IMMUNOSUPPRESSIVE THERAPY FOR PRIMARY BILIARY CIRRHOSIS

IMMUNOSUPPRESSIEVE BEHANDELING VAN PRIMAIRE BILIAIRE CIRRHOSE

IMMUNOSUPPRESSIVE THERAPY FOR PRIMARY BILIARY CIRRHOSIS

IMMUNOSUPPRESSIEVE BEHANDELING VAN PRIMAIRE BILIAIRE CIRRHOSE

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam,
op gezag van de rector magnificus
Prof. dr. C.J. Rijnvos
en volgens besluit van het college van Dekanen.
De openbare verdediging zal plaats vinden op
woensdag 15 april 1992 om 15.45 uur

door

RUDOLF BEUKERS

geboren te Schiedam

Promotiecommissie

Promotor : *Prof. dr. S.W. Schalm*

Overige leden: *Prof. dr. J.C. Birkenhäger*
Prof. dr. F.T. Bosman
Prof. J.H.P. Wilson

Publicatie van dit proefschrift werd mogelijk gemaakt door Inpharzam Nederland B.V., Merck Sharp & Dohme B.V., SmithKline Beecham Farma, Sandoz B.V. en Astra Pharmaceutica B.V.

CONTENTS

GENERAL INTRODUCTION

1. PRIMARY BILIARY CIRRHOSIS

1.1 Introduction. Primary biliary cirrhosis (PBC) is a chronic liver disease characterized by an inflammatory process that destroys the intrahepatic bile ducts, usually leading to progressive cholestasis, biliary cirrhosis and eventually death due to hepatic failure or the complications of portal hypertension. The disease is most common among middle-aged women.

1.2 Clinical and biochemical features. PBC usually has an insidious onset with pruritus as the most common symptom at presentation (1-4). Subsequently the symptoms and signs of cholestatic liver disease develop. Other clinical features include: fatigue, right upper abdominal pain, arthralgia, hyperpigmentation and xanthelasmas. Some patients, however, first present with signs of portal hypertension, such as variceal bleeding or ascites (3,4). Moreover in an increasing number of cases a diagnosis of PBC has been established for asymptomatic patients on the basis of abnormal liver function tests or a positive test for antimitochondrial antibodies (AMA) (5,6). PBC is frequently associated with other immunopathological conditions, including autoimmune thyroid disease and systemic disorders such as Sjögren's syndrome and scleroderma (2-5). In the later stages of the disease hepatic osteodystrophy and complications due to portal hypertension may occur.
Biochemically and serologically PBC is characterized by elevated serum alkaline phosphatase and IgM levels and a positive test for AMA (2), the latter being found for over 90% of the patients (7). More recently several subtypes of AMA have been described; the subtype which is specific for PBC has been labeled M2. These anti-M2 antibodies are directed against antigens on the inner membrane of mitochondria identified as enzymes (8). The classical diagnostic criteria for PBC are an elevated serum alkaline phosphatase level, a positive test for AMA and a typical bile duct lesion in the liver biopsy (see below). Extrahepatic bile duct obstruction should be excluded. Another diagnostic system of major and minor criteria has been proposed; it yields two levels of accuracy: definite or probable PBC (9).

1.3 Histopathology. Histologically four stages of PBC can be recognized (10,11): in stage I there is focal destruction of septal and interlobular bile ducts by a dense mononuclear cell infiltrate. Lymph follicle and granuloma formation may be seen. The morphological changes in stage I disease are considered to be highly characteristic of PBC (florid duct lesion) (10). Stage II is characterized by spread of the

infiltrate into the periportal parenchyma and proliferation of small bile ducts. In stage III inflammation is less prominent, bile ducts disappear and fibrosis ensues, eventually leading to cirrhosis (stage IV). In stage IV, features of all four stages can be present simultaneously.

Although the term chronic non-suppurative destructive cholangitis has been proposed for the precirrhotic stages of the disease (12), primary biliary cirrhosis - which in fact only refers to the last stage - is still the term most frequently used.

Hepatic copper concentrations are usually elevated and may reach the levels found in Wilson's disease. The subcellular copper distribution however differs from that in Wilson's disease. The copper accumulation in PBC has been attributed to chronic cholestasis and is thought to play no role in its pathogenesis (13).

1.4 Etiology and pathogenesis. The etiology and pathogenesis of PBC have not yet been elucidated. The association of PBC with disorders with a presumed autoimmune cause, the composition of the inflammatory infiltrate invading the bile ducts and periportal hepatocytes and the numerous immune abnormalities, however, provide evidence that an immune-mediated process plays a role in the pathogenesis of this disease.

Several abnormalities of both the humoral and cellular immune systems have been described in PBC, including raised serum immunoglobulin levels (especially IgM), serum autoantibodies (e.g. AMA), activation of the complement system, circulating immune complex-like material, defective immune complex clearance by Kupffer cells and functional defects of T-lymphocytes and natural killer cells (14). Many of these alterations may be secondary features. The defective in-vitro suppressor T-cell function in PBC, reported by several authors (14-17), might be more important because impairment of suppressor T-cell function is considered to be an essential factor in the development of clinically overt autoimmune disease (18,19). Involvement of genetic factors in the pathogenesis of PBC is suggested by the occasional familial occurrence of the disease (20). In addition, healthy first-degree relatives of patients with PBC are more likely than normal controls to have sero-immunological abnormalities (20,21) and a defective in-vitro suppressor T-cell function (22).

Finally, the strong female predominance in PBC suggests that hormonal factors may also be implicated in the pathogenesis.

The initial destructive bile duct lesion in PBC is probably caused by cytotoxic

T-lymphocytes. Subsets of mononuclear cells can be identified by monoclonal antibodies against cell surface proteins. CD4-positive lymphocytes (CD4+cells) consist of helper T-cells and cytotoxic T-cells, whereas CD8-positive lymphocytes (CD8+cells) are cytotoxic T-cells and T-cells with suppressor activity. T-cells become activated when they recognize a nonself-antigen bound to major histocompatibility (MHC) self-antigens on the surface of another cell. Class I MHC antigens are found on all nucleated cells and are recognized by CD8+cells. Class II antigens, that play an important role in the presentation of antigens to CD4+cells, are normally restricted to macrophages and other antigen-presenting cells, B-lymphocytes, activated T-lymphocytes and vascular endothelium.
Analysis of the mononuclear infiltrate in PBC has demonstrated that activated T-lymphocytes are predominant in portal tracts (CD4+cells being the major subset) and areas of piecemeal necrosis (CD4+ and CD8+cells; CD8+cells may outnumber CD4+cells) (23-26). Of particular interest is the aberrant expression of class II (25-27) and an increased expression of class I MHC antigens (25, 27) on biliary epithelium in PBC.

Although there is at present no conclusive evidence, the following hypothesis is attractive because most of the immunological and immuno-histological features of PBC then fit with theories on the pathogenesis of other autoimmune disorders, such as autoimmune thyroid disease (19) (figure). An as yet unknown exogenous factor may induce aberrant expression of class II MHC antigens on bile duct epithelial cells which then may act as antigen-presenting cells, presenting their own surface antigens. These surface antigens may also be altered by exogenous factors or may cross-react with foreign antigens, e.g. bacterial antigens as has been proposed by Hopf and co-workers (28,29). Subsequently, this presentation of bile duct epithelial antigens in the context of class II MHC antigens will activate helper T-lymphocytes, which in turn activate cytotoxic T-cells and stimulate B-lymphocytes to produce autoantibodies. The increased expression of class I MHC antigens may lead to amplification of cytotoxic T-cell responses. If the activation of helper T-lymphocytes and the subsequent humoral and cellular immune responses are not controlled because the suppressor T-cell function is defective, clinically significant autoimmune disease - i.e "autoimmune cholangitis" - may develop. Genetic factors that determine the susceptibility to disease might be the facility with which individuals express class II

MHC antigens on bile duct epithelium after exogenous stimulation (25) or the impairment of the suppressor T-cell function (22).

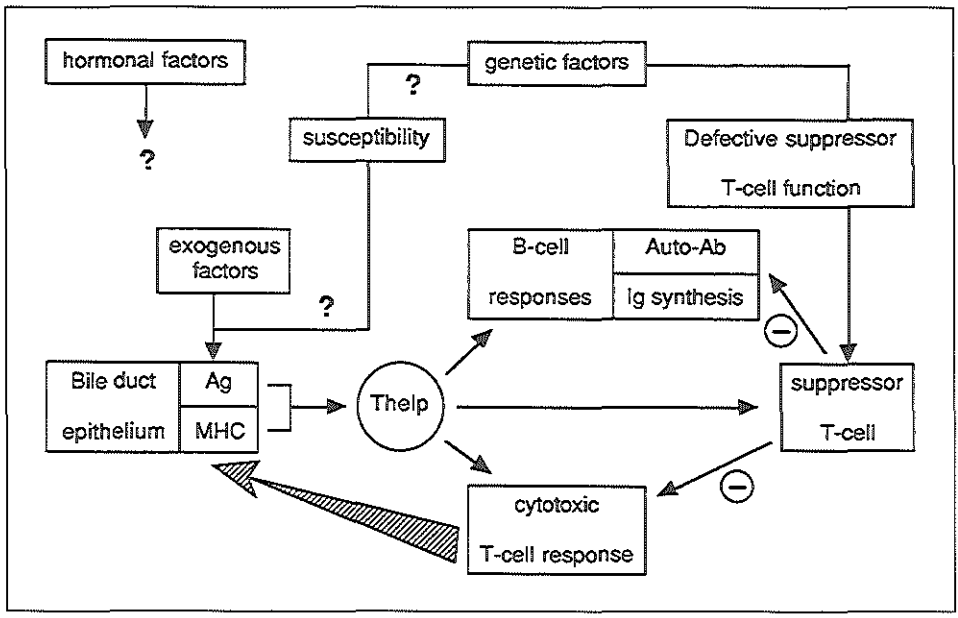

Figure: Hypothesis on the pathogenesis of primary biliary cirrhosis; see section 1.4 for explanation.
T_{help}: helper T-cell; Ag: bile duct epithelial antigens; MHC: major histocompatibility antigen; Ig synthesis: synthesis of immunoglobulins; Auto-ab: auto-antibodies.

1.5 Treatment. Assuming that PBC is an autoimmune disease and considering the progressive fibrosis and copper overload observed in the late stages of PBC, many uncontrolled and controlled trials have focussed on evaluation of immunosuppressive, antifibrotic and cupruretic therapy. Controlled trials have been conducted with corticosteroids, penicillamine, azathioprine, colchicine, chlorambucil, cyclosporin A and ursodeoxycholic acid. The results of these studies, summarized in table 1, are discussed below.

1.5.1 Corticosteroids. The first mention of corticosteroid therapy for PBC consisted of anecdotal reports on jaundiced patients with late stage disease (1,30,31). The results were equivocal, but at any rate corticosteroids appeared to have no significant effect on the clinical and histological progression of the disease in these patients. Moreover, these studies demonstrated rapid progression of metabolic bone disease with vertebral fractures in some cases (30,31). Mainly as a result

of these uncontrolled studies corticosteroids were long considered to be ineffective and contraindicated in PBC (2,32).

In a small controlled trial by Taal et al. prednisone (10 mg) combined with a low dose of penicillamine (250 mg) for six months appeared to be superior to penicillamine or placebo, significantly improving symptoms and biochemical values (9). No side-effects were reported, but the effect of prednisone on bones was not evaluated.

The first controlled trial of prednisolone as single drug therapy for PBC by Mitchison et al. was not published until 1989 (33). Prednisolone treatment (10 mg maintenance dose) for 1 year resulted in a significant symptomatic, biochemical and histological improvement, although progression of the metabolic bone disease was also noticed.

1.5.2 Penicillamine. The rationale for using penicillamine to treat PBC was its cupruretic effect and its well-known efficacy in treating the hepatic copper overload in Wilson's disease. In addition, penicillamine appeared to have antifibrotic and immunosuppressive properties.

Seven placebo-controlled trials (34-41) and one dose-controlled trial (42) to evaluate the therapeutic efficacy of penicillamine in PBC have been conducted. A total of 739 patients has been studied, the dose of penicillamine varying from 250-1000 mg per day, and follow-up from one to over five years. Although penicillamine decreased hepatic copper levels, the drug was found to have no beneficial effect on symptoms, histological progression and survival. The effect on the biochemical liver function tests varied. Moreover, a high incidence of side-effects was reported, necessitating discontinuation of the drug in up to 46% of the treated patients.

1.5.3 Azathioprine. The administration of the immunosuppressive drug azathioprine to treat PBC has been the subject of two controlled trials (43,44). In the first study, by Heathcote et al. (43), no significant effect of azathioprine on symptoms, biochemical parameters or histological progression was demonstrated, but there was a trend toward prolonged survival after three years of therapy.

In a second larger placebo-controlled study, Christensen et al. (44), also could not show improvement in the biochemical and histological parameters. The authors did not report on symptoms. A significant, though clinically small, improvement in mean survival of 20 months was found for the azathioprine-treated group. This significant difference in survival was however only reached after statistical adjustment

for an imbalance in bilirubin levels between the treated and control groups. Azathioprine was well tolerated in both studies.

1.5.4 Colchicine. Three placebo-controlled studies evaluated the effect of the antiinflammatory and antifibrotic drug colchicine (45-47). Symptoms were not improved by colchicine treatment in two studies (45,46) and were not evaluated in the other (47). The three studies demonstrated improved liver function tests but histological progression was not retarded by colchicine. Kaplan et al. (45) found a significant increase in survival in the colchicine-treated group, whereas Warnes et al. (47) reported only a trend toward improved survival which did not reach significance. The Mount Sinaï group, however, recently reported no beneficial effect of colchicine on survival in their patients (48). Side-effects of colchicine therapy were minimal.

1.5.5 Chlorambucil. Use of the alkylating drug chlorambucil to treat PBC was studied by Hoofnagle et al. (49). There was no report on symptoms but the biochemical liver function tests were favorably influenced by chlorambucil treatment. Histological progression was not prevented; survival was not evaluated. Bone marrow suppression was the major side-effect necessitating discontinuation of the drug in one third of the treated patients.

1.5.6 Cyclosporin A. See 3.5

1.5.7 Ursodeoxycholic acid. Long-term intrahepatic retention of the major human hydrophobic bile acids, as in chronic cholestasis, causes liver cell damage. During ursodeoxycholic acid (UDCA) treatment this non-hepatotoxic hydrophilic bile acid becomes the main constituent of the bile acid pool. This was the rationale for the use of UDCA in PBC, as was first reported by Poupon et al. (50). Furthermore, UDCA appears to have cytoprotective properties (51,52) and it may influence the aberrant expression of class I MHC antigens on hepatocytes in PBC (53). Since the first uncontrolled study by Poupon and co-workers (50), the (interim) results of four placebo-controlled trials have been published (51,54-57). In general, UDCA in a daily dose of 10-15 mg/kg significantly reduces pruritus and the parameters of cholestasis (bilirubin, alkaline phosphatase, γ-glutamyl transpeptidase) and hepatic inflammation (aminotransferases). In two studies serum IgM levels were also

significantly affected (51,56,57). The two-year results of the largest study thus far, carried out by Poupon et al. (57), do not support those of Hadziyannis et al., who suggest that early improvement in clinical and biochemical features might not be maintained in the long run - especially in patients with late stage disease (54). Histological data are limited. Two studies showed histological improvement in UDCA-treated patients (51,57), but Hadziyannis et al. reported that there was no beneficial effect on the histological parameters (54). In addition, Wiesner et al. found histological progression in patients treated with UDCA, despite clinical and biochemical improvement (58). At present, the long-term effect of UDCA treatment of PBC, especially with respect to histological progression and survival, remains to be established. The toxicity of UDCA is remarkably low.

1.5.8 Supportive treatment and liver transplantation. Because there is no effective causal therapy for PBC medical management is limited to supportive care, such as nutritional measures (supplementation of fat-soluble vitamins, adequate intake of calcium), treatment of pruritus (cholestyramine) and management of the complications of portal hypertension (such as variceal sclerotherapy).

In end-stage disease liver transplantation must be considered. Patients with PBC are relatively good candidates for liver transplantation, with a reported 5-year survival rate after transplantation of 66% (59). Moreover, the large majority of surviving patients achieve social and vocational rehabilitation (59,60).

1.6 Prognosis. The clinical course and prognosis of PBC are highly variable. Early studies reported an average survival after diagnosis of 5 years (1). Because a diagnosis of PBC is made more frequently in patients with earlier stages of the disease the prognosis has improved: the reported average survival is now more than 11 years after diagnosis for symptomatic patients (4). Overall survival for asymptomatic patients is significantly better than for those with symptomatic disease but is diminished compared with a matched normal population (61-63). However, asymptomatic patients with histological stage I disease may comprise a subgroup with an excellent prognosis (61). In symptomatic patients, advanced age, hepatomegaly, elevated serum bilirubin levels, decreased serum albumin levels and cirrhosis each correlate with shortened survival (4,44,64-66). The most predictive prognostic factor is serum bilirubin (44,65). Once the serum bilirubin has exceeded 100 µmol/l the average life expectancy is limited to two years (65). Various mathematical models

have been devised to improve assessment of the prognosis (44,64,67). These models may be helpful in the management of PBC patients, for instance for decisions concerning the timing of liver transplantation. The most common causes of death in PBC are hepatic failure and the complications of portal hypertension.

Table 1: Controlled trials in PBC.

Author (references)	Patients randomized	Follow-up (months)*	Symptomatic improvement	Biochemical improvement **	Histological progression retarded	Survival improved
Prednisolone:						
Mitchison (33)	36	12	+	+	?	not evaluated
Penicillamine:						
Triger (34)	35	24	not evaluated	-	?	-
Epstein (35,36)	98	66	not evaluated	+	-	-
Bassendine (37)	59	37	not evaluated	+	-	-
Matloff (38)	52	28	-	trend	-	-
Teal (39)	23	12	-	-	-	not evaluated
Dickson (40)	227	50	-	+	-	-
Neuberger (41)	189	24	-	-	-	-
Bodenheimer (42)	56	36	not evaluated	+	-	not evaluated
Azathioprine:						
Heathcote (43)	45	36	-	-	-	trend
Christensen (44)	248	48	not evaluated	-	-	+*
Colchicine:						
Kaplan (45)	60	24	-	+	-	+
Bodenheimer (46,48)	57	33	-	+	-	-
Wernes (47)	64	23	not evaluated	+	-	trend
Chlorambucil:						
Hoofnagle (49)	24	52	not evaluated	+	-	not evaluated
UDCA:						
Leuschner (51)	20	9	+	+	?	not evaluated
Hadziyannis (54)	50	19	+	+	-	not evaluated
Italian Multicenter (55)	86	8	+	+	not evaluated	not evaluated
Poupon (56,57)	148	24	+	+	?	not evaluated
Cyclosporin A:						
Wiesner (117)	29	12	+	+	trend	not evaluated
Minuk (118)	12	12	-**	+	-	not evaluated

* mean or median follow up
** significant improvement of one or more of the following: bilirubin, ASAT, alkaline phosphatase, albumin
* after statistical adjustment
** symptoms progressed after discontinuation of CyA

2. METABOLIC BONE DISEASE IN PRIMARY BILIARY CIRRHOSIS

Like other chronic, especially cholestatic, liver diseases PBC may be complicated by metabolic bone disease (hepatic osteodystrophy). The histopathological lesions encountered in hepatic osteodystrophy are osteomalacia, osteoporosis or a combination of the two.

2.1 Osteomalacia. Osteomalacia is characterized by defective mineralization of bone; the only accurate way to diagnose this defect is examination of undecalcified bone biopsies, preferably by means of tetracycline double-labelling (68).

2.1.1 Prevalence of osteomalacia in PBC. In earlier studies the prevalence of osteomalacia in PBC appeared to be quite variable and sometimes rather high. Most of these studies, however, involved selected groups of patients, often with longstanding cholestasis, jaundice and steatorrhea (69,70), while inadequate histological criteria were used for diagnosis (32,69-71). In more recent studies, in which the recognized histological criteria were applied, various series of patients with PBC were investigated: osteomalacia could not be demonstrated at the time of diagnosis (72), in premenopausal women (73), or in patients selected for bone disease (74) and not even in those with jaundice (73,74) or subnormal vitamin D levels (75) (table 2).

Table 2: prevalence of osteomalacia in PBC.

Author (reference)	N	patient characteristics	prevalence
Mitchison (72)	33	at the time of diagnosis of PBC	0%
Hodgson (73)	15	premenopausal women	0%
Matloff (74)	10	selected for bone disease	0%
Herlong (75)	15	73% with subnormal vit-D	0%

2.1.2 Etiology of osteomalacia in PBC. When osteomalacia occurs in PBC it is related to vitamin D deficiency (68). The factors which can cause vitamin D deficiency in PBC are listed in table 3. Decreased sun exposure appears to be the

main cause (68,76). Impaired vitamin D intake because of anorexia or a low fat diet and impaired availability due to fat malabsorption may contribute to the problem in late stages of the disease (77,78). The 25-hydroxylation fuction of the liver, important as the first step in the synthesis of the active vitamin D metabolite 1, 25-dihydroxy-vitamin D, is maintained for a long time in the course of the disease and only becomes an additional factor in end-stage disease (79-81). Jaundice does not appear to affect vitamin D synthesis in the skin (79).

Table 3: possible factors implicated in vitamin D deficiency in PBC.

Synthesis in the skin:	decreased secondary to low sun exposure
Intake/availability:	decreased secondary to anorexia or low fat diet; intestinal malabsorption
Metabolism:	impaired 25-hydroxylation; increased urinary or fecal excretion of metabolites

2.1.3 Treatment of osteomalacia in PBC. Osteomalacia in PBC can be treated effectively by oral or parenteral administration of vitamin D or its metabolites (69,79,80).

2.2 Osteoporosis. There is general agreement that osteoporosis is the more common and clinically most important lesion in hepatic osteodystrophy (68). Osteoporosis is a heterogeneous skeletal disorder characterized by loss of bone mineral mass, which may result in non-traumatic fractures at typical sites such as the vertebrae, hip and forearm (82). Trabecular bone is metabolically more active than cortical bone and is therefore more susceptible to factors that influence bone metabolism. Because of this, cortical and trabecular bone are affected at different rates by osteoporosis (82). In PBC both trabecular (73,74,83) and cortical (83,84) osteoporosis occur, but trabecular bone loss, especially in the spine, is clinically more important (68).

2.2.1 Diagnosis of osteoporosis. According to most investigators trabecular bone volume measurements in iliac crest biopsies are not necessarily representative of events at clinically relevant sites such as the spine (68). Recently

non-invasive techniques have been developed to measure the bone mineral content of both the peripheral and the axial skeleton with high accuracy and reproducibility. One of these techniques is photon absorptiometry. Photons are absorbed by bone and, to a much lesser extent, by soft tissues. When a beam of photons passes through bone, the amount of photon energy that is absorbed is proportional to the amount of mineral in the bone. In single photon absorptiometry (SPA) the source of photons generates photons with a single energy. SPA can only produce accurate measurements of the skeleton when there is very little soft tissue, as in the forearm. In dual photon absorptiometry (DPA) the source produces photons with two energies, making it possible to adjust for variable amounts of soft tissue surrounding the skeletal part of interest. DPA, therefore, can be applied to measure the bone mineral density of the spine or hip (85). At present DPA of the spine has been used in only one study on osteoporosis in PBC (73). In most studies histomorphometry of iliac crest biopsies and in some SPA of the forearm was used to assess the bone status in patients with PBC (69,72,74,83,86).

2.2.2 Prevalence of osteoporosis in PBC. When osteoporosis is defined as a trabecular bone volume in an iliac crest biopsy that is more than two standard deviations less than the mean value for controls, matched for age and sex, the prevalence of osteoporosis in PBC varies in the literature from 0-17% (table 4).

Table 4: prevalence of osteoporosis in PBC.

Author (reference)	N	patient characteristics	prevalence
Mitchison (72)	33	at the time of diagnosis of PBC	0%
Cuthbert (86)	11		9%
Hodgson (73)	15	premenopausal women	15%
Stellon (83)	36	patients with cholestatic liver disease, 33 of whom had PBC	17%

2.2.3 Etiology and pathogenesis of osteoporosis in PBC. The cause of osteoporosis in hepatic osteodystrophy is unknown. Postmenopausal status, immobilization, malabsorption of calcium and the use of corticosteroids are all factors which

can cause osteoporosis and, therefore, may be involved in the pathogenesis of this bone disease in chronic liver disease.

The majority of patients with PBC are indeed postmenopausal women, but obviously osteoporosis also occurs in premenopausal patients (73). The absorption of calcium may be impaired in PBC (70,72,74,75), even without vitamin D deficiency, because of the formation of intraluminal calcium soaps (when steatorrhea exists), the use of cholestyramine or intestinal mucosal changes related to portal hypertension. Prolonged immobilization has been shown to cause vertebral bone loss (87). Finally, corticosteroids cause osteoporosis by impairing osteoblast function, promoting bone resorption and inhibiting the intestinal absorption of calcium (88,89). Few data on the effect of corticosteroids on the bones of patients with PBC have been published. Among 33 patients, 6 of whom were receiving or had received corticosteroids, Stellon et al. found a significantly greater loss of trabecular and cortical bone for the corticosteroid-treated group (all female) compared with other female patients (83). Furthermore, Mitchison et al. reported an increase in bone loss equal to twice the expected rate in patients treated with prednisolone (10 mg maintenance dose) for one year (33).

An important factor in the pathogenesis of osteoporosis in PBC appears to be low bone turnover. Several investigators have come to this conclusion on the basis of histomorphometrical data (73,74,90) and/or low osteocalcin levels (73,91). Osteocalcin (or bone Gla-protein) is a bone-specific protein which is produced by osteoblasts and is thought to reflect osteoblast function (73); its formation depends on vitamin D activity and the carboxylation of its glutamine residues on vitamin K.

From the studies of osteodystrophy in patients with PBC it is clear that the above-mentioned factors or conditions known to cause osteoporosis cannot explain the occurrence of osteoporosis in all patients with this complication. Postmenopausal status, immobilization, malabsorption of calcium and the use of corticosteroids may all contribute to the severity of the osteoporosis in PBC, but it is very likely that there is some other (probably liver disease-related) factor involved in the pathogenesis of this frequently debilitating complication.

2.2.4 Treatment of osteoporosis in PBC. At present there is no specific therapy or prophylaxis for osteoporosis in patients with PBC. In any case vitamin D is not effective (69,70,74,75). Calcium salts, administered as hydroxyapatite, have

been shown to be beneficial for cortical osteoporosis in PBC (84), but the clinical relevance of this therapy for spinal osteoporosis remains unclear. Other therapeutic regimens such as estrogens, sodium fluoride and biphosphonates, which may be effective in the prevention or treatment of other forms of osteoporosis (82,92,93), have not been studied in hepatic osteodystrophy. Liver transplantation may eventually reverse -at least partially- the loss of vertebral bone mass in patients with PBC (94).

3. CYCLOSPORIN A

3.1 Characteristics. CyA is the first of a new generation of immunosuppressive drugs (95). The compound, extracted from the soil fungus Tolypocladium inflatum Gams, and its unique immunosuppressive properties were first described by Borel and co-workers in the 1970s (96). CyA is a lipophilic cyclic peptide consisting of eleven amino acids, including several N-methylated and one new amino acid. For clinical use CyA is stabilized with castor oil and olive oil vehicles for intravenous and oral administration, respectively. CyA is extensively metabolized by the liver, primarily through demethylation and hydroxylation, and its metabolites are excreted into bile (97). Only a very small portion of unchanged CyA is recovered from urine. The metabolites of CyA probably do not contribute to either the immunosuppressive effects of the parent drug or its major toxic effect (i.e. nephrotoxicity) (98). There is a marked variability in the intestinal absorption, hepatic metabolism and excretion of CyA. Because of its lipophilic nature, the intestinal absorption of CyA depends on a normal bile flow into the gut, whereas its biotransformation in the liver may be influenced by hepatic disease or drugs interfering with hepatic metabolism (97).

3.2 Mechanisms of action (95,99,100). CyA is a unique immunosuppressive drug because it exerts its action almost exclusively on T-lymphocytes, although some (mainly T-cell dependent) B-cell responses also appear to be sensitive. CyA has no effect on myelopoeietic tissues. T-lymphocytes are activated by exposure to antigens and interaction with antigen presenting cells (e.g. macrophages). Activated helper T-cells release growth factors such as interleukin (IL-2), a lymphokine that stimulates the proliferation of activated cytotoxic T-cells. In addition, helper T-cells produce factors which amplify activated suppressor T-cell populations, necessary for down-regulation of the evolving immune response. The main mechanism of action of CyA is inhibition of the synthesis and release of IL-2 and other lymphokines (e.g.

γ-interferon and B-cell stimulating factor) by helper T-cells as well as the activation of precurser cytotoxic T-cells. Therefore, CyA influences the early phases of the immune response. CyA does not, however, affect the activation and amplification of regulatory suppressor T-cells. The precise intracellular site of action of CyA on the lymphocyte is unknown. CyA seems to interfere with calcium-dependent pathways, subsequent to the intracellular rise in calcium after antigen binding, thereby aborting the translation of messenger RNA which codes for lymphokines.

3.3 Clinical efficacy. CyA has been used extensively in the management of patients receiving organ transplants. In renal transplantation CyA appeared to be superior to conventional immunosuppressive therapy for graft and patient survival (101,102). The drug has been used successfully in bone marrow transplantation for the prevention of graft rejection and the treatment of graft-versus-host disease (103). In addition, the advent of CyA has had a major impact on transplantation results for other organs, such as the heart and liver (95,103).

Because of its specific immunosuppressive action CyA has been tried for the treatment of a variety of established or presumed autoimmune diseases (104), especially when T-cell responses are thought to be involved in the tissue damage (105). CyA has proven to be effective in the treatment of uveïtis, psoriasis, reumatoid arthritis, type I diabetes mellitus of recent onset and some forms of nephrotic syndrome (95).

3.4 Side-effects. Nephrotoxicity, the most frequent and clinically most important side-effect of CyA, is encountered in transplant recipients as well as patients with an autoimmune disease (95,103,106-112). Early nephrotoxicity is functional rather than structural. Its presumed cause is an imbalance between the vasodilator prostacyclin and its vasoconstrictive antagonist thromboxane A_2 in renal cortical tissue, leading to an increased renal vascular resistance (95,106). Histopathological changes in acute CyA-induced nephrotoxicity are absent or consist only of minimal tubular abnormalities (108). Acute nephrotoxicity is related to high CyA blood levels (108) and in general reversible (106).

Chronic nephrotoxicity is associated with the development of structural alterations which are potentially irreversible, including tubular atrophy, hyalinosis of arterioles and interstitial fibrosis (108). These morphological abnormalities have been found in

renal (108) and heart (107) transplant recipients, as well as patients on long-term CyA for autoimmune diseases (110-112). The clinical course of nephrotoxicity associated with the long-term use of CyA is generally benign, the reduction in renal function being non-progressive (101,102,109) and at least partially reversible (95,109). Progression to end-stage renal failure, however, has been reported (107). In addition, chronic changes in renal morphology have been found in patients with a normal renal function at the time of biopsy (110). Therefore, clinical assessment of the renal function may underestimate the extent of the morphological renal damage.
CyA-induced nephrotoxicity may be enhanced by the concomitant use of other nephrotoxic drugs (e.g. aminoglycosides, non-steroidal anti-inflammatory drugs) or the presence of hypertension (103). With respect to CyA-induced nephrotoxicity in autoimmune disease, Dieterle et al. (109) have reported on 465 patients collected from several studies of autoimmune diseases of various etiologies. In these studies patients were treated for six to more than 24 months. Renal function, expressed as the mean calculated creatinine clearance, decreased significantly in these patients within one month but stabilized about six months after initiation of therapy. The extent of renal dysfunction appeared to be greatest among patients with high initial CyA doses and correspondingly higher blood levels and those suffering from reumatoid arthritis or uveïtis. The impairment of renal function was largely reversible. Eight weeks after CyA was stopped, the mean decrease in creatinine clearance from baseline was only 4%. Reversibility was unfavorably influenced by more extensive acute nephrotoxicity, older age and longer duration of therapy. The possible role of the extent of acute renal injury in the development of chronic nephrotoxicity was also stressed by Palestine et al. (110). These investigators, who evaluated the effect of CyA in patients with uveïtis, found that the length of time that the serum creatinine exceeded the baseline value by more than 50 per cent was a good predictor of abnormal renal morphology.

Another side-effect related to the use of CyA is hypertension, the pathogenesis of which is unclear. The incidence of CyA-related hypertension is greater among renal and heart transplant recipients (103,107) than patients with autoimmune disease (109,113). This drug-induced hypertension may necessitate antihypertensive treatment but is usually reversible. Abnormal laboratory tests, associated with the use of CyA, include hyperkalemia, hyperuricemia, mild normochronic normocytic anemia and liver function disturbances, especially hyperbilirubinemia (95,113). Other

side-effects are common but in general dose-dependent, reversible and of minor clinical importance. These include hirsutism, hyper- or paresthesia, gingival hyperplasia, tremor and gastrointestinal complaints (95,113). In accordance with the specificity of its immunosuppressive action, CyA does not increase the risk of bacterial and fungal infections (95). Finally, CyA does not promote the development of de novo neoplasms (114).

3.5 Cyclosporin A in primary biliary cirrhosis; rationale for its use.

The possible role of T-lymphocytes in the hepatobiliary inflammatory process in PBC and the T-lymphocyte specific action of CyA make this drug potentially beneficial for patients with this chronic liver disease. In addition, the impaired concanavalin A-induced suppression of immunoglobulin production - one of the immunological abnormalities reported in PBC - was corrected in vitro by incubating mononuclear cells with CyA at a concentration of 250-500 ng/ml (16). The same immunological defect was found to be corrected in vivo by treating PBC patients with CyA in a dose of 2-4 mg/kg daily (115).

The first data on CyA therapy for PBC were published in 1980 by Routhier et al. (116). In an uncontrolled study 6 patients were treated with CyA in a dose of 10 mg/kg for 8 months. A significant decrease in alkaline phosphatase and aspartate aminotransferase (ASAT) levels was observed, but nephrotoxicity precluded continuation of the drug in all cases.

More recently the data on placebo-controlled trials to evaluate CyA for the treatment of PBC have been published. Wiesner et al. studied 29 non-cirrhotic patients with PBC, 19 receiving low-dose CyA therapy and the others a placebo (117). After 12 months there was a significant decrease in symptoms (pruritus, fatigue) and biochemical (bilirubin, alkaline phosphatase, ASAT) and immunological (serum IgM, IgG) values in the CyA-treated group compared with the controls. Furthermore, CyA beneficially influenced liver histology. Although there was a marked increase in serum creatinine levels in the majority of the treated patients as well as a rise in blood pressure in nearly half of them, the authors reported that these side-effects could be controlled by dose adjustment.

Minuk et al. conducted a small trial with 12 unselected patients with symptomatic PBC (118). In CyA recipients symptoms did not change during therapy but fatigue increased and well-being decreased after the drug was stopped. Cholestatic liver

enzymes (alkaline phosphatase and γ-glutamyl transpeptidase) decreased significantly but immunological (serum IgM) and histological parameters were not affected. Nephrotoxicity appeared to be the main side-effect, according to the mean serum creatinine levels which increased by 51%; creatinine clearance values, however, remained unchanged.

4. AIMS OF THE THESIS

Lack of knowledge about the specific cause and pathogenesis of PBC and its chronic and unpredictable course make it difficult to conduct and evaluate therapeutic studies. Moreover, it is hard to predict which patient will develop progressive disease and therefore is most likely to benefit from therapeutic intervention.
From what is known about the possible pathogenetic mechanisms involved in PBC, it seems reasonable to attempt to treat patients with antiinflammatory or immunosuppressive drugs. Results with drugs commonly used to treat autoimmune diseases, however, have been disappointing. The statement that corticosteroids are not only ineffective but also contraindicated in PBC is based mainly on theoretical considerations and not on clinical data. It was our clinical impression that - although not as effective as in chronic active autoimmune hepatitis - corticosteroids do have a beneficial effect on symptoms and biochemical parameters in PBC. This was supported by data from studies discussed in section 1.5.1 (9,33). In addition, it was our impression that low-dose corticosteroid therapy did not cause major clinical metabolic bone disease in our patients with PBC.

The studies described in this thesis focus on three items:

a. The possible role of CyA in the treatment of PBC. Firstly, we tried to find out whether CyA is effective in treating PBC, which dose would be appropriate and whether there is a subgroup of PBC patients that would particularly benefit from CyA therapy (chapter II). Secondly, we investigated whether the efficacy of CyA in the treatment of PBC could be enhanced by selecting patients on the basis of data from the first study and by adding a low dose of prednisone (chapter III). Finally - because CyA is metabolized almost entirely in the liver - we studied the pharmacokinetics of the drug in patients with non-endstage PBC and a control group of patients with a presumably normal liver, in this case patients with skin diseases (chapter IV).

b. The extent of metabolic bone disease and the possible role of corticosteroid therapy in our patients with PBC (chapter V).

c. The value of type III procollagen amino propeptide as a predictor for progressive disease. Looking for a parameter that would indicate which patients will develop progressive disease we retrospectively studied the levels of the precursor peptide of collagen type III, i.e. the aminoterminal procollagen peptide type III (PIIIP), in patients with histologically progressive disease compared with those with non-progressive early disease (chapter VI).

In chapter VII a general discussion of immunosuppressive therapy is given, including a proposition for future clinical trials on PBC.

5. REFERENCES

1. Sherlock S. Primary biliary cirrhosis (chronic intrahepatic obstructive jaundice). Gastroenterology 1959;37:574-86.
2. Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. N Engl J Med 1973;289:674-8.
3. Christensen E, Crowe J, Doniach D, Popper H, Ranek L, Rodés J et al. Clinical pattern and course of disease in primary biliary cirrhosis based on an analysis of 236 patients. Gastroenterology 1980;78:236-46.
4. 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.
5. James O, Macklon AF, Watson AJ. Primary biliary cirrhosis - a revised clinical spectrum. Lancet 1981;I:1278-81.
6. Beswick DR, Klatskin G, Boyer JL. Asymptomatic primary biliary cirrhosis. Gastroenterology 1985;89:267-71.
7. Munoz LE, Thomas HC, Scheuer PJ, Doniach D, Sherlock S. Is mitochondial antibody diagnostic of primary biliary cirrhosis? Gut 1981;22:136-40.
8. Manns M. Auto-antibodies and antigens in liver diseases-updated. J Hepatol 1989;9:272-80.
9. Taal BG. Studies in primary biliary cirrhosis. Thesis. Neth J Med 1984;24, suppl.I:1-32.
10. Scheuer PJ. Primary biliary cirrhosis. Proc Roy Soc Med 1967;60:1257-60.

11. Ludwig J, Dickson ER, McDonald GSA. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Path Anat and Histol 1978;379:103-12.
12. Rubin E, Schaffner F, Popper H. Primary biliary cirrhosis. Am J Path 1965;46:387-400.
13. Janssens AR. Copper metabolism in primary biliary cirrhosis. Thesis 1983.
14. James SP, Hoofnagle JH, Strober W, Jones EA. Primary biliary cirrhosis: a model autoimmune disease. Ann Int Med 1983;99:500-12.
15. James SP, Elson CO, Jones EA, Strober W. Abnormal regulation of immunoglobulin synthesis in vitro in primary biliary cirrhosis. Gastroenterology 1980;79:242-54.
16. Al-Aghbar MNA, Alexander GJM, Nouri-Aria KT, Neuberger J, Eddleston ALWF, Williams R. In vitro effect of Cyclosporin A on immunoglobulin production and concanavalin A induced suppression in primary biliary cirrhosis. Gut 1986;27:317-23.
17. Zetterman RK, Woltjen JA. Suppressor cell activity in primary biliary cirrhosis. Dig Dis Sc 1980;25:104-7.
18. Paronetto F, Colucci G, Colombo M. Lymphocytes in liver disease. In: Progress in liver disease. Popper H and Schaffner F, eds. Grune & Stratton Inc., Orlando 1986;191-208.
19. Bottazzo GF, Pujol-Borrell R, Hanafusa T, Feldmann M. Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. Lancet 1983;II:1115-9.
20. Jaup BH, Zettergren LSW. Familial occurrence of primary biliary cirrhosis associated with hypergammaglobulinemia in descendants: a family study. Gastroenterology 1980;78:549-55.
21. Galbraith RM, Smith M, MacKenzie RM, Tee DE, Doniach D, Williams R. High prevelance of sero-immunologic abnormalities in relatives of patients with active chronic hepatitis or primary biliary cirrhosis. N Engl J Med 1974;290:63-9.
22. Miller KB, Sepersky RA, Brown KM, Goldberg MJ, Kaplan MM. Genetic abnormalities of immunoregulation in primary biliary cirrhosis. Am J Med 1983;75:75-80.

23. Eggink HF, Houthoff HJ, Huitema S, Gips CH, Poppema S. Cellular and humoral immune reactions in chronic active liverdisease. I. Lymphocyte subsets in liver biopsies of patients with untreated idiopathic autoimmune hepatitis, chronic active hepatitis B and primary biliary cirrhosis. Clin Exp Immunol 1982;50:17-24.

24. Yamada G, Hyodo I, Tobe K, Mizuno M, Nishihara T, Kobayashi T et al. Ultrastructural immunocytochemical analysis of lymphocytes infiltrating bile duct epithelium in primary biliary cirrhosis. Hepatology 1986;6:385-91.

25. Ballardini G, Bianchi FB, Doniach D, Mirakian R, Pisi E, Bottazo GF. Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. Lancet 1984;II:1009-13.

26. Colucci G, Schaffner F, Paronetto F. Lymphocyte subpopulations and distribution of HLA antigen in liver biopsies of patients with primary biliary cirrhosis. Hepatology 1984;4:1087 (abstract).

27. Van den Oord JJ, Sciot R, Desmet VJ. Expression of MHC products by normal and abnormal bile duct epithelium. J Hepatol 1986;3:310-7.

28. 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.

29. 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:1419-22.

30. Hoffbauer FW. Primary biliary cirrhosis: observations on the natural course of the disease in 25 women. Am J Dig Dis 1960;5:348-83.

31. 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.

32. Long RG, Meinhard E, Skinner RK, Varghese Z, Wills MR, Sherlock S. Clinical, biochemical and histological studies of osteomalacia, osteoporosis, and parathyroid function in chronic liver disease. Gut 1978;19:85-90.

33. 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.

34. Triger DR, Manifold IH, Cloke P, Underwood JCE. D-penicillamine in primary biliary cirrhosis: two year results of a single centre, double-blind controlled trial. Gut 1980;21:919-20 (abstract).

35. 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.

36. Epstein O, Cook DG, Jain S, McIntyre N, Sherlock S. D-penicillamine and clinical trials in PBC. Hepatology 1984;4:1032 (abstract).

37. Bassendine MF, Macklon AF, Mulcahy R, James OFW. Controlled trial of high and low dose D-penicillamine in primary biliary cirrhosis (PBC): results at three years. Gut 1982;23:909 (abstract).

38. 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.

39. 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.

40. 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.

41. 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.

42. 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.

43. Heathcote J, Ross A, Sherlock S. A prospective controlled trial of azathioprine in primary biliary cirrhosis. Gastroenterology 1976;70:656-60.

44. 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.

45. 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.

46. Bodenheimer H, Schaffner F, Pezullo J. Evaluation of colchicine therapy in primary biliary cirrhosis. Gastroenterology 1988;95:124-9.

47. 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.

48. Zifroni A, Schaffner F. Long-term follow-up of patients with primary biliary cirrhosis (PBC) on colchicine therapy. Hepatology 1990;12:843 (abstract).

49. 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.

50. 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.

51. 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 trial. Gastroenterology 1989;97:1268-74.

52. Güldütuna S, Imhof M, Hoffmann T, Zimmer G, Leuschner U. Ursodeoxycholic acid (UDCA) protects basolateral liver plasma membranes (blLPM) against toxic bile salts. Hepatology 1990;12:997 (abstract).

53. Calmus Y, Gane P, Rouger Ph, Poupon R. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-5.

54. Hadziyannis SJ, Hadziyannis ES, Makris A. A randomized controlled trial of ursodeoxycholic acid (UDCA) in primary biliary cirrhosis (PBC). Hepatology 1989;10:580 (abstract).

55. Italian multicenter project for UDCA treatment in PBC. Ursodeoxycholic acid (UDCA) for symptomatic primary biliary cirrhosis (PBC): a double-blind multicenter trial. J Hepatol 1989;9:S44 (abstract).

56. Poupon RE, Eschwège E, Poupon R, the UDCA-PBC study group. Ursodeoxycholic acid for the treatment of primary biliary cirrhosis. J Hepatol 1990;11:16-21.

57. Poupon RE, Balkau B, Eschwège E, Poupon R, the UDCA-PBC study group. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Eng J Med 1991;324:1548-54.

58. Wiesner RH, Jorgenson R, Perdigoto R. Ursodeoxycholic acid therapy for primary biliary cirrhosis: progression of disease despite near normalization of biochemical liver tests. Hepatology 1990;12:843 (abstract).

59. Esquivel CO, Van Thiel DH, Demetris AJ, Bernardos A, Iwatsuki S, Markus B et al. Transplantation for primary biliary cirrhosis. Gastroenterology 1988;94:1207-16.

60. 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.

61. Nyberg A, Lööf L. Primary biliary cirrhosis: clinical features and outcome, with special reference to asymptomatic disease. Scand J Gastroenterol 1989;24:57-64.

62. Balasubramaniam K, Grambsch PM, Wiesner RH, Lindor KD, Dickson ER. Diminished survival in asymptomatic primary biliary cirrhosis. Gastroenterology 1990;98:1567-71.

63. 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;99:778-84.

64. Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989;10:1-7.

65. Shapiro JH, Smith H, Schaffner F. Serum bilirubin: a prognostic factor in primary biliary cirrhosis. Gut 1979;20:137-40.

66. Portmann B, Popper H, Neuberger J, Williams R. Sequential and diagnostic features in primary biliary cirrhosis based on serial histologic study in 209 patients. Gastroenterology 1985;88:1777-90.

67. Neuberger JM. Predicting the prognosis of primary biliary cirrhosis. Gut 1989;30:1519-22.

68. Compston JE. Hepatic osteodystrophy: vitamin D metabolism in patients with liver disease. Gut 1986;27:1073-90.

69. Reed JS, Meredith SC, Nemchausky BA, Rosenberg IH, Boyer JL. Bone disease in primary biliary cirrhosis: reversal of osteomalacia with oral 25-hydroxyvitamin D. Gastroenterology 1980;78:512-7.

70. Kehayoglou AK, Agnew JE, Holdsworth CD, Whelton MJ, Sherlock S. Bone disease and calciumabsorption in primary biliary cirrhosis. Lancet 1968;I:715-9.

71. Ajdukiewicz AB, Agnew JE, Byers PD, Wills MR, Sherlock S. The relief of bone pain in primary biliary cirrhosis with calciuminfusions. Gut 1974;15:788-93.
72. Mitchison HC, Malcolm AJ, Bassendine MF, James OFW. Metabolic bone disease in primary biliary cirrhosis at presentation. Gastroenterology 1988;94:463-70.
73. Hodgson SF, Dickson ER, Wahner HW, Johnson KA, Mann KG, Riggs BL. Bone loss and reduced osteoblast function in primary biliary cirrhosis. Ann Int Med 1985;103:855-60.
74. Matloff DS, Kaplan MM, Neer RM, Goldberg MJ, Bitman W, Wolfe HJ. Osteoporosis in primary biliary cirrhosis: effects of 25-hydroxyvitamin D_3 treatment. Gastroenterology 1982;83:97-102.
75. Herlong HF, Recker RR, Maddrey WC. Bone disease in primary biliary cirrhosis: histologic features and response to 25-hydroxyvitamin D. Gastroenterology 1982;83:103-8.
76. Long RG. Hepatic osteodystrophy: outlook good but some problems unsolved. Gastroenterology 1980;78:644-7.
77. Barragry JM, Long RG, France MW, Wills MR, Boucher BJ, Sherlock S. Intestinal absorption of cholecalciferol in alcoholic liver disease and primary biliary cirrhosis. Gut 1979;20:559-64.
78. Kaplan MM, Elta GH, Furie B, Sadowski JA, Russell RM. Fat-soluble vitamin nutriture in primary biliary cirrhosis. Gastroenterology 1988;95:787-92.
79. Davies M, Mawer EB, Klass HJ, Lumb GA, Berry JL, Warnes TW. Vitamin D deficiency, osteomalacia, and primary biliary cirrhosis. Response to orally administered vitamin D_3. Dig Dis Sc 1983;28:145-53.
80. Compston JE, Horton LWL, Thompson RPH. Treatment of osteomalacia associated with primary biliary cirrhosis with parenteral vitamin D_2 or oral 25-hydroxyvitamin D_3. Gut 1979;20:133-6.
81. Skinner RK, Long RG, Sherlock S, Wills MR. 25-Hydroxylation of vitamin D in primary biliary cirrhosis. Lancet 1977;I:720-1.
82. Riggs BL, Melton LJ. Involutional osteoporosis. N Engl J Med 1986;314:1676-86.
83. Stellon AJ, Davies A, Compston JE, Williams R. Osteoporosis in chronic cholestatic liver disease. Quart J Med 1985;57:783-90.

84. Epstein O, Kato Y, Dick R, Sherlock S. Vitamin D, hydroxyapatite, and calciumgluconate in treatment of cortical bone thinning in postmenopausal women with primary biliary cirrhosis. Am J Clin Nutr 1982;36:426-30.

85. Health and public policy committee, American college of physicians. Bone mineral densitometry. Ann Int Med 1987;107:932-6.

86. Cuthbert JA, Pak ChYC, Zerwekh JE, Glass KD, Combes B. Bone disease in primary biliary cirrhosis: increased bone resorption and turnover in the absence of osteoporosis or osteomalacia. Hepatology 1984;4:1-8.

87. Krolner B, Toft B. Vertebral bone loss: an unneeded side effect of therapeutic bed rest. Clin Sc 1983;64:537-40.

88. Peck W, Gennari C, Raisz L, Meunier P, Ritz E, Krane S et al. Corticosteroids and bone. Calcif Tissue Int 1984;36:4-7.

89. Braun JJ, Birkenhäger-Frenkel DH, Rietveld AH, Juttman JR, Visser TJ, Birkenhäger JC. Influence of 1 α-(OH)D3 administration on bone and bone mineral metabolism in patients on chronic glucocorticoid treatment: a double blind controlled study. Clin Endocrinol 1983;19:265-73.

90. Stellon AJ, Webb A, Compston J, Williams R. Low bone turnover state in primary biliary cirrhosis. Hepatology 1987;7:137-42.

91. Fonseca V, Epstein O, Gill DS, Menon RK, Thomas M, McIntyre N et al. Hyperparathyreoidism and low serum osteocalcin despite vitamin D replacement in primary biliary cirrhosis. J Clin Endocrinol Metab 1987;64:873-7.

92. Valkema R, Vismans FJFE, Papapoulos SE, Pauwels EKJ, Bijvoet OLM. Maintained improvement in calcium balance and bone mineral content in patients with osteoporosis treated with the biphosphonate APD. Bone and Mineral 1989;5:183-92.

93. Reid IR, King AR, Alexander CJ, Ibbertson HK. Prevention of steroid-induced osteoporosis with (3-amino-1-hydroxypropylidene)-1, 1-biphosphonate (APD). Lancet 1988;I:143-6.

94. Wiesner RH, Dickson ER, Hodgson SF, Wahner H. The effect of liver transplantation on bone mineral density in primary biliary cirrhosis. Hepatology 1987;5:1049 (abstract).

95. Kahan BD. Cyclosporine. N Engl J Med 1989; 321: 1725-38.

96. Borel JF, Feuer C, Gubler HU, Stähelin H. Biological effects of cyclosporin A: a new antilymphocytic agent. Agents and Actions 1976;6:468-75.

97. Ptachcinski RJ, Venkataramanan R, Burckart GJ. Clinical pharmacokinetics of cyclosporin. Clin Pharmacok 1986;11:107-32.

98. Ryffel B, Foxwell BMJ, Mihatsch MJ, Donatsch P, Maurer G. Biologic significance of cyclosporine metabolites. Transpl Proceed 1988;20 (suppl 2):575-84.

99. Hess AD, Esa AH, Colombani PM. Mechanisms of action of cyclosporine: effect on cells of the immune system and on subcellular events in T cell activation. Transpl Proceed 1988;20 (suppl.2):29-40.

100. Borel JF, Ryffel B. The mechanism of action of ciclosporin: a continuing puzzle. In: Ciclosporin in autoimmune diseases. Schindler R. Ed. Springer-Verlag Berlin 1985;24-32.

101. Merion RM, White DJG, Thiru S, Evans DB, Calne RY. Cyclosporine: five year's experience in cadaveric renal transplantation. N Engl J Med 1984;310:148-54.

102. The Canadian multicentre transplant study group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. N Engl J Med 1986;314:1219-25.

103. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporine: a new immunosuppressive agent for organ transplantation. Ann Int Med 1984;101:667-82.

104. Bach JF. Cyclosporine in autoimmunity. Transpl Proceed 1988;20 (suppl 4):379-81.

105. Talal N. Cyclosporine as an immunosuppressive agent for autoimmune disease: theoretical concepts and therapeutic strategies. Transpl Proceed 1988;20 (suppl 4):11-5.

106. Myers BD. Cyclosporine nephrotoxicity. Kidney Int 1986;30:964-74.

107. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. N Engl J Med 1984;311:699-705.

108. Mihatsch MJ, Thiel G, Ryffel B. Cyclosporine-associated nephropathy. In: Ciclosporin in autoimmune diseases. Schindler R. Ed. Springer-Verlag Berlin 1985;50-8.

109. Dieterle A, Abeywickrama K, Graffenried B von. Nephrotoxicity and hypertension in patients with autoimmune disease treated with cyclosporine. Transpl Proceed 1988 (suppl. 4);20:349-55.

110. Palestine AG, Austin III HA, Balow JE, Antonovych TT, Sabnis SG, Preuss HG et al. Renal histopathologic alterations in patients treated with cyclosporine for uveitis. N Engl J Med 1986;314:1293-8.

111. Svenson K, Bohman SO, Hälgren R. Clinical effects and renal side effects in patients with autoimmune diseases treated with ciclosporin (CyA). In: Ciclosporin in autoimmune diseases. Schindler R. Ed. Springer-Verlag Berlin 1985;74-9.

112. Nahman NS, Cosio FG, Kolkin S, Mendell JR, Sharma HM. Cyclosporine nephrotoxicity without major organ transplantation. Ann Int Med 1987;106:400-2.

113. Palestine AG, Nussenblatt RB, Chan CC. Side effects of systemic cyclosporine in patients not undergoing transplantation. Am J Med 1984;77:652-6.

114. Penn I. Cancers following cyclosporine therapy. Transplantation 1987;43:32-5.

115. Al-Aghbar MNA, Alexander GJM, Neuberger J, Nouri-Aria KT, Eddleston ALWF, Williams R. Effect of cyclosporin A on suppressor cell function in vivo and in vitro in primary biliary cirrhosis. Gut 1983;24:A970 (abstract).

116. Routhier G, Epstein O, Janossy G, Thomas HC, Sherlock S, Kung PC et al. Effects of cyclosporin A on suppressor and inducer T-lymphocytes in primary biliary cirrhosis. Lancet 1980;II:1223-6.

117. 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;322:1419-24.

118. Minuk GY, Bohme CE, Burgess E, Hershfield NB, Kelly JK, Shaffer EA et al. Pilot study of cyclosporin A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988;95:1356-63.

CYCLOSPORIN A IN PRIMARY BILIARY CIRRHOSIS

A pilot study of ten patients.

R. Beukers, S.W. Schalm

The contents of this study have been published as part of the paper entitled:
Effect of cyclosporine and cyclosporine plus prednisone in primary biliary cirrhosis.
Transplant Proceed 1988; 20 (suppl. 4): 340-343

SUMMARY

Ten patients with symptomatic primary biliary cirrhosis (PBC) were treated for six months with cyclosporin A (CyA); the initial dose was 5 mg/kg. Five patients were icteric (stage IV), five were anicteric (stages I-IV). CyA therapy was discontinued in one case because of chronic diarrhea. For the remaining nine patients CyA treatment had no marked effect on symptoms. The mean decrease in the alkaline phosphatase level was 16% ($p<0.05$), but the results of other liver function tests did not change. Serum IgM decreased from an average of 7.2 to 5.9 g/l ($p=0.05$). The main side-effect was nephrotoxicity, which could be controlled by close monitoring of CyA trough levels in plasma and dose adjustment. The mean CyA dose decreased in the course of the six-month period to 2.8 mg/kg.

CyA treatment did not prevent further clinical deterioration in patients with end-stage disease. In some anicteric PBC patients, however, treatment had a beneficial effect on the abnormal biochemical and immunological parameters, which disappeared after discontinuation of CyA.

Our data warrant more extensive studies on the efficacy of long-term low-dose CyA therapy for anicteric PBC patients with signs of active or progressive disease.

INTRODUCTION

Although the etiology of primary biliary cirrhosis (PBC) is unknown, immunological mechanisms may play an important role in its pathogenesis (1). However, treatment with immunosuppressive agents such as D-penicillamine (2) and azathioprine (3) was without clinically important benefit. No controlled trials have been carried out to evaluate the results of corticosteroid therapy, mainly because these drugs were presumed to be contraindicated in view of possible aggravation of osteodystrophy. Cyclosporin A (CyA) is a new T lymphocyte-specific immunosuppressive agent with proven benefit in transplant recipients. The efficacy of CyA in autoimmune disease is now being investigated in centers all over the world. Encouraging results of CyA therapy for PBC were reported by Routhier and co-workers (4); however their dose of 10 mg/kg led to a nephrotoxicity which precluded continuation of treatment in all cases. We studied the effect of a six-month course of CyA in 10 symptomatic patients with PBC. To reduce nephrotoxicity we chose a lower initial dose and monitored the CyA levels.

PATIENTS AND METHODS

Ten symptomatic patients with PBC received CyA in an initial dose of 5 mg/kg for six months, and were then closely followed for another six months. The diagnosis of PBC was established according to classical criteria (i.e. alkaline phosphatase more than two times the upper limit of normal, a positive test for antimitochondrial antibodies and demonstration of typical bile duct lesion in the liver biopsy) in all cases.

The median age was 47.5 years (range: 29-56 years) and the median duration of disease was 7.5 years (range: 3-13 years). Five patients were icteric, all with stage IV disease. The histological stage of the five anicteric patients ranged from I-IV. Seven patients had previously received immunosuppressive agents which, except for prednisone (10 mg/day), had been discontinued for at least six months at the time of this study. Four patients continued to take prednisone during the trial period. Three patients had never received immunosuppressive therapy before.

The effect of CyA treatment on symptoms (general well-being, fatigue, pruritus), biochemical (alkaline phosphatase, bilirubin and ASAT levels) and immunological (serum IgM and IgG levels, Clq binding assay) measurements and histology was evaluated. Patients were also monitored to determine the toxicity and side-effects of CyA. Symptoms were scored from 0 to 3, corresponding to no, mild, moderate or severe symptoms, respectively. Liver biopsies were taken before and - whenever possible - after treatment with CyA. The biopsies were examined without prior knowledge by an experienced hepatic pathologist for fibrosis (score: 0 to 4) and mononuclear cell infiltration (density and invasion of periportal parenchyma; score: 0 to 3). CyA trough levels were measured in plasma (separated from blood cells at 37°C (5)) and whole blood by radioimmunoassay. On the basis of the CyA trough plasma levels and the clinical and biochemical side-effects, the CyA dose was adjusted in the course of the six-month period to an average of 2.8 mg/kg (range: 2.0 - 5.0 mg/kg).

The changes in symptom scores and biochemical and immunological variables were analyzed by the Wilcoxon signed rank test for paired data. Probability values ≤ 0.05 were considered significant.

RESULTS

In one case, an icteric 46-year-old female patient with stage IV disease, CyA therapy was discontinued after four months because of chronic diarrhea associated with a lack of therapeutic CyA levels, possibly related to malabsorption of the drug. Therefore the effect of CyA on PBC could be evaluated in nine patients (table 1). No obvious effect of CyA treatment on general well-being, fatigue or pruritus was observed. The mean serum alkaline phosphatase levels decreased significantly ($p<0.05$) from 317 to 266 U/l (upper limit of normal: 45 U/l), an effect observed in icteric as well as anicteric patients. Neither the bilirubin nor the ASAT levels were significantly affected. The mean serum IgM levels decreased from 7.2 to 5.9 g/l ($p=0.05$). Circulating immune complex levels (according to the Clq binding assay) and serum IgG levels remained unchanged. All patients underwent a liver biopsy before initiation of CyA treatment. Nine out of the ten patients completed the six-month course of CyA therapy. A biopsy could be taken at the end of the trial period in five cases. After six months of treatment with CyA, the density of portal and periportal mononuclear infiltration had decreased in four patients and remained unchanged in one. The spread of the infiltrate into the periportal parenchyma had decreased in three patients and was unchanged in two. CyA treatment did not influence the degree of fibrosis in four patients. In one case the score for fibrosis had decreased after CyA treatment. None of the liver specimens taken after therapy showed progression of either fibrosis or mononuclear cell infiltration.

Nephrotoxicity, as indicated by an elevated serum creatinine level, was the main side-effect and was encountered in all patients. The mean (±SD) increase in serum creatinine levels compared with initial values was 63 (±60) per cent after three months ($p=0.008$) and 41 (±30) per cent after six months ($p=0.008$). Futhermore, the maximum increase in creatinine levels ranged from 23 to 157 per cent (mean ±SD: 83 ±48) and exceeded 100 per cent in three cases (patients 2, 5 and 6; table 1). There was no clear correlation between CyA trough levels and serum creatinine for the total group (fig. 1). For the individual patient however a relationship between CyA dose, CyA levels and serum creatinine could be observed. Other side-effects included gastrointestinal complaints in four cases and hirsutism, gingival hyperplasia and tremor each in one case (table 1).

CyA treatment did not prevent further clinical deterioration in the three patients with end-stage disease. One patient with end-stage disease died of liver failure four

Table 1: Effect on laboratory test results and side-effects of CyA treatment for patients with primary biliary cirrhosis.

Patient	sex	age (yr)	histological stage	before treatment			after treatment			side-effects°
				bili (< 12 umol/l)	alk.ph. (< 45 U/l)	IgM (< 2.8 G/l)	bili (< 12 umol/l)	alk.ph. (< 45 U/l)	IgM (< 2.8 G/l)	
1	F•	56	II	16	586	4.4	46	496	4.8	hisurtism
2	F•	44	IV	230	303	3.5	190	276	2.4	
3	F•	50	IV	391	516	1.5	322	401	1.6	nausea
4	F	56	III	10	196	7.8	12	109	5.5	
5	M	29	IV	70	268	17.7	42	166	12.9	anorexia tremor
6	F	50	IV	155	249	3.1	141	155	4.3	
7	F	46	IV	7	222	12.2	10	179	10.6	
8	F	42	I	14	265	4.3	15	350	3.0	nausea
9	F•	54	III	37	247	10.2	40	266	7.6	anorexia gingival-hyperplasia
mean (SD)				103 (133)	317** (137)	7.2* (5.3)	91 (107)	266** (129)	5.9* (3.8)	

• prednisone (10 mg/kg) continued during trial
* p = 0.05 (Wilcoxon test)
** p < 0.05 (Wilcoxon test)
° other than nephrotoxicity

alk.ph.: alkaline phosphatase

months after discontinuation of CyA treatment. The other two end-stage patients exhibited progressive elevation of the bilirubin level and a further impairment of protein synthesis; in one case this led to liver transplantation eight months after CyA treatment. Some of the patients in earlier stages of the disease, however, seem to

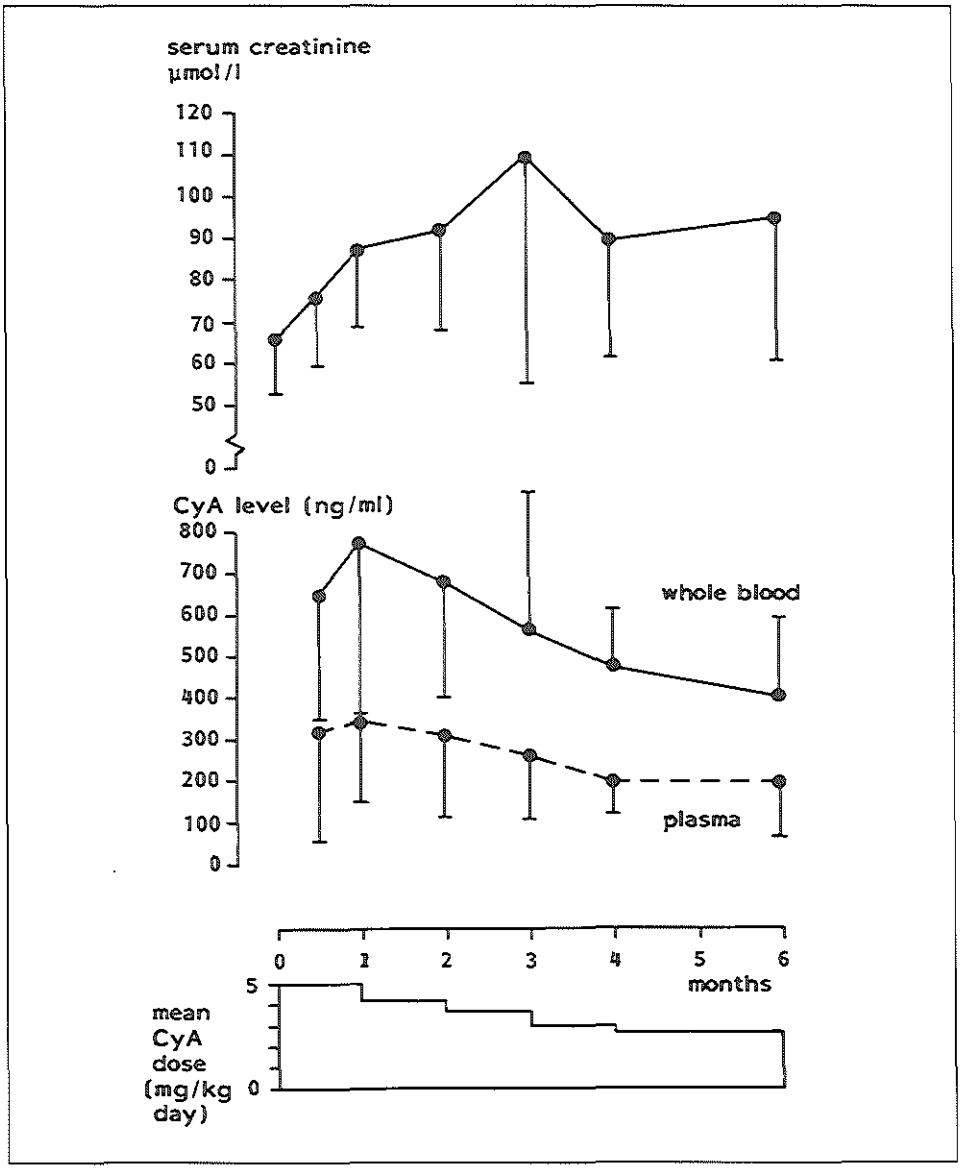

Figure 1: Relationship between serum creatinine, CyA dose and CyA trough levels in blood and plasma for 9 patients with PBC (mean ± SD).

have benefitted from CyA treatment, as illustrated by the data on a 56-year-old woman with stage III PBC (fig. 2). In this patient a decrease in alkaline phosphatase, ASAT, serum immunoglobulins and circulating immune complex levels was obtained during CyA treatment. After discontinuation of CyA however all measurements returned to pretreatment levels.

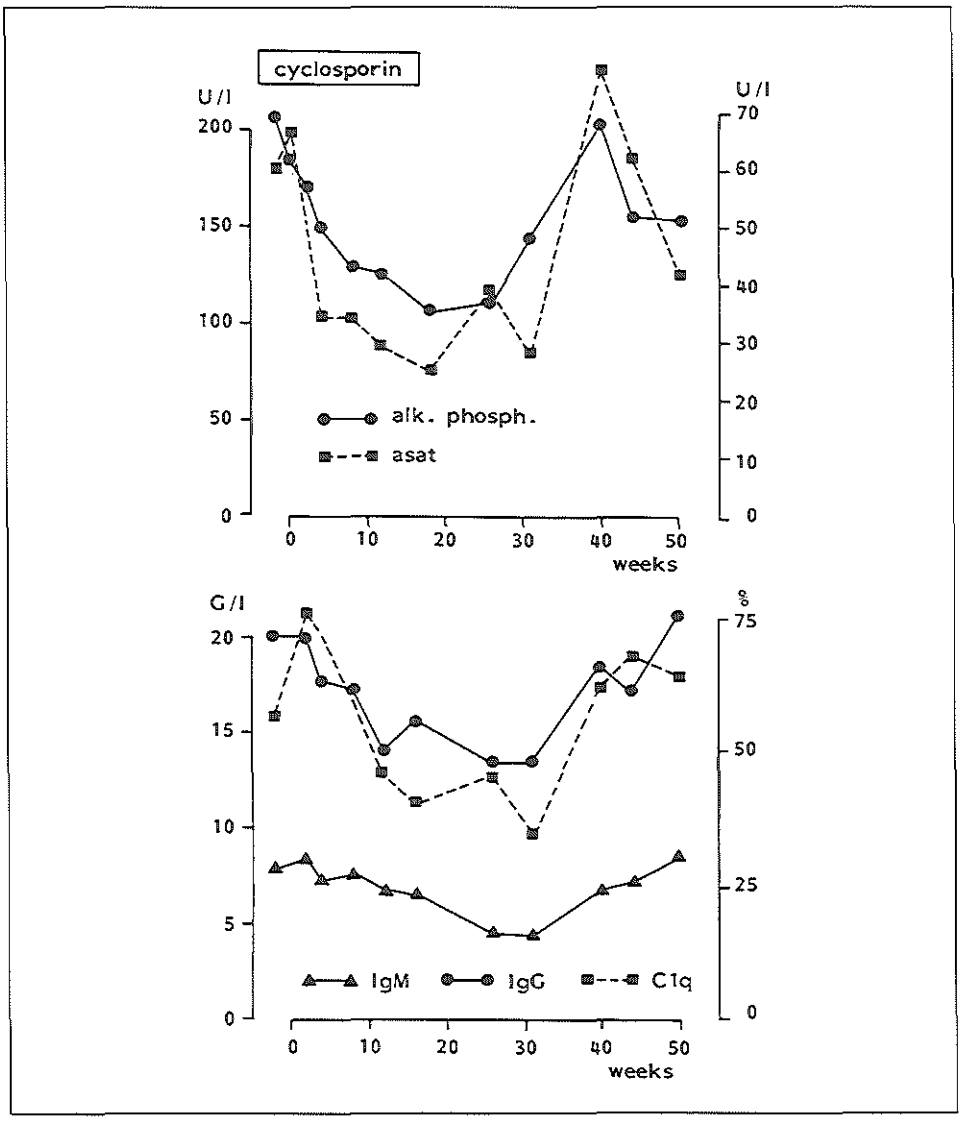

Figure 2: Effect of CyA treatment on biochemical and immunological parameters for a 56-year-old woman with PBC (stage III) of 7 years duration.

DISCUSSION

The ultimate goal of therapeutic trials for PBC is prolongation of survival. The effect on survival is, however, difficult to assess because of the slow and variable course of this disease. We believe that a therapeutic agent that effectively prolongs survival when administered for long periods will have striking effects on clinical, biochemical and histological parameters when given for a short period. Although a small patient series carries the risk of a type B error, it is our opinion that striking results will still become obvious in such studies.

Administration of CyA for six months had no obvious immediate effect on symptoms in this group of patients with different stages of PBC. A significant decrease in alkaline phosphatase levels was observed, but - in contrast to an earlier report (4) - we found no effect on ASAT levels. In addition CyA induced a small, but significant, decrease in IgM levels.

In view of the specific influence of CyA on T lymphocytes, one would expect mononuclear cell infiltration in diseased tissue to decrease during CyA treatment. Indeed we found that the density of portal and periportal mononuclear cell infiltration was lower in four out of the five patients from whom liver biopsies could be taken before and after therapy, while the extent of the infiltrate was reduced in three. No progression of either the portal and periportal mononuclear cell infiltration or the fibrosis was observed.

Nephrotoxicity was the main side-effect, as has been reported by others (4). Serum creatinine rose markedly (fig.1) at rather low doses of CyA, compared to the doses used in organ transplantation. The magnitude of the nephrotoxic effect was variable, dose-dependent and reversible in most patients. In some cases, serum creatinine did not return to pretreatment levels. Presumably concomitant use of diuretics and further impairment of liver function also contributed to the impairment of renal function in these patients. Nephrotoxicity was the reason to discontinue CyA treatment - temporarily - in one case only. It has been stated that nephrotoxicity precludes the administration of CyA to PBC patients (4), but in our patients this side-effect could be controlled by close monitoring of the serum creatinine and CyA trough levels and reduction of the CyA dose.

Asymptomatic patients were excluded from the study because of the uncertain benefits and the potential toxicity of the drug. There was no preselection of the symptomatic patients included in this study as far as the clinical stage of the disease

is concerned. For end- stage patients the clinical course was not altered by CyA treatment. For these patients, in whom liver cell damage appears to be self-perpetuating because of irreversible cholestasis, no medical treatment is likely to be of benefit. But our data suggest that anicteric patients with PBC, in particular those with active or progressive but not end-stage disease (such as the patient of fig. 2), may benefit from CyA treatment. A six-month course does not appear to have a permanent effect on the course of this chronic liver disease. Although the overall efficacy of CyA treatment for this group of PBC patients was not impressive, we conclude that more extensive studies on the effects of long-term low-dose CyA in anicteric patients with PBC are warranted.

ACKNOWLEDGEMENTS

We are indebted to A.J.H. de Boer for his technical assistance.

This study was supported by Sandoz Ltd., Basel, Switzerland

REFERENCES

1. James SP, Hoofnagle JH, Strober W, Jones EA. Primary biliary cirrhosis: a model autoimmune disease. Ann Int Med 1983;99:500-12.
2. James O. D-penicillamine for primary biliary cirrhosis. Gut 1985;26:109-13.
3. 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.
4. Routhier G, Epstein O, Janossy G, Thomas HC, Sherlock S, Kung PC et al. Effects of cyclosporin A on suppressor and inducer T lymphocytes in primary biliary cirrhosis. Lancet 1980;II:1223-26.
5. Berg JWO van den, Verhoef ML, Boer AJH de, Schalm SW. Cyclosporin A assay: condition for sampling and processing of blood. Clin Chem Acta 1985;147:291-7.

PILOT STUDY ON THE COMBINATION OF CYCLOSPORIN A AND PREDNISONE FOR THE TREATMENT OF SYMPTOMATIC PRIMARY BILIARY CIRRHOSIS

R. Beukers, S.W. Schalm

Submitted for publication.

SUMMARY

In a pilot study we examined the efficacy and toxicity of the therapeutic combination of cyclosporin A and prednisone in primary biliary cirrhosis. Primary goal of the study was the induction of symptomatic and biochemical remission of the disease. Ten patients with symptomatic primary biliary cirrhosis, with active and/or progressive but not end-stage disease, were treated with cyclosporin A (2 mg/kg initially) and prednisone (30 mg initially; 10 mg maintenance dose) for 12 to 24 months.

There was one withdrawal due to side-effects and one patient died after seven months. For the eight patients who completed the course combination therapy resulted in a significant improvement in symptoms and mean serum levels of alkaline phosphatase (maximum decrease from initial value, 46%), ASAT (38%) and immunoglobulins (IgM, 42% and IgG, 37%). However, the primary goal of the study, i.e. the induction of a sustained symptomatic and biochemical remission, was not achieved in any of the patients. After discontinuation of cyclosporin A symptom scores, liver blood tests and immunoglobulin levels tended to return to pretreatment levels, although the changes were only significant for IgG. The main side-effect attributable to cyclosporin A was a mean maximum increase in serum creatinine levels of 30%. Nephrotoxicity appeared to be largely reversible but in three cases the serum creatinine levels did not return to baseline values after the discontinuation of cyclosporin A. In conclusion, combination treatment with cyclosporin A and prednisone did not induce symptomatic and biochemical remission of primary biliary cirrhosis. Because of its nephrotoxicity cyclosporin A is less suitable as first line therapy for primary biliary cirrhosis. Other less toxic regimens of combination therapy, which could include corticosteroids, merit further study.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic inflammatory liver disease for which a therapeutic modality leading to clinical, biochemical and histological remission has not yet been found. The etiology of PBC is unknown but immunological mechanisms probably play an important role in its pathogenesis (1). However, treatment with anti-inflammatory or immunosuppressive agents did not produce clinically important results, while the use of some of these drugs was associated with an unacceptable incidence of side-effects (2).

The first reports on corticosteroid therapy for PBC mainly concerned jaundiced patients with advanced disease. In these patients steroid treatment had a minimal or no effect on the clinical or histological progression of the disease, on the one hand, and appeared to aggravate metabolic bone disease, on the other (3,4). For this reason corticosteroids were long considered to be ineffective and contraindicated in PBC (5,6). Just recently the first controlled trial on prednisolone as single drug therapy for PBC was reported (7). Prednisolone treatment for one year resulted in significant symptomatic, biochemical and histological improvement, but concern about metabolic bone disease remained (8). The T-lymphocyte-specific immunosuppressive agent cyclosporin A (CyA) could also be therapeutic, because cytotoxic T-cell reactions are thought to be involved in the hepatobiliary damage in PBC (9). This hypothesis was supported by reports on the correction by CyA of the defective suppressor T-cell function in PBC (10,11). In addition, small uncontrolled (12,13) and - more recently - placebo-controlled (14,15) studies have shown that CyA treatment may have a favorable effect on symptoms, liver blood tests and immunological variables in symptomatic PBC.

Assessment of the efficacy of medical treatment of PBC is hampered by the broad variation in the clinical course of the disease and its prognosis. Patients with early asymptomatic disease may have a favorable prognosis (16). In these patients the potential benefit of medical therapy is difficult to evaluate and might not outweigh the side-effects associated with long-term treatment with anti-inflammatory or immunosuppressive drugs. In contrast, in advanced disease no medical treatment is expected to be effective and liver transplantation should be considered. The patients between these two groups, i.e. those with symptomatic active disease but without complications, are most likely to benefit from therapeutic intervention. Furthermore, we believe that - as in autoimmune chronic active hepatitis - the most important short-term goal of the treatment of PBC is the induction of clinical, biochemical and histological remission of the inflammatory process, because in all likelihood this is a prerequisite for an ultimate improvement in survival. The results of the above-mentioned studies on CyA and prednisolone, however, suggest that neither of these drugs alone is efficacious in this respect. The combination of CyA with corticosteroids may be expected to have at least additional immunosuppressive effects on the inflammatory process in PBC, but more side-effects are also a possibility. To

evaluate the efficacy and toxicity of the therapeutic combination CyA and prednisone in PBC, we conducted a pilot study of patients with active and/or progressive but not end-stage disease.

PATIENTS AND METHODS

Patient selection. Ten symptomatic patients with progressive and/or active disease were selected. In all cases the diagnosis of PBC was based on the typical clinical features, cholestatic liver blood tests, a positive test for antimitochondrial antibodies and liver histology consistent with or diagnostic for the disease (5,17). Patients were considered to have clinically progressive disease when in the previous year serum bilirubin levels increased from normal (below 17 μmol/l) to abnormal (above 34 μmol/l), the latter being confirmed by a repeat serum determination after three months, or histologically progressive disease when repeated liver biopsies showed progression from early (i.e. stage I and II) to late stage disease (i.e. stage III and IV) (17). The disease was considered active when serum aspartate aminotransferase (ASAT) levels exceeded five times the upper limit of normal or marked immunological activity (i.e. serum immunoglobulin M>5x, or serum immunoglobulin G>2x, or C1q binding assay>5x) was observed. Patients over 70 years of age or with serum bilirubin levels above 100 μmol/l, symptoms of portal hypertension (variceal bleeding, ascites) or impaired renal function were excluded. The patient data and characteristics at entry are given in table 1. Seven patients had been treated previously with penicillamine, azathioprine, colchicine, prednisone, cyclosporin A or a combination of these drugs. All previous anti-inflammatory or immunosuppressive drugs were discontinued at least three months before entry.

Treatment. Treatment was divided into three periods: induction (first three months), maintenance (at least nine months) and CyA withdrawal (six months). In the first two periods patients received CyA and prednisone combination therapy, in the third period prednisone monotherapy. In the induction period prednisone was started as a single morning dose of 30 mg and subsequently tapered within six weeks to a dose of 10 mg, which was maintained throughout the study. Cyclosporin A was administered orally in two divided doses (initial daily dose: 2 mg/kg). The dose was decreased by 1 mg/kg if whole blood trough levels measured by radioimmunoassay exceeded 800 ng/ml, serum creatinine levels increased to more than 50 per cent of the pretreatment level or serious side-effects developed. The CyA dose

Table 1: Patient characteristics at entry.

Patient No.	Sex	Age (yr)	Histological stage	Duration of PBC (yr)	Inclusion criteria	Symptom score	Bilirubin (μmol/l) N<17	Alkaline phosphatase (U/l) N<45	ASAT (U/l) N<30	IgM (G/l) N<2.8	IgG (G/l) N<18.0	C_1q-binding (%) N<7
1	F	57	IV	2	im	3	10	155	52	7.8	18.6	66
2	M	29	IV	9	im/ASAT	2	44	252	162	20.2	19.8	20
3	F	44	III	6	hist	3	9	170	35	5.2	9.7	7
4	F	52	II	4.5	im	5	14	340	63	3.3	19.2	30
5	F	67	II	4.5	im	5	8	272	52	12.3	11.3	19
6	F	43	II	1	im	3	7	297	87	5.8	35.3	46
7	F	58	IV	5.5	bili	4	39	396	64	10.9	18.8	32
8	M	62	III	6	im/hist	6	29	543	58	27.1	20.7	26
9	F	62	IV	3.5	bili	1	58	240	59	1.8	11.9	3
10	F	60	IV	5.5	im	2	19	573	52	6.7	25.5	76
mean		53		4.8		3.4	24	324	68	10.1	19.1	33

Inclusion criteria: im = immunological activity; ASAT = elevated serum aspartate aminotransferase; bili = elevated serum bilirubin;

hist = histological progression.

IgM = serum immunoglobulin M; IgG = serum immunoglobulin G.

was increased by 1 mg/kg if whole blood trough levels were below 200 ng/ml. In addition, to ensure a maximum effect of CyA therapy, the dose was increased if liver blood tests and/or immunological variables showed no further improvement on two occasions with a three-month interval. However, in all cases the CyA dose was increased only if permitted according to trough levels, renal function and drug tolerance. CyA therapy was continued for at least twelve months and then discontinued as soon as no further improvement in liver blood tests and immunological variables was observed, despite dose adjustments for optimum CyA effect. After discontinuation of CyA prednisone was continued for six months.

Investigations. The following symptoms were graded from 0-3 (corresponding to no, mild, moderate and severe): fatigue, pruritus and arthralgia. For each patient the scores for these three symptoms were added to achieve a symptom score, which consequently ranged from 0-9. Serum levels of bilirubin, alkaline phosphatase, ASAT, creatinine, immunoglobulin M (IgM) and G (IgG) and circulating immune complexes, as well as whole blood CyA trough levels were measured at regular intervals. Baseline clinical, biochemical and immunological variables were determined prior to treatment on two occasions within a three-month period; the means of these two measurements were used as the pretreatment values (table 1). Routine biochemical and immunological parameters were assessed by standard techniques. The C1q binding assay was used to determine circulating immune complexes. CyA trough levels were measured in whole blood by the polyclonal radioimmunoassay (Sandoz Ltd, Basle). Liver biopsies, obtained prior to the study and at the end of CyA therapy, were graded according to Scheuer (17) by one experienced hepatic pathologist. Pre- and post-treatment biopsies were also compared to assess changes in the intensity of portal and periportal inflammation (graded from none to severe) and the degree of fibrosis (graded from 0 (no fibrosis) to 4 (cirrhosis)).

Remission. Clinical remission was defined as the absence of symptoms related to PBC (symptom score 0), biochemical remission as normalization of serum bilirubin, IgM and ASAT levels and alkaline phosphatase levels below 1.5 times the upper limit of normal. The absence of (peri)portal inflammation or restriction of the inflammatory changes to the portal tracts without bile duct destruction was considered to represent histological remission.

Calculations and statistical methods. Changes in liver blood tests, immunological variables and serum creatinine were expressed as the percentage change in pretreatment values. After conversion to percentage change these data and the changes in symptom scores were analyzed by means of the Wilcoxon signed rank test for paired data. Probability values ≤0.05 were considered significant. In addition, 95 per cent confidence intervals (95% CI) were calculated.

RESULTS

Two patients (9 and 10; table 1) did not complete the first two treatment periods. In the case of patient 9 prednisone was discontinued after three weeks because of dizziness and muscular weakness and CyA after four weeks because of abdominal pain. These presumed side-effects subsided after discontinuation of the drugs. During combination therapy this patient had noticed a marked decrease in pruritus. Serum bilirubin increased from 58 to 90 μmol/l, whereas the alkaline phosphatase level dropped from 240 to 194 U/l and serum IgG from 11.9 to 9.3 G/l. Other biochemical parameters did not change. Alkaline phosphatase and IgG levels returned to pretreatment values after medication was stopped, while serum bilirubin increased progressively. This patient died of liver failure 18 months after entering the study. Patient 10 died of an unknown cause seven months after entry. During therapy she reported improvement in pruritus. After six months of therapy serum bilirubin had increased from 19 to 31 μmol/l, alkaline phosphatase from 573 to 826 U/l and ASAT from 52 to 60 U/l. In contrast, all immunological variables decreased (IgG from 25.5 to 16.2 G/l, IgM from 6.7 to 5.9 G/l and the C1q binding assay from 76 to 37 per cent). In eight patients the effect of combination therapy could be evaluated. The median duration of CyA therapy was 16.5 months (range: 12-24).

Induction and maintenance period. Initially there was a rapid and significant decrease in symptoms, biochemical values (except serum bilirubin) and immunological parameters (figures 1-3). Three months after initiation of therapy, the decrease in symptom score compared with the initial value was 2.5 (95% CI: 1.1-3.9). Serum alkaline phosphatase levels had decreased by 38.4 per cent (95% CI: 18.6-58.2) and ASAT levels by 28.1 per cent (95% CI: 11.1-45.1). Serum bilirubin levels did not change. In addition serum IgM and IgG levels decreased by 27.1 per cent (95% CI: 5.6-48.5) and 35.3 per cent (95% CI: 21.7-48.9), respectively, and C1q binding values by 51.3 per cent (95% CI: 40.0-62.6).

During the maintenance period no further significant changes in symptoms and biochemical and immunological values were observed, although there was a trend toward an increase in serum bilirubin levels. Three patients (patients 2, 5 and 6, table 1) were asymptomatic at one or more follow-up examinations. This symptomatic remission, however, was maintained for a longer period only in patient 6. None of the patients met the criteria for a biochemical remission.

CyA-withdrawal. After discontinuation of CyA the symptom score and alkaline phosphatase and ASAT levels tended to rise, but these changes were not statistically significant. However, six months after CyA withdrawal, serum bilirubin levels had decreased by 39.7 per cent (95% CI: 9.5-69.9). Of the immunological parameters only the IgG levels had increased significantly by 9.3 per cent (95% CI: 3.0-15.6).

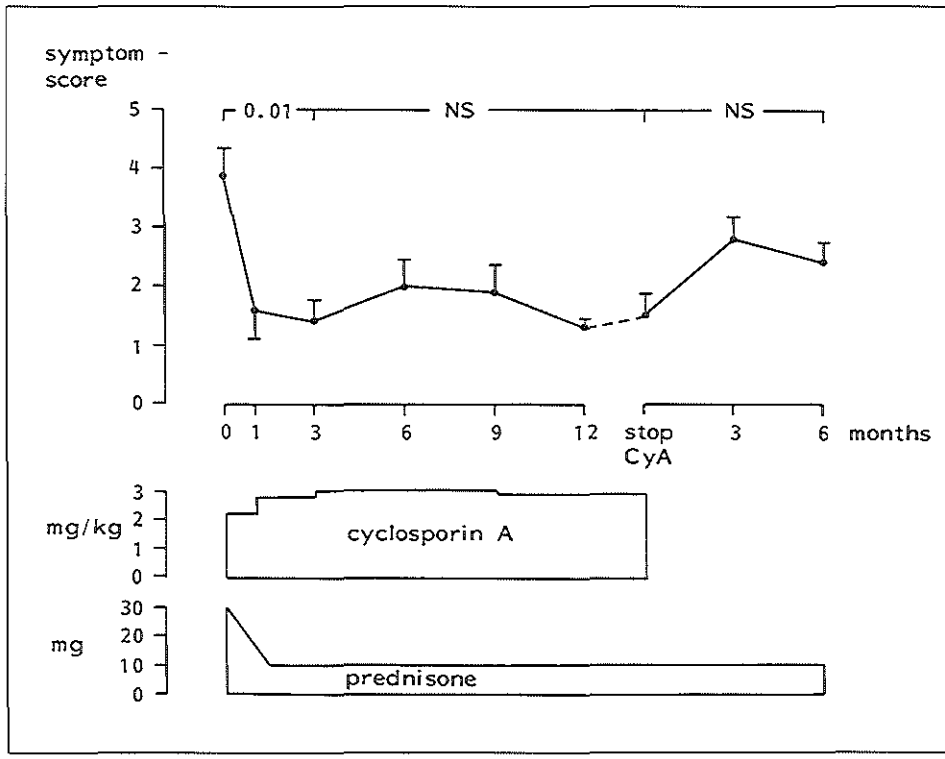

Fig. 1: Mean (± SEM) symptom scores during combination therapy with cyclosporin A and prednisone and after subsequent discontinuation of cyclosporin A. In this and following figures p-values are indicated at the top of the graphs.

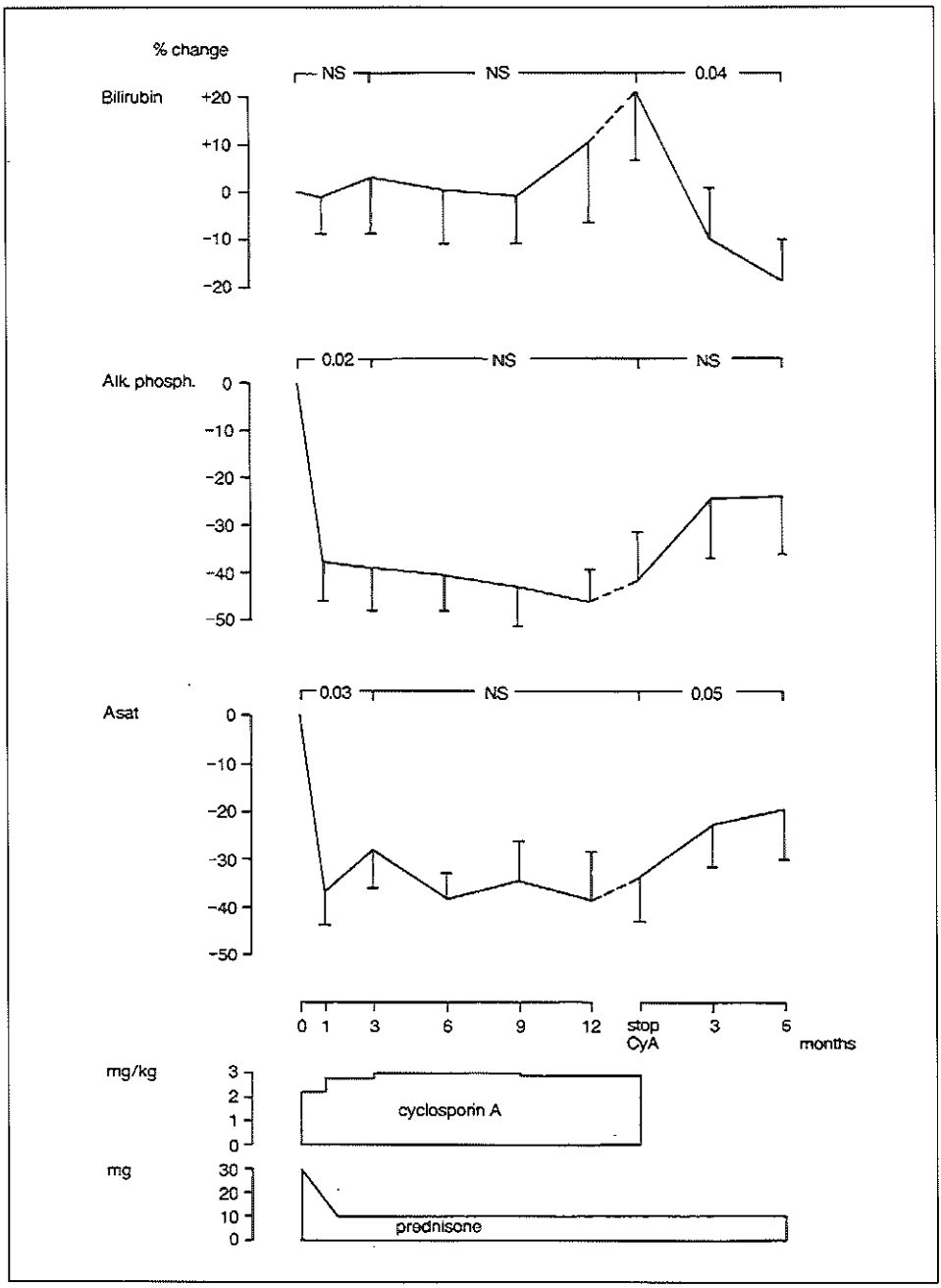

Fig. 2: Mean (± SEM) changes in liver blood tests during combination therapy with cyclosporin A and prednisone and after subsequent discontinuation of cyclosporin A.

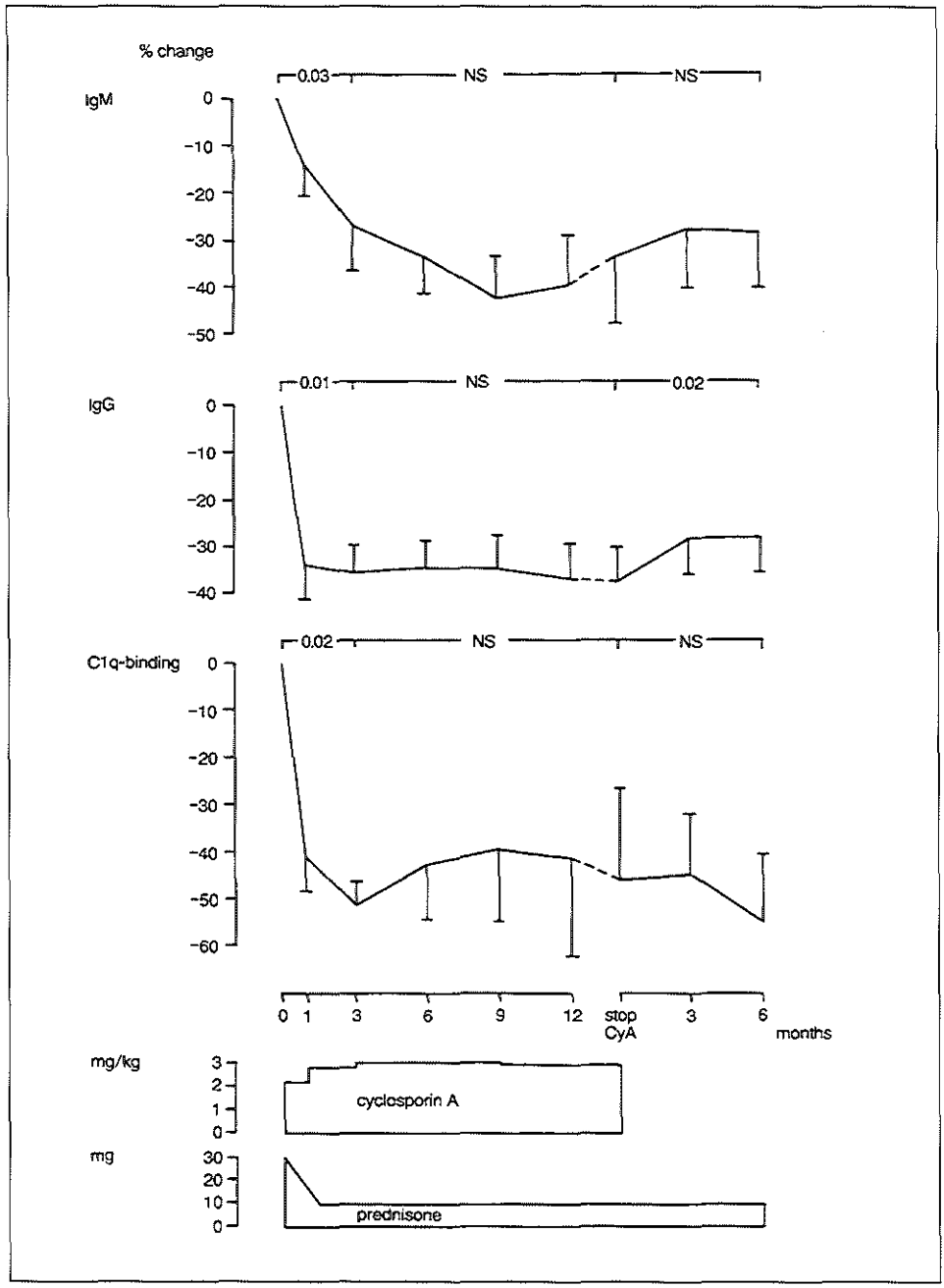

Fig. 3: Mean (± SEM) changes in serum immunoglobulin levels and C1q binding assay value during combination therapy with cyclosporin A and prednisone and after subsequent discontinuation of cyclosporin A.

Effect on histology. The effect of combination therapy on liver histology could be evaluated in seven patients. Liver biopsies were not obtained from patient 2 because a previous procedure was complicated by severe hemobilia. Liver specimens were obtained within five months of entry from five patients. For patients 5 and 6 liver specimens obtained one year before entry were taken as the initial biopsies. These two patients had received no specific treatment for PBC between biopsy and entry. The changes in liver histology are shown in figure 4. After treatment with CyA and prednisone fibrosis had increased in three cases, remained unchanged in another three and was considered less in one. Periportal inflammation remained mild to moderate in two cases and slightly increased in one but subsided in four patients. In three of the latter cases only a minimal cellular infiltration, confined to the portal tracts without damaging the bile ducts remained; nevertheless fibrosis increased in two of these patients.

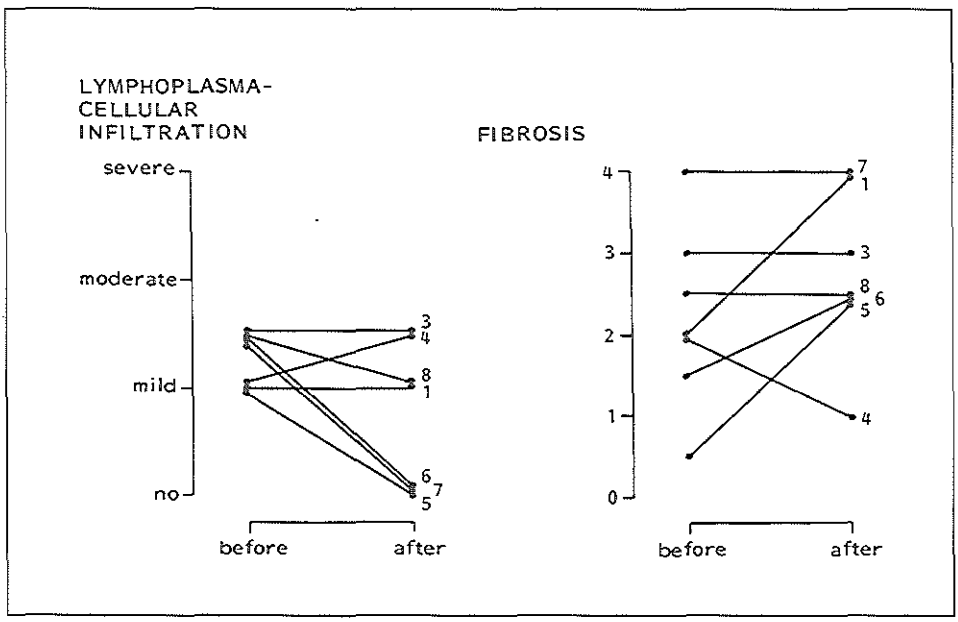

Fig. 4: Changes in histological features of the liver biopsies from seven patients with primary biliary cirrhosis treated with cyclosporin A and prednisone for 12 to 24 months. The numbers in the graphs indicate patient numbers (see table 1).

Side-effects. The main side-effect attributable to CyA was nephrotoxicity. The CyA dose was adjusted in the course of treatment to an average of 3.0 mg/kg (range: 2.0-5.2 mg/kg). The individual mean CyA trough levels in whole blood ranged from 146-422 ng/ml. During CyA therapy serum creatinine levels increased in all cases. In the induction period the mean creatinine level increased significantly by 18.0 per cent (95% CI: 8.3-27.6) and remained significantly elevated for the duration of the treatment (fig. 5). The maximum increase in serum creatinine ranged from 18.3 to 37.3 per cent of the initial values (mean: 30.3 per cent) and exceeded 30 per cent in five cases. Six months after discontinuation of CyA the mean creatinine level had decreased by 14.2 per cent (95% CI: 5.0-23.3) and the mean increase with respect to the initial value was reduced to less than 10 per cent (fig. 5). Serum creatinine levels returned to pretreatment levels within 3-16 months in five cases, but in three cases an 8-15 per cent increase with respect to initial values was still observed after 16 months.

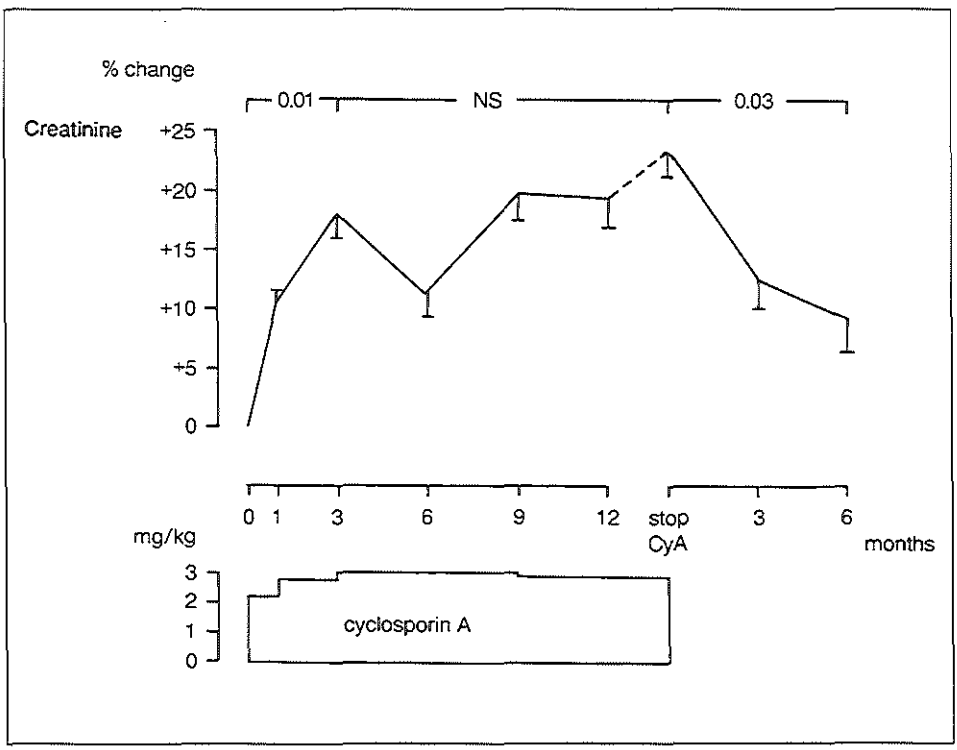

Fig. 5: Mean (± SEM) changes in serum creatinine levels during and after treatment with cyclosporin A.

Other side-effects included impaired control of preexisting hypertension in two cases, hirsutism in two cases and tremor in one case. All of these side-effects subsided after CyA was stopped. The main side-effect of prednisone was considerable weight gain in one case. During this study the possible effect of prednisone on the bones was not evaluated.

DISCUSSION

Treatment of PBC with a combination of CyA and prednisone seems attractive because these drugs influence immune responses in different ways and both have been shown to act beneficially in PBC in placebo-controlled, albeit small, studies. Placebo-controlled studies on CyA single drug treatment of non-cirrhotic patients have been reported by Wiesner et al. (14) and unselected patients with PBC by Minuk et al. (15). CyA treatment for one year appeared to improve symptoms and abnormal liver blood tests. In addition, Wiesner et al. (14) reported that the serum immunoglobulin levels and histology were favorably affected. Mitchison et al. (7) conducted the first placebo-controlled trial on prednisolone as single drug therapy for PBC. Patients with very early (stage I) or clinically advanced disease were excluded. Treatment with prednisolone (10 mg maintenance dose) for one year resulted in symptomatic, biochemical and histological improvement. Changes were greatest in non-cirrhotic patients. None of these studies, however, based their assessment of therapeutic efficacy on criteria for clinical, biochemical and histological remission.

In our selected patients with PBC the combination of CyA and prednisone resulted in a significant reduction in symptoms and serum parameters of cholestasis (alkaline phosphatase), hepatic inflammation (ASAT) and immunological activity (immunoglobulins, C1q binding assay). Maximal improvement was achieved within three months of initiation of therapy and persisted for the duration of the treatment. Our data on the histological changes induced by combination therapy should be considered with caution because of the possibility of sampling error. In addition, pre-treatment liver specimens were obtained one year prior to treatment in two cases. Nevertheless, our results suggest a beneficial effect of this regimen on hepatic inflammatory changes, which is in agreement with some (7,13,14) but not all (15) reports on single drug treatment of PBC with CyA or prednisolone.

As stated earlier, we believe that any treatment that significantly alters the course of the disease should at least have a significant effect on the symptoms, biochemical

abnormalities and histological changes. Our study was not placebo-controlled but a spontaneous sustained improvement in symptoms, liver blood tests and immunological variables, to the extent observed in our patients during combination therapy, is exceptional in PBC. Furthermore, our results are by and large comparable with those of the above-mentioned placebo-controlled studies by Wiesner et al. (14) and Minuk et al. (15) on single drug treatment with CyA and Mitchison et al. (7) on single drug treatment with prednisolone. However, despite our favorable results, the primary goal of our study - i.e. the induction of a remission - was not achieved.
After discontinuation of CyA the symptom score and the biochemical and immunological parameters tended to increase, suggesting that improvement could only partially be maintained by prednisone monotherapy; these changes were, however, only significant for IgG levels. CyA may cause mild hyperbilirubinemia (18), which probably explains the changes in serum bilirubin levels observed during and after CyA therapy.

One patient was withdrawn early from the study because of adverse effects and another died at home of an unknown cause. Among the remaining patients nephrotoxicity was the main side-effect of CyA, as has been reported by others (14,15). Although individual mean CyA blood concentrations were in the low to median therapeutic range and none of the patients had toxic blood levels (i.e. above 800 ng/ml) during treatment, renal dysfunction occurred in all cases. The reduction in renal function was moderate, not progressive and largely reversible, but in three cases serum creatinine levels had not returned to baseline levels more than one year after discontinuation of CyA. None of these patients had hypertension and only one received simultaneously another potentially nephrotoxic drug, i.e. a thiazide diuretic. The fact that elevated serum creatinine levels had not returned to baseline values more than twelve months after discontinuation of CyA therapy for PBC was also reported by Minuk et al. (15). The nephrotoxic effects of CyA have been well documented in transplant recipients (19) as well as patients treated with CyA for autoimmune disease (20). Early nephrotoxicity is functional, dose-related and reversible (21). Chronic CyA-related nephrotoxicity is in general not progressive (20,22) and at least partially reversible (18,20). However, after long-term use of CyA structural and potentially irreversible changes in renal morphology may develop (23,24). We, therefore, share the concern expressed by Minuk et al. (15) and Wiesner et al. (14)

about the long-term safety of CyA in PBC.

None of our patients developed de novo hypertension, but adjustment of therapy for preexisting hypertension was necessary in two cases. Other side-effects observed in our patients were clinically unimportant and similar to those reported by others (19,25).

We did not study the effects of CyA and prednisone on bone mass in our patients. CyA might influence bone metabolism because it was found to induce osteopenia in rats (18). The clinical relevance of these findings, however, remains unclear. Mitchison et al. (7) reported a significant drop in trabecular bone volume (measured in iliac crest biopsies) and femoral bone mineral density (assessed by photon absorptiometry) in patients treated for PBC with prednisolone for one year. Bone loss appeared to be greatest in the first two months of treatment, when higher doses of corticosteroids were administered. In contrast, we found spinal bone mineral density to be related to the histological stage of the disease rather than the administration of low dose prednisone (10 mg) in 55 unselected female patients with longstanding PBC (26). Similar data have been reported for patients with chronic liver disease of various etiologies (27). In patients with rheumatoid arthritis low-dose corticosteroids also did not significantly diminish bone mineral content (28). Therefore, the effect on bone of low-dose maintenance treatment with corticosteroids may be of minor clinical importance.

We conclude that, although CyA combined with prednisone appears to be effective in reducing symptoms, abnormal liver blood tests and immunological variables in patients with PBC, this treatment regimen does not lead to clinical and biochemical remission and therefore is unlikely to significantly alter the course of the disease. Furthermore, because the outlook after liver transplantation for patients with PBC is still improving (29), one should be cautious about administering drugs which may irreversibly impair the functioning of other vital organs, thereby decreasing the possibility of future transplantation. Therefore, the toxicity of CyA with its uncertain sequelae with respect to renal function makes the drug unsuitable for long-term treatment of PBC. Regimens of combination therapy which include low-dose corticosteroids merit further study.

REFERENCES

1. James SP, Hoofnagle JH, Strober W, Jones EA. Primary biliary cirrhosis: a model autoimmune disease. Ann Int Med 1983;99:500-12.
2. Wiesner RH, Grambsch PM, Lindor KD, Ludwig J, Dickson ER. Clinical and statistical analyses of new and evolving therapies for primary biliary cirrhosis. Hepatology 1988;8:668-76.
3. Hoffbauer FW. Primary biliary cirrhosis: observations on the natural course of the disease in 25 women. Am J Dig Dis 1960;5:348-83.
4. 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.
5. Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. N Engl J Med 1973;289:674-8.
6. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1987;316:521-8.
7. 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.
8. Combes B. Prednisolone for primary biliary cirrhosis - good news, bad news. Hepatology 1989;10:511-3.
9. Paronetto F, Colucci G, Colombo M. Lymphocytes in liver disease. In: Progressin liver disease. Popper H and Schaffner F, eds. Grune & Stratton Inc., Orlando 1986;191-208.
10. Al-Aghbar MNA, Alexander GJM, Nouri-Aria KT, Neuberger J, Eddleston ALWF, Williams R. In vitro effect of cyclosporin A on immunoglobulin production and concanavalin A induced suppression in primary biliary cirrhosis. Gut 1986;27:317-23.
11. Alexander GJM, Al-Aghbar MNA, Nouri-Aria KT, Neuberger J, Eddleston ALWF, Williams R. Correction of the suppressor cell defect of PBC by cyclosporin A (abstract). Liver 1984;4:74.
12. Routhier G, Epstein O, Janossy G, Thomas HC, Sherlock S, Kung PC et al. Effects of cyclosporin A on suppressor and inducer T-lymphocytes in primary biliary cirrhosis. Lancet 1980;II:1223-6.

13. Beukers R, Schalm SW. Effect of cyclosporine and cyclosporine plus prednisoe in primary biliary cirrhosis. Transpl Proceed 1988 (suppl. 4);20:340-3.
14. 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;322:1419-24.
15. Minuk GY, Bohme CE, Burgess E, Hershfield NB, Kelly JK, Shaffer EA et al. Pilot study of cyclosporin A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988;95:1356-63.
16. Nyberg A, Lööf L. Primary biliary cirrhosis: clinical features and outcome, with special reference to asymptomatic disease. Scand J Gastroenterol 1989;24:57-4.
17. Scheuer PJ. Primary biliary cirrhosis. Proc Roy Soc Med 1967;60:1257-60.
18. Kahan BD. Cyclosporine. N Engl J Med 1989;321:1725-38.
19. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporine: a new immunosuppressive agent for organ transplantation. Ann Int Med 1984;101:667-82.
20. Dieterle A, Abeywickrama K, Graffenried B von. Nephrotoxicity and hypertension in patients with autoimmune disease treated with cyclosporine. Transpl Proceed 1988 (suppl. 4);20:349-55.
21. Myers BD. Cyclosporine nephrotoxicity. Kidney Int 1986;30:964-74.
22. The Canadian multicentre transplant study group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. N Engl J Med 1986;314:1219-25.
23. Mihatsch MJ, Thiel G, Ryffel B. Ciclosporin-associated nephropathy. In: Ciclosporin in autoimmune diseases. Schindler R. Ed. Springer-Verlag Berlin 1985;50-8.
24. Palestine AG, Austin III HA, Balow JE, Antonovych TT, Sabnis SG, Preuss HG et al. Renal histopathologic alterations in patients treated with cyclosporine for uveitis. N Engl J Med 1986;314:1293-8.
25. Palestine AG, Nussenblatt RB, Chan CC. Side effects of systemic cyclosporine in patients not undergoing transplantation. Am J Med 1984;77:652-6.
26. Berkum FNR van, 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.

27. Diamond T, Stiel D, Lunzer M, Wilkinson M, Roche J, Posen S. Osteoporosis and skeletal fractures in chronic liver disease. Gut 1990;31:82-7.

28. Sambrook PN, Eisman JA, Yeates MG, Pocock NA, Eberl S, Champion GD. Osteoporosis in rheumatoid arthritis: safety of low-dose corticosteroids. Ann Rheum Dis 1986;45:950-3.

29. Markus BH, Dickson ER, Grambsch PM, Fleming TR, Mazzaferro V, Klintmalm GBG et al. Efficacy of liver transplantation in patients with primary biliary cirhosis. N Engl J Med 1989;320:1709-13.

APPENDIX TO CHAPTER III: EFFECT OF PREDNISONE WITHDRAWAL

Six months after the discontinuation of CyA, prednisone was slowly tapered to zero in four months. The patients were followed for another six months without treatment.

Results: The protocol was violated in the case of patient 2 because in this period he experienced two episodes of infection (respiratory tract infection and a septicemia) which meant that the prednisone dose had to be increased temporarily. Therefore, the effect of tapering and discontinuation of prednisone could be evaluated in seven patients. The course of alkaline phosphatase, ASAT and immunoglobulin serum levels for individual patients is shown in the table. Four patients (1, 3, 6 and 7; group A, table) completed this part of the study as planned. They reported no obvious change in symptoms. Alkaline phosphatase levels, however, tended to rise in all four cases; ASAT and immunoglobulin levels did not change in patients 1 and 3 but were clearly increased in patients 6 and 7. In patients 4, 5 and 8 (group B, table) prednisone was tapered to zero according to the protocol but was subsequently reinstituted for various reasons three months, seven weeks and two weeks after discontinuation, respectively. Patient 4 had severe debilitating right-sided coxalgia with only minimal radiological changes before entry. Symptoms disappeared completely during induction and maintenance treatment. During tapering and after discontinuaton of prednisone, however, arthralgia returned to the pretreatment level accompanied by increasing fatigue and pruritus and a marked increase in ASAT and IgG levels. Symptoms and changes in biochemistry subsided after resumption of prednisone therapy (30 mg/day). Patient 5 complained of joint and muscular pains during tapering of prednisone. These symptoms as well as liver blood tests and immunoglobulin levels increased after prednisone was stopped. There was a marked reduction in symptoms and laboratory abnormalities after resumption of prednisone (15 mg/day). Patient 8 complained of pruritus, malaise and moderate joint pains during tapering and after discontinuation of prednisone. There was an increase in alkaline phosphatase, ASAT and immunoglobulin levels. In addition, serum bilirubin rose from 14 to 30 µmol/l. The symptoms and changes in laboratory values subsided after resumption of prednisone therapy (20 mg/day).

Table 1: The effect of prednisone withdrawal in seven patients with primary biliary cirrhosis and the resumption of prednisone therapy in three, on liver blood tests and serum immunoglobulin levels.

Patient No.	Variable	Values at time			
		1	2	3	4
Group A:					
1	A.Ph.	130	200		302
	ASAT	37	53		46
	IgM	4.3	4.6		2.9
	IgG	11.4	11.3		12.0
3	A.Ph.	181	127		305
	ASAT	46	34		34
	IgM	4.7	3.3		4.1
	IgG	10.8	9.5		10.3
6	A.Ph.	93	200		181
	ASAT	27	82		113
	IgM	4.0	5.2		6.0
	IgG	15.0	21.4		35.1
7	A.Ph.	292	242		366
	ASAT	68	54		70
	IgM	8.2	9.6		12.0
	IgG	13.8	14.7		17.0
Group B:					
4	A.Ph.	286	330	248	306
	ASAT	64	155	141	50
	IgM	4.3	4.2	4.4	4.7
	IgG	18.5	21.4	34.9	18.7
5	A.Ph.	125	264	377	211
	ASAT	31	50	52	34
	IgM	4.0	6.8	8.7	6.5
	IgG	7.4	8.9	10.6	9.0
8	A.Ph.	272	412	–	296
	ASAT	39	55	–	42
	IgM	11.5	18.7	–	12.3
	IgG	12.9	16.0	–	15.3

A.Ph. = alkaline phosphatase (U/l); ASAT = aspartate aminotransferase (U/l); IgM = immunoglobulin M (G/l); IgG = immunoglobulin G (G/l). Time 1 = the start of tapering of prednisone; time 2 = prednisone tapered to zero; time 3 = before resumption of prednisone therapy (group B); time 4 = six months with (group B) or without (group A) prednisone. See table 1 of chapter III for normal values.

Comment: Since prednisone therapy had to be resumed in three of the seven patients, two small subgroups could be evaluated. The main feature of the patients who resumed prednisone therapy was a rapid increase in symptoms during tapering and after discontinuation of the drug. It is noteworthy that these patients (patients 4, 5 and 8) also had the highest pretreatment symptom scores (see chapter III, table 1). There was no clear difference in the course of biochemical and immunological values between the two subgroups. These data confirm our earlier clinical findings that prednisone has a favorable effect on the - sometimes debilitating - symptoms of patients with PBC (Gastroenterology 1980;79:1058).

ORAL PHARMACOKINETICS OF CYCLOSPORIN A IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS AND PATIENTS WITH SKIN DISEASES

R. Beukers, S. de Rave, J.W.O. van den Berg, S.W. Schalm

Submitted for publication.

SUMMARY

The pharmacokinetics of cyclosporin A (CyA) after oral administration were studied in seven patients with non-end stage primary biliary cirrhosis (PBC) without previous CyA treatment (group I), a control group of nine patients with skin diseases (mainly psoriasis; group II) and six patients with non-end stage PBC after prolonged CyA treatment (group III). Whole blood concentrations of CyA and its metabolites were measured using a non-specific (N) radioimmunoassay (RIA); in a majority of the cases CyA concentrations were also estimated using a RIA specific (S) for the parent drug. No difference in CyA absorption was observed between patients with PBC and those with a skin disease. The mean values for the area under the blood concentration-time curve for the first six hours after the test dose (AUC_{0-6}) and the maximal blood concentrations (C_{max}) were significantly higher for group I compared with group II patients (p=0.007 and 0.03, respectively), but the time to maximal blood concentrations (t_{cmax}) did not differ. There was a trend toward higher mean AUC_{0-6} (p=0.08) and C_{max} (p=0.08) values for group III compared with group I patients, probably due to saturation of the peripheral compartment. T_{cmax} values were not influenced by prolonged CyA treatment. The ratio of CyA whole blood concentrations measured by the non-specific and specific RIA's (N/S ratio) increased with time, reflecting metabolite formation, without obvious differences between the three groups. The mean N/S ratio of the AUC_{0-6} values was approximately 2.0 for all groups. These data suggest that CyA absorption and its biotransformation in the liver are not impaired in patients with non-end stage PBC and that both are not affected by prolonged treatment.

INTRODUCTION

Cyclosporin A (CyA) is an immunosuppressive drug which exerts its action mainly by inhibition of the synthesis and release of interleukin 2, thereby primarily affecting cytotoxic T-cell responses (1). CyA has been applied extensively and with success for the management of patients receiving organ transplants (2). In addition, because of its specific immunosuppressive action, CyA has been tried for the treatment of a variety of established or presumed autoimmune diseases (3), especially when T-cell responses are thought to be involved in the tissue damage (4). Indeed, CyA appears to be an effective drug for the treatment of some of these diseases, such as psoriasis and uveïtis (3,5,6). Primary biliary cirrhosis (PBC) is a chronic chole-

static liver disease of unknown origin, which is typically encountered in middle-aged women and may eventually lead to biliary cirrhosis and death due to the complications of portal hypertension or hepatic failure (7). Although immunological mechanisms are thought to play an important role in its pathogenesis (8), the results of treatment of PBC with conventional immunosuppressive drugs have been disappointing (9). CyA has been investigated as a therapeutic modality for PBC in two controlled trials and was found to improve symptoms and abnormal liver blood tests (10,11).

The most important side-effect of CyA is nephrotoxicity (12). CyA is metabolized almost entirely in the liver and its metabolites are excreted in bile. Only a very small portion of unchanged CyA may be recovered from the urine. There is a marked variation in the intestinal absorption, hepatic metabolism and excretion of CyA (13). Because CyA is fat-soluble, its intestinal absorption depends on a normal bile flow into the gut, whereas its biotranformation in the liver may be influenced by hepatic disease or drugs that interfere with hepatic metabolism (13). Therefore, the pharmacokinetics of CyA in patients with PBC could be different from those in patients with normal liver function and bile flow. To investigate this, we performed pharmacokinetic studies of CyA in patients with non-end stage PBC and a control group of patients with a presumably normal liver function, in this case patients with various skin diseases - mainly psoriasis. In addition, to determine the effect of long-term use on the pharmacokinetic parameters of CyA, we studied patients with PBC before treatment and patients treated with CyA for 6 up to 20 months. In all cases CyA levels were determined in whole blood samples with the original non-specific radioimmunoassay (RIA) (Sandoz Ltd., Basel). The polyclonal antibodies in this RIA, however, cross-react with the metabolites of CyA and therefore are unable to differentiate between the parent drug and its metabolites (14). Since CyA metabolism might be impaired in chronic liver disease, we also determined CyA pharmacokinetics in a large proportion of the patients (table 1) with the newly available specific RIA based on monoclonal antibodies that recognize only the parent drug (14).

MATERIAL AND METHODS

Patients. CyA pharmacokinetics were studied in seven patients with PBC without previous CyA therapy (group I), nine patients with skin diseases (mainly psoriasis, group II) and six patients with PBC during CyA treatment (group III). One

patient included in group I, who was thought to have stage IV PBC on the basis of clinical, biochemical and histological data, eventually was shown to have primary sclerosing cholangitis. Because the chronic cholestatic character of this disease is comparable to that of PBC, this patient was not excluded from the study. All patients with PBC were about to take part or were taking part in pilot studies on the efficacy of CyA or CyA/prednisone combination therapy (15). The patients with psoriasis had severe disease and were about to participate in a study on CyA treatment for psoriasis resistant to regular therapy (5). Group II included three patients with

Table 1: Patient characteristics.

	Group I (n=7)	Group II (n=9)	Group III (n=6)
Sex (F/M)	6/1	5/4	5/1
Age (yrs)	55 (43-62)	48 (24-72)	56 (42-64)
Weight (kg)	61 (45-76)	71 (51-89)	62 (43-78)
Duration of disease (yrs)	6 (1-14)	10 (1-42)	4 (2-7)
Bilirubin (μmol/l) (N<17)	10 (7-48)	8 (4-11)	18 (11-59)
Hematocrit(%)	39 (33-41)	43 (32-50)	41 (35-47)
Histological stage III/IV (n) (for PBC patients)	5		5
Previous CyA therapy (n)	0	0	6
Duration 6 months (n)			3
Duration 16-20 months (n)			3
Daily dose (mg/kg)			2.4 (1.6-2.6)
Additional specific RIA (n)	4	5	3

Age, weight, duration of disease, bilirubin, hematocrit and daily CyA dose are expressed as median and the range.

non-psoriatic skin disorders (prurigo nodularis, chronic discoid lupus erythematodes, Behçet's disease). They all had debilitating disease that had not responded sufficiently to extensive previous treatment; therefore a trial of CyA therapy was under consideration. At the time of the study none of the patients received medication known to have a potential influence on the pharmacokinetics of CyA, except for prednisone (13) (group I:3;group II:2; group III:4 patients). The patient characteristics are summarized in table 1.

Methods. After an overnight fast, 1 ml of a CyA solution (100 mg/ml) was administered in 150 ml of milk. Care was taken that all CyA was ingested. The patients consumed a low-fat breakfast 30 minutes afterwards, but otherwise only liquids were allowed until the end of the study.

Patients already on CyA therapy received the test dose of CyA approximately twelve hours after their last regular dose.

Various blood samples were collected from an indwelling venous cannula into EDTA containing tubes 0, 1/2 and one hour after the test dose and every hour for six hours (eleven patients), eight hours (five patients), nine hours (two patients), ten hours (one patient), twelve hours (two patients) or thirteen hours (one patient). All samples were deep-frozen until analysis. In all cases CyA levels were measured in whole blood by the non-specific polyclonal CyA RIA (Sandoz Polyclonal RIA kit). In addition, CyA levels were measured by the specific monoclonal antibody CyA RIA (Sandimmune kit) in whole blood samples from twelve patients (table 1). All samples were assayed in duplicate.

The following pharmacokinetic parameters were determined: the maximal concentration (C_{max}) and the time to reach this maximum (t_{cmax}). Unfortunately, for the majority of our patients the number of samples appeared to be too small to be able to calculate the elimination half-life reliably and, consequently, to estimate the area under the concentration versus time curve (AUC). Therefore, by using the trapezoid rule, only the AUC for the period zero to six hours after the test dose was calculated (AUC_{0-6}). Furthermore, the difference between the CyA concentrations measured by the non-specific polyclonal RIA and the specific monoclonal RIA was expressed as a non-specific/specific RIA ratio (N/S ratio). Finally, we studied absorption patterns. On the basis of the C_{max} and t_{cmax} values we defined the following absorption patterns: rapid absorption (t_{cmax} $\leq$3 hours and C_{max} $\geq$400 ng/ml), delayed

absorption (t_{cmax} >3 hours and C_{max} ≥400 ng/ml) and poor absorption (all CyA concentrations <250 ng/ml). If a second peak concentration of more than 400 ng/ml was observed, the profile was considered biphasic.

Differences in patient characteristics as well as C_{max}, t_{cmax} and AUC_{0-6} values were tested with the Wilcoxon test for unpaired data. Probability values <0.05 were considered significant.

RESULTS

The values of C_{max}, t_{cmax}, AUC_{0-6} and the N/S ratio of the AUC_{0-6} values as well as the observed absorption patterns are presented in table 2. Absorption was poor in four patients, two of whom (one in group I and one in group II) were excluded from further calculations because all CyA concentrations over a seven hour period were below 200 and 150 ng/ml, respectively. Absorption was rapid in thirteen patients and delayed in five. One of the rapid absorbers in group III had a biphasic profile with peak CyA concentrations of 760 ng/ml at two and seven hours.

Group I versus group II. The patients of group I had significantly higher serum bilirubin levels (p=0.03) and lower hematocrit values (p=0.03) compared with group II patients. In addition, the patients of group II had a higher bodyweight and, consequently, received a lower relative CyA test dose (median 1.41 versus 1.65 mg/kg for group I patients), but this trend did not reach significance (p=0.15). Mean C_{max} and AUC_{0-6} values were higher for group I patients (p=0.03 and 0.007, respectively). Mean t_{cmax} values did not differ between the two groups.

Group I versus group III. These groups did not differ in age, bodyweight, serum bilirubin and hematocrit values. The mean C_{max} and AUC_{0-6} values tended to be higher in group III (p=0.08). The mean t_{cmax} values did not differ between the two groups.

Ratio of the non-specific and specific RIA values. The mean N/S ratios of the AUC_{0-6} values were approximately 2.0 for all three groups, but the number of data was too small for statistical analysis.

The N/S ratio of the CyA concentrations are plotted against time in figure 1. There was an obvious increase in N/S ratio with time in all three groups. In addition, for the three patients of group III for whom this ratio could be calculated, the N/S ratio

of the CyA concentrations 30 minutes after the test dose (mean 2.4) was higher than that at one hour (not shown in the figure).

Table 2: Pharmacokinetic parameters derived from cyclosporin A blood concentrations measured by the non-specific (N) and the specific (S) RIA.

Patients	C_{max} (ng/ml)	t_{cmax} (h)	AUC_{0-6} (ng.ml^{-1}.h) non-specific RIA	specific RIA	N/S-RIA ratio	Absorption* pattern
Group I						
1.	440	2	1562	668	2.3	R
2.	830	3	2899	1512	1.9	R
3.	660	3	2550	1126	2.3	R
4.	560	2	2066	1334	1.6	R
5.**	550	2	2113			R
6.	650	4	1870			D
Mean	615	2.7	2177		2.0	
SD	132	0.8	479		0.4	
Group II						
7.	600	2	1897	791	2.4	R
8.	420	3	1453	854	1.7	R
9.	450	2	1493	806	1.9	R
10.	250	1	1069	563	1.9	P
11.	455	5	1294	1270	1.0	D
12.	490	4	1647			D
13.	250	5	1051			P
14.	550	4	1495			D
Mean	433	3.3	1425		1.8	
SD	127	1.5	284		0.5	
Group III						
15.	940	3	4840	1532	3.2	R
16.	590	1	2678	2027	1.3	R
17.	890	2	5528	2269	2.4	R
18.	760	2 (7)°	3290			B
19.	940	2	4790			R
20.	580	7	890			D
Mean	783	2.8	3669		2.3	
SD	167	2.1	1728		0.9	

* R = rapid, D = delayed, P = poor and B = biphasic absorption profile.
** This patient appeared to have stage IV primary sclerosing cholangitis.
° Time of second peak in brackets.

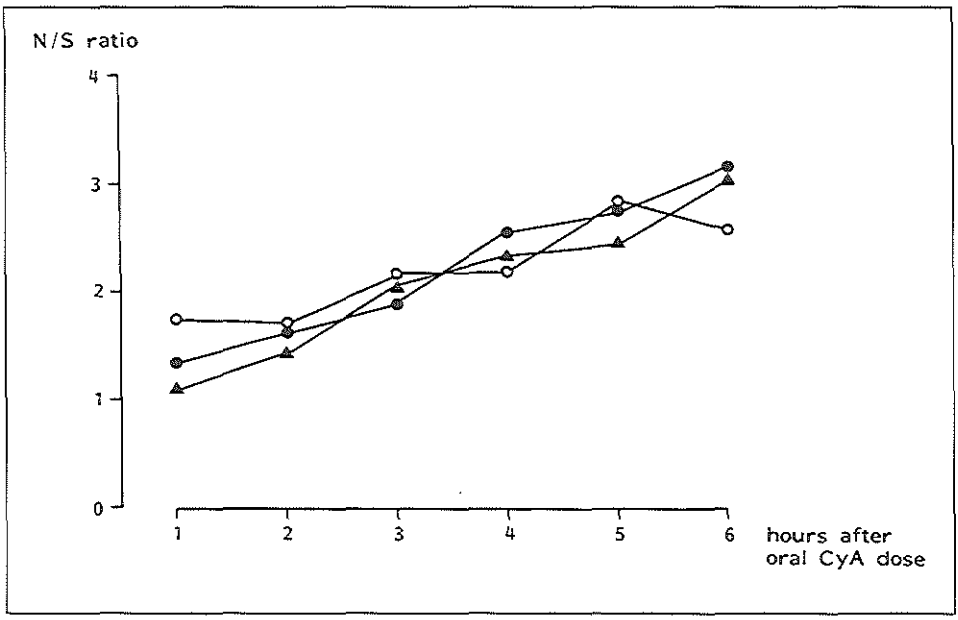

Figure 1: Mean ratios of CyA levels measured in whole blood by the non-specific polyclonal (N) and the specific monoclonal (S) radioimmunoassay after oral administration of CyA.

DISCUSSION

Most pharmacokinetic data on CyA have been obtained by means of the non-specific polyclonal RIA and/or high performance liquid chromatography (HPLC) using blood from organ transplant recipients (13). The polyclonal RIA measures both the parent drug and its metabolites, although the latter most probably do not contribute to either the immunosuppressive effect of CyA or its nephrotoxicity (16). The parent drug can be measured specifically by HPLC or, more recently, the specific monoclonal RIA (14), which is less expensive and easier to use than HPLC. Measurements by the monoclonal RIA closely parallel the levels obtained by HPLC (14). Very few data on CyA pharmacokinetics in patients with PBC have been reported (17, 18). Robson et al. studied CyA pharmacokinetics in the steady state after oral administration to ten patients with PBC, the majority of whom had histologically late stage disease (17). The mean values of the pharmacokinetic parameters, as measured by the polyclonal RIA and HPLC, were similar to the results of previous studies of transplant recipients. Comparable data have been reported by De Groen et al., who studied oral CyA pharmacokinetics by HPLC in twenty patients with early

stage disease (18). The aims of our study were to find out whether there is a difference in the absorption and metabolism of CyA between patients with PBC and a control group of patients with a presumably normal gastrointestinal and liver function and to investigate the influence of prolonged CyA therapy on its absorption and metabolism in PBC. For parent drug measurements we used the monoclonal RIA.

The absorption of CyA is variable and depends on several factors such as gastric emptying, intestinal transit time and bile flow (13,19). Our patients exhibited several different absorption patterns, as has been reported by others (20,21). Most patients had a normal rapid absorption profile. Delayed or poor absorption was found for both patients with skin diseases and those with PBC. In PBC impaired absorption of CyA could be expected as a result of diminished bile flow and subsequent fat malabsorption. However, fat malabsorption generally becomes clinically manifest only in late stage disease that is accompanied by severe cholestasis. Among our patients with PBC, most of whom had histologically late stage disease, serum bilirubin levels were elevated in only three patients of both group I and III (patients 3, 5, 6 and 15, 17, 19 respectively; table 2). The one patient in group I with CyA malabsorption, who was excluded from the calculations, had a normal serum bilirubin level. For the patients with skin diseases, delayed or poor absorption could not be attributed to concomitant diseases or medication. Therefore, an explanation for the delayed or poor absorption in some of the patients with PBC and the majority of those with skin diseases is lacking. One patient had a biphasic absorption profile. Secondary peaks in the blood concentration versus time curve are well known but as yet unexplained. Secondary peaks cannot be the result of enterohepatically recycled CyA or its metabolites, since these peaks have also been demonstrated by HPLC (20,21) and unchanged CyA is excreted in bile in only very small amounts (13).

The mean values of C_{max} and AUC_{0-6} were significantly lower for group II patients compared with group I. This cannot be explained by the difference in hematocrit but may partially be attributed to the higher mean bodyweight and consequently lower relative CyA test dose in the former group. Taking into account the lower test dose of CyA used, the C_{max} and t_{cmax} values were comparable to those reported by others for PBC (17,18) and transplant recipients (13,22).

There was an obvious trend toward higher C_{max} and AUC_{0-6} values for the patients with PBC, who had used CyA for up to twenty months (group III) compared to those without previous CyA treatment (group I). This has also been reported for

transplant recipients (22,23) and is thought to be related to a progressive saturation of the peripheral compartment during continued treatment (21,22). In addition, a time dependent increase in CyA absorption has been postulated (23,24). As a consequence, dose reduction during continued treatment is necessary to avoid excessive CyA blood concentrations (18,23). This is in accordance with our own experience with CyA therapy for PBC (unpublished results). In agreement with previous data on transplant recipients (22,23), the time needed to reach the maximum concentration was not influenced by prolonged CyA treatment.

CyA is metabolized almost entirely in the liver. Therefore, CyA metabolism may be influenced by hepatic disease. Unfortunately, our data do not allow calculation of metabolic parameters such as drug clearance and elimination half-life. Nevertheless, we believe that the AUC for the first six hours after the test dose and, especially, the rate of formation of metabolites provide an indication of CyA metabolism in our patients. The ratio of CyA levels measured by the polyclonal and monoclonal RIA's reflects metabolite formation, especially when followed in time. The mean N/S ratio of the AUC_{0-6} values was approximately 2.0 and apparently did not differ between the three groups. This value agrees with the ratio of AUC_{0-24} values determined by polyclonal RIA and HPLC for patients with PBC reported by Robson et al. (17). More interesting is the N/S ratio of CyA levels when plotted against time. As shown in figure 1, this ratio increases progressively with time. Similar data have been obtained for transplant recipients (22) and hemodialysis patients (25), using HPLC for measurement of the parent drug. More importantly, our data indicate that the biotransformation of CyA in our patients with PBC is not different from that in patients without liver disease and is not influenced by prolonged CyA treatment. It should be noted that liver function was not greatly impaired in our patients with PBC, in view of the serum bilirubin levels discussed above and the normal serum albumin levels found for all but three patients (all group I; albumin levels slightly decreased: 31-33 G/l). The higher N/S ratio at 30 minutes compared with one hour after the test dose for group III patients probably reflects the presence of CyA metabolites in the serum of these patients before the test dose.

In conclusion, our results indicate that there is no difference in CyA absorption and its biotransformation in the liver between patients with non-end stage PBC and patients without liver disease. Furthermore, although prolonged CyA treatment

probably leads to saturation of the peripheral compartment, necessitating dose adjustment, biotransformation of the drug in the liver does not seem to be affected by continued administration. Because of individual variations in the absorption and metabolism of CyA, measurement of CyA trough levels, preferably by the specific monoclonal RIA, is recommended.

ACKNOWLEDGEMENTS

We wish to thank F. Heule for his cooperation with regard to the dermatological patients and A.J. de Boer and G. Voortman for their technical assistance.

REFERENCES

1. Hess AD, Esa AH, Colombani PM. Mechanisms of action of cyclosporin: effect on cells of the immune system and on subcellular events in T cell activation. Transpl Proceed 1988;20 (suppl.2):29-40.
2. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporin: a new immunosuppressive agent for organ transplantation. Ann Int Med 1984;101:667-82.
3. Bach JF. Cyclosporin in autoimmunity. Transpl Proceed 1988;20 (suppl 4):379-81.
4. Talal N. Cyclosporin as an immunosuppressive agent for autoimmune disease: theoretical concepts and therapeutic strategies. Transpl Proceed 1988;20 (suppl 4):11-5.
5. Joost Th van, Heule F, Stolz E, Beukers R. Short-term use of cyclosporin A in severe psoriasis. Br J Dermatol 1986;114:615-20.
6. Heule F, Meinardi MMHM, Joost Th van, Bos JD. Low-dose cyclosporin effective in severe psoriasis: a double-blind study. Transpl Proceed 1988;20 (suppl 4):32-41.
7. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1987;316:521-8.
8. James SP, Hoofnagle JH, Strober W, Jones EA. Primary biliary cirrhosis: a model autoimmune disease. Ann Int Med 1983;99:500-12.
9. Wiesner RH, Grambsch PM, Lindor KD, Ludwig J, Dickson ER. Clinical and statistical analyses of new and evolving therapies for primary biliary cirrhosis. Hepatology 1988;8:668-76.

10. Minuk GY, Bohme CE, Burgess E, Hershfield NB, Kelly JK, Shaffer EA et al. Pilot study of cyclosporin A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988;95:1356-63.
11. Wiesner RH, Ludwig J, Lindor KD, Jorgensen RA, Baldus WP, Homburger HA et al. A controlled trial of cyclosporin in the treatment of primary biliary cirrhosis. N Engl J Med 1990;322:1419-24.
12. Kahan BD. Cyclosporin. N Engl J Med 1989;321:1725-38.
13. Ptachcinski RJ, Venkataramanan R, Burckart GJ. Clinical pharmacokinetics of cyclosporin. Clin Pharmacok 1986;11:107-32.
14. Keown PA. Optimizing cyclosporin therapy: dose, levels and monitoring. Transpl Proceed 1988;20 (suppl 2):382-9.
15. Beukers R, Schalm SW. Effect of cyclosporin and cyclosporin plus prednisone in primary biliary cirrhosis. Transpl Proceed 1988;20 (suppl. 4):340-3.
16. Ryffel B, Foxwell BMJ, Mihatsch MJ, Donatsch P, Maurer G. Biologic significance of cyclosporin metabolites. Transpl Proceed 1988;20 (suppl 2):575-84.
17. Robson S, Neuberger J, Keller HP, Abisch E, Niederberger W, Graffenried B von et al. Pharmacokinetic study of cyclosporinA (Sandimmun) in patients with primary biliary cirrhosis. Br J Clin Pharmac 1984;18:627-31.
18. Groen PC de, McCallum DK, Moyer TP, Wiesner RH. Pharmacokinetics of cyclosporin in patients with primary biliary cirrhosis. Transpl Proceed 1988;20 (suppl. 2):509-11.
19. Naoumov NV, Tredger JM, Steward CM, O'Grady JG, Grevel J, Niven A et al. Cyclosporin A pharmacokinetics in liver transplant recipients in relation to biliary T-tube clamping and liver dysfunction. Gut 1989;30:391-6.
20. Philips TM, Karmi SA, Frantz SC, Henriques HF. Absorption profiles of renal allograft recipients receiving oral doses of cyclosporin: a pharmacokinetic study. Transpl Proceed 1988;20 (suppl. 2):457-61.
21. Clardy CW, Schroeder TJ, Myre SA, Wadhwa NK, Pesce AJ, First MR et al. Clinical variability of cyclosporin pharmacokinetics in adult and pediatric patients after renal, cardiac, hepatic, and bone-marrow transplants. Clin Chem 1988;34:2012-5.
22. Keown PA, Stiller CR, Laupacis AL, Howson W, Coles R, Stawecki M et al. The effects and side effects of cyclosporin: relationship to drug pharmacokinetics. Transpl Proceed 1982;14:659-61.

23. Wilms HWF, Straeten V, Lison AE. Different pharmacokinetics of cyclosporin A early and late after renal transplantation. Transpl Proceed 1988;20 (suppl. 2):481-4.

24. Kahan BD, Ried M, Newburger J. Pharmacokinetics of cyclosporin in human renal transplantation. Transpl Proceed 1983;15:446-53.

25. Follath F, Wenk M, Vozeh S, Thiel G, Brunner F, Loertscher R et al. Intravenous cyclosporin kinetics in renal failure. Clin Pharmacol Ther 1983;34:638-43.

BONE MASS IN WOMEN WITH PRIMARY BILIARY CIRRHOSIS: THE RELATION WITH HISTOLOGICAL STAGE AND USE OF GLUCOCORTICOIDS

F.N.R. van Berkum[1], R.Beukers[2], J.C. Birkenhäger[1], P.P.M. Kooij[3], S.W. Schalm[2], H.A.P. Pols[1].

Departments of Internal Medicine III and Clinical Endocrinology[1], Internal Medicine II and Hepatology[2] and Nuclear Medicine[3], University Hospital Dijkzigt, Rotterdam.

Gastroenterology 1990;99:1134-39.

ABBREVIATIONS USED IN THIS PAPER:

BMD: Bone mineral density

DPA_{spine}: Dual photonabsorptiometry of the lumbar spine

Ha: Hydroxyapatite

OH-prol: Hydroxyproline

$1,25\text{-}(OH)_2D_3$: 1,25-dihydroxyvitamin D_3

$25\text{-}(OH)D_3$: 25-hydroxyvitamin D_3

PBC: Primary biliary cirrhosis

PTH: Parathyroid hormone

SPA_{dist}: Single photonabsorptiometry of the distal forearm

SPA_{prox}: Single photonabsorptiometry of the proximal forearm

SUMMARY

To assess the impact of primary biliary cirrhosis on bone mass in general and the relative importance of the stage of the liver disease and of treatment with glucocorticoids for the possible development of osteoporosis, bone mineral mass was measured by single and dual photon-absorptiometry in 55 unselected female patients with longstanding primary biliary cirrhosis. Although most of the patients had a bone mineral density within the normal range, the bone mineral densities of the lumbar spine, distal and proximal forearm were 8% ($p<0.004$), 8% ($p<0.03$) and 5% (NS) respectively, lower than in age-matched healthy females.

Multiple regression analysis showed that the histological stage of the liver disease (early versus late stage) was an independent determinant of axial bone mineral density, whereas the use of glucocorticoids resulted in only a moderate and not significant bone loss.

Serum calcium proved to be significantly lower in the patients with late stage primary biliary cirrhosis than in those with early stage disease, whereas no significant differences were found in these groups with regard to several biochemical parameters of bone metabolism.

In conclusion, in patients with primary biliary cirrhosis, bone loss was only moderate and related to the histological stage. The effect of low-dose glucocorticoids on bone mass seemed not significant.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic disease of the liver typically encountered in middle aged women; it is characterized by an inflammatory process affecting the intrahepatic bile ducts. This inflammation is accompanied by cholestasis and may eventually result in biliary cirrhosis and death of hepatic failure (1).

Like other cholestatic liver diseases, PBC may be complicated by metabolic bone disease. Both osteomalacia and osteopenia have been described in PBC (2-12). Nowadays, it is generally agreed that osteoporosis is the more common and clinically more important lesion in PBC. In patients with PBC, prevalence rates of osteoporosis, based on histological criteria, have been reported to vary from 0-17% (8,10,11,13). The cause of osteoporosis in PBC is unknown.

Currently there is no effective treatment for PBC and results with various immunosuppressive or anti-inflammatory drugs have been disappointing (14). Especially in

PBC glucocorticoids are assumed to aggravate osteoporosis (1,15), but this has never been extensively studied.

The current study investigated the determinants of bone mineral density in 55 unselected female patients with PBC. Furthermore, we investigated whether glucocorticoids cause a further decrease of the bone mineral density in PBC, and if so, to what extent.

MATERIALS AND METHODS

Patients. From 1973 to 1988 a diagnosis of PBC was made in our department in 102 patients (89 women). Fourteen patients (all women) were lost to follow up and 22 (18 women) died. Of the 66 patients still followed up, 57 are women. Of these women one was excluded because of severe polymyositis and consequent immobilisation; another was excluded because incomplete data were obtained. The remaining 55 patients (aged 39-75 years) were entered to the study. All patients' liver biopsy results were consistent with PBC, and all had positive tests for antimitochondrial antibodies. None of the patients had evidence of bone disease in their history or at physical examination. All patients received a diet containing at least 1.5 g of calcium daily, and vitamin D_3 (400 IU/day orally) was supplemented, when indicated by serum 25-hydroxyvitamin D_3 (25-(OH)D_3) levels (6 patients). In principle, therefore, all patients may be considered to have had an adequate intake of calcium and vitamin D.

Thirty patients had been treated for their PBC with D-penicillamine, azathioprine, colchicine, cyclosporin A, prednisone or combinations of these drugs. Of these patients only five had been treated with azathioprine or cyclosporin A. Twenty-three patients had received glucocorticoids or were still treated with glucocorticoids. All patients were initially administered 30 mg prednisone which was tapered off within 6 weeks to a maintenance dose of 10 mg. The mean duration of treatment was 6.3 years (range, 0.3-14.5 years). For the calculation of the cumulative glucocorticoid dose, one patient with chronic asthma was excluded because reliable data could not be obtained.

To determine the relationship between the histological stage of PBC (assessed according to Scheuer (16)) and parameters of bone metabolism and bone mineral density (BMD), the patients were divided into groups of early stage (stage I and II, n=30) or late stage (stage III and IV, n=25) disease (Table 1). Furthermore, the effects of treat-

Table 1: Patient characteristics, bone mineral densities and biochemical data according to the classification based on histological stage of the liver disease.

	Early stages	Late stages	p	Normal values
No.	30	25		
Age (yr)	59.2 ± 7.6	56.1 ± 9.7	N.S.	
Height (cm)	162.6 ± 7.8	161.1 ± 5.7	N.S.	
Mean menopausal age*	48.3	50.2	N.S.	
Weight (kg)	68.1 ± 11.8	61.7 ± 7.6	<0.05	
Quetelet index (kg/cm²)	25.7 ± 4.10	23.7 ± 3.1	N.S.	
Duration of PBC (yr)	8.2 ± 3.6	7.2 ± 2.9	N.S.	
Corticoids (n)	12	11		
cumulative dose (g)	25.8 ± 25.5	22.8 ± 15.0	N.S.	
Bone Mineral Density**				
DPA_{spine} (%)	94.8 ± 14.7	85.4 ± 10.6		
SPA_{dist} (%)	96.1 ± 20.2	85.8 ± 17.5		
SPA_{prox} (%)	100.2 ± 17.2	91.5 ± 16.2		
Serum				
Bilirubin (μmol/l)	15 ± 17	34 ± 44	0.05	2-12
Calcium (mmol/l)•	2.35 ± 0.07	2.29 ± 0.15	0.002	2.20-2.65
Albumin (g/l)	42.6 ± 2.1	39.2 ± 6.2	N.S.	36-48
PTH (pg/ml)	19 ± 10	20 ± 10	N.S.	10-55
Osteocalcin (ng/ml)	3.5 ± 1.4	3.5 ± 1.6	N.S.	1.8-6.6
25-(OH)D_3 (nmol/l)	63.2 ± 46.3	70.9 ± 41.0	N.S.	>30
1,25-$(OH)_2D_3$ (pmol/l)	61.2 ± 18.9	59.3 ± 17.6	N.S.	40-101
Urine				
Calcium/creatinine (mol/mol)	0.30 ± 0.16	0.35 ± 0.22	N.S.	0.28 ± 0.06
OH-prol/creatinine (mol/mol)	0.02 ± 0.01	0.02 ± 0.009	N.S.	0.02 ± 0.005
TMP/GFR (mmol/l)	1.1 ± 0.2	1.1 ± 0.2	N.S.	0.81-1.35

Note: The values are expressed as mean ± SD. The cumulative dose of corticoids is expressed as prednisone equivalents.

* For both groups, the mean age at menopause was calculated.

** The BMDs are presented as percentages of age-matched control subjects.

• Corrected for serum albumin. For statistical evaluation, multiple linear regression analysis was used as described in Materials and Methods.

ment with glucocorticoids on the same parameters were studied, by subdividing the PBC patients in a group who used or had used glucocorticoids (n=23) and a group who had never used this type of drug (n=32) (Table 2). Informed consent was obtained from all participants.

Table 2: Patient characteristics, bone mineral densities and biochemical data according to the classification based on use of glucocorticoids.

	No steroids	Steroids	p
No.	32	23	
Age (yr)	59.2 ± 8.5	55.9 ± 8.7	N.S.
Height (cm)	160 ± 6.1	163 ± 7.5	N.S.
Mean menopausal age*	48.9	49.0	N.S.
Weight (kg)	64.7 ± 8.4	65.9 ± 13.4	N.S.
Quetelet index (kg/cm²)	25.1 ± 3.1	24.5 ± 8.7	N.S.
Duration of PBC (yr)	6.9 ± 3.0	8.9 ± 3.5	N.S.
Early/late stage	19/13	11/12	
Bone Mineral Density**			
DPA_{spine} (%)	92.9 ± 15.5	87.5 ± 10.6	
SPA_{dist} (%)	95.5 ± 21.9	86.1 ± 14.9	
SPA_{prox} (%)	101.1 ± 18.8	90.0 ± 12.6	
Serum			
Bilirubin (μmol/l)	24 ± 40	23 ± 23	N.S.
Calcium (mmol/l)•	2.35 ± 0.16	2.34 ± 0.81	N.S.
Albumin (g/l)	40.9 ± 5.8	41.4 ± 2.6	N.S.
PTH (pg/ml)	23 ± 7	27 ± 12	N.S.
Osteocalcin (ng/ml)	3.7 ± 1.4	3.2 ± 1.5	N.S.
25-$(OH)D_3$ (nmol/l)	58.2 ± 21.3	78.0 ± 61.4	N.S.
1,25-$(OH)_2D_3$ (pmol/l)	61.9 ± 18.9	58.2 ± 17.8	N.S.
Urine			
Calcium/creatinine (mol/mol)	0.33 ± 0.20	0.32 ± 0.17	N.S.
OH-prol/creatinine (mol/mol)	0.02 ± 0.09	0.03 ± 0.01	N.S.
TMP/GFR (mmol/l)	1.1 ± 0.2	1.1 ± 0.2	N.S.

Note: The values are expressed as mean ± SD.

* For both groups, the mean age at menopause was calculated.

** The BMDs are presented as percentages of age-matched control subjects.

• Corrected for serum albumin. For statistical evaluation, multiple linear regression analysis was used as described in Materials and Methods. For normal values see Table 1.

Biochemistry. Serum concentrations of calcium, creatinine, inorganic phosphorus, bilirubin and albumin were measured by standard methods. Serum calcium levels were corrected for the serum albumin concentration as described by Payne et al. (17). Urinary hydroxyproline (OH-prol) excretion was measured according to a previously described method (18). Serum 25-$(OH)D_3$ levels were measured by a competitive protein binding assay, whereas 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), immunoreactive intact parathyroid hormone (PTH(1-84)) and osteocalcin were measured with commercially available kits (ICNSTAR corporation, Stillwater, Minnesota).

Assessment of bone mineral mass. All patients were measured with photon absorptiometry as described elsewhere (19). Peripheral bone measurements were carried out at the right forearm according to the method described by Nilas et al. (20), using a Nuclear Data 1100a bone density scanner. Measurements were performed both distally (SPA_{dist}) and proximally (SPA_{prox}). With this technique, the bone measured distally consists of trabecular bone in a higher proportion than when measured proximally (21). The results are expressed as BMD in arbitrary units (U/cm^2). In our laboratory, the coefficient of variation based on 50 duplicate measurements in normal subjects, is 1.9% for the distal site and 1.0% for the proximal site.

For the dual energy photon absorptiometric measurements of the lumbar spine (DPA_{spine}), L2-4 was the region of interest.Measurements were carried out with a Novo BMC-lab 22a scanning device as described by Krolner and Nielsen (22). Results were expressed as grams hydroxyapatite (Ha) per cm^2 (BMD). The coefficient of variation calculated on the basis of duplicate measurements of the lumbar BMD of 20 patients with osteoporosis in our laboratory is 2.3%.

Statistical methods. Bone densitometric values of the PBC patients (n=55) were compared with values obtained from a group of age-matched healthy subjects, randomly sampled from a reference group described previously (19). Differences were analyzed by Mann-Whitney's test. Multiple linear regression analyses were performed to evaluate the dependency of the various densitometric values on age, histological stage of PBC and the use of glucocorticoids. In this way, the influence of disease stage, glucocorticoids and age, respectively, on bone mass was evaluated independently. Adding the variables (corrected) serum calcium and Quetelet-index (or weight) in the regression model had no significant influence. The participants were classified to subgroups according to the use of glucocorticoids and to the histological stage. The Mann-Whitney test was used to compare the differences among the various parameters in the subgroups.

RESULTS

All patients. Mean BMDs at both the distal region of the forearm and in the lumbar spine were 8% lower in patients with PBC than in normal women of the same age ($p<0.03$ and $p<0.004$, respectively, Table 3). However, no significant difference was observed in the proximal forearm. In Figure 1 A-C the axial and peripheral

BMD values of the patients are plotted against age. Although the mean DPA_{spine} and SPA_{dist} were significantly lower, most of the individual BMD values of the patients are within the normal range. For all biochemical parameters no differences between the total PBC group and our reference values were found, including vitamin D metabolites, PTH and osteocalcin.

Table 3: Bone mineral densities in patients with PBC and age-matched control subjects.

	DPA_{spine}	SPA_{dist}	SPA_{prox}
Control subjects (n = 55)	0.85 ± 0.14	0.97 ± 0.019	1.30 ± 0.25
PBC (n = 55)	0.78 ± 0.12	0.89 ± 0.20	1.24 ± 0.22
p value	<0.004	<0.03	0.18

Note: BMDs are expressed in grams hydroxyapatite or units/cm² standard deviation (see Methods). The reference group is also described in the Materials and Methods section.

Early and late stage. Using the criteria of Scheuer (16), the patients were subdivided in groups with early stages (I and II) and late stages of PBC (III and IV). With this approach the only significant difference in clinical characteristics between these two groups was weight, which was lower in the late stage group (Table 1). Late stage PBC appeared not to be associated with a longer duration of the liver disease. Of the biochemical parameters measured, only bilirubin was higher in the late stage group, whereas on the other hand serum calcium and corrected calcium levels were significantly lower in this group (Table 1). Using the multiple regression method, we observed a tendency to lower age-corrected peripheral and axial BMD values in the late stage group. However, this difference reached significance ($p<0.002$) only for DPA_{spine}. Weight or Quetelet index could not be identified as independent determinants of BMD in these groups.

Glucocorticoids. The patients with PBC who used or had used glucocorticoids were also compared with those who had never used this type of drug. As shown in Table 2 no significant differences between both groups could be observed. Also, the cumulative dose of glucocorticoids was not correlated with the histological

stage of PBC. Nevertheless, the steroid group tended to have lower age-corrected BMDs than the non-steroid group. Multiple regression analysis indicated that for SPA_{prox} this trend just reached significance (p=0.05).

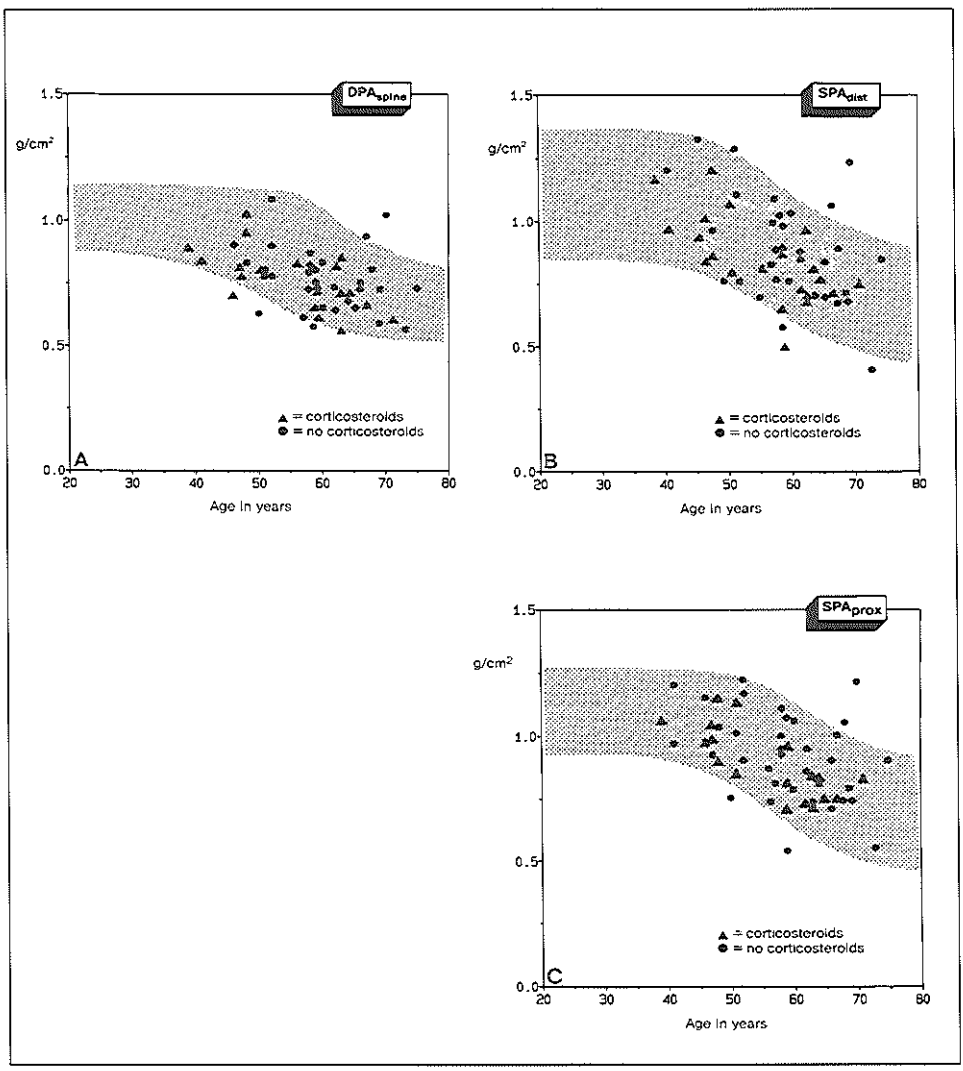

Figure 1: Bone mineral densities of (A) lumbar spine (DPA_{spine}) and (B) distal (SPA_{dist}) and (C) proximal (SPA_{prox}) forearm in 55 patients with PBC. Shaded area indicates the 5th to 95th percentiles determined for 171 normal women. ▲ Patients who used or had used corticosteroids; ● patients who never used corticosteroids.

DISCUSSION

The results presented in this report confirm earlier reports that the peripheral BMD at the distal forearm is lower in female patients with PBC than in age-matched control subjects (6,8). Furthermore, axial BMD, another parameter of trabecular bone mass, was also significantly lower in the patients with PBC. Only BMD measurements at the proximal forearm, largely reflecting cortical bone, did not show a significant difference with our reference group. Therefore, these results suggest a preferential loss of trabecular bone compared with cortical bone in PBC-related bone disease.

However, our findings are not in agreement with the severe form of bone loss as described by Hodgson et al. (10). These investigators reported that in approximately 50% of their patients with PBC, axial BMD values were even below the theoretical fracture threshold, whereas we found a mean reduction of BMD of only 8% both in the lumbar spine and distally in the forearm compared with the results in age-matched control subjects. This discrepancy may be explained by differences in the populations of patients studied and in the statistical methods used. For instance, Hodgson et al. used a linear relationship for the age-related axial bone loss in control subjects, whereas the current report used the more common non-linear function with an accelerated bone loss around the menopause (Fig. 1A-C and reference 19).

At the presentation of PBC, there is little evidence for metabolic bone disease (13); it remains to be seen whether early substitution with vitamin D and calcium, as applied in our patients, prevents bone loss. In this respect, the available data are not conclusive, because only the effect of relatively short-term treatment with vitamin D on bone loss has been reported (4,5).

The importance of the histological stage of PBC for bone mass is illustrated by the significant difference between the axial BMD of our early stage and late stage groups. To our knowledge this is the first report of such a relationship. Another interesting observation in the current study was the slightly but significantly lower mean serum calcium level in patients with histological evidence of late stage PBC. This was not accompanied by differences in the concentration of vitamin D metabolites or PTH. The latter observation might indicate a certain degree of hypoparathyroidism in this patient group, because the lower mean serum calcium level should have resulted in a higher mean PTH concentration. Also in other studies normal or even

subnormal serum immunoreactive PTH levels have been found (5,6).
Unfortunately, we had no opportunity to measure parameters of calcium absorption to investigate a possible relationship with serum calcium levels and bone mass. However, it has been reported that chronic intestinal calcium malabsorption seems to be implicated in the pathogenesis of bone loss in patients with PBC (6). Consequently, our findings could be a reflection of this phenomenon.

Several recent studies have shown that not osteomalacia, but osteoporosis in the most frequently found metabolic bone disease in patients with PBC (5-12). Furthermore, histological analyses of bone biopsies have shown impaired osteoblastic function with decreased bone formation (9,10). Although we have not performed bone biopsies, the slightly lowered serum osteocalcin levels in our PBC patients, as compared with reference values, do not indicate a severely impaired osteoblast function. This is in agreement with the very moderate lowering of bone mass in our patients.
The recently proposed hypothesis that toxic substances related to hepatic disease and cholestasis play a role in PBC-related bone disease remains attractive (10,12). For instance, copper and bile salts are known to have cytotoxic effects and are found in high concentrations in hepatocytes and other tissues of patients with PBC (10). In patients with Wilson's disease copper may be implicated in the development of hypoparathyroidism (23). Toxic substances might not only interfere with osteoblast activity but might also depress parathyroid function (24,25).

Several investigators consider the use of glucocorticoids in the treatment of PBC disadvantageous because of the induced bone loss (1,15). However, our observations do not indicate a clinically important long-term influence of glucocorticoids, in the doses used, on bone mass or biochemical parameters of bone turnover. A recent study by Diamond et al. (12) of patients with hepatic osteodystrophy points in the same direction. An explanation for this finding could be that most of our patients were kept on a relatively low maintenance dose of prednisone (10 mg daily). We did not observe lower serum osteocalcin levels as others did in glucocorticoid-treated patients (26). However, we have to emphasize that not all patients in the steroid group were treated at the time of measurement. Because only a limited number of patients has been treated with azathioprine or cyclosporin A, it may be assumed that the potential negative effects of these substances on bone mass could

not have influenced our overall results significantly.

In patients with rheumatoid arthritis low-dose glucocorticoids also did not significantly diminish bone mineral content (27). Similar data were obtained by our group in patients with chronic obstructive lung disease (28). In this respect, the importance of the cumulative dose of corticoids has been stressed by Dykman et al. (29). Indeed, in our patients the mean cumulative dose was below the critical level (30 g equivalent of prednisone) indicated by these authors for the occurrence of fractures. Therefore, we believe that relatively low doses do not strongly accelerate the progression of osteoporosis in patients with PBC. This conclusion may have relevance for future studies, because long-term prospective controlled trials of corticosteroids in PBC are lacking.

In conclusion, our study does not indicate the occurrence of a severe degree of bone loss in our patients with PBC, although we found a moderately lower BMD in patients with a late histological stage of PBC. Furthermore, the assumed deleterious effect of long-term (low dose) glucocorticoids on BMD seemed to be minor in our patients. It remains to be determined whether the early substitution with vitamin D and calcium in our patients has had a beneficial effect. Finally, our data provide evidence for a lower serum calcium in the late stage group. This phenomenon has to be studied in more detail, especially with regard to parathyroid function.

REFERENCES

1. Kaplan MM. Primary biliary cirrhosis. N Eng J Med 1987;316:521-8.
2. Atkinson M, Nordin BEC, Sherlock S. Malabsorption and bone disease in prolonged obstructive jaundice. Q J Med 1956;25:299-312.
3. Long RC, Meinhard E, Skinner RK, Varghese Z, Wills MR, Sherlock S. Clinical, biochemical and histological studies of osteomalacia, osteoporosis and parathyroid function in chronic liver disease. Gut 1978;19:85-90.
4. Reed JS, Meredith SC, Nemchausy BA, Rosenberg IH, Boyer JL. Bone disease in primary biliary cirrhosis: Reversal of osteomalacia with oral 25-hydroxyvitamin D. Gastroenterology 1980;78:512-7.
5. Herlong HF, Recker RR, Maddrey WC. Bone disease in primary biliary cirrhosis: Histologic features and response to 25-hydroxyvitamin D. Gastroenterology 1982;83:103-8.

6. Matloff DS, Kaplan MM, Neer RM, Goldberg MJ, Bitman W, Wolfe HJ. Osteoporosis in primary biliary cirrhosis: Effects of 25-hydroxyvitamin D_3 treatment. Gastroenterology 1982;83:97-102.
7. Cuthbert JA, Pak CYC, Zerwekh JE, Glass KD, Combes B. Bone disease in primary biliary cirrhosis: Increased bone resorption and turnover in the absence of osteoporosis or osteomalacia. Hepatology 1984;4:1-8.
8. Stellon AJ, Davies A, Compston J, Williams R. Osteoporosis in chronic cholestatic liver disease. Q J Med 1985;57:783-90.
9. Stellon AJ, Webb A, Compston J, Williams R. Low bone turnover state in primary biliary cirrhosis. Hepatology 1987;7:137-42.
10. Hodgson SF, Dickson ER, Wahner HW, Johnson KA, Mann KG, Riggs BL. Bone loss and reduced osteoblast function in primary biliary cirrhosis. Ann Int Med 1985;103:855-60.
11. Compston JE. Hepatic osteodystrophy: Vitamin D metabolism in patients with liver disease. Gut 1986;27:1073-90.
12. Diamond TH, Stiel D, Lunzer M, McDowall D, Eckstein RP, Posen S. Hepatic osteodystrophy. Static and dynamic bone histomorphometry and serum bone Gla-protein in 80 patients with chronic liver disease. Gastroenterology 1989:96:213-21.
13. Mitchison HC, Malcolm AJ, Bassedine MF, James OFW. Metabolic bone disease in primary biliary cirrhosis at presentation. Gastroenterology 1988;94:463-70.
14. Wiesner RH, Grambsch PM, Lindor KD, Ludwig J, Dickson ER. Clinical and statistical analyses of new and evolving therapies for primary biliary cirrhosis. Hepatology 1988;8:668-76.
15. Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. N Eng J Med 1973;289:674-8.
16. Scheuer PJ. Primary biliary cirrhosis. Proc Roy Soc Med 1967;60:1257-60.
17. Payne RB, Little AJ, William RB. Interpretation of serum calcium in patients with abnormal serum proteins. Br Med J 1973;4:643-6.
18. Goverde BC, Veenkamp FNJ. Routine assay of total urinary hydroxyproline based on resin catalyzed analysis. Clin Chim Acta 1972;41:29-40.

19. Berkum van FNR, Pols HAP, Kooij PPM, Birkenhager JC. Peripheral and axial bone mass in Dutch women. Relationship to age and menopausal state. Neth J Med 1988;32:226-34.
20. Nilas L, Borg J, Gotfredsen A, Christiansen C. Comparison of single-and dual-photon absorptiometry in postmenopausal bone mineral loss. J Nucl Med 1985;26:1257-62.
21. Schlenker RA. Percentages of cortical and trabecular bone mass in the radius and ulna. In: Mazess RB (ed) Third international conference on bone mineral measurement. Am J Roentg 1976;126:1309-12.
22. Krolner B, Nielsen P. Measurement of bone mineral content (BMC) of the lumbar spine I. Theory and application of a new two dimensional dual-photon attenuation method. Scan J Clin Lab Invest 1980;40:653-63.
23. Carpenter TO, Carnes DL, Anast CS. Hypoparathyroidism in Wilson's disease. N Engl J Med 1983;309:873-7.
24. Cournot-Witmer GC, Zingraff J, Plachott JJ, Escaig F, Lefevre R, Boumati P et al. Aluminum localization in bone from hemodialyzed patients: Relationship to matrix mineralization. Kidney Int 1981;20:375-85.
25. Slatopolsky E. The interaction of parathyroid hormone and aluminum in renal osteodystrophy. Kidney Int 1987;31:842-54.
26. Reid IR, Chapman GE, Fraser TRC, Davies AD, Surus AS, Meyer J et al. Low serum osteocalcin levels in glucocorticoid-treated asthmatics. J Clin Endocrinol Metab 1986;62:379-83.
27. Sambrook PN, Eisman JA, Yeates MG, Pocock NA, Eberl S, Champion GD. Osteoporosis in rheumatoid arthritis: Safety of low dose corticosteroids. Ann Rheum Dis 1986;45:950-3.
28. Berkum van FNR, Pols HAP, Braun JJ, Heysteeg M, Kooy PPM, Birkenhager JC. Glucocorticoid induced bone loss and treatment with 1a-hydroxyvitamin D_3: A placebo controlled double blind trial. In: Osteoporosis. Christiansen C (ed), Osteopress ApS, Denmark 1987;87-9.
29. Dykman TR, Gluck OS, Murphy WA, Hahn TJ, Hahn BH. Evaluation of factors associated with glucocorticoid-induced osteopenia in patients with rheumatic disease. Arthritis Rheum 1985;28:361-7.

SERIAL DETERMINATION OF TYPE III PROCOLLAGEN AMINO PROPEPTIDE SERUM LEVELS IN PATIENTS WITH HISTOLOGICALLY PROGRESSIVE AND NON-PROGRESSIVE PRIMARY BILIARY CIRRHOSIS

R. Beukers, R.A.A. van Zanten, S.W. Schalm

Journal of Hepatology 1992;14:22-9.

SUMMARY

We examined the value of serum procollagen III amino propeptide (PIIIP) for predicting histological progression of primary biliary cirrhosis (PBC). Serial PIIIP measurements were obtained for 9 patients with histologically progressive PBC and 9 patients with histologically stable early disease, assessed by repeated liver biopsies and followed for up to 13 years. The means of the follow-up PIIIP concentrations were elevated in 39% of the cases; moreover, PIIIP levels were elevated at least once during follow-up in 72% of the cases. Mean follow-up PIIIP concentrations did not differ significantly between progressive and non-progressive patients. In addition, in the progressive group, histological progression was not reflected by PIIIP levels. No difference was found between the serum PIIIP levels corresponding to the histological stages I, II and III. The individual coefficients of the correlation between serum PIIIP and biochemical variables (bilirubin, alkaline phospatase, ASAT, albumin) and histology showed a wide distribution without a consistent trend towards positive or negative. Treatment with cyclosporin A or cyclosporin A combined with prednisone did not influence serum PIIIP levels. Treatment with penicillamine combined with prednisone, however, resulted in a significant decrease in PIIIP concentrations ($p<0.05$).

We conclude that serum PIIIP measurements are of no value for predicting histological progression of PBC.

INTRODUCTION

Hepatic fibrosis, a common and important feature of chronic liver disease, is characterized by an increased synthesis and deposition of collagen, mainly types I and III, in the intercellular spaces (1). In liver disease with enhanced fibrogenic activity an increased synthesis of type III collagen precedes that of type I (2). During the extracellular formation of type III collagen from type III procollagen, the amino terminal peptide of the procollagen molecule is split off enzymatically and released into the circulation. In recent years a sensitive radioimmunoassay (RIA) for this type III procollagen amino propeptide (PIIIP) has been developed (3). Increased serum PIIIP levels have been reported in acute and chronic viral hepatitis (3,4), alcoholic liver disease (3,5,6), chronic active hepatitis (6,7) and primary biliary cirrhosis (6,8-13). Serum PIIIP levels in liver disease probably reflect active fibrogenesis rather than the extent of fibrous tissue in the liver (5,14).

Chronic non-suppurative cholangiolytic hepatitis, usually called primary biliary cirrhosis (PBC) even in stages without characteristics of cirrhosis, runs a variable course. The disease may progress to liver cirrhosis with complications of portal hypertension and death from hepatic failure, but it also may run a benign course with a normal life expectancy for asymptomatic patients (15). Apart from liver transplantation in end-stage disease, effective treatment for PBC is still not available (16). Theoretically patients with active and progressive disease could benefit the most from therapeutic intervention. Parameters to identify such patients, however, are lacking. Serum bilirubin has proven to be an important prognostic factor (17, 18), but rising serum bilirubin levels and jaundice develop only late in the course of the disease. Histologically the later stages of the disease are characterized by increasing fibrosis (stage III) and eventually cirrhosis (stage IV) (19). Several authors have suggested that serum PIIIP concentrations provide independent prognostic information (10-12), although this has been disputed (13). In the former studies, however, PIIIP levels were only determined at a single point in time. Data on serial PIIIP measurements for individual patients are scarse (9,13). Patients with histologically progressive PBC may be expected to have increased hepatic fibrogenic activity. Therefore, during follow-up these patients may show elevated serum PIIIP levels compared with patients with histologically stable disease. To assess the value of serum PIIIP measurements for predicting histological progression of PBC, we studied serial PIIIP levels in patients with histologically progressive disease compared with those with histologically stable early PBC, all of whom were followed for up to 13 years. In addition the effects of three different therapeutic regimens on serum PIIIP concentrations were investigated in several small series of patients.

MATERIAL AND METHODS

Patients. The patients in this study comprise nine patients selected for histologically progressive PBC, i.e. progression from early stage (I and II) to late stage (III and IV) disease, and nine patients with early stage disease without histological progression as assessed by repeated liver biopsies. They will be referred to as the progressive group and non-progressive group, respectively. The diagnosis of PBC was established according to clinical, biochemical and histological criteria (15) in all cases. Entry and follow-up liver biopsies were taken blindly or under laparoscopic control with a Tru-Cut needle and classified according to Scheuer (19) by one expe-

rienced hepatic pathologist. The timing and results of the liver biopsies for individual patients are shown in table 1. For each patient of the progressive group, except patient 5, at least two liver biopsies were available for assessment of the phase of early stage disease, thereby for the most part circumventing the problem of sampling error. During follow-up a total of 52 and 39 liver specimens were obtained from the patients of the progressive and non-progressive group, respectively. Entry data, including liver histology, were obtained at the time of diagnosis or - in referred cases - at the time of the first examination in our hospital. The patient data and characteristics at entry are given in table 2. The two groups did not differ significantly. All patients of the progressive group and seven patients with non-progressive disease had symptoms attributable to PBC, including fatigue, pruritus or jaundice or symptoms of portal hypertension. During follow-up three patients in the non-progressive group (patient 10, 15 and 17; table 1) and all of the progressive group received one or more treatment courses with colchicine, azathioprine, penicillamine, prednisone, cyclosporin A or combinations of these drugs.

The patients were evaluated clinically, biochemically and histologically at regular intervals. Additional serum samples were stored at -20°C for future analysis. Clinical progression, defined as a definite rise in serum bilirubin (i.e. above 34 μmol/l, confirmed on at least two occasions), progressive jaundice or the development of signs and symptoms of portal hypertension, was observed in four patients from the progressive group (patients 2, 5, 7 and 9; table 1). The clinical follow-up further illustrates the progressive nature of the disease in these patients. Patients 2 and 7 died of liver failure seven and eleven years after diagnosis, respectively; patient 9 underwent liver transplantation seven years after diagnosis but died four months later; patient 5 is still alive but has developed esophageal varices and is suffering from progressive ascites.

The non-progressive patients were followed by means of histology and/or serum PIIIP determinations for 4-9.5 years (table 2), but clinically for 8.5-11.5 years (mean: 10 years). None of these patients showed clinical progression as defined above. At the end of the clinical follow-up all non-progressive patients had normal serum bilirubin levels (mean: 8 μmol/l; range 5-14). Furthermore, mean serum levels of aspartate aminotransferase (30 U/l; range 17-49), immunoglobulin G (13.5 G/l; range: 8.0-17.4) and albumin (43 G/l; range: 40-45) were normal, but mean serum immu-

Table 1: Timing and results of sequential liver biopsies in patients with histologically progressive and non-progressive PBC.

	Patient No.	Sex	Age (yr)*	Histological stage at: Entry	1	2	3	4	5	6	7	8	9 years
Progressive Group	1	M	58	1		2		3		3			
	2	F	39	1	2	2 3	3	3	3		3		
	3	F	31	1	1	1				2		3	
	4	F	63	1	2		1			3			
	5	F	55	2	3 2		4						
	6	F	38	2	2 3		3			3		3	
	7	F	38	1	1	1	1	2 2	2		3	3	
	8	F	38	1		1	1 3			3			2
	9	F	45	2 1	3 3			4 4					
Non-progressive Group	10	F	54	1	2	1				2		2	
	11	F	52	2	1	1	2						
	12	F	60	1	1	1	1	1					
	13	F	50	1	2	1						2	
	14	F	42	1 2	2	2	2						
	15	F	49	1			2	2					
	16	F	49	1	1		1				1		
	17	F	57	2 2	1 1			2					1
	18	F	53	2	1					1			

For practical purposes the histological stages of PBC are presented in this table in Arabic numerals.

* Age at entry.

Table 2: Patients characteristics.

variable	normal values	progressive group (n=9)	non-progressive group (n=9)
Entry data *:			
sex (F/M)		8/1	9/0
age (yr)		39 (31-63)	53 (43-61)
bilirubin (μmol/l)	<17	11 (5-90)	9 (5-26)
alkaline phosphatase (U/l)	18-45	174 (70-470)	93 (51-418)
ASAT (U/l)	5-30	85 (22-148)	40 (25-156)
PIIIP (ng/ml)	5.6-12.8	12.1 (8.9-20.8)	11.1 (7.1-14.8)
Follow-up data:			
duration (yr)**		7 (6-13)	8 (4-9.5)
medical treatment (n)		9	3
clinical progression (n)		4	0
mortality (n)		3	0

Age, duration and biochemical variables are expressed as median values (range)

ASAT: aspartate aminotransferase.

* Entry data were not significantly different.

** Duration of follow-up by means of histology and/or PIIIP determinations.

noglobulin M levels were moderately elevated (3,5 G/l; range: 1.6-5.6). As to be expected, alkaline phosphatase levels were elevated in all cases (mean: 108 U/l; range: 62-200), but the mean level was not increased compared with that at entry. Seven of the nine non-progressive patients were examined during the last two years of clinical follow-up for signs of portal hypertension by means of endoscopy, radiology or ultrasonography with negative results in all cases.

Data to study the effect of anti-inflammatory and immunsuppressive drug therapy on PIIIP levels were available for seven patients of the progressive group and one non-progressive patient. Similar data could be obtained from four additional patients with PBC not included in the progressive or non-progressive group. In all of these cases serum samples and liver biopsies were taken before and after treatment. The following drugs or combinations of drugs were studied, each in five patients (one patient underwent two, another patient all three treatment regimens): penicillamine (250 mg) plus prednisone (10 mg) for 12 months, cyclosporin A (2-5 mg/kg) for 6 months and cyclosporin A plus prednisone for 16-24 months.

Laboratory investigations. Bilirubin, alkaline phosphatase and aspartate aminotransferase (ASAT) levels in the serum were assessed by standard techniques. Serum PIIIP concentrations were measured by radioimmunoassay, using a commercially available kit (Behringwerke, Frankfurt, F.R.G.). The RIA was performed as described by Rohde et al. (3), with modifications suggested by the manufacturer. The concentration of PIIIP was calculated using a 50% intercept method (20). All tests were carried out in duplicate and a standard serum was run together with all determinations for quality control. For the standard serum the mean value of 23 determinations was 16.5 ng/ml (manufacturer's value 17.0 ng/ml). The inter-assay coefficient of variation was 11%. Determinations of PIIIP were done in sera frozen at -20°C for up to 12 years. Storage at -20°C for up to 2 years (3,21) or at -70°C (11) does not influence PIIIP concentrations. The mean serum PIIIP level plus and minus two standard deviations for 16 normal controls was used as normal reference range (mean ± SD: 9.2 ± 1.8 ng/ml).

Statistical methods. Differences between groups were tested with the Wilcoxon rank test for unpaired data and within groups with the Wilcoxon rank test for paired data. The association between serum PIIIP concentrations and serum levels of aspartate aminotransferase, alkaline phosphatase, bilirubin and albumin as well as histological stage was evaluated by means of the Spearman correlation coefficient (r).

RESULTS

The follow-up data on serum PIIIP levels for patients from both groups are depicted in figure 1. For the progressive group the same data, rearranged according to an artificial point in time indicating the transition from histologically early to late stage disease, are shown in figure 2. The time of the histological transition is given by the median time between the last early stage and the first late stage liver specimen for each patient. No consistent pattern in the course of PIIIP levels could be recognized in either the progressive group or the non-progressive group (fig. 1). The same holds for the PIIIP levels corresponding to early stage disease compared with those corresponding to late stage disease for patients of the progressive group (fig. 2). For each patient the mean of all serum PIIIP levels obtained during follow-up was calculated. No significant difference in these mean PIIIP levels between the progressive group and non-progressive group was observed (table 3). Two patients of the non-progressive group and five of the progressive group had mean serum PIIIP levels above the upper limit of normal (12.8 ng/ml). Elevated PIIIP levels occured at least once during follow-up for four patients of the non-progressive group and all those with histologically progressive disease.
Furthermore, in the progressive group the means of the PIIIP levels during early stage disease were compared with the means during late stage disease. For one patient no PIIIP levels were obtained during early stage disease. In the other eight patients the mean PIIIP levels did not change significantly with histological progression (fig. 3).

To investigate whether the serum PIIIP levels at a given time reflect the histological stage of PBC, we compared the peptide levels corresponding to the different stages of the disease. Mean values were used in case a patient had more than one PIIIP value corresponding to a given histological stage. PIIIP levels corresponding to stage IV disease were available for only two patients. Therefore, only stage I, II and III were compared. We found no significant differences between mean PIIIP levels ($\pm$ SD) corresponding to stage I (11.7 $\pm$ 4.2 ng/ml), stage II (15.0 $\pm$ 8.1 ng/ml) and stage III (13.2 $\pm$ 5.8 ng/ml) disease (Wilcoxon test for paired data).

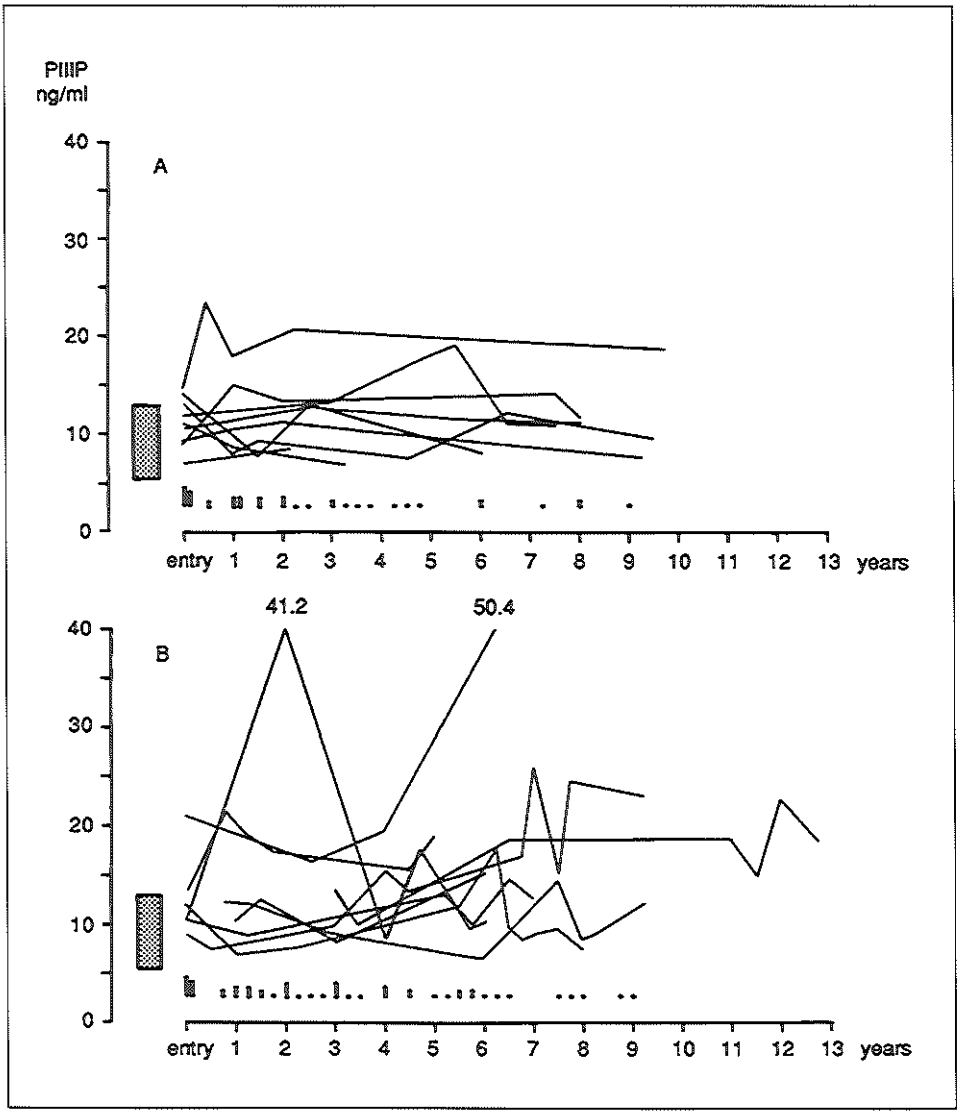

Fig. 1: Follow-up serum PIIIP concentrations in patients with (A) histologically non-progressive and (B) progressive PBC. Each dot at the bottom of the graphs represents a liver biopsy. In this and following figures the hatched bar indicates the normal range.

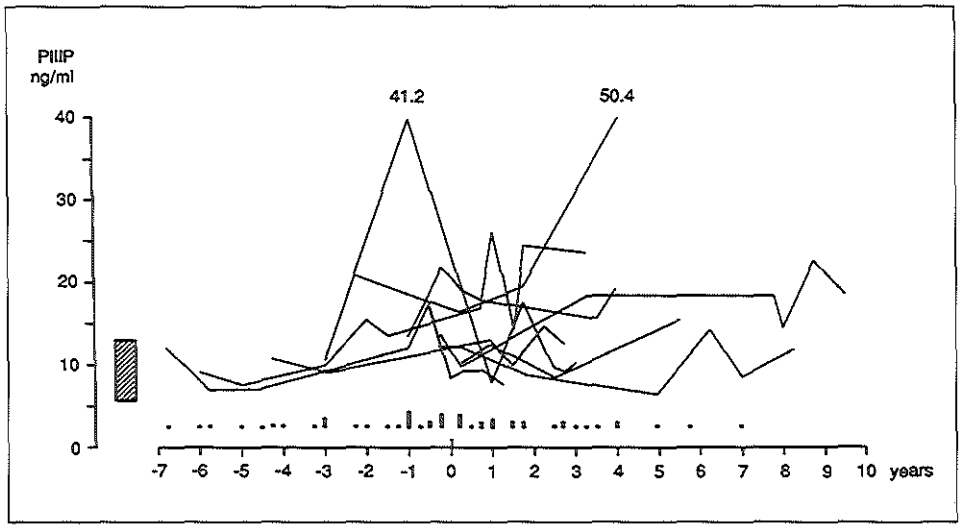

Fig. 2: Follow-up serum PIIIP concentrations in patients with histologically progressive PBC. The data are arranged according to an artificial histological turning point (time zero), separating early from late stage disease. Each dot at the bottom of the graph represents a liver biopsy.

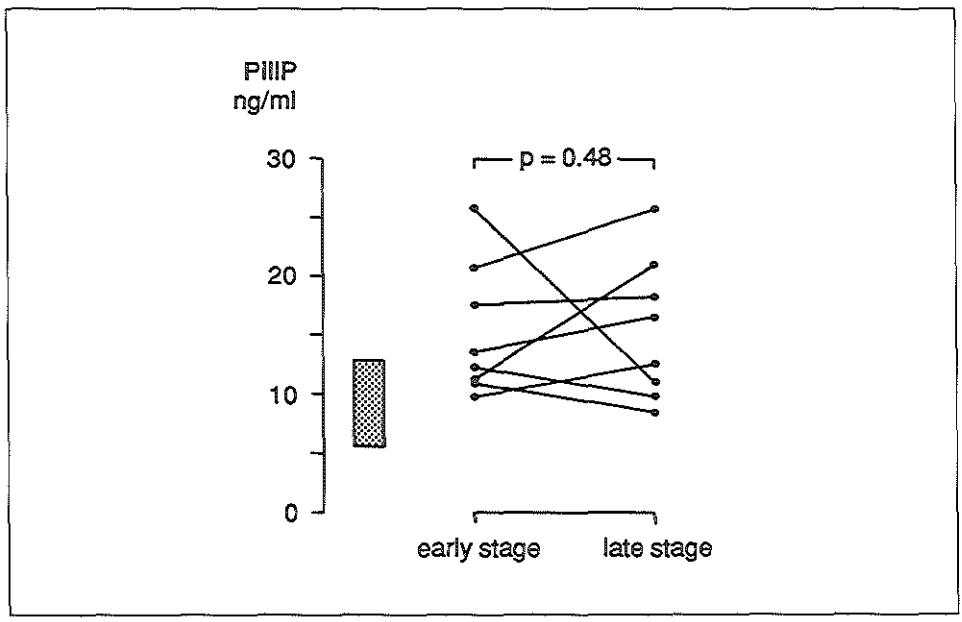

Fig. 3: Mean serum PIIIP concentrations found for early stage disease compared with those found for late stage disease in 8 patients with histologically progressive PBC.

Table 3: Means of follow-up serum PIIIP concentrations in patients with histologically progessive and non-progessive PBC.

Patient	PIIIP concentrations (N: 5.6-12.8 ng/ml)		
	number of values	mean values*	SEM
Progressive group			
1	9	14.5	3.5
2	5	24.8	6.5
3	11	9.7	0.9
4	6	11.7	0.9
5	5	11.6	1.4
6	9	10.2	0.8
7	11	16.5	1.9
8	9	16.2	1.2
9	6	17.7	1.2
Non-progressive group			
10	4	11.6	0.4
11	5	9.7	0.6
12	3	7.8	0.5
13	5	12.6	1.1
14	5	19.0	1.6
15	6	14.0	1.4
16	3	8.8	1.3
17	7	9.9	0.8
18	4	10.8	1.6

* mean values for the two groups were not significantly different (p=0.10; Wilcoxon test).

SEM: standard error of the mean.

For each patient the correlations between PIIIP levels and corresponding bilirubin, alkaline phosphatase, ASAT and albumin concentrations as well as histological stage were calculated. The median number of observations available for correlation was 6 (range: 3-11) for each variable tested. The correlation coefficients appeared to be widely distributed without a consistent trend towards a positive or negative correlation for either of the variables (fig. 4).

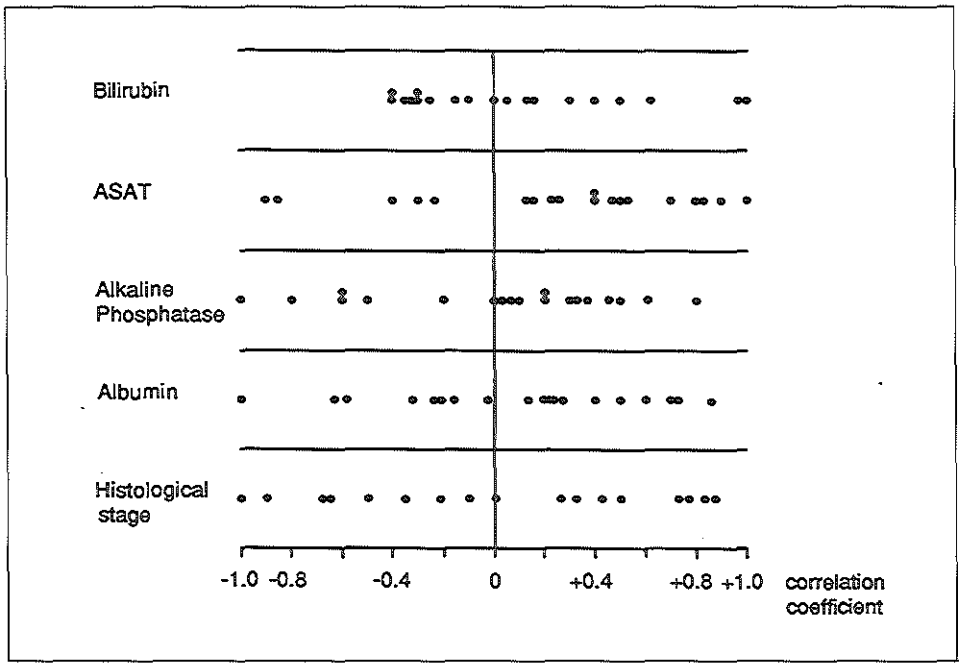

Fig. 4: Distribution of the correlation coefficients found for 18 patients with PBC indicating the correlation between serum PIIIP levels and corresponding biochemical values and histology.

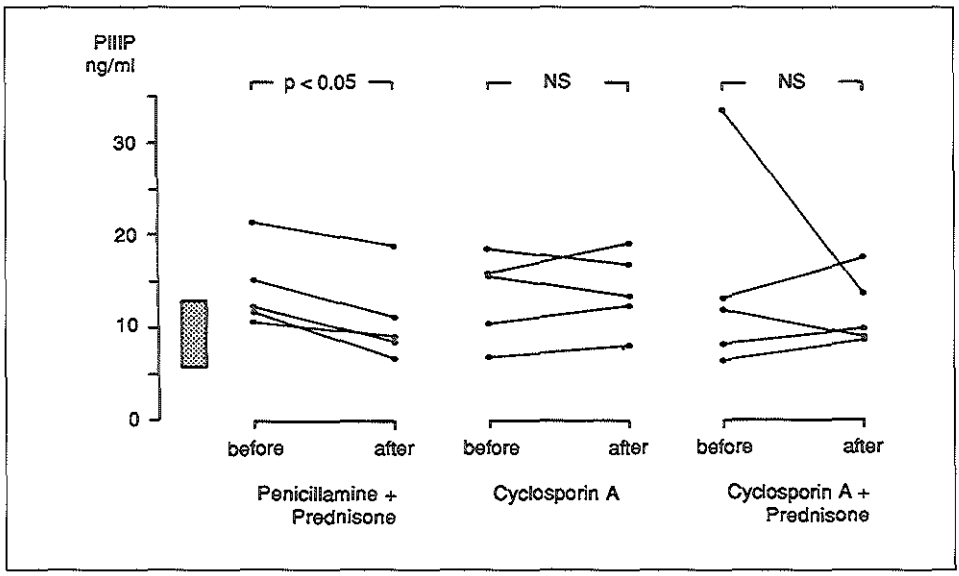

Fig. 5: Effect on serum PIIIP concentrations of three immunosuppressive and anti-inflammatory treatment regimens in patients with PBC. NS=statistically not significant.

The effects on PIIIP levels of three different treatment regimens are shown in figure 5. In these small series of patients only penicillamine combined with prednisone resulted in a significant decrease in PIIIP concentrations.

DISCUSSION

Serum PIIIP levels have been found to be elevated in acute and chronic liver diseases of various etiologies (3-13). Some investigators have reported a good correlation between serum PIIIP and established fibrosis (6,12), while others concluded that the peptide levels primarily reflect active fibrogenesis (5,14,22) or necrosis and inflammation (3,23). Furthermore, serum PIIIP has been proposed as a useful marker of disease activity in autoimmune chronic active hepatitis (7) and as a prognostic factor in PBC (10-12).

Several investigators have reported on serum PIIIP levels in patients with PBC (6,8-13). Savolainen et al. found elevated PIIIP concentrations in the majority of their 21 patients (8). The peptide levels exceeded the upper limit of normal in three-quarters of the 24 patients studied by Eriksson et al. Mean serum PIIIP levels, however, were significantly elevated in symptomatic patients only (10). Niemelä et al. reported elevated peptide levels in all 11 patients with histologically late stage PBC, whereas 6 of the 11 patients with early stage disease in their series had normal PIIIP levels (11). In the largest series reported so far, Babbs et al. found that the serum PIIIP levels were elevated in 73% of the 63 patients studied (12). In all of these series PIIIP measurements were carried out at a single point in time. Few data are available on follow-up determinations of serum PIIIP for the same patients. Weigand et al. followed PIIIP levels in four patients with PBC for up to seven years. The initially increased peptide levels remained elevated in all cases (9). In contrast, during a mean follow-up of 42 months PIIIP concentrations dropped from the initial value in 10 out of 18 patients in the series reported by Mutimer et al. (13). In addition, PIIIP levels decreased prior to death in five of the seven patients with a fatal outcome of their disease.

In the present series, to our knowledge the largest report on serial PIIIP measurements in PBC, mean peptide levels during follow-up exceeded the upper limit of normal in 39% of the patients, whereas serum PIIIP was elevated at least once during follow up in 72%. As stated earlier, in PBC patients with histologically progressive disease and therefore active fibrogenesis, one would expect serum PIIIP levels to be elevated. Although the peptide levels of patients of the progressive

group were elevated more often during follow-up than those of the non-progressive group, the means of the individual values did not differ between the groups. Moreover, progression from histologically early to late stage disease was not reflected in serum PIIIP levels. We selected our patients on the basis of histological criteria, i.e. histological progression versus stable disease. All patients included in the progressive group had been treated with immunosuppressive or anti-inflammatory drugs. In contrast, only three of the non-progressive patients had been treated medically. Therefore, incorrect patient selection due to sampling error as well as drug treatment could have influenced our results. The hazard of sampling error applies especially to the inclusion of patients in the progressive group, i.e. to an erroneous histological diagnosis of early stage disease in a patient with late stage disease. We believe that we have overcome this problem by establishing the phase of early stage disease in these patients on the basis of at least two liver biopsies in all but one case. Once a biopsy showed stage III or IV, a patient was considered to have late stage disease. If in such a patient a follow-up biopsy showed early stage disease (like in patient 5 and 8), we considered this to be a sampling error, because spontaneous or medically induced regression from fibrotic to non-fibrotic stages does not occur in PBC. The correctness of our selection appears to be supported by the clinical progression of four patients, three of whom died, in the progressive group, versus none in the non-progressive group. Although tested in small series of patients, penicillamine (8,9) and azathioprine and prednisolone (9) have been reported to have no influence on serum PIIIP levels. In agreement with others (24) we observed no effect on serum PIIIP due to treatment with cyclosporin A. The same applies for cyclosporin A combined with prednisone. Only treatment with the combination penicillamine and prednisone for one year resulted in a significant decrease in PIIIP levels. Five out of nine patients of the progressive group, but none of the non-progressive group had been treated in the past with this combination of drugs; apparently this did not prevent histological progression in the former group. Ninety percent of the serum samples for PIIIP determination in these five patients were obtained before or more than one year after treatment. In two cases one serum sample was taken during treatment; in a third patient one sample was taken during and another three months after treatment. Therefore we believe it to be unlikely that our results have been influenced by medical treatment.

We found no significant difference between PIIIP levels corresponding to the different histological stages. In contrast, other investigators did find a positive correlation between PIIIP levels and histology with significantly lower peptide levels in early disease. In these studies, however, the overlap between groups was considerable (12, 13). Therefore, we believe that PIIIP is not a reliable marker of the histological stage of PBC.

Serum PIIIP has been reported to correlate with parameters of cholestasis (alkaline phosphatase, bilirubin) (11-13), hepatic necrosis and inflammation (ASAT) (11-13) and protein synthesis (albumin) (11,13) in PBC as well as other liver diseases (3,4,6). Although statistically significant, the correlations in all these studies were weak. Because serial data were available for all patients in our series, we were able to calculate individual correlation coefficients. Of course, our results may have been influenced by the relatively small number of observations per patient. The wide distribution of the correlation coefficients without an obvious trend towards positive or negative for either of the variables tested, however, throws doubt upon the relationship of PIIIP with cholestasis and hepatic necrosis, inflammation or protein synthesis.

Babbs et al., using the Cox proportional hazard model, concluded that serum PIIIP is an independent prognostic variable in PBC (12). Although this seems to be supported by data from other investigators (10,11), the conclusions of these studies were criticized in a recent report by Mutimer et al. (13). Our data do not allow firm conclusions on the value of serum PIIIP for predicting the prognosis for PBC. Nevertheless, there are arguments to assume that, despite the lack of difference in PIIIP levels, prognosis is worse for patients included in the progressive group compared with those in the non-progressive group. Firstly there is the unfavorable clinical outcome for some patients in the former group, in contrast to the absence of clinical deterioration in the latter. Secondly, several, although not all, authors have reported histologically late stage disease, i.e. cirrhosis, to be correlated with shortened survival (18,25,26).

We conclude that serum PIIIP level determinations are of no value for predicting histological progression of PBC. This could be due to the complexity of collagen metabolism and variations in PIIIP clearance (13). Another explanation could be that during the slow process of progression to the fibrotic stages of the disease, active

hepatic fibrogenesis results in increments in serum PIIIP concentrations which are too small to be detected with the present methods.

ACKNOWLEDGEMENTS

We wish to thank R.E.W. van Leeuwen for his technical assistance.

REFERENCES

1. Rojkind M, Giambrone MA, Biempica L. Collagen types in normal and cirrhotic liver. Gastroenterology 1979; 76: 710-719.
2. Wick G, Brunner H, Penner E, Timpl R. The diagnostic application of specific antiprocollagen sera. Int Archs Allergy Appl Immun 1978; 56: 316-324.
3. Rohde H, Vargas L, Hahn E, Kalbfleisch H, Bruguera M, Timpl R. Radioimmunoassay for type III procollagen peptide and its application to human liver disease. Eur J Clin Invest 1979; 9: 451-459.
4. Chang T-T, Lin H-C, Lee S-D, Tsai Y-T, Lee F-Y, Jeng F-S, Wu J-C, Yeh PS-H, Lo K-J. Clinical significance of serum type-III procollagen aminopropeptide in hepatitis B virus-related liver diseases. Scand J Gastroenterol 1989; 24: 533-538.
5. Torres-Salinas M, Parés A, Caballeria J, Jiménez W, Heredia D, Bruguera M, Rodés J. Serum procollagen type III peptide as a marker of hepatic fibrogenesis in alcoholic hepatitis. Gastroenterology 1986; 90: 1241-1246.
6. 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: 830-834.
7. McCullough AJ, Stassen WN, Wiesner RH, Czaja AJ. Serum type III procollagen peptide concentrations in severe chronic active hepatitis: relationship to cirrhosis and disease activity. Hepatology 1987; 7: 49-54.
8. Savolainen E-R, Miettinen TA, Pikkarainen P, Salaspuro MP, Kivirikko KI. Enzymes of collagen synthesis and type III procollagen aminopropeptide in the evaluation of D-penicillamine and medroxyprogesterone treatments of primary biliary cirrhosis. Gut 1983; 24: 136-142.
9. Weigand K, Zaugg P-Y, Frei A, Zimmermann A. Long-term follow-up of serum N-terminal propeptide of collagen type III levels in patients with chronic liver disease. Hepatology 1984; 4: 835-838.

10. Eriksson S, Zettervall O. The N-terminal propeptide of collagen type III in serum as a prognostic indicator in primary biliary cirrhosis. J Hepatol 1986; 2: 370-378.
11. Niemelä O, Risteli L, Sotaniemi EA, Stenbäck F, Risteli J. Serum basement membrane and type III procollagen-related antigens in primary biliary cirrhosis. J Hepatol 1988; 6: 307-314.
12. Babbs C, Hunt LP, Haboubi NY, Smith A, Rowan BP, Warnes TW. Type III procollagen peptide: a marker of disease activity in primary biliary cirrhosis. Lancet 1988; I: 1021-1024.
13. Mutimer DJ, Bassendine MF, Kelly P, James OFW. Is measurement of type III procollagen amino propeptide useful in primary biliary cirrhosis? J Hepatol 1989; 9: 184-189.
14. Heredia D, Caballeria J, Parés A, Jiménez W, Torres M, Bruguera M, Rodés J. Serum procollagen type III peptide does not reflect hepatic collagen content in alcoholics with inactive cirrhosis. J Hepatol 1985 (Suppl 2): S252 (abstract).
15. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1987; 316: 521-528.
16. Wiesner RH, Grambsch PM, Lindor KD, Ludwig J, Dickson ER. Clinical and statistical analyses of new and evolving therapies for primary biliary cirrhosis. Hepatology 1988; 8: 668-676.
17. Shapiro JM, Smith H, Schaffner F. Serum bilirubin: a prognostic factor in primary biliary cirrhosis. Gut 1979; 20: 137-140.
18. Christensen E, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B, Doniach D, Ranek L, Tygstrup N, Williams R. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Gastroenterology 1985; 89: 1084-1091.
19. Scheuer PJ. Primary biliary cirrhosis. Proc Roy Soc Med 1967; 60: 1257-1260.
20. Hahn EG. Blood analysis for liver fibrosis. J Hepatol 1984; 1: 67-73.
21. Trivedi P, Cheeseman P, Portmann B, Hegarty J, Mowat AP. Variation in serum type III procollagen peptide with age in healthy subjects and its comparative value in the assessment of disease activity in children and adults with chronic active hepatitis. Eur J Clin Invest 1985; 15: 69-74.
22. Galambos MR, Collins DC, Galambos JT. A radioimmunoassay procedure for type III procollagen: its use in the detection of hepatic fibrosis. Hepatology 1985; 5: 38-42.

23. Surrenti C, Casini A, Milani S, Ambu S, Ceccatelli P, D'Agata A. Is determination of serum N-terminal procollagen type III peptide (sPIIIP) a marker of hepatic fibrosis? Dig Dis Sc 1987; 32: 705-709.

24. Minuk GY, Bohme CE, Burgess E, Hershfield NB, Kelly JK, Shaffer EA, Sutherland LR, Rosendaal G van. Pilot study of cyclosporin A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988; 95: 1356-1363.

25. Roll J, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl Med 1983; 308: 1-7.

26. Portmann B, Popper H, Neuberger J, Williams R. Sequential and diagnostic features in primary biliary cirrhosis based on serial histologic study in 209 patients. Gastroenterology 1985; 88: 1777-1790.

IMMUNOSUPPRESSIVE THERAPY FOR PRIMARY BILIARY CIRRHOSIS

R. Beukers, S.W. Schalm

Journal of Hepatology 1992;14:1-6.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by an inflammatory process that destroys interlobular and septal bile ducts, usually leading to progressive cholestasis, biliary cirrhosis and eventually death from the complications of portal hypertension or hepatic failure (1). The etiology of the disease is unknown, but there is considerable clinical, serological and histological evidence that immune mechanisms play a major role in the damage of hepatobiliary tissue (1-6).

The primary lesion in PBC is thought to be attributable to an attack of cytotoxic T-lymphocytes on antigens of the bile duct epithelium. Bile duct epithelial antigens may be altered by exogenous factors or crossreact with foreign, e.g. bacterial (7,8), antigens. T-cell clones may be activated by recognition of these antigens in connection with class II major histocompatibility (MHC) antigens, which are expressed by the bile duct epithelium in PBC (9,10). The defective T-suppresor cell function, encountered in PBC (3,4), may facilitate uncontrolled immune responses.

In view of this hypothesis on the pathogenesis of PBC, it seems justifiable to use immunosuppressive or anti-inflammatory drugs for treatment. Indeed, during the past fifteen years numerous controlled trials on the efficacy of several such drugs have been conducted (11-27). Although the methodology of these studies has been the subject of criticism, one can conclude that none of these drugs has proven to be totally effective (28). Moreover, assessment of the efficacy of medical treatment of PBC is hampered by wide variations in the clinical course of the disease and its prognosis. In addition, it has been a matter of debate whether asymptomatic patients should be included in therapeutic trials, since their prognosis is presumed to be good (29).

In recent years several review articles on the clinical characteristics and/or treatment of PBC (1,28,30) have been published. The present review focuses on immunomodulating and immunosuppressive therapy and issues such as the assessment of treatment efficacy and patient and drug selection, which we believe to be important for the design of future therapeutic trials in PBC.

ASSESSMENT OF TREATMENT EFFICACY

The long-term goal of the medical treatment of PBC is to prevent histological progression and the development of portal hypertension and thus to eliminate the

need for liver transplantation or premature death due to hepatic failure. Because of the variable and often slowly progressive course of the disease, the effect of therapy on progression and survival is difficult to assess, requiring a large number of patients and a long follow-up.

We believe that the only way to significantly alter the course of the disease is to achieve clinical, biochemical and histological remission of the hepatobiliary inflammatory process. We consider this to be the most important short-term goal. Since no generally accepted definition of remission exists for PBC, our proposed guidelines are presented in table 1.

Table 1: Definitions of remission in primary biliary cirrhosis.

Symptomatic remission:	- no PBC-related symptoms, in particular pruritus and fatigue - ability to resume normal daily activities
Biochemical remission:	- normalization of bilirubin, ASAT and serum IgM - alkaline phosphatase <1.5 x the upper limit of normal
Histological remission:	- inflammation absent or restricted to the portal tracts without bile duct destruction

Our definition of clinical remission includes the disappearance of fatigue and pruritus (the most frequent symptoms of PBC (1)) and the ability to resume normal daily activities. Symptoms arising from associated autoimmune disorders are not included. For biochemical remission normalization of serum bilirubin (the most important prognostic factor), aspartate aminotransferase (a marker of hepatic inflammation) and serum IgM (a parameter of immunological activity) is required. We accept a maximum elevation for serum alkaline phosphatase levels of 1.5 times the upper limit of normal, because the histopathological features of the disease (31) are such that normalization might not be possible despite remission of the inflammatory process.

Since symptoms and biochemistry do not always reflect histological changes, the establishment of histological remission is essential. Whether the elemination of the

inflammation will eventually prevent histological progression probably depends on the extent of irreversible tissue damage already present. Extensive fibrosis and loss of bile ducts may promote further liver damage, even when active inflammation is absent. Nevertheless, one may assume that remission of the inflammation will at least delay histological progression.

PATIENT SELECTION

Patients with PBC can be roughly divided into three groups. The first group is characterized by the presence of symptomatic portal hypertension and progressive jaundice and is considered to have end-stage disease and a poor prognosis. Once serum bilirubin exceeds 100 µmol/l the average life expectancy is reduced to two years (32). For these patients no medical treatment can be expected to be effective and liver transplantation is regarded as the treatment of choice (33).

The second group of patients has the symptoms but not the complications of liver disease. In these patients the disease usually runs a progressive course and life expectancy is estimated to be five to ten years from diagnosis (30,34). There is general agreement that some form of therapeutic intervention to halt progresssion of the disease in this group is urgently required (30,34).

Finally, the third group has asymptomatic disease. Although these patients are reported to have a normal life expectancy and, therefore, should not be enrolled in clinical trials (35), more recent studies indicate that prognosis may not be normal (36-38). In table 2 four long-term follow-up studies on the clinical outcome of asymptomatic PBC are summarized. Although there were probably differences in the populations studied, partly due to differences in referral pattern (29,38), we can conclude that a considerable number of asymptomatic patients eventually become symptomatic (35-38) and progress to cirrhosis (37). Overall survival for asymptomatic patients is significantly better than for patients with symptomatic disease (36) but is diminished compared to a matched normal population (36-38). This rate may only become apparent after five to twelve years of follow-up (36,37). Furthermore, once asymptomatic patients develop symptoms, their prognosis is similar to patients with symptoms at presentation (38). Only in the Yale series was survival for asymptomatic patients similar to the general population (35), but those results may have been influenced by the relatively small number of patients (29). The findings of studies concerning the prognostic value of histological features at presentation, such as

Table 2: Prognosis of patients with asymptomatic primary biliary cirrhosis.

Centre (ref.)	Period	Asympt. patients N (% of total)	Late stage (III/IV)	Follow-up (yr)*	Patients becoming symptomatic	Survival**
Yale (35)	1960-1978	36 (13)	50%	11.4	42%	=
Uppsala (36)	1979-1986	56 (70°)	34%	9.5	37%	↓
Mayo Clinic (37)	1974-1984	73 (17)	61%	7.6	89%	↓
New Castle (38)	1969-1987	70 (39)	40%	5.8	27%	↓

* median follow-up from diagnosis; in Mayo Clinic-series mean follow-up from referral to Mayo Clinic

** compared with a normal control population

° fatigue was not considered a symptom of PBC; if fatigue was included, 59% of the patients were asymptomatic

granulomas or cirrhosis, are equivocal. However, in accordance with observations in our clinic, the Uppsala group found that asymptomatic patients with histological stage I disease may comprise a subgroup with an excellent prognosis (36).
We, therefore, conclude that only patients with end-stage PBC and asymptomatic patients with stage I disease should be excluded from clinical trials.

DRUG SELECTION

Considering the natural history of the disease, any medical treatment of PBC must be suitable for long-term use. Potentially effective drugs with a high incidence of side-effects will not be accepted by patients. Furthermore, one must be cautious about administering drugs which may irreversibly impair vital organ functions, thereby decreasing the possibility of a future liver transplantation. These considerations should be taken into account in future treatment trials for PBC.

Single drug treatment: results of controlled studies. To date, randomized controlled trials have been conducted with the following immunosuppressive or immunomodulating drugs: penicillamine, azathioprine, colchicine, chlorambucil, cyclosporine and prednisolone (11-27). The assessment of therapeutic efficacy was not based on the criteria for clinical, biochemical and histological remission in any of these studies.

Penicillamine, a drug with antifibrotic, immunomodulating and copper-chelating properties, was studied in eight controlled trials (11-18). Not only was this drug ineffective, it was also associated with a high incidence of side-effects, necessitating discontinuation in up to 40% of the cases.

Azathioprine has been studied in two controlled trials (19,20). Although in one of these studies treatment with azathioprine was associated with a statistically significant, though clinically unimportant, improvement in survival (20), a substantial number of patients was lost to follow-up. In addition, in both studies azathioprine did not affect symptoms, biochemical abnormalities or histological progression.

The anti-inflammatory and antifibrotic drug colchicine has been evaluated in three trials (21-23). Colchicine had no effect on symptoms but improved abnormal liver blood tests. One study reported a significant increase in survival for colchicine-treated patients (21). Histological progression, however, was not prevented and the difference in survival was no longer significant when the 'intention to treat' rule was applied (28).

The alkylating agent chlorambucil was evaluated in one controlled trial (24). Chlorambucil treatment favorably influenced liver function tests and inflammatory changes in liver histology. Histological progression, however, was not retarded. Survival was not analyzed. Bone marrow toxicity precluded further use of the drug in four of the thirteen treated patients.

Cyclosporine, an immunosuppressive drug which specially influences T-lymphocyte mediated immune responses, was evaluated for the treatment of PBC in two studies (25,26). Cyclosporine appears to improve symptoms and biochemical abnormalities. Histology was not affected in one study (25), but the results of the Mayo Clinic study indicate that cyclosporine might beneficially influence histological progression (26). In both studies nephrotoxicity was the major side-effect of cyclosporine treatment. The nephrotoxic effects of cyclosporine have been well documented in both transplant recipients (39) and patients with auto-immune diseases (40). Early nephrotoxicity is functional, dose-related and reversible (41). Chronic cyclosporine-related nephrotoxicity is usually not progressive (40,42) and at least partially reversible (40,43). However, after long-term administration of cyclosporine structural and potentially irreversible changes in renal morphology can develop (44,45). In some of our PBC patients treated with cyclosporine, serum creatinine levels did not completely return to baseline, even twelve months after the drug had been discontinued (unpublished results). Comparable data have been reported by Minuk et al. (25). The nephrotoxicity of cyclosporine, therefore, raises concern about the long-term safety of this agent in PBC.

Finally, in one controlled trial (27), prednisolone treatment resulted in improvement of both symptoms and abnormal liver tests. There was histological improvement in some cases, but the effect on histological progression and survival was not analyzed. However, prednisolone therapy (initial dose 30 mg/day; maintenance dose 10 mg/day) for one year was associated with an increased bone loss equal to approximately twice the expected rate (27). Bone loss appeared to be greatest in the first two months of treatment, when higher doses of prednisolone were used. In contrast, the effect on bone of low-dose prednisolone (10 mg/day) is probably of minor clinical importance. Indeed, we found that the mineral density of the spine was not related to the administration of low-dose prednisone in 55 unselected female patients with longstanding PBC (46). Similar findings have been reported for patients with chronic liver disease of various etiologies (47) and patients with reumatoid arthritis (48).

Single drug treatment: uncontrolled studies. The administration of low weekly doses of methotrexate (MTX) is a well-established approach to the treatment of reumatoid arthritis and refractory psoriasis. Recently, this 'pulse therapy' (15 mg per week, in three divided doses over a 24 h period) was applied for the treatment of PBC (49,50).

In an uncontrolled study of sixteen patients MTX therapy for 12-34 months resulted in symptomatic, biochemical and histological improvement. In addition, the data suggested that MTX might induce clinical, biochemical and even histological remission in patients with early disease. The response, however, in cirrhotic patients seems to be minimal. The only complication was thrombocytopenia in two cases. However, some reserve seems warranted. Firstly, this was an uncontrolled study of a small number of patients. Secondly, MTX is a potentially hepatotoxic drug (51). Although it has been stated that, in the absence of alcohol consumption, low dose weekly MTX therapy rarely causes clinically significant liver damage (52,53), the hepatotoxicity of long-term MTX treatment in patients with pre-existing chronic liver disease is as yet unknown.

Another infrequent but unpredictable and potentially serious complication of MTX therapy is acute pneumonitis (54).

Combination therapy. The theoretical advantages of the combination of two or more potentially effective drugs are improvement of efficacy and a mutual dose-sparing effect. However, more side-effects are also a possibility. No controlled trials have been reported on immunosuppressive combination therapy for PBC. We conducted an uncontrolled pilot study on the combination of low dose cyclosporine (2 mg/kg initial daily dose) and prednisone (10 mg maintenance dose)(55). Ten patients with symptomatic, non-end stage disease were treated for 12-24 months. There was significant improvement in symptoms and biochemical abnormalities. One patient achieved a symptomatic remission during treatment, but none met our criteria of a biochemical remission. In addition, although periportal inflammation was reduced to the level of a histological remission in three patients, fibrosis increased in two of these cases. Side-effects were similar to those of cyclosporine or prednisone single-drug therapy.

The concept of combination treatment remains attractive. Besides the combination of two immunosuppressive drugs, the combination of one of these drugs

(e.g. prednisolone) and other potentially effective agents of low toxicity, such as ursodeoxycholic acid (UDCA), should also be considered. UDCA, a drug which has cytoprotective properties (56,57) and might influence the aberrant expression of class I MHC antigens on hepatocytes in PBC (58), has improved symptoms and biochemical abnormalities in PBC (56,59).

New immunosuppressive drugs. There is no doubt that in time, newly developed immunosuppressive drugs, such as new cyclosporine-derivates and FK 506, will become available for clinical trials in autoimmune liver diseases. FK 506 is a T-cell specific immunosuppressant, structurally distinct from cyclosporine but with similar, although more potent, immunosuppressive properties (60). It also has a hepatotropic effect and is particularly successful in the management of liver transplantation (60).

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE THERAPEUTIC TRIALS

Our present knowledge of the pathogenetic mechanisms in PBC and the results obtained with some of the above-mentioned immunosuppressive and immunomodulating agents warrant further research for an effective and non-toxic immunosuppressive treatment regimen. Most single drugs studied thus far are of limited use or unsuitable for the treatment of PBC due to low efficacy (penicillamine, azathioprine, colchicine) or high toxicity (penicillamine, chlorambucil). We believe that, in view of its nephrotoxicity and the uncertain sequelae, cyclosporine must also be considered unsafe for long-term use. Furthermore, cyclosporine treatment is costly and its efficacy appears to be only moderate.

The results of prednisolone are promising but higher doses are probably needed to induce and maintain a remission, undoubtedly at the cost of accelerated bone loss. Steroid-sparing may, however, be achieved by the addition of azathioprine. Moreover, since steroid-induced bone loss may be prevented by biphosphonates (61), the treatment of PBC patients with a combination of prednisolone, azathioprine and biphosphonates, under regular monitoring of bone density at relevant sites (e.g. by dual photon absorptiometry of the spine), might be an attractive and interesting subject for further study.

The results of MTX therapy need to be confirmed in controlled trials and more needs to be known about its hepatoxicity in chronic liver disease before its widespread use

in PBC can be advocated.

The options for future therapeutic trials in PBC are summarized in table 3.

Table 3: Options for future therapeutic trials in primary biliary cirrhosis.

I Low-toxicity immunosuppressive therapy, e.g:
- prednisolone* + azathioprine
- methotrexate pulse therapy?
- new immunosuppressive drugs (e.g: FK 506; new cyclosporine-derivatives)?

II Combination of I and other drugs, e.g:
- ursodeoxycholic acid

**under biphosphonate protection*

REFERENCES

1. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1987;316:521-8.
2. James SP, Hoofnagle JH, Strober W, Jones EA. Primary biliary cirrhosis: a model autoimmune disease. Ann Int Med 1983;99:500-12.
3. James SP, Elson CO, Jones EA, Strober W. Abnormal regulation of immunoglobulin synthesis in vitro in primary biliary cirrhosis. Gastroenterology 1980;79:242-54.
4. Zetterman RK, Woltjen JA. Suppressor cell activity in primary biliary cirrhosis. Dig Dis Sc 1980;25:104-7.
5. Yamada G, Hyodo I, Tobe K, Mizuno M, Nishihara T, Kobayashi T et al. Ultrastructural immunocytochemical analysis of lymphocytes infiltrating bile duct epithelium in primary biliary cirrhosis. Hepatology 1986;6:385-91.
6. Paronetto F, Colucci G, Colombo M. Lymphocytes in liver disease. In: Progress in liver disease. Popper H, Schaffner F, eds. Grune & Stratton Inc., Orlando 1986;191-208.
7. 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.
8. 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:1419-22.

9. Ballardini G, Bianchi FB, Doniach D, Mirakian R, Pisi E, Bottazo GF. Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. Lancet 1984;II:1009-13.
10. Sprengler U, Pape GR, Hoffmann RM, Johnson JP, Eisenberg J, Paumgartner G et al. 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.
11. Triger DR, Manifold IH, Cloke P, Underwood JCE. D-penicillamine in primary biliary cirrhosis: two year results of a single centre, double-blind controlled trial. Gut 1980;21:919-20 (abstract).
12. 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:1275-77.
13. Bassendine MF, Macklon AF, Mulcahy R, James OFW. Controlled trial of high and low dose D-penicillamine in primary biliary cirrhosis (PBC): results at three years. Gut 1982;23:909 (abstract).
14. 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.
15. 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.
16. 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;312:1011-15.
17. 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.
18. 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.
19. Heathcote J, Ross A, Sherlock S. A prospective controlled trial of azathioprine in primary biliary cirrhosis. Gastroenterology 1976;70:656-60.

20. 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.
21. 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.
22. Bodenheimer H, Schaffner F, Pezzullo J. Evaluation of colchicine therapy in primary biliary cirrhosis. Gastroenterology 1988;95:124-9.
23. 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.
24. 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.
25. Minuk GY, Bohme CE, Burgess E, Hershfield NB, Kelly JK, Shaffer EA et al. Pilot study of cyclosporin A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988;95:1356-63.
26. 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 Eng J Med 1990;322:1419-24.
27. 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;10:420-29.
28. Wiesner RH, Grambsch PM, Lindor KD, Ludwig J, Dickson ER. Clinical and statistical analyses of new and evolving therapies for primary biliary cirrhosis. Hepatology 1988;8:668-76.
29. Kaplan MM. Survival in asymptomatic primary biliary cirrhosis. Gastroenterology 1990;98:1707-09.
30. Epstein O. Review: the treatment of primary biliary cirrhosis. Aliment Pharmacol Therap 1988;2:1-12.
31. Scheuer PJ. Primary biliary cirrhosis. Proc Roy Soc Med 1967;60:1257-60.
32. Shapiro JH, Smith H, Schaffner F. Serum bilirubin: a prognostic factor in primary biliary cirrhosis. Gut 1979;20:137-40.

33. 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.
34. James O. D-penicillamine for primary biliary cirrhosis. Gut 1985;26:109-13.
35. Beswick DR, Klatskin G, Boyer JL. Asymptomatic primary biliary cirrhosis. Gastroenterology 1985;89:267-71.
36. Nyberg A, Lööf L. Primary biliary cirrhosis: clinical features and outcome, with special reference to asymptomatic disease. Scand J Gastroenterol 1989;24:57-64.
37. Balasubramaniam K, Grambsch PM, Wiesner RH, Lindor KD, Dickson ER. Diminished survival in asymptomatic primary biliary cirrhosis. Gastroenterology 1990;98:1567-71.
38. 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;99:778-84.
39. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporine: a new immunosuppressive agent for organ transplantation. Ann Int Med 1984;101:667-82.
40. Dieterle A, Abeywickrama K, Graffenried B von. Nephrotoxicity and hypertension in patients with autoimmune disease treated with cyclosporine. Transpl Proceed 1988 (suppl. 4);20:349-55.
41. Myers BD. Cyclosporine nephrotoxicity. Kidney Int 1986;30:964-74.
42. The Canadian multicentre transplant study group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. N Engl J Med 1986;314:1219-25.
43. Kahan BD. Cyclosporine. N Engl J Med 1989;321:1725-38.
44. Mihatsch MJ, Thiel G, Ryffel B. Ciclosporin-associated nephropathy. In: Ciclosporin in autoimmune diseases. Schindler R. Ed. Springer-Verlag Berlin 1985;50-8.
45. Palestine AG, Austin III HA, Balow JE, Antonovych TT, Sabnis SG, Preuss HG et al. Renal histopathologic alterations in patients treated with cyclosporine for uveitis. N Engl J Med 1986;314:1293-8.

46. Berkum FNR van, 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;99:1134-39.

47. Diamond T, Stiel D, Lunzer M, Wilkinson M, Roche J, Posen S. Osteoporosis and skeletal fractures in chronic liver disease. Gut 1990;31:82-7.

48. Sambrook PN, Eisman JA, Yeates MG, Pocock NA, Eberl S, Champion GD. Osteoporosis in rheumatoid arthritis: safety of low-dose corticosteroids. Ann Rheum Dis 1986;45:950-3.

49. Kaplan MM, Knox TA. Effective treatment of pre-cirrhotic primary biliary cirrhosis (PBC) with methotrexate (MTX): remission in some. Hepatology 1989;10:585 (abstract).

50. Kaplan MM. Methotrexate treatment of chronic cholestatic liver diseases: friend or foe? Quart J Med 1989;72:757-61.

51. Zakim D, Boyer TD, editors. Hepatology. Philidelphia: W.B. Saunders Company, 1990;773-4.

52. Lanse SB, Arnold GL, Gowans JDC, Kaplan MM. Low incidence of hepatotoxicity associated with long-term, low-dose oral methotrexate in treatment of refractory psoriasis, psoriatic arthritis, and rheumatoid arthritis. Dig Dis Sc 1985;30:104-9.

53. Kremer JM, Lee JK. The safety and efficacy of the use of methotrexate in long-term therapy for rheumatoid arthritis. Arthritis Rheum 1986;29:822-31.

54. Ridley MG, Wolfe CS, Mathews JA. Life threatening acute pneumonitis during low dose methotrexate treatment for rheumatoid arthritis: a case report and review of the literature. Ann Rheum Dis 1988;47:784-8.

55. Beukers R, Schalm SW. Effect of cyclosporine and cyclosporine plus prednisone in primary biliary cirrhosis. Transpl Proceed 1988;20 (suppl. 4);340-3.

56. 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 trial. Gastroenterology 1989;97:1268-74.

57. Güldütuna S, Imhof M, Hoffmann T, Zimmer G, Leuschner U. Ursodeoxycholic acid (UDCA) protects basolateral liver plasma membranes (blPM) against toxic bile salts. Hepatology 1990;12:997 (abstract).

58. Calmus Y, Gane P, Rouger Ph, Poupon R. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-5.

59. 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 Eng J Med 1991;324:1548-54.

60. Macleod AM, Thomson AW. FK 506: an immunosuppressant for the 1990s? Lancet 1991;337:25-7.

61. Reid IR, King AR, Alexander CJ, Ibbertson HK. Prevention of steroid-induced osteoporosis with (3-amino-1-hydroxypropylidene)-1,1-biphosphonate (APD). Lancet 1988;I:143-6.

SUMMARY

This thesis describes several aspects of immunosuppressive therapy for primary biliary cirrhosis (PBC).

Chapter I. This chapter gives a general introduction to PBC, the metabolic bone diseases associated with this disease, and the immunosuppressive agent cyclosporin A (CyA).

PBC is a chronic cholestatic liver disease of unknown origin, characterized by an inflammatory process that destroys interlobular and septal bile ducts. This usually leads to progressive cholestasis, biliary cirrhosis and eventually death due to the complications of portal hypertension or hepatic failure. Although there is considerable evidence that immunological mechanisms play a major role in the pathogenesis of PBC, the results of treatment with conventional immunosuppressive drugs have been disappointing. Except for liver transplantation, there is at present no therapy for PBC which can significantly alter the course of the disease and improve survival.

PBC may be complicated by metabolic bone disease, especially osteoporosis. For this reason corticosteroids were long considered to be contraindicated for the treatment of PBC. The etiology of osteoporosis in PBC is unknown but low bone turnover appears to be an important factor in its pathogenesis.

CyA is an immunosuppressive drug which exerts its action mainly by inhibition of the synthesis and release of interleukin 2, thereby primarely affecting T-cell responses. CyA has been applied extensively and with success for the management of patients receiving organ transplants. In addition, CyA has been tried for the treatment of a variety of established or presumed autoimmune diseases. Because cytotoxic T-cell reactions are thought to be involved in the hepatobiliary damage in PBC, CyA could be of therapeutic benefit. This hypothesis was supported by the results of in vitro studies on the effect of CyA on the T-cell functions of patients with PBC. The most important side-effect of CyA is nephrotoxicity.

The studies described in this thesis focus on three items:

- the possible role of CyA in the treatment of PBC (chapter II, III and IV);
- the extent of metabolic bone disease and the possible role of corticosteroid therapy in our patients with PBC (chapter V);

- the value of type III procollagen amino propeptide - a precursor peptide of collagen type III - as a predictor of progressive disease (chapter VI).

Chapter II. In this chapter the results are presented of a pilot study on CyA therapy for unselected patients with PBC. Ten patients were treated for six months; the initial CyA dose was 5 mg/kg. Six patients had cirrhosis (stage IV disease), five of whom were icteric. CyA therapy was discontinued in one case because of chronic diarrhea. For the remaining nine patients CyA treatment had no marked effect on symptoms. Mean serum alkaline phosphatase levels decreased significantly but the results of other liver function tests did not change. In addition, a significant decrease in serum IgM levels was observed. The main side-effect was nephrotoxicity, which could be controlled by close monitoring of CyA trough levels in plasma and dose adjustment. CyA treatment did not prevent further clinical deterioration in patients with end-stage disease. In some anicteric PBC patients, however, treatment had a beneficial effect on the abnormal biochemical and immunological parameters, which disappeared after discontinuation of CyA. We concluded that the efficacy of long-term low-dose CyA therapy for PBC patients with active and/or progressive but not end-stage disease merited further study.

Chapter III. Based on the results of our first study, we conducted a second pilot study on low-dose CyA therapy for symptomatic PBC in ten patients with active and/or progressive disease; those with end-stage disease were excluded. To enhance the efficacy of the treatment, CyA (2 mg/kg initially) was combined with prednisone (30 mg initially; 10 mg maintenance dose) and continued for at least twelve months. Primary goal of the study was the induction of symptomatic and biochemical remission of the disease, because we believe this to be a prerequisite for an ultimate improvement in survival. There was one withdrawal due to side-effects and one patient died after seven months. For the eight patients who completed the course combination therapy resulted in a significant improvement in symptoms and mean serum levels of alkaline phosphatase (maximum decrease from initial value, 46%), ASAT (38%) and immunoglobulins (IgM, 42% and IgG, 37%). However, the primary goal of the study, i.e. the induction of a sustained symptomatic and biochemical remission, was not achieved in any of the patients. After discontinuation of CyA symptom scores, liver blood tests and immunoglobulin levels tended to return to pretreatment levels, although the changes were only significant for IgG. The main

side-effect attributable to CyA was a mean maximum increase in serum creatinine levels of 30%. Nephrotoxicity appeared to be largely reversible but in three cases the serum creatinine levels did not return to baseline values after the discontinuation of CyA. In conclusion, combination treatment with CyA and prednisone did not induce symptomatic and biochemical remission of PBC. The nephrotoxicity of CyA makes the drug unsuitable for long-term treatment of PBC. Other less toxic regimens of combination therapy, which could include corticosteroids, merit further study.

Chapter IV. Because CyA is fat-soluble, its intestinal absorption depends on a normal bile flow into the gut. Furthermore, CyA is metabolized almost entirely in the liver and its metabolites are excreted in bile. Therefore, the pharmacokinetics of CyA in patients with PBC could be different from those in patients with normal liver function and bile flow. To investigate this, we studied the pharmacokinetics of CyA in seven patients with non-end stage PBC without previous CyA treatment (group I) and a control group of nine patients with skin diseases (mainly psoriasis; group II). In addition, to determine the effect of long-term use on the pharmacokinetic parameters of CyA, we studied six patients with non-end stage PBC after prolonged CyA treatment (group III). Whole blood concentrations of CyA and its metabolites were measured using a non-specific (N) radioimmunoassay (RIA); in a majority of the cases CyA concentrations were also estimated using a RIA specific (S) for the parent drug. No difference in CyA absorption was observed between patients with PBC and those with a skin disease. The mean values for the area under the blood concentration-time curve for the first six hours after the test dose (AUC_{0-6}) and the maximal blood concentrations (C_{max}) were significantly higher for group I compared with group II patients, but the time to maximal blood concentrations (t_{cmax}) did not differ. There was a trend toward higher mean AUC_{0-6} and C_{max} values for group III compared with group I patients, probably due to saturation of the peripheral compartment. T_{cmax} values were not influenced by prolonged CyA treatment. The ratio of CyA whole blood concentrations measured by the non-specific and specific RIA's (N/S ratio) increased with time, reflecting metabolite formation, without obvious differences between the three groups. The mean N/S ratio of the AUC_{0-6} values was approximately 2.0 for all groups. These data suggest that CyA absorption and its biotransformation in the liver are not impaired in patients with non-end stage PBC

and that both are not affected by prolonged treatment.

Chapter V. To assess the impact of primary biliary cirrhosis on bone mass in general and the relative importance of the stage of the liver disease and of treatment with glucocorticoids for the possible development of osteoporosis, bone mineral mass was measured by single and dual photon-absorptiometry in 55 unselected female patients with longstanding PBC. Although most of the patients had a bone mineral density within the normal range, the bone mineral densities of the lumbar spine, distal and proximal forearm were 8%, 8% and 5%, respectively, lower than in age-matched healthy females; these differences were statistically significant for the lumbar spine and distal forearm. Multiple regression analysis showed that the histological stage of the liver disease (early versus late stage) was an independent determinant of axial bone mineral density, whereas the use of glucocorticoids resulted in only a moderate and not significant bone loss. Serum calcium proved to be significantly lower in the patients with late stage PBC than in those with early stage disease, whereas no significant differences were found in these groups with regard to several biochemical parameters of bone metabolism. In conclusion, in patients with PBC, bone loss was only moderate and related to the histological stage; such a relationship has not been reported before. The effect of low-dose glucocorticoids on bone mass seemed not significant.

Chapter VI. It is likely that patients with active and/or progressive PBC can benefit the most from therapeutic intervention. Serological parameters to assess progressive disease, however, are lacking. Because the later stages of the disease are histologically characterized by increasing fibrosis, we examined the value of serum procollagen III amino propeptide (PIIIP) for predicting histological progression of PBC. This propeptide is split enzymatically from the procollagen molecule during the formation of type III collagen. Serial PIIIP measurements were obtained for 9 patients with histologically progressive PBC and 9 patients with histologically stable early disease, assessed by repeated liver biopsies and followed for up to 13 years. The means of the follow-up PIIIP concentrations were elevated in 39% of the cases; moreover, PIIIP levels were elevated at least once during follow-up in 72% of the cases. Mean follow-up PIIIP concentrations did not differ significantly between progressive and non-progressive patients. In addition, in the progressive group, histological progression was not reflected by PIIIP levels. No difference was found between the serum

PIIIP levels corresponding to the histological stages I, II and III. The individual coefficients of the correlation between serum PIIIP and biochemical variables (bilirubin, alkaline phospatase, ASAT, albumin) and histology showed a wide distribution without a consistent trend towards positive or negative. Treatment with cyclosporin A or cyclosporin A combined with prednisone did not influence serum PIIIP levels. Treatment with penicillamine combined with prednisone, however, resulted in a significant decrease in PIIIP concentrations.
We concluded that serum PIIIP measurements are of no value for predicting histological progression of PBC.

Chapter VII. In this chapter a general discussion of immunosuppressive therapy is given, including a proposition for future clinical trials on PBC.

SAMENVATTING

Hoofdstuk 1. In dit inleidende hoofdstuk wordt een beschrijving gegeven van primaire biliaire cirrhose (PBC), de bij deze leverziekte voorkomende metabole botziekten en het immunosuppressieve middel cyclosporine A (CyA).
PBC is een chronische cholestatische leverziekte waarvan de oorzaak niet bekend is. PBC wordt gekenmerkt door een ontstekingsproces dat interlobulaire en septale galgangen aantast. Dit leidt meestal tot progressieve cholestase, biliaire cirrhose en uiteindelijk de dood als gevolg van de complicaties van portale hypertensie of leverinsufficiëntie. Ofschoon er veel aanwijzingen zijn dat immunologische mechanismen een belangrijke rol spelen in de pathogenese van PBC, zijn de resultaten van de behandeling met conventionele immunosuppressieve middelen teleurstellend gebleken. Afgezien van levertransplantatie is er momenteel geen behandeling voor PBC, die het beloop van de ziekte significant kan beïnvloeden en daarmee de overleving verbeteren.

PBC kan gecompliceerd worden door metabole botziekten, met name osteoporose. Dit is de reden waarom corticosteroiden lange tijd gecontra-indiceerd werden geacht voor de behandeling van PBC. De etiologie van osteoporose in PBC is niet bekend, maar een lage bot-turnover lijkt een belangrijke pathogenetische factor te zijn.

CyA is een immunosuppressieve stof waarvan de werking voornamelijk berust op een remming van de synthese en afgifte van interleukine 2, waardoor vooral T-cel reacties worden beïnvloed. CyA is op grote schaal en met veel succes toegepast bij de behandeling van transplantatiepatiënten. Bovendien is CyA uitgeprobeerd bij de behandeling van verscheidene auto-immuun ziekten. Omdat verondersteld wordt dat T-cel reacties een belangrijke rol spelen in de aantasting van de lever in PBC, zou CyA van therapeutische waarde kunnen zijn. Deze hypothese werd gesteund door de resultaten van in vitro studies naar het effect van CyA op de T-cel functies van patiënten met PBC. Nefrotoxiciteit is de belangrijkste bijwerking van CyA.

De in dit proefschrift beschreven studies richten zich op een drietal onderwerpen:

- de mogelijke rol van CyA in de behandeling van PBC (hoofdstukken 2, 3 en 4);
- het vóórkomen en de ernst van metabole botziekten bij onze patiënten met PBC en de mogelijke rol hierbij van de behandeling met corticosteroiden (hoofdstuk 5);
- de waarde van type III procollageen amino-propeptide (een precursor-peptide van type III collageen) voor het voorspellen van een progressief ziektebeloop (hoofdstuk 6).

Hoofdstuk 2. Dit hoofdstuk geeft de resultaten van een "pilot" studie naar het effect van de behandeling met CyA van een groep niet-geselecteerde patiënten met PBC. Tien patiënten werden gedurende zes maanden behandeld; de aanvangsdosis van CyA was 5 mg/kg. Zes patiënten hadden levercirrhose (PBC stadium IV), waarvan er vijf icterisch waren. Bij één patiënt werd de behandeling afgebroken wegens chronische diarree. Voor de overige negen patiënten had behandeling met CyA geen duidelijk effect op de symptomen. De gemiddelde alkalische fosfatase spiegels daalden significant, maar de overige leverfuncties veranderden niet. Bovendien was er een significante daling van het serum IgM gehalte. De voornaamste bijwerking van CyA was nefrotoxiciteit. Behandeling met CyA kon verdere klinische achteruitgang van patiënten, die zich in het eindstadium van de ziekte bevonden, niet voorkomen. Bij sommige niet-icterische patiënten had de behandeling echter een gunstige invloed op de afwijkende biochemische en immunologische parameters. Dit effect verdween na het staken van CyA. Wij kwamen tot de conclusie dat het effect van lange termijn behandeling met een lage dosis CyA het bestuderen waard zou zijn bij patiënten met een actieve en/of progressieve PBC, maar nog geen eind-stadium van de ziekte.

Hoofdstuk 3. Op grond van de resultaten van ons eerste onderzoek verrichtten wij een tweede "pilot" studie naar de werkzaamheid van de behandeling met CyA van tien symptomatische PBC patiënten met een actieve en/of progressieve ziekte. Patiënten met een eind-stadium PBC werden van het onderzoek uitgesloten. Om de werkzaamheid van de behandeling te vergroten werd CyA (aanvangsdosis: 2 mg/kg) gecombineerd met prednison (aanvangsdosis: 30 mg; onderhoudsdosis: 10 mg) en gecontinueerd gedurende minstens twaalf maanden. Het voornaamste doel van de behandeling was om een symptomatische en biochemische remissie van de

ziekte te bewerkstelligen. Wij zijn van mening dat dit een voorwaarde is voor een uiteindelijke verbetering van de overleving. Eén patiënt trok zich terug uit het onderzoek wegens bijwerkingen en één patiënt overleed na zeven maanden. Bij de overige acht patiënten resulteerde de combinatie-therapie in een significante verbetering van de symptomen en daling van de gemiddelde serumspiegels van het alkalische fosfatase (maximale daling ten opzichte van de uitgangswaarde: 46%), ASAT (38%) en immuunglobulines (IgM: 42% en IgG: 37%). Echter, het hoofddoel van de studie, de inductie van een symptomatische en biochemische remissie, werd bij geen van de patiënten bereikt. Na het staken van CyA neigden de symptoom-scores, leverfuncties en immuunglobulinegehaltes terug te keren naar de uitgangswaarden. Deze veranderingen waren alleen significant voor IgG. De voornaamste bijwerking van CyA was een gemiddelde maximum stijging van de creatinine spiegels van 30%. De nefrotoxiciteit bleek grotendeels reversibel, maar bij drie patiënten daalde het creatininegehalte niet tot de uitgangswaarde na het staken van CyA. Concluderend bleek het met een combinatie van CyA en prednison niet mogelijk een symptomatische en biochemische remissie van PBC te induceren. De nefrotoxiciteit van CyA maakt dit middel ongeschikt voor langdurige behandeling van PBC.

Hoofdstuk 4. CyA is vetoplosbaar. De absorptie van CyA in de darm is dan ook afhankelijk van een normale galafvloed. Bovendien wordt CyA vrijwel geheel in de lever gemetaboliseerd en worden de metabolieten in de gal uitgescheiden. De farmacokinetiek van CyA bij patiënten met PBC zou daarom kunnen verschillen van die bij patiënten met een normale leverfunctie en galafvloed. Wij bestudeerden de farmacokinetiek van CyA bij patiënten met PBC, die zich nog niet in het eind-stadium van de ziekte bevonden. Vergeleken werden patiënten met PBC zonder voorafgaande behandeling met CyA (n=7; groep I), een controlegroep van patiënten met huidziekten (voornamelijk psoriasis; n=9; groep II) en patiënten met PBC na langdurige behandeling met CyA (n=6; groep III). CyA spiegels (moederstof en metabolieten) werden gemeten in vol bloed met een niet-specifieke (N) radioimmunoassay (RIA). Bij het merendeel van de patiënten werden CyA spiegels ook gemeten met een RIA die specifiek (S) is voor de moederstof. Er werd geen verschil in CyA absorptie gevonden tussen de patiënten met PBC en die met huidziekten. De gemiddelde waarden van de "area under the curve" over de eerste zes uur na de testdosis (AUC_{0-6}) en de maximale bloedspiegels (C_{max}) waren significant hoger voor groep

I vergeleken met groep II patiënten. Het tijdstip van de maximale bloedspiegel (t_{cmax}) was niet verschillend tussen de twee groepen. Er was een neiging tot hogere gemiddelde AUC_{0-6} en C_{max} waarden voor groep III vergeleken met groep I patiënten, waarschijnlijk als gevolg van verzadiging van het perifere compartiment. Daarentegen werden de tcmax waarden niet beïnvloed door langdurige CyA behandeling. De verhouding tussen de CyA spiegels gemeten met de niet-specifieke en specifieke RIA (N/S ratio) nam toe in de tijd, duidend op de vorming van metabolieten. Wat dit betreft waren er geen verschillen tussen de drie groepen. De gemiddelde N/S ratio van de AUC_{0-6} waarden was voor alle groepen ongeveer 2.0. Deze gegevens suggereren dat de absorptie en de biotransformatie in de lever van CyA niet verminderd zijn bij patiënten met (niet eind-stadium) PBC. Bovendien lijken beide niet beïnvloed te worden door langdurige CyA behandeling.

Hoofdstuk 5. Het doel van de in dit hoofdstuk beschreven studie was om de invloed van PBC op de botmassa in het algemeen vast te stellen en tevens het relatieve belang van het stadium van de ziekte en het gebruik van corticosteroiden voor de eventuele ontwikkeling van osteoporose. Bij 55 niet-geselecteerde vrouwelijke patiënten met een langbestaande PBC werd de botmassa gemeten met behulp van "single photon" en "dual photon" absorptiometrie. Alhoewel voor de meeste patiënten de botmassa binnen de normale spreiding lag, was de botmassa gemeten in de lumbale wervelkolom, de distale en proximale onderarm respectievelijk 8%, 8% en 5% lager dan van gezonde vrouwen van vergelijkbare leeftijd. Deze verschillen waren significant voor de lumbale wervelkolom en de distale onderarm. Met multipele regressie analyse kon worden aangetoond dat het histologisch stadium van de leverziekte een onafhankelijk determinant was van de axiale botmassa. Het gebruik van corticosteroiden resulteerde echter in een matig en niet significant verlies aan botmassa. Het gecorrigeerde serum calcium bleek significant lager te zijn bij patiënten met een histologisch laat stadium PBC, vergeleken met patiënten met een vroeg stadium. Er waren tussen deze groepen echter geen verschillen wat betreft verscheidene parameters van het botmetabolisme. Wij concludeerden dat bij onze patiënten met PBC het verlies aan botmassa slechts matig was en gerelateerd aan het histologisch stadium. Een dergelijke relatie is niet eerder gerapporteerd. Het effect van een lage dosis corticosteroiden op de botmassa leek daarentegen klinisch niet van belang.

Hoofdstuk 6. Het is aannemelijk dat patiënten met een actieve en/of progressieve PBC het meest baat kunnen hebben bij therapeutische beïnvloeding van hun ziekte. Er zijn echter geen serologische parameters om ziekteprogressie vast te stellen. Omdat de latere stadia van PBC histologisch gekenmerkt worden door een toenemende fibrose, bestudeerden wij de waarde van het serum procollageen III amino-propeptide (PIIIP) voor het voorspellen van histologische progressie. Dit propeptide wordt tijdens de vorming van type III collageen enzymatisch van het procollageen molecuul afgesplitst. Seriële PIIIP bepalingen werden verricht bij negen patiënten met een histologisch progressieve PBC en negen patiënten met een histologisch stationair vroeg stadium van de ziekte. Het histologisch beloop werd vastgesteld aan de hand van herhaalde leverbiopsieën gedurende een follow-up van maximaal dertien jaar. Bij 39% van de patiënten was het gemiddelde van de follow-up PIIIP serumspiegels verhoogd. Bovendien waren in 72% van de gevallen de PIIIP spiegels tenminste eenmaal tijdens de onderzoeksperiode verhoogd. De gemiddelde follow-up PIIIP spiegels waren niet sifnificant verschillend tussen de progressieve en niet-progressieve patiënten. Verder werd in de progressieve groep de histologische progressie niet weerspiegeld in PIIIP spiegels. Er werd geen verschil gevonden in de PIIIP spiegels corresponderend met de histologische stadia I, II en III. De individuele coëfficienten van de correlatie tussen PIIIP en biochemische variabelen (bilirubine, alkalische fosfatase, ASAT en albumine) en histologie vertoonden een grote spreiding, zonder een duidelijke neiging naar positief of negatief. Behandeling met CyA of CyA gecombineerd met prednison had geen invloed op de PIIIP spiegels. Behandeling met penicillamine gecombineerd met prednison, daarentegen, resulteerde in een significante daling van het PIIIP gehalte. Wij concludeerden dat serum PIIIP-bepalingen geen waarde hebben voor het voorspellen van histologische progressie van PBC.

Hoofdstuk 7. In dit hoofdstuk wordt een algemene beschouwing gegeven over immunosuppressieve therapie voor PBC, waarin opgenomen een voorstel voor toekomstige therapeutische studies.

DANKWOORD

Het in dit proefschrift beschreven onderzoek werd verricht op de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Dijkzigt te Rotterdam. Graag wil ik een ieder die op enigerlei wijze heeft bijgedragen aan de totstandkoming van dit proefschrift hartelijk danken. Enkelen wil ik in het bijzonder noemen.

Mijn promotor prof. dr. Solko Schalm wist mij telkens op het juiste moment weer te motiveren en bleef vertrouwen hebben in de goede afloop. Zijn kritische kanttekeningen en voorgestelde wijzigingen, hoewel niet zelden in eerste instantie aanleiding gevend tot enige frustatie, leidden zonder uitzondering tot een beter resultaat.
Ik ben hem hiervoor zeer erkentelijk.
Henk van Buuren dank ik voor zijn snelle en kritische beoordeling van enkele hoofdstukken en zijn hulp bij de bewerking van de resultaten.
Met plezier kijk ik terug op de vruchtbare samenwerking met Huib Pols en Frank van Berkum met betrekking tot het onderzoek naar de botstatus van onze PBC-patiënten.
Mijn mede-auteurs Wim van den Berg, Sjoerd de Rave en René van Zanten, dank ik voor hun waardevolle bijdragen.
Statistische adviezen kreeg ik van ir. W. Hop van de afdeling Biostatistiek en Jan Boot hielp mij met de uitwerking daarvan.
Op het laboratorium Inwendige Geneeskunde II werden de cyclosporine-bepalingen verricht door Angelo de Boer en Gardi Voortman en de procollageenpeptide-bepalingen door Rick van Leeuwen. De metingen van de minerale botmassa werden op de afdeling Nucleaire Geneeskunde verricht door Peter Kooij.
Het manuscript (met uitzondering van hoofdstuk 5) werd nauwgezet uitgetypt door Marian van Noord. De Engelse tekst (met uitzondering van hoofdstuk 5) werd gecorrigeerd door mevrouw G.P. Bieger. Willeke Beukman verleende onmisbare secretariële hulp. Mijn broer Harry verzorgde de grafische vormgeving van het proefschrift.
Prof. dr. F.T. Bosman, prof. dr. J.C. Birkenhäger en prof. J.H.P. Wilson dank ik voor hun beoordeling van het manuscript en hun bereidheid om zitting te nemen in de promotiecommissie.
Tenslotte ben ik mijn "maten" zeer erkentelijk voor het feit dat zij mijn bij de sollicitatie gedane voorspelling, dat de voltooiing van mijn proefschrift nog ruim een jaar zou vergen (het werden er uiteindelijk bijna vijf), al bij voorbaat met een korreltje zout namen.

CURRICULUM VITAE

Ruud Beukers werd op 8 juni 1954 geboren te Schiedam. Daar behaalde hij aan de scholengemeenschap Spieringshoek in 1972 het eindexamen gymnasium β. Dat zelfde jaar begon hij de studie geneeskunde aan de Erasmus Universiteit te Rotterdam; het doctoraal examen werd behaald in 1977 en het artsexamen in februari 1979.

Van maart 1979 tot maart 1982 volgde hij de opleiding tot internist in het Rode Kruis Ziekenhuis in Den Haag (opleider: Dr. J. Roos). Vanaf maart 1982 werd de opleiding voortgezet op de afdeling Interne Geneeskunde II (tevens Gastroenterologie en Hepatologie) van het Academisch Ziekenhuis Dijkzigt te Rotterdam (opleider: Prof. Dr. M. Frenkel). In het laatste jaar van de opleiding werd onder leiding van Prof. Dr. S.W. Schalm een aanvang gemaakt met het in dit proefschrift beschreven onderzoek. Na inschrijving in het specialistenregister in maart 1984 was hij tot juli 1987 werkzaam op de onderafdeling Gastroenterologie van de afdeling Interne Geneeskunde II (hoofd: M. van Blankenstein). Sinds 1 juli 1987 maakt hij deel uit van de maatschap internisten en longartsen van het Drechtsteden Ziekenhuis lokatie Refaja in Dordrecht, met speciale aandacht voor gastroenterologie en hepatologie.