to 5.0%). Using multiple regression, the change in BMC did not appear to be dependent on initial bone mass, sex or age. There were no significant differences in changes of femoral bone mass (mean difference between both groups: 0.3%, 95%-confidence interval: -25 to +26%).

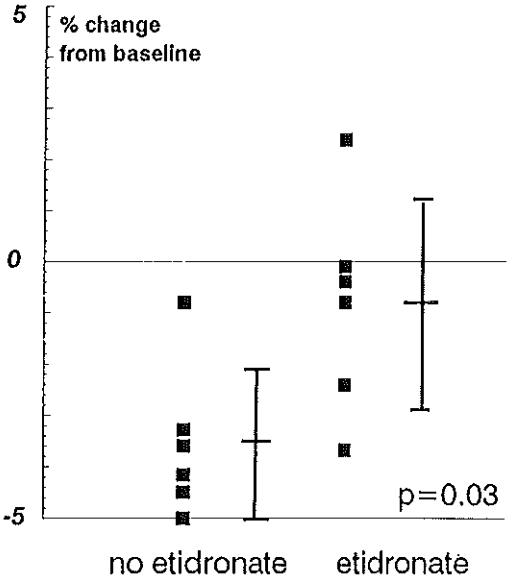


Figure 2.
Changes in vertebral Bone Mineral Content as percentage change from baseline after 1 year of prednisone treatment for patients with and without etidronate treatment. Bars indicate means with 95%-confidence limits.

Table 3. **Biochemical data (mean ± SEM) at entry and after 1 year of prednisone treatment.**

	etidronate (n=6)		no etidronate (n=6)	
	Baseline	1 year	Baseline	1 year
Serum:				
Osteocalcine (N 1.8-6.6 µg/L)	3.0 ± 0.8	1.9 ± 0.5	2.3 ± 0.7	2.0 ± 0.5
PICP (N 50-170 µg/L)	93 ± 9	70 ± 7	116 ± 9	103 ± 10
PTH (N 10-55 ng/L)	31 ± 7	28 ± 5	21 ± 5	19 ± 4
Calcium[1] (N 2.20-2.7 mmol/L)	2.3 ± 0.1	2.0 ± 0.3	2.3 ± 0.1	2.2 ± 0.1
25-(OH)D (N >30 nmol/L)	51 ± 5	54 ± 3	49 ± 8	56 ± 5
1,25-$(OH)_2$D (N 39-102 pmol/L)	82 ± 17	92 ± 15	65 ± 8	59 ± 7
Urine:				
D-pyr/creatinine (nmol/mmol)	5.2 ± 0.8	4.3 ± 0.6	5.4 ± 1.8	4.9 ± 1.2
Calcium/creatinine (mmol/mmol)	0.24 ± 0.07	0.25 ± 0.08	0.21 ± 0.09	0.18 ± 0.04

[1] Corrected for serum albumin
N = normal range

No significant differences in changes of serum levels of osteocalcin, PICP, calcium, PTH or vitamin D metabolites, and urinary ratios of deoxypyridinoline/creatinine and calcium/creatinine were noted between both treatment groups. In table 3 the values at baseline and after 12 months of treatment are presented.

In the patients who were not treated with prednisone/ azathioprine, no significant differences were found between patients with (n=7) or without (n=5) etidronate, with respect to changes in BMC (lumbar spine: -1.1% ± 1.4 vs. -0.1% ± 2.6; femoral neck: -1.5% ± 4.4 vs. -2.8 % ± 2.4, respectively) and biochemical parameters.

DISCUSSION

This study indicates that cyclical etidronate may prevent steroid-induced bone loss in PBC patients. The mean annual spinal bone loss has been reported to be 2% in a North-American PBC population (2), while a decrease of 1.3% was previously found in our PBC population (unpublished data). Corticosteroid-induced bone loss has been shown to be maximal during the first year of treatment (6,16,17), whereas the effect of long-term, low-dose treatment seems mild if present at all (7,18-20). Indeed, Mitchison et al. showed a doubled rate of bone loss during the first year of low-dose glucocorticoid treatment in PBC patients (6), which is in agreement with our results. The lumbar bone loss of 0.8% in etidronate treated patients as compared to 3.5% in the untreated patients, suggests not only reduction but complete prevention of steroid-induced bone loss. Although the number of patients is relatively small and considering the precision error of DXA, the presented confidence limits and the magnitude of the observed difference plead in favour of a reliable finding.

Corticosteroids are thought to exert their negative effect on bone status by both decreasing bone formation and increasing bone resorption. The metabolically active trabecular bone seems to be more prone to the steroid effects than cortical bone[21]. This is in agreement with the accelerated bone loss observed at the site of the lumbar spine (mainly trabecular bone) but not in the femoral neck (mainly cortical bone). This may also explain the absence, as noted in accordance with others (10,12,22), of an explicit effect of bisphosphonate treatment on femoral bone mass. Moreover, etidronate did not clearly affect bone mass in patients not treated with prednisone. This may not be surprising as etidronate is an antiresorptive drug, while decreased bone formation seems to be a major factor involved in PBC associated osteoporosis, particularly in patients with clinically less advanced disease (3,23), as included in our study. In regard of this, we found subnormal osteocalcine levels at entry in one third of the patients. However, possible type II errors cannot be excluded.

Changes in biochemical markers varied considerably and no significant differences were found, although there appeared to be a

tendency towards a somewhat more pronounced inhibition of bone turnover in the etidronate treated patients. Cortico-steroids have been shown to reduce osteocalcine and PICP (15), and to affect calcium and PTH metabolism (17), but none of these effects could be established in our study.

In agreement with a previous report from our center (18), bone status in our patients was not as problematic as found by other groups (24). Apart from differences in diet and life style, the relatively normal bone mass in our study may be due to the rather mild disease of our patients, which may be explained by the previous UDCA treatment (25), as well as by the exclusion of patients with very severe PBC (Child-Pugh Class B/C), who are considered unlikely to benefit from any medical treatment and should be considered for liver transplantation. Furthermore, our patient group included pre- and postmenopausal women as well as males. These patients, however, appear to be equally susceptible to the glucocorticoid effects on bone mass (21,26).

Patients in the prednisone group who received etidronate had a lower mean APh than those without etidronate. APh levels, however, are not related to bone loss in chronic liver diseases (23,27). Other, more important markers of disease severity (histological stage, bilirubin levels), were comparable in both groups. UDCA treatment, which was used by all patients, does not seem to affect bone mass (28). As for azathioprine, given in combination with prednisone, we are not aware of any data regarding the effect of this drug on the bone status.

Although this study is small and relatively short, we believe our findings are important, particularly in view of the recently reported disease modifying effects of corticosteroid treatment in PBC (5-7,29). Etidronate appears to obviate the main drawback of this therapy, i.e. enhanced bone loss, and thus questions the prevailing ban on corticosteroids in PBC. Larger studies are needed to define the effect of etidronate, or other currently available bisphosphonates, on bone mass and fracture rate during more prolonged corticosteroid treatment.

REFERENCES

1. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1987;316:521-528.
2. Eastell R, Dickson ER, Hodgson SF, Wiesner RH, Porayko MK, Wahner HW, Cedel SL et al. Rates of vertebral bone loss before and after liver transplantation in women with primary biliary cirrhosis. Hepatology 1991;14:296-300.
3. Hodgson SF, Dickson ER, Eastell R, Eriksen EF, Bryant SC, Riggs BL. Rates of cancellous bone remodeling and turnover in osteopenia associated with primary biliary cirrhosis. Bone 1993;14;819-827.
4. Sherlock S, Dooley J. Diseases of the liver and biliary system. Ed 9. London: Blackwell Scientific Publications, 1993:237-248.
5. Wolfhagen FHJ, Van Buuren HR, Schalm SW. Combined treatment with ursodeoxycholic acid and prednisone in primary biliary cirrhosis. Neth J Med 1994;44:84-90.
6. Mitchison HC, Bassendine MF, Malcolm AJ, Watson AJ, Record CO, James OFW. A pilot, double-blind controlled 1-year trial of prednisolone treatment in primary biliary cirrhosis: hepatic improvement but greater bone loss. Hepatology 1989;10:420-429.
7. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OFW. A controlled trial of prednisolone treatment in primary biliary cirrhosis; three year results. J Hepatol 1992;15:336-344.
8. Compston JE. The therapeutic use of bisphosphonates. BMJ 1994;309:711-715.
9. Storm T, Thamsborg G, Steiniche T, Genant HK, Sorensen OH. Effect of intermittent cyclical etidronate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. N Engl J Med 1990;322:1265-1271.
10. Watts NB, Harris ST, Genant HK, Wasnich RD, Miller PD, Jackson RD, Licata AA et al. Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. N Engl J Med 1990;323:73-79.
11. Reid IR, Alexander CJ, King AR, Ibbertson HK. Prevention of steroid induced osteoporosis with (3-amino-1-hydroxypropylididene)-,1-bisphosphonate (APD). Lancet 1988:i:143-146.
12. Gallagher SJ, Fenner JA, Anderson K, Bryden FM, Banham SW, Logue FC, Cowan RA et al. Intravenous pamidronate in the treatment of osteoporosis associated with corticosteroid dependent lung disease: an open pilot study. Thorax 1992;47:932-936.
13. Mulder H, Struys A. Intermittent cyclical etidronate in the prevention of corticosteroid-induced bone loss. Br J Rheumatol 1994;33:348-350.
14. Taal BG, Schalm SW, Ten Kate FWJ, Hermans J, Geertzen GM, Feltkamp BEW. Clinical diagnosis of primary biliary cirrhosis: a classification based on major and minor criteria. Hepato-gastroenterol 1983;30:178-182.
15. Delmas PD. Biochemical markers of bone turnover. J Bone Min Res 1993;8:S549-555.
16. Locascio V, Bonucci E, Imbimbo B, Ballantani P, Adami S, Milani S, Tartarotti D et al. Bone loss in response to long term glucocorticoid therapy. Bone Miner 1990;8:39-51.
17. Gennari C, Vitelli R. Glucocorticoid-induced osteoporosis. Clin Rheum Dis 1986;12:637-654.
18. Van Berkum FNR, Beukers R, Birkenhäger JC, Kooij PPM, Schalm SW, Pols HAP. Bone mass in women with primary biliary cirrhosis; the relation with histological stage and use of corticosteroids. Gastroenterology 1990;99:1134-1139.
19. Sambrook PN, Cohen ML, Eisman JA, Pocock NA, Champion GD, Yeates MG. Effects of low dose corticosteroids on bone mass in rheumatoid arthritis: a longitudinal study. Ann Rheum Dis 1989;48:535-538.
20. Clements D, Compston JE, Rhodes J, Evans WD, Smith PM. Low dose corticosteroids in chronic active hepatitis do not adversely affect spinal bone. Eur J Gastroenterol Hepatol 1993;5:543-547.
21. Reid IA, Grey AB. Corticosteroid osteoporosis. Baillière's Clinical Rheumatology 1993;7:573-588.

22. Ott SM. Clinical effects of bisphosphonates in involutional osteoporosis. J Bone Min Res 1993;8(suppl.2):S597-606.
23. Guañabens N, Parés A, Mariñoso L, Brancós MA, Piera C, Serrano S, Rivera F et al. Factors influencing the development of metabolic bone disease in primary biliary cirrhosis. Am J Gastroenterol 1990;85:1356-1362.
24. Hodgson SF, Dickson ER, Wahner HW, Johnson KA, Mann KG, Riggs BL. Bone loss and reduced bone osteoblast function in primary biliary cirrhosis. Ann Intern Med 1985;103:855-860.
25. Poupon RE, Balkau B, Eschwège E, Poupon R and the UDCA-PBC Study Group. A multicenter controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Engl J Med 1991;324:1548-1554.
26. Stellon AJ, Davies A, Compston J, Williams R. Bone loss in autoimmune chronic active hepatitis on maintenance corticosteroid therapy. Gastroenterology 1985;89:1078-1083.
27. Bonkovsky HL, Hawkins M, Steinberg K, Hersh T, Galambos JT, Henderson JM, Millikan WJ et al. Prevalence and prediction of osteopenia in chronic liver disease. Hepatology 1990;12:273-280.
28. Lindor KD, Janes CH, Crippin JS, Jorgensen RA, Dickson ER. Bone disease in primary biliary cirrhosis: does ursodeoxycholic acid make a difference. Hepatology 1995;21:389-392.
29. Wolfhagen FHJ, Van Buuren HR, Van Berge Henegouwen GP, Van Hattum J, Den Ouden JW, Kerbert MJ, Smit AM et al. and the Dutch Multicenter PBC Study Group. A randomized, placebo-controlled trial with prednisone/azathioprine in addition to ursodeoxycholic acid in primary biliary cirrhosis [Abstract]. J Hepatol 1994;21:S49.

CHAPTER IX

-DISCUSSION-

THE TREATMENT OF PRIMARY BILIARY CIRRHOSIS A.D 1995

Frank H.J. Wolfhagen, Henk R. van Buuren, Solko W. Schalm

Department of Internal Medicine II, University Hospital Dijkzigt, Rotterdam

Parts of this chapter have been published in: Van Buuren HR, Wolfhagen FHJ, Schalm SW. Treatment with ursodeoxycholic acid, as monotherapy and in combination with other agents, in primary biliary cirrhosis. In: Cholestatic Liver Diseases: New strategies for prevention and treatment of hepatobiliary and cholestatic liver diseases. Eds. Van Berge Henegouwen GP, De Groote J, Van Hoek B, Matern S, Stockbrügger RW. Kluwer Academic Publishers, Dordrecht 1994; pp 236-45.

1. INTRODUCTION

Primary Biliary Cirrhosis (PBC) is a chronic, cholestatic autoimmune liver disease of unknown origin characterized by a non-suppurative destruction of interlobular and septal bile ducts (1). As a consequence of the bile duct destruction, endogenous bile salts will accumulate and hepatotoxic species, such as chenodeoxycholic acid, will cause further liver damage (2,3). The majority of patients will develop biliary liver cirrhosis, which may eventually lead to death or liver transplantation. The natural course of the disease is variable and protracted and there is no proven method to predict, in an early phase of the disease, which patient will eventually have a fatal outcome.

Once patients reach end stage disease, liver transplantation should be considered and PBC nowadays is one of the main indications for this operation (4).

Medical treatment, directed at halting disease progression, has been largely disappointing (5-25). Table 1 provides a summary of randomized controlled trials, which have evaluated the efficacy of a wide variety of drugs in PBC.

The experiences with ursodeoxycholic acid, which was found to improve clinical, biochemical and histological features of the disease, revived the hope for an effective medical treatment for PBC. However, ursodeoxycholic acid is thought to act mainly on the secondary, bile salt mediated damage; therefore the drug is unlikely to induce disease remissions or affect the natural course of the disease in a major way (26). A combination of ursodeoxycholic acid with other drugs, preferably immunosuppressives, might be more effective (2,26-29).

In the following we will discuss whether, during the last 5 years, UDCA has lived up to its promises. Furthermore the results of treatment regimens combining UDCA with other drugs, and future treatment options will be discussed.

Table 1. **Controlled (single) drug trials in Primary Biliary Cirrhosis, other than ursodeoxycholic acid; effects on symptoms, liver tests, histology and survival (or need of liver transplantation). N.e. = not evaluable/evaluated; + = improvement; ± = trend or not consistent; - = no improvement. The degree of intolerance has been estimated, based on the percentage of withdrawals/drop-outs in the treatment group as compared to the placebo group (++ = >10% and >2x the percentage in the placebo group;+ = >10% and >1.5x;- = ≤10% or ≤1.5x).**

Drug (references)	No. of studies	Total no. patients	Improvement *symptoms*	*liver functions*	*histology*	*survival*	Intolerance
Penicillamine(5-12)	8	740	-	±	-	-	++
Colchicine (13-15)	3	181	-	±	-	±	+
Chlorambucil (16)	1	24	n.e.	+	-	n.e.	++
Malotilate (17)	1	101	n.e.	+	+	-	++
Thalidomide (18)	1	18	-	-	-	n.e.	++
Methotrexate[1] (19)	1	87	-	+	+	n.e.	n.e
Prednisolone (20)	1	36	+	+	+	n.e.	-
Azathioprine (21)	1	45	+	±	-	n.e.	+
(22)	1[2]	248	+	n.e	n.e.	+ (5 yr: 41%↓†)[3]	-
Cyclosporin (23,24)	2	41	±	+	-	n.e.	+
(25)	1[2]	349	+	+	-	+ (5 yr: 39%↓†/Tx)	+

[1] = MTX vs. colchicine (ongoing);
[2] = presented more explicitly as they are the only studies in which survival could be evaluated with confidence;
[3] = after adjustment for unequal bilirubin levels at entry;
↓†/Tx = reduction in death/liver transplantation.

2. UDCA

A large quantity of clinical as well as basic research with regard to UDCA has been published during the last years. Apart from gallstone disease and PBC, UDCA has been administered with encouraging results in a wide variety of disorders, mostly with a cholestatic component, such as primary sclerosing cholangitis, cystic fibrosis, hepatitis C, alcoholic liver disease, graft versus host disease and in the prevention of liver graft rejection (31). Here, we will focus on the use of UDCA in PBC.

2.1 Working mechanism

The precise working mechanism of UDCA is still not completely understood, but several actions seem to be involved. Support has mainly been found for three concepts, i.e. modulation of the bile salt pool, direct hepatoprotection and immunomodulation (31).

Hydrophobic bile salts can induce cell damage and cholestasis (32,33). The hydrophilic UDCA has a higher critical micellar concentration and lower cell surface activity than endogenous hydrophobic bile acids. These properties result in a lower cell damaging capacity of UDCA (3,31,34). Therefore, enrichment of the bile acid pool with UDCA at the expense of hydrophobic bile acids is likely to reduce hepatic damage. Indeed, during UDCA administration UDCA conjugates form the largest proportion of bile salts in bile as well as in serum (3,35,36). UDCA treatment results in decreased reabsorption of endogenous bile acids from the gut (37-39); furthermore it enhances the elimination of bile salts, both by improving hepatocellular bile salt excretion (40) and by producing a bicarbonate-rich hypercholeresis, probably resulting from cholehepatic recycling of protonated UDCA (31). The latter mechanism, though, has only been documented in rats and seems of minor significance in humans (41-43).

Several workers found the decrease in endogenous bile acids to be mainly at the expense of cholic acid, a relatively hydrophilic bile acid, while chenodeoxycholic acid, the main hydrophobic constituent of the bile acid pool, remained unaltered (37,44). In contrast to these reports Poupon et al. recently found UDCA to decrease the proportion of

chenodeoxycholic acid in 150 patients participating in a placebo controlled trial with UDCA (45). Analysis of the biliary bile salt composition in 27 patients with PBC and PSC (36) showed a significant decrease in the hydrophobicity index according to Heuman (46). So, although not all studies are univocal, a net shift towards hydrophilicity in the bile acid pool seems likely.

UDCA also seems to exert direct, hepatoprotective effects. It has been shown that UDCA reduces the cytolysis induced by other bile salts (31-33,43,47). In vitro studies showed that one potential cytoprotective mechanism may be the binding of UDCA to cellular membranes, thus decreasing membrane polarization and solubilization caused by hydrophobic bile salts (48,49). Whether the basolateral membrane (48) or the canalicular membrane (49) is the major site of this cytoprotective action is still controversial. In support of the latter we found that biochemical improvement was related to the proportion of UDCA in bile but not in serum (36). Furthermore there is evidence that UDCA may also have hepatoprotective effects at the intracellular level; UDCA has been reported to reduce the impairment of mitochondrial function by hepatotoxic bile salts (50), to decrease intracellular concentrations of toxic endogenous bile salts by improving their hepatic excretion (40), and to modulate intracellular Ca^{2+} metabolism (51).

Accumulating data indicate that UDCA also has immunomodulating properties. The first indication was the fact that UDCA significantly reduced increased serum IgM levels in PBC (52). Then Calmus reported that UDCA reduces the aberrant HLA-1 expression on hepatocytes (53). In PBC, aberrant hepatocytic HLA-1 expression, as well as increased HLA-II expression on biliary epithelium, has been documented (54,55). HLA antigens are essential in the antigen recognition in immune processes. HLA-1 antigens are required in the lysis of target cells by cytotoxic T-lymphocytes while T-helper cells can only recognize antigens in association with HLA-II (56). Increased HLA-I, but not HLA-II expression, may be secondary to cholestasis as it has also been found in extrahepatic obstruction (57). The recent reports that, in contrast to Calmus' findings, UDCA also reduces HLA-

II expression on biliary epithelium, is therefore even more interesting (58,59).

In vitro and in vivo studies have found UDCA to decrease the production of cytokines and immunoglobulins by mononuclear cells as well as lymphocyte proliferation (60,61). Normalisation of reduced levels of interleukin-2 and surface expression of dipeptidylpeptidase IV (CD26), an activation marker of lymphocytes, also suggest immunomodulating mechanisms (62).

Recently the role of intercellular adhesion molecule-1 (ICAM-1) in immune reactions has been recognized. ICAM-1 and its lymphocytic ligand LFA-1 (Lymphocyte Function-associated Antigen 1) are important in lymphocyte migration towards and adhesion to target cells. In inflammatory circumstances, cytokines induce and enhance ICAM-1 cell surface expression on lymphocytes and a variety of other cell types (63,64). In PBC, increased ICAM-1 expression has been found on hepatocytes, and more specifically, biliary epithelium (65).

Biologically active circulating soluble forms of ICAM-1 (sICAM-1) have been identified, which can be shedded by activated lymphocytes and, as has only recently been shown, by other cells such as endothelial cells (66) and hepatocytes (67), but apparently not biliary epithelium (68). sICAM-1 levels are very high in PBC and correlate with stage and severity of disease (68,69). Although the exact origin and role of sICAM-1 is still unknown, it has been suggested that sICAM-1 levels may be useful in the investigation, diagnosis and monitoring of inflammatory and immune disorders, and several studies in a variety of diseases have supported this idea (70-75). We found that UDCA appears to reduce sICAM-1 levels in PBC, and that further reduction can be achieved by combined prednisone/azathioprine therapy (76). In contrast, methotrexate did not reduce sICAM-1 levels in PBC (77).

The significance of these immunomodulating properties remains to be established, as they appear very heterogenous and it is not clear to what extent they are due to direct interference with the immune system, or to reduction of immune aberrations secondary to cholestasis (78).

2.2 Effects on liver function tests, symptoms and histology.

Regarding PBC, a large number of controlled studies has now been published confirming the initial observations of Leuschner and co-workers (79) that UDCA improves liver function tests, particularly alkaline phosphatase, gamma-glutamyl transferase and transaminases (52, 80-88; table 2). UDCA also seems to lower or stabilize serum bilirubin. Furthermore serum IgM, which is thought to reflect immune reactivity, also decreases during UDCA treatment.

Poupon in 1991 (52) claimed an effect of UDCA on pruritus, however this finding failed to reach significance. The results of other trials also indicate at most mild improvement of pruritus by UDCA as compared to placebo (81,85-88). Only Hadzyannis et al. (82) found significantly greater ameliorations of pruritus in the UDCA treated patients. Of the six studies reporting on fatigue, again, Hadzyannis was the only one who noted a favourable effect of UDCA.

Six groups reported data on histology (52,80,82,86-88). None of them could report a convincing effect on fibrosis and histological progression. Four groups looked at a variety of histological features. One did not find any difference between groups (82), three found differences in favour of UDCA (52,80,88), although in one these did not reach significance (80). Differences were noted for bile duct paucity and proliferation, degree of portal and lobular inflammation and piece meal necrosis.

Some authors have suggested lower or no efficacy of UDCA in patients with cirrhosis (82,89). We found that, in patients with Child-Pugh Class A cirrhosis, UDCA treatment induces a clinical and biochemical response comparable to that in non-cirrhotic patients. Therefore, based on the available data, UDCA should not be denied to these patients (90).

Table 2. **Controlled trials (published as full article) with ursodeoxycholic acid in Primary Biliary Cirrhosis: effects on symptoms, liver tests, histology, disease progression and survival/need of liver transplantation (n.e. = not evaluable/evaluated;+ = improvement; ± = trend or not consistent;- = no improvement)**

Author year (ref)	Dose (mg/day)	No. of patients (n=)	Follow up (months)	Symptoms	Liver functions [bilirubin]	Histology *inflammation/fibrosis*	Signs of progression[1]	Survival/ liver Tx
Leuschner 1989 (80)	10/kg	20	9	±	+ [n.e.]	± / -	n.e.	n.e.
Oka 1990 (81)	600	45	6	-	+ [-]	n.e / n.e.	-	n.e.
Hadzyannis 1991 (82)	12-15/kg	50	mean 29	+	+ [±]	- / -	-	-/-
Poupon 1991/1994 (52,83)	13-15/kg	146	48[2]	±	+ [+]	+ / -	+	-/+
Hwang 1993 (84)	600	12	3[3]	±	+ [+]	n.e. / n.e.	n.e.	n.e.
Battezzati 1993 (85)	500	88	6	-	+ [+]	n.e. / n.e.	-	n.e.
Turner 1994 (86)	10/kg	46	24	-	+ [+]	- / ±	-	-/-
Lindor 1994 (87)	13-15/kg	180	mean 24	-	+ [+]	n.e. / -	+	-/-
Heathcote 1994 (88)	14/kg	222	24	-	+ [+]	+ / -	-	-/-

[1] signs of progression: hepatic decompensation e.g. ascites, variceal bleeding, encephalopathy

[2] last 2 years uncontrolled

[3] cross-over design with 3 month-periods

2.3 Tolerance and adverse effects

UDCA is generally considered to be a very safe drug with virtually no side effects. In the published controlled trials (52,80-88), 5% of UDCA treated patients reported adverse effects, mostly (transient) diarrhea or worsening of pruritus. Three percent of these patients discontinued treatment. Moreover, comparable percentages of side effects and withdrawals were noted in the placebo treated patients (4 and 3% respectively).

However, some workers observed acceleration of the disease in cirrhotic PBC patients (91,92). Review of these reports indicate that most of these patients had clinically advanced disease. In contrast, UDCA appears safe in Child-Pugh class A patients (90). We believe UDCA is contraindicated in patients with decompensated cirrhosis (Child-Pugh Class B or C) (93), because of the improbability of beneficial effects and possible deterioration. Liver transplantation should be the primary consideration in these patients.

2.4. Effect on disease progression

Several relatively large placebo-controlled trials (83,87,88) have indicated that treatment failures (endpoints such as rise in bilirubin, development of ascites, variceal bleeding, progression to cirrhosis, death or liver transplantation) occur about twice as often in placebo treated patients than in UDCA treated patients (figure 1). These findings were confirmed by a recent meta-analysis of UDCA treatment in PBC (94). However, a clear improvement in survival and reduction in need for transplantation could not be substantiated yet. Poupon noted a significant reduction in the need of liver transplantation in those patients who had received 4 years of UDCA as compared to those who had received placebo during the initial 2 years followed by 2 years of UDCA treatment (83). These results should be interpreted cautiously as the decision to perform liver transplantation is rather subjective and the trial was not double blind at that stage.

It should be stressed that all of these studies had relatively short follow-up periods (up to 4 years) and lacked the power to assess effects on survival. Hopefully, the pending meta-analysis of the raw data of four large controlled studies (83,87,88,95) will shed more light in the dark.

2.5. Alternative assessment of treatment efficacy

The golden standards for the assessment of therapeutic efficacy are improvement of survival (and, in case of PBC, reduction of need for liver transplantation) and quality of life. The relative rarity of PBC and its unpredictable and protracted natural course (often over 10-20 years) limit the feasibility to assess survival in controlled trials. Therefore alternative endpoints or markers indicative of treatment efficacy are warranted to identify potentially effective treatments. However, positive results still require subsequent confirmation by long term controlled studies (96,97).

The most important prognostic markers currently in use are bilirubin, albumin and clotting parameters, or scores and indexes containing -more or less- all of these variables (22,93,98-100). Recently published models offer the possibility to adjust the estimated prognosis during the course of the disease (99,100). However, these

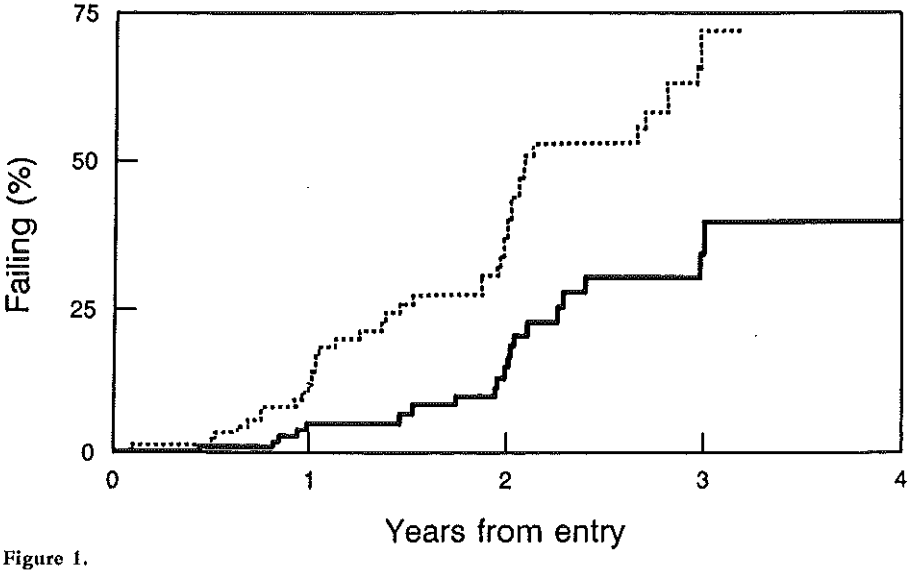


Figure 1.
Time to treatment failure (defined as: death, liver transplantation, histological progression by 2 stages or to cirrhosis; development of varices, ascites, or encephalopathy; doubling of serum bilirubin to > 1.5 mg/dl; marked worsening of fatigue or pruritus; drug intolerance; voluntary withdrawal) for 89 patients treated with UDCA (—) compared to 91 patients with placebo (- - -). P=0.0003, log rank test. (Taken from reference 87 with permission of the authors)

models have been developed in untreated patients and whether they are also applicable to treated patients is not clear yet. UDCA may lower serum bilirubin, the major component of all models, by increasing its biliary excretion; therefore, the prognostic value of these models may be limited in patients taking UDCA (97). Despite these considerations, these models seem useful in the difficult process of timing of liver transplantation.

Patients are likely to benefit from medical treatment only during the early phase of the disease. The above mentioned prognostic indicators only become disturbed in the later stages of the disease. Therefore, other indicators of efficacy of medical treatments are required.

One alternative may be to study the potential of a treatment to induce complete disease remission (27). This is a well-established concept in hematological diseases such as leukemia and malignant lymphomas, and in autoimmunological disorders like chronic autoimmune hepatitis (101). When using criteria for remission, as proposed by Beukers and Schalm (27), less than 5% of PBC patients will achieve a complete remission during UDCA treatment (102). However, these strict criteria are arbitrary and await further validation.

Markers of fibrogenesis, like procollagen-III-propeptide (P-III-P) and hyaluronic acid, may be appropiate alternatives (103). Particularly P-III-P has been reported by several independent groups to have prognostic significance with regard to survival and development of complications of liver disease (104-106). Measurements of P-III-P and hyaluronic acid levels in one uncontrolled study showed no effect of UDCA treatment (89).

The use of histological features, like degree of fibrosis, as surrogate parameters is hampered by sampling error and the invasiveness of the procedure. Moreover, improvements in bile ductular and inflammatory aspects, as reported for UDCA, lack prognostic significance.

Dynamic liver function tests (e.g. bromosulpthalein clearance test, aminopyrine breath test, galactose excretion test) may become important prognostic parameters, but at this moment their significance in patients with non-advanced disease is unclear (97).

In the future, other parameters such as sICAM-1 may also show to be suitable for monitoring treatment effects in PBC.

2.6. How to administer UDCA?

The optimal way of UDCA administration has not been completely elucidated yet; there is a large variation in the doses used by different groups (table 2). Although one short-term, cross-over study in a small group of PBC patients indicated no extra benefit of 750 mg UDCA over 500 mg per day (107), good dose response studies in cholestatic disorders have not been performed and the dosage of 10-15 mg/kg as is being used now may be suboptimal. For example in cystic fibrosis the optimal dosage seems to be 20 mg/kg/d (108).

Furthermore, it was recently suggested by some authors, that a divided daily dose should be applied, since in experiments with a single gift of UDCA the proportion absorbed in the ileum decreased with higher doses (109). We found no difference between a divided dose and a single bedtime dose with regard to biochemical improvements or biliary UDCA enrichment during chronic UDCA administration (36); therefore, in view of the better patient compliance, a single bedtime dose, taken with a small snack to improve absorption, appears to be preferable.

Further studies to establish the optimal way of UDCA administration may lead to an increased therapeutic efficacy.

3 COMBINATION THERAPY WITH UDCA

In view of the limited effects of single drug therapy in PBC, it has been suggested to turn to the evaluation of combinations of drugs with at least partial efficacy in the treatment of PBC (27-29)

Next to UDCA a number of drugs, e.g. chlorambucil, cyclosporine, prednisone, azathioprine, methotrexate, colchicine and malotilate have been shown to have beneficial effects in PBC as evidenced by (at least) amelioration of liver function abnormalities. The toxicity of the immunosuppressive drugs chlorambucil (bone marrow depression) and cyclosporine (nefrotoxicity) seems to outweigh the beneficial effects and these agents are therefore considered less suitable options (16,23-25,110). Another drug with immunosuppressive properties, thalidomide, has been tried in PBC but showed no favoura-

ble effects on symptoms, liver tests and histology (18).

Recently, a placebo-controlled study evaluating malotilate (17), a drug with presumed hepatoprotective and antifibrotic properties, documented moderate improvements of laboratory parameters and histological inflammation. Adverse effects were relatively frequent and one patient developed a serious toxic hepatitis. Moreover, malotilate is not commercially available outside Japan.

Some authors have already reported data on combinations of UDCA with methotrexate (114-116), colchicine (117-121) and prednisone (26)(table 3). Methotrexate may be effective in early stage PBC as has been suggested by several small studies (111-113). Kaplan evaluated the combination of methotrexate (15 mg weekly) with UDCA in 14 PBC patients and found additional effects on biochemistry, fatigue and itching (115). Buscher et al. (116) also studied the effects of methotrexate added to previous treatment with UDCA. Alkaline phosphatase improved in 6 of the 8 patients. As reported by others, transaminases initially rose but subsequently decreased. Symptoms also improved after initial deterioration. However, methotrexate may have severe adverse effects. PBC patients seem to be particularly susceptible to interstitial pneumonitis and this has been reported in up to 14% of treated patients (122).

Colchicine, a drug with anti-inflammatory and antifibrotic properties, is well tolerated and has been shown to improve biochemistry in PBC. Moreover some authors have previously suggested slowing of progression (13,15), which was not supported by a study with long term follow-up of colchicine treated patients (123). In contrast to one uncontrolled study (121), four placebo-controlled trials did not show clear additional effects of colchicine to UDCA with respect to symptoms and biochemistry (117-120).

Scarce data in mostly advanced PBC patients indicated that major improvements could not be induced by corticosteroid treatment (124-126). It has even been suggested that response to prednisone is an important determinator in the differential diagnosis of PBC and autoimmune hepatitis (127). Moreover prednisone has long been considered contraindicated in PBC, mainly because of its negative effects on the

bone status (128). The partial effect of immunosuppression in PBC, may be related to the role of accumulated toxic bile salts in liver damage, which is not obviated by immunosuppression. With respect to bone mass, more recent data have shown that, although bone loss is enhanced during the first year of treatment (129-131), negative effects on bone during long term treatment with low dose corticosteroids seem to be absent or mild (19,132-134). Furthermore, steroid induced bone loss in PBC appears to be prevented by cyclical etidronate, an anti-bone resorptive drug belonging to the class of bisphosphonates (135). Similar observations with bisphosphonates have been made in other steroid treated disorders (136-138).

Another way to minimize adverse effects while maintaining the immunosuppressive potential is to combine low doses of prednisone and azathioprine. Azathioprine has immunosuppressive properties and has been shown to improve survival in PBC, albeit to a minor extent (21).

A combination of prednisone (initially 30 mg/d reduced in 2 months to 10 mg/d maintenance dose), and azathioprine (50 mg/d) was added to UDCA in a placebo-controlled study in 36 patients who had not achieved remission with UDCA alone (139). Pruritus, alkaline phosphatase, ASAT, IgM, P-III-P, soluble ICAM-1 as well as histological inflammation improved significantly on top of the previous effects of UDCA alone. As mentioned above, steroid enhanced bone loss seemed to be prevented with cyclical etidronate. Weight gain was the major adverse effect. In 3 prednisone treated patients cosmetic changes were considered problematic and were accompanied by increases in blood pressure; two patients in the placebo group were withdrawn because they became in need of liver transplantation while in the treatment group 3 patients withdrew within one month (2 general malaise, 1 spontaneous bacterial peritonitis). In a chronic disease like PBC, a high induction dose may not be necessary and in future studies the risk of adverse events may be reduced by abolishing this high dose. Based on the short term benefit/risk ratio larger, long term controlled studies with this regimen, under bisphosphonate protection, seem indicated to assess its effect on disease progression (139).

Table 3. **Studies evaluating the addition of other agents to UDCA in PBC**

Agents	Author	Year	Reference	Type of study	No of patients	Follow-up (months)	Additional beneficial effects to UDCA (symptoms,biochemistry,histology)	
Methotrexate	Kaplan	1992	115	Open	14	mean 17	suggestive	(s,b)
	Buscher	1993	116	Open	8	6	suggestive	(s,b)
Colchicine	Raedsch	1992	117	RCT	22	12	no	(s,b,h)
	Podda	1992	118(A)	RCT	88	?[1]	no	(s,b)
	Poupon	1994	119(A)	RCT	74	24	no	(s,b,h)[2]
	Goddard	1994	120(A)	RCT	57	mean 15	no	(s,b)[3]
	Shibata	1992	121	Open	12	24	suggestive	(b)
Prednisone + Azathioprine	Wolfhagen	1995	139	RCT	36	12	yes	(s,b,h)

RCT = randomized placebo-controlled trial
(A) = published in abstract form
[1] ongoing trial
[2] bromsulphatelein clearance improved in colchicine group ($p<0.01$)
[3] four randomized groups; UDCA, colchicine, UDCA+colchicine, placebo; lower decrease of albumine in UDCA+colchicine treated group ($p=0.02$)

4 FUTURE TREATMENT OPTIONS

To establish a specific, curative medical therapy for PBC, increased knowledge on the etiology and pathophysiology of the disease and identification of the antigens involved in the primary immune attack is needed. However, as long as this knowledge is not available, therapy should aim at optimal suppression of disease activity and halting progression, preferably in an early stage of the disease. Such treatments should have a low toxicity profile, like UDCA.

In this respect, we believe that the long term evaluation of the combination of UDCA with corticosteroids and azathioprine under bisphosphonate protection is promising. It remains to be established whether it is preferable to replace prednisone by newly synthesized corticosteroids with (presumed) relatively bone sparing properties (e.g. deflazacort) in PBC (140-142).

Evaluation of other drug combinations could be worthwhile too. Methotrexate is still an option, but the problem of the high incidence of interstitial pneumonitis should be adressed. Combination with cyclosporin-like immunosuppressives such as FK 506 or new antifibrotic drugs (HOE 077) may also be attractive for future research.

Advances in molecular biology may disclose new potential treatment options aiming at specific factors involved in inflammatory, immunological and fibrogenetic processes like cytokines and adhesion molecules (143-151).

Experimental research in animals has indicated that autoimmune disorders may be cured by bone marrow transplantation (152). Several cases of remissions of autoimmune diseases (psoriasis, arthritis, ulcerative colitis) following bone marrow transplantation for a coexisting disorder, have been reported (153-155).

To obtain an optimal benefit/risk ratio, treatment should preferably be instituted in an early stage of the disease, in particular before cirrhosis has developed. Furthermore a method to predict, in an early stage, whether a patient will show progressive disease would be very valuable, especially when considering more invasive interventions such as bone marrow transplantation. The development of such models is therefore of utmost importance.

5 CONCLUSION

During the last five years there has been substantial progress in the medical treatment of PBC. UDCA tends to reduce symptoms, improves liver tests and histological features and seems to slow down disease progression, albeit to a modest extent. Moreover, UDCA is a very safe drug. Despite its limitations, these properties justify the use of UDCA as current standard treatment in non-advanced PBC.

Furthermore, in contrast with previous believe, prednisone, when given in combination with azathioprine and UDCA, has been shown to have clear beneficial effects in PBC while enhanced bone loss appears to be manageable by the use of bisphosphonates. We believe that our current results justify the initiation of long term studies to evaluate this triple treatment regimen with UDCA, prednisone and azathioprine.

Further research regarding the etiology and pathophysiology of the disease is required to enable the eventual development of a curative treatment for PBC, other than liver transplantation.

REFERENCES

1. Kaplan MM. Primary Biliary Cirrhosis. N Engl J Med 1987;316:521-8.
2. De Caestecker JS, Jazrawi RP, Petronei ML, Northfield TC. Ursodeoxycholic acid in chronic liver disease. Gut 1991;32:1061-5.
3. Hofmann AF. Bile acid hepatotoxicity and the rationale of UDCA therapy in chronic cholestatic liver diseases: some hypotheses. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990; pp.13-33.
4. Markus BH, Dickson ER, Grambsch PM, Fleming TR, Mazzaferro V, Klintmalm GBG et al. Efficacy of liver transplantation in patients with primary biliary cirrhosis. N Engl J Med 1989;320:1709-13.
5. Triger DR, Manifold IH, Underwood JCE. D-Penicillamine in primary biliary cirrhosis: two year results of a single centre, double blind controlled trial. Gut 1980;21:919-20.
6. Epstein O, Jain S, Lee RG, Cook DG, Boss AM, Scheuer PJ et al. D-penicillamine treatment improves survival in primary biliary cirrhosis. Lancet 1981:i:1275-7.
7. Bassendine MF, Macklon AF, Mulcahy R, James OFW. Controlled trial of high and low dose D-penicillamine in primary biliary cirrhosis: results at 3 years. Gut 1982;23:909 (Abstract).
8. Matloff DS, Alpert E, Resnick RH, Kaplan MM. A prospective trial of D-penicillamine in primary biliary cirrhosis. N Engl J Med 1982;306:319-26.
9. Taal BG, Schalm SW, Ten Kate FWJ, Van Berge Henegouwen GP, Brandt KH. Low therapeutic value of D-penicillamine in a short term prospective trial in primary biliary cirrhosis. Liver 1983;3:345-52.
10. Dickson ER, Fleming TR, Wiesner RH, Baldus WP, Fleming TR, Ludwig JL et al. Trial on penicillamine in advanced primary biliary cirrhosis. N Engl J Med 1985;312:1011-5.
11. Neuberger J, Christensen E, Portmann B, Cabballeria J, Rodes J, Ranek L et al. Double-blind controlled trial of D-penicillamine in patients with primary biliary cirrhosis. Gut 1985;26:114-9.
12. Bodenheimer HC, Schaffner F, Sternlieb J, Klion FM, Vernace S, Pezzulo J. A prospective clinical trial of D-penicillamine in the treatment of primary biliary cirrhosis. Hepatology 1985;5:1139-42.
13. Kaplan MM, Alling DW, Zimmerman HJ, Zimmerman HJ, Wolfe HJ, Speresky RA, Hirsch GS et al. A prospective trial of colchicine for primary biliary cirrhosis. N Engl J Med 1986:315:1448-54.
14. Bodenheimer H, Schaffner F, Pezullo J. Evaluation of colchicine therapy in primary biliary cirrhosis. Gastroenterology 1988;95:124-9.
15. Warnes TW, Smith A. Lee FI, Haboubi NY, Johnson PJ, Hunt L. A controlled trial of colchicine in primary biliary cirrhosis. J Hepatol 1987:5:1-7.
16. Hoofnagle JH, Davis GL, Schafer DF, Peters M, Avigan MI, Pappas SC et al. Randomized trial of chlorambucil for primary biliary cirrhosis. Gastroenterology 1986;91:1327-34.
17. Van Buuren HR, Schalm SW, Triger DR, James OFW and the European Multicentre Study Group. The results of a randomized double blind controlled trial evaluating malotilate in primary biliary cirrhosis, J Hepatol 1993;17:227-35.
18. McCormick PA, Scott F, Epstein O, Burroughs AK, Scheuer PJ, McIntyre N. Thalidomide as therapy for primary biliary cirrhosis: a double-blind, placebo controlled study. J Hepatol 1994;21:496-9.
19. Kaplan M, Schmid C, McKusick A, Provenzale D, Sharma A, Sepe T. Double blind trial of methotrexate versus colchicine in primary biliary cirrhosis. Hepatology 1993; :176A (Abstract)
20. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OFW. A controlled trial of prednisolone treatment in primary biliary cirrhosis: three-year results. J Hepatol 1992;15:336-44.
21. Heathcote J, Ross A, Sherlock S. A prospective controlled trial of azathioprine in primary biliary cirrhosis. Gastroenterology 1976;70:656-60.
22. Christensen E, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B et al. Beneficial effect

of azathioprine and prediction of prognosis in primary biliary cirrhosis: final results of an international trial. Gastroenterology 1985;89:1084-91.
23. Minuk GY, Bohme CE, Burgess E, Hershfield NB, Kelly JK, Shaffer EA et al. Pilotstudy of cyclosporine A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988;95:1356-63.
24. Wiesner RH, Ludwig J, Lindor KD, Jorgensen RA, Baldus WP, Homburger HA et al. A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. N Engl J Med 1990;32:119-24.
25. Lombard M, Portmann B, Neuberger J, Williams R, Tygstrup N, Ranek L et al. Cyclosporin A treatment in primary biliary cirrhosis: results of a long-term placebo controlled trial. Gastroenterology 1993;104:519-26.
26. Wolfhagen FHJ, Van Buuren HR, Schalm SW. Combined treatment with ursodeoxycholic acid and prednisone in primary biliary cirrhosis. Neth J Med 1994;44:84-90.
27. Beukers R, Schalm SW. Immunosuppressive therapy for primary biliary cirrhosis. J Hepatol 1992;14: 1-6.
28. Kaplan MM. New strategies needed for treatment of primary biliary cirrhosis. Gastroenterology 1993;104:651-3.
29. Jansen PLM. Antifibrotic therapy of liver cirrhosis, with special reference to primary biliary cirrhosis. Neth J Med 1992;40:209-14.
30. Rubin AR, Kowalski TE, Khandelwal M, Malet PF. Ursodiol for hepatobiliary disorders. Ann Intern Med 1994;121:207-18.
31. Van Erpecum KJ, Van de Meeberg PC, Van Berge Henegouwen GP. Rationale for therapy with ursodeoxycholic acid in patients with cholestatic liver disease. Neth J Med 1993;43:233-8.
32. Heuman DM, Mills AS, McCall J, Hylemon PB, Pandak WM, Vlahcevic ZR. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts. Gastroenterology 1991;100:203-11.
33. Galle PR, Theilmann L, Raedsch R, Otto G, Stiehl A. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human cultures. Hepatology 1990;12:486-91.
34. Queneau P-E, Montet J-C. Hepatoprotection by hydrophilic bile salts. J Hepatol 1994;21:260-8.
35. Stiehl A, Rudolph G, Raedsch R, Möller B, Hopf U, Lotterer E et al. Ursodeoxycholic acid-induced changes of plasma and urinary bile acids in patients with primary biliary cirrhosis. Hepatology 1990;492-7.
36. Van de Meeberg PC, Wolfhagen FHJ, Van Erpecum KJ, Salemans JMJJ, Tangerman A, Schalm SW, Van Berge Henegouwen GP. Single or multiple dose ursodeoxycholic acid for cholestatic liver disease: relation with biliary enrichment and biochemical response. Submitted.
37. Stiehl A, Raedsch R, Rudolph G. Acute effects of ursodeoxycholic acid and chenodeoxycholic acid on the small intestinal absorption of bile acids. Gastroenterology 1990;98:424-8.
38. Marteau P, Chazouilléres O, Myara A, Jian R, Rambaud JC, Poupon R. Effect of chronic administration of ursodeoxycholic acid in the ileal absorption of endogenous bile acids in man. Hepatology 1990;12:1206-8.
39. Eusufzai S, Ericsson S, Cederlund T, Einarsson K, Angelin B. Effect of ursodeoxycholic acid treatment on ileal absorption of bile acids in man as determined by the SeHCAT test. Gut 1991;32: 1044-8.
40. Jazrawi RP, De Caestecker JS, Goggin PM, Brittern AJ, Joseph AEA, Maxwell JD et al. Kinetics of hepatic handling in cholestatic liver disease: effect of ursodeoxycholic acid. Gastroenterology 1993;106:134-42.
41. Erlinger S, Dumont M. Influence of ursodeoxycholic acid on bile secretion. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990 ; pp.35-42.
42. Kitani K, Kanai S. Effect of ursodeoxycolate on the bile flow in the rat. Life Sci 1982;31:1973-5.

43. Heuman DM. Hepatoprotective properties of ursodeoxycholic acid. Gastroenterology 1993;104:1865-70.
44. Crosignani A, Podda M, Battezzati PM et al. Changes in bile acid composition in patients with primary biliary cirrhosis induced by ursodeoxycholic acid administration. Hepatology 1991;14:1000-7.
45. Poupon RE, Chrétien Y, Poupon R, Paumgartner G. Serum bile acids in primary biliary cirrhosis: effect of ursodeoxycholic acid therapy. Hepatology 1993;17:599-604.
46. Heuman DM. Quantitative estimation of the hydrophylic-hydrophobic balance of mixed bile salt solutions. J Lipid Res 1989;30:719-30.
47. Kitani K. Hepatoprotective effect of ursodeoxycholate in experimental animals. In: Paumgartner G, Stiehl A, Barbara L, Roda E, eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht: Kluwer Academic, 1990 ; pp.43-60.
48. Güldütuna S, Zimmer G, Imhof M, Bhatti S, You T, Leuschner U. Molecular aspects of membrane stabilization by ursodeoxycholate. Gastroenterology 1993;104:1736-44.
49. Heuman DM, Bajaj R. Ursodeoxycholate conjugates protect against disruption of cholesterol-rich membranes by bile salts. Gastroenterology 1994;106;1333-41.
50. Krähenbühl S, Fischer S, Talos C, Reichen J. Ursodeoxycholate protects oxidative mitochondrial metabolism from bile acid toxicity: dose response study in isolated rat liver mitochondria. Hepatology 1994;20:1595-1601.
51. Beuers U, Nathanson MH, Boyer JL. Effects of tauroursodeoxycholic acid on cytosolic Ca^{2+} signals in isolated rat hepatocytes. Gastroenterology 1993;104:604-612.
52. Poupon RE, Balkau B, Eschwège E, Poupon R and the UDCA-PBC Study Group. A multicenter controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Engl J Med 1991;324:1548-54.
53. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-5.
54. Ballardini G, Mirakian R, Bianchi FB, Pisi E, Doniach B, Bottazzo GF. Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. Lancet 1984;2:1009-13.
55. Barbatis C, Woods J, Morton JA, Fleming KA, McMichael A, McGee JOD. Immunohistochemical analysis of HLA(A,B,C) antigens in liver disease using a monoclonal antibody. Gut 1981; 22:985-91.
56. Rose NR. The concept of autoimmunity and autoimmune disease. In: Krawitt EL, Wiesner RH, eds. Autoimmune liver disease. Raven Press, New York, 1991; pp 1-20.
57. Calmus Y, Arvieux C, Gane P, Boucher E, Nordlinger B, Rouger P et al. Cholestasis induces major histocompatibility complex class I expression in hepatocytes. Gastroenterology 1992;102:1371-7.
58. Lo SK, Chapman RW, Dooley JS, Fleming KA. Aberrant HLA-DR antigen expression by bile duct epithelium in primary sclerosing cholangitis is down-regulated by ursodeoxycholic acid. Gastroenterology 1993;4:A943 (Abstract).
59. Leuschner U, Dienes HP, Güldütuna S, Birkenfeld G, Leuschner M. Ursodeoxycholic acid influences immune parameters in patients with primary biliary cirrhosis. Hepatology 1990;12:no 477(abstract)
60. Yoshikawa M, Tsujii T, Matsumura K, Yamao J, Matsumura Y, Kubo R et al. Immunomodulatory effects of ursodeoxycholic acid on immune responses. Hepatology 1992;16:358-64.
61. Lacaille F, Paradis K. The immunosuppressive effect of ursodeoxycholic acid: a comparative in vitro study on human peripheral blood mononuclear cells. Hepatology 1993;18:165-72.
62. Kürtschiev D, Subat S, Adler D, Schentke K-U. Immunomodulating effect of ursodeoxycholic acid therapy in patients with primary biliary cirrhosis. J Hepatol 1993;18:373-7.

63. Adams DH, Shaw S. Leucocyte-endothelial interactions and regulation of leucocyte migration. Lancet 1994;343:831-6.
64. Singer TA. Adhesion of the immune system. Nature 1990;346:425-34.
65. Adams DH, Hubscher SG, Shaw J, Johnson GD, Babbs C, Rothlein R, Neuberger JM. Increased expression of Intercellular Adhesion Molecule 1 on bile ducts in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 1991;14:426-31.
66. Leeuwenberg JFM, Smeets EF, Neefjes JJ, Shaffer MA, Cinek T, Jeunhomme TMAA et al. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. Immunology 1992;77:543-9.
67. Thomson AW, Satoh S, Nüssler AK, Tamura K, Woo J, Gavaler J, Van Thiel DH. Circulating intercellular adhesion-molecule (ICAM-1) in autoimmune liver disease and evidence for the production of ICAM-1 by cytokine-stimulated human leucocytes. Clin Exp Immunol 1994;95:83-90.
68. Adams DH, Mainolfi E, Burra P, Neuberger JM, Ayres R, Elias E, Rothlein R. Detection of circulating Adhesion Molecule-1 in chronic liver diseases. Hepatology 1992;16:810-4.
69. Lim AG, Jazrawi RP, Ahmed HA, Levy JH, Zuin M, Douds AC, Maxwell JD, Northfield TC. Soluble Intercellular Adhesion Molecule-1 in primary biliary cirrhosis: relationship with disease stage, immune activity and cholestasis. Hepatology 1994;20:882-8.
70. Seth R, Raymond FD, Makgoba MW. Circulating ICAM-1 isoforms: diagnostic prospects for inflammatory and immune disorders. Lancet 1991;338:83-4.
71. Adams DH, Mainolfi E, Elias E, Neuberger JM, Rothlein R. Detection of circulating intercellular adhesion molecule-1 after liver transplantation – evidence of local release within the liver during graft rejection. Transplantation 1993;55:83-7.
72. Heufelder AE, Bahn RS. Soluble intercellular adhesion molecule-1 (sICAM-1) in sera of patients with Graves' ophtalmology and thyroid diseases. Clin Exp immunol 1993;92:296-302.
73. Nielsen OH, Langholz E, Hendel J, Brynskov J. Circulating soluble intercellular adhesion molecule-1 (sICAM-1) in active inflammatory bowel disease. Dig Dis Sci 1994;39:1918-23.
74. Kling E, Bieg S, Boehme M, Scherbaum WA. Circulating intercellular adhesion molecule-1 as a new activity marker in patients with lupus erythematosus. Clin Investig 1993;71:299-304.
75. Zöhrens G, Armbrust T, Pirzer U, Meyer zum Büschenfelde KH, Ramadori G. Intercellular adhsion molecul-1 concentration in sera of patients with acute and chronic liver disease: relationship to disease activity and cirrhosis. Hepatology 1993;18:798-802.
76. Lim AG, Wolfhagen FHJ, Verma A, Van Buuren HR, Jazrawi RP, Northfield TC, Schalm SW. Soluble intercellular adhesion molecule 1 in primary biliary cirrhosis: effect of ursodeoxycholic acid and immunosuppressive therapy. Submitted.
77. Bergasa NV, Newman W, Rothlein R, Jones EA, Adams DH. Serum levels of soluble adhesion molecules (ICAM-1, VCAM-1 and E-selectin) are markedly elevated in primary biliary cirrhosis (PBC) and unaffected by low dose oral methotrexate. Gastroenterology 1993;104:A877 (abstract).
78. Calmus Y, Weill B, Ozier Y, Chéreau C, Houssin D, Poupon R. Immunosuppressive properties of chenodeoxycholic and ursodeoxycholic acids in the mouse. Gastroenterology 1992;103:617-21.
79. Leuschner U, Leuschner M, Sieratzki J, Kurtz W and Huebner K. Gallstone dissolution with ursodeoxycholic acid in patients with chronic active hepatitis and two years follow-up. A pilot-study. Dig Dis Sci, 1985;30:642-9.
80. Leuschner U, Fischer H, Kurtz W, Güldütuna S, Huebner K, Hellstern A et al. Ursodeoxycholic acid in primary biliary cirrhosis; results of a controlled double-blind study. Gastroenterology 1989;97:1268-74.
81. Oka H, Toda G, Ikeda Y, Hashimoto N, Hasamuru Y, Kamimura T et al. A multi-center double-blind controlled trial of ursodeoxycholic acid for primary biliary cirrhosis. Gastroenterol Jpn 1990;25:774-80.
82. Hadziyannis SJ, Hadziyannis ES, Lianidou E, Makris A. Long-term treatment of primary biliary

cirrhosis with ursodeoxycholic acid: the third year of a controlled trial. In: Bile acids as therapeutic agents. Eds: Paumgartner G, Stiehl A, Gerok W. Kluwer, Dordrecht 1991; pp. 287-96.
83. Poupon RE, Poupon R, Balkau B and the UDCA-PBC Study Group. Ursodiol for the long term treatment of primary biliary cirrhosis. N Engl J Med 1994;330:1342-7.
84. Hwang SJ, Chan CY, Lee SD, Wu JC, Tsay SH, Lo KJ. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis: a short-term, randomized, double-blind controlled, cross-over study with long-term follow up. J Gastroenterol Hepatol 1993;8:217-23.
85. Battezzati PM, Podda M, Bianchi R and the Italian Multicenter Group for the Study of UDCA in PBC. Ursodeoxycholic acid for symptomatic primary biliary cirrhosis: preliminary analysis of a double-blind controlled multicenter trial. J Hepatol 1993;17:332-8.
86. Turner IB, Myszor M, Mitchison HC, Bennett MK, Burt AD, James OFW. A two year controlled trial examining the effectiveness of ursodeoxycholic acid in primary biliary cirrhosis. J Gastroenterol & Hepatol 1994;9:162-8.
87. Lindor KD, Dickson ER, Baldus WB, Jorgensen RA, Ludwig J, Murtaugh PA et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-90.
88. Heathcote EJL, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN et al. The Canadian multi-centre double-blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.
89. Floreani A, Zappalà F, Mazzetto M, Naccarato R, Plebani M, Chiaramonte M. Different response to ursodeoxycholic acid in primary biliary cirrhosis according to severity of disease. Dig Dis Sci 1994;39:9-14.
90. Wolfhagen FHJ, Van Buuren HR, Van Berge Henegouwen GP, Ten Kate FJW, Den Ouden JW, Kerbert MJ et al. Ursodeoxycholic acid in stage IV primary biliary cirrhosis. Submitted
91. Vogel W, Kathrein H, Judmaier G, Braunsteiner H. Deterioration of primary biliary cirrhosis during treatment with ursodeoxycholic acid. Lancet 1988;i:1163.
92. Kneppelhout JC, Mulder CJJ, Van Berge Henegouwen GP, DE Vries RA. Brandt KH. Ursodeoxycholic acid treatment in primary biliary cirrhosis with the emphasis on late stage disease. Neth J Med 1992;41;11-6.
93. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646.
94. Simko V, Shoukry M, Prego V. Ursodeoxycholic therapy in chronic liver disease: a meta-analysis in primary biliary cirrhosis. Am J Gastroenterol 1994;89:392-8.
95. Combes B, Carithers RL, Maddrey WC, Munoz SJ, McDonald MF, Garcia-Tsao G et al. A randomized double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1993;18:A175 (Abstract).
96. Fleming TR. Surrogate markers in AIDS and cancer trials. Stat Med (in press).
97. Reichen J. Pharmacologic treatment of cholestasis. Sem Liver Dis 1993;13:302-15.
98. Wiesner RH, Porayko MK, Dickson ER, Gores GJ, LaRusso NF, Hay JE et al. Selection and timing of liver transplantation in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 1992; 1290-9.
99. Christensen E, Altman DG, Neuberger J, De Stavola BL, Tygstrup N, Williams R. Updating prognosis in primary biliary cirrhosis using a time dependent Cox regression model. Gastroenterology 1993;105:1865-76.
100. Murtaugh PA, Dickson ER, Van Dam GM, Malinchoc M, Grambsch PM, Langworthy AL et al. Primary biliary cirrhosis: prediction of short-term survival based on repeated patient visits. Hepatology 1994;20:126-134.
101. Davis GL, Czaja AJ. Immediate and long term results of cortiocteroid therapy for severe idiopathic chronic active hepatits. In: Chronic Active Hepatitis - The Mayo Clinic Experience. Eds. Czaja AJ, Dickson ER. Marcel dekker Inc, New york, 1986, pp.269-83.

102. Wolfhagen FHJ,Van Buuren HR, Schalm SW,Ten Kate FJW,Van Hattum J, Den Ouden JW et al. Can ursodeoxycholic acid induce disease remission in primary biliary cirrhosis. J Hepatol 1995;22:381
103. Schuppan D. Connective tissue polypeptides in serum as parameters to monitor antifibrotic treatment in hepatic fibrosis. J Hepatol 1991;13 (suppl.3):S17-26.
104. Eriksson S, Zetterval O. The N-terminal propeptide of collagen type III in serum as prognostic indicator in primary biliary cirrhosis. J Hepatol 1986;2:370-8.
105. Babbs C, Hunt LP, Haroubi NY, Smith A, Rowas BP, Warnes TW. Type III procollagen peptide: a marker of disease activity and prognosis in primary biliary cirrhosis. Lancet 1988;i:1021-4.
106. Poupon RE, Balkau B, Guéchot J, Heintzmann E Predictive factors in ursodeoxycholic acid treated patients with primary biliary cirrhosis: role of serum markers of connective tissue. Hepatology 1994;19:635-40.
107. Podda M, Ghezzi C, Battezzati PM, Bertolini E, Crosignani A, Petroni ML et al. Effect of different doses of ursodeoxycholic acid in chronic liver disease. Dig Dis Sci 1989;34 (suppl): 59S-65S.
108. Colombo C, Crosignani A, Assaisso M, Battezzati PM, Podda M, Giunta A et al. Ursodeoxycholic acid therapy in Cystic Fibrosis-associated liver disease: a dose response study. Hepatology 1992;16:924-30.
109. Walker S, Rudolph G, Raedsch R, Stiehl A. Intestinal absorption of ursodeoxycholic acid with extrahepatic biliary obstruction and bile drainage. Gastroenterology 1992;102:610-5.
110. Beukers R, Schalm SW. Effects of cyclosporine and cyclosporine plus prednisone in primary biliary cirrhosis. Transplant Proceed 1988; 20(suppl.4):340-3.
111. Kaplan MM, Knox TA. Treatment for primary biliary cirrhosis with low dose weekly methotrexate. Gastroenterology 1991;101:1332-8.
112. Weber P, Scheurlen M, Wiedmann KH. Methotrexate ameliorates disease in patients with early primary biliary cirrhosis. Gastroenterology 1991:100:A810 (Abstract).
113. Bergasa NV, Hoofnagle JH, Axiotis CA, Rabin L, Park Y, Jones EA. Oral methotrexate for primary biliary cirrhosis: preliminary report. Gastroenterology 1991;100:A720 (Abstract).
114. Van Steenbergen W, Sciot R, Van Eyken P, Desmet V, Fevery J. Methotrexate alone or in combination with ursodeoxycholic acid as possible treatment in primary biliary cirrhosis. In: Cholestatic Liver Diseases: New strategies for prevention and treatment of hepatobiliary and cholestatic liver diseases. Eds. Van Berge Henegouwen GP, De Groote J, Van Hoek B, Matern S, Stockbrügger RW. Kluwer Academic Publishers, Dordrecht 1994; pp 246-54.
115. Kaplan MM. The therapeutic effects of ursodiol and methotrexate are additive and well tolerated in primary biliary cirrhosis. Hepatology 1992;16:92A (Abstract).
116. Buscher H-P, Zietzschmann Y, Gerok W. Positive responses to methotrexate and ursodeoxycholic acid in patients with primary biliary cirrhosis responding insufficiently to ursodeoxycholic acid alone. J Hepatol 1993;18:9-14.
117. Raedsch R, Stiehl A, Walker S, Scherrmann JM, Kommerell B. Kombinierte Ursodeoxycholsäure plus Colchizin-Behandlung bei primär biliärer Zirrhose: Ergebnisse einer Placebo-kontrollierten Dopplelblindstudie. Z Gastroenterol 1992;30:55-7.
118. Podda M and the Italian multicenter group for the study of UDCA in PBC. Long-term effects of the administration of ursodeoxycholic acid (UDCA) alone or with colchicine in patients with primary biliary cirrhosis (PBC). A double-blind multicenter study. Proceedings of the XII international Falk bile acid meeting. Basel 1992,50-1.
119. Poupon RE, Niard AM, Huet PM, Miguet JP, Mathieu-Chandelier C, Doffoël M et al. A randomized trial comparing the combination ursodeoxycholic acid (UDCA) and colchicine to UDCA alone in primary biliary cirrhosis. Hepatology 1994;20:151A (Abstract).
120. Goddard CJR, Hunt L, Smith A, Fallowfield G, Rowan B, Warnes TW. A trial of ursodeoxycholic acid and colchicine in primary biliary cirrhosis. Hepatology 1994;20:151A (Abstract).

121. Shibata J, Fujiyama S, Honda Y, Sato T. Combination therapy with ursodeoxycholic acid and colchicine for primary biliary cirrhosis. J Gastroenterol Hepatol 1992;7:277-82.
122. Sharma A, Provenzale D, McKusick A, Kaplan MM. Interstitial pneumonitis after low-dose methotrexate therapy in primary biliary cirrhosis. Gastroenterology 1994;107:266-70.
123. Zifroni A, Schaffner F. Long-term follow-up of patients with primary biliary cirrhosis on colchicine therapy. Hepatology 1991;14:990-3.
124. Sherlock S. Primary biliary cirrhosis (chronic intrahepatic obstructive jaundice). Gastroenterology 1959;37:574-86.
125. Howat HT, Ralston AJ, Varley H, Wilson JAC. The late results of long- term treatment of primary biliary cirrhosis by corticosteroids. Rev Int Hepatol 1966;16:227-38.
126. Hoffbauer FW. Primary biliary cirrhosis: observations on the natural course of the disease in 25 women. Am J Dig Dis 1960;5:348-83.
127. Geubel AP, Baggenstoss AH, Summerskill WHJ. Responses to treatment can differentiate chronic active liver disease with cholangitic featueres from the primary biliary cirrhosis syndrome. Gastroenterology 1976;71:444-9.
128. Sherlock S, Dooley J. Diseases of the liver and biliary system. Blackwell Scientific publications, 9th ed. 1993:pp 236-48.
129. Mitchison HC, Bassendine MF, Malcolm MF, Watson AJ, Record CO, James OFW. A pilot double-blind, controlled one year trial of prednisolone treatment in primary biliary cirrhosis: hepatic improvement but greater bone loss. Hepatology 1989;10:420-9.
130. Gennari C, Vitelli R. Glucocorticoid-induced osteoporosis. Clin Rheum Dis 1986;637-54.
131. Locascio V, Bonucci E, Imbimbo B, Ballantani P, Adami S, Milani S et al. Bone loss in response to long term glucocorticoid therapy. Bone Miner 1990;8:39-51.
132. Sambrook PN, Cohen ML, Eisman JA, Pocock NA, Champion GD, Yeates MG. Effects of low dose corticosteroids on bone mass in rheumatoid arthritis: a longitudinal study. Ann Rheum Dis 1989;48:535-8.
133. Clements D, Compston JE, Rhodes J, Evans WD, Smith PM. Low-dose corticosteroids in chronic active hepatitis do not adversely affect spinal bone. Eur J Gastroenterol & Hepatol 1993;5:543-7.
134. Berkum van FNR, Beukers R, Birkenhäger JC, Kooij PPM, Schalm SW, Pols HAP. Bone mass in women with primary biliary cirrhosis; the relation with histological stage and use of corticosteroids. Gastroenterology 1990;99:1134-9.
135. Wolfhagen FHJ, Van Buuren HR, Hop WCJ, Den Ouden JW, Van Leeuwen JPTM, Schalm SW, Pols HAP. Cyclical etidronate in the prevention of bone loss in corticosteroid treated primary biliary cirrhosis. Submitted.
136. Reid IR, Alexander CJ, King AR, Ibbertson HK. Prevention of steroid-induced osteoporosis with (3-amino-1-hydroxypropylididene)-1,1-bisphosphonate (APD). Lancet 1988:i;143-6.
137. Gallagher SJ, Fenner JA, Anderson K, Bryden FM, Banham SW, Logue FC et al. Intravenous pamidronate in the treatment of osteoporosis associated with corticosteroid dependent lung disease: an open pilot study. Thorax 1992;47:932-6.
138. Mulder H, Struys A. Intermittent cyclical etidronate in the prevention of corticosteroid-induced bone loss. Br J Rheumatol 1994;33:348-50.
139. Wolfhagen FHJ, Van Buuren HR, Van Berge Henegouwen GP, Ten Kate FJW, Hop WCJ, Kerbert MJ et al. Triple therapy with ursodeoxycholic acid, prednisone and azathioprine in primary biliary cirrhosis: benefits and risks (this thesis).
140. Loftus J, Allen R, Hesp R, David J, Reid DM, Wright DJ et al. Randomized, double-blind trial or deflazacort versus prednisone in juvenile chronic (or rheumatoid) arthritis: a relatively bone sparing effect of deflazacort. Br J Rheumatol 1993;32 (suppl.2):31-8.
141. Olgaard K, Storm T, Van Wowern N, Daugaard H, Egfjord M, Lewin E et al. Glucocorticoid-induced osteoporosis in the lumbar spine, forearm, and mandible of nephrotic patients: a double-blind study on the high dose, long-term effects of prednisone versus deflazacort. Calcif Tissue Int 1992;50:490-7.

142. Avioli LV. Potency ratio - a brief synopsis. Br J Rheumatol 1993;32 (suppl. 2):24-6.
143. Powrie F, Coffman RL. Cytokine regulation of T-cell function: potential for therapeutic intervention. Immunology Today 1993;14:270-4.
144. Waldmann TA. The Il-2/IL-2 receptor system: a target for rational immune intervention. Immunology Today 1993;14:264-9.
145. Nguyen MT, Herrine SK, Zern MA. Cytokine involvement in the liver. Current Opinion in Gastroenterology 1994;10;277-84.
146. Friedman S. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. N Engl J Med 1993;328:1828-35.
147. Kovacs E. Fibrogenic cytokines: the role of immune mediators in the development of scar tissue. Immunology Today 1991;12:17-23.
148. Castilla A, Prieto J, Fausto N. Transforming growth factor beta-1 and beta-2 in chronic liver disease. N Engl J Med 1991;324:933-40.
149. Isobe M, Yagita H, Okumura K, Ihara A. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. Science 1992;255:1125-8.
150. Waldmann H, Cobbold S. The use of monoclonal antibodies to achieve immunological tolerance. Immunology Today 1993;14:246-51.
151. Hutchings P, O'Reilly L, Parisch NM, Waldmann H, Cooke A. The use of non-depleting anti-CD4 monoclonal antibody to re-establish tolerance to beta cells in NOD mice. Eur J Immunol 1991;22:1913-8.
152. Van Bekkum DW. BMT in experimental autoimmune diseases. Bone Marrow Transplant 1993;11;183-7.
153. Eedy DJ, Burrows D, Bridges JM et al. Clearance of severe psoriasis after allogeneic bone marrow transplantation. Br Med J 1990;300:908-9.
154. Liu Yin JA, Jowitt SN. Resolution of immune-mediated diseases following allogeneic bone marrow transplantation. Bone Marrow Transplant 1992;9:31-3.
155. Jacobs P, Vincent MD, Martell RW. Prolonged remission of severe refractory rheumatoid arthritis following allogeneic bone marrow transplantation for drug induced aplastic anaemia. Bone Marrow Transplant 1986;1;237-9.

SUMMARY

The studies presented in this thesis aimed at exploring the benefits and risks of the treatment of primary biliary cirrhosis (PBC) with ursodeoxycholic acid as monotherapy, and in combination with prednisone and azathioprine. These studies were performed in the context of a Dutch multicenter project which was initiated in 1990.

Chapter I

This chapter presents a general introduction regarding the clinical picture and pathophysiology of this enigmatic disease, with a more detailed review of the therapeutic state of the art in 1990. While liver transplantation had become a life saving therapy for patients with end-stage PBC, efforts with a large variety of drugs, to discover an effective medical therapy had been in vain. At that time the first promising controlled trials on ursodeoxycholic acid were published. Based on the supposed pathophysiology of PBC and the action mechanisms of UDCA, the rationale of combining UDCA with immunosuppressive drugs is elucidated. Moreover, the aims of this thesis are described in this chapter.

Chapter II

Here we aimed at assessing how many PBC patients would achieve a disease remission during a 1-year course of UDCA (ca. 10 mg/kg/day). In doing so, incompletely responding patients, who were considered potential candidates for further trials, could be identified. In spite of the observed beneficial effects on symptoms and liver tests, less than 5% of the 110 treated patients achieved a complete remission according to our criteria.

Chapter III

Several authors had found the response to UDCA treatment to be minor or absent in small, heterogenous groups of PBC patients with cirrhosis. Moreover, UDCA had been reported to induce disease deterioration in cirrhotic PBC patients. In this chapter we compared

the tolerance and response to UDCA in PBC patients with Child Pugh Class A cirrhosis (n=20) and patients without cirrhosis (n=39). No differences in response were found between both groups and none of the cirrhotic patients showed clear worsening of the disease.

Chapter IV

It had been advocated that UDCA should be given in multiple small doses during the day rather than in one single gift. We compared biochemical response and biliary UDCA enrichment in 27 patients with cholestatic liver diseases (PBC, PSC), who were randomized to receive UDCA either according to a divided dose or a single dose regimen. UDCA enrichment correlated with biochemical improvements of alkaline phosphatase, transaminases and γ-glutamine transferase. There were no differences in biochemical response or biliary enrichment between both treatment regimens.

Chapter V

This retrospective study in 7 PBC patients rendered the first data regarding the synergistic effects of UDCA and immunosuppressive treatment on symptoms and liver tests. These findings led to the the initiation of the studies described in the following chapters.

Chapter VI

Here an interim-analysis is reported of 36 patients participating in a 1-year, double-blind, placebo-controlled trial with prednisone and azathioprine, given in addition to UDCA. In the treatment group, but not in the placebo group, clear and significant improvements in itching, markers of liver cell necrosis, cholestasis, fibrogenesis and immune activity, as well as in inflammatory features on liver biopsy were noted. These effects were obtained on top of ameliorations previously induced by UDCA monotherapy. In the treatment group 3 patients were withdrawn from treatment because of adverse events compared to 2 patients in the placebo group, who were withdrawn following disease progression and eventual referral for liver transplantation. Corticosteroid induced bone loss seemed to be prevented by the use of bisphos-

phonates (see chapter 8). The short term benefit/risk ratio appeared to justify further long term trials with this triple treatment regimen.

Chapter VII

Soluble Intercellular Adhesion Molecule-1 (sICAM-1) in serum has been proposed as a marker of disease activity in immune diseases, such as PBC. We studied sICAM-1 levels in 24 patients before and during UDCA therapy and during additional prednisone/azathioprine or placebo treatment. sICAM-1 concentrations at entry correlated with histological stage, bilirubin and transaminases, but not with a marker of lymphocyte activation, i.e. soluble Interleukin-2 receptor. This suggests a local (hepatic) rather than a lymphocytic origin of sICAM-1 in PBC. UDCA diminished sICAM-concentrations by a median of 20%, indicating an immunomodulatory effect of this drug. Further decreases (median 25%) were achieved by prednisone and azathioprine but not by placebo.

Chapter VIII

To assess whether cyclical etidronate could reduce corticosteroid induced bone loss in PBC, 24 patients participating in the trial described in chapter 6 were randomized to receive either cyclical etidronate + calcium or calcium alone. Bone Mineral Content in the lumbar spine and proximal femur were monitored using Dual Energy X-ray Absorptiometry. In the prednisone treated patients etidronate appeared to prevent the enhancement of vertebral bone loss by corticosteroid treatment. No differences in bone loss between placebo treated patients with or without etidronate were noted. Femoral bone loss was comparable in all groups. These data indicate that the main drawback of corticosteroid therapy in PBC, increased bone loss, can be obviated by bisphosphonates.

Chapter IX

In this chapter the advances regarding the treatment of PBC during the last 5 years and future therapeutic options are discussed.

SAMENVATTING

In dit proefschrift worden een aantal studies beschreven met betrekking tot de effecten en bijwerkingen van behandeling van primaire biliaire cirrose (PBC) patiënten met ursodeoxycholzuur (UDCA) alleen, alswel in combinatie met prednison en azathioprine. Deze studies werden uitgevoerd binnen een Nederlands multicentrisch projekt dat werd gestart in 1990.

Hoofdstuk I

Dit hoofdstuk geeft een algemene introductie met betrekking tot de kliniek en pathofysiologie van deze raadselachtige ziekte, met een meer gedetailleerd overzicht van de stand van zaken rond de behandeling van PBC anno 1990. De levertransplantatie had zich ontwikkeld tot een effectieve, levensverlengende ingreep voor patiënten in het eind-stadium van de ziekte. Daarentegen waren alle pogingen om een effectieve medicamenteuze behandeling voor PBC te vinden tevergeefs gebleken. Rond die tijd, echter, kwamen de eerste veelbelovende data van gecontroleerde trials met UDCA beschikbaar. Op basis van de veronderstelde pathofysiologie van PBC en de werkingsmechanismen van UDCA wordt de rationale van gecombineerde behandeling van UDCA met immunosuppressie toegelicht. Tevens worden de doelen van dit proefschrift beschreven.

Hoofdstuk II

In deze studie wilden wij bepalen hoeveel patiënten met PBC in remissie zouden komen gedurende 1 jaar UDCA behandeling (ca. 10 mg/kg/dag). Op deze manier konden tevens die patiënten die niet of slechts gedeeltelijk op UDCA respondeerden worden geïdentificeerd. Deze zouden dan in aanmerking kunnen komen voor verdere behandelingsstudies. Ondanks de duidelijk positieve effecten op klachten en levertesten, voldeed na 1 jaar minder dan 5% van de patiënten aan onze criteria voor complete ziekte remissie.

Hoofdstuk III

Verschillende onderzoekers hadden geen of slechts een geringe respons op UDCA gevonden in kleine aantallen patiënten met PBC stadium IV (cirrose). Ook was door enkele auteurs verslechtering van de ziekte gerapporteerd, die zij weten aan het gebruik van UDCA. Wij vergeleken de tolerantie van, en de respons op UDCA in PBC patiënten met gecompenseerde (Child-Pugh Class A) cirrose (n=20) en patiënten zonder cirrose (n=39). Wij vonden geen verschillen m.b.t. respons tussen beide groepen. In geen van de cirrose patiënten trad een duidelijke verslechtering van de ziekte op.

Hoofdstuk IV

Men heeft geopperd dat UDCA beter kan worden toegediend in over de dag verdeelde giften dan in een enkelvoudige dosis. Wij vergeleken de biochemische respons en biliaire UDCA verrijking in 27 patiënten met cholestatische leverziekten (PBC, PSC), die gedurende 3 maanden werden gerandomizeerd naar een multipel of enkelvoudig UDCA doseringsschema. De biliaire UDCA verrijking correleerde met de afnames van alkalisch fosfatase, γ-glutamine transferase en transaminases. Er waren geen verschillen in biochemische respons en biliaire UDCA verrijking.

Hoofdstuk V

Deze retrospectieve studie in 7 PBC patiënten toonde voor het eerst synergistische effecten van UDCA en immunosuppressieve behandeling. Deze bevindingen leidden tot de start van de studies zoals beschreven in de volgende hoofdstukken.

Hoofdstuk VI

Hierin wordt een interim analyse gerapporteerd van 36 patiënten, deelnemend aan een 1 jaar durende, dubbelblinde, placebo-gecontroleerde studie met prednison en azathioprine, toegevoegd aan eerdere UDCA behandeling. In de behandelingsgroep, maar niet in de placebo-groep, werden duidelijke, significante verbeteringen in jeuk, serologische testen voor levercelverval, cholestase, fibrogenese en immuunactiviteit, en in ontstekingsverschijnselen op het leverbiopt

gevonden. Deze effecten werden bereikt bovenop de verbeteringen verkregen door de voorafgaande behandeling met UDCA alleen. In de behandelingsgroep stopten 3 patiënten met de behandeling vanwege bijwerkingen, terwijl in de placebo groep 2 patiënten stopten wegens ziekteprogressie en, uiteindelijk, verwijzing voor levertransplantatie. Toename van botverlies door prednison leek te worden voorkomen met behulp van cyclische behandeling met het bisphosphonaat etidronaat (zie hoofdstuk 8). Deze korte termijn bevindingen lijken de opzet en uitvoer van verdere langdurige studies met dit behandelingsregimen te rechtvaardigen.

Hoofdstuk VII

Men heeft geopperd dat oplosbare vormen van het Intercellulaire Adhesie Molecuul-1 (sICAM-1) een parameter voor ziekte activiteit bij immunologische aandoeningen, zoals PBC, zou kunnen zijn. Wij onderzochten sICAM-1 concentraties in 24 patiënten voor en gedurende UDCA therapie en gedurende additionele behandeling met prednison/azathioprine of placebo. sICAM-1 concentraties voor start correleerden met histologisch stadium, bilirubine en transaminase spiegels, maar niet met de concentratie van oplosbare Interleukine-2 receptoren, een parameter voor lymfocyt-activatie. Dit wijst op een lokale (hepatische) en niet zozeer een lymfocytaire oorsprong van sICAM-1 bij PBC. UDCA verlaagde de sICAM-1 concentraties met een mediaan van 20%, hetgeen een immunomodulerend effect van UDCA suggereert. Verdere verlaging van sICAM-1 spiegels (mediaan 25%) werd bereikt met prednison en azathioprine maar niet met placebo.

Hoofdstuk VIII

Om te bepalen of cyclisch etidronaat het corticosteroïd geïnduceerde botverlies bij PBC zou kunnen verminderen, werden 24 patiënten deelnemend aan de studie, zoals beschreven in hoofdstuk 6, gerandomizeerd naar behandeling met etidronaat+calcium (in cycli van 3 maanden) of calcium alleen. De mineraalinhoud van de wervelkolom en proximale femur werd vervolgd met behulp van Dual Energy X-ray Absorptiometry. In de groep patiënten die werd behandeld met predni-

son/azathioprine bleek behandeling met etidronaat het corticosteroïd-geïnduceerd botverlies in de wervelkolom te voorkomen. Er werden geen verschillen in botverlies gevonden tussen patiënten in de placebo groep met of zonder etidronaat. Het botverlies in de heup was vergelijkbaar in alle groepen. Deze gegevens suggereren dat het voornaamste nadeel van corticosteroidenbehandeling bij PBC, namelijk toegenomen botverlies, ondervangen kan worden met behulp van bisphosphonaten.

Hoofdstuk IX

In dit hoofdstuk worden de vorderingen gedurende de laatste vijf jaar met betrekking tot de behandeling van PBC besproken. Tevens wordt ingegaan op toekomstige behandelingsmogelijkheden.

DE NEDERLANDSE MULTICENTER PBC STUDIE GROEP
(d.d. 1-1-1995)

Academisch Ziekenhuis Rotterdam:	H.R. van Buuren
	H.J. Metselaar
	S.W. Schalm
	F.H.J. Wolfhagen
Academisch Ziekenhuis Utrecht:	G.P. van Berge Henegouwen
	J. van Hattum
	P.C. van de Meeberg
Academisch Ziekenhuis Groningen:	I.J. Klompmaker
	E.B. Haagsma
Academisch Ziekenhuis Maastricht:	W. Hameeteman
	R.P.R. Adang
Vrije Universiteit, Amsterdam:	C.E. Friedberg
Canisius Ziekenhuis, Nijmegen:	F.A.L.M. Eskens
	P. Stadhouders
	R.W. de Koning
Carolus Ziekenhuis, Den Bosch:	E.W. van der Hoek
	A.M. Smit
St. Jans Ziekenhuis, Weert:	J.G.S. Breed
	F.M. Lalisang
Medisch Centrum, Leeuwarden:	J.W. Kappelle
	P. Spoelstra
Medisch Spectrum Twente, Enschede:	M.J. Kerbert-Dreteler
	J.H. van Lijf
Rode Kruis Ziekenhuis, Den Haag:	D. Overbosch
	R.M. Valentijn
Rijnstate Ziekenhuis, Arnhem:	J.E. Thies
	R.A. de Vries
Drechtsteden Ziekenhuis, Dordrecht:	A. van Vliet
	A.E.G. Lückers

Ysselmeer Ziekenhuizen, Lelystad:	S.A. Peters P.L. Tjepkema
Maasland Ziekenhuis, Sittard/Geleen:	L.G.J.B. Engels B.J. Looij
Sophia Ziekenhuis, Zwolle:	P.M. Blom van Assendelft T. Thobé
Kennemer Gasthuis, Haarlem:	J. Ferwerda
Zuiderziekenhuis, Rotterdam:	P.L. Batenburg
Bleuland Ziekenhuis, Gouda:	K.J. Heering
St.Franciscus Gasthuis, Rotterdam:	J.W. den Ouden-Muller
Reinier de Graaf Ziekenhuis, Delft:	J. Scherpenisse
Bethesda Ziekenhuis, Hoogeveen:	J.C. Thijs
St.Maartens Ziekenhuis, Venlo:	V.M.C. Verstappen
St.Lucas Ziekenhuis, Amsterdam:	M.M. Vidakovic-Vukic
Twenteborg Ziekenhuis, Almelo:	R.A.A. van Zanten
Juliana-Lucas Ziekenhuis, Apeldoorn:	R. Robijn
Ziekenhuis de Baronie, Breda:	M.C.M. Rijk
Willem-Alexander Ziekenhuis, Den Bosch:	Th.J.M. van Ditzhuijsen
Westeinde Ziekenhuis, Den Haag:	S.D.J. van de Werf
Merwede Ziekenhuis, Dordrecht:	W. Lesterhuis
St. Jansdal, Harderwijk:	J.N. Groen
West Fries Gasthuis, Hoorn:	R. Zwetbroek
St. Franciscus Ziekenhuis, Roosendaal:	L.R.K.W. Lie
Schieland Ziekenhuis, Schiedam:	J.J. Braun
Nieuwe Spittaal, Zutphen:	O.A. Dobbenburgh

Trial monitors: F.H.J. Wolfhagen en H.R. van Buuren, Academisch Ziekenhuis Dijkzigt, Rotterdam

Patholoog-anatoom: Prof. dr. F.J.W. ten Kate, Academisch Medisch Centrum, Amsterdam

Statisticus: Ir. W.C.J. Hop, Erasmus Universiteit, Rotterdam

Trial secretaresses (in chronologische volgorde):

Kim van de Haar
Annelies Roos-Schipper
Marja Bakker
Willeke Beukman

Steering Committee: Dr. R. Beukers, Drechtsteden Ziekenhuis, Dordrecht
Prof. dr. G.P. van Berge Henegouwen, Academisch Ziekenhuis Utrecht
Prof. J.H.P. Wilson, Academisch Ziekenhuis Dijkzigt, Rotterdam

Studie sponsors: Zambon Nederland BV
Nederlandse Lever Darm Stichting
Wellcome Pharmaceuticals BV
Procter & Gamble Pharmaceuticals NV

Verdere financiële bijdrages:
Lamepro BV
Tramedico BV
Duphar Nederland BV
Glaxo BV

DANKWOORD

Het in dit proefschrift beschreven onderzoek werd niet alleen verricht in het Academisch Ziekenhuis Rotterdam, maar in vele klinieken - academisch en perifeer, groot en klein - in heel Nederland. Bij zeldzame ziekten zoals PBC telt elke patiënt en alleen samenwerking kan leiden tot goed en zinvol onderzoek. Ik wil dan ook graag alle artsen en patiënten bedanken die deelnamen en -nemen aan het Multicentrische PBC Project. Ik hoop dat allen enthousiast zullen blijven participeren aan dit en soortgelijke projecten. Ook financiële support is onontbeerlijk en ik ben de diverse sponsors dan ook zeer erkentelijk voor hun steun. Met name Zambon Nederland BV en in het bijzonder Gerrit Paarlberg wil ik bedanken voor de organisatorische diensten met betrekking tot de Nederlandse Multicenter PBC studie.

Een aantal mensen zou ik in het bijzonder willen noemen. Op de eerste plaats Henk van Buuren, niet alleen mijn bezielde begeleider, maar eeuwige steun en toeverlaat; altijd had hij tijd voor mij, vaak ten koste van zijn eigen drukke programma; zijn optimisme en enthousiasme vormden regelmatig een goed tegenwicht voor mijn pessimistische buien. Henk, ik zal ons sigaartje en ons borreltje (na zessen, hoor!) missen.

Mijn promotor Prof. dr. Solko Schalm die mij de mogelijkheid heeft geboden om dit proefschrift tot stand te brengen, zij het af en toe met bloed, zweet en tranen. Dank ook voor de duwtjes (en soms een flinke zet?) in de juiste richting.

Ook mijn Utrechtse promotor, Prof. dr. Gerard van Berge Henegouwen, ben ik zeer erkentelijk voor zijn enthousiasme, zijn belangstelling en met name ook zijn snelle beoordeling van de vele protocolvoorstellen en manuscripten.

Dan wil ik mijn dank betuigen aan twee personen, die mijns inziens hèt prototype van de ware wetenschapper personifiëren, gezien de lichtelijk wanordelijke toestand op hun werkkamer (overigens sterk gelijkend op de chaos op mijn eigen kamer). Ten eerste Ir. Wim Hop,

van de afdeling biostatistiek van de Erasmus Universiteit, bij wie ik onder het genot van een lekker "shaggie" menig statistisch wonder geboren zag worden. Ten tweede Prof. dr. Fiebo ten Kate, patholoog-antoom, met wie ik, onder het genot van een lekker bakje koffie in het AMC of bij hem thuis, vele uren biopten heb zitten bekijken, tot de hoofdpijn er op volgde. Hier wil ik ook Paul Dillenburg noemen, die in het AMC onze biopten ordende en mee beoordeelde.

De dames van het trialbureau - waarvan ik er enkele letterlijk heb "versleten" blijkens het tempo waarin zij onze afdeling verlieten zodra zij met mij moesten werken - verdienen ook een woord van dank: Marja, Annelies, Kim, en verder Willeke, Juliëtte, Marjan, Carin, Ellis, Nicole, en natuurlijk ook Stanny de Roode, met wie ik, samen met Henk, een jaar lang het berghok van de afdeling heb mogen delen. Zij stak mij vaak een hart onder de riem en hield me daarnaast op de hoogte van alle afdelingsroddels en -nieuwtjes.

Ook de dames van de polikliniek heb ik waarschijnlijk meer dan eens tot wanhoop gebracht, o.a. met mijn vroege spreekuur, waar best (heel graag, dus) van afgeweken mocht worden. Frequent waren ook hun (vaak succesvolle) razzia's op onze kamer, met als doel het opsporen van missende statussen.

Alle medewerkers van de Balansafdeling (o sorry, Clinical Research Unit) wil ik eveneens hartelijk bedanken voor de wijze waarop zij hebben geholpen om de trials goed te laten verlopen, ondanks het feit dat patiënten nog wel eens totaal onverwacht op de Unit verschenen.

Jan Boot, bedankt voor je altijd snelle reactie op elk wanhopig telefoontje dat van achter mijn computer vandaan kwam.

Jopie Hensen, die de botmassametingen verrichtte wil ik een pluim geven voor de uiterst flexibele wijze waarop zij toch elke keer weer op korte termijn een plaatsje wist te creëeren voor mijn trial-patiënten, ondanks haar overvolle agenda. Ook Cock Buurman en Gert-Jan van de Bemd (laboratorium Inwendige Geneeskunde III) wil ik danken voor het snelle en adequate bepalen van de diverse bot-ombouw markers.

Dr. Huib Pols, bedank ik voor zijn inbreng en begeleiding m.b.t.

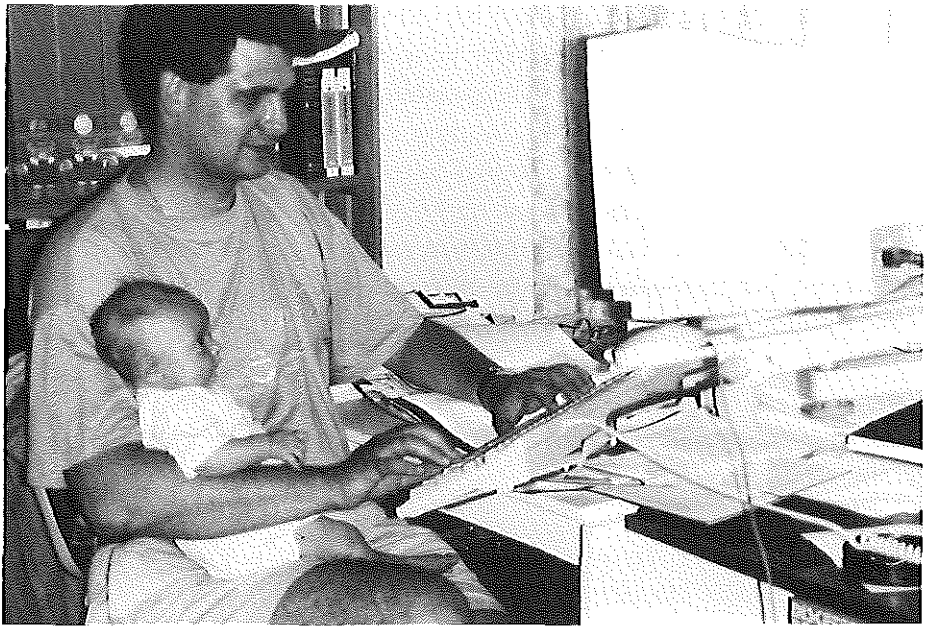
de "botstudie" en Ingrid de Graaf en Lidwien Hanff, apothekers in het Academisch Ziekenhuis Rotterdam voor de prettige samenwerking.

Monica wil ik even speciaal noemen, voor al de moeite die zij zich heeft getroost om voor mij biopten en verslagen uit de diepste spelonken van het pathologie archief te voorschijn te halen.

Prof. dr. F.T. Bosman, Prof. dr. J.C. Birkenhäger en Prof. J.H.P. Wilson dank ik heel hartelijk voor hun snelle beoordeling van het manuscript en hun bereidheid om zich tijdens de Paasdagen van 1995 te verpozen met zo'n 250 bladzijden PBC.

I would like to thank dr. Guan Lim, London, for the pleasant cooperation. Maybe our work helped to bring the U.K. a little bit closer to the continent.

De twee voor mij meest belangrijke personen wil ik nog even speciaal noemen. Harriët, jij hebt mijn twijfels en zorgen van zeer dichtbij moeten meemaken en altijd was je me daarin tot steun.Vaak was ik ver weg, diep in gedachten verzonken. Ik hoop dat ik nu, na de afsluiting van deze periode, ook geestelijk weer meer bij je zal zijn. Dan, iemand die mij thuis vaak bij mijn schrijf- en rekenwerk heeft bijgestaan, wiens niet te evenaren adviezen ik altijd graag ter harte heb genomen, en die de zon, die ik achter mijn computer gezeten niet zien kon, binnenbracht met haar lach ...



Laura

Tot slot, het aantal mensen dat een bijdrage levert aan een onderzoek zoals dit is ontzettend groot; daarom besluit ik zoals dagelijks vele malen te horen is op Radio 3: "... en iedereen die ik vergeten ben."

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 13 januari 1964 te Spaubeek. Na het afleggen van het eindexamen Gymnasium-β in 1982 aan de Scholengemeenschap St. Michiel te Geleen, vervulde hij zijn dienstplicht bij de Geneeskundige troepen in Hilversum en Oirschot. In 1984 begon hij zijn studie Geneeskunde aan de Rijksuniversiteit Limburg, te Maastricht. De studie kende enkele korte onderbrekingen voor stages in het buitenland (Zweden, Kenya). Tijdens zijn co-assistentschappen werkte hij als Ambulance-verpleegkundige bij de Gemeenschappelijke Gezondheidsdienst, te Heerlen.

Na zijn afstuderen in 1990 werkte hij een half jaar lang als poort-arts in het St. Gregorius Ziekenhuis te Brunssum en als docent Ziekte-leer aan de Centrale School voor Gezondheidszorg te Heerlen.

Vanaf juni 1991 was hij gedurende 3½ jaar als arts-onderzoeker werkzaam op de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Dijkzigt te Rotterdam (hoofd Prof. J.H.P. Wilson). In deze hoedanigheid fungeerde hij als coördinator en mede-initiator van de Nederlandse Multicentrische Primaire Biliaire Cirrose en Primaire Scleroserende Cholangitis projecten (Projectleiders: Drs. H.R. van Buuren, Prof. dr. S.W. Schalm en Prof. dr. G.P. van Berge Henegouwen). Onder leiding van Prof. dr. S.W. Schalm en Prof. dr. G.P. van Berge Henegouwen kwam hieruit dit proefschrift tot stand. Tijdens deze roerige periode verraste hij de burgerlijk stand van zijn woonplaats Breda met een huwelijk en werd hij vader van een dochter.

Op 1 mei 1995 begon hij de opleiding tot internist in het Ikazia Ziekenhuis te Rotterdam (opleider Dr. R.J.T. Ouwendijk).