Primary biliary cirrhosis: Diagnostic and therapeutic aspects

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COLOFON

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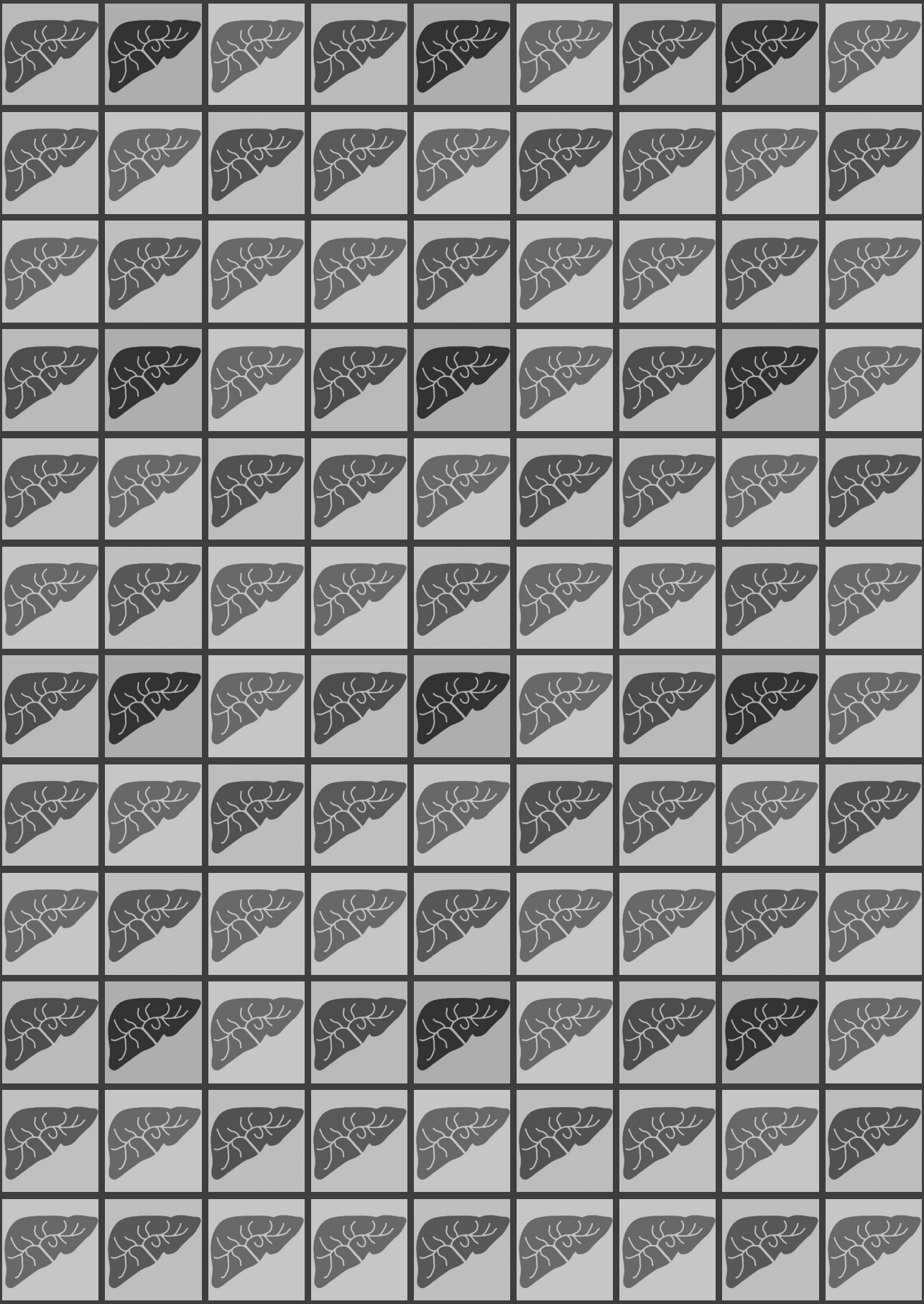

Introduction and outline of the thesis

Edith M.M. Kuiper, Jannie W. den Ouden-Muller, Henk R. van Buuren.

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INTRODUCTION

Primary Biliary Cirrhosis (PBC) is a relatively rare cholestatic liver disease. The first case was described by Addison and Gull in 1851. The name PBC is generally accepted, however in fact this is a misnomer since cirrhosis is found in a minority of patients. PBC is one of the most frequent causes of the "vanishing bile duct syndrome", characterized by cholestasis due to destruction and progressive disappearance of small intrahepatic bile ducts. Until a few decades ago no effective medical treatment was available and the prognosis for patients with symptomatic disease was poor, with reported survival less than 10 years. The pathogenesis of PBC has still not been resolved, but nowadays an effective therapy is available and this has significantly improved the prospects for patients with this condition.

EPIDEMIOLOGY

The vast majority (90%) of PBC patients is female and middle aged [2]. While the disease can occur in all ethnic and racial groups, the worldwide prevalence varies widely. In Canada and Australia the reported prevalence varies from 22 to 51 per million inhabitants whereas the disease seems much rarer in Africa and India [3]. Substantial higher prevalences were found in Spain (195/million), Finland (195/million) and Great Britain (251/million). In the north of the US the highest prevalence was described; 402 patients per million inhabitants. Based on this study the estimated number of new cases was 3500 per year [4]. In the Netherlands the incidence and prevalence are unknown. Based on the above mentioned European data the number of cases may vary from 2000 to 4000.
The incidence of PBC may be rising. It is unknown whether this can be explained by a true increase, by growing familiarity with PBC or rather by changes in diagnostic activity.

PATHOGENESIS

Based on numerous clinical, histological and immunological characteristics PBC is classified as an auto-immune disease. The presence of disease specific auto-antibodies and an elevated incidence of associated diseases like Sjögren's syndrome, CREST syndrome and celiac disease are in agreement with this hypothesis. The

prevailing theory is that environmental substances or micro-organisms trigger an inadequate immune response against the epithelium of the bile ducts in genetically predisposed people. Many potential triggers have been described, including one of the chemical constituents of hair dye or nail polish, cigarette smoke, exogenous estrogens and infection with E. Coli, Chlamydia, mycobacterium gordonae or retroviruses [5]. However, none of these factors has been proven to be causative. The relatively high incidence of PBC among first degree relatives suggests a genetic component but only a weak association has been described with certain HLA haplotypes like HLA-DR8 [6].

Almost pathognomomic for PBC is the presence of antimitochondrial antibodies (AMA), which can be detected in up to 95% of patients. These antibodies are highly specific, however a role in the pathogenesis seems unlikely. AMA are antibodies directed against antigens on the inner membrane of mitochondria. The majority of the antibodies react against the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2). This antigen is recognized by autoreactive T-cells in the liver, present around bile ducts. It is unknown how PDC-E2 and other mitochondrial antigens activate the immunological response that ultimately leads to destruction of cholangiocytes and disappearance of bile ducts. Destruction of bile ducts causes cholestasis (stasis of bile). Superimposed on immunologically mediated damage accumulation of cytotoxic bile contributes to the necro-inflammatory process which is further augmented by increased hepatocellular HLA-expression. Prieto et al. showed that the activity of the chloride-bicarbonate anion exchangers, the transporter which regulates the bicarbonate secretion in the bile ducts, is decreased in PBC patients [7]. Together with the decrease in receptor activity both immunological and serological features characteristic for PBC manifest, like AMA-positivity [8].

Histology shows cholangitis in the portal triads, sometimes accompanied by granulomas and a variable increase in the number of lymphocytes and plasma cells. In more advanced stages progressive fibrosis develops, finally leading to cirrhosis, associated with severe ductopenia, intrahepatic bile stasis and cupper accumulation. Sometimes severe ductopenia is present in early stages, when no or minimal fibrosis is present [9].

CLINICAL FEATURES AND NATURAL HISTORY

Currently, the majority of PBC patients (50-60%) are asymptomatic at the time of diagnosis [10]. Most frequent symptoms are fatigue and pruritus, less frequent are aspecific arthritis or arthralgia, upper quadrant abdominal pain and dry eyes and mouth (Sjögren's syndrome). The severity of symptoms is not related to severity of disease. However in advanced stages additional symptoms like icterus, xanthelasmata, ascites, edema, variceal bleeding, encephalopathy, vitamin deficiencies, osteoporotic vertebra fractures and malnutrition may occur. Despite a high prevalence of hyperlipoproteinemia (especially hypercholesterolemia) in PBC patients, an increased cardiovascular risk has not been documented [11]. Hypercholesterolaemia is related to an increased lipoprotein X, a LDL-particle whereas HDL-cholesterol levels are usually normal or elevated.
The incidence of hepatocellular carcinoma is increased, although the risk is not as high as in viral hepatitis. The most frequently used prognostic model to predict survival is the Mayo risk model, including age, bilirubin- and albumin level, protrombin time and presence of edema [12]. Reported median survival in earlier reports was 10-16 yr for symptom-free patients and 7 years for PBC patients with symptoms [13]. Nowadays the prognosis is better, possibly thanks to early diagnosis and effective treatment.

DIAGNOSIS

PBC should be considered in middle-aged women presenting with fatigue, pruritus, arthralgia and/or cholestasis. According to the EASL practice guidelines PBC can be diagnosed in adult patients with otherwise unexplained elevation of alkaline phosphatase and presence of AMA (titer 1:40) and/or AMA type M2. A liver biopsy is not essential for diagnosis but allows activity and stage to be assessed. Liver biopsy is particularly important when PBC is considered in the absence of AMA, or when there is possible concurrent autoimmune hepatitis [14].
In the majority of PBC patients, histology is characteristic [15]. IgM levels are frequently elevated, a characteristic laboratory feature that is diagnostically helpful in atypical cases. In 30% of patients antinuclear antibodies (ANA) are present, especially the diagnostic specificity of anti-sp100 and anti-gp210 subtypes is high [16]. However, these tests cannot be performed in the majority of Dutch laboratories.

5-10% of PBC patients have an overlap syndrome with autoimmune hepatitis (AIH). Recognition of this overlap syndrome (OS) is important since treatment with corticosteroids improves prognosis significantly. The diagnosis of AIH-OS should be considered if AST levels are >5 times the upper limit of normal (ULN), if antibodies (ANA, anti smooth muscle) are present and if IgG levels are elevated (1.5-3 times ULN) [17].

TREATMENT

Despite the autoimmune character of the disease, the response to immunosuppressive therapy is disappointing. Until now ursodeoxycholic acid (UDCA) is the only registered and accepted treatment. The standard dose is 13-15mg/kg/day [18]. The efficacy of a single dose UDCA is comparable to multiple-dosing. UDCA is a hydrophilic bile acid that is naturally present in human bile. Side effects are (virtually) absent. The working mechanism has not completely been elucidated but several potential beneficial effects have been described: UDCA may have immune-modulating, cytoprotective and anti-apoptotic effects, lowers hydrophobic bile acid levels and thereby toxicity of bile and stimulates impaired biliary secretion.
Despite the consensus that UDCA improves biochemical and histological features, some discussion regarding the potential of UDCA to alter the natural course of the disease remains. The number of patients in most RCT's has been too small, and the duration too short, to draw definitive conclusions. Also, considerable variation in population, treatment period and doses among several trials has contributed to scientific controversy and uncertainty with respect to the true efficacy of UDCA therapy.
The results of meta-analyses of randomized controlled trials are contradictory [19; 20]. The only meta-analysis of individual patient data from the largest trials, that also used adequate UDCA doses, demonstrated improved transplantation-free survival for UDCA treated patients [19]. Also, the results of several prospective long-term cohort studies suggest that UDCA constitutes an effective therapy [21; 22].
Unfortunately, UDCA is not very effective in alleviating symptoms of the disease. For cholestatic pruritus several treatment options are available including anion exchange resins (cholestyramine, colesevelam), rifampicin, naltrexone and sertraline [23]. Antihistaminics are not effective.

Chronic fatigue is an important clinical problem. There is no evidence that fatigue is related to depression. Also, antidepressive drugs are ineffective [24; 25]. An interesting new option is modafinil, an agent registered for the treatment of narcolepsy [26]. However, the positive effects observed in an open label study await confirmation by controlled studies. Treatment with UDCA decreases cholesterol levels. If high cholesterol levels persist despite UDCA treatment, treatment should be considered if additional cardiovascular risk factors are present.

SURVEILLANCE AND LIVER TRANSPLANTATION

The AASLD recommends surveillance for HCC in PBC patients if cirrhosis is present and the annual risk is at least 1.5%. Transplantation should be considered in patients with progressive liver failure, as indicated by Child-Pugh scores ≥7, presence of ascites, variceal bleeding or encephalopathy. After transplantation PBC may recur but this rarely causes significant clinical problems. The prognosis following transplantation is good with reported 5-year-survival rates of about 80%.

AIMS OF THE THESIS

Ursodeoxycholic acid (UDCA) is the only established and accepted medical treatment for PBC [27]. Treatment has been claimed to decrease disease activity and prevent progression to more advanced stages, particularly when therapy is instituted early [28; 29]. In contrast, a favorable effect in advanced disease has not been well documented. A number of studies have defined a potential correlation between the magnitude of biochemical response to UDCA treatment and the evolution of the disease. In a large cohort study, Pares et al. found that a decrease of ALP levels greater than 40% from baseline values, or a normal level, after 1 year of treatment was associated with improved prognosis [21]. Corpechot et al. proposed alternative criteria for biochemical response, based on bilirubin, AST and ALP levels [22].
We assessed the long-term prognosis of patients with PBC and evaluated the prognostic significance of biochemical response to UDCA treatment, according to the previously proposed criteria and according to changes in baseline bilirubin and albumin, in a population of prospectively followed PBC patients **(chapter 2)**. The beneficial effect of UDCA on laboratory parameters of cholestasis and inflammation, including serum bilirubin, alkaline phosphatase, AST and ALT has been well

established as well as the lowering effect on serum IgM levels. Since the evolution of laboratory parameters beyond 6 years UDCA therapy has not been reported it is uncertain whether the initial beneficial effects are maintained during more prolonged follow-up. Therefore, we evaluated the long term evolution of liver biochemical and immunological variables in PBC patients treated with UDCA **(chapter 3)**.

In PBC patients with non-advanced disease who are treated with ursodeoxycholic acid survival appears to be comparable to the general population and causes of death are mainly non-liver related [30]. The overall incidence of hepatocellular carcinoma (HCC) among PBC patients varies from 0.7% to 3.8% [31; 32] while an incidence of up to 14% has been reported for those with cirrhotic disease [32]. Several risk factors for the development of HCC have been reported, including histological stage, gender, age, presence of portal hypertension and history of blood transfusion [33; 34]. Ideally, surveillance programs should be limited to those patients carrying a significant risk for HCC and these patients should be easily identifiable to prevent burden of medical resources. Also, tumours should be detected at an early stage. Today, surveillance for HCC is solely recommended for PBC patients with cirrhosis [35]. This strategy, however, has inherent disadvantages, the most important being the necessity of repeated diagnostic procedures, such as liver biopsy, in the majority of cases who present with non-cirrhotic disease. Further, liver biopsy is associated with sampling error and interpretation difficulties. We aimed to evaluate the epidemiology of PBC related mortality focussing on HCC in a large cohort of prospectively followed patients and to define early risk factors for the development of HCC **(chapter 4)**.

In the past PBC was one of the main indications for liver transplantation (LTX), whereas nowadays, the proportion of patients receiving a transplant for PBC has decreased to around 10% [36-38]. This could possibly be due to an increase in the number of patients transplanted for other indications, to a decrease in the need for transplantation in PBC or to a combination of these factors. A decrease in need for LTX in PBC could correspond with improved transplantation-free survival for UDCA treated PBC patients, particularly for those with a favorable biochemical response upon treatment. The impression exists that in addition to a proportional decrease in the need for LTX the absolute number of patients with PBC receiving transplantation over time is gradually falling. However, detailed reports on time trends in the absolute number of LTX for PBC are sparse [36; 38]. We studied transplant patterns for PBC and changes over the past 20 years in the Netherlands **(chapter 5)**.

PBC and autoimmune hepatitis (AIH) are immune-mediated chronic liver diseases with clear differences in clinical, biochemical, serological and histological features [39; 40]. Mainly due to the high diagnostic sensitivity and specificity of antimitochondrial antibodies (AMA) the diagnosis of PBC is usually straightforward. Although AIH can be diagnosed without much difficulty in patients with classical presentation, in clinical practice the diagnosis is frequently difficult. PBC and AIH can occur simultaneously [41; 42] or consecutively [43; 44]. Up till now standardization of diagnostic criteria for overlap syndrome has not been achieved and the long-term prognosis of the syndrome is poorly defined [45]. Several problems are encountered in diagnosing patients with possible overlap syndromes. These include features of AIH being found in many patients with PBC, such as (mild) interface hepatitis, elevated IgG serum levels and presence of anti-smooth muscle antibodies. Also, patients with AIH may have features of PBC such as biliary abnormalities, elevated serum IgM levels and AMA-positivity. Chazouillères et al. proposed detailed diagnostic criteria for the PBC-AIH overlap syndrome (Paris criteria) [42], but these criteria had never been validated. For AIH diagnostic numerical scoring systems have been developed by the International Autoimmune Hepatitis Group [46-50], but these scoring systems were not designed for diagnosing AIH in patients with PBC. This is illustrated by the fact that presence of AMA or biliary changes on liver biopsy have a negative impact on the diagnostic scores. Recently simplified criteria for AIH were proposed, and in this scoring system presence of AMA or histological features of PBC are no longer taken into account [51]. We aimed to evaluate the value and accuracy of the Paris criteria and of the revised and simplified autoimmune hepatitis scoring systems in the diagnosis of the PBC-AIH overlap syndrome. A further aim was to assess the long-term prognosis of patients with this syndrome **(chapter 6)**.

Pruritus is a frequent and debilitating symptom in cholestatic liver disease [52]. Although the pathophysiology of pruritus secondary to cholestasis remains largely unknown it is widely assumed that bile acids are etiologically involved [20; 53]. The principal pharmacological treatment options currently available and recommended in recent guidelines [13] are cholestyramine [54; 55] (a non-absorbable bile-acid binding resin), rifampicin [56; 57], naltrexone [58; 59] and sertraline [60]. However, the efficacy of these drugs is variable and side-effects are common. Therefore, treatment of cholestatic pruritus is currently often problematic and unsatisfactory and alternative treatment options are warranted.

Colesevelam (Cholestagel®) is a bile acid sequestrant taken in tablet form that hydrates to a gel and is being used for the treatment of hypercholesterolemia. This agent differs from other sequestrants in that the hydrophilic polymer backbone has abundant hydrophobic side chains facilitating the binding of bile acids. Also, it is better tolerated than other bile acid sequestrants [61; 62]. Until now, efficacy of colesevelam for ameliorating the pruritus of cholestasis was only explored in a small open study [63], which showed that colesevelam was effective in ameliorating pruritus in 5 out of 8 patients. We decided to assess the effect of colesevelam on cholestatic pruritus in a double-blind randomized placebo-controlled trial **(chapter 7)**.

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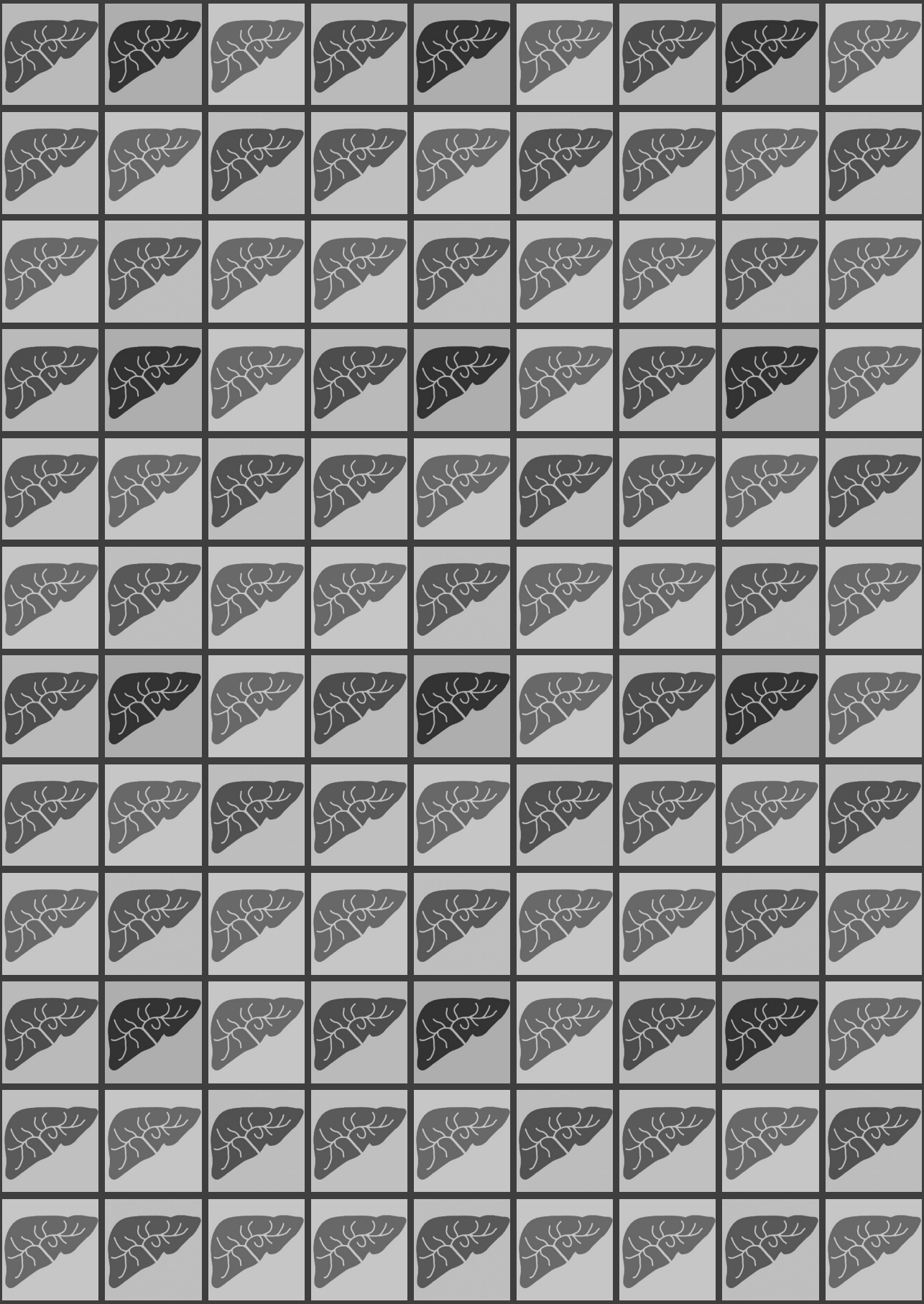

Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid

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ABSTRACT

Background and Aims

Ursodeoxycholic Acid (UDCA) improves laboratory liver tests in patients with Primary Biliary Cirrhosis (PBC). Few studies have assessed prognostic significance of biochemical data collected following UDCA treatment. We performed a prospective, multicenter study of PBC patients treated with UDCA to compare prognosis with biochemical response.

Patients and Methods

PBC was classified as early (pre-treatment bilirubin and albumin levels normal), moderately advanced (one abnormal) or advanced (both abnormal). Biochemical response was defined as proposed by Pares (decrease in alkaline phosphatase (ALP) levels >40% of baseline or normal levels), Corpechot (CO: ALP<3-fold the upper limit of normal (ULN); aspartate aminotransferase levels <2-fold the ULN, bilirubin<1-fold the ULN) and our group (RD: normalization of abnormal bilirubin and/or albumin).

Results

The study included 375 patients and median follow-up time was 9.7 (1.0–17.3) years. The prognosis for early PBC was comparable with that of the Dutch population and better than predicted by Mayo risk score. Survival of responders was better than of non-responders, according to CO and RD criteria ($p<0.001$). Prognosis of early PBC was comparable for responders and non-responders; prognosis of responders was significantly better in those with (moderately) advanced disease.

Conclusions

Prognosis for UDCA-treated patients with early PBC is comparable to that of the general population. Survival of those with advanced PBC with biochemical response to UDCA is significantly better than for non-responders. Thus, UDCA may be of benefit irrespective of the stage of disease. Prognostic information, based on bilirubin and albumin levels, is superior to that provided by ALP levels.

INTRODUCTION

Ursodeoxycholic acid (UDCA) is the only accepted medical treatment for primary biliary cirrhosis (PBC) [1]. UDCA has consistent and significant, albeit variable, effects on liver biochemical and immunological parameters of PBC [2-5]: Treatment has been claimed to decrease disease activity and prevent progression to more advanced stages, particularly when therapy is instituted early [6; 7]. In contrast, a favorable effect in advanced disease has not been well documented.

A number of studies have defined a potential correlation between the magnitude of biochemical response to UDCA treatment and the evolution of the disease. Angulo et al reported a more favorable prognosis for responders, defined as patients with a Mayo Risk Score <4.5 or alkaline phosphatase < 2-fold the upper limit of normal after 6 months of treatment [8]. In a large cohort study, Pares et al. found that a decrease of ALP levels greater than 40% from baseline values, or a normal level, after 1 year of treatment was associated with improved prognosis [9]. Corpechot et al. proposed alternative criteria for biochemical response, based on bilirubin, AST and ALP levels [10]. In a preceding study we found that serum bilirubin and albumin are independent predictors of survival in PBC patients treated long-term with UDCA [11]. This study did not evaluate possible changes in prognostic status related to treatment-induced alterations of these laboratory parameters.

The aim of this study was to assess the long-term prognosis of patients with PBC treated with UDCA and to analyze the correlation with laboratory findings following treatment. More specific, we wanted to evaluate the prognostic significance of biochemical response to UDCA treatment, according to previously proposed criteria and according to changes in baseline bilirubin and albumin, in a population of prospectively followed PBC patients.

PATIENTS AND METHODS

The Dutch Multicenter PBC Study is a prospective cohort study of patients with PBC with participation of 7 university and 39 general hospitals. Inclusion started in January 1990 and follow-up data until April 2007 were analyzed. A definite diagnosis of PBC was made in the presence of 2 major plus 2 minor criteria or 1 major plus 4 minor criteria. Major criteria were AMA titer ≥1:20 and compatible liver biopsy, minor criteria were pruritus, jaundice, ALP ≥2-fold the upper

limit of normal, serum IgM >2.8g/l and positive Schirmer test [12]. Available liver biopsies were reviewed according to Ludwig et al [13]. Exclusion criteria were age > 75, pregnancy, evidence of extra hepatic biliary disease, autoimmune-overlap syndrome or use of immunosuppressive drugs, concomitant disorders limiting life expectancy and decompensated PBC, defined as Child-Pugh class B or C cirrhosis. Following inclusion, patients started with UDCA therapy 13 - 15 mg/kg/day [5; 14]. Follow-up data were collected at 3-month intervals in the first year and at yearly intervals thereafter. At each visit general physical examination and measurement of bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, IgG, IgM and total cholesterol was performed. Liver tests were reported as the ratio of the test result to the upper or lower limit of normal (ULN, LLN) for the laboratory performing the test.

Definitions and statistical analysis

Based on serum bilirubin and albumin values at entry the disease was classified as early (both bilirubin and albumin normal), moderately advanced (one parameter normal) and advanced (both parameters abnormal) [11].

Survival was analyzed as transplantation-free survival (end-points: liver transplantation and death) and survival free of liver-related death or transplantation (end-points liver-related death and transplantation; censored non-liver related death). Liver related death was defined as: death because of liver failure or hepatocellular carcinoma or death occurring within 2 months of an episode of variceal bleeding, spontaneous bacterial peritonitis, hepatorenal syndrome, or hepatic encephalopathy. Patients lost to follow-up were censored at the time of their last visit. For time-to-event analysis we applied the Kaplan-Meier method and compared groups using the log-rank test. Observed survival was compared with expected survival from matched gender-, age- and calendar time-specific death rates for the general Dutch population using life table method and Wilcoxon (Gehan) test. A test for a single proportion with continuity correction was used to evaluate differences between observed survival and survival predicted by the Mayo model (MRS) [15]. Biochemical response to UDCA was evaluated 1 year after start of treatment and subsequently at any moment in time according to 3 definitions: 1: criteria proposed by Corpechot et al. (CO) : ALP <3 x the upper limit of normal (ULN) and AST <2 x ULN and bilirubin <1 x ULN, 2: Pares criteria (PA): ALP decrease >40% of baseline values or normal levels [9; 10], 3: we analyzed the prognostic significance

of alterations in bilirubin and albumin concentrations. Biochemical response was defined by normal bilirubin and albumin concentrations after treatment with UDCA when one or both parameters were abnormal before treatment, or as normal bilirubin or albumin concentration after treatment when both were abnormal at entry. These criteria are further referred to as Rotterdam (RD) criteria. Serum bilirubin and/or albumin concentrations were not available for 21 patients at baseline and 33 patients at 1 year. In 10 additional patients either AST or ALP was absent. The remaining 311 patients were comparable to the total group regarding patient characteristics and number of events. Patients with and without biochemical response at 1 year were compared using parametric and non-parametric tests. Univariate and multivariate Cox-regression analyses were performed at baseline for multiple variables and at 1 year to compare CO, PA and RD criteria. Furthermore to evaluate biochemical response at any moment in time according to CO, PA and RD criteria Cox-regression analysis with time-dependent variable was used. A p-value <0.05 (two-tailed) was considered statistically significant. Statistical package SPSS 15.0 was used to perform analyses.

RESULTS

Patients

The baseline patient characteristics are summarized in Table 1. The study population comprised 375 (89% female) patients, with a mean age of 54.7 years, who were followed for a median period of 9.7 (range 1-17.3) years. Following inclusion, the total number of study visits with collection of laboratory and clinical data was 4776, the average number of data collections was 9 per patient. Sixteen (4%) patients were lost to follow-up. The majority of patients (60%) suffered from early PBC. Regarding histology, 65% of available liver biopsies were classified Ludwig stage I or II disease.

Survival

The total number of events was 91; 69 (18%) patients died, 22 (6%) patients underwent liver transplantation. Thirty-four events occurred in early PBC, 35 and 22 in moderately advanced and advanced PBC, resp. Causes of death were mainly non-liver related for patients with early PBC: in this subgroup only 18% (6/34) died from liver related causes or underwent liver transplantation. Liver related death and

Table 1. Baseline patient characteristics (n=375)

Age (years; mean ± SD)	54.7 ± 10.9
Sex (male/female)	40 (11%)/335 (89%)
AMA +	350 (93%)
ALP (ULN, range)	2.6 (0.4-13.7)
AST (ULN, range)	1.6 (0.3-7.4)
ALT (ULN, range)	1.8 (0.2-12.1)
Bilirubin (ULN, range)	0.7 (0.1-5.6)
Albumin (LLN, range)	1.1 (0.3-7.2)
IgM (ULN, range)	1.9 (0.2-17.5)
IgG (ULN, range)	1.0 (0.1-2.9)
MRS (mean ± SD)	4.1 ± 1.0
Histological stage*	
I	69 (18%)
II	92 (25%)
III	52 (14%)
IV	34 (9%)
Not available	128 (34%)
Prognostic class	
Early PBC	225 (60%)
Moderately advanced PBC	95 (25%)
Advanced PBC	34 (9%)
Not available	21 (6%)

* Liver biopsy within 1 year of entry and/or established cirrhosis.
ULN: ratio test result to upper limit of normal range
LLN: ratio test result to lower limit of normal range
MRS: Mayo Risk Score

liver transplantation occurred relatively more often in moderately advanced and advanced PBC: 37% (13/35) and 91% (20/22), resp.

Transplantation-free survival was 100% after 1 yr, 90% after 5 yr, 78% after 10 yr and 67% after 15 yr (Figure 1). Transplantation-free survival for early PBC (n=225) was 97% after 5 yr, 88% after 10 yr and 75% after 15 yr, for moderately advanced PBC (n=95) this was 85%, 66% and 58% resp. and for advanced PBC (n=34) 55%, 34% and 25% resp. Survival free of liver-related death or transplantation was 99.7% after 1 yr, 93% after 5 yr, 88% after 10 yr and 84% after 15 yr. Survival free of liver-related death or transplantation for early PBC was 99%, 97% and 96% for 5-, 10-, and 15-yr-survival, resp. For moderately advanced PBC this was 89%, 79% and 72%, for advanced PBC 55%, 40% and 30%. Overall survival was significantly decreased

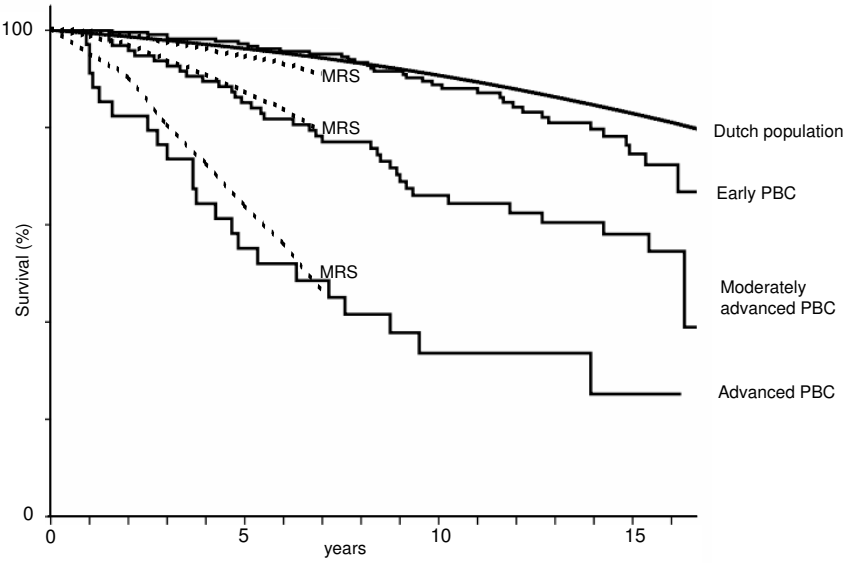

Figure 1. Survival of early, moderately advanced and advanced PBC compared to a sample of the Dutch population, matched for gender and age. Observed prognosis is compared to prognosis predicted by the Mayo Risk Score (MRS). Survival for early PBC was comparable to survival of the Dutch population (p=0.254), survival for (moderately) advanced PBC was significantly worse (p<0.001). For early PBC, observed survival was better than predicted survival (p<0.001), in moderately advanced PBC and advanced PBC the MRS predicted survival adequately (p=0.90 and p=0.23, resp).

compared with survival of a sample of the standardized Dutch population (p<0.001). However, survival for early PBC (normal bilirubin and albumin at baseline) was comparable with survival of the general population (p=0.254). Observed survival for early PBC was significantly improved compared with survival without UDCA therapy as predicted by MRS (p<0.001). For patients with (moderately) advanced disease survival was significantly decreased compared with survival of the general population (p<0.001) and adequately predicted by MRS (p=0.90 and p=0.23) (Figure 1).

Prognostic significance of biochemical response to UDCA

CO and PA criteria

Prognosis of patients treated with UDCA was evaluated according to the results of laboratory liver tests after one year and at subsequent visits. This analysis included 311 patients since entry and/or 1-year results of either bilirubin, albumin, ALP or

Table 2. Proportion responders before and after 1 year treatment with UDCA both for the total cohort and for different prognostic classes

		pre-treatment	After 1 year
	criteria	n (%)	n (%)
Total cohort	CO	120 (39)	206 (66)
(n=311)	PA	21 (7)	192 (62)
	RD		236 (76)
Early PBC	CO	10 (57)	159 (83)
(n=191)	PA	18 (9)	128 (67)
	RD		179 (94)
Moderately advanced PBC	CO	12 (14)	39 (45)
(n= 87)	PA	3 (3)	48 (55)
	RD		40 (46)
Advanced PBC	CO	0 (0)	8 (24)
(n= 33)	PA	0 (0)	16(48)
	RD		17 (52)

AST were not available for 64/375 (17%) patients. 15-yr survival for this group of patients was: 72% for early PBC, 61% for moderately advanced PBC and 23% for advanced PBC and comparable to the total cohort (75%, 58% and 25% resp). At entry (before treatment with UDCA) 120/311 (39%) patients already met the CO criteria for biochemical response and 21 (7%) met the PA criteria. After 1 year treatment, the number of patients with biochemical response according to CO increased to 206 (66%) and according to PA to 192 (62%) (Table 2). Considering laboratory liver tests and liver histology, patients with biochemical response according to CO criteria had less severe disease at baseline than patients not responding. Using PA criteria, patients with and without response were highly comparable at entry (table 3). Transplantation-free survival following one year was better for responders to treatment, according to both CO and PA criteria ($p<0.001$ and $p=0.052$ resp) (Figure 2).

For patients with early PBC, survival for responders and non-responders was comparable (CO: $p=0.35$; PA: $p=0.96$). In moderately advanced and advanced PBC differences between responders and non-responders were significant, in favor of responders, using CO criteria but not when using PA criteria (Figure 2).

In moderately advanced PBC, survival for responders using CO criteria was 79% after 10 years and 75% after 15 years. For non-responders this was 57% and 48%,

Table 3. Baseline characteristics of patients with and without biochemical response (according to Corpechot, Pares and Rotterdam criteria) following 1 year treatment with UDCA

	Response acc. to Corpechot		Response acc. to Pares		Response acc. to Rotterdam	
	Yes (n=206)	**No** (n=105)	**Yes** (n=192)	**No** (n=119)	**Yes** (n=236)	**No** (n=75)
Age (years; mean ± SD)	56±11	52±11	55±11	55±11	55±11	55±11
ALP (ULN/range)	2.3 (0.4-8.3)‡	4.2 (0.8-13.7)	2.6 (0.4-13.7)	2.7 (0.7-12.2)	2.5 (0.4-13.7)‡	3.0 (0.9-13.0)
AST (ULN/range)	1.3 (0.3-6.7)‡	2.2 (0.8-7.4)	1.6 (0.3-7.1)	1.7 (0.3-7.4)	1.5 (0.3-6.7)‡	2.2 (0.4-7.4)
ALT (ULN/range)	1.6 (0.2-10.1)‡	2.6 (0.6-12.1)	1.9 (0.2-10.0)	1.8 (0.3-12.1)	1.8 (0.2-12.1)	2.1 (0.3-8.1)
Bilirubin (ULN/range)	0.6 (0.1-2.9)‡	1.1 (0.3-5.3)	0.7 (0.1-5.6)	0.7 (0.1-5.2)	0.6 (0.1-4.4)‡	1.3 (0.2-5.6)
Albumin (LLN/range)	1.1 (0.3-1.5)‡	1.1 (0.5-1.4)	1.1 (0.3-1.5)	1.1 (0.7-1.5)	1.2 (0.5-1.5)‡	1.0 (0.3-1.5)
MRS (mean ± SD)	3.8±0.8‡	4.7±1.3	4.0±1.0‡	4.2±1.2	3.8±0.9‡	4.9±1.2
Histological stage*						
I	44 (22%)	8 (8%)	34 (18%)	18 (15%)	46 (19%)	6 (8%)
II	46 (22%)	31 (29%)	44 (23%)	33 (27%)	66 (28%)	11 (15%)
III	28 (14%)	20 (19%)	32 (16%)	16 (13%)	32 (14%)	16 (21%)
IV	13 (6%)	20 (19%)	17 (9%)	16 (13%)	15 (6%)	18 (24%)
not available	75 (36%)	26 (25%)	65 (34%)	36 (30%)	77 (33%)	24 (32%)
Prognostic class PBC						
Early	159 (77%)	32 (30%)	128 (67%)	63 (53%)	179 (76%)	12 (16%)
Moderately advanced	39 (19%)	48 (46%)	48 (25%)	39 (33%)	40 (17%)	47 (63%)
Advanced	8 (4%)	25 (24%)	16 (8%)	17 (14%)	17 (7%)	16 (21%)

* Data for patients with established cirrhosis and/or with liver biopsy within 1 year of entry
‡ Statistical significant difference p>0.05 between responders and non-responders
ULN: ratio test result to upper limit of normal range
LLN: ratio test result to lower limit of normal range
MRS: Mayo Risk score

resp (p= 0.015). In advanced PBC, survival was 63% after 10 and 15 years for responders and 20% and 14% for non-responders (p=0.027).

Serum bilirubin and albumin (RD) criteria

After 1 year the RD response criteria were met in 76% of patients (Table 2). Responders differed significantly from non-responders and had lower pre-treatment bilirubin, ALP, AST and Mayo risk scores and higher albumin levels (Table 3). Survival of patients responding to UDCA treatment according to RD criteria was significantly

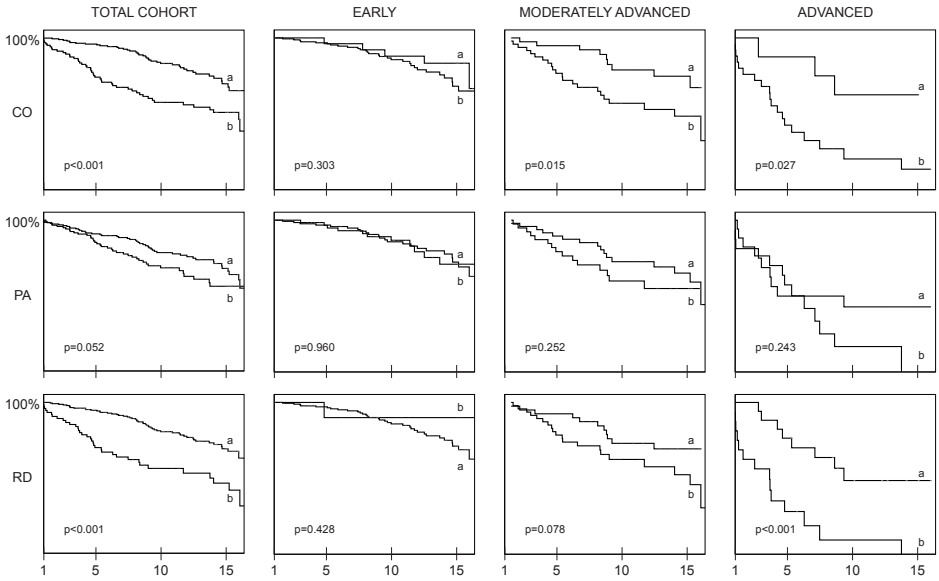

Figure 2. Kaplan-Meier plots of survival free of transplantation for UDCA-treated PBC patients according to biochemical response at 1 year defined by Corpechot (CO), Pares (PA) and Rotterdam (RD) criteria. Analysis starts after 1 year treatment with UDCA. Responders: a, non-responders: b.

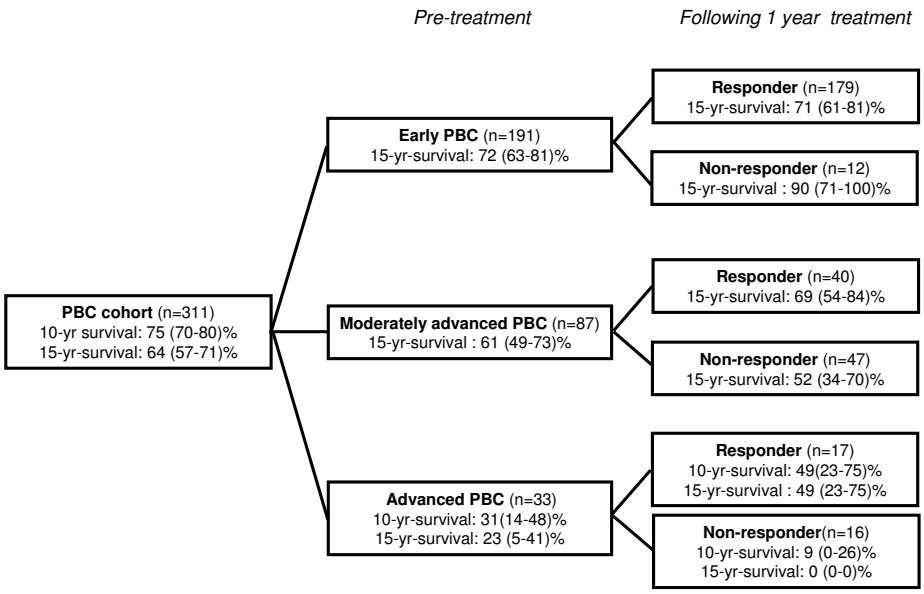

Figure 3. Survival (%(CI)) for different prognostic classes before treatment and for responders and non-responders (according to Rotterdam criteria) following 1 year treatment with UDCA.

better than for patients not responding (p<0.001) (Figure 2). Following treatment with UDCA during 1 year, transplantation-free survival for responders (n=236) and non-responders (n=75) after 5 year was 95% and 70%, after 10 year 81% and 56% and after 15 year 69% and 47%, respectively. The majority of patients had normal bilirubin and albumin levels at entry and following 1 year UDCA treatment (responders in early PBC). Survival for these patients, calculated at entry and after 1 year, was comparable (Figure 3). For patients with moderately advanced PBC (n=87) changes in prognostic status after treatment were observed. After 1 year UDCA treatment 40 of these patients fulfilled criteria of response, observed 15-yr survival for this group was 69%. This was significantly better compared to non-responders (52%) and comparable to survival for the early PBC group (71%). Similarly, prognostic status of 17 of 33 patients with advanced PBC clearly improved following UDCA treatment while prognosis for patients without response was worse compared to prognosis before treatment. Further analysis showed that biochemical response occurring after more prolonged follow-up, irrespective of the time interval from entry, was similarly associated with improved prognosis.

Univariate and multivariate analysis of prognostic factors at entry

In order to determine factors influencing survival univariate analysis was performed concerning the following baseline factors: age, sex, weight, AMA, total serum bilirubin, serum albumin, ALP, ALT, AST, AST/ALT, platelet count, serum cholesterol, IgG, IgM, the presence or absence of cirrhosis and prognostic class (early/moderately advanced/advanced PBC). Bilirubin, albumin, AST, AST/ALT ratio, platelet count, cirrhosis and prognostic class were all significantly associated with transplantation-free survival and survival free of liver-related death or transplantation. In addition, age was significantly associated with transplantation-free survival, sex and ALT with survival free of liver-related death (Table 2). In multivariate analysis elevated bilirubin (≥1ULN), decreased albumin (≤1LLN), age, cirrhosis and prognostic class before start of UDCA were independent risk factors significantly (p<0.001) associated with transplantation-free survival. In non-liver related death only age at entry was significantly associated with survival.

Univariate and multivariate analysis of prognostic factors after 1 year

In univariate analysis CO, RD and age were significantly associated with transplantation-free survival (p<0.001). PA was not significantly associated (p=0.056), but was

also included in the multivariate analysis. In the multivariate analysis CO ($p<0.001$), RD ($p=0.03$) and age ($p<0.001$) remained statistically significant. CO is of additional value to RD and vice versa.

DISCUSSION

The results of this study show that biochemical improvements in serum bilirubin and albumin following treatment with UDCA are associated with improved prognosis for patients with PBC. More specifically, our data suggest that long-term prognosis, irrespective of the severity of the disease, improves if UDCA treatment results in normalization of previously abnormal bilirubin and/or albumin concentrations.

Bilirubin and albumin concentrations are often normal in newly diagnosed PBC. In the present study levels were normal in 60% of patients (early PBC), and this a priori prevented occurrence of treatment response in terms of further improvement. This implies that the term 'biochemical response' as used in the context of this study is arbitrary and could suggest that bilirubin and albumin are less suitable for assessing response to treatment. In contrast, ALP levels were normal in only 7% of patients. The overall prognostic usefulness of bilirubin and albumin as response criteria, however, was clearly superior to using ALP, as indicated by the results of uni- and multivariate analysis. Obviously, this finding awaits confirmation in an independent cohort study.

This study confirms previous reports that prognosis in UDCA treated PBC is also predicted by ALP and by levels of ALP, AST and bilirubin after 1 year of treatment [8; 9]. In the present study the prognostic information provided by (changes in) bilirubin, albumin and AST levels was superior to that provided by ALP. Our data are highly comparable to those of Pares et al with respect to the number of patients with normal ALP before treatment (7% vs. 8%), the percentage responders (62% vs. 60%) and the finding that survival was significantly better for responders. The difference in survival between responders and non-responders in our study, however, was markedly less than reported in the Spanish study. In an independent large sample of PBC patients, this study also confirms the usefulness and prognostic significance of the biochemical response criteria developed by Corpechot et al. When we applied these criteria, the 1-year response rate to UDCA in our cohort was 66% versus 61% in the French cohort, the 10-year survival for responders was 79% versus 90% and for non-responders 57% versus 51%. The differences between our results and

those reported by the Spanish and French groups can possibly be explained by differences in patient populations. In particular, regarding liver histological stage, albumin, bilirubin and Mayo risk score, patients in our cohort seemed to have more advanced disease than those included in the Spanish cohort and somewhat less advanced disease than those in the French cohort. In our population of PBC patients predicting prognosis using the biochemical response criteria of Corpechot et al. was at least as reliable as using the Rotterdam response criteria.

However, since the Rotterdam model is easier to apply we would prefer to use these criteria.The prospective character of our study, the size of the study population and the prolonged duration of follow-up are factors likely to contribute to the reliability of the findings. We also included non-selected patients i.e. all cases diagnosed and followed in a large number of general and university hospitals. Therefore, the study sample is probably representative for the total population of PBC patients in our country. Difficulties included missing study variables, especially bilirubin, albumin, AST and ALP concentrations were not available for 17% of patients, and a 4% drop-out of patients. It seems unlikely, however, that these shortcomings have influenced the results in a major way, since the remaining 311 patients were highly comparable to the total group regarding baseline characteristics and relative number of events.

Additionally to Pares and Corpechot Criteria, it would have been interesting to calculate the Mayo Risk Score 6 months after start of therapy to define responders and non-responders as suggested by Angulo et al [8]. However, our data-set with respect to the prothrombin time and edema status at that time was incomplete and precluded calculation of the Mayo risk score.

Estimating prognosis in PBC is of key importance in managing and counseling patients. The present study shows that treatment induced changes may help to fine-tune predictions regarding the course of the disease, enabling selection of individuals with unfavorable prognosis for alternative or additional therapy. At present, however, no established second line treatment has been defined for PBC and further studies are indicated to further explore potential benefits of agents such as nor-UDCA, 6-alpha-cholic acid and budesonide [16]. Obviously, accurate prediction of prognosis is also important with respect to other aspects of patient care such as scheduling follow-up visits and control examinations.

In a previous study we found that (pre-treatment) bilirubin and albumin values are powerful predictors of long-term prognosis of UDCA-treated PBC [11]. The present analysis, which was based on a significantly larger cohort with considerably longer

follow-up time, confirms that patients with excellent, moderate and poor prognosis can be identified based on (ab)normal bilirubin and albumin levels. Our results are also in agreement with earlier reports that survival for the majority of UDCA treated PBC patients, i.e. patients with non-advanced disease, does not significantly differ from that of an age- and sex matched population [9; 11; 17; 18]. We further confirm previous findings that the Mayo model applied before the start of treatment underestimates survival in UDCA treated patients, particularly for patients with normal bilirubin and albumin concentrations [9; 17; 18]. This observation is compatible with a therapeutic effect of UDCA, especially in early PBC [7; 9; 17-19]. Moreover, the present study found that prognosis for 57/120 (48%) patients with (moderately) advanced PBC and responding to UDCA improved markedly, a finding compatible with therapeutic effects of UDCA not only in early but also in advanced disease. Apart from the question whether UDCA is effective in PBC, these data indicate that biochemical response criteria can be used for optimizing prognostication.

In conclusion, the prognosis for the majority of patients with PBC, i.e. those presenting with early disease, is excellent and comparable with that of the general population when treatment with UDCA is instituted. Prognosis for UDCA patients with more advanced disease is clearly worse than that of the general population and can be differentiated based on the biochemical findings during treatment with UDCA. Our study suggests that prognosis based on albumin and bilirubin levels is more accurate than prognosis based on changes in ALP. Normalization of abnormal albumin and bilirubin concentrations following UDCA treatment is associated with improved prognosis. Therefore, our data are compatible with a therapeutic effect of UDCA in PBC, irrespective of the stage of the disease.

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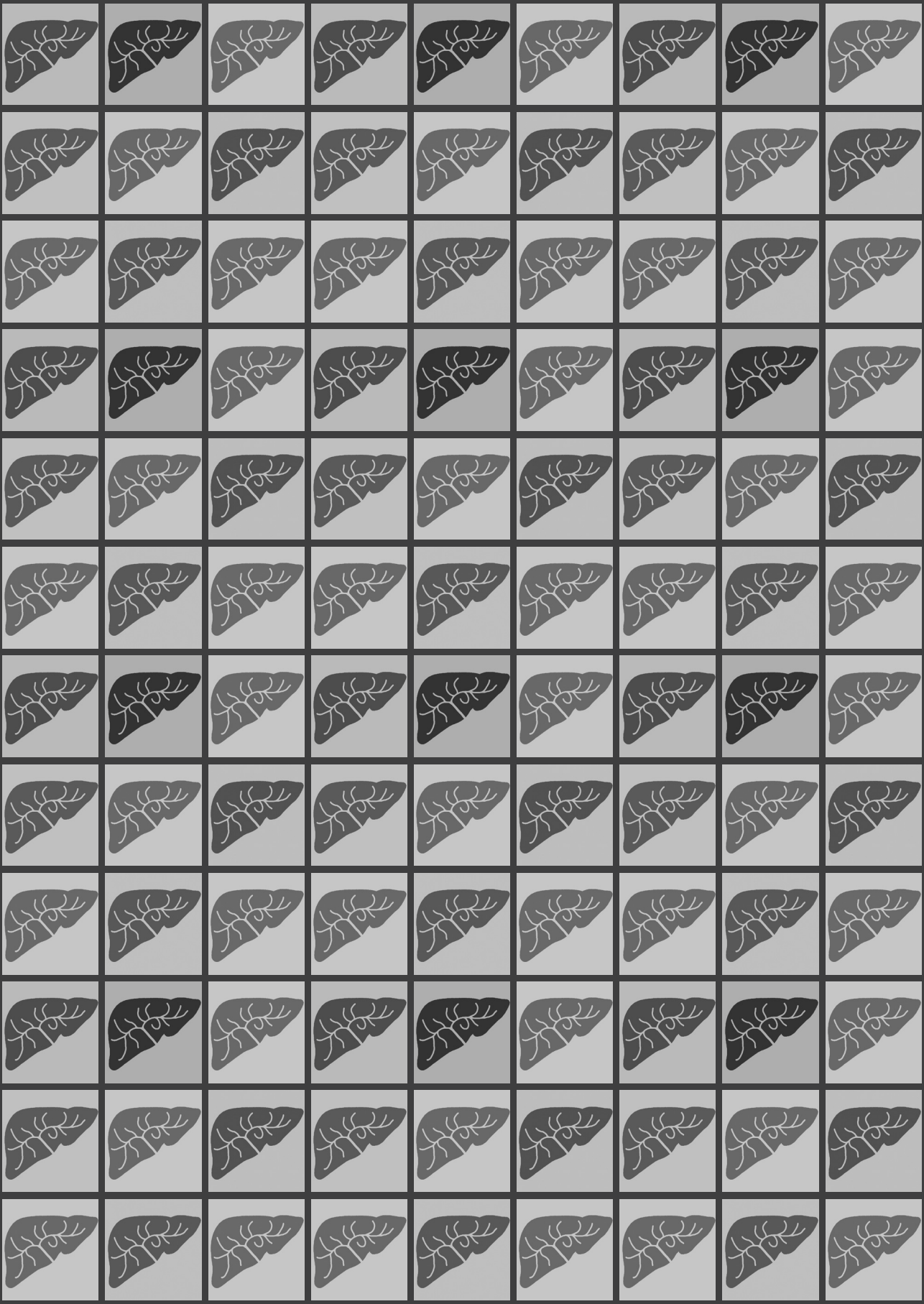

The long-term effect of ursodeoxycholic acid on laboratory liver parameters in biochemically non-advanced primary biliary cirrhosis

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ABSTRACT

Background and aims

Ursodeoxycholic acid (UDCA) has an established effect on liver biochemistries in Primary Biliary Cirrhosis (PBC). Few studies have evaluated long-term laboratory treatment effects and data beyond 6 years are not available. The aim of this study was to assess the long-term evolution of liver biochemistries during prolonged treatment with UDCA in biochemically non-advanced PBC.

Patients and methods

Prospective multicenter cohort study of patients with PBC with pre-treatment normal bilirubin and albumin, treated with UDCA 13 - 15 mg/kg/day. At yearly intervals, follow-up data including serum bilirubin, alkaline phosphatase (ALP), transaminases, albumin and IgM were collected. Data were analyzed with a repeated measurement model.

Results

225 patients were included and followed during a median period of 10.3 years. Following 1 year treatment with UDCA 36-100% of the total biochemical improvement was achieved, the maximum response was observed after 3 years. After initial improvements, bilirubin and AST levels increased and albumin levels significantly decreased after 6-10 years. However, these changes were of limited magnitude. The beneficial effects on ALT and ALP were maintained while IgM continued to decrease.

Conclusion

In non-advanced PBC the biochemical response to UDCA is maintained up to 15 years. The long-term evolution of bilirubin, albumin and ALT differs from that of ALP and AST. The mean IgM level normalised and levels continued to decrease during the period of follow-up.

INTRODUCTION

Primary Biliary Cirrhosis (PBC) is a chronic cholestatic liver disease and the medical treatment of choice is ursodeoxycholic acid (UDCA) [1; 2]. In principle this treatment is maintained lifelong. The beneficial effect of UDCA on laboratory parameters of cholestasis and inflammation, including serum bilirubin, alkaline phosphatase (ALP), aspartate amino transferase (AST) and alanine transaminase (ALT) have been well established as well as the lowering effect on serum IgM levels [3; 4]. Further, several groups have reported that the magnitude of the biochemical response to treatment at one year provides important prognostic information. The definition of response of Pares et al. was based on a 40% reduction or normalization of ALP levels, the definition of Corpechot et al. included ALP, AST and bilirubin levels and the Rotterdam definition included bilirubin and albumin levels. Irrespective of the definition for response that was used the prognosis of responders was significantly better than that of non-responders [5-7].

Since the evolution of laboratory parameters beyond 6 years UDCA therapy has not been reported in the literature for a large cohort of patients, it is uncertain whether the initial beneficial effects are maintained during more prolonged follow-up. The aim of this study was to evaluate the long term evolution of liver biochemical and immunological variables in PBC patients treated with UDCA.

PATIENTS AND METHODS

The Dutch Multicenter PBC Study is a prospective cohort study of patients with PBC with participation of 7 university and 39 general hospitals. Inclusion started in January 1990 and follow-up data until April 2007 were analyzed [5].

Since PBC is a slowly progressive disease, laboratory variables reflecting liver function are expected to worsen over time. Furthermore, patients with most pronounced abnormalities are more likely to drop out because of liver-related death or liver transplantation than patients with less advanced disease. During long-term follow-up this could, in theory, have a normalizing effect on mean levels of variables, such as bilirubin and albumin, while in reality the opposite occurs. We intentionally wanted to limit this study to a homogenous cohort and thus to patients with an expected low drop out risk. Based on earlier findings, patients with normal bilirubin and albumin levels before treatment have an excellent prognosis [5]. Therefore, for

the purpose of this study we selected, from the total cohort of patients, cases with non-advanced disease, defined by pre-treatment normal serum bilirubin and albumin levels.

A definite diagnosis of PBC was made in the presence of 2 major plus 2 minor criteria or 1 major plus 4 minor criteria. Major criteria were AMA titer ≥1:20 and compatible liver biopsy, minor criteria were pruritus, jaundice, ALP ≥2-fold the upper limit of normal, serum IgM >2.8g/l and positive Schirmer test [8]. Available liver biopsies were reviewed according to Ludwig et al. [9]. Exclusion criteria were age > 75, pregnancy, evidence of extra hepatic biliary disease, autoimmune-overlap syndrome or use of immunosuppressive drugs, concomitant disorders limiting life expectancy and decompensated PBC, defined as Child-Pugh class B or C cirrhosis. Following inclusion, patients started with UDCA therapy 13 - 15 mg/kg/day [10]. Laboratory data (serum levels of bilirubin, ALP, AST, ALT, albumin and IgM) were collected at 3-month intervals in the first year and at yearly intervals thereafter. These variables were reported as the ratio of the test result to the upper or lower limit of normal (ULN, LLN) for the laboratory performing the test, in order to deal with variability in the reference ranges in the numerous participating hospitals.

Statistical analysis

The evolution of laboratory parameters was analyzed using a repeated measurement model for longitudinal data. A p-value <0.05 (two-tailed) was considered statistically significant. Statistical package SPSS 15.0 and SAS 9.1 were used to perform analyses.

RESULTS

Patients

The baseline patient characteristics are summarized in Table 1. The study population comprised 225 (92% female) PBC patients, with a mean age of 54 years, who were followed for a median period of 10.3 (range 1.1-17.3) years. Following inclusion, the total number of study visits with collection of laboratory and clinical data was 2886, the average number of data collections was 13 per patient. Regarding histology, 46% of available liver biopsies were classified Ludwig stage I or II disease.

During follow-up 6/225 (3%) patients died from liver related causes or underwent liver transplantation while 28 (12%) died from a non liver related cause.

Table 1. Baseline patient characteristics (n=225)

Age (years; mean ± SD)	54 ± 11
Sex (male/female)	18 (8%) / 207 (92%)
AMA +	209 (93%)
ALP (ULN, range)	2.3 (0.4-11.5)
AST (ULN, range)	1.3 (0.3-4.1)
ALT (ULN, range)	1.7 (0.2-10.0)
Bilirubin (ULN, range)	0.6 (0.1-1.0)
Albumin (LLN, range)	1.2 (1.0-7.2)
IgM (ULN, range)	1.7 (0.2-12.8)
MRS (mean ± SD)	3.6 ± 0.7
Histological stage*	
I	53 (24%)
II	50 (22%)
III	29 (13%)
IV	8 (4%)
Not available	85 (37%)

* Liver biopsy within 1 year of entry and/or established cirrhosis.
ULN: ratio test result to upper limit of normal range
LLN: ratio test result to lower limit of normal range
MRS: Mayo Risk Score

Long-term evolution of laboratory variables

57/225 (25%) of patients had a complete (ie normal bilirubin, albumin, ALP, AST, ALT and IgM) and sustained biochemical response upon treatment with UDCA. Marked rises in transaminases, with levels > 5 x normal, were observed in two cases. In both cases this was a single observation and further follow-up levels were comparable with those before these flares.

Figure 1a-f shows the long term evolution of serum bilirubin, albumin, ALP, AST, ALT and IgM respectively. The mean serum bilirubin level was normal at entry (Figure 1a). A statistically significant decrease at 1 year was noted that was not followed by a further decrease at 2 years. This was a temporary event and bilirubin gradually increased during subsequent years. The levels at 3 years were comparable to those at entry and thereafter a further continuous increase was observed, resulting in levels from 10 years onwards that were significantly higher (p=0.04) compared to levels at entry. However in absolute terms these changes were relatively modest and levels remained within normal limits up to 15 years.

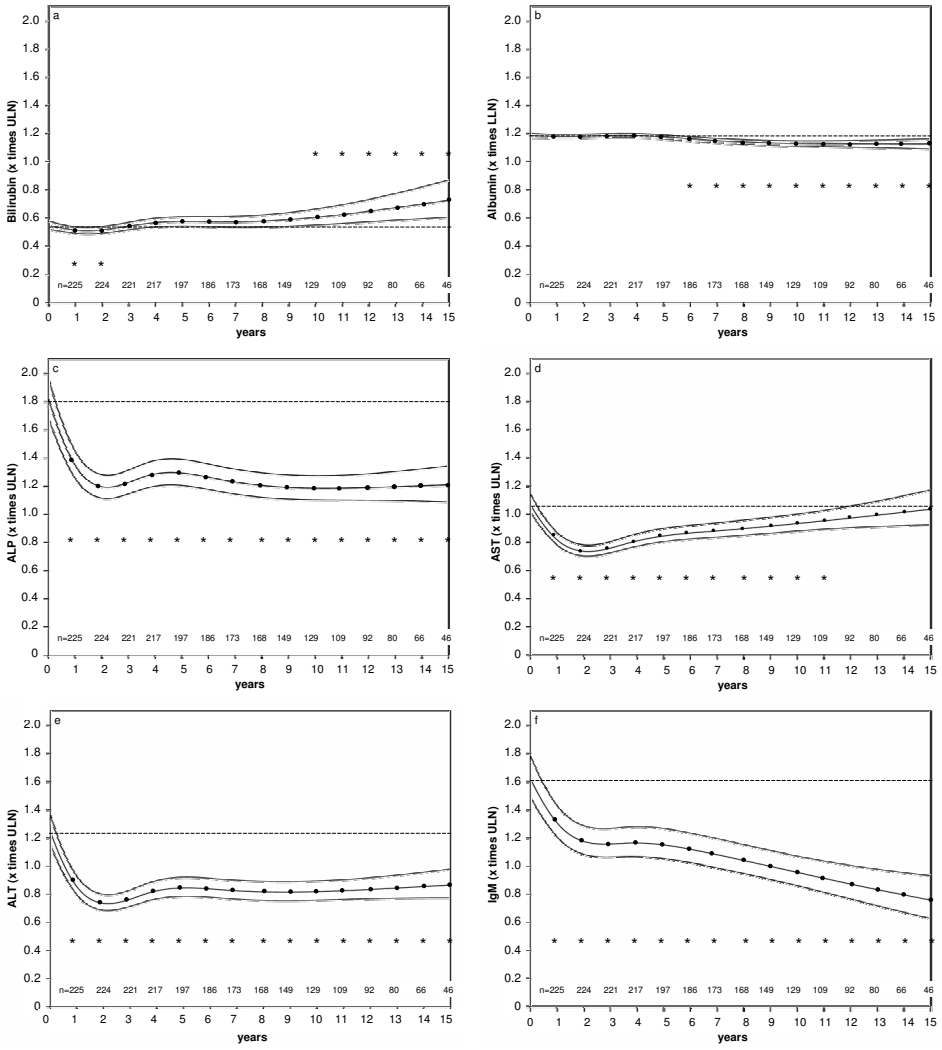

Figure 1. Serum bilirubin (a), albumin (b), ALP (c), AST (d), ALT (e) and IgM (f) levels with 95% confidence levels over time for patients with PBC and normal serum bilirubin and albumin levels. The values at T=0 were obtained before UDCA treatment started. Asterixes indicate statistically significant differences with the values at T=0.

During the first 2 years of treatment a marked and significant decrease in levels of ALP, AST, ALT and IgM was observed (Figure 1c-1f). The maximum decrease in ALP, AST and ALT was observed at 3 years, followed by steady levels of ALP and ALT in subsequent years. In contrast, levels of AST gradually returned to entry values after 3 years and after 12 years the difference with the values at entry was no

longer statistically significant (p=0.14). Serum levels of IgM, however, continued to decrease, with normal levels from 9 years onwards.
Serum albumin levels, which were normal before entry, did not change during the first 5 years (Figure 1b). During subsequent follow-up albumin significantly decreased, although quantitative changes were minor, and stabilized from 10 years onwards. Albumin levels remained within normal limits throughout the study period.

DISCUSSION

This study shows that in patients with biochemically non-advanced PBC the initial treatment effect of UDCA on ALP, ALT and IgM levels is maintained up to 15 years. Intriguingly, IgM levels continued to decrease throughout the study while the decrease in AST levels was only temporary. During prolonged treatment bilirubin increases and albumin decreases, but in absolute terms the observed quantitative changes were minor and mean levels of these parameters reflecting liver function remained within normal limits.
Previous reports on the early biochemical response after initiating UDCA therapy are in agreement with the present findings [3; 4; 11]. This study confirms that the most marked laboratory effects are observed within 1-3 years. The evolution of IgM is a clear exception as levels continue to decrease and tend to normalise over time. There are virtually no previous studies evaluating the biochemical treatment effects of UDCA beyond five years. Already in 1994 Leuschner et al reported on 22 patients who were treated up to 12 years, however only 3 cases were followed beyond 10 years [12]. With respect to ALT, ALP and IgM the results of this study were comparable with our results. Leuschner et al also noticed that withdrawal of UDCA even after more than 6 years was followed by immediate biochemical rebound.
An intriguing finding was the divergent course of AST and ALT during prolonged UDCA treatment. Since UDCA treatment resulted in a stable decrease in ALT but only temporally lowered AST levels, the AST/ALT ratio gradually increased over time. Nyblom et al. found this ratio to be associated with the presence or development of cirrhosis [13]. However, the AST/ALT ratio was not found to be of prognostic significance, a finding in agreement with the results of a previous study by our group [14]. An association between cirrhosis and high AST/ALT ratio has also been reported for other liver diseases such as viral hepatitis and non-alcoholic steatohepatitis [15; 16].
Together with the observed slowly increasing bilirubin and decreasing albumin

levels these data may indicate slow progression of PBC, despite UDCA therapy, towards (more advanced) cirrhosis. However, bilirubin and albumin levels remained within the normal range during follow-up. As patients in our cohort did not undergo follow-up liver biopsies we are unable to further analyse the histological evolution. A previous large cohort study in France has documented gradual development of cirrhosis in UDCA treated patients [17]. The investigators found, using a Markov model analysis, that the median time to develop cirrhosis was 25 years for patients with histological Ludwig stage I disease, 20 years for stage II and 4 years for stage III. In summary, the evolution of AST and ALT (ratio) is compatible with gradual development of cirrhosis during UDCA therapy, but existing evidence suggests that these parameters are not clinically helpful in predicting prognosis.

The significance of the continued effect of UDCA on IgM is not clear. Although IgM levels are characteristically elevated in PBC, and can be helpful in diagnosis, the pathophysiological role of IgM has not been elucidated. A recent study found that UDCA may have a direct effect on IgM production [18]. Numerous previous studies have reported that UDCA decreases serum IgM levels. Interestingly, we found that IgM continues to decrease during prolonged treatment and normalizes in a large proportion of patients.

Nowadays the majority of cases with PBC is diagnosed in the early phase of the disease and presents with normal serum bilirubin and albumin. Obviously, a limitation of our study is that the results only apply to this population of biochemically non-advanced PBC. This was a deliberate choice in order to assess the evolution of liver biochemistries during UDCA therapy in a homogenous population. The prospective character of the study, the size of the study population, the long duration of follow-up and the frequent collection of biochemical data are factors likely to contribute to the reliability of the results.

In conclusion, the biochemical response to UDCA is maintained in PBC patients who were treated up to 17 years. Although the evolution of bilirubin, albumin and transaminases is compatible with progression of the disease, the absolute changes were minor and probably of little clinical significance.

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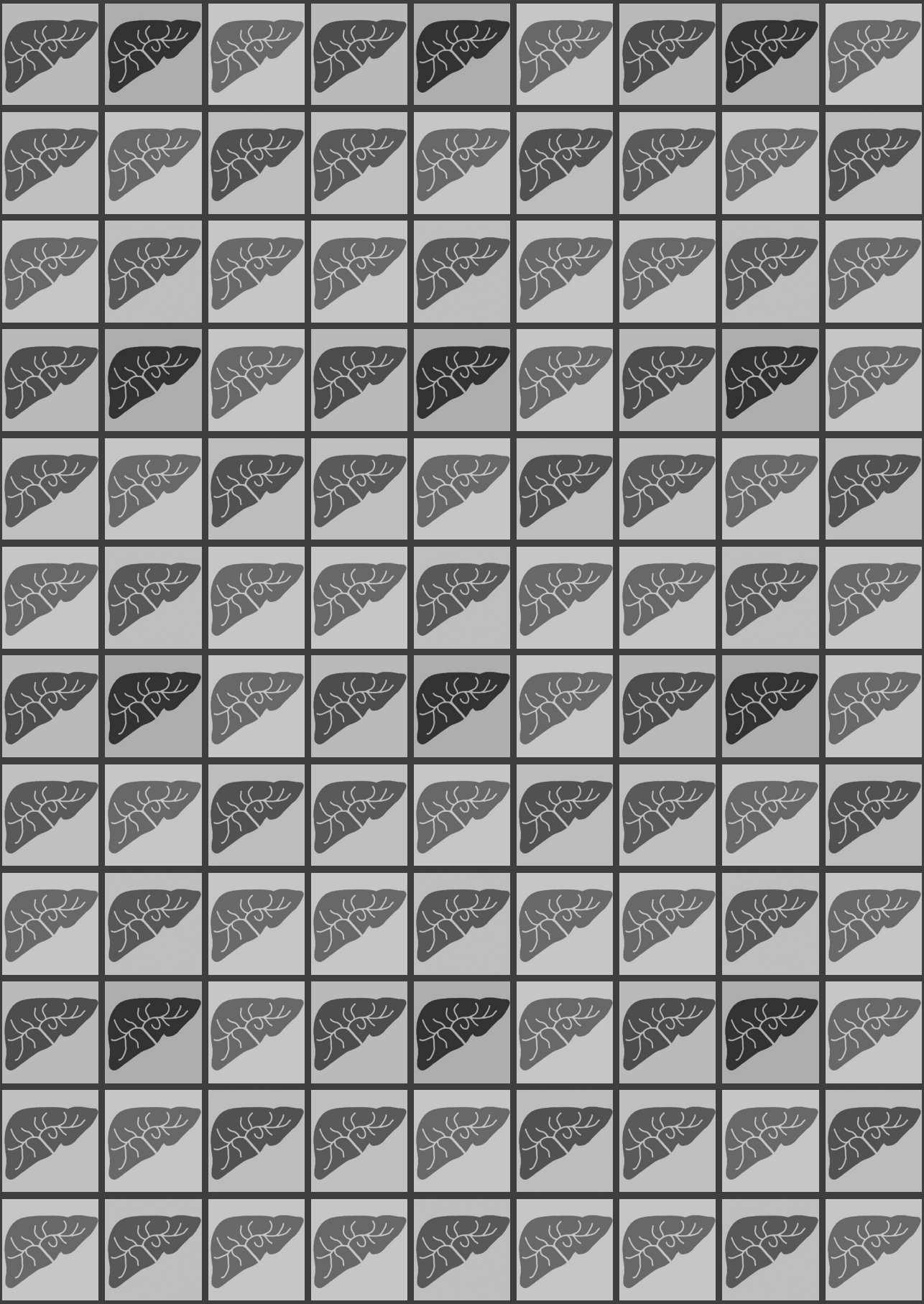

Relatively high risk for hepatocellular carcinoma in patients with primary biliary cirrhosis not responding to ursodeoxycholic acid

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ABSTRACT

Background and aims

The reported incidence of hepatocellular carcinoma (HCC) among patients with Primary Biliary Cirrhosis (PBC) varies from 0.7-3.8% while in cirrhotic patients the risk is considerably higher. Age, male sex, cirrhosis and portal hypertension are reported risk factors. It has been suggested that ursodeoxycholic acid (UDCA) may protect against HCC. We aimed to define risk factors for the development of HCC at the time of PBC diagnosis and to identify, among patients treated long-term with UDCA, a subgroup that could benefit from screening.

Patients and Methods

Prospective multicenter cohort study of patients with established PBC treated with UDCA 13-15 mg/kg/day. Age, sex, AMA, bilirubin, albumin, ALP, ALT, AST, cirrhosis, portal hypertension, Mayo Risk Score, prognostic class (based on bilirubin and albumin levels) and response to UDCA (normalization of bilirubin and/or albumin levels) were analyzed as potential risk factors in Cox regression analysis.

Results

375 patients were included, median follow-up was 9.7 years. HCC occurred in 9 patients, corresponding with an annual incidence of 0.2%. The factor significantly associated with the development of HCC was response to UDCA ($p<0.001$). The risk for HCC was highest in the group of non-responders to UDCA: the 10yr incidence of HCC was 9% and the 15yr incidence was 20%. The number needed to screen in this subgroup was 11.

Conclusions

In UDCA treated PBC patients the risk of HCC is relatively low. The main risk factor for HCC in the present study was absence of biochemical response to UDCA after 1 year treatment.

INTRODUCTION

Primary Biliary Cirrhosis (PBC) is a chronic cholestatic liver disease which predominantly occurs in middle-aged women. In patients with non-advanced disease who are treated with ursodeoxycholicacid survival appears to be comparable to the general population and causes of death are mainly nonliver related [1]. The overall incidence of hepatocellular carcinoma (HCC) among PBC patients varies from 0.7% to 3.8% [2; 3] while an incidence of up to 14% has been reported for those with cirrhotic disease [3] (Table 1). Limited data are available on a possible protective effect of treatment with UDCA [4].

Table 1. Reported annual and cumulative incidences of HCC in patients with PBC

year	1st author	Time period	patients	UDCA	Follow-up	annual incidence		cumulative incidence	
			N		years	Total %	III/IV*) %	Total %	III/IV*) %
1994	Farinati [18]	1976-1981	89	?	5.5/4.3	0.4	1.4	2.2	6.1
1997	Jones [20]	1975-1995	667	?	7.3	0.3	0.8	2.4	5.9
1999	Nijhawan [14]	1976-1985	1689	?	4.4	0.2	-	0.7	-
2001	Caballeria [17]	1977-1996	140	?	5.6	0.6	2.0	3.6	11.1
2001	Shibuya [4]	1980-1998	396	?	3.6	1.0	3.4	3.5	12.3
2002	Findor [19]	1978-1998	292	?	3.0	0.5	1.1	1.3	3.4
2003	Floreani [21]	1973-1999	170	?	6.2	0.5	-	3.0	-
2007	Jackson [2]	1987-2002	395	yes	3.7	0.2	-	0.7	-
2008	Deutsch [3]	1987-2005	212	partly	6.0/7.0	0.6	2.1	3.8	14.5
Present study		1990-2007	375	Yes	9.7	0.2	0.2	2.4	1.9

*) histological stage III (marked liver fibrosis) and IV (cirrhosis)
? no details on UDCA treatment

Several risk factors for HCC have been reported, including histological stage, gender, age, presence of portal hypertension and history of blood transfusion [3-5]. Ideally, surveillance programs should be limited to those patients carrying a significant risk for HCC and these patients should be easily identifiable to prevent burden of medical resources. Also, tumors should be detected at an early stage.
Today, surveillance for HCC is solely recommended for PBC patients with cirrhosis [6]. This strategy, however, has inherent disadvantages, the most important being the necessity of repeated diagnostic procedures, such as liver biopsy, in the majority of cases who present with non-cirrhotic disease.

Further, liver biopsy is associated with sampling error and interpretation difficulties. Silveira et al. developed a model to predict HCC based on age, gender, portal hypertension status and history of blood transfusions, but the criterion of portal hypertension has the same disadvantages as histological status and the model has not been validated yet [5].

The aim of the present study was to evaluate epidemiology of PBC related mortality focused on HCC in a large UDCA-treated cohort. We aimed to define risk factors for the development of HCC early in the disease process in order to identify a subgroup of PBC patients who could most likely benefit from surveillance.

PATIENTS AND METHODS

The Dutch Multicenter PBC Study is a multicentre prospective cohort study of PBC patients. The primary goal was to analyze long-term prognosis, in particular survival and causes of death, in patients treated with UDCA. Inclusion started in January 1990 and follow-up data until April 2007 were analyzed [1]. A definite diagnosis of PBC was made in the presence of 2 major plus 2 minor criteria or 1 major plus 4 minor criteria. Major criteria were AMA titer ≥1:20 and compatible liver biopsy, minor criteria were pruritus, jaundice, ALP ≥2-fold the upper limit of normal, serum IgM >2.8g/l and positive Schirmer test [7]. Available liver biopsies were reviewed according to Ludwig et al [8]. Exclusion criteria were previous or current treatment with UDCA, age > 75, pregnancy, evidence of extra hepatic biliary disease, autoimmune-overlap syndrome or use of immunosuppressive drugs, concomitant disorders limiting life expectancy and decompensated PBC, defined as Child-Pugh class B or C cirrhosis. Patients were eligible irrespective of the interval between diagnosis and inclusion. Following inclusion, patients started with UDCA therapy 13 - 15 mg/kg/day [9]. Follow-up data with respect to HCC, other complications, liver transplantation and (causes of) death were collected at 3-month intervals in the first year and at yearly intervals thereafter. At each visit general physical examination and laboratory studies including bilirubin, albumin, alkaline phosphatase (ALP), aspartate

aminotransferase (AST), alanine aminotransferase (ALT), albumin, IgG, IgM and total cholesterol were performed. Liver tests were reported as the ratio of the test result to the upper or lower limit of normal (ULN, LLN) for the laboratory performing the test.

Information on the causes of death was retrieved from hospital case records. In a limited number of cases, for instance if patients were lost to follow-up, family doctors or other hospitals were contacted.
The cohort study started well before the first consensus guidelines on HCC screening appeared. Therefore, with respect to screening liver imaging studies and measurement of fetoprotein levels were performed according to the policy of the individual participating hospitals.

Definitions and statistical analysis

Survival was analyzed as transplantation-free survival (end-points: liver transplantation and death). Liver related death was defined as: death because of liver failure or HCC or death occurring within 2 months of an episode of variceal bleeding, spontaneous bacterial peritonitis, hepatorenal syndrome, or hepatic encephalopathy. Patients lost to follow-up were censored at the time of their last visit. Survival was compared to survival of the Dutch population, matched for gender, age and time of observation. Data for the Dutch population were derived from an official demographic data base. For time-to-event analysis we applied the Kaplan-Meier method and compared groups using the log-rank test.
Possible risk factors for HCC at diagnosis were evaluated using univariate and multivariate cox regression analysis. We calculated the estimated risk to develop HCC based on the Silveira model.
Based on serum bilirubin and albumin values the following prognostic classes were defined: early PBC (both bilirubin and albumin normal), moderately advanced PBC (one parameter normal) or advanced PBC (both parameters abnormal) [10]. Further, based on Mayo Risk Scores, the disease was classified as low risk (score <4.5) or high-risk (score ≥4.5) [11]. Portal hypertension was defined as presence of ascites and/or a history of variceal bleeding. Biochemical response to UDCA treatment was defined as normal bilirubin and albumin concentrations after 1 year treatment with UDCA when one or both parameters were abnormal before treatment, or as normal bilirubin or albumin concentration after treatment when both were abnormal at entry. Patients with persistently normal levels were also regarded as responders to therapy [1]. The occurrence of events during follow-up, including HCC, liver transplantation and death, was expressed as the cumulative incidence (CI) and the annual incidence (AI). CI represents the cumulative or total incidence over the complete follow-up period, whereas AI represents the mean annual incidence.

A p-value <0.05 (two-tailed) was considered statistically significant. Statistical package SPSS 15.0 was used to perform analyses.

RESULTS

Patients

The baseline patient characteristics are summarized in Table 2. The study population comprised 375 (89% female) patients, with a mean age of 54.7 years, who were followed for a median period of 9.7 (range 1-17.3) years. Sixteen (4%) patients were lost to follow-up. Following inclusion, the total number of study visits with collection of laboratory and clinical data was 4776; the average number of data collections was 9 per patient. The majority of patients (60%) suffered from early PBC. 65% (n=161) of available liver biopsies were histologically classified as Ludwig stage I or II disease.

Table 2. Baseline patient characteristics (n= 375)

Age (years; mean ± SD)	54.7 ± 10.9
Sex (male/female)	40 (11%)/335 (89%)
AMA +	350 (93%)
ALP (ULN, range)	2.7 (0.4-13.0)
AST (ULN, range)	1.6 (0.3-7.4)
ALT (ULN, range)	2.0 (0.3-12.1)
Bilirubin (ULN, range)	0.7 (0.1-5.6)
Albumin (LLN, range)	1.1 (0.3-7.2)
IgM (ULN, range)	1.9 (0.2-17.5)
IgG (ULN, range)	1.0 (0.1-2.9)
Mayo Risk Score (mean ± SD)	4.1 ± 1.0
Histological stage	
I	69 (18%)
II	92 (25%)
III	52 (14%)
IV	34 (9%)
Not availble	128 (34%)
Prognostic class	
Early PBC	225 (60%)
Moderately advanced PBC	95 (25%)
Advanced PBC	34 (9%)
Not available	21 (6%)

Survival

Compared with a sample of the general Dutch population, transplantation free survival of our PBC cohort was significantly lower ($p<0.001$). Survival of the total PBC cohort was 67%, for the standardized Dutch population the 15-yr survival rate was 80%. The incidence of non-liver related death in our cohort was comparable tot that in the general population and the difference in survival can be explained by liver related causes, assuming liver related death and non liver related death are independent events (Figure 1).

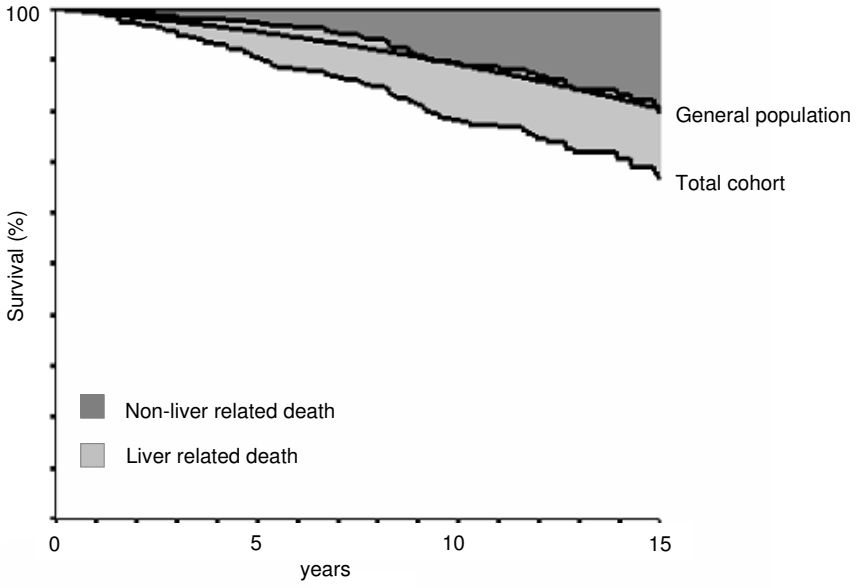

Figure 1. Survival of the cohort of 375 patients with PBC compared with a matched sample of the Dutch population. Survival of patients with PBC was clearly lower than in the general population. The excess mortality in PBC is attributable to liver-related causes of death (light grey area). The incidence of non-liver related death (dark grey area) was comparable for both populations (the line between liver related death and non-liver related death was calculated, assuming that liver related death and non-liver related death are not related).

Causes of death

During follow-up 69/375 (18%) patients died and 22 (6%) underwent liver transplantation (LTX). The number of deaths and transplantations was 34 in patients with early PBC, 35 in moderately advanced PBC and 22 in advanced PBC. Causes of death were mainly non-liver related for patients with early PBC: in this subgroup

only 18% (6/34) died from liver related causes or underwent LTX. Liver related death and LTX occurred relatively more often in moderately advanced and advanced PBC: 63% (22/35) and 91% (20/22), respectively. The annual incidence of liver related death and transplantation was 0.3%, 2.4% and 8.8% for early, moderately advanced and advanced PBC (Table 3). HCC accounted for 33% (2/6) of liver related causes of death in early PBC, for 27% (6/22) in moderately advanced PBC and 5% (1/20) in advanced PBC. The corresponding annual incidences were 0.1%, 0.6% and 0.4% (Table 3).

Table 3. Causes of death in a cohort of 375 UDCA-treated PBC patients

	Early PBC (N=225)	Moderately advanced PBC (N=95)	Advanced PBC (N=34)
Age at entry (yrs; mean ± SD)	54 ± 11	55 ± 11	57 ± 10
Follow-up (months; mean ± SD)	124 ± 50	118 ± 55	80 ± 56
N liver related death/transplantation (AI)	6 (0.3)	22 (2.4)	20 (8.8)
Liver failure	4 (0.2)	16 (1.7)	20 (8.8)*
HCC	2 (0.1)	6 (0.6)	-
N non liver related death (AI)	28 (1.2)	13 (1.4)	2 (0.9)
Cardio-respiratory disease	11 (0.5)	2 (0.2)	1 (0.4)
Cerebrovascular accident	3 (0.1)	4 (0.4)	-
Non-hepatic malignancy	8 (0.3)	1 (0.1)	-
Other causes	6 (0.3)	6 (0.6)	1 (0.4)
N alive at end of study	180	56	11
Age at time of death (yrs; mean ± SD)	72 (9)	63 (13)	65 (10)

AI annual incidence

* 1 patient died from liver failure, HCC was detected in autopsy material.

HCC cases

During follow-up 9 cases of HCC were diagnosed (Table 4). Seven of these patients were female, the mean age at the diagnosis of PBC was 57 ± 9 years and at the diagnosis of HCC 66 ± 11 years. At HCC diagnosis 33% (3/9) was more than 70 years old. The time from PBC diagnosis to the development of HCC was 16± 5 (mean ± SD) years.

Diagnosis was made by the characteristic findings on MRI or CT imaging in combination with markedly elevated (and rising) alpha-fetoprotein (AFP) levels. In 4 of the 9 cases with HCC diagnosis was confirmed by histology of needle biopsies (n=2), surgical explant specimen (n=1) or autopsy material (n=1).

Table 4. Details of 9 patients with PBC who developed HCC

Case	1	2	3	4	5	6	7	8	9	Total
Female sex	no	no	yes	yes	yes	yes	yes	yes	yes	78%
Age at diagnosis PBC (years)	56	57	42	47	48	62	58	37	39	50±9
Age at diagnosis HCC (years)	76	68	50	66	63	78	78	50	60	66±11
Interval diagnosis PBC – HCC (years)	20	11	7	19	14	16	20	13	21	16±5
Mayo Risk Score										
- at diagnosis of PBC	4.6	4.8	5.6	3.7	6.1	4.9	5.2	4.6	4.4	4.9±0.7
- at diagnosis of HCC	-	5.5	5.7	4.9	9.8	9.0	8.0	-	5.8	7.0±5.8
Histological stage PBC										
- at diagnosis PBC	-	III	III	II	III	n.a.	II	II	IV	3.4±1.3
- at diagnosis HCC	IV	III	IV	IV	IV	III	IV	IV	IV	3.8±0.4
Portal hypertension	no	yes	yes	yes	yes	yes	yes	n.a.	yes	88%
History Blood transfusion	no	yes	no	no	no	no	no	n.a.	no	13%
Body Mass Index	22	27	22	25	19	30	26	30	22	25±4
Other liver disease	no	no	no	no	no	Yes +	no	no	no	11%
Nodules	2	1	1	2	1	1	2	1	1	1.3±0.5
Diameter (mm)*	80	-	5	37	24	25	68	75	25	42±28
AFP levels (ULN)										
- at diagnosis PBC	-	0.2	0.6	-	0.8	-	0.7	-	0.7	0.6±0.2
- at diagnosis HCC	55.6	56.7	12.9	112.1	11.2	3.3	23.1	n.a.	72.3	42±40
Predicted risk for HCC (%) **	5.9	46.7	5.6	15.6	15.6	64.8	64.8	-	15.6	28±24
Prognostic class ***										
- at diagnosis PBC	2	1	3	1	2	2	2	2	2	1.9±0.6
- at diagnosis HCC	3	3	3	n.a.	3	3	3	n.a.	3	3
Responder to UDCA	no	yes	no	yes	no	no	no	no	no	22%
Treatment	none	none	LTX	RFA	none	none	none	none	PEI	-
Status	Dead	Dead	Alive	Dead	Dead	Dead	Dead	Dead	Dead	-
Survival (months)	10	14	133	23	0	1	35	23	9	13±12

n.a.: not available RFA: radio frequency ablation PEI: percutaneous ethanol injection

\+ previous hepatitis B, HBsag negative.

* of the largest tumour nodule

** according to Silveira [18]

*** 1= early PBC 2= moderately advanced PBC 3=advanced PBC

At PBC diagnosis the Mayo Risk Score was ≥4.5 in 78% of the patients, at HCC diagnosis this was 100%. Initially, liver histology showed Ludwig stage III or IV in 4/7 cases, but at the time of HCC diagnosis all 9 patients had progressed to stage III or IV. Three patients were diagnosed with multifocal HCC. The mean diameter of the largest tumour was 42 ± 28 mm (SD). At the time of HCC diagnosis AFP levels were elevated in all cases, varying from 3 to 112 times the upper limit of normal.

The mean predicted probability to develop HCC according to the Silveira model, based on sex, age, presence of portal hypertension and history of blood transfusion, was 28 ± 24% (SD) [5].

According to pre-treatment bilirubin and albumin levels, 7 of the 9 HCC cases were categorised as moderately advanced or advanced PBC at diagnosis (78%). All seven patients were non-responders to treatment with UDCA, based on Rotterdam criteria.

According to the Paris [12] and Barcelona [13] criteria for response to UDCA treatment, 1/9 (11%) and 6/9 (67%), respectively of our PBC patients with HCC were non-responders. However, the 2 patients not responding according to the Rotterdam criteria were responders according to the Barcelona criteria. Thus, responders identified by one set of criteria are not identical to responders when other criteria are used, and the way biochemical response is defined is obviously of major importance when interpreting the results of the present and comparable studies.

All but one patient died within 3 years of HCC diagnosis. One patient underwent liver transplantation and was alive 133 months later.

Risk factors HCC

To determine risk factors for the development of HCC, univariate analysis was performed concerning the following baseline variables: age, sex, AMA, total serum bilirubin, serum albumin, ALP, ALT, AST, the presence or absence of cirrhosis and portal hypertension, Mayo Risk Score and prognostic class (early/moderately advanced/advanced PBC). Bilirubin (p=0.001), AST (p=0.003), prognostic class (p=0.017) and Mayo Risk Score (p=0.004) were significantly associated factors. In multivariate analysis no factor remained statistically significant.

In univariate analysis at 1 year after diagnosis, response to UDCA was significantly associated with the development of HCC (p=0.001).

In the total population the annual incidence of HCC was 0.2% and the cumulative incidence was 2.4% (9/375) (Figure 2a). The incidence of HCC was comparable

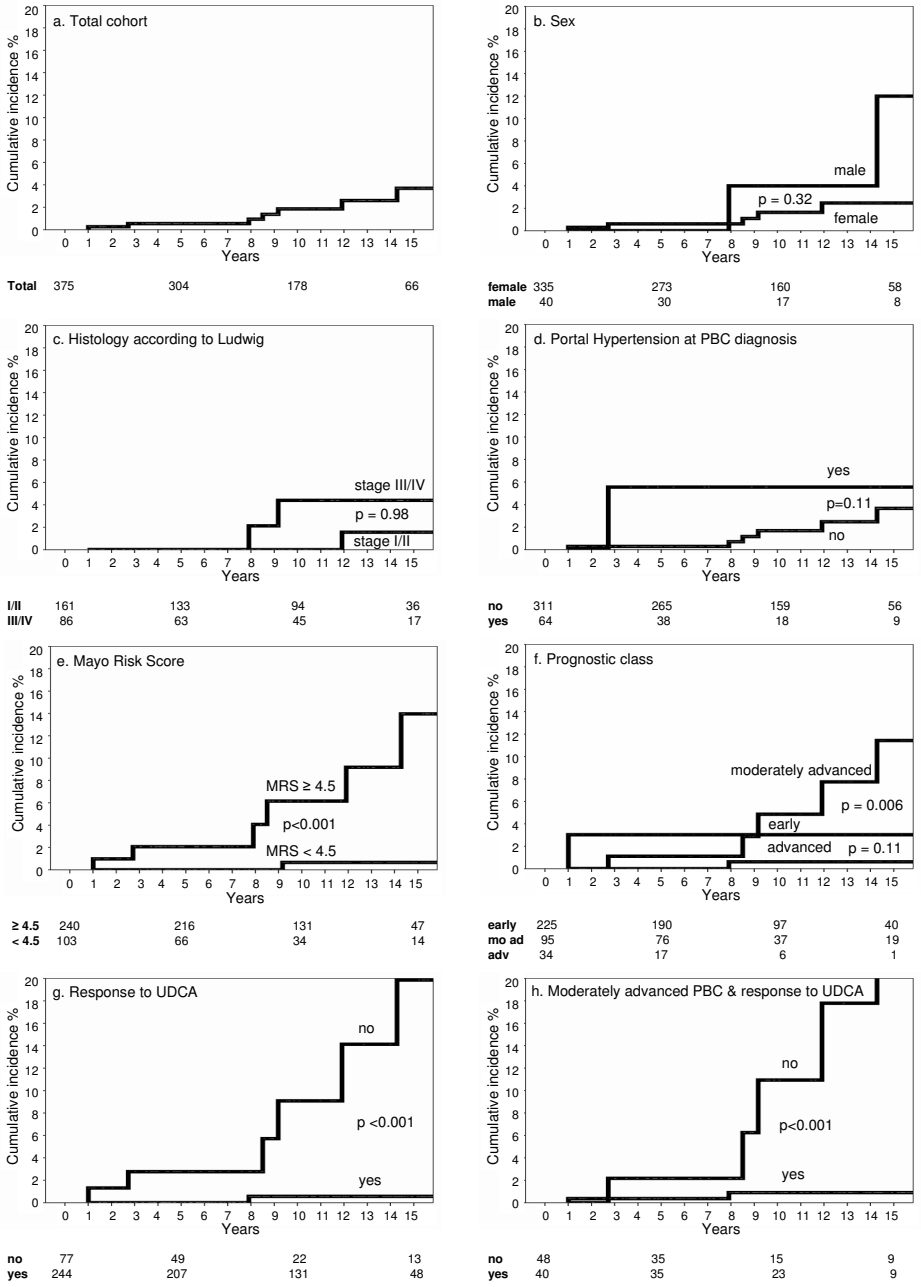

Figure 2. Kaplan Meier plots showing the cumulative incidence of HCC in the total group of patients with PBC (a) and in subgroups of patients according to gender (b), histological stage (c), presence of portal hypertension (d), Mayo Risk Score (e), prognostic class at the time of PBC diagnosis (f), response to UDCA following 1 year of treatment (g) and and the combination of moderately advanced PBC and response to UDCA (h). The number of patients at risk is shown under the X-axis.

for males and females (p=0.32), for Ludwig stage I/II and III/IV (p=0.98) and for presence and absence of portal hypertension (p=0.112).
Mayo Risk Scores ≥4.5 and moderately advanced PBC were significant risk factors for HCC (Figure 2e and 2f). The highest incidence of HCC was found in patients who were non-responders to UDCA treatment (Figure 2g).
The annual incidence of HCC in patients with cirrhosis (n=86) was 0.2%, in patients with Mayo Risk Scores ≥4.5 (n=96) 1%, in patients with moderately advanced PBC (n=95) 0.7% and in patients not responding to UDCA 1.4%. In patients with moderately advanced disease who failed to respond to UDCA (n=48), the annual risk was 1.5%. In 78% of HCC cases the Mayo Risk Score was ≥4.5. Hypothetically, when this criterion would have been used for initiating a screening program, and assuming 100% sensitivity of the program, the number needed to screen (NNS) to identify one case with HCC would have been 14. Similarly, the NNS in moderately advanced PBC cases would have been 16, in UDCA non-responders 11 and in moderately advanced PBC and UDCA non-response 8.

DISCUSSION

This cohort study of 375 UDCA treated PBC patients found a total incidence of HCC of 2.4% after a median follow-up of 9.7 years. The annual incidence of HCC was 0.2% and this is the lowest reported risks of HCC in PBC. However, a comparable incidence of HCC in PBC was found by two other studies [2; 14]. Nineteen percent of liver related death was secondary to HCC and this was responsible for 30% of liver related mortality in patients with early or moderately advanced PBC. Thus, although the absolute number of cases with HCC was only nine, HCC was a significant cause of death secondary to liver disease. The most important risk factor for future development of HCC in the present study was non-response to UDCA treatment, which was noted in 7 of the 9 HCC cases. In the sub-group of patients not responding to UDCA the incidence of HCC was 9% after 10 years and 20% after 15 years. The a posteriori calculated number needed to screen to identify a case of HCC in this population was 11. In the group of patients with advanced PBC only one case with HCC was observed, this was an incidental finding at autopsy of patients who died from liver failure. The low incidence of HCC in this subgroup might be explained by the severity of disease leading to liver related death and liver transplantation preceding potential development of HCC.

Although this study included a relatively large population followed prospectively during a substantial time period, the absolute number of HCC cases was low. Therefore, the results should be interpreted cautiously, and our finding that non-response to UDCA is a main risk factor for HCC awaits confirmation by other studies. In the present study the screening strategy was left to the individual participating centers. In theory a uniform, standardized program could have been more effective in identifying cases with HCC. It seems unlikely, however, that this would have resulted in identifying substantially more cases, given the strong association between HCC and clinical symptoms, albeit at variable points in time of its natural history.

This study did not confirm the major prognostic importance of cirrhosis for future HCC. It should be noted, however, that histological staging at entry was possible in only two-thirds of the population. Possibly this may have resulted in lack of power for cirrhosis in the multivariate Cox regression analysis. Therefore, the results of this study should not be interpreted as providing proof for cirrhosis not being a major risk factor for HCC.

A potential major advantage of using non-response to UDCA for selecting patients for screening procedures for HCC is that such patients can be recognized early, that is after one year treatment with UDCA. Also, the necessary data are easily available and do not require particular (invasive) diagnostic procedures such as liver biopsy or gastrointestinal endoscopy. We previously found that nonresponders in comparison with responders had higher pre-treatment serum levels of bilirubin, ALP and AST, higher Mayo Risk Scores, and histologically more advanced disease. Survival for nonresponders was significantly worse than for responders [1]. Hypothetically, ongoing inflammatory activity and cholestasis superimposed on significant liver fibrosis or cirrhosis are the most important drivers of carcinogenesis in UDCA non-responsive patients. For the time being, however, it remains speculative what biological mechanism could explain the high cancer risk in UDCA non-responders, also considering the significantly lower or absent prognostic significance of cirrhosis, portal hypertension and Mayo Risk Score.

Two of the nine patients developing HCC were responders to UDCA. Both patients had biochemically non-advanced disease at entry, as reflected by normal serum bilirubin and albumin levels. We were unable to identify particular factors posing these patients at an increased risk for HCC.

Obviously, further studies are required to confirm the prognostic significance of non-response to UDCA according to our criteria and to determine whether

non-response according to other, in particular the Barcelona [13], criteria is a risk factor for HCC.

We could not confirm the usefulness of the Silveira risk model for HCC [15]. This model was developed using a case control design and found that older age, male sex, history of blood transfusion and any sign of portal hypertension indicated a high risk of HCC. Our patients with HCC were comparable to the Mayo clinic patients at the time of HCC diagnosis with respect to age, BMI, alcohol consumption, histological stage at PBC diagnosis, presence of portal hypertension and Mayo Risk Score. However, the duration from PBC diagnosis until the development of HCC was significantly longer in our population compared to the Mayo clinic patients (93±13 months versus 192±60 months), the proportion male patients was lower (41% versus 22%), and our patients received less often blood transfusions (59% versus 13%). Possible explanations for these diverging results are a retrospective versus a prospective study design, geographical differences and a potential protective or delaying effect of UDCA in the development of HCC. A protective effect of UDCA was also suggested by Jackson et al, who reported a 3-fold increased risk for HCC in treated PBC patients versus an 8-fold increased risk in untreated patients in comparison to the general population [2]. Another study including treated and non-treated patients found that UDCA was not independently associated with the development of HCC [3].

According to the AASLD and EASL practice guidelines surveillance programs in subjects with cirrhosis should be considered when the risk of HCC is at least 1.5% per year [6; 16]. The AASLD guideline proposed screening in PBC patients with cirrhosis based on previous findings of a risk for HCC that was comparable to that in hepatitis C cirrhosis [17]. We found an annual incidence of HCC in individuals with cirrhosis of only 0.2% and this would clearly argue against screening in this population. Several other studies also reported a risk for HCC lower than 1.5% per year in patients with advanced histological stages [18-20]. In our study the only population carrying the proposed minimal 1.5% annual risk for HCC was the group of patients with moderately advanced PBC who failed to respond to UDCA. However, 3 of our 9 HCC cases would not have been selected for screening according to these criteria. A slightly lower annual HCC risk of 1.4% was established for patients not responding to UDCA. Our data therefore suggest that the population most likely to benefit from screening would be patients who fail to respond to UDCA.

In conclusion, this multicentre prospective study found a relatively low risk for HCC in patients who were all treated long-term with UDCA. We could not confirm the prognostic importance of male sex, cirrhosis, portal hypertension and a history of blood transfusion. The strongest risk factor for HCC in our population was absence of biochemical response after 1 year treatment with UDCA. In this subgroup the 15 year incidence of HCC was 20% and the number needed to screen was 11.
Further studies are required to confirm that the simple criterion of non-response to UDCA treatment is superior to cirrhosis as the criterion for selecting individuals with PBC for screening programs.

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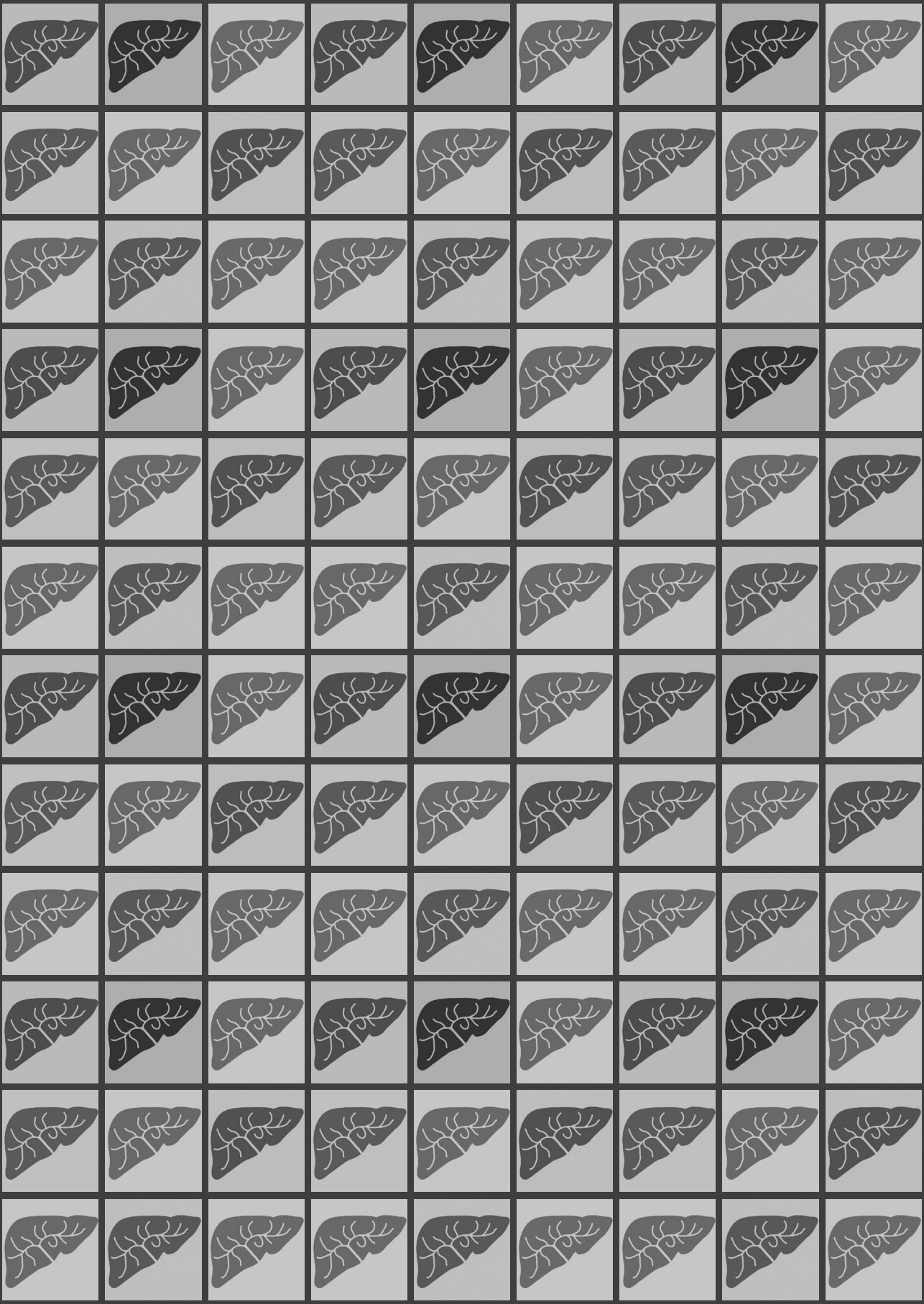

Trends in liver transplantation for primary biliary cirrhosis in the Netherlands 1988-2008

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ABSTRACT

Background and aims

A decrease in the need for liver transplantations (LTX) in Primary Biliary Cirrhosis (PBC), possibly related to treatment with ursodeoxycholic acid (UDCA), has been reported in the USA and UK. The aim of this study was to assess LTX requirements in PBC over the past 20 years in the Netherlands.

Patients and methods

Analysis of PBC transplant data of the Dutch Organ Transplant Registry during the period 1988-2008, including both absolute and proportional numbers. The indication for LTX was categorized as liver failure, hepatocellular carcinoma or poor quality of life (severe fatigue or pruritus). Data were analyzed for two decades: 1.1.1988-31.12.1997 (1st) and 1.1.1998-31.12.2007 (2nd). The severity of disease was quantified using MELD scores. To fit lines which show trends over time we applied a linear regression model.

Results

A total of 110 patients (87% women) was placed on the waiting list. 105 patients were transplanted (1st: 61, 2nd: 44), 5 (5%) died while listed. The absolute annual number of LTX for PBC slightly decreased during the 20 year period, the proportional number decreased significantly. At the time of LTX the mean age was 53.6 yrs. (1st: 53.4, 2nd: 53.8), the mean MELD score 13.9 (1st:14.5, 2nd:13.0). The median interval from diagnosis to LTX was 90.5 months (1st:86.5, 2nd: 93.5). 69% of patients was treated with UDCA (1st 38%, 2nd 82%).

Conclusions

Over the past 20 years the absolute number of LTX for PBC in the Netherlands showed a tendency to decrease whereas the proportional decrease was significant. There was a trend over time toward earlier transplantation.

INTRODUCTION

In the past Primary Biliary Cirrhosis (PBC) was one of the main indications for liver transplantation (LTX), whereas nowadays, the proportion of patients receiving a transplant for PBC has decreased to around 10% [1-3]. This could possibly be due to an increase in the number of patients transplanted for other indications, to a decrease in the need for transplantation in PBC or to a combination of these factors. A decrease in need for LTX in PBC could correspond with the finding of several recent long-term cohort studies suggesting improved transplantation-free survival for ursodeoxycholic acid (UDCA) treated PBC patients, particularly for those with a favorable biochemical response upon treatment [4-6]. The impression exists that in addition to a proportional decrease in the need for LTX the absolute number of patients with PBC receiving transplantation over time is gradually falling. However, detailed reports on time trends in the absolute number of LTX for PBC are sparse [1; 2]. We aimed to describe transplant patterns for PBC and changes over the past 20 years in the Netherlands.

PATIENTS AND METHODS

We performed a retrospective analysis of all patients who underwent LTX in the Netherlands in the period 01.01.1988 until 31.12.2007. Only first liver transplantations were included for analysis. Patients who were listed and/or transplanted during this period were identified using the Dutch Organ Transplant Registry. Name, date of birth, indication and date of LTX were obtained from this database. Additional individual data were extracted from medical records in the 3 liver transplant centers in the Netherlands: the University Medical Centers in Groningen, Leiden and Rotterdam.

Laboratory data collected included alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, albumin and creatinin levels, and platelet count, prothrombin time and INR, at the time of PBC diagnosis, at the time of placement on the waiting list and at the time of surgery. Most laboratory parameters were reported as the ratio of the test result to the upper or lower limit of normal (ULN, LLN) for the laboratory performing the test.

Definitions and statistical analysis

A definite diagnosis of PBC was made in the presence of 2 major plus 2 minor criteria or 1 major plus 4 minor criteria. Major criteria were AMA titer ≥1:20 and compatible liver biopsy, minor criteria were pruritus, jaundice, ALP ≥2-fold the upper limit of normal, serum IgM >2.8g/l and a positive Schirmer test (test to assess tear production) [7].

We divided the time span into the decades 1.1.1988-31.12.1997 and 1.1.1998-31.12.2007. Based on serum bilirubin, INR and creatinin the UNOS MELD score (Model for End-Stage Liver Disease, www.mayoclinic.org/meld/mayomodel6.html) was calculated. This model is widely used to determine prognosis in patients with liver disease [8; 9]. In the Netherlands the MELD score is used for stratifying patients and allocating organs since December 2006. In addition, the disease was classified based on serum albumin and bilirubin levels, resulting in classes of early (both parameters normal), moderately advanced (one parameter normal, one abnormal) and advanced (both parameters abnormal) PBC [5]. Particularly for patients transplanted in the first decade reliable data on edema were not uniformly available, precluding calculation of the Mayo Risk Score [10].

Serum bilirubin and/or albumin concentrations were not available for 28 patients at the time of diagnosis but were complete at the time of placement on the waiting list and at the time of surgery. At the time of diagnosis the MELD score could be calculated for 40 patients. However, data were available for all patients at the time of listing and transplantation.

The indications for liver transplantation were divided into three subgroups: 1. liver failure including treatment-resistant ascites and spontaneous bacterial peritonitis, recurrent variceal bleeding, progressive muscle wasting and encephalopathy, 2. hepatocellular carcinoma and 3. poor quality of life due to intractable pruritus or severe chronic fatigue.

UDCA-treatment was classified as "yes" if the patient had used 13-15 mg/kg/day UDCA for at least 2 years, as "no" if UDCA was never used or for a period less than 2 years.

Linear regression models were applied to fit lines which show trends over time for the number of LTX, severity of disease and age at the time of LTX. A P value of less than .05 was considered significant.

Table 1. Details of patients at the time of PBC diagnosis, placement on the waiting list and transplantation.

	PBC diagnosis		Waiting list		Transplantation	
	1st decade 1988-1997	2nd decade 1998-2008	1st decade 1988-1997	2nd decade 1998-2008	1st decade 1988-1997	2nd decade 1998-2008
Age (mean ± SD)	45.4 ± 7.2	45.4 ± 7.6	53.1 ± 7.6	53.0 ± 7.1	53.4 ± 7.6	53.8 ± 7.2
Female n (%)	56 (92)	36 (81)	56 (92)	36 (81)	56 (92)	36 (81)
UDCA n (%)	23 (38) started	36 (82) started	23 (38)	36 (82)	23 (38)	36 (82)
Bilirubin (ULN)	1.4(0.2-16.1)*)	1.0(0.4-6.6)*)	6.3(0.5-37.7)*)	3.6(0.5-29.6)*)	7.4 (0.4-37.7)	6.4 (0.5-43.1)
Albumin (LLN)	1.1 (0.7-2.6)	1.1 (0.8-2.0)	0.8 (0.5-6.5)	0.9 (0.6-1.2)	0.8 (0.5-6.5)	0.9 (0.3-1.3)
ALP (ULN)	5.0(1.1-19.4)*)	4.3(1.1-9.1)*)	4.5 (0.4-14.5)	2.8 (0.4-16.2)	4.1(0.4-12.6)#)	2.3(0.2-7.9)#)
AST (ULN)	2.4 (0.6-8.9)	2.1 (0.6-10.8)	3.3 (0.6-8.9)	2.8 (0.6-14.7)	2.9 (0.5-26.3)	3.0 (0.8-19.4)
ALT (ULN)	3.3 (0.7-9.3)	2.7 (0.9-14.3)	2.4 (0.4-9.3)	2.3 (0.6-12.8)	2.5 (0.2-14.6)	2.4 (0.4-18.2)
Creatinin (ULN)	0.6 (0.5-0.8)	0.7 (0.5-1.3)	0.7 (0.1-1.6)	0.7 (0.4-2.5)	0.7 (0.1-1.6)	0.7 (0.5-2.7)
INR	0.9(0.7-1.9)*)	0.8(0.6-1.0)*)	1.0 (0.7-2.4)	1.0 (0.7-1.7)	1.1 (0.7-2.8)	1.0 (0.7-2.2)
MELD (mean ± SD)	7.3 ± 2.7	7.0 ± 2.1	12.4 ± 5.1*)	14.5 ± 6.0*)	14.5 ± 6.0	13.0 ± 6.6
Prognostic class n (%)						
Early	11 (18)	18 (41)	0 (0)	3 (7)	2 (3)	4 (9)
Moderately advanced	26 (43)	7 (16)	12 (20)	11 (25)	8 (13)	11 (25)
Advanced	11 (18)	10 (23)	49 (80)	30 (68)	51 (84)	29 (66)
Insufficient data	13 (21)	9 (20)	0 (0)	0 (0)	0 (0)	0 (0)

(#: Early vs Moderately advanced vs Advanced)

*) p<0.05#) p<0.001

Laboratory values are expressed as the median of the number of times the upper or lower limit of normal (range between brackets).

RESULTS

Patients

Baseline patient characteristics are summarized in Table 1.

In total 105 PBC patients had a first liver transplant during the study period, eighty-eight percent was female; the mean age at the time of LTX was 53.6 years. The median time from PBC diagnosis to the moment of LTX was 90.5 months; the median time on the waiting list was 4.1 months.

Changes in transplantation patterns over time

In the first decade (1.1.1988-31.12.1997) 61 PBC patients were transplanted compared to 44 patients in the second decade (1.1.1998-31.12.2007). In the first decade PBC was the cause of liver disease in 11.7% of individuals undergoing transplantation compared to 4.5% in the second decade.

Figure 1 visualizes a trend to a decrease in the absolute numbers of LTX for PBC, including 5 patients who died on the waiting list (p=0.17), the proportional decrease

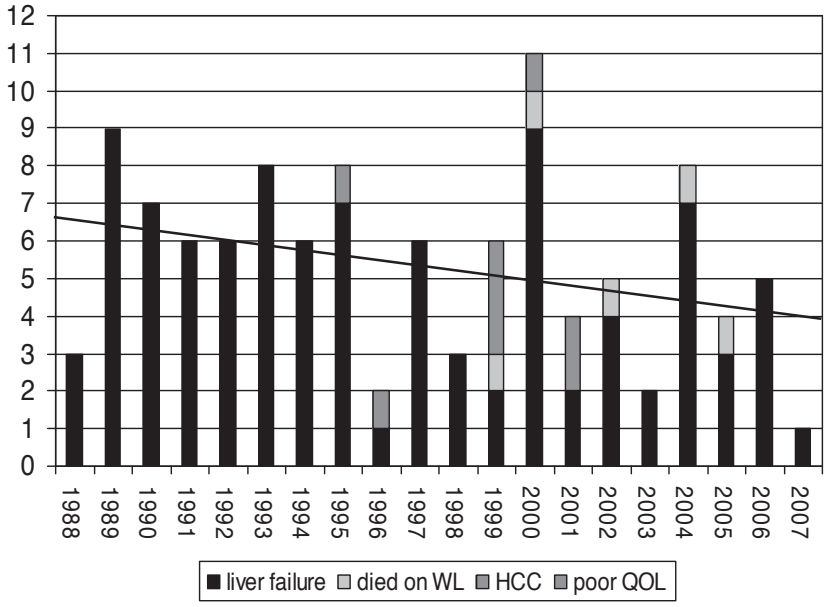

Figure 1. Indications, waiting list mortality and annual absolute number of first-time liver transplantations in PBC in the Netherlands from 1988 to 2008. The linear interpolation line indicates a decrease over time.

is significant ($p<0.001$) (Figure 2). As shown in Table 1, the age of patients at the time of PBC diagnosis, listing and transplantation was comparable for the two decades. Bilirubin and ALP levels were lower in the second decade both at the time of PBC diagnosis and at the time of listing and surgery. Also, the MELD scores and the proportion of patients with advanced PBC at the time of transplantation decreased over time, $p=0.14$ and $p=0.03$ respectively (trend-test).

The median interval from PBC diagnosis to the moment of LTX was 86.5 months in the first decade, and 93.5 months in the second decade. The median time on the waiting list was 2.3 months and 8.5 months, respectively.

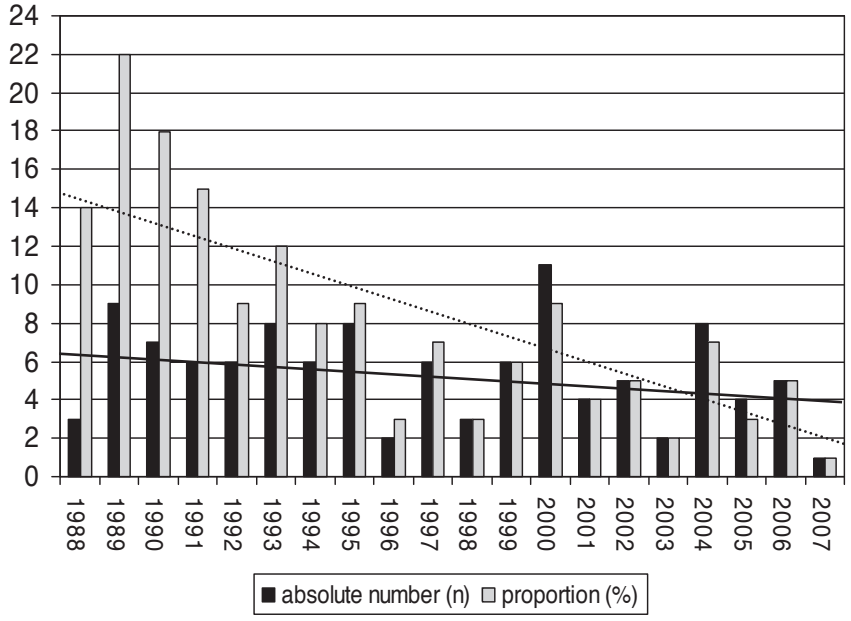

Figure 2. Annual absolute number of first-time liver transplantations versus proportional number in the Netherlands from 1988 to 2008. The linear interpolation lines indicate a decrease over time.

LTX & indication

The indication for LTX was liver failure in 97 (92%) cases (Figure 1). 1 (1%) patient underwent liver transplantation for the presence of hepatocellular carcinoma and 7 (7%) patients for poor quality of life due to fatigue or pruritus. In the latter group, the mean age at the time of LTX was 48.3 years, the median interval between diagnosis and transplantation was 58.8 months, and the median time on the waiting list was

6.0 months. 6/7 (86%) of patients grafted for poor quality of life were transplanted early in the second decade (Figure 1).

Mortality on the waiting list

In total, 5/110 (5%) patients (4 female) died on the liver transplant waiting list. Their mean age at the time of listing was 55.4 years, the mean MELD score was 11.3 and the median time from listing to death was 4.9 months. All deaths occurred between 1998 and 2008, corresponding with 10% (5/49) waiting list mortality in that period. Causes of death were liver failure in 4 cases and heart failure in 1.

UDCA Treatment

In the first decade 38% of patients listed for transplantation had been treated with UDCA for at least 2 years versus 82% in the second decade.
For the total group of patients the (mean) time they had been treated with UDCA increased from 21.5 months in the first decade to 71.1 months in the second decade. The mean duration of UDCA treatment for the entire period analyzed was 42.3 months.

DISCUSSION

The results of this study show that both the absolute and the relative number of patients who received a liver transplantation for the indication PBC has fallen during the past 20 years in the Netherlands. The severity of disease at the time of transplantation, as indicated by serum levels of bilirubin and albumin and by MELD scores, slightly decreased while the age of the patients at the time of listing and transplantation remained unchanged.
Our results are in agreement with a recent study from the USA [1]. From 1995 to 2006 the authors observed a significant decrease in the need for liver transplantation in PBC while the total number of transplantations increased and the number of transplantations for another chronic cholestatic liver disease, namely PSC, remained stable. The introduction of UDCA as standard therapy of PBC was considered the most likely explanation for the observed decline. Our data are also compatible with a single center study from Birmingham UK, reporting a decrease in the absolute and proportional number of transplantations for PBC between 1983 and 1999. The authors also noted less advanced disease at the time of transplantation for PBC

over time [2]. We cannot confirm another finding of this study, namely that the age of patients at the moment of transplantation tends to increase.

A possible explanation for our findings remains uncertain. Obviously, the actual number of liver transplantations for PBC is influenced by numerous complex factors including the incidence and natural history of the disease over time, the availability of effective medical treatment, referral patterns, selection criteria and the availability of donor organs. The incidence of PBC in our country has not been defined and therefore the potential importance of changes in time cannot be addressed properly. A noticeable increased incidence of PBC has been documented in the UK while other data suggest that the incidence worldwide at least seems stable but may be increasing [11; 12]. If this also applies to our country an increase or stable number of transplantations - and not a decrease - would have been more likely. It has been established that ursodeoxycholic acid has a beneficial effect in PBC and may improve prognosis, in particular in cases showing a clear biochemical response to treatment [5].

Given the almost uniform treatment of PBC with UDCA during the last 10-15 years in the Netherlands, the use of UDCA could well be a factor contributing to the observed changes. However, it is obvious that the present study was neither designed nor suitable to adequately assess the efficacy of UDCA in PBC. In the absence of data on possible changes in referral patterns for liver transplantation the significance of this factor remains unknown. With respect to selection criteria in general there was a change over time towards acceptance of older patients. However, our data show that the age at the time of listing and at the time of surgery in PBC patients in fact did not change during the 20 year study period, similar to findings in the USA [1]. Only few individuals were transplanted for other indications than liver failure, and thus changes in selection criteria are not likely to be involved in the observed trend toward less transplantation for PBC.

This was a retrospective study and the absolute number of patients was limited. We recognize that we were unable to assess all potential factors influencing the need and actual number of liver transplantations performed in the Netherlands. On the other hand we included all patients listed and/or transplanted for PBC in our country and were able to collect and analyze relevant detailed data for all cases.

In conclusion, our study indicates that both the absolute and the relative number of liver transplantations for PBC tended to decrease during the past two decades. The general introduction of UDCA as the standard treatment for PBC may be a factor

explaining this trend over time. However, other mechanisms could well be involved given the many complex factors determining the eventual number of patients referred for, and finally undergoing, transplantation.

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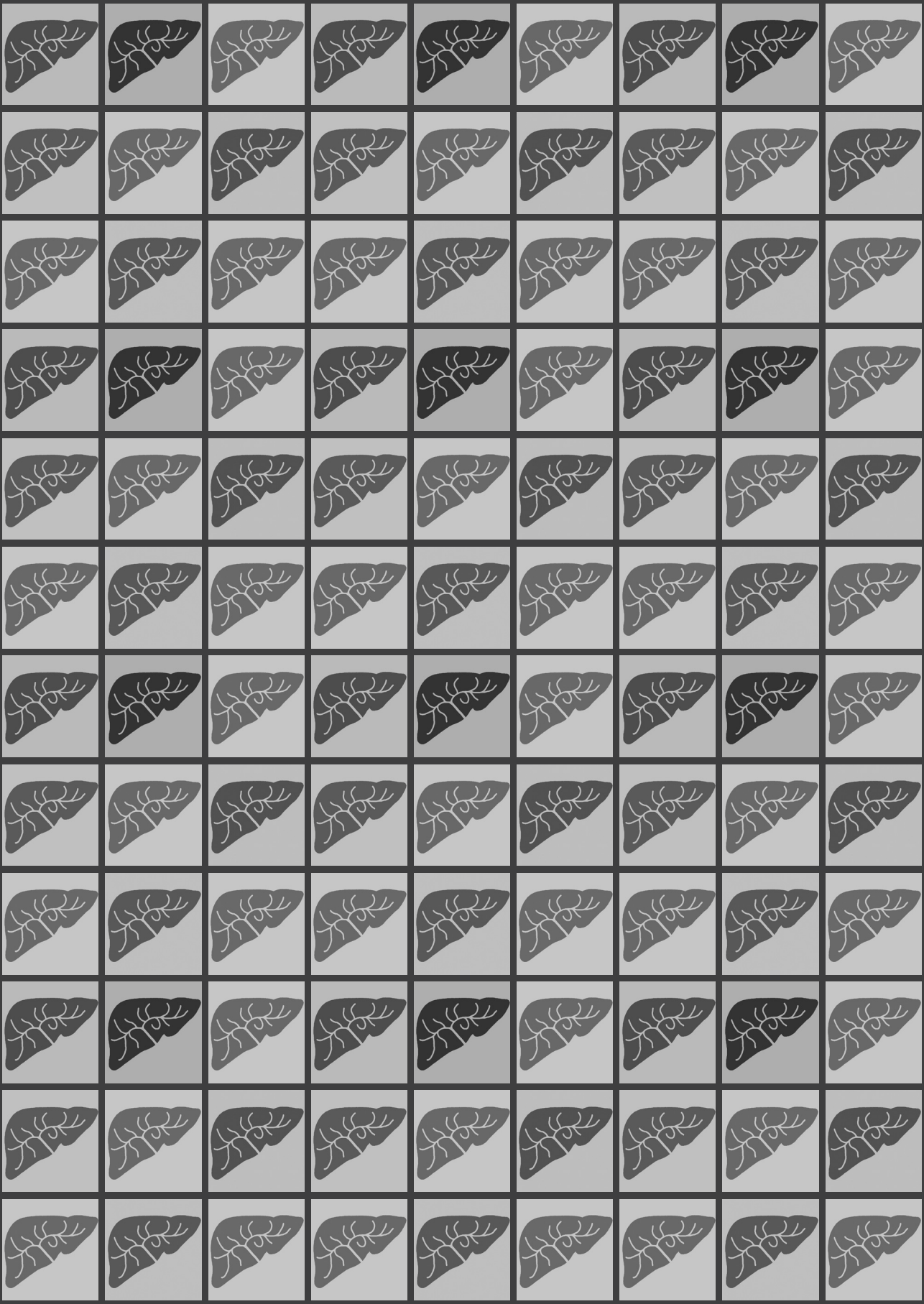

Paris criteria are effective in diagnosis of primary biliary cirrhosis and autoimmune hepatitis overlap syndrome

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ABSTRACT

Background and aims

Primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) differ in clinical, laboratory, and histological features as well as in response to therapy. A small subgroup of patients has an 'overlap syndrome' with features of both diseases, although there is no consensus on its definition or diagnostic criteria. We evaluated the significance of the criteria used to diagnose PBC-AIH overlap syndrome.

Patients and Methods

This retrospective, single-centre study included all patients diagnosed with PBC, AIH, or PBC-AIH overlap syndrome, based on the Paris criteria, since January 1990 (n=134); patients were followed for 9.7±3.7 years. The 3 groups were compared for their clinical, laboratory, and histological features. Patients with overlap syndrome or PBC were graded by the revised and simplified AIH scoring systems, to assess the ability of this system to properly identify AIH cases.

Results

The sensitivity and specificity of the Paris criteria for diagnosing the overlap syndrome were 92% and 97%, respectively. The sensitivity and specificity of the AIH scoring systems were considerably lower. Among patients with the overlap syndrome, 10-year-transplantation-free survival rate was 92%.

Conclusions

The Paris diagnostic criteria detect overlap syndrome (PBC and AIH) with high levels of sensitivity and specificity. The clinical value of the revised and simplified AIH scoring system is not as reliable. Patients with PBC-AIH overlap syndrome have a 92% rate of 10-year-transplantation-free survival.

INTRODUCTION

Primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) are immune-mediated chronic liver diseases with clear differences in clinical, biochemical, serological and histological features [1; 2]. Mainly due to the high diagnostic sensitivity and specificity of antimitochondrial antibodies (AMA) the diagnosis of PBC is usually straightforward. Although AIH can be diagnosed without much difficulty in patients with classical presentation, in clinical practice the diagnosis is frequently difficult and not straightforward.

PBC and AIH can occur simultaneously [3; 4] or consecutively [5; 6]. Up till now standardization of diagnostic criteria for overlap syndrome has not been achieved and the long-term prognosis of the syndrome is poorly defined [7]. Several problems are encountered in diagnosing patients with possible overlap syndromes. These include features of AIH being found in many patients with PBC, such as (mild) interface hepatitis, elevated IgG serum levels and presence of anti-smooth muscle antibodies. Also, patients with AIH may have features of PBC such as biliary abnormalities, elevated serum IgM levels and AMA-positivity. Chazouillères et al. proposed detailed diagnostic criteria for the PBC-AIH overlap syndrome (Paris criteria) [4], but to our knowledge these have never been validated.

For AIH diagnostic numerical scoring systems have been developed by the International Autoimmune Hepatitis Group [8-12]. These scoring systems were not designed for diagnosing AIH in patients with PBC. This is illustrated by the fact that presence of AMA or biliary changes on liver biopsy has a negative impact on the diagnostic scores. Recently simplified criteria for AIH were proposed, and in this scoring system presence of AMA or histological features of PBC were no longer taken into account [13].

In this study we aimed to evaluate the value and accuracy of the Paris criteria and of the revised and simplified autoimmune hepatitis scoring systems in the diagnosis of the PBC-AIH overlap syndrome.

PATIENTS AND METHODS

Patients

For this retrospective study we collected and analyzed data of all patients diagnosed with PBC, AIH and the overlap syndrome between January 1990 and January

2008 and who were followed up in our hospital, a referral centre for liver disease and transplantation. For PBC patients data were retrieved from the prospective database of the Dutch Multicenter PBC cohort [14]. For AIH patients an automated search in the hospital patient registration system was performed for "autoimmune hepatitis". Only patients satisfying the criteria of the revised AIH score for probable or definite AIH before or after treatment were included [8]. Patients with an overlap syndrome were identified by reviewing the PBC database, which includes data on patients with a clinical diagnosis of both PBC and AIH. Exclusion criteria were age >75 years, pregnancy, evidence of extra hepatic biliary disease, concomitant disorders limiting life expectancy, decompensated liver disease, defined as Child Pugh B or C cirrhosis and patients with PSC, as were the exclusion criteria for the Dutch PBC cohort.

Then we tested whether these patients satisfied the Paris criteria for overlap syndrome [4]. These require presence of at least two of the three accepted criteria for diagnosis of PBC and AIH. The diagnostic criteria for PBC are: 1. serum ALP ≥ 2-fold the upper limit of normal (ULN) or serum GGT levels ≥ 5-fold ULN; 2. positive test for AMA; 3. liver biopsy specimen showing florid bile duct lesions. The diagnostic criteria for AIH are: 1. serum ALT levels ≥ 5-fold ULN; 2. IgG levels ≥ 2-fold ULN or a positive test for SMA; and 3. liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis . Subsequently, the PBC and overlap cases, as defined by the Paris criteria, were graded by the revised (before treatment) and the simplified AIH scoring systems [8; 13].

Data collected from medical records included date of diagnosis and start of therapy, serological studies at diagnosis including AMA (positive if titre ≥1:20), antinuclear antibodies (ANA) (positive if titre >1:40) and smooth muscle antigen (SMA) (positive if titre >1:40), laboratory results including alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine aminotransferase (ALT), bilirubin, albumin, platelet count, cholesterol, IgG and IgM.

Available biopsies at diagnosis were blindly reviewed (PEZ). The histological activity index was used to semi-quantify interface hepatitis (0-4), extent of portal inflammation (0-4) and fibrosis (Ishak 0-6). Bile ducts were categorised as: 1. no biliary changes, 2. non-destructive cholangitis, 3. destructive cholangitis (including florid bile duct lesions) or 4. ductopenia. Destructive cholangitis was further classified as florid bile duct lesion when granulomatous destruction of interlobular and septal bile ducts was observed. Biopsies were also assessed according to Ludwig [15].

Finally, biopsies were classified either as 1. atypical AIH, 2. compatible with AIH or 3. typical AIH, according to the degree of interface hepatitis, lymphoplasmacytic infiltrate and rosetting as previously described [13].

All patients with PBC were treated with ursodeoxycholic acid 13-15 mg/kg/day while patients with AIH were treated with immunosuppressive therapy, in the large majority of cases with prednisone and/or azathioprine. The patients with overlap syndrome were all treated with a combination of prednisone, azathioprine and ursodeoxycholic acid.

Statistical analysis

Quantitative data were compared using parametric and non-parametric tests. Survival was analyzed as transplantation-free survival (end-points: liver transplantation and death). For time-to-event analysis we applied the Kaplan-Meier method. A p-value <0.05 (two-tailed) was considered statistically significant. Statistical package SPSS 15.0 was used to perform analyses.

RESULTS

One hundred thirty-four patients, 17 males and 117 females with a mean age of 46 (SD ± 15) years were included. The mean duration of follow-up was 9.7 (SD ± 3.7) years. Follow-up till death, liver transplantation or end of the study was complete for all subjects.

Among the 134 patients included in the study, we identified 12 patients with a clinical diagnosis of overlap syndrome. In all cases PBC and AIH had been diagnosed simultaneously. These patients were characterized by relatively high serum transaminases and IgG levels and prominent histological features of AIH. Eleven (92%) of these patients met Chazouillères criteria for overlap syndrome. The single patient not meeting these criteria was a female with ALP >2 ULN, ALT >5 ULN and liver biopsy showing a florid bile duct lesion, and features compatible but not characteristic for AIH, she tested negative for AMA and SMA and IgG levels were normal.

We identified 65/134 patients with AIH according to the revised criteria for probable and definite AIH. Four (6%) of these patients satisfied the Paris criteria for overlap syndrome. In contrast, none of the 57/134 PBC cases, as defined by Taal [16], suffered

from an overlap syndrome according to these criteria. The resulting sensitivity of the Paris criteria for the overlap syndrome was 92% and the specificity was 97%. Altogether, 15 patients fulfilled the Paris criteria, 12 patients fulfilled two out of three criteria for PBC and two out of three criteria for AIH, the remaining 3 patients fulfilled 3 criteria for PBC and two criteria for AIH.

Comparison of patients with overlap syndrome, PBC and AIH

Using the Paris criteria as the gold standard for the diagnosis of overlap syndrome, the 132 study subjects were (re)allocated to groups with PBC (n=57, 43%), AIH (n=62, 46%) and overlap syndrome (n=15, 11%).

As shown in Table 1, overlap patients were comparable to PBC patients regarding ALP levels and to AIH patients regarding ALT and bilirubin levels at diagnosis. Albumin levels were comparable for all groups. AST, IgM and IgG levels in patients with overlap syndrome were in between levels for PBC and AIH.

Table 1. Baseline patient characteristics

	PBC (n=57)	Overlap syndrome (n=15)	AIH (n=62)
Age (yrs; mean ± SD)	51 ± 10*	43 ± 15	42 ± 18
Male/Female (n,%)	3(5)/54(95)	3(20)/12(80)	11(18)/51(82)
Follow-up (yrs; median)	9.4	8.6	9.3
AMA positive (n,%)	49 (86)*	9 (60)	6 (10)**
Titer (1:n, median)	640 (0-20480)*	80 (0-2560)	0 (0-1280)**
ANA positive (n,%)	18 (32)*	10 (67)	40 (65)
Titer (1:n, median)	0 (0-5120)*	320 (0-10240)	80 (0-5120)
SMA positive (n,%)	3 (5)*	4 (27)	20 (32)
Titer (1:n, median)	0 (0-640)*	0 (0-640)	0 (0-2560)
ALP (ULN, range)	2.5 (0.6-7.8)	2.6 (1.6-9.5)	1.3 (0.5-9.4)**
AST (ULN, range)	1.6 (0.3-4.7)**	6.2 (1.4-60.7)	14.6 (0.7-114.7)*
ALT (ULN, range)	1.9 (0.4-8.6)**	11.2 (1.9-104.7)	14.7 (0.6-101.1)
Bilirubin (ULN, range)	0.8 (0.2-4.2)**	1.5 (0.6-27.9)	2.3 (0.2-50.8)
Albumin (LLN, range)	1.2 (1.0-1.4)	1.1 (0.7-1.5)	1.1 (0.6-1.4)
IgM (ULN, range)	1.7 (0.5-9.2)	1.1 (0.1-6.3)	0.9 (0.2-3.2)**
IgG (ULN, range)	1.0 (0.6-2.0)	1.9 (0.6-3.3)	1.7 (0.8-6.6)*

* Significant difference compared to overlap syndrome p=0.05-p=0.001
** Significant difference compared to overlap syndrome; p<0.001
ULN: ratio test result to upper limit of normal range
LLN: ratio test result to lower limit of normal range

Serology

Antimitochondrial antibodies (AMA) were detected in 60% of patients with overlap syndrome and in 86% of patients with PBC, but also in 10% of patients with AIH. Titers were highest in PBC and lowest in AIH. Antinuclear antibodies (ANA) were identified in 67% of the overlap cases, and smooth muscle antibodies (SMA) in 27%. This was comparable for patients with AIH (65% and 32% respectively) (Table 1).

Histology

Florid bile duct lesions were observed in 67% of the overlap patients, and this was more frequent than in patients with PBC (33%) and AIH (6%) Conversely, the total number of biliary changes, including non-destructive cholangitis, destructive cholangitis (including florid bile duct lesions) and ductopenia, was almost comparable for PBC (74%), overlap syndrome (87%) and AIH (72%).
Histological findings were compatible with, or typical for, AIH in 100% of patients with overlap syndrome, 60% of patients with PBC and 81% with AIH (Table 2).

Table 2. Histological findings in patients with PBC, overlap syndrome and AIH.

	PBC (n=57)	Overlap syndrome (n=15)	AIH (n=62)
	n (%)	n (%)	n (%)
non-destructive cholangitis	6 (11)	0 (0)	26 (42)
Florid bile duct lesion	19 (33)	10 (67)	4 (6)
ductopenia	8 (14)	2 (13)	4 (6)
atypical AIH	10 (18)	0 (0)	8 (13)
compatible AIH	22 (39)	2 (13)	24 (39)
typical AIH	12 (21)	13 (87)	26 (42)
Ishak fibrosis score 0	1 (2)	0 (0)	0 (0)
1	8 (14)	0 (0)	3 (5)
2	8 (14)	2 (13)	6 (10)
3	17 (30)	6 (40)	19 (31)
4	3 (5)	2 (13)	11 (18)
5	4 (7)	2 (13)	7 (11)
6	2 (4)	3 (20)	9 (15)

Diagnostic performance of revised and simplified AIH scores

Patients with PBC and with the overlap syndrome were graded according to the revised and simplified AIH scoring systems and the sensitivity and specificity of the

resulting scores for identifying individuals with AIH were determined (Table 3). For definitive AIH, the sensitivity of the revised system was 0% and that of the simplified system was 40%. For definitive and probable AIH together, the sensitivity was 60% and 73%, respectively. The specificity of the simplified scoring system for definitive AIH was 80% and this was comparable to that of the revised scoring system (79%).

Table 3. Diagnostic performance of tests for overlap syndrome of PBC and AIH

	rAIH *)		sAIH *)	
	definite	def + prob	definite	def + prob
Sensitivity	0%	60%	40%	73%
Specificity	79%	83%	80%	78%

*) in patients with PBC and OS
def: definitive AIH
prob: probable AIH

Survival

During follow-up 4 patients died from liver related causes and 9 from other causes. Seven patients underwent liver transplantation. In patients with overlap syndrome the 5-year-transplantation free survival was 100%, in PBC patients 96% and in AIH patients 100%. 10-year-survival was 92% for the overlap syndrome, 81% for PBC and 88% for AIH.

DISCUSSION

As far as we are aware, this is the first study attempting to validate the Paris criteria [4] for identifying patients with an overlap syndrome of PBC and AIH. Our results suggest that these criteria are valuable and may be superior to the revised and also the simplified autoimmune hepatitis scoring systems for recognizing patients with both PBC and AIH. This study also indicates that the long-term prognosis of patients with overlap syndrome is good.

Studies on PBC – AIH overlap syndrome are severely hindered by the lack of a generally accepted diagnostic gold standard. As a consequence, the diagnostic criteria for overlap syndrome in previous studies varied widely and included the revised AIH scoring system [17] and the Paris criteria [4] applied in groups of patients with PBC, and variable sets of criteria in groups of patients with autoimmune liver disease [15; 18; 19]. For assessing the usefulness of the Paris criteria we arbitrarily considered

patients who we believed - based on clinical, laboratory and histological findings but without applying strict diagnostic criteria – to represent cases with overlap syndrome as the reference group. Interestingly, the diagnostic accuracy of the Paris criteria for patient groups classified accordingly proved to be high. For assessing the diagnostic performance of the revised and simplified autoimmune hepatitis scoring systems, patients with AIH could not be taken into account since this group was selected using the revised scoring system. Therefore, a direct comparison of the diagnostic accuracy of the Paris criteria and the AIH scoring systems was not possible from a methodological point of view. As a consequence, we feel that our results with respect to the diagnostic value of these scores should be considered as indicative.

Thus far, all studies on the PBC-AIH overlap syndrome had a retrospective character and have been relatively small in terms of patient number and duration of follow-up. These characteristics also apply to the present study although the duration of follow-up was nearly ten years. A difficulty in our study was the retrospective allocation of 4 cases to the overlap group. These patients, originally diagnosed with AIH, had not been diagnosed and treated as having PBC. Therefore, the group of patients with overlap syndrome, as defined by the Paris criteria, was not completely homogenous with respect to treatment.

Not unexpectedly, the sensitivity of the simplified AIH scoring system for overlap syndrome was better than that of the revised system. This can be explained by negative scores incurred from biliary abnormalities on histological assessment or presence of AMA using the revised system. The specificity of the revised system was comparable to that of the simplified system. In this context it should be kept in mind that these scoring systems were not specifically devised for diagnosing overlap syndromes of AIH and other disorders.

Few reported data are available on the optimal therapy and long term prognosis of the PBC- AIH overlap syndrome. Several case series indicate that the therapy of choice is a combination of immunosuppressive drugs and ursodeoxycholic acid [3-5; 20]. The long term course of the PBC-AIH overlap has not been well defined. Silveira et al reported that the 10-year transplantation-free survival rate was about 55%, which was significantly shorter than for patients with PBC [17]. In another recent series the 10-year survival rate was about 85% and this was comparable with survival for AIH [18]. We found a 10-year survival of 92% for patients with the overlap syndrome. A possible explanation for this good prognosis may be the systematic

use of combined ursodeoxycholic acid/immunosuppressive treatment [20]. However, it must be stressed that it is difficult to compare these studies given the fundamental differences in diagnostic criteria. Clearly, more studies are needed, in particular studies using uniform diagnostic criteria.

AMA were detected in 10% of our patients with AIH. These findings are comparable to those reported by other groups who detected AMA in 12-18% of patients with AIH not considered to suffer from an overlap syndrome [21; 22]. The response to therapy and long-term clinical course were found to be indicative for AIH and no evolution to a cholestatic syndrome was reported. In AIH AMA can be present permanently or temporarily and AMA may also appear in individuals who tested negative on earlier occasions [21]. These data indicate that AMA positive AIH is not rare and further suggest that it does not represent a separate clinical entity.

The blind re-evaluation of liver biopsies revealed that in nearly 50 percent of individuals with PBC liver histology showed destructive cholangitis. In other reported series this percentage varied from 20% to 65% [3; 23; 24]. We also found a surprisingly high prevalence of bile duct changes in our patients with AIH. A total of 72% had some type of biliary abnormality, including non-destructive cholangitis in 43% and destructive cholangitis in 24%, key features of PBC. This is in agreement with a previous study of Czaja et al [25], who found that patients with AIH with and without biliary changes do not differ regarding clinical features or treatment response. In that study, however, which included only patients with AIH, the overall prevalence of biliary changes was 24% and that of destructive cholangitis 7%, frequencies considerably lower than in the present study. We have no clear explanation for these diverging results. All patients with AIH fulfilled accepted diagnostic criteria and, with respect to gender, age, serum liver test results and immunological findings, were comparable with groups of patients with AIH reported in literature [26-28].

In conclusion, we attempted to confirm the value of the diagnostic Paris criteria for the overlap syndrome of PBC and AIH. Our results confirm that these criteria are useful and suggest that they may be more helpful than the numerical AIH scoring systems, which were not created for diagnosing overlap syndromes. This is completely in line with the recently published EASL clinical practice guidelines for the management of cholestatic liver disease [29]. International consensus on the diagnostic criteria for overlap syndromes is absolutely necessary.

From a practical point of view, diagnosing an overlap syndrome should have therapeutic consequences. Based on our experience, we believe that the Paris criteria

should be sincerely considered as the diagnostic criteria of choice. Our data show that the prognosis of patients with overlapping PBC and AIH is excellent.

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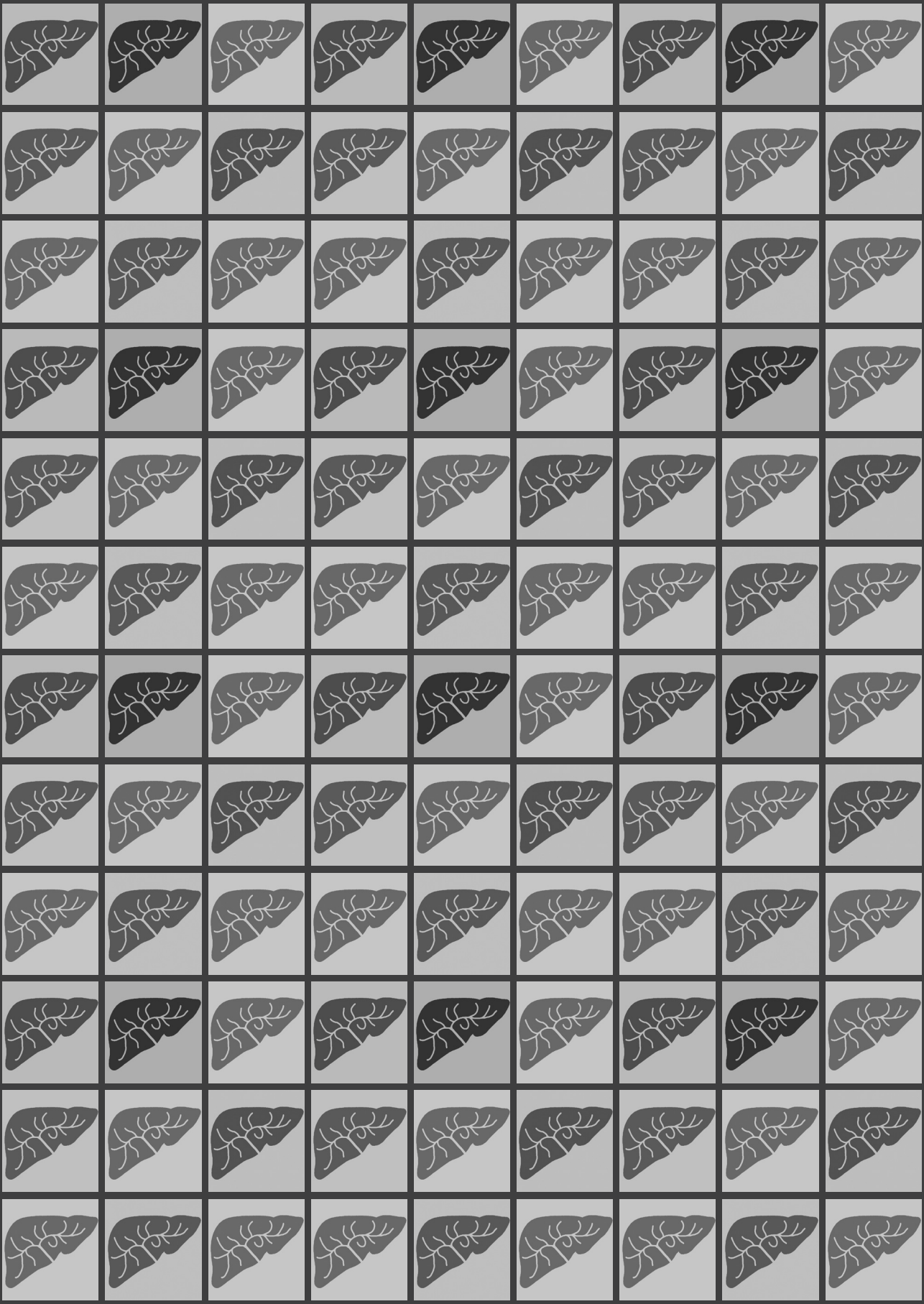

The potent bile acid sequestrant colesevelam is not effective in cholestatic pruritus: Results of a double-blind, randomized, placebo-controlled trial

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ABSTRACT

Background and Aims

Colesevelam is an anion exchange resin with a 7-fold higher bile acid binding capacity and fewer side effects than cholestyramine, the current first line treatment option in cholestatic pruritus. The aim of this trial was to compare the effect of colesevelam with placebo in patients with cholestatic pruritus.

Patients and Methods

In a randomized, double-blind, investigator-initiated multicentre trial, patients with cholestatic pruritus, both treatment naïve and previously treated, received 1875 mg of colesevelam or identical placebo twice daily during three weeks. The effect on pruritus was assessed by daily visual analogue scales (VAS), quality of life scores (QOL) and evaluation of cutaneous scratch lesions. The predefined primary endpoint was the proportion of patients with at least a 40% reduction in VAS pruritus scores.

Results

38 patients were included, of which 35 patients were evaluable: 17 colesevelam, 18 placebo, 22 females, 8 treatment naïve, primary biliary cirrhosis n=14, primary sclerosing cholangitis n=14. Mean serum bile acid level was comparable between groups before treatment (p=0.74), and significantly different after treatment (p=0.01) in favor of patients treated with colesevelam. 36% of patients in the colesevelam group reached the primary endpoint compared to 35% in the placebo group (p=1.0). There were no significant differences between the groups with respect to pruritus scores, QOL scores and severity of cutaneous scratch lesions. Mild side effects occurred in one colesevelam and four placebo treated patients.

Conclusions

Although colesevelam significantly decreased serum bile acid levels, this trial was unable to demonstrate that it was more effective than placebo in alleviating the severity of pruritus of cholestasis.

INTRODUCTION

Pruritus is a frequent and debilitating symptom in cholestatic liver disease [1]. Although the pathophysiology of pruritus secondary to cholestasis remains largely unknown it is widely assumed that bile acids are etiologically involved [2; 3]. The principal pharmacological treatment options currently available and recommended in recent guidelines [4] are cholestyramine [5; 6] (a non-absorbable bile-acid binding resin), rifampicin [7; 8], naltrexone [9; 10] and sertraline [11]. However, the efficacy of these drugs is variable and side-effects are common. Cholestyramine frequently causes constipation and nausea, rifampicin is known for its potential hepatotoxicity and patients on naltrexone may experience symptoms of an endogenous opioid-withdrawal syndrome. Therefore, treatment of cholestatic pruritus is currently often problematic and unsatisfactory and alternative treatment options are warranted.
Colesevelam (Cholestagel®) is a bile acid sequestrant taken in tablet form that hydrates to a gel and is being used for the treatment of hypercholesterolemia. This agent differs from other sequestrants in that the hydrophilic polymer backbone has abundant hydrophobic side chains facilitating the binding of bile acids. Also, it is better tolerated than other bile acid sequestrants such as cholestyramine and colestipol [12; 13]: specifically, no clinically significant difference was shown between placebo and colesevelam with regard to gastrointestinal complaints such as flatulence, constipation, diarrhoea, nausea and dyspepsia [14]. Until now, efficacy of colesevelam for ameliorating the pruritus of cholestasis was only explored in a small open study [15], which showed that colesevelam was effective in ameliorating pruritus in 5 out of 8 patients. The aim of the current trial was to assess the effect of colesevelam on cholestatic pruritus in a double-blind randomized placebo-controlled trial.

PATIENTS AND METHODS

This study is a double-blind, randomized, placebo-controlled, multicentre trial. The aim was to assess the efficacy of colesevelam versus placebo in cholestatic pruritus. Inclusion started in September 2008 and the follow-up was completed in October 2009. With approval of their medical ethical committees, three Dutch university hospitals participated in this trial; Erasmus University Medical Centre Rotterdam (EMC), University Medical Centre Utrecht (UMCU) and University Medical Centre Amsterdam (AMC). The trial was registered at www.clinicaltrials.gov (number

NCT00756171) and conducted, recorded and reported in compliance with ICH GCP and national regulations. All consecutive patients with cholestatic pruritus, both treatment naïve and previously treated, who visited our out-patient clinic, were asked to participate. Inclusion criteria were presence of pruritus of any severity as a result of any cholestatic disorder, age >18 years and informed consent. Exclusion criteria were use of cholestyramine within three weeks before the evaluation, pregnancy, no ability to understand or speak the Dutch language, malignancy was present or life expectancy less than 6 months.

Patients received Cholestagel ® (colesevelam HCL), a hydrophilic water-insoluble non-absorbable agent, or placebo tablets of identical shape and taste, taken orally in a dose of 3 tablets of 625 mg twice daily. The total individual treatment period was 21 days. Randomization was centralized, using opaque serial numbered envelopes prepared by the trial statistician. Participants were assigned to one of the 2 arms according to a standard randomization schedule 1:1, in blocks of 4 and stratified per trial centre (EMC, AMC and UMCU). Both participants and investigators were blinded. Participants visited the outpatient clinic 3 times; the first time for screening, the second time before start of treatment, the third time at the end of treatment. After 1 week of treatment they were contacted by telephone to address potential problems and questions and to assess potential side effects. Patients were further instructed to call the principle investigator when necessary.

The intensity of symptoms was quantified by means of Visual Analogue Scales (VAS) in the morning and in the evening. The VAS pruritus score varied from score 0 (no pruritus) to 10 (severe pruritus). Fatigue and quality of sleep were evaluated using comparable VAS scores. Patients were asked to complete the VAS pruritus scores starting 7 days before the actual start or treatment (T=0). The severity of pruritus was further quantified by open categorized questions, and by describing the nature and extent of cutaneous scratch lesions, classified as follows: 1. excoriations (mild), 2. plaques (moderate), 3. nodules (severe) or 4. indifferent scars. Additionally, using identical cameras with the same settings, photographs were taken from the front and backside of the extremities and the trunk before treatment and at the end of treatment. The severity of skin lesions was scored based on these photographs by an experienced dermatologist (HBT), who was blinded with respect to treatment allocation and sequence of photographs. The participants completed two validated Quality of life scores (Short Form (SF)-36 and the Liver Disease Symptom Index (LDSI) 2.0) before and after treatment [16; 17]. Finally, laboratory investigations,

including total serum bile acids, total bilirubin, albumin, alkaline phosphatase (ALP), ASAT and ALAT were performed before and at the end of treatment. Possible adverse events were assessed after 7 and 21 days of treatment.
When patients were using UDCA this treatment was continued. To prevent interference with the absorption of UDCA and other drugs including levothyroxine, glyburide and oral contraceptives, a minimal interval of 4 hours between the intake of the study medication and these drugs was advised. Antipruritic drugs other than the study medication were stopped, with a wash-out period of at least 3 weeks. However, patients were allowed to continue rifampicin and naltrexone in a stable dose if they felt these agents were of (some) benefit. No other antipruritic drug was allowed during the trial when patients experienced worsening of pruritus.

Definitions and statistical methods

The predefined primary endpoint of this study was the proportion of patients per group with at least a 40% reduction of pruritus based on VAS scores by comparing the mean scores on day T=18,19 and 20 with the mean scores from T=-2, -1 and 0. Secondary endpoints were an improvement in quality of life scores and a reduction in severity of scratch lesions after 21 days in comparison to pre-treatment scores.
The power calculation (Fisher's exact test) was based on the primary outcome of 40% reduction in the severity of pruritus. Based on a study of Mayo et al [11] we expected this outcome in at least 50% of patients treated with colesevelam and in 5% of those treated with placebo. To detect this difference with a significance level of 0.05 and a power of 80%, and using a two-tailed test 17 participants had to be included in each treatment arm. Considering a 10% drop-out rate, the total number to be included was 38 patients. An interim analysis was not performed. Both a modified intention-to-treat analysis and a per protocol analyses were performed. Statistical differences were evaluated for 2 groups by both parametric and non-parametric tests. A p-value <.05 (two-tailed) was considered statistically significant. SPSS 15.0 was used to perform analyses.

RESULTS

Patients

We included and randomized a total number of 38 patients, of which 35 patients were analyzed since 1 patient withdrew participation after randomization and before

start of treatment, 1 patient stopped the intake of naltrexone during the trial period and 1 patient was unable to fill out the questionnaires. Both a modified intention to treat analysis (n=36) and a per protocol analysis (n=35) were performed, the results were concordant. For matters of clarity we decided to describe the 35 patients randomized and treated according to protocol.

Of these remaining 35 participants 17 were treated with colesevelam and 18 with placebo. Eight patients were treatment naïve while 27 patients had already been treated with one or more antipruritic drugs. Symptoms had been present for a median period of 24 (range 1-360) months. All 35 participants completed the trial. The collection of study data including questionnaires, VAS scores and laboratory studies was complete.

Both groups were comparable regarding age, baseline biochemistries and use of ursodeoxycholic acid (table 1). Regarding etiology, however, the majority of patients with primary biliary cirrhosis (PBC) was assigned to the placebo group (10/14). Conversely, the majority of the patients with primary sclerosing cholangitis (PSC) were assigned to the colesevelam group (10/14). Since PBC is a disease mostly affecting females while PSC predominantly affects males, this distribution explains the observed difference in male/female ratio between the two groups. Other aetiologies of cholestatic pruritus were alcoholic cirrhosis, cirrhotic hepatitis C, biliary atresia, sarcoidosis hepatis and ABCB4 (MDR-3) deficiency in one case each. In 2 cases the aetiology of the liver disease was cryptogenic. No patient reported an atopic constitution.

At entry, all participants graded the severity of pruritus as severe. In most patients (89%) pruritus was most severe in the evening and/or at night. Scratch lesions of any type or severity were present in 55% of cases. These lesions were found primarily on the extremities and the back. On average 10-30% of the total body area showed abnormalities secondary to scratching. In 34% of cases excoriations were seen, in 37% plaques, in 26% noduli and in 23% scars.

The use of other antipruritic medication was equal for the two treatment arms, with 2 patients in each group using naltrexone with incomplete effect. Side effects, no more than mild stool changes, were reported by 4 patients in the placebo group and by 1 in the colesevelam group. Dose reduction was not necessary; all patients continued the treatment during the 3-week period. The reported trial medication intake was 100 percent.

Table 1 Baseline characteristics

	Colesevelam N=17	Placebo N=18
Sex (male/female)	8 / 9	5 / 13
Age (years; mean, SD)	50 [13]	54 [13]
Etiology (PBC / PSC / other)	4 / 10 / 3	10 / 4 / 4
Bilirubin (ULN, SD)	2.1 (2.8)	2.0 (2.4)
ALP (ULN, SD)	2.5 (2.3)	1.9 (1.2)
Albumin (LLN, SD)	1.1 (0.2)	1.1 (0.2)
AST (ULN, SD)	2.2 (1.7)	2.2 (1.5)
ALT (ULN, SD)	2.0 (1.5)	1.6 (1.0)
MELD score (mean, SD)	7.2 (5.5)	6.8 (4.8)
Concurrent antipruritic treatment (rifampicin / naltrexone / sertaline)	0 / 2 / 0	0 / 2 / 0
n patients treated with UDCA	16	15
UDCA (mg/kg/day, mean, SD)	14.9 (6.0)	16.2 (4.0)
Total serum bile acids (umol/L, range) [#])	140 (32-440)	155 (9-550)

[#] normal <10 umol/L

Primary outcome

The primary outcome, the proportion of patients with at least a 40% reduction of pruritus VAS score after treatment did not show any significant difference between the colesevelam and placebo group. In the colesevelam group 36% of patients reached the defined 40% reduction of the pruritus VAS score in the morning versus 35% in the placebo group (p=1.0). Regarding the VAS pruritus score in the evening, a 40% reduction was noted in 40% and 50% of colesevelam and placebo treated patients, respectively (p=0.74).

According to an open categorized question, 100% of participants experienced severe pruritus before treatment. At the end of the treatment period, 76% of the colesevelam treated patients and 72 % of the placebo treated patients reported severe pruritus.

Regarding quality of sleep and fatigue VAS scores no statistically significant differences were found.

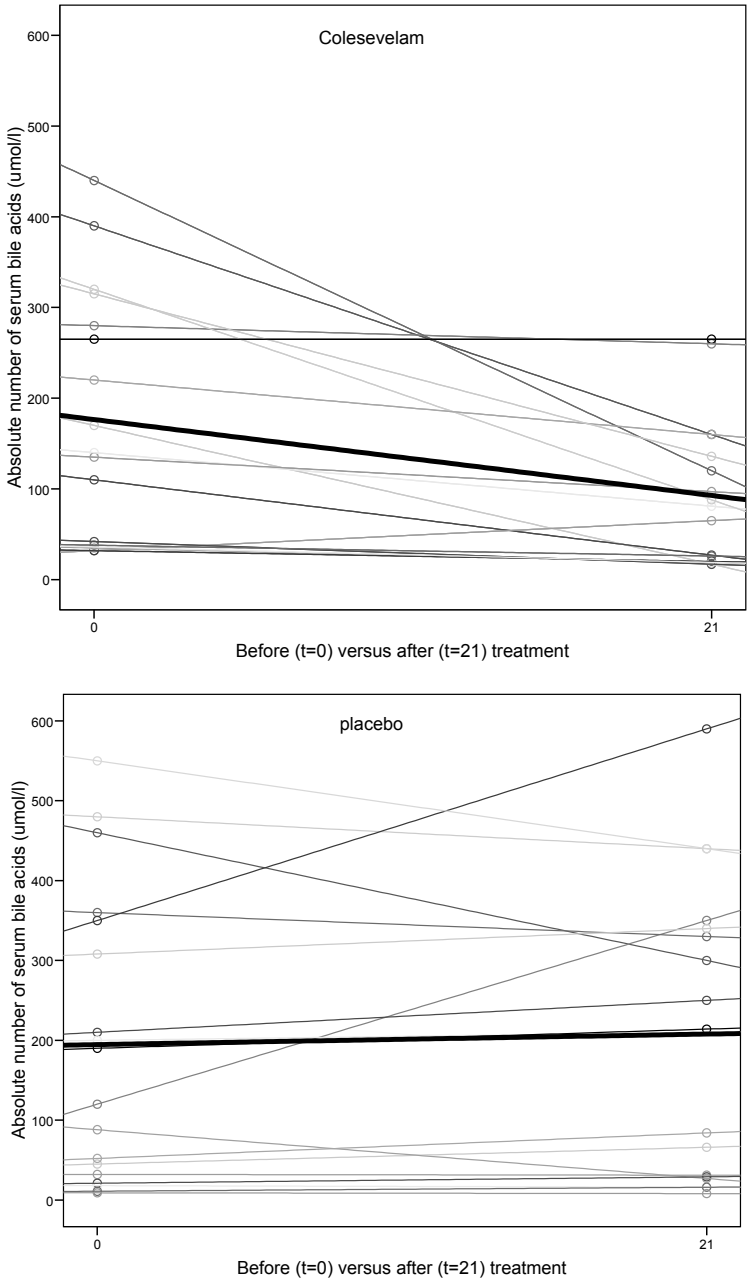


Figure 1. Individual serum bile acid levels before and after treatment for colesevelam (left figure) and placebo (right figure). The vet black line represents the interpolation line.

Serum bile acid and bilirubin levels before and after treatment

The median total serum bile acid level at baseline was 140 and 155 umol/l for the colesevelam and placebo group, respectively (p=0.74). During treatment levels decreased significantly in the colesevelam group to 73umol/l (p=0.003), whereas levels tended to increase (212 umol/l) in the placebo group (p=0.67) (Figure 1).

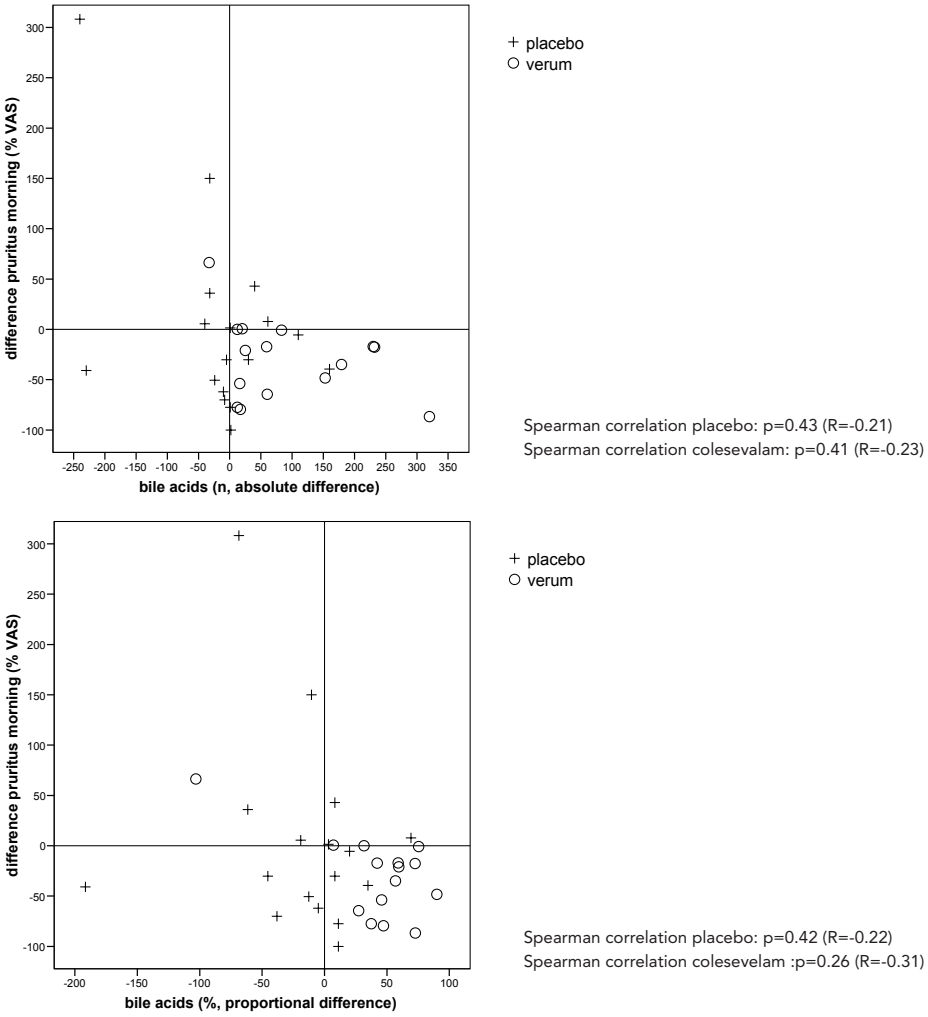


Figure 2. Absolute (upper figure) and proportional (lower figure) changes (T=0 vs T=21 days) in morning VAS pruritus scores and serum bile acid levels for individuals treated with colesevelam and placebo

After treatment, the serum bile acid level was significantly lower in the colesevelam group, compared to placebo (p=0.01).

Figure 2 shows the relation between changes in morning pruritus scores and changes in serum bile acid levels. In the majority of patients pruritus scores decreased, and this was associated with slightly increased mean serum bile acid levels in the placebo group and reduced levels in the colesevelam group. There was no significant correlation between these changes in either group (Spearman test). Bilirubin levels were comparable for placebo (1.1 x ULN) and colesevelam (1.8 x ULN) treated patients, both before (p=0.96) and after (p=0.27) treatment. Also, serum levels of ALP and transminases remained unchanged and were comparable for both groups.

Individual VAS scores

The individual pruritus VAS scores during the study are shown in Figure 3. The positive change in the mean morning pruritus VAS scores during the study period was statistically significant for colesevelam (p=0.01) but not for placebo (p=0.37). However, the difference between the colesevelam and placebo group was not statistically different (p=0.18).

The mean evening VAS scores decreased significantly in both the colesevelam (p=0.01) and placebo (p=0.03) groups, however there was no statistical difference between the two groups (p=0.70). As shown in Figure 4, in the majority of patients the pruritus VAS score decreased. Overall, the magnitude of a positive response (decrease in pruritus VAS score) was comparable for patients treated with colesevelam and placebo.

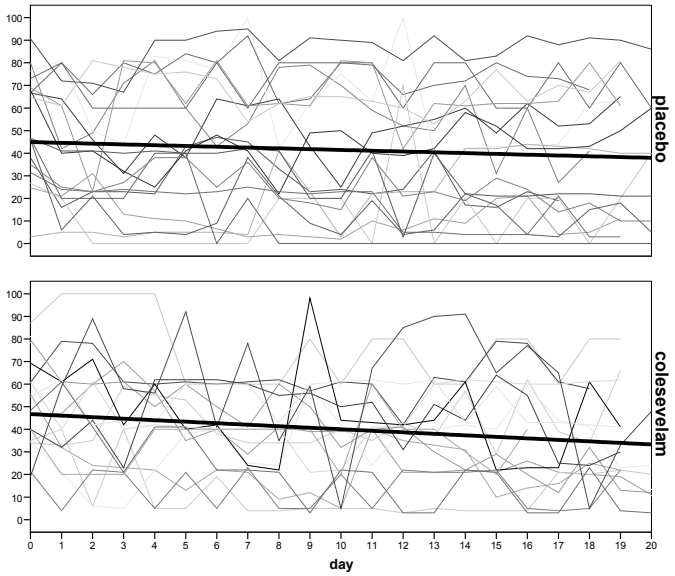
Dermatologic assessment

The dermatologic assessment, based on photographs taken before and after treatment, showed no significant differences between the two groups at entry with respect to the nature and severity of scratch lesions (p=1.0). Also, no significant changes were noted within or between the groups at the end of the trial (p=0.35).

Quality of life scores

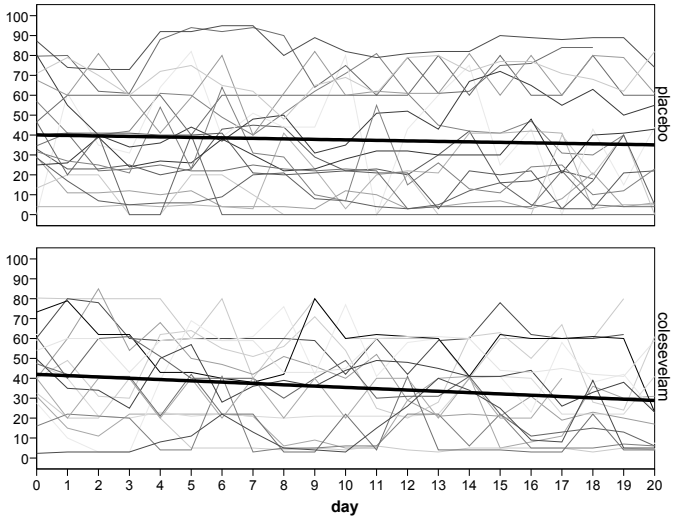
No statistically significant changes were found regarding the domains physical functioning (p=0.67), role physical functioning (p=0.50), bodily pain (p=1.00), general health (p=0.48), vitality (p=0.90), social functioning (p=0.37), emotional functioning (p=0.17) or mental health (p=0.26) of the Short Form 36 questionnaire,

a. VAS score morning.



interpolation line placebo p=0.03
interpolation line colesevelam p=0.01
colesevelam versus placebo p=0.70

b. VAS score evening.



interpolation line placebo p=0.37
interpolation line colesevelam 0.01
Colesevelam versus placebo p=0.18

Figure 3. Individual VAS pruritus scores

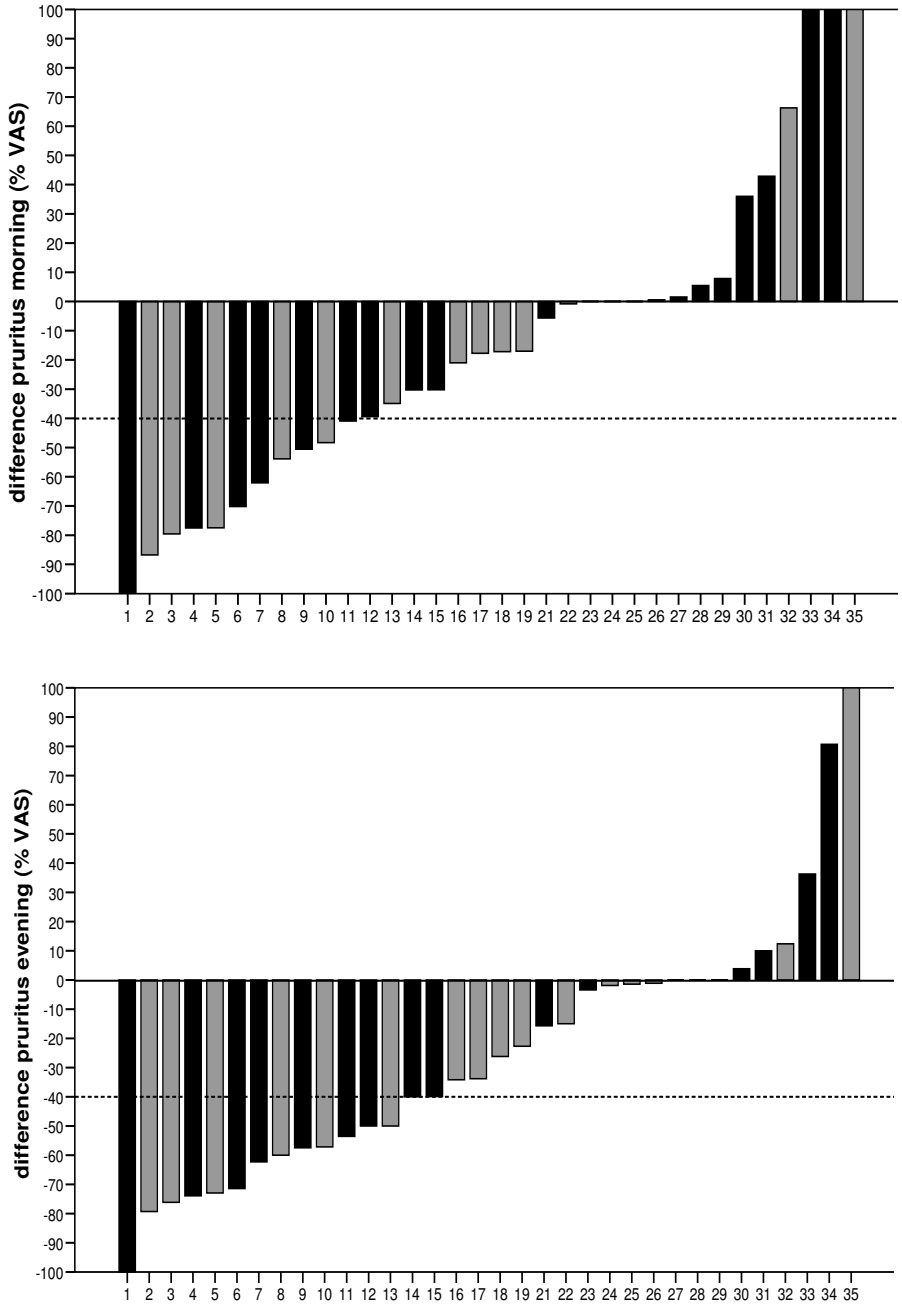


Figure 4. Individual proportional differences in VAS score for pruritus in the morning and in the evening.

The grey bars represent participants treated with colesevelam (n=17), the black bars represent participants treated with placebo (n=18).

before versus after treatment in the Colesevelam group. Both treatment groups were comparable before and after treatment. The Liver disease symptom index 2.0 revealed no significant differences either.

DISCUSSION

This trial shows that colesevelam significantly lowers serum bile acid levels in cholestatic individuals with pruritus but is not more effective than placebo in alleviating pruritus. Although patients treated with colesevelam experienced less pruritus this was also the case in patients treated with placebo, and there was no difference in the number of participants with a significant response or in the magnitude of symptom reduction between both groups. We were also unable to demonstrate a clear relation between a decrease in serum bile acids and a reduction in pruritus. Since the study population consisted in the majority of patients not or insufficiently responding to other treatments, our results do not address the efficacy of colesevelam as a first line therapy.

This is the first randomized controlled trial to assess the efficacy of colesevelam in cholestatic pruritus and the second trial evaluating bile acid sequestrants for this indication. In an earlier open study with 8 participants that was only published in abstract form, colesevelam had a beneficial effect on cholestatic pruritus in 5 out of 8 patients while side effects were absent [15]. The present study only confirms observations of this and other studies that colesevelam is well tolerated and seems free of major adverse treatments effects [12; 14].

There are several potential explanations for the observed lack of efficacy of colesevelam. First this drug may not interfere with the mechanisms eventually resulting in the perception of pruritus. Since serum bile acid levels decreased in all but one person treated with colesevelam, the present study may suggest that (intestinal) bile acids are not of key importance in the pathogenesis of pruritus [2; 18]. During colesevelam treatment serum bile acid levels decreased by nearly 50%. However, in the majority of cases these levels remained markedly elevated. Therefore, an alternative explanation for the negative results of the trial may be that the decrease in serum bile acid levels was not enough to have an impact on the severity of pruritus. The negative trial result could also be related to insufficient compliance. However, all participants were highly motivated to participate in this study and the reported intake of the study medication was 100%. The observed and expected effect of

colesevelam, but not placebo on serum bile acid levels also indicates that non-compliance was highly unlikely. The trial may also have been too small to detect beneficial treatment effects. However, the basis of the power size calculation seems reasonable and we were able to recruit and analyse the required number of patients. Our inability to document a beneficial effect of colesevelam could also be related to the selected endpoints, particularly the percentage responders with a minimal 40% decrease in the severity of pruritus over a 3 week period. The decrease in pruritus score is relatively low compared with reported response rates of about 60% for bile acid sequestrants [6; 15]. However, our analysis, as illustrated in Figure 4, shows that choosing other cut-off levels would not have changed the results in any way. The trial may also have been too short in order to detect an effect on pruritus. We chose a three week treatment duration because we felt uncomfortable to withhold patients treatment for more than 6 (wash off cholestyramine and duration of trial) weeks. Moreover, longer treatment with possibly placebo was expected a major obstacle to participation and thus would have negative impact on recruitment. Also, nasobiliary drainage has an immediate effect on pruritus [18; 19], suggesting that an effect of colesevelam on enterohepatic circulation of an undefined pruritogen would at least have become apparent within three weeks. The majority of our patients experienced severe pruritus for which they had already been treated with agents including cholestyramine, naltrexone or rifampicin. Possibly the majority of the patients included may have suffered from truly refractory pruritus and the results may have been different in populations with other characteristics, for instance patients with less severe pruritus or exclusively previously untreated patients. We cannot completely exclude this possibility.

It is intriguing why the results of the present trial are negative while cholestyramine, a drug with markedly weaker bile acid binding capacity, is generally believed to be an effective drug that is usually prescribed as a first treatment option. In theory, cholestyramine could have other effects than colesevelam, for instance more potent binding of intestinal or biliary constituents undergoing an enterohepatic circulation (other than bile acids) involved in the pathogenesis of pruritus. Currently there are no data to support or refute this possibility. The rationale for the clinical use of cholestyramine is mainly based on empirical experience. The only double blind randomized trial with cholestyramine was reported by Di Padova et al. already more than 25 years ago [6]. This study, with only 10 participants, found a significant beneficial effect of cholestyramine compared to placebo (p=0.01) A positive linear

relationship between itching and serum bile acids was also demonstrated. Other studies reporting beneficial effects of cholestyramine were not placebo-controlled [5; 20]. The scientific basis for use of cholestyramine as a treatment for cholestatic pruritus is therefore weak at best [21]. The present study was the first adequately powered trial evaluating anion exchanger resins in cholestatic pruritus. The number of included patients was higher than that reported by any other comparable trials [10; 11]. Furthermore, several complimentary methods were used to assess treatment effects, and all analyses revealed consistent results. A weak aspect of the study was the unequal distribution of liver disease aetiologies over the treatment groups. This imbalance in etiology is also reflected in gender distribution. It seems unlikely, however, that these features have significantly influenced the main results of the trial.

In conclusion, this randomized placebo controlled trial shows that colesevelam is not more effective than placebo in the treatment of cholestatic pruritus.

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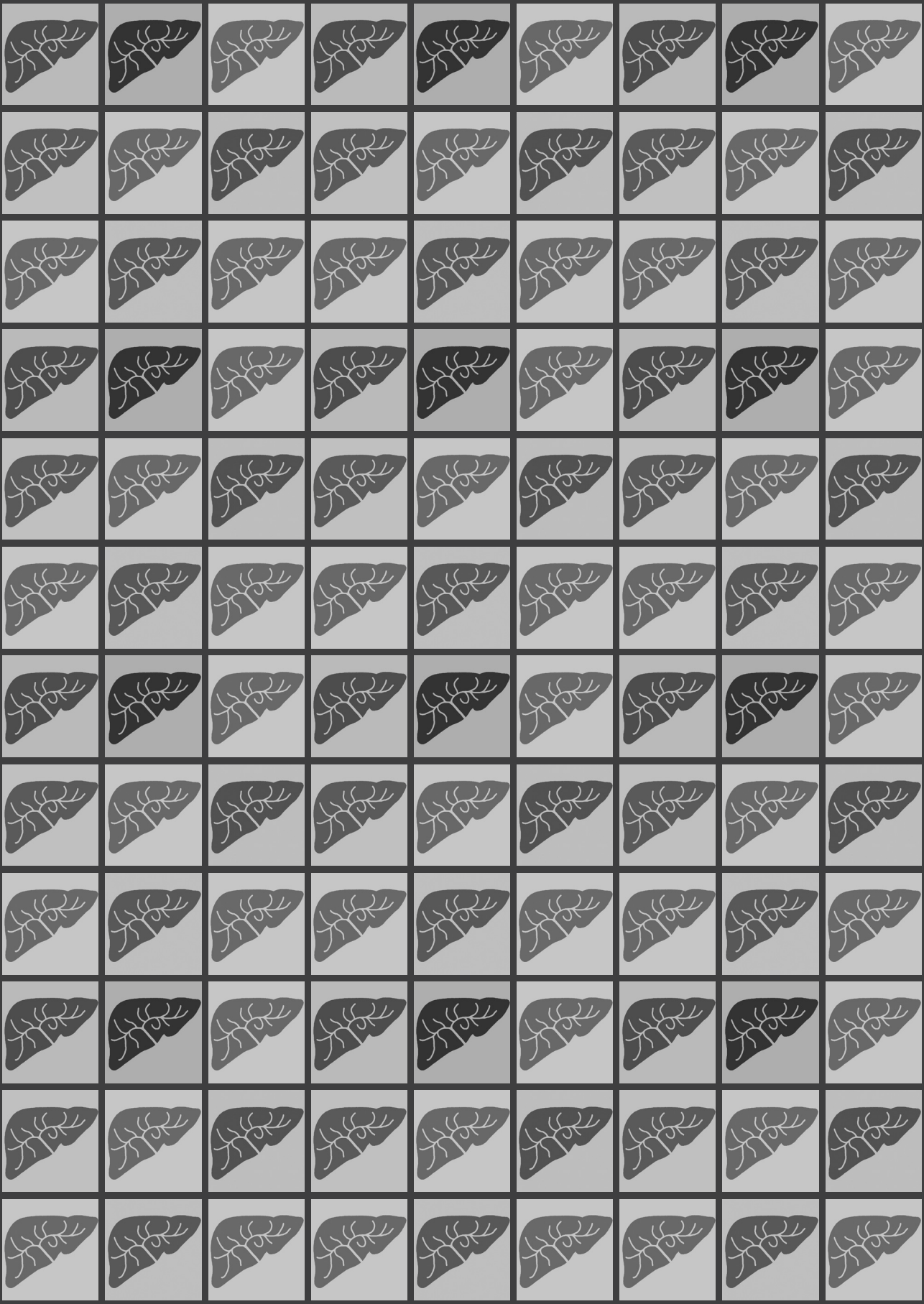

Summary and discussion

Chapter 1 provides an overview of the epidemiology, prognosis, pathogenesis and laboratory characteristics in Primary biliary cirrhosis (PBC). Further this chapter summarizes the current available therapies including liver transplantation.

PBC AND UDCA TREATMENT

Biochemical response and prognosis

In **chapter 2** we describe the biochemical improvements in serum bilirubin and albumin following treatment with UDCA which are associated with improved prognosis for patients with PBC [1]. More specifically, our data suggest that long-term prognosis, irrespective of the severity of the disease, improves if UDCA treatment results in normalization of previously abnormal bilirubin and/or albumin concentrations (based on these criteria patients are classified as either responder or non-responder). Following treatment with UDCA during 1 year, transplantation-free survival for responders and non-responders after 5 years was 95% and 70%, after 10 years 81% and 56% and after 15 years 69% and 47%, respectively.

Corpechot et al. proposed another definition for biochemical response, including ALP, AST and bilirubin levels [2]. When applied to our data, these criteria were useful, of prognostic significance and as reliable as the definition based on bilirubin and albumin. However, since these criteria are easier to apply we prefer the definition based on bilirubin and albumin levels. **Chapter 2** further confirms previous findings that the Mayo model applied before the start of treatment underestimates survival in UDCA treated patients, particularly for patients with normal bilirubin and albumin concentrations at the time of diagnosis [3; 4]. This observation is compatible with a therapeutic effect of UDCA, especially in early PBC (i.e. normal bilirubin and albumin levels at the time of diagnosis). Moreover, the present study found that prognosis for 48% of patients with (moderately) advanced PBC and responding to UDCA improved markedly, a finding compatible with therapeutic effects of UDCA not only in early but also in advanced disease.

Apart from the question whether UDCA is effective in PBC, these data indicate that biochemical response criteria can be used for optimizing prognostication. The prognosis for the majority of patients with PBC, i.e. those presenting with early disease, is excellent and comparable with that of the general population when treatment with UDCA is instituted. Prognosis for UDCA patients with more advanced disease is clearly worse than that of the general population and can be differentiated based

on the biochemical findings during treatment with UDCA. Normalization of abnormal albumin and bilirubin concentrations following UDCA treatment is associated with improved prognosis. Therefore, our data are compatible with a therapeutic effect of UDCA in PBC, irrespective of the stage of the disease.
A placebo-controlled study would be the golden standard to prove efficacy. However, a long term placebo controlled trial comparing UDCA to placebo in PBC patients is not feasible. UDCA non-responders represent a selection of individuals with unfavourable prognosis who may be candidate for alternative or additional therapy. At present, however, no established second line treatment has been defined for PBC and further studies are indicated to further explore potential benefits of agents such as nor-UDCA, obeticholic acid and budesonide.

Long term response to UDCA

Chapter 3 confirms previous reports that UDCA therapy has a beneficial effect on the serum liver tests bilirubin, albumin, alkaline phosphatase (ALP), ASAT and ALAT and that the effect is maximal within 2 – 3 years [5-7]. In addition, our data indicate that in patients with biochemically non-advanced PBC the initial treatment effect on ALP, ALT and IgM levels is maintained up to 15 years. Intriguingly, the decrease of AST levels was found to be temporary. During prolonged treatment bilirubin increases and albumin decreases, but in absolute terms the observed quantitative changes were minor and mean levels remained within normal limits. During UDCA treatment IgM levels continue to decrease up to 15 years. Although the evolution of bilirubin, albumin and transaminases is compatible with progression of the disease, the absolute changes over time were minor and probably of little clinical significance.

PBC AND HEPATOCELLULAR CARCINOMA

Chapter 4 reports that the total incidence of HCC in a cohort of 375 UDCA treated PBC patients was 2.4% after a median follow-up of 9.7 years. The annual incidence of HCC was 0.2%. Nineteen percent of liver related death was secondary to HCC and this was responsible for 30% of liver related mortality in patients with early or moderately advanced PBC. Thus, although the absolute number of cases with HCC was only nine, HCC was a significant cause of death secondary to liver disease. The most important risk factor for future development of HCC in the present study was

non-response to UDCA treatment, which was noted in 7 of the 9 HCC cases. In the sub-group of patients not responding to UDCA the incidence of HCC was 9% after 10 years and 20% after 15 years. The a posteriori calculated number needed to screen to identify a case of HCC in this population was 11. In the group of patients with advanced PBC only one case with HCC was observed. The low incidence of HCC in this subgroup might be explained by the severity of disease leading to liver related death and liver transplantation preceding potential development of HCC. Hypothetically, ongoing inflammatory activity and cholestasis superimposed on significant liver fibrosis or cirrhosis are the most important drivers of carcinogenesis in UDCA non-responsive patients. For the time being, however, it remains speculative what biological mechanism could explain the high cancer risk in UDCA non-responders.

According to the AASLD and EASL practice guidelines surveillance programs in subjects with cirrhosis should be considered when the risk of HCC is at least 1.5% per year [5; 8]. We found an annual incidence of HCC in individuals with cirrhosis (at the time of diagnosis) of only 0.2% and this would clearly argue against screening in this population. For patients not responding to UDCA the annual incidence of HCC was 1.4% in our cohort. These data suggest that the population most likely to benefit from screening would be patients who fail to respond to UDCA. Further (prospective) studies are required to confirm that the simple, non-invasive criterion of non-response to UDCA treatment is superior to cirrhosis as the criterion for selecting individuals with PBC for screening programs.

PBC AND LIVER TRANSPLANTATION

Chapter 5 shows that in the Netherlands both the absolute and the relative number of patients who received a liver transplantation for PBC has fallen during the past 20 years. The severity of disease at the time of transplantation slightly decreased while the age of the patients at the time of listing and transplantation remained unchanged.

Given the almost uniform treatment of PBC with UDCA during the past 10-15 years in the Netherlands, the use of UDCA could well be a factor contributing to the observed changes. However, it is obvious that the present study was neither designed nor suitable to adequately assess the efficacy of UDCA in PBC. Other mechanisms, like the referral pattern, indications for liver transplantation and

selection criteria, could well be involved given the many complex factors determining the eventual number of patients undergoing transplantation.

PBC AND AUTOIMMUNE HEPATITIS OVERLAP SYNDROME

5-10% of PBC patients have an overlap syndrome with autoimmune hepatitis (AIH). Recognition of this overlap syndrome is important since treatment with corticosteroids improves prognosis significantly [9].

Diagnostic criteria

In **chapter 6** the first study attempting to validate the Paris criteria for identifying patients with an overlap syndrome of PBC and AIH is described [10]. (Paris criteria: presence of at least two of the three accepted criteria for diagnosis of PBC and AIH. The diagnostic criteria for PBC are: 1. serum ALP ≥ 2-fold the upper limit of normal (ULN) or serum GGT levels ≥ 5-fold ULN; 2. positive test for AMA; 3. liver biopsy specimen showing florid bile duct lesions. The diagnostic criteria for AIH are: 1. serum ALT levels ≥ 5-fold ULN; 2. IgG levels ≥ 2-fold ULN or a positive test for SMA; and 3. liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis) [11].

Our results suggest that the Paris criteria are valuable and may be superior to the revised and also the simplified autoimmune hepatitis scoring systems for recognizing patients with both PBC and AIH.

Not unexpectedly, the sensitivity of the simplified AIH scoring system for overlap syndrome was better than that of the revised system [12; 13]. This can be explained by negative scores incurred from biliary abnormalities on histological assessment or presence of AMA using the revised system. The specificity of the revised system was comparable to that of the simplified system. In this context it should be kept in mind that these scoring systems were not specifically devised for diagnosing overlap syndromes of AIH and other disorders.

Our results confirm that the Paris criteria are useful and suggest that they may be more helpful than the numerical AIH scoring systems which were not created for diagnosing overlap syndromes. This is completely in line with the recently published EASL clinical practice guidelines for the management of cholestatic liver disease [5]. International consensus on the diagnostic criteria for overlap syndromes is absolutely necessary.

Studies on PBC – AIH overlap syndrome are severely hindered by the lack of a generally accepted diagnostic gold standard. As a consequence, the diagnostic criteria for overlap syndrome in previous studies varied widely.

Histology

The blind re-evaluation of liver biopsies revealed that in nearly 50 percent of individuals with PBC liver histology showed destructive cholangitis. In other reported series this percentage varied from 20% to 65% [14; 15]. We also found a surprisingly high prevalence of bile duct changes in our patients with AIH. A total of 72% had some type of biliary abnormality, including non-destructive cholangitis in 43% and destructive cholangitis in 24%, key features of PBC.

PBC AND SYMPTOMS; CHOLESTATIC PRURITUS

Chapter 7 shows that colesevelam, a potent bile acid sequestrant, significantly lowers serum bile acid levels in individuals with PBC and other cholestatic conditions, but is not more effective than placebo in alleviating pruritus. Although patients treated with colesevelam experienced less pruritus this was also the case in patients treated with placebo, and there was no difference in the number of participants with a significant response or in the magnitude of symptom reduction between both groups. Moreover, there was no clear relation between a decrease in serum bile acid levels and a reduction in pruritus.

This is the first randomized controlled trial to assess the efficacy of colesevelam in cholestatic pruritus and the second trial evaluating bile acid sequestrants for this indication. It is intriguing why the results of the present trial are negative while cholestyramine, a drug with markedly weaker bile acid binding capacity, is generally believed to be an effective drug that is usually prescribed as a first treatment option [16]. The rationale for the clinical use of cholestyramine is mainly based on empirical experience [17; 18]. The scientific basis for use of cholestyramine as a treatment for cholestatic pruritus is therefore weak at best. Future studies focussing on unravelling the pathogenesis of cholestatic pruritus are eagerly needed as these will hopefully result in identifying new therapeutic approaches.

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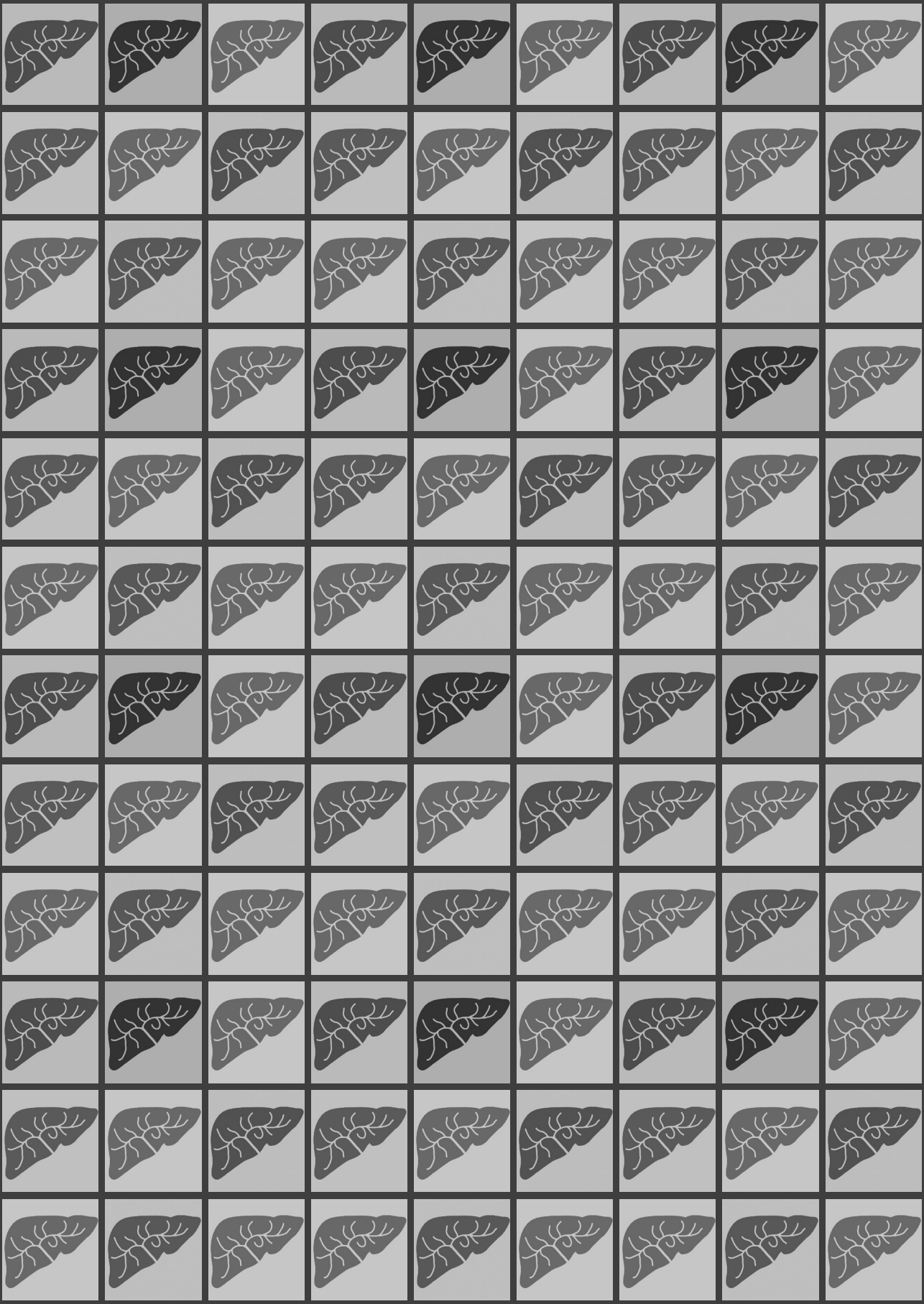

Samenvatting en discussie

Hoofdstuk 1 geeft een overzicht van de epidemiologie, pathogenese en kliniek van primaire biliaire cirrhose (PBC). Dit hoofdstuk vat bovendien de huidige behandelmogelijkheden samen, inclusief levertransplantatie.

PBC EN BEHANDELING MET UDCA

Biochemische respons en prognose

In **hoofdstuk 2** wordt de prognostische betekenis van biochemische respons op ursodeoxycholzuur (UDCA) beschreven [1]. De data laten zien dat voor met UDCA behandelde PBC patiënten de prognose op lange termijn verbetert, wanneer behandeling na 1 jaar resulteert in normalisering van voor de behandeling afwijkende serum bilirubine en/of albumine concentraties (responders). Na 1 jaar behandeling met UDCA was de transplantatie-vrije overleving voor responders en non-responders na 5 jaar respectievelijk 95% en 70%, na 10 jaar 81% en 56% en na 15 jaar 69% en 47%.

Corpechot et al. verrichtten eerder een soortgelijke analyse waarbij biochemische respons echter gedefinieerd werd op basis van veranderingen van het alkalisch fosfatase, ASAT en bilirubine gehalte [2]. Wanneer we deze responscriteria toepassen op onze data resulteert dit in een even goed prognostisch onderscheid tussen responders en non-responders dan bij gebruik van de albumine en bilirubine criteria. Wij hebben een voorkeur voor de laatste omdat deze gemakkelijker toe te passen zijn.

In **hoofdstuk 2** wordt tevens beschreven dat het bekende Mayo prognostische model voor PBC de overleving van patiënten die behandeld worden met UDCA onderschat, vooral voor de groep patiënten met een normaal bilirubine en albumine ten tijde van diagnose [3; 4]. Deze constatering komt overeen met een therapeutisch effect van UDCA, voornamelijk in deze patiëntengroep. Bovendien vonden wij dat behandeling ook de prognose verbetert voor patiënten met een meer gevorderde (gelet op afwijkend bilirubine en/of albuminegehalte) leverziekte, Deze gegevens suggereren dat UDCA een gunstig effect kan hebben ongeacht het stadium van de ziekte.

Los van de vraag of UDCA werkzaam is bij PBC patiënten, laten deze data zien dat biochemische respons criteria gebruikt kunnen worden om de prognose verder te specificeren.

Voor de meerderheid van de patiënten met PBC (bilirubine en albumine normaal) is de levensverwachting uitstekend en vergelijkbaar met die van de normale Nederlandse bevolking wanneer behandeling met UDCA wordt ingesteld. De overleving van patiënten met een gevorderde PBC (bilirubine en/of albumine afwijkend) is slechter dan die van de Nederlandse bevolking, maar zoals hierboven beschreven kan er gedifferentieerd worden op basis van de biochemische respons op UDCA.
Normalisering van bij diagnose afwijkend albumine en bilirubine dankzij behandeling met UDCA is geassocieerd met een verbeterde prognose en indirect met een therapeutisch effect van UDCA.
Placebo-gecontroleerde studies zijn de gouden standaard voor geneesmiddelonderzoek. Echter nieuwe, grotere en langduriger placebo-gecontroleerde studies dan die al verricht zijn naar het effect van UDCA ten opzichte van placebo zijn niet meer haalbaar.
Onze gegevens suggereren dat patiënten die geen respons tonen bij behandeling met UDCA een slechtere prognose hebben en daarom kandidaat zouden kunnen zijn voor aanvullende therapie.
Helaas is er vandaag de dag geen bewezen nuttige 2e lijns behandeling voor PBC beschikbaar. Aanvullende studies zijn nodig om het effect van nieuwe geneesmiddelen, zoals nor-UDCA, obetichol zuur en budesonide te testen.
Het langetermijneffect van UDCA op biochemische leverparameters
Hoofdstuk 3 bevestigt dat behandeling met UDCA een gunstig effect heeft op de gebruikelijke laboratorium levertesten, zoals bilirubine, albumine, alkalische fosfatase, ASAT en ALAT en dat het maximale effect bereikt wordt na 2 tot 3 jaar behandeling [5-7].
De effecten bij langdurig voortgezette behandeling zijn tot nu toe niet goed bekend. Wij vonden dat bij patiënten met een vroeg stadium van PBC het initiële effect op alkalische fosfatase, ALAT en IgM bij een behandelingsduur tot 15 jaar behouden bljift. Het effect op ASAT daarentegen was slechts tijdelijk. Tijdens langdurig voortgezette behandeling stijgt het bilirubine en daalt het albumine, maar deze veranderingen zijn minimaal en de waarden blijven binnen de grenzen van normaal. Opmerkelijk is dat IgM concentraties ook na jaren blijven dalen en bij vele patiënten normaal worden. Hoewel de veranderingen in bilirubine, albumine en transaminasen passen bij progressie van ziekte, zijn de absolute veranderingen klein en hebben deze waarschijnlijk weinig klinische betekenis.

PBC EN HEPATOCELLULAIR CARCINOOM

Hoofdstuk 4 laat de totale incidentie van hepatocelluair carcinoom (HCC) in een cohort met 375 met UDCA-behandelde PBC patienten zien. Deze was bij een mediane follow-up van 9.7 jaar 2,4%. De jaarlijkse incidentie was 0.2%. 19% van alle lever gerelateerde doodsoorzaken was ten gevolge van HCC en in de ernstiger stadia van PBC maakt HCC 30% uit van de lever gerelateerde mortaliteit. Hoewel het absolute aantal patiënten met HCC in deze studie slechts 9 was, is HCC dus toch verantwoordelijk voor een substantieel deel van de puur levergerelateerde sterfte. De belangrijkste risicofactor voor het ontwikkelen van HCC was in de huidige studie het niet reageren op behandeling met UDCA, gedefinieerd zoals beschreven. De respons ontbrak bij 7 van de 9 patiënten met HCC. In deze subgroep van patiënten (non-responders) was de incidentie van HCC 9% na 10 jaar en 20% na 15 jaar.
Het achteraf berekende aantal patiënten dat gescreend zou moeten worden om 1 patiënt met HCC op te sporen was 11 voor deze subgroep.
In de groep patiënten met gevorderde PBC (bilirubine en albumine afwijkend) werd slechts 1 geval met HCC gezien. De lage incidentie in deze subgroep van patiënten met een ernstige leverziekte kan misschien verklaard worden door een combinatie van ernst van de ziekte en snel overlijden danwel levertransplantatie voordat HCC zich kan ontwikkelen. Hypothetisch zou het zo kunnen zijn dat continue ontstekingsactiviteit en cholestase in een lever met fibrose of zelfs cirrose de belangrijkste factoren zijn in de carcinogenese bij non-responders. Echter, dit is pure speculatie.
Volgens de Europese en Amerikaanse richtlijnen moet surveillance voor HCC overwogen worden bij patiënten met cirrose wanneer het risico om HCC te ontwikkelen tenminste 1.5% per jaar is [5; 8].
De incidentie van HCC bij patiënten met cirrose (ten tijde van diagnose), was in ons cohort slechts 0.2%. Voor UDCA non-responders was de incidentie 1.4%. Op basis hiervan zou surveillance vooral bij deze groep daarom nuttig kunnen zijn.
Nieuwe, prospectieve studies zijn nodig om te bevestigen dat het simpele, niet invasieve biochemische responscriterium beter is dan de aanwezigheid van cirrose om patiënten te selecteren die baat zouden kunnen hebben bij een screeningsprogramma.

PBC EN LEVER TRANSPLANTATIE

Hoofdstuk 5 toont dat in Nederland zowel het absolute als het relatieve aantal lever transplantaties voor PBC in de afgelopen 20 jaar in geringe mate lijkt te dalen. De ernst van de ziekte ten tijde van transplantatie nam in de loop van de jaren iets af terwijl de leeftijd ten tijde van transplantatie hetzelfde bleef. In tegenstelling tot de beginjaren van de levertransplantatieprogramma's in Nederland (en elders) vormt PBC kwantitatief niet een belangrijke indicatie voor transplantatie
Gezien het feit dat bijna alle PBC patiënten in Nederland de afgelopen 10-15 jaar behandeld zijn met UDCA, is het mogelijk dat UDCA een rol speelt bij de daling in het aantal transplantaties. Echter, onze studieopzet was geenszins geschikt om dit op betrouwbare wijze te onderzoeken.

PBC - AUTOIMMUUN HEPATITIS OVERLAP SYNDROOM

5-10% van de PBC patiënten heeft een overlap syndroom met autoimmuun hepatitis (AIH). Herkenning van het overlap syndroom is belangrijk omdat behandeling met corticosteroïden de prognose duidelijk verbetert [9]. Het stellen van de diagnose AIH bij patiënten met PBC is evenwel notoir lastig. In **Hoofdstuk 6** wordt de eerste studie beschreven waarin getracht wordt de "Parijs criteria" te valideren. Deze criteria zijn ontwikkeld om de diagnose PBC-AIH overlap syndroom te stellen [10]. Volgens de criteria moet tenminste aan 2 van de 3 criteria voldaan worden, voor zowel de diagnose PBC als AIH. De overlap criteria voor PBC zijn: 1. serum ALP ≥ 2x de bovengrens van normaal of serum yGT ≥ 5x ULN 2. AMA positiviteit 3. een leverbiopt met een floride galganglaesie. De overlap criteria voor AIH zijn: 1. serum ALAT ≥ 5x de bovengrens van normaal 2. IgG ≥ 2x de bovengrens van normaal of positiviteit van antistoffen tegen glad spierweefsel 3. een leverbiopt met "piecemeal" necrose [11].
Onze resultaten laten zien dat de "Parijs criteria" geschikt zijn om de diagnose PBC-AIH-overlap syndroom te stellen, en beter lijken te voldoen dan de "revised" en "simplified" autoimmuun hepatitis score systemen [12;13].
Bij ''blinde" herbeoordeling van lever biopten (PBC, AIH en overlap syndroom) werd bij bijna 50% van de PBC patiënten destructieve cholangitis gezien. In andere series werd dit bij 20-65% van de patiënten gevonden [14; 15]. De prevalentie van galgang veranderingen bij AIH patiënten was verrassend hoog. In totaal had 72%

van de patiënten in meerdere of mindere mate een galgang afwijking; variërend van non-destructieve cholangitis in 43% van de gevallen tot destructieve cholangitis – een typisch kenmerk van PBC - in 24% van de gevallen. Deze data geven aan dat histologische criteria op zich weinig behulpzaam zijn bij het stellen van de diagnose overlapsyndroom.
Onze bevindingen betreffende de diagnostische waarde van de Parijs criteria ondersteunen hetgeen hierover gesteld is in de onlangs gepubliceerde Europese richtlijnen over cholestatische leverziekten [5]. Internationale consensus over de diagnostische criteria is van groot belang. Gepubliceerde studies naar PBC-AIH-overlap syndroom zijn moeilijk met elkaar te vergelijken omdat verschillende diagnostische criteria toegepast zijn en een gouden standaard ontbreekt.

PBC EN SYMPTOMEN; CHOLESTATISCHE JEUK

Hoofdstuk 7 beschrijft dat colesevelam, een intestinale galzuur binder, de serum galzuren concentratie significant verlaagt bij patiënten met PBC en andere cholestatische aandoeningen, maar niet effectiever is dan placebo bij het verlichten van jeuk. Hoewel patiënten behandeld met colesevelam minder jeuk ervaarden, was dit ook het geval bij patiënten die met placebo behandeld werden. Er was bovendien geen verschil tussen beide groepen met betrekking tot het aantal patiënten met een significante respons en de mate van jeuk reductie, ook was er geen duidelijke relatie tussen een daling van de serum galzuurspiegel en de afname van jeuk.
Dit is de eerste gerandomiseerde placebo gecontroleerde studie naar het effect van colesevelam op cholestatische jeuk en pas de tweede studie naar het effect van galzuurbinders voor deze indicatie. Het is een interessante vraag waarom de resultaten van de huidige studie negatief zijn, terwijl cholestyramine, een medicijn met een zwakkere capaciteit om galzuren te binden, universeel gezien wordt als de 1e lijns behandeling [16]. Het gebruik van cholestyramine is vooral gebaseerd op klinische ervaring [17; 18], de wetenschappelijke onderbouwing is echter zeer beperkt. Toekomstige studies naar de pathogenese van cholestatische jeuk en daarmee naar nieuwe behandelingsmogelijkheden zijn noodzakelijk.

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PHD PORTFOLIO

International conferences: oral presentations

2010 The potent bile acid sequestrant Colesevelam is not effective in cholestatic pruritus.
Dutch Society of Hepatology. Veldhoven, the Netherlands.

2009 The potent bile acid sequestrant Colesevelam is not effective in cholestatic pruritus.
Liver Day. Rotterdam, the Netherlands.
The long-term effect of ursodeoxycholic acid on laboratory liver parameters in biochemically non-advanced primary biliary cirrhosis.
Dutch Society of Hepatology. Veldhoven, the Netherlands.
Relatively high risk for hepatocellular carcinoma in patients with primary biliary cirrhosis not responding to ursodeoxycholic acid.
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Trends in liver transplantation for primary biliary cirrhosis in the Netherlands 1988-2008.
Dutch Society of Hepatology. Veldhoven, the Netherlands.

2008 New therapies in cholestatic pruritus.
Diner Pensant. Utrecht, the Netherlands.
Improved prognosis for patients with primary biliary cirrhosis showing relevant biochemical response to UDCA. Results of a multicenter long-term cohort study involving 375 patients.
Dutch Society of Hepatology. Veldhoven, the Netherlands.
Patients with early PBC predominantly die from non-liver related causes
Dutch Society of Hepatology. Veldhoven, the Netherlands.
Efficacy of nasobiliary drainage for refractory cholestatic pruritus
Dutch Society of Hepatology. Veldhoven, the Netherlands.
Prognosis of 375 ursodeoxycholic acid-treated patients with primary biliary cirrhosis. A follow-up to 17-yrs.
Dutch Society of Hepatology. Veldhoven, the Netherlands.
Prognosis of 375 ursodeoxycholic acid-treated patients with primary biliary cirrhosis. A follow-up to 17-yrs.
43th meeting EASL. Milan, Italy.

International conferences: poster presentations

2009 The long-term effect of ursodeoxycholic acid on laboratory liver parameters in biochemically non-advanced primary biliary cirrhosis.
AASLD liver meeting, Boston, USA.
Relatively high risk for hepatocellular carcinoma in patients with primary biliary cirrhosis not responding to ursodeoxycholic acid.
AASLD liver meeting, Boston, USA.
Trends in liver transplantation for primary biliary cirrhosis in the Netherlands 1988-2008.
AASLD liver meeting, Boston, USA.

2008 Normalization of serum bilirubin and/or albumin levels following treatment with UDCA is associated with improved prognosis for patients with moderately advanced and advanced primary biliary cirrhosis
AASLD liver meeting, San Francisco, USA.
Improved prognosis for patients with primary biliary cirrhosis showing relevant biochemical response to UDCA. Results of a multicenter long-term cohort study involving 375 patients.
AASLD liver meeting, San Francisco, USA.
Patients with early PBC predominantly die from non liver related causes
43th meeting EASL. Milan, Italy.

Memberships

2009 American Gastroenterological Association
2006 Dutch Society of Gastroenterology
2006 Dutch Society of Hepatology

Peer reviewer activities

2009 Hepatology

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CURRICULUM VITAE

Edith Kuiper werd geboren op 1 december 1981 in Margraten. Na het behalen van haar gymnasium diploma aan het Sint Maartens College te Maastricht in 2000 startte zij met de studie geneeskunde aan de Universiteit Maastricht. Zij liep co-schappen onder andere in Zuid-Afrika, Trinidad & Tobago en Malta. Na het behalen van haar artsexamen startte zij in 2006 haar promotieonderzoek met het onderwerp "primaire biliaire cirrose" op de afdeling Maag-, Darm- en Leverziekten van het Erasmus MC in Rotterdam onder begeleiding van haar promotor professor H.L.A. Janssen en haar co-promotor dr. H.R. van Buuren. In 2008 ontving zij tijdens het Europese lever congres (EASL) de "Best Clinical Abstract Award" en van de Nederlandse Vereniging voor Hepatologie de "beste voordrachts-prijs". Per 1 april 2009 is zij gestart met de opleiding tot MDL-arts vanuit het Erasmus MC (opleider: dr. R.A. de Man), thans in vooropleiding in het Reinier de Graaf Gasthuis in Delft (opleider: dr. E.F.M. Posthuma). Ze woont samen met Pieter Soetaert in Rotterdam.